ISSN 2220-3230 (online)

World Journal of *Transplantation*

World J Transplant 2017 February 24; 7(1): 1-102

Published by Baishideng Publishing Group Inc

WJT World Journal of Transplantation

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

Editorial Board

2016-2019

The *World Journal of Transplantation* Editorial Board consists of 361 members, representing a team of worldwide experts in transplantation. They are from 43 countries, including Argentina (1), Australia (7), Austria (3), Belgium (4), Brazil (7), Bulgaria (1), Canada (13), China (32), Cuba (1), Czech Republic (1), Denmark (1), Finland (1), France (4), Georgia (1), Germany (14), Greece (5), Hungary (2), India (7), Iran (7), Israel (4), Italy (33), Japan (18), Jordan (1), Macedonia (1), Mexico (2), Morocco (1), Netherlands (4), Nigeria (1), Norway (1), Pakistan (1), Poland (2), Qatar (1), Saudi Arabia (3), Singapore (1), South Korea (16), Spain (9), Sweden (1), Switzerland (3), Thailand (2), Tunisia (1), Turkey (6), United Kingdom (17), and United States (120).

WJT World Journal of Transplantation

Contents

Bimonthly Volume 7 Number 1 February 24, 2017

CASE REPORT

World Journal of Transplantation
Volume 7 Number 1 February 24, 2017

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Transplantation*, Michael A Ramsay, MD, Professor, Department of Anesthesiology, Baylor University Medical Center, Dallas, TX 75240, 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, PubMed Central.

FLYLEAF

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Ya-Jing Lu*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Fang-Fang Ji*
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL
World Journal of Transplantation

ISSN
ISSN 2220-3230 (online)

LAUNCH DATE
December 24, 2011

FREQUENCY
Bimonthly

EDITORIAL OFFICE
Xiu-Xia Song, Director

World Journal of Transplantation
Baishideng Publishing Group Inc
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
February 24, 2017

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Transplantation

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Transplant 2017 February 24; 7(1): 1-25

DOI: 10.5500/wjt.v7.i1.1

ISSN 2220-3230 (online)

REVIEW

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

Marina Burgos da Silva, Flavia Franco da Cunha, Fernanda Fernandes Terra, Niels Olsen Saraiva Camara

Marina Burgos da Silva, Fernanda Fernandes Terra, Niels Olsen Saraiva Camara, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo 05508-900, Brazil

Flavia Franco da Cunha, Niels Olsen Saraiva Camara, Nephrology Division, Department of Medicine, Federal University of São Paulo, São Paulo 04039-032, Brazil

Niels Olsen Saraiva Camara, Renal Pathophysiology Laboratory, Faculty of Medicine, University of São Paulo, São Paulo 01246-903, Brazil

Author contributions: da Silva MB, da Cunha FF and Terra FF contributed equally to this work; da Silva MB, da Cunha FF and Terra FF performed the research and wrote the paper; Camara NOS analyzed the paper, discussed the topic and supervised the publication of this review.

Supported by São Paulo Research Foundation - FAPESP, Nos. 2012/23347-3, 2014/14147-6, 2012/02270-2 and CNPq.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Manuscript source: Invited manuscript

Correspondence to: Niels Olsen Saraiva Camara, MD, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, 1730 Av. Prof Lineu Prestes - Cidade Universitária, São Paulo 05508-900, Brazil. niels@icb.usp.br
Telephone: +55-11-30917388
Fax: +55-11-30917324

Received: August 15, 2016
Peer-review started: August 23, 2016
First decision: September 28, 2016
Revised: November 16, 2016
Accepted: December 7, 2016
Article in press: December 9, 2016
Published online: February 24, 2017

Abstract

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

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

tolerance

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

da Silva MB, da Cunha FF, Terra FF, Camara NOS. Old game, new players: Linking classical theories to new trends in transplant immunology. *World J Transplant* 2017; 7(1): 1-25 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i1/1.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i1.1

INTRODUCTION

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

ALLOANTIGENS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

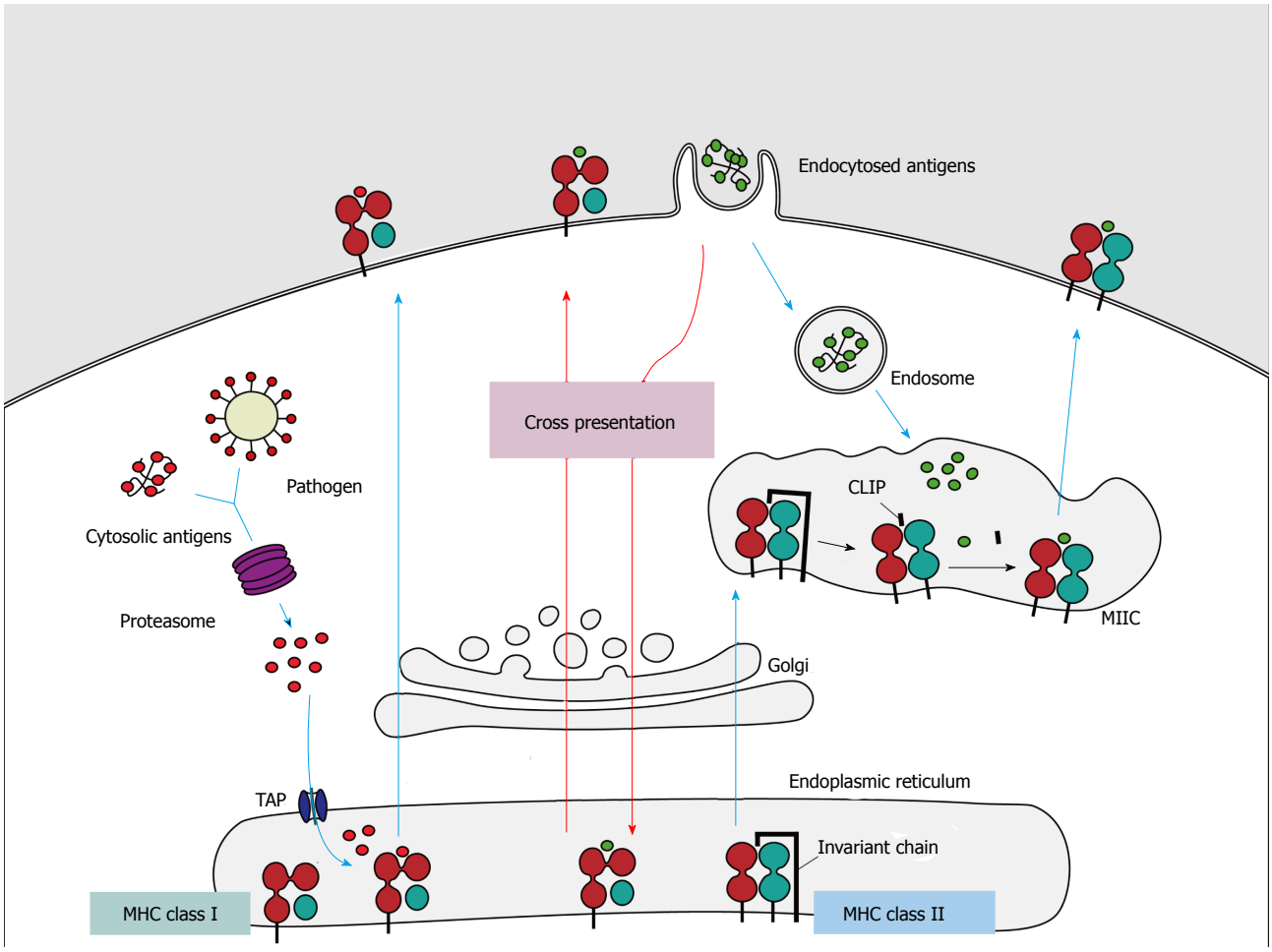


Figure 1 Major histocompatibility complex class Ⅰ and Ⅱ pathways. (1) MHC class Ⅰ molecules present peptides derived from proteins presented in the cytosol of endogenous or pathogen origin. The proteasome breaks down these proteins into peptides, which are then translocated to ER by the transporter associated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

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

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

ANTIGEN PRESENTATION IN TRANSPLANTATION

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

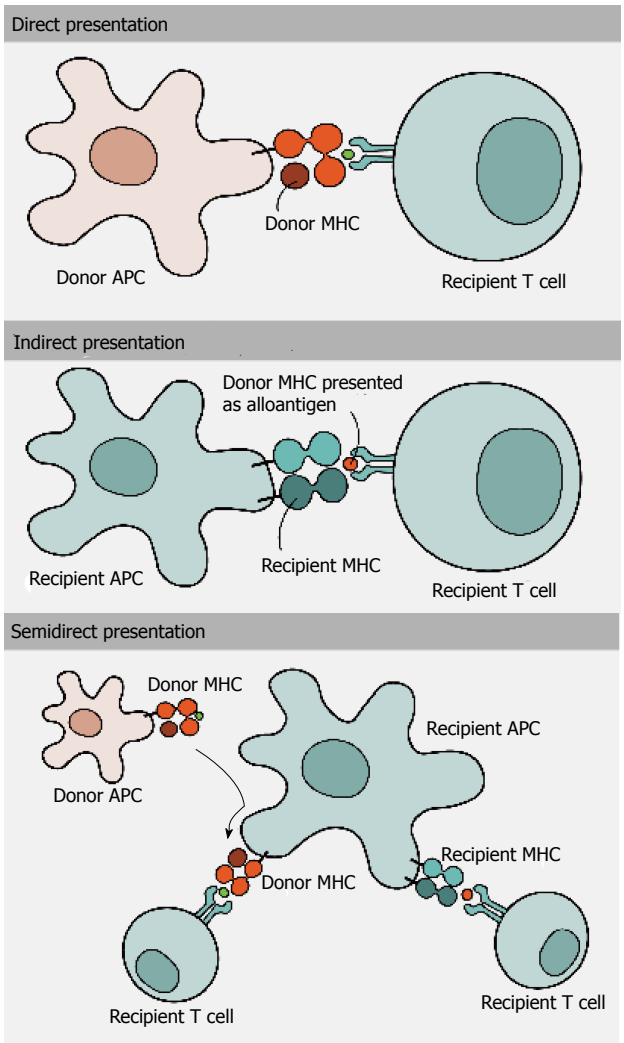


Figure 2 Antigen presentation and allorecognition. T cells can recognize alloantigens by three different pathways of allorecognition: (1) Direct pathway involves the recognition of intact donor MHC molecules on the cell surface of donor APCs by recipient T cells; (2) In contrast, in indirect pathway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

alloantigens expressed by the donor.

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

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

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

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

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

ALLOGENEIC REJECTION: THE CLASSICAL VIEW

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

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

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

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

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

ALLOGENEIC REJECTION: AN UPDATED VIEW

Innate immunity in graft rejection

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

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

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

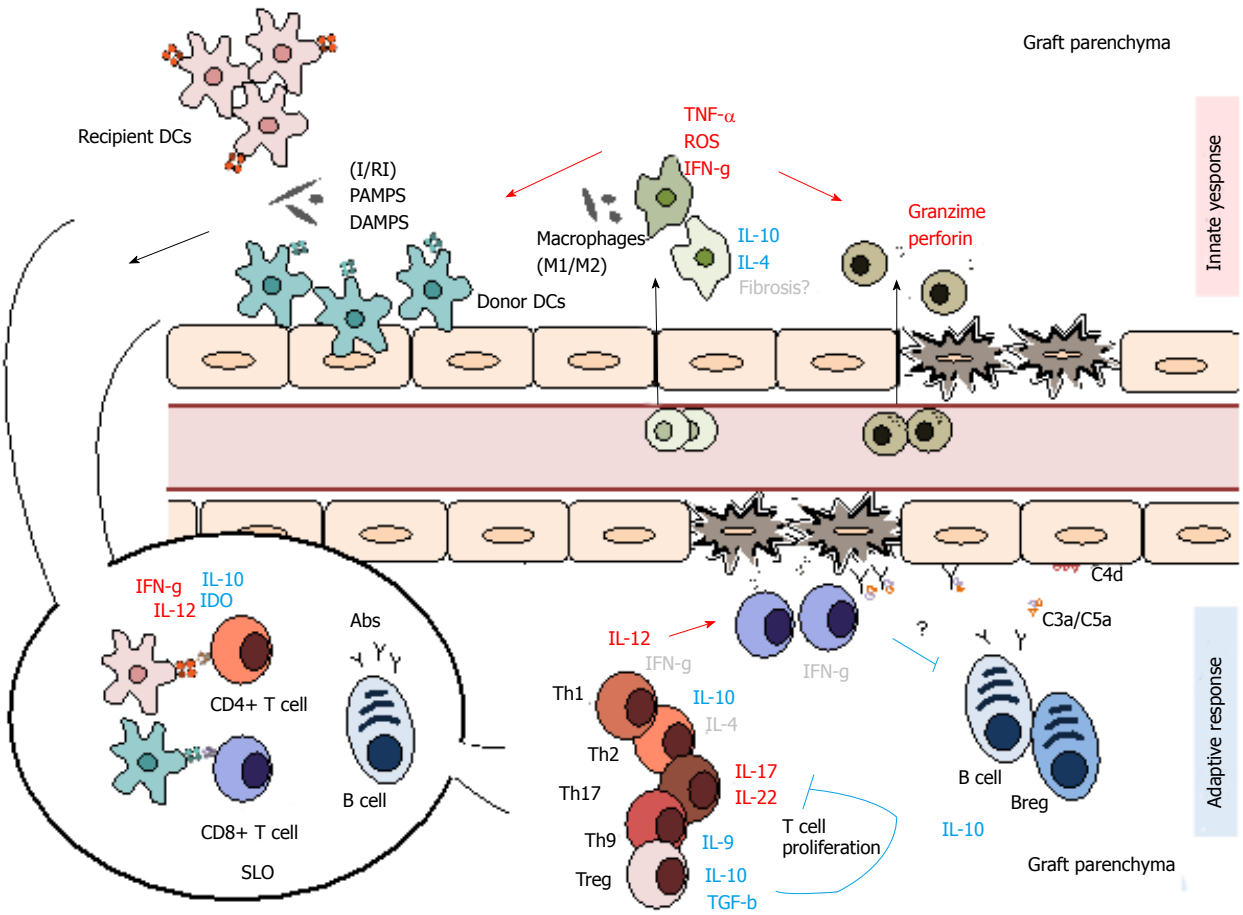


Figure 3 Summary of the main innate and adaptive mediators of graft rejection. Alloimmune rejection is a multifaceted process that involves both innate and adaptive mediators. Initial tissue damage is mostly mediated by innate participants as macrophages and NK cells along with dendritic cells, which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

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

Macrophages

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

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

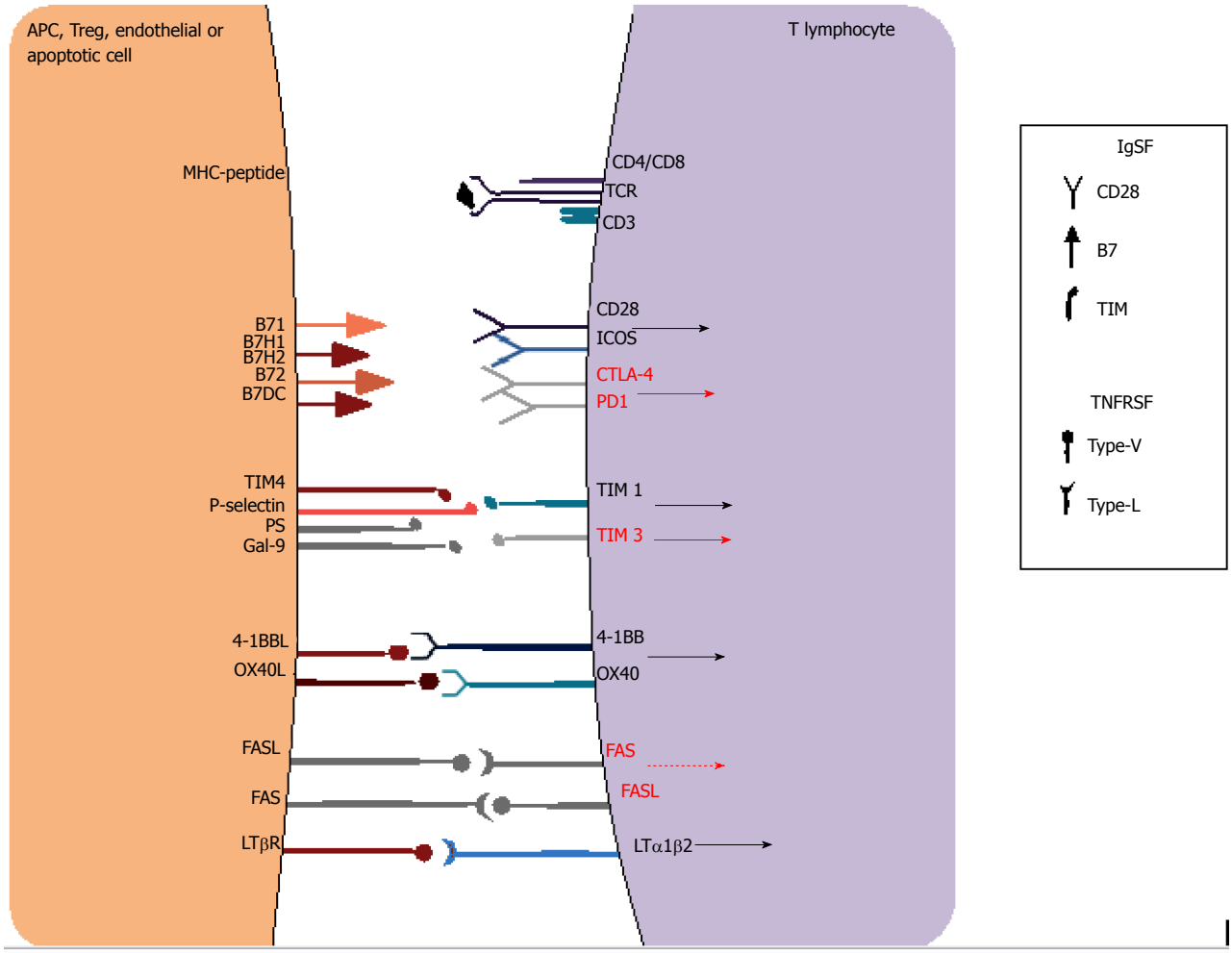


Figure 4 Examples of co-stimulatory receptors in immune synapse interactions. T cell activation depends not only on the antigen-specific signals provided by MHC-TCR signaling but also on a complex balance of co-stimulatory signals that may enhance or inhibit activation. These receptors are mainly classified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

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

NK cells

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

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

NKT cells

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

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

DCs

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

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

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

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

ADAPTIVE IMMUNITY

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

T cells

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

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

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

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

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

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

Cytotoxic T cells

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

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

Memory T cells

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

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

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

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

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

B cells

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

Antibody-mediated rejection and complement

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

IMMUNOSUPPRESSIVE DRUGS AND TOLERANCE

Immunosuppression

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

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

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

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

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

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

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

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

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

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

Tolerance

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

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

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

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

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

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

Regulatory T cells

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

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

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

DCs

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

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

Regulatory B cells

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

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

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

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

Mesenchymal stromal cells

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

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

Fetal tolerance

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

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

CONCLUSION

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

REFERENCES

1 **Gibson T**, Medawar PB. The fate of skin homografts in man. *J Anat* 1943; **77**: 299-310.4 [PMID: 17104936]

2 **Medawar PB**. The behaviour and fate of skin autografts and skin homografts in rabbits: A report to the War Wounds Committee of the Medical Research Council. *J Anat* 1944; **78**: 176-199 [PMID: 17104960]

3 **Mitchison NA**. Passive transfer of transplantation immunity. *Proc R Soc Lond B Biol Sci* 1954; **142**: 72-87 [PMID: 13145632]

4 **Billingham RE**, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. *Nature* 1953; **172**: 603-606 [PMID: 13099277]

5 **Sherman LA**, Chattopadhyay S. The molecular basis of allorecognition. *Annu Rev Immunol* 1993; **11**: 385-402 [PMID: 8476567 DOI: 10.1146/annurev.iy.11.040193.002125]

6 **Hernandez-Fuentes MP**, Baker RJ, Lechler RI. The alloresponse.

Rev Immunogenet 1999; **1**: 282-296 [PMID: 11256420]
7 **Gorer P**. The genetic and antigenic basis of tumor transplantation. *J Pathol Bacteriol* 1937; **44**: 691-697
8 **Guild WR**, Harrison JH, Merrill JP, Murray J. Successful homotransplantation of the kidney in an identical twin. *Trans Am Clin Climatol Assoc* 1955; **67**: 167-173 [PMID: 13360847]
9 **Michon L**, Hamburger J, Oeconomos N, Delinotte P, Richet G, Vaysse J, Antoine B. [An attempted kidney transplantation in man: medical and biological aspects]. *Presse Med* 1953; **61**: 1419-1423 [PMID: 13120746]
10 **Afzali B**, Lombardi G, Lechler RI. Pathways of major histocompatibility complex allorecognition. *Curr Opin Organ Transplant* 2008; **13**: 438-444 [PMID: 18685342 DOI: 10.1097/MOT.0b013e328309ee31]
11 **Snell GD**. Methods for the study of histocompatibility genes. *J Genet* 1948; **49**: 87-108 [PMID: 18893744]
12 **Klein J**, Sato A. The HLA system. First of two parts. *N Engl J Med* 2000; **343**: 702-709 [PMID: 10974135 DOI: 10.1056/NEJM200009073431006]
13 **King A**, Hiby SE, Gardner L, Joseph S, Bowen JM, Verma S, Burrows TD, Loke YW. Recognition of trophoblast HLA class I molecules by decidual NK cell receptors--a review. *Placenta* 2000; **21** Suppl A: S81-S85 [PMID: 10831129]
14 **Bodmer JG**, Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, Hansen JA, Mach B, Mayr WR, Parham P, Petersdorf EW, Sasazuki T, Schreuder GM, Strominger JL, Svejgaard A, Terasaki PI. Nomenclature for factors of the HLA system, 1998. *Tissue Antigens* 1999; **53**: 407-446 [PMID: 10321590]
15 **Chinen J**, Buckley RH. Transplantation immunology: solid organ and bone marrow. *J Allergy Clin Immunol* 2010; **125**: S324-S335 [PMID: 20176267 DOI: 10.1016/j.jaci.2009.11.014]
16 **Doxiadis II**, Smits JM, Schreuder GM, Persijn GG, van Houwelingen HC, van Rood JJ, Claas FH. Association between specific HLA combinations and probability of kidney allograft loss: the taboo concept. *Lancet* 1996; **348**: 850-853 [PMID: 8826810]
17 **Opelz G**. Correlation of HLA matching with kidney graft survival in patients with or without cyclosporine treatment. *Transplantation* 1985; **40**: 240-243 [PMID: 3898488]
18 **Gilks WR**, Bradley BA, Gore SM, Klouda PT. Substantial benefits of tissue matching in renal transplantation. *Transplantation* 1987; **43**: 669-674 [PMID: 3554659]
19 **Doxiadis II**, de Fijter JW, Mallat MJ, Haasnoot GW, Ringers J, Persijn GG, Claas FH. Simpler and equitable allocation of kidneys from postmortem donors primarily based on full HLA-DR compatibility. *Transplantation* 2007; **83**: 1207-1213 [PMID: 17496537 DOI: 10.1097/01.tp.0000261108.27421.bc]
20 **Reisaeter AV**, Leivestad T, Vartdal F, Spurkland A, Fauchald P, Brekke IB, Thorsby E. A strong impact of matching for a limited number of HLA-DR antigens on graft survival and rejection episodes: a single-center study of first cadaveric kidneys to nonsensitized recipients. *Transplantation* 1998; **66**: 523-528 [PMID: 9734498]
21 **Luz JG**, Huang M, Garcia KC, Rudolph MG, Apostolopoulos V, Teyton L, Wilson IA. Structural comparison of allogeneic and syngeneic T cell receptor-peptide-major histocompatibility complex complexes: a buried alloreactive mutation subtly alters peptide presentation substantially increasing V(beta) Interactions. *J Exp Med* 2002; **195**: 1175-1186 [PMID: 11994422]
22 **Germain RN**. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. *Cell* 1994; **76**: 287-299 [PMID: 8293464]
23 **York IA**, Rock KL. Antigen processing and presentation by the class I major histocompatibility complex. *Annu Rev Immunol* 1996; **14**: 369-396 [PMID: 8717519 DOI: 10.1146/annurev.immunol.14.1.369]
24 **Chaplin DD**. 1. Overview of the human immune response. *J Allergy Clin Immunol* 2006; **117**: S430-S435 [PMID: 16455341 DOI: 10.1016/j.jaci.2005.09.034]
25 **Fellay J**, Shianna KV, Ge D, Colombo S, Ledergerber B, Weale M, Zhang K, Gumbs C, Castagna A, Cossarizza A, Cozzi-Lepri A, De Luca A, Easterbrook P, Francioli P, Mallal S, Martinez-Picado J, Miro JM, Obel N, Smith JP, Wyniger J, Descombes P, Antonarakis SE, Letvin NL, McMichael AJ, Haynes BF, Telenti A, Goldstein DB. A whole-genome association study of major determinants for host control of HIV-1. *Science* 2007; **317**: 944-947 [PMID: 17641165 DOI: 10.1126/science.1143767]
26 **Fellermann K**, Stange DE, Schaeffeler E, Schmalzl H, Wehkamp J, Bevins CL, Reinisch W, Teml A, Schwab M, Lichter P, Radlwimmer B, Stange EF. A chromosome 8 gene-cluster polymorphism with low human beta-defensin 2 gene copy number predisposes to Crohn disease of the colon. *Am J Hum Genet* 2006; **79**: 439-448 [PMID: 16909382 DOI: 10.1086/505915]
27 **Barcellos LF**, Sawcer S, Ramsay PP, Baranzini SE, Thomson G, Briggs F, Cree BC, Begovich AB, Villoslada P, Montalban X, Uccelli A, Savettieri G, Lincoln RR, DeLoa C, Haines JL, Pericak-Vance MA, Compston A, Hauser SL, Oksenberg JR. Heterogeneity at the HLA-DRB1 locus and risk for multiple sclerosis. *Hum Mol Genet* 2006; **15**: 2813-2824 [PMID: 16905561 DOI: 10.1093/hmg/ddl223]
28 **Bahram S**, Bresnahan M, Geraghty DE, Spies T. A second lineage of mammalian major histocompatibility complex class I genes. *Proc Natl Acad Sci USA* 1994; **91**: 6259-6263 [PMID: 8022771 DOI: 10.1073/pnas.91.14.6259]
29 **Racca AL**, Veaute CM, Bailat AS, Gaite L, Arriola M, Hajos SE, Malan Borel IS. Expression of HLA-G and MICA mRNA in renal allograft. *Transpl Immunol* 2009; **21**: 10-12 [PMID: 19193353 DOI: 10.1016/j.trim.2009.01.002]
30 **Tonnerre P**, Gérard N, Chatelais M, Charreau B. MICA gene polymorphism in kidney allografts and possible impact of functionally relevant variants. *Transplant Proc* 2010; **42**: 4318-4321 [PMID: 21168690 DOI: 10.1016/j.transproceed.2010.09.118]
31 **Zwirner N**, Fuertes MB, Girart MV, Domaica CI, Rossi LE. Immunobiology of the human MHC class I chain-related gene A (MICA): from transplantation immunology to tumour immune escape. *Inmunología* 2006: 378-385
32 **Stastny P**. Introduction: MICA/MICB in innate immunity, adaptive immunity, autoimmunity, cancer, and in the immune response to transplants. *Hum Immunol* 2006; **67**: 141-144 [PMID: 16698435 DOI: 10.1016/j.humimm.2006.02.019]
33 **Sumitran-Holgersson S**. Relevance of MICA and other non-HLA antibodies in clinical transplantation. *Curr Opin Immunol* 2008; **20**: 607-613 [PMID: 18675346 DOI: 10.1016/j.coi.2008.07.005]
34 **Cobbold SP**. Rejecting minors--it's all in the presentation. *Transplantation* 2011; **91**: 152-153 [PMID: 21079548 DOI: 10.1097/TP.0b013e318201ac7b]
35 **Mizutani K**, Terasaki P, Bignon JD, Hourmant M, Cesbron-Gautier A, Shih RN, Pei R, Lee J, Ozawa M. Association of kidney transplant failure and antibodies against MICA. *Hum Immunol* 2006; **67**: 683-691 [PMID: 17002898 DOI: 10.1016/j.humimm.2006.06.002]
36 **Martin LH**, Calabi F, Milstein C. Isolation of CD1 genes: a family of major histocompatibility complex-related differentiation antigens. *Proc Natl Acad Sci USA* 1986; **83**: 9154-9158 [PMID: 3097645 DOI: 10.1073/pnas.83.23.9154]
37 **Calabi F**, Milstein C. A novel family of human major histocompatibility complex-related genes not mapping to chromosome 6. *Nature* 1986; **323**: 540-543 [PMID: 3093894 DOI: 10.1038/323540a0]
38 **Sonoda KH**, Taniguchi M, Stein-Streilein J. Long-term survival of corneal allografts is dependent on intact CD1d-reactive NKT cells. *J Immunol* 2002; **168**: 2028-2034 [PMID: 11823540 DOI: 10.4049/jimmunol.168.4.2028]
39 **Wang B**, Chun T, Rulifson IC, Exley M, Balk SP, Wang CR. Human CD1d functions as a transplantation antigen and a restriction element in mice. *J Immunol* 2001; **166**: 3829-3836 [PMID: 11238626 DOI: 10.4049/jimmunol.166.6.3829]
40 **Sumitran-Karuppan S**, Tyden G, Reinholt F, Berg U, Moller E. Hyperacute rejections of two consecutive renal allografts and early loss of the third transplant caused by non-HLA antibodies specific for endothelial cells. *Transpl Immunol* 1997; **5**: 321-327 [PMID: 9504155 DOI: 10.1016/S0966-3274(97)80016-0]
41 **Perrey C**, Brenchley PE, Johnson RW, Martin S. An association between antibodies specific for endothelial cells and renal transplant failure. *Transpl Immunol* 1998; **6**: 101-106 [PMID: 9777698 DOI: 10.1016/S0966-3274(98)80024-5]

42 **Faulk WP**, Rose M, Meroni PL, Del Papa N, Torry RJ, Labarrere CA, Busing K, Crisp SJ, Dunn MJ, Nelson DR. Antibodies to endothelial cells identify myocardial damage and predict development of coronary artery disease in patients with transplanted hearts. *Hum Immunol* 1999; **60**: 826-832 [PMID: 10527389 DOI: 10.1016/S0198-8859(99)00056-7]

43 **Barth R**, Counce S, Smith P, Snell GD. Strong and weak histocompatibility gene differences in mice and their role in the rejection of homografts of tumors and skin. *Ann Surg* 1956; **144**: 198-204 [PMID: 13355191 DOI: 10.1097/00000658-195608000-00009]

44 **Simpson E**, Scott D, James E, Lombardi G, Cwynarski K, Dazzi F, Millrain M, Dyson PJ. Minor H antigens: genes and peptides. *Transpl Immunol* 2002; **10**: 115-123 [PMID: 12216941 DOI: 10.1016/S0966-3274(02)00057-6]

45 **Wettstein PJ**. Immunodominance in the T-cell response to multiple non-H-2 histocompatibility antigens. II. Observation of a hierarchy among dominant antigens. *Immunogenetics* 1986; **24**: 24-31 [PMID: 2426194 DOI: 10.1007/BF00372294]

46 **Claas FH**, Paul LC, van Es LA, van Rood JJ. Antibodies against donor antigens on endothelial cells and monocytes in eluates of rejected kidney allografts. *Tissue Antigens* 1980; **15**: 19-24 [PMID: 12735328 DOI: 10.1111/j.1399-0039.1980.tb00880.x]

47 **Goulmy E**. Minor histocompatibility antigens: from transplantation problems to therapy of cancer. *Hum Immunol* 2006; **67**: 433-438 [PMID: 16728266 DOI: 10.1016/j.humimm.2006.03.012]

48 **Goulmy E**, Gratama JW, Blokland E, Zwaan FE, van Rood JJ. A minor transplantation antigen detected by MHC-restricted cytotoxic T lymphocytes during graft-versus-host disease. *Nature* 1983; **302**: 159-161 [PMID: 6186923]

49 **Loveland B**, Wang CR, Yonekawa H, Hermel E, Lindahl KF. Maternally transmitted histocompatibility antigen of mice: a hydrophobic peptide of a mitochondrially encoded protein. *Cell* 1990; **60**: 971-980 [PMID: 2317868 DOI: 10.1038/302159a0]

50 **Bhuyan PK**, Young LL, Lindahl KF, Butcher GW. Identification of the rat maternally transmitted minor histocompatibility antigen. *J Immunol* 1997; **158**: 3753-3760 [PMID: 9103440]

51 **Game DS**, Lechler RI. Pathways of allorecognition: implications for transplantation tolerance. *Transpl Immunol* 2002; **10**: 101-108 [PMID: 12216939 DOI: 10.1016/S0966-3274(02)00055-2]

52 **Otten HG**, van den Bosch JM, van Ginkel WG, van Loon M, van de Graaf EA. Identification of non-HLA target antigens recognized after lung transplantation. *J Heart Lung Transplant* 2006; **25**: 1425-1430 [PMID: 17178336 DOI: 10.1016/j.healun.2006.09.022]

53 **Grandtnerová B**, Laca L, Jahnová E, Horváthová M, Baláz V, Hovoricová B, Hudec P, Zarnovicanová M. Hyperacute rejection of living related kidney graft caused by IgG endothelial specific antibodies with a negative monocyte cross-match. *Ann Transplant* 2002; **7**: 52-54 [PMID: 12854349]

54 **Sumitran-Holgersson S**, Ge X, Karrar A, Xu B, Nava S, Broomé U, Nowak G, Ericzon BG. A novel mechanism of liver allograft rejection facilitated by antibodies to liver sinusoidal endothelial cells. *Hepatology* 2004; **40**: 1211-1221 [PMID: 15486937 DOI: 10.1002/hep.20434]

55 **Mickelson EM**, Fefer A, Storb R, Thomas ED. Correlation of the relative response index with marrow graft rejection in patients with aplastic anemia. *Transplantation* 1976; **22**: 294-302 [PMID: 135386]

56 **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]

57 **Suchin EJ**, Langmuir PB, Palmer E, Sayegh MH, Wells AD, Turka LA. Quantifying the frequency of alloreactive T cells in vivo: new answers to an old question. *J Immunol* 2001; **166**: 973-981 [PMID: 11145675]

58 **Barroso M**, Tucker H, Drake L, Nichol K, Drake JR. Antigen-B Cell Receptor Complexes Associate with Intracellular major histocompatibility complex (MHC) Class II Molecules. *J Biol Chem* 2015; **290**: 27101-27112 [PMID: 26400081 DOI: 10.1074/jbc.M115.649582]

59 **Hobeika E**, Nielsen PJ, Medgyesi D. Signaling mechanisms regulating B-lymphocyte activation and tolerance. *J Mol Med* (Berl) 2015; **93**: 143-158 [PMID: 25627575 DOI: 10.1007/s00109-015-1252-8]

60 **Lanzavecchia A**. Antigen-specific interaction between T and B cells. *Nature* 1985; **314**: 537-539 [PMID: 3157869]

61 **Warrens AN**, Lombardi G, Lechler RI. Presentation and recognition of major and minor histocompatibility antigens. *Transpl Immunol* 1994; **2**: 103-107 [PMID: 7953301]

62 **Matzinger P**, Bevan MJ. Hypothesis: why do so many lymphocytes respond to major histocompatibility antigens? *Cell Immunol* 1977; **29**: 1-5 [PMID: 300293]

63 **Kourilsky P**, Chaouat G, Rabourdin-Combe C, Claverie JM. Working principles in the immune system implied by the "peptidic self" model. *Proc Natl Acad Sci USA* 1987; **84**: 3400-3404 [PMID: 3494999]

64 **Afzali B**, Lechler RI, Hernandez-Fuentes MP. Allorecognition and the alloresponse: clinical implications. *Tissue Antigens* 2007; **69**: 545-556 [PMID: 17498264 DOI: 10.1111/j.1399-0039.2007.00834.x]

65 **Shoskes DA**, Wood KJ. Indirect presentation of MHC antigens in transplantation. *Immunol Today* 1994; **15**: 32-38 [PMID: 8136009 DOI: 10.1016/0167-5699(94)90023-X]

66 **Auchincloss H**, Lee R, Shea S, Markowitz JS, Grusby MJ, Glimcher LH. The role of "indirect" recognition in initiating rejection of skin grafts from major histocompatibility complex class II-deficient mice. *Proc Natl Acad Sci USA* 1993; **90**: 3373-3377 [PMID: 8475083]

67 **Taylor AL**, Negus SL, Negus M, Bolton EM, Bradley JA, Pettigrew GJ. Pathways of helper CD4 T cell allorecognition in generating alloantibody and CD8 T cell alloimmunity. *Transplantation* 2007; **83**: 931-937 [PMID: 17460565 DOI: 10.1097/01.tp.0000257960.07783.e3]

68 **Steele DJ**, Laufer TM, Smiley ST, Ando Y, Grusby MJ, Glimcher LH, Auchincloss H. Two levels of help for B cell alloantibody production. *J Exp Med* 1996; **183**: 699-703 [PMID: 8627185]

69 **Baker RJ**, Hernandez-Fuentes MP, Brookes PA, Chaudhry AN, Lechler RI. The role of the allograft in the induction of donor-specific T cell hyporesponsiveness. *Transplantation* 2001; **72**: 480-485 [PMID: 11502979]

70 **Mason PD**, Robinson CM, Lechler RI. Detection of donor-specific hyporesponsiveness following late failure of human renal allografts. *Kidney Int* 1996; **50**: 1019-1025 [PMID: 8872979]

71 **Hornick PI**, Mason PD, Baker RJ, Hernandez-Fuentes M, Frasca L, Lombardi G, Taylor K, Weng L, Rose ML, Yacoub MH, Batchelor R, Lechler RI. Significant frequencies of T cells with indirect anti-donor specificity in heart graft recipients with chronic rejection. *Circulation* 2000; **101**: 2405-2410 [PMID: 10821818]

72 **Herrera OB**, Golshayan D, Tibbott R, Salcido Ochoa F, James MJ, Marelli-Berg FM, Lechler RI. A novel pathway of alloantigen presentation by dendritic cells. *J Immunol* 2004; **173**: 4828-4837 [PMID: 15470023]

73 **Game DS**, Rogers NJ, Lechler RI. Acquisition of HLA-DR and costimulatory molecules by T cells from allogeneic antigen presenting cells. *Am J Transplant* 2005; **5**: 1614-1625 [PMID: 15943619 DOI: 10.1111/j.1600-6143.2005.00916.x]

74 **Harshyne LA**, Watkins SC, Gambotto A, Barratt-Boyes SM. Dendritic cells acquire antigens from live cells for cross-presentation to CTL. *J Immunol* 2001; **166**: 3717-3723 [PMID: 11238612]

75 **Morelli AE**, Larregina AT, Shufesky WJ, Sullivan ML, Stolz DB, Papworth GD, Zahorchak AF, Logar AJ, Wang Z, Watkins SC, Falo LD, Thomson AW. Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. *Blood* 2004; **104**: 3257-3266 [PMID: 15284116 DOI: 10.1182/blood-2004-03-0824]

76 **Denzer K**, van Eijk M, Kleijmeer MJ, Jakobson E, de Groot C, Geuze HJ. Follicular dendritic cells carry MHC class II-expressing microvesicles at their surface. *J Immunol* 2000; **165**: 1259-1265 [PMID: 10903724]

77 **Rinaldo CR**, Piazza P. Virus infection of dendritic cells: portal for host invasion and host defense. *Trends Microbiol* 2004; **12**: 337-345 [PMID: 15223061 DOI: 10.1016/j.tim.2004.05.003]

78 **Dalheimer SL**, Richards DM, Mueller DL. Sharing of class I MHC molecules between donor and host promotes the infiltration of allografts by mHAg-reactive CD8 T cells. *Am J Transplant* 2005; **5**: 832-838 [PMID: 15760409 DOI: 10.1111/j.1600-6143.2005.00752.x]

79 **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]

80 **Grau V**, Garn H, Bette M, Spener F, Steiniger B, Gemsa D, Stehling O. Induction of epidermal fatty acid binding protein in intravascular monocytes of renal allografts. *Transplantation* 2003; **75**: 685-688 [PMID: 12640310 DOI: 10.1097/01.TP.0000052591.91653.52]

81 **Gröne HJ**, Weber C, Weber KS, Gröne EF, Rabelink T, Klier CM, Wells TN, Proudfood AE, Schlöndorff D, Nelson PJ. Met-RANTES reduces vascular and tubular damage during acute renal transplant rejection: blocking monocyte arrest and recruitment. *FASEB J* 1999; **13**: 1371-1383 [PMID: 10428761]

82 **Ciubotariu R**, Liu Z, Colovai AI, Ho E, Itescu S, Ravalli S, Hardy MA, Cortesini R, Rose EA, Suciu-Foca N. Persistent allopeptide reactivity and epitope spreading in chronic rejection of organ allografts. *J Clin Invest* 1998; **101**: 398-405 [PMID: 9435312 DOI: 10.1172/JCI1117]

83 **Vella JP**, Spadafora-Ferreira M, Murphy B, Alexander SI, Harmon W, Carpenter CB, Sayegh MH. Indirect allorecognition of major histocompatibility complex allopeptides in human renal transplant recipients with chronic graft dysfunction. *Transplantation* 1997; **64**: 795-800 [PMID: 9326400]

84 **Gueler F**, Gwinner W, Schwarz A, Haller H. Long-term effects of acute ischemia and reperfusion injury. *Kidney Int* 2004; **66**: 523-527 [PMID: 15253702 DOI: 10.1111/j.1523-1755.2004.761_11.x]

85 **Menke J**, Sollinger D, Schamberger B, Heemann U, Lutz J. The effect of ischemia/reperfusion on the kidney graft. *Curr Opin Organ Transplant* 2014; **19**: 395-400 [PMID: 24905021 DOI: 10.1097/MOT.0000000000000090]

86 **Oberbarnscheidt MH**, Zeng Q, Li Q, Dai H, Williams AL, Shlomchik WD, Rothstein DM, Lakkis FG. Non-self recognition by monocytes initiates allograft rejection. *J Clin Invest* 2014; **124**: 3579-3589 [PMID: 24983319 DOI: 10.1172/JCI74370]

87 **Zecher D**, van Rooijen N, Rothstein DM, Shlomchik WD, Lakkis FG. An innate response to allogeneic nonself mediated by monocytes. *J Immunol* 2009; **183**: 7810-7816 [PMID: 19923456 DOI: 10.4049/jimmunol.0902194]

88 **Cooper MA**, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci USA* 2009; **106**: 1915-1919 [PMID: 19181844 DOI: 10.1073/pnas.0813192106]

89 **Bingaman AW**, Ha J, Waitze SY, Durham MM, Cho HR, Tucker-Burden C, Hendrix R, Cowan SR, Pearson TC, Larsen CP. Vigorous allograft rejection in the absence of danger. *J Immunol* 2000; **164**: 3065-3071 [PMID: 10706695]

90 **Anderson CC**, Carroll JM, Gallucci S, Ridge JP, Cheever AW, Matzinger P. Testing time-, ignorance-, and danger-based models of tolerance. *J Immunol* 2001; **166**: 3663-3671 [PMID: 11238605]

91 **Rodriguez-Manzanet R**, Meyers JH, Balasubramanian S, Slavik J, Kassam N, Dardalhon V, Greenfield EA, Anderson AC, Sobel RA, Hafler DA, Strom TB, Kuchroo VK. TIM-4 expressed on APCs induces T cell expansion and survival. *J Immunol* 2008; **180**: 4706-4713 [PMID: 18354194]

92 **Moran AE**, Kovacsovics-Bankowski M, Weinberg AD. The TNFRs OX40, 4-1BB, and CD40 as targets for cancer immunotherapy. *Curr Opin Immunol* 2013; **25**: 230-237 [PMID: 23414607 DOI: 10.1016/j.coi.2013.01.004]

93 **Pilat N**, Sayegh MH, Wekerle T. Costimulatory pathways in transplantation. *Semin Immunol* 2011; **23**: 293-303 [PMID: 21616680 DOI: 10.1016/j.smim.2011.04.002]

94 **Chen L**, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol* 2013; **13**: 227-242 [PMID: 23470321 DOI: 10.1038/nri3405]

95 **Tinckam KJ**, Djurdjev O, Magil AB. Glomerular monocytes predict worse outcomes after acute renal allograft rejection independent of C4d status. *Kidney Int* 2005; **68**: 1866-1874 [PMID: 16164665 DOI: 10.1111/j.1523-1755.2005.00606.x]

96 **Grimm PC**, McKenna R, Nickerson P, Russell ME, Gough J, Gospodarek E, Liu B, Jeffery J, Rush DN. Clinical rejection is distinguished from subclinical rejection by increased infiltration by a population of activated macrophages. *J Am Soc Nephrol* 1999; **10**: 1582-1589 [PMID: 10405215]

97 **Grandaliano G**, Gesualdo L, Ranieri E, Monno R, Stallone G, Schena FP. Monocyte chemotactic peptide-1 expression and monocyte infiltration in acute renal transplant rejection. *Transplantation* 1997; **63**: 414-420 [PMID: 9039933]

98 **Lan HY**, Yang N, Brown FG, Isbel NM, Nikolic-Paterson DJ, Mu W, Metz CN, Bacher M, Atkins RC, Bucala R. Macrophage migration inhibitory factor expression in human renal allograft rejection. *Transplantation* 1998; **66**: 1465-1471 [PMID: 9869087]

99 **Le Meur Y**, Jose MD, Mu W, Atkins RC, Chadban SJ. Macrophage colony-stimulating factor expression and macrophage accumulation in renal allograft rejection. *Transplantation* 2002; **73**: 1318-1324 [PMID: 11981428]

100 **Jose MD**, Le Meur Y, Atkins RC, Chadban SJ. Blockade of macrophage colony-stimulating factor reduces macrophage proliferation and accumulation in renal allograft rejection. *Am J Transplant* 2003; **3**: 294-300 [PMID: 12614284]

101 **Hoffmann U**, Bergler T, Segerer S, Rümmele P, Krüger B, Banas MC, Reinhold S, Banas B, Krämer BK. Impact of chemokine receptor CX3CR1 in human renal allograft rejection. *Transpl Immunol* 2010; **23**: 204-208 [PMID: 20600902 DOI: 10.1016/j.trim.2010.06.006]

102 **Kakuta Y**, Okumi M, Miyagawa S, Tsutahara K, Abe T, Yazawa K, Matsunami K, Otsuka H, Takahara S, Nonomura N. Blocking of CCR5 and CXCR3 suppresses the infiltration of macrophages in acute renal allograft rejection. *Transplantation* 2012; **93**: 24-31 [PMID: 22124337 DOI: 10.1097/TP.0b013e31823aa585]

103 **Girlanda R**, Kleiner DE, Duan Z, Ford EA, Wright EC, Mannon RB, Kirk AD. Monocyte infiltration and kidney allograft dysfunction during acute rejection. *Am J Transplant* 2008; **8**: 600-607 [PMID: 18294156 DOI: 10.1111/j.1600-6143.2007.02109.x]

104 **Zhang PL**, Malek SK, Prichard JW, Lin F, Yahya TM, Schwartzman MS, Latsha RP, Skaletsky M, Norfolk ER, Brown RE, Hartle JE, Potdar S. Monocyte-mediated acute renal rejection after combined treatment with preoperative Campath-1H (alemtuzumab) and postoperative immunosuppression. *Ann Clin Lab Sci* 2004; **34**: 209-213 [PMID: 15228236]

105 **Laskin DL**, Sunil VR, Gardner CR, Laskin JD. Macrophages and tissue injury: agents of defense or destruction? *Annu Rev Pharmacol Toxicol* 2011; **51**: 267-288 [PMID: 20887196 DOI: 10.1146/annurev.pharmtox.010909.105812]

106 **West AP**, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, Walsh MC, Choi Y, Shadel GS, Ghosh S. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature* 2011; **472**: 476-480 [PMID: 21525932 DOI: 10.1038/nature09973]

107 **Italiani P**, Boraschi D. From Monocytes to M1/M2 Macrophages: Phenotypical vs. Functional Differentiation. *Front Immunol* 2014; **5**: 514 [PMID: 25368618 DOI: 10.3389/fimmu.2014.00514]

108 **Hutchinson JA**, Riquelme P, Sawitzki B, Tomiuk S, Miqueu P, Zuhayra M, Oberg HH, Pascher A, Lützen U, Janssen U, Broichhausen C, Renders L, Thaiss F, Scheuermann E, Henze E, Volk HD, Chatenoud L, Lechler RI, Wood KJ, Kabelitz D, Schlitt HJ, Geissler EK, Fändrich F. Cutting Edge: Immunological consequences and trafficking of human regulatory macrophages administered to renal transplant recipients. *J Immunol* 2011; **187**: 2072-2078 [PMID: 21804023 DOI: 10.4049/jimmunol.1100762]

109 **Pilmore HL**, Painter DM, Bishop GA, McCaughan GW, Eris JM. Early up-regulation of macrophages and myofibroblasts: a new marker for development of chronic renal allograft rejection. *Transplantation* 2000; **69**: 2658-2662 [PMID: 10910290]

110 **Vitalone MJ**, O'Connell PJ, Wavamunno M, Fung CL, Chapman JR, Nankivell BJ. Transcriptome changes of chronic tubulointerstitial damage in early kidney transplantation. *Transplantation* 2010; **89**: 537-547 [PMID: 20147884 DOI: 10.1097/TP.0b013e3181ca7389]

111 **Toki D**, Zhang W, Hor KL, Liuwantara D, Alexander SI, Yi Z, Sharma R, Chapman JR, Nankivell BJ, Murphy B, O'Connell PJ. The role of macrophages in the development of human renal allograft fibrosis

in the first year after transplantation. *Am J Transplant* 2014; **14**: 2126-2136 [PMID: 25307039 DOI: 10.1111/ajt.12803]

112 **Long EO**, Rajagopalan S. Stress signals activate natural killer cells. *J Exp Med* 2002; **196**: 1399-1402 [PMID: 12461075]

113 **Lim O**, Jung MY, Hwang YK, Shin EC. Present and Future of Allogeneic Natural Killer Cell Therapy. *Front Immunol* 2015; **6**: 286 [PMID: 26089823 DOI: 10.3389/fimmu.2015.00286]

114 **Long EO**, Kim HS, Liu D, Peterson ME, Rajagopalan S. Controlling natural killer cell responses: integration of signals for activation and inhibition. *Annu Rev Immunol* 2013; **31**: 227-258 [PMID: 23516982 DOI: 10.1146/annurev-immunol-020711-075005]

115 **Alnabhan R**, Madrigal A, Saudemont A. Differential activation of cord blood and peripheral blood natural killer cells by cytokines. *Cytotherapy* 2015; **17**: 73-85 [PMID: 25248279 DOI: 10.1016/j.jcyt.2014.08.003]

116 **Foley B**, Cooley S, Verneris MR, Curtsinger J, Luo X, Waller EK, Weisdorf DJ, Miller JS. NK cell education after allogeneic transplantation: dissociation between recovery of cytokine-producing and cytotoxic functions. *Blood* 2011; **118**: 2784-2792 [PMID: 21757615 DOI: 10.1182/blood-2011-04-347070]

117 **Hamby K**, Trexler A, Pearson TC, Larsen CP, Rigby MR, Kean LS. NK cells rapidly reject allogeneic bone marrow in the spleen through a perforin- and Ly49D-dependent, but NKG2D-independent mechanism. *Am J Transplant* 2007; **7**: 1884-1896 [PMID: 17617852 DOI: 10.1111/j.1600-6143.2007.01864.x]

118 **Chow T**, Whiteley J, Li M, Rogers IM. The transfer of host MHC class I protein protects donor cells from NK cell and macrophage-mediated rejection during hematopoietic stem cell transplantation and engraftment in mice. *Stem Cells* 2013; **31**: 2242-2252 [PMID: 23818226 DOI: 10.1002/stem.1458]

119 **Noval Rivas M**, Hazzan M, Weatherly K, Gaudray F, Salmon I, Braun MY. NK cell regulation of CD4 T cell-mediated graft-versus-host disease. *J Immunol* 2010; **184**: 6790-6798 [PMID: 20488796 DOI: 10.4049/jimmunol.0902598]

120 **Olson JA**, Leveson-Gower DB, Gill S, Baker J, Beilhack A, Negrin RS. NK cells mediate reduction of GVHD by inhibiting activated, alloreactive T cells while retaining GVT effects. *Blood* 2010; **115**: 4293-4301 [PMID: 20233969 DOI: 10.1182/blood-2009-05-222190]

121 **Hüber CM**, Doisne JM, Colucci F. IL-12/15/18-preactivated NK cells suppress GvHD in a mouse model of mismatched hematopoietic cell transplantation. *Eur J Immunol* 2015; **45**: 1727-1735 [PMID: 25778912 DOI: 10.1002/eji.201445200]

122 **Hu B**, Bao G, Zhang Y, Lin D, Wu Y, Wu D, Liu H. Donor NK Cells and IL-15 promoted engraftment in nonmyeloablative allogeneic bone marrow transplantation. *J Immunol* 2012; **189**: 1661-1670 [PMID: 22798668 DOI: 10.4049/jimmunol.1103199]

123 **Godfrey DI**, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L. NKT cells: what's in a name? *Nat Rev Immunol* 2004; **4**: 231-237 [PMID: 15039760 DOI: 10.1038/nri1309]

124 **Yang YF**, Tomura M, Ono S, Hamaoka T, Fujiwara H. Requirement for IFN-gamma in IL-12 production induced by collaboration between v(alpha)14(+) NKT cells and antigen-presenting cells. *Int Immunol* 2000; **12**: 1669-1675 [PMID: 11099306]

125 **Monteiro M**, Graca L. Response to comment on "Induced IL-17-producing invariant NKT cells require activation in presence of TGF-β and IL-1β". *J Immunol* 2013; **190**: 5910-5911 [PMID: 23749965 DOI: 10.4049/jimmunol.1390033]

126 **Di Pietro C**, Falcone M. The role of invariant NKT cells in organ-specific autoimmunity. *Front Biosci* (Landmark Ed) 2014; **19**: 1240-1250 [PMID: 24896348]

127 **Singh AK**, Gaur P, Das SN. Natural killer T cell anergy, co-stimulatory molecules and immunotherapeutic interventions. *Hum Immunol* 2014; **75**: 250-260 [PMID: 24373798 DOI: 10.1016/j.humimm.2013.12.004]

128 **Yang SH**, Jin JZ, Lee SH, Park H, Kim CH, Lee DS, Kim S, Chung NH, Kim YS. Role of NKT cells in allogeneic islet graft survival. *Clin Immunol* 2007; **124**: 258-266 [PMID: 17662658 DOI: 10.1016/j.clim.2007.06.003]

129 **Ikehara Y**, Yasunami Y, Kodama S, Maki T, Nakano M, Nakayama T, Taniguchi M, Ikeda S. CD4(+) Valpha14 natural killer T cells are essential for acceptance of rat islet xenografts in mice. *J Clin Invest* 2000; **105**: 1761-1767 [PMID: 10862791 DOI: 10.1172/JCI8922]

130 **Seino KI**, Fukao K, Muramoto K, Yanagisawa K, Takada Y, Kakuta S, Iwakura Y, Van Kaer L, Takeda K, Nakayama T, Taniguchi M, Bashuda H, Yagita H, Okumura K. Requirement for natural killer T (NKT) cells in the induction of allograft tolerance. *Proc Natl Acad Sci USA* 2001; **98**: 2577-2581 [PMID: 11226281 DOI: 10.1073/pnas.041608298]

131 **Jiang X**, Kojo S, Harada M, Ohkohchi N, Taniguchi M, Seino KI. Mechanism of NKT cell-mediated transplant tolerance. *Am J Transplant* 2007; **7**: 1482-1490 [PMID: 17511678 DOI: 10.1111/j.1600-6143.2007.01827.x]

132 **Hongo D**, Tang X, Dutt S, Nador RG, Strober S. Interactions between NKT cells and Tregs are required for tolerance to combined bone marrow and organ transplants. *Blood* 2012; **119**: 1581-1589 [PMID: 22174155 DOI: 10.1182/blood-2011-08-371948]

133 **Kim JH**, Choi EY, Chung DH. Donor bone marrow type II (non-Valpha14Jalpha18 CD1d-restricted) NKT cells suppress graft-versus-host disease by producing IFN-gamma and IL-4. *J Immunol* 2007; **179**: 6579-6587 [PMID: 17982047]

134 **Cella M**, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, Colonna M. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med* 1999; **5**: 919-923 [PMID: 10426316 DOI: 10.1038/11360]

135 **Piqueras B**, Connolly J, Freitas H, Palucka AK, Banchereau J. Upon viral exposure, myeloid and plasmacytoid dendritic cells produce 3 waves of distinct chemokines to recruit immune effectors. *Blood* 2006; **107**: 2613-2618 [PMID: 16317096 DOI: 10.1182/blood-2005-07-2965]

136 **Markey KA**, Banovic T, Kuns RD, Olver SD, Don AL, Raffelt NC, Wilson YA, Raggatt LJ, Pettit AR, Bromberg JS, Hill GR, MacDonald KP. Conventional dendritic cells are the critical donor APC presenting alloantigen after experimental bone marrow transplantation. *Blood* 2009; **113**: 5644-5649 [PMID: 19336758 DOI: 10.1182/blood-2008-12-191833]

137 **Weber M**, Rudolph B, Stein P, Yogev N, Bosmann M, Schild H, Radsak MP. Host-derived CD8 dendritic cells protect against acute graft-versus-host disease after experimental allogeneic bone marrow transplantation. *Biol Blood Marrow Transplant* 2014; **20**: 1696-1704 [PMID: 25132527 DOI: 10.1016/j.bbmt.2014.08.005]

138 **Toubai T**, Malter C, Tawara I, Liu C, Nieves E, Lowler KP, Sun Y, Reddy P. Immunization with host-type CD8{alpha}+ dendritic cells reduces experimental acute GVHD in an IL-10-dependent manner. *Blood* 2010; **115**: 724-735 [PMID: 19965670 DOI: 10.1182/blood-2009-06-229708]

139 **Belladonna ML**, Volpi C, Bianchi R, Vacca C, Orabona C, Pallotta MT, Boon L, Gizzi S, Fioretti MC, Grohmann U, Puccetti P. Cutting edge: Autocrine TGF-beta sustains default tolerogenesis by IDO-competent dendritic cells. *J Immunol* 2008; **181**: 5194-5198 [PMID: 18832670]

140 **O'Connell PJ**, Li W, Wang Z, Specht SM, Logar AJ, Thomson AW. Immature and mature CD8alpha+ dendritic cells prolong the survival of vascularized heart allografts. *J Immunol* 2002; **168**: 143-154 [PMID: 11751957]

141 **Cheong C**, Matos I, Choi JH, Dandamudi DB, Shrestha E, Longhi MP, Jeffrey KL, Anthony RM, Kluger C, Nchinda G, Koh H, Rodriguez A, Idoyaga J, Pack M, Velinzon K, Park CG, Steinman RM. Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209(+) dendritic cells for immune T cell areas. *Cell* 2010; **143**: 416-429 [PMID: 21029863 DOI: 10.1016/j.cell.2010.09.039]

142 **Chow KV**, Lew AM, Sutherland RM, Zhan Y. Monocyte-Derived Dendritic Cells Promote Th Polarization, whereas Conventional Dendritic Cells Promote Th Proliferation. *J Immunol* 2016; **196**: 624-636 [PMID: 26663720 DOI: 10.4049/jimmunol.1501202]

143 **Münz C**, Dao T, Ferlazzo G, de Cos MA, Goodman K, Young JW. Mature myeloid dendritic cell subsets have distinct roles for activation and viability of circulating human natural killer cells. *Blood* 2005; **105**: 266-273 [PMID: 15331446 DOI: 10.1182/blood-2004-06-2492]

144 **Kolumam GA**, Thomas S, Thompson LJ, Sprent J, Murali-Krishna K. Type I interferons act directly on CD8 T cells to allow clonal expansion and memory formation in response to viral infection. *J*

Exp Med 2005; **202**: 637-650 [PMID: 16129706 DOI: 10.1084/jem.20050821]

145 **Famulski KS**, Einecke G, Reeve J, Ramassar V, Allanach K, Mueller T, Hidalgo LG, Zhu LF, Halloran PF. Changes in the transcriptome in allograft rejection: IFN-gamma-induced transcripts in mouse kidney allografts. *Am J Transplant* 2006; **6**: 1342-1354 [PMID: 16686758 DOI: 10.1111/j.1600-6143.2006.01337.x]

146 **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 DOI: 10.1097/01.TP.0000144057.31799.6A]

147 **Halloran PF**, Afrouzian M, Ramassar V, Urmson J, Zhu LF, Helms LM, Solez K, Kneteman NM. Interferon-gamma acts directly on rejecting renal allografts to prevent graft necrosis. *Am J Pathol* 2001; **158**: 215-226 [PMID: 11141495 DOI: 10.1016/S0002-9440(10)63960-0]

148 **Famulski KS**, Sis B, Billesberger L, Halloran PF. Interferon-gamma and donor MHC class I control alternative macrophage activation and activin expression in rejecting kidney allografts: a shift in the Th1-Th2 paradigm. *Am J Transplant* 2008; **8**: 547-556 [PMID: 18294151 DOI: 10.1111/j.1600-6143.2007.02118.x]

149 **Brok HP**, Vossen JM, Heidt PJ. IFN-gamma-mediated prevention of graft-versus-host disease: pharmacodynamic studies and influence on proliferative capacity of chimeric spleen cells. *Bone Marrow Transplant* 1998; **22**: 1005-1010 [PMID: 9849699 DOI: 10.1038/sj.bmt.1701478]

150 **Welniak LA**, Blazar BR, Anver MR, Wiltrout RH, Murphy WJ. Opposing roles of interferon-gamma on CD4+ T cell-mediated graft-versus-host disease: effects of conditioning. *Biol Blood Marrow Transplant* 2000; **6**: 604-612 [PMID: 11128810 DOI: 10.1016/S1083-8791(00)70025-5]

151 **Koenecke C**, Lee CW, Thamm K, Föhse L, Schafferus M, Mittrücker HW, Floess S, Huehn J, Ganser A, Förster R, Prinz I. IFN-γ production by allogeneic Foxp3+ regulatory T cells is essential for preventing experimental graft-versus-host disease. *J Immunol* 2012; **189**: 2890-2896 [PMID: 22869903 DOI: 10.4049/jimmunol.1200413]

152 **Bushell A**, Niimi M, Morris PJ, Wood KJ. Evidence for immune regulation in the induction of transplantation tolerance: a conditional but limited role for IL-4. *J Immunol* 1999; **162**: 1359-1366 [PMID: 9973390]

153 **Mariotti J**, Foley J, Ryan K, Buxhoeveden N, Kapoor V, Amarnath S, Fowler DH. Graft rejection as a Th1-type process amenable to regulation by donor Th2-type cells through an interleukin-4/STAT6 pathway. *Blood* 2008; **112**: 4765-4775 [PMID: 18625883 DOI: 10.1182/blood-2008-05-154278]

154 **Onodera K**, Hancock WW, Graser E, Lehmann M, Sayegh MH, Strom TB, Volk HD, Kupiec-Weglinski JW. Type 2 helper T cell-type cytokines and the development of "infectious" tolerance in rat cardiac allograft recipients. *J Immunol* 1997; **158**: 1572-1581 [PMID: 9029092]

155 **Sadeghi M**, Daniel V, Weimer R, Wiesel M, Hergesell O, Opelz G. Pre-transplant Th1 and post-transplant Th2 cytokine patterns are associated with early acute rejection in renal transplant recipients. *Clin Transplant* 2003; **17**: 151-157 [PMID: 12709083 DOI: 10.1034/j.1399-0012.2003.00037.x]

156 **Sabet-Baktach M**, Eggenhofer E, Rovira J, Renner P, Lantow M, Farkas SA, Malaisé M, Edtinger K, Shaotang Z, Koehl GE, Dahlke MH, Schlitt HJ, Geissler EK, Kroemer A. Double deficiency for RORγt and T-bet drives Th2-mediated allograft rejection in mice. *J Immunol* 2013; **191**: 4440-4446 [PMID: 24058178 DOI: 10.4049/jimmunol.1301741]

157 **Karczewski J**, Karczewski M, Glyda M, Wiktorowicz K. Role of TH1/TH2 cytokines in kidney allograft rejection. *Transplant Proc* 2008; **40**: 3390-3392 [PMID: 19100396 DOI: 10.1016/j.transproceed.2008.07.125]

158 **Kwan T**, Chadban SJ, Ma J, Bao S, Alexander SI, Wu H. IL-17 deficiency attenuates allograft injury and prolongs survival in a murine model of fully MHC-mismatched renal allograft transplantation. *Am J Transplant* 2015; **15**: 1555-1567 [PMID: 25824574 DOI: 10.1111/ajt.13140]

159 **Snell GI**, Levvey BJ, Zheng L, Bailey M, Orsida B, Williams TJ, Kotsimbos TC. Interleukin-17 and airway inflammation: a longitudinal airway biopsy study after lung transplantation. *J Heart Lung Transplant* 2007; **26**: 669-674 [PMID: 17613395 DOI: 10.1016/j.healun.2007.05.004]

160 **Tsaur I**, Gasser M, Aviles B, Lutz J, Lutz L, Grimm M, Lange V, Lopau K, Heemann U, Germer CT, Chandraker A, Waaga-Gasser AM. Donor antigen-specific regulatory T-cell function affects outcome in kidney transplant recipients. *Kidney Int* 2011; **79**: 1005-1012 [PMID: 21270769 DOI: 10.1038/ki.2010.533]

161 **Sugimoto K**, Itoh T, Takita M, Shimoda M, Chujo D, SoRelle JA, Naziruddin B, Levy MF, Shimada M, Matsumoto S. Improving allogeneic islet transplantation by suppressing Th17 and enhancing Treg with histone deacetylase inhibitors. *Transpl Int* 2014; **27**: 408-415 [PMID: 24410777 DOI: 10.1111/tri.12265]

162 **Vanaudenaerde BM**, De Vleeschauwer SI, Vos R, Meyts I, Bullens DM, Reynders V, Wuyts WA, Van Raemdonck DE, Dupont LJ, Verleden GM. The role of the IL23/IL17 axis in bronchiolitis obliterans syndrome after lung transplantation. *Am J Transplant* 2008; **8**: 1911-1920 [PMID: 18786233 DOI: 10.1111/j.1600-6143.2008.02321.x]

163 **Malard F**, Bossard C, Brissot E, Chevallier P, Guillaume T, Delaunay J, Mosnier JF, Moreau P, Grégoire M, Gaugler B, Mohty M. Increased Th17/Treg ratio in chronic liver GVHD. *Bone Marrow Transplant* 2014; **49**: 539-544 [PMID: 24419519 DOI: 10.1038/bmt.2013.215]

164 **Bommiasamy H**. IL-22 Is Critical To The Pathogenesis Of Cutaneous Gvhd Mediated By Th17 Cells. *Blood* 2013; **122**: 4482

165 **Socié G**, Ritz J. Current issues in chronic graft-versus-host disease. *Blood* 2014; **124**: 374-384 [PMID: 24914139 DOI: 10.1182/blood-2014-01-514752]

166 **Ramming A**, Druzd D, Leipe J, Schulze-Koops H, Skapenko A. Maturation-related histone modifications in the PU.1 promoter regulate Th9-cell development. *Blood* 2012; **119**: 4665-4674 [PMID: 22446486 DOI: 10.1182/blood-2011-11-392589]

167 **Mangus CW**, Massey PR, Fowler DH, Amarnath S. Rapamycin resistant murine th9 cells have a stable in vivo phenotype and inhibit graft-versus-host reactivity. *PLoS One* 2013; **8**: e72305 [PMID: 23991087 DOI: 10.1371/journal.pone.0072305]

168 **Ramadan A**, Zhang J, Griesenauer B, Kapur R, Hanenberg H, Sun J, Kaplan M, Paczesny S. IL-33/ST2 activation of IL-9-secreting T cells alters the balance of fatal immunity and tumor immunity (TRAN1P.926). *J Immunol* 2015; **194** (1 Supplement): 140.148-140.148

169 **Halamay KE**, Kirkman RL, Sun L, Yamada A, Fragoso RC, Shimizu K, Mitchell RN, McKay DB. CD8 T cells are sufficient to mediate allorecognition and allograft rejection. *Cell Immunol* 2002; **216**: 6-14 [PMID: 12381345 DOI: 10.1016/S0008-8749(02)00530-0]

170 **Stenger S**, Hanson DA, Teitelbaum R, Dewan P, Niazi KR, Froelich CJ, Ganz T, Thoma-Uszynski S, Melián A, Bogdan C, Porcelli SA, Bloom BR, Krensky AM, Modlin RL. An antimicrobial activity of cytolytic T cells mediated by granulysin. *Science* 1998; **282**: 121-125 [PMID: 9756476 DOI: 10.1126/science.282.5386.121]

171 **Thomas WD**, Hersey P. TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis in Fas ligand-resistant melanoma cells and mediates CD4 T cell killing of target cells. *J Immunol* 1998; **161**: 2195-2200 [PMID: 9725211]

172 **Kayagaki N**, Yamaguchi N, Nakayama M, Kawasaki A, Akiba H, Okumura K, Yagita H. Involvement of TNF-related apoptosis-inducing ligand in human CD4+ T cell-mediated cytotoxicity. *J Immunol* 1999; **162**: 2639-2647 [PMID: 10072506]

173 **Krams SM**, Hayashi M, Fox CK, Villanueva JC, Whitmer KJ, Burns W, Esquivel CO, Martinez OM. CD8+ cells are not necessary for allograft rejection or the induction of apoptosis in an experimental model of small intestinal transplantation. *J Immunol* 1998; **160**: 3673-3680 [PMID: 9558067]

174 **Lowry RP**, Forbes RD, Blackburn JH, Marghesco DM. Immune mechanisms in organ allograft rejection. V. Pivotal role of the cytotoxic-suppressor T cell subset in the rejection of heart grafts

bearing isolated class I disparities in the inbred rat. *Transplantation* 1985; **40**: 545-550 [PMID: 3904091 DOI: 10.1097/00007890-198511000-00014]

175 **Haspot F**, Li HW, Lucas CL, Fehr T, Beyaz S, Sykes M. Allospecific rejection of MHC class I-deficient bone marrow by CD8 T cells. *Am J Transplant* 2014; **14**: 49-58 [PMID: 24304495 DOI: 10.1111/ajt.12525]

176 **Zimmerer JM**, Pham TA, Wright CL, Tobin KJ, Sanghavi PB, Elzein SM, Sanders VM, Bumgardner GL. Alloprimed CD8(+) T cells regulate alloantibody and eliminate alloprimed B cells through perforin- and FasL-dependent mechanisms. *Am J Transplant* 2014; **14**: 295-304 [PMID: 24472191 DOI: 10.1111/ajt.12565]

177 **Schürch CM**, Riether C, Ochsenbein AF. Cytotoxic CD8+ T cells stimulate hematopoietic progenitors by promoting cytokine release from bone marrow mesenchymal stromal cells. *Cell Stem Cell* 2014; **14**: 460-472 [PMID: 24561082 DOI: 10.1016/j.stem.2014.01.002]

178 **Fowler DH**, Gress RE. Th2 and Tc2 cells in the regulation of GVHD, GVL, and graft rejection: considerations for the allogeneic transplantation therapy of leukemia and lymphoma. *Leuk Lymphoma* 2000; **38**: 221-234 [PMID: 10830730 DOI: 10.3109/10428190009087014]

179 **Erdmann AA**, Jung U, Foley JE, Toda Y, Fowler DH. Co-stimulated/Tc2 cells abrogate murine marrow graft rejection. *Biol Blood Marrow Transplant* 2004; **10**: 604-613 [PMID: 15319772 DOI: 10.1016/j.bbmt.2004.06.006]

180 **Jung U**, Foley JE, Erdmann AA, Eckhaus MA, Fowler DH. CD3/CD28-costimulated T1 and T2 subsets: differential in vivo allosensitization generates distinct GVT and GVHD effects. *Blood* 2003; **102**: 3439-3446 [PMID: 12855580 DOI: 10.1182/blood-2002-12-3936]

181 **Masopust D**, Vezys V, Marzo AL, Lefrançois L. Preferential localization of effector memory cells in nonlymphoid tissue. *Science* 2001; **291**: 2413-2417 [PMID: 11264538 DOI: 10.1126/science.1058867]

182 **Obhrai JS**, Oberbarnscheidt MH, Hand TW, Diggs L, Chalasani G, Lakkis FG. Effector T cell differentiation and memory T cell maintenance outside secondary lymphoid organs. *J Immunol* 2006; **176**: 4051-4058 [PMID: 16547240]

183 **Chang JT**, Wherry EJ, Goldrath AW. Molecular regulation of effector and memory T cell differentiation. *Nat Immunol* 2014; **15**: 1104-1115 [PMID: 25396352 DOI: 10.1038/ni.3031]

184 **London CA**, Lodge MP, Abbas AK. Functional responses and costimulator dependence of memory CD4+ T cells. *J Immunol* 2000; **164**: 265-272 [PMID: 10605020]

185 **Harrington LE**, Janowski KM, Oliver JR, Zajac AJ, Weaver CT. Memory CD4 T cells emerge from effector T-cell progenitors. *Nature* 2008; **452**: 356-360 [PMID: 18322463 DOI: 10.1038/nature06672]

186 **Li J**, Huston G, Swain SL. IL-7 promotes the transition of CD4 effectors to persistent memory cells. *J Exp Med* 2003; **198**: 1807-1815 [PMID: 14676295 DOI: 10.1084/jem.20030725]

187 **Kaech SM**, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nat Immunol* 2003; **4**: 1191-1198 [PMID: 14625547 DOI: 10.1038/ni1009]

188 **Valujskikh A**, Li XC. Frontiers in nephrology: T cell memory as a barrier to transplant tolerance. *J Am Soc Nephrol* 2007; **18**: 2252-2261 [PMID: 17634436 DOI: 10.1681/ASN.2007020151]

189 **Chalasani G**, Dai Z, Konieczny BT, Baddoura FK, Lakkis FG. Recall and propagation of allospecific memory T cells independent of secondary lymphoid organs. *Proc Natl Acad Sci USA* 2002; **99**: 6175-6180 [PMID: 11983909 DOI: 10.1073/pnas.092596999]

190 **Pearl JP**, Parris J, Hale DA, Hoffmann SC, Bernstein WB, McCoy KL, Swanson SJ, Mannon RB, Roederer M, Kirk AD. Immunocompetent T-cells with a memory-like phenotype are the dominant cell type following antibody-mediated T-cell depletion. *Am J Transplant* 2005; **5**: 465-474 [PMID: 15707400 DOI: 10.1111/j.1600-6143.2005.00759.x]

191 **Vu MD**, Clarkson MR, Yagita H, Turka LA, Sayegh MH, Li XC. Critical, but conditional, role of OX40 in memory T cell-mediated rejection. *J Immunol* 2006; **176**: 1394-1401 [PMID: 16424166]

192 **Minamimura K**, Sato K, Yagita H, Tanaka T, Arii S, Maki T. Strategies to induce marked prolongation of secondary skin allograft survival in alloantigen-primed mice. *Am J Transplant* 2008; **8**: 761-772 [PMID: 18261171 DOI: 10.1111/j.1600-6143.2007.02143.x]

193 **Yang J**, Brook MO, Carvalho-Gaspar M, Zhang J, Ramon HE, Sayegh MH, Wood KJ, Turka LA, Jones ND. Allograft rejection mediated by memory T cells is resistant to regulation. *Proc Natl Acad Sci USA* 2007; **104**: 19954-19959 [PMID: 18042727 DOI: 10.1073/pnas.0704397104]

194 **Li XC**, Kloc M, Ghobrial RM. Memory T cells in transplantation - progress and challenges. *Curr Opin Organ Transplant* 2013; **18**: 387-392 [PMID: 23838642 DOI: 10.1097/MOT.0b013e3283626130]

195 **Heeger PS**, Greenspan NS, Kuhlenschmidt S, Dejelo C, Hricik DE, Schulak JA, Tary-Lehmann M. Pretransplant frequency of donor-specific, IFN-gamma-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. *J Immunol* 1999; **163**: 2267-2275 [PMID: 10438971]

196 **Nadazdin O**, Boskovic S, Murakami T, Tocco G, Smith RN, Colvin RB, Sachs DH, Allan J, Madsen JC, Kawai T, Cosimi AB, Benichou G. Host alloreactive memory T cells influence tolerance to kidney allografts in nonhuman primates. *Sci Transl Med* 2011; **3**: 86ra51 [PMID: 21653831 DOI: 10.1126/scitranslmed.3002093]

197 **Wu Z**, Bensinger SJ, Zhang J, Chen C, Yuan X, Huang X, Markmann JF, Kassaee A, Rosengard BR, Hancock WW, Sayegh MH, Turka LA. Homeostatic proliferation is a barrier to transplantation tolerance. *Nat Med* 2004; **10**: 87-92 [PMID: 14647496 DOI: 10.1038/nm965]

198 **Goldrath AW**, Bogatzki LY, Bevan MJ. Naive T cells transiently acquire a memory-like phenotype during homeostasis-driven proliferation. *J Exp Med* 2000; **192**: 557-564 [PMID: 10952725]

199 **Sener A**, Tang AL, Farber DL. Memory T-cell predominance following T-cell depletional therapy derives from homeostatic expansion of naive T cells. *Am J Transplant* 2009; **9**: 2615-2623 [PMID: 19775313 DOI: 10.1111/j.1600-6143.2009.02820.x]

200 **Taylor DK**, Neujahr D, Turka LA. Heterologous immunity and homeostatic proliferation as barriers to tolerance. *Curr Opin Immunol* 2004; **16**: 558-564 [PMID: 15341999 DOI: 10.1016/j.coi.2004.07.007]

201 **Selin LK**, Brehm MA. Frontiers in nephrology: heterologous immunity, T cell cross-reactivity, and alloreactivity. *J Am Soc Nephrol* 2007; **18**: 2268-2277 [PMID: 17634431 DOI: 10.1681/ASN.2007030295]

202 **Koyama I**, Nadazdin O, Boskovic S, Ochiai T, Smith RN, Sykes M, Sogawa H, Murakami T, Strom TB, Colvin RB, Sachs DH, Benichou G, Cosimi AB, Kawai T. Depletion of CD8 memory T cells for induction of tolerance of a previously transplanted kidney allograft. *Am J Transplant* 2007; **7**: 1055-1061 [PMID: 17286617 DOI: 10.1111/j.1600-6143.2006.01703.x]

203 **Krummey SM**, Ford ML. Heterogeneity within T Cell Memory: Implications for Transplant Tolerance. *Front Immunol* 2012; **3**: 36 [PMID: 22566919 DOI: 10.3389/fimmu.2012.00036]

204 **Afzali B**, Mitchell PJ, Scottà C, Canavan J, Edozie FC, Fazekasova H, Lord GM, John S, Barber LD, Hernandez-Fuentes MP, Lechler RI, Lombardi G. Relative resistance of human CD4(+) memory T cells to suppression by CD4(+) CD25(+) regulatory T cells. *Am J Transplant* 2011; **11**: 1734-1742 [PMID: 21749646 DOI: 10.1111/j.1600-6143.2011.03635.x]

205 **Yamada Y**, Boskovic S, Aoyama A, Murakami T, Putheti P, Smith RN, Ochiai T, Nadazdin O, Koyama I, Boenisch O, Najafian N, Bhasin MK, Colvin RB, Madsen JC, Strom TB, Sachs DH, Benichou G, Cosimi AB, Kawai T. Overcoming memory T-cell responses for induction of delayed tolerance in nonhuman primates. *Am J Transplant* 2012; **12**: 330-340 [PMID: 22053723 DOI: 10.1111/j.1600-6143.2011.03795.x]

206 **Khandelwal P**, Lane A, Chaturvedi V, Owsley E, Davies SM, Marmer D, Filipovich AH, Jordan MB, Marsh RA. Peripheral Blood CD38 Bright CD8+ Effector Memory T Cells Predict Acute Graft-versus-Host Disease. *Biol Blood Marrow Transplant* 2015; **21**: 1215-1222 [PMID: 25881755 DOI: 10.1016/j.bbmt.2015.04.010]

207 **Schultz KR**, Paquet J, Bader S, HayGlass KT. Requirement for B cells in T cell priming to minor histocompatibility antigens and development of graft-versus-host disease. *Bone Marrow Transplant*

1995; **16**: 289-295 [PMID: 7581150]
208 **Iori AP**, Torelli GF, De Propris MS, Milano F, Pupella S, Gozzer M, Mancini F, Milani ML, Intoppa S, Cerretti R, Lucarelli B, Valle V, Malandruccolo L, Iannella E, Arleo E, Guarini A, Foà R. B-cell concentration in the apheretic product predicts acute graft-versus-host disease and treatment-related mortality of allogeneic peripheral blood stem cell transplantation. *Transplantation* 2008; **85**: 386-390 [PMID: 18322430 DOI: 10.1097/TP.0b013e3181622e36]
209 **Patriarca F**, Skert C, Sperotto A, Zaja F, Falleti E, Mestroni R, Kikic F, Calistri E, Filì C, Geromin A, Cerno M, Fanin R. The development of autoantibodies after allogeneic stem cell transplantation is related with chronic graft-vs-host disease and immune recovery. *Exp Hematol* 2006; **34**: 389-396 [PMID: 16543073 DOI: 10.1016/j.exphem.2005.12.011]
210 **Svegliati S**, Olivieri A, Campelli N, Luchetti M, Poloni A, Trappolini S, Moroncini G, Bacigalupo A, Leoni P, Avvedimento EV, Gabrielli A. Stimulatory autoantibodies to PDGF receptor in patients with extensive chronic graft-versus-host disease. *Blood* 2007; **110**: 237-241 [PMID: 17363728 DOI: 10.1182/blood-2007-01-071043]
211 **Miklos DB**, Kim HT, Miller KH, Guo L, Zorn E, Lee SJ, Hochberg EP, Wu CJ, Alyea EP, Cutler C, Ho V, Soiffer RJ, Antin JH, Ritz J. Antibody responses to H-Y minor histocompatibility antigens correlate with chronic graft-versus-host disease and disease remission. *Blood* 2005; **105**: 2973-2978 [PMID: 15613541 DOI: 10.1182/blood-2004-09-3660]
212 **Zeng Q**, Ng YH, Singh T, Jiang K, Sheriff KA, Ippolito R, Zahalka S, Li Q, Randhawa P, Hoffman RA, Ramaswami B, Lund FE, Chalasani G. B cells mediate chronic allograft rejection independently of antibody production. *J Clin Invest* 2014; **124**: 1052-1056 [PMID: 24509079 DOI: 10.1172/JCI70084]
213 **Lefaucheur C**, Loupy A, Hill GS, Andrade J, Nochy D, Antoine C, Gautreau C, Charron D, Glotz D, Suberbielle-Boissel C. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. *J Am Soc Nephrol* 2010; **21**: 1398-1406 [PMID: 20634297 DOI: 10.1681/ASN.2009101065]
214 **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]
215 **Peng Q**, Li K, Wang N, Li Q, Asgari E, Lu B, Woodruff TM, Sacks SH, Zhou W. Dendritic cell function in allostimulation is modulated by C5aR signaling. *J Immunol* 2009; **183**: 6058-6068 [PMID: 19864610 DOI: 10.4049/jimmunol.0804186]
216 **Strainic MG**, Shevach EM, An F, Lin F, Medof ME. Absence of signaling into CD4+ cells via C3aR and C5aR enables autoinductive TGF-β1 signaling and induction of Foxp3+ regulatory T cells. *Nat Immunol* 2013; **14**: 162-171 [PMID: 23263555 DOI: 10.1038/ni.2499]
217 **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]
218 **Csomor E**, Bajtay Z, Sándor N, Kristóf K, Arlaud GJ, Thiel S, Erdei A. Complement protein C1q induces maturation of human dendritic cells. *Mol Immunol* 2007; **44**: 3389-3397 [PMID: 17383729 DOI: 10.1016/j.molimm.2007.02.014]
219 **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]
220 **Halloran PF**. Immunosuppressive drugs for kidney transplantation. *N Engl J Med* 2004; **351**: 2715-2729 [PMID: 15616206 DOI: 10.1056/NEJMra033540]
221 **Merrill JP**, Murray JE, Takacs FJ, Hager EB, Wilson RE, Dammin GJ. Successful transplantation of kidney from a human cadaver. *JAMA* 1963; **185**: 347-353 [PMID: 13935048]
222 Organ Procurement and Transplantation Network and Scientific Registry of Transplant Recipients 2010 data report. *Am J Transplant* 2012; **12** Suppl 1: 1-156 [PMID: 22107249 DOI: 10.1111/j.1600-6143.2011.03886.x]
223 **van Gelder T**, van Schaik RH, Hesselink DA. Pharmacogenetics and immunosuppressive drugs in solid organ transplantation. *Nat Rev Nephrol* 2014; **10**: 725-731 [PMID: 25247332 DOI: 10.1038/nrneph.2014.172]
224 **Konidari A**, Matary WE. Use of thiopurines in inflammatory bowel disease: Safety issues. *World J Gastrointest Pharmacol Ther* 2014; **5**: 63-76 [PMID: 24868487 DOI: 10.4292/wjgpt.v5.i2.63]
225 **Hariharan S**, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med* 2000; **342**: 605-612 [PMID: 10699159 DOI: 10.1056/NEJM200003023420901]
226 **Tedesco D**, Haragsim L. Cyclosporine: a review. *J Transplant* 2012; **2012**: 230386 [PMID: 22263104 DOI: 10.1155/2012/230386]
227 **Rao A**, Luo C, Hogan PG. Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol* 1997; **15**: 707-747 [PMID: 9143705 DOI: 10.1146/annurev.immunol.15.1.707]
228 **Zununi Vahed S**, Ardalan M, Samadi N, Omidi Y. Pharmacogenetics and drug-induced nephrotoxicity in renal transplant recipients. *Bioimpacts* 2015; **5**: 45-54 [PMID: 25901296 DOI: 10.15171/bi.2015.12]
229 **Nankivell BJ**, Borrows RJ, Fung CL, O'Connell PJ, Chapman JR, Allen RD. Calcineurin inhibitor nephrotoxicity: longitudinal assessment by protocol histology. *Transplantation* 2004; **78**: 557-565 [PMID: 15446315]
230 **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]
231 **Bloom RD**, Reese PP. Chronic kidney disease after nonrenal solid-organ transplantation. *J Am Soc Nephrol* 2007; **18**: 3031-3041 [PMID: 18039925 DOI: 10.1681/ASN.2007040394]
232 **Harris TE**, Lawrence JC. TOR signaling. *Sci STKE* 2003; **2003**: re15 [PMID: 14668532 DOI: 10.1126/stke.2122003re15]
233 **Battaglia M**, Stabilini A, Migliavacca B, Horejs-Hoeck J, Kaupper T, Roncarolo MG. Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. *J Immunol* 2006; **177**: 8338-8347 [PMID: 17142730]
234 **Zeiser R**, Leveson-Gower DB, Zambricki EA, Kambham N, Beilhack A, Loh J, Hou JZ, Negrin RS. Differential impact of mammalian target of rapamycin inhibition on CD4+CD25+Foxp3+ regulatory T cells compared with conventional CD4+ T cells. *Blood* 2008; **111**: 453-462 [PMID: 17967941 DOI: 10.1182/blood-2007-06-094482]
235 **Staatz CE**, Tett SE. Pharmacology and toxicology of mycophenolate in organ transplant recipients: an update. *Arch Toxicol* 2014; **88**: 1351-1389 [PMID: 24792322 DOI: 10.1007/s00204-014-1247-1]
236 **Satyananda V**, Shapiro R. Belatacept in kidney transplantation. *Curr Opin Organ Transplant* 2014; **19**: 573-577 [PMID: 25333833 DOI: 10.1097/MOT.0000000000000134]
237 **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]
238 **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]
239 **Masson P**, Henderson L, Chapman JR, Craig JC, Webster AC. Belatacept for kidney transplant recipients. *Cochrane Database Syst Rev* 2014; **11**: CD010699 [PMID: 25416857 DOI: 10.1002/14651858.CD010699.pub2]
240 **Okimura K**, Maeta K, Kobayashi N, Goto M, Kano N, Ishihara T, Ishikawa T, Tsumura H, Ueno A, Miyao Y, Sakuma S, Kinugasa F, Takahashi N, Miura T. Characterization of ASKP1240, a fully human antibody targeting human CD40 with potent immunosuppressive effects. *Am J Transplant* 2014; **14**: 1290-1299 [PMID: 24731050 DOI:

10.1111/ajt.12678]

241 **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]

242 **Vincenti F**, Silva HT, Busque S, O'Connell PJ, Russ G, Budde K, Yoshida A, Tortorici MA, Lamba M, Lawendy N, Wang W, Chan G. Evaluation of the effect of tofacitinib exposure on outcomes in kidney transplant patients. *Am J Transplant* 2015; **15**: 1644-1653 [PMID: 25649117 DOI: 10.1111/ajt.13181]

243 **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]

244 **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]

245 **Pascher A**, De Simone P, Pratschke J, Salamé E, Pirenne J, Isoneimi H, Bijarnia M, Krishnan I, Klupp J. Protein kinase C inhibitor sotrastaurin in de novo liver transplant recipients: a randomized phase II trial. *Am J Transplant* 2015; **15**: 1283-1292 [PMID: 25677074 DOI: 10.1111/ajt.13175]

246 **Lopez M**, Clarkson MR, Albin M, Sayegh MH, Najafian N. A novel mechanism of action for anti-thymocyte globulin: induction of CD4+CD25+Foxp3+ regulatory T cells. *J Am Soc Nephrol* 2006; **17**: 2844-2853 [PMID: 16914538 DOI: 10.1681/ASN.2006050422]

247 **Bloom DD**, Chang Z, Fechner JH, Dar W, Polster SP, Pascual J, Turka LA, Knechtle SJ. CD4+ CD25+ FOXP3+ regulatory T cells increase de novo in kidney transplant patients after immunodepletion with Campath-1H. *Am J Transplant* 2008; **8**: 793-802 [PMID: 18261176 DOI: 10.1111/j.1600-6143.2007.02134.x]

248 **Heidt S**, Hester J, Shankar S, Friend PJ, Wood KJ. B cell repopulation after alemtuzumab induction-transient increase in transitional B cells and long-term dominance of naïve B cells. *Am J Transplant* 2012; **12**: 1784-1792 [PMID: 22420490 DOI: 10.1111/j.1600-6143.2012.04012.x]

249 **Al-Homsi AS**, Feng Y, Duffner U, Al Malki MM, Goodyke A, Cole K, Muilenburg M, Abdel-Mageed A. Bortezomib for the prevention and treatment of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Exp Hematol* 2016; **44**: 771-777 [PMID: 27224851 DOI: 10.1016/j.exphem.2016.05.005]

250 **Al-Homsi AS**, Lai Z, Roy TS, Kouttab N. Effect of novel proteasome and immunoproteasome inhibitors on dendritic cell maturation, function, and expression of IκB and NFκB. *Transpl Immunol* 2013; **29**: 1-6 [PMID: 24103732 DOI: 10.1016/j.trim.2013.09.011]

251 **Berges C**, Haberstock H, Fuchs D, Miltz M, Sadeghi M, Opelz G, Daniel V, Naujokat C. Proteasome inhibition suppresses essential immune functions of human CD4+ T cells. *Immunology* 2008; **124**: 234-246 [PMID: 18217957 DOI: 10.1111/j.1365-2567.2007.02761.x]

252 **Verbrugge SE**, Scheper RJ, Lems WF, de Gruijl TD, Jansen G. Proteasome inhibitors as experimental therapeutics of autoimmune diseases. *Arthritis Res Ther* 2015; **17**: 17 [PMID: 25889583 DOI: 10.1186/s13075-015-0529-1]

253 **Sarno G**, Muscogiuri G, De Rosa P. New-onset diabetes after kidney transplantation: prevalence, risk factors, and management. *Transplantation* 2012; **93**: 1189-1195 [PMID: 22475764 DOI: 10.1097/TP.0b013e31824db97d]

254 **Bottomley MJ**, Harden PN. Update on the long-term complications of renal transplantation. *Br Med Bull* 2013; **106**: 117-134 [PMID: 23645842 DOI: 10.1093/bmb/ldt012]

255 **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]

256 **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]

257 **Evans RW**, Applegate WH, Briscoe DM, Cohen DJ, Rorick CC, Murphy BT, Madsen JC. Cost-related immunosuppressive medication nonadherence among kidney transplant recipients. *Clin J Am Soc Nephrol* 2010; **5**: 2323-2328 [PMID: 20847093 DOI: 10.2215/CJN.04220510]

258 **Saidi RF**, Hejazii Kenari SK. Clinical transplantation and tolerance: are we there yet? *Int J Organ Transplant Med* 2014; **5**: 137-145 [PMID: 25426282]

259 **Xing Y**, Hogquist KA. T-cell tolerance: central and peripheral. *Cold Spring Harb Perspect Biol* 2012; **4**: pii: a006957 [PMID: 22661634 DOI: 10.1101/cshperspect.a006957]

260 **Girlanda R**, Kirk AD. Frontiers in nephrology: immune tolerance to allografts in humans. *J Am Soc Nephrol* 2007; **18**: 2242-2251 [PMID: 17634435 DOI: 10.1681/ASN.2007020180]

261 **Alex Bishop G**, Bertolino PD, Bowen DG, McCaughan GW. Tolerance in liver transplantation. *Best Pract Res Clin Gastroenterol* 2012; **26**: 73-84 [PMID: 22482527 DOI: 10.1016/j.bpg.2012.01.003]

262 **Fehr T**, Sykes M. Clinical experience with mixed chimerism to induce transplantation tolerance. *Transpl Int* 2008; **21**: 1118-1135 [PMID: 18954364 DOI: 10.1111/j.1432-2277.2008.00783.x]

263 **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]

264 **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]

265 **Alexander SI**, Smith N, Hu M, Verran D, Shun A, Dorney S, Smith A, Webster B, Shaw PJ, Lammi A, Stormon MO. Chimerism and tolerance in a recipient of a deceased-donor liver transplant. *N Engl J Med* 2008; **358**: 369-374 [PMID: 18216357 DOI: 10.1056/NEJMoa0707255]

266 **Kawai T**, Cosimi AB, Sachs DH. Preclinical and clinical studies on the induction of renal allograft tolerance through transient mixed chimerism. *Curr Opin Organ Transplant* 2011; **16**: 366-371 [PMID: 21666482 DOI: 10.1097/MOT.0b013e3283484b2c]

267 **Scandling JD**, Busque S, Dejbakhsh-Jones S, Benike C, Millan MT, Shizuru JA, Hoppe RT, Lowsky R, Engleman EG, Strober S. Tolerance and chimerism after renal and hematopoietic-cell transplantation. *N Engl J Med* 2008; **358**: 362-368 [PMID: 18216356 DOI: 10.1056/NEJMoa074191]

268 **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]

269 **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]

270 **Wood KJ**, Bushell A, Hester J. Regulatory immune cells in transplantation. *Nat Rev Immunol* 2012; **12**: 417-430 [PMID: 22627860 DOI: 10.1038/nri3227]

271 **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]

272 **Mazariegos GV**, Zahorchak AF, Reyes J, Ostrowski L, Flynn B, Zeevi A, Thomson AW. Dendritic cell subset ratio in peripheral blood correlates with successful withdrawal of immunosuppression in liver transplant patients. *Am J Transplant* 2003; **3**: 689-696 [PMID: 12780560]

273 **Newell KA**, Asare A, Kirk AD, Gisler TD, Bourcier K, Suthanthiran M, Burlingham WJ, Marks WH, Sanz I, Lechler RI, Hernandez-Fuentes MP, Turka LA, Seyfert-Margolis VL. Identification of a B cell signature associated with renal transplant tolerance in humans. *J Clin Invest* 2010; **120**: 1836-1847 [PMID: 20501946 DOI: 10.1172/JCI39933]

274 **Shabir S**, Girdlestone J, Briggs D, Kaul B, Smith H, Daga S, Chand S, Jham S, Navarrete C, Harper L, Ball S, Borrows R. Transitional B lymphocytes are associated with protection from kidney allograft rejection: a prospective study. *Am J Transplant* 2015; **15**: 1384-1391 [PMID: 25808898 DOI: 10.1111/ajt.13122]

275 **Singer BD**, King LS, D'Alessio FR. Regulatory T cells as immunotherapy. *Front Immunol* 2014; **5**: 46 [PMID: 24575095 DOI: 10.3389/fimmu.2014.00046]

276 **Stolp J**, Turka LA, Wood KJ. B cells with immune-regulating function in transplantation. *Nat Rev Nephrol* 2014; **10**: 389-397 [PMID: 24846332 DOI: 10.1038/nrneph.2014.80]

277 **Ezzelarab M**, Thomson AW. Tolerogenic dendritic cells and their role in transplantation. *Semin Immunol* 2011; **23**: 252-263 [PMID: 21741270 DOI: 10.1016/j.smim.2011.06.007]

278 **Gordon JR**, Ma Y, Churchman L, Gordon SA, Dawicki W. Regulatory dendritic cells for immunotherapy in immunologic diseases. *Front Immunol* 2014; **5**: 7 [PMID: 24550907 DOI: 10.3389/fimmu.2014.00007]

279 **Benoist C**, Mathis D. Treg cells, life history, and diversity. *Cold Spring Harb Perspect Biol* 2012; **4**: a007021 [PMID: 22952391 DOI: 10.1101/cshperspect.a007021]

280 **Geiger TL**, Tauro S. Nature and nurture in Foxp3(+) regulatory T cell development, stability, and function. *Hum Immunol* 2012; **73**: 232-239 [PMID: 22240298 DOI: 10.1016/j.humimm.2011.12.012]

281 **Feuerer M**, Hill JA, Kretschmer K, von Boehmer H, Mathis D, Benoist C. Genomic definition of multiple ex vivo regulatory T cell subphenotypes. *Proc Natl Acad Sci USA* 2010; **107**: 5919-5924 [PMID: 20231436 DOI: 10.1073/pnas.1002006107]

282 **Verhagen J**, Wegner A, Wraith DC. Extra-thymically induced T regulatory cell subsets: the optimal target for antigen-specific immunotherapy. *Immunology* 2015; **145**: 171-181 [PMID: 25716063 DOI: 10.1111/imm.12458]

283 **Issa F**, Chandrasekharan D, Wood KJ. Regulatory T cells as modulators of chronic allograft dysfunction. *Curr Opin Immunol* 2011; **23**: 648-654 [PMID: 21752619 DOI: 10.1016/j.coi.2011.06.005]

284 **Francis RS**, Feng G, Tha-In T, Lyons IS, Wood KJ, Bushell A. Induction of transplantation tolerance converts potential effector T cells into graft-protective regulatory T cells. *Eur J Immunol* 2011; **41**: 726-738 [PMID: 21243638 DOI: 10.1002/eji.201040509]

285 **Wing JB**, Sakaguchi S. Multiple treg suppressive modules and their adaptability. *Front Immunol* 2012; **3**: 178 [PMID: 22754556 DOI: 10.3389/fimmu.2012.00178]

286 **Joffre O**, Santolaria T, Calise D, Al Saati T, Hudrisier D, Romagnoli P, van Meerwijk JP. Prevention of acute and chronic allograft rejection with CD4+CD25+Foxp3+ regulatory T lymphocytes. *Nat Med* 2008; **14**: 88-92 [PMID: 18066074 DOI: 10.1038/nm1688]

287 **Pilat N**, Klaus C, Gattringer M, Jaeckel E, Wrba F, Golshayan D, Baranyi U, Wekerle T. Therapeutic efficacy of polyclonal tregs does not require rapamycin in a low-dose irradiation bone marrow transplantation model. *Transplantation* 2011; **92**: 280-288 [PMID: 21697774 DOI: 10.1097/TP.0b013e3182241133]

288 **Joffre O**, Gorsse N, Romagnoli P, Hudrisier D, van Meerwijk JP. Induction of antigen-specific tolerance to bone marrow allografts with CD4+CD25+ T lymphocytes. *Blood* 2004; **103**: 4216-4221 [PMID: 14976053 DOI: 10.1182/blood-2004-01-0005]

289 **Shin HJ**, Baker J, Leveson-Gower DB, Smith AT, Sega EI, Negrin RS. Rapamycin and IL-2 reduce lethal acute graft-versus-host disease associated with increased expansion of donor type CD4+CD25+Foxp3+ regulatory T cells. *Blood* 2011; **118**: 2342-2350 [PMID: 21734238 DOI: 10.1182/blood-2010-10-313684]

290 **Hester J**, Schiopu A, Nadig SN, Wood KJ. Low-dose rapamycin treatment increases the ability of human regulatory T cells to inhibit transplant arteriosclerosis in vivo. *Am J Transplant* 2012; **12**: 2008-2016 [PMID: 22500984 DOI: 10.1111/j.1600-6143.2012.04065.x]

291 **Moore C**, Tejon G, Fuentes C, Hidalgo Y, Bono MR, Maldonado P, Fernandez R, Wood KJ, Fierro JA, Rosemblatt M, Sauma D, Bushell A. Alloreactive regulatory T cells generated with retinoic acid prevent skin allograft rejection. *Eur J Immunol* 2015; **45**: 452-463 [PMID: 25381698 DOI: 10.1002/eji.201444743]

292 **Kingsley CI**, Karim M, Bushell AR, Wood KJ. CD25+CD4+ regulatory T cells prevent graft rejection: CTLA-4- and IL-10-dependent immunoregulation of alloresponses. *J Immunol* 2002; **168**: 1080-1086 [PMID: 11801641]

293 **Nadig SN**, Wieckiewicz J, Wu DC, Warnecke G, Zhang W, Luo S, Schiopu A, Taggart DP, Wood KJ. In vivo prevention of transplant arteriosclerosis by ex vivo-expanded human regulatory T cells. *Nat Med* 2010; **16**: 809-813 [PMID: 20473306 DOI: 10.1038/nm.2154]

294 **Sagoo P**, Ali N, Garg G, Nestle FO, Lechler RI, Lombardi G. Human regulatory T cells with alloantigen specificity are more potent inhibitors of alloimmune skin graft damage than polyclonal regulatory T cells. *Sci Transl Med* 2011; **3**: 83ra42 [PMID: 21593402 DOI: 10.1126/scitranslmed.3002076]

295 **Issa F**, Hester J, Goto R, Nadig SN, Goodacre TE, Wood K. Ex vivo-expanded human regulatory T cells prevent the rejection of skin allografts in a humanized mouse model. *Transplantation* 2010; **90**: 1321-1327 [PMID: 21048528 DOI: 10.1097/TP.0b013e3181ff8772]

296 **Nguyen VH**, Shashidhar S, Chang DS, Ho L, Kambham N, Bachmann M, Brown JM, Negrin RS. The impact of regulatory T cells on T-cell immunity following hematopoietic cell transplantation. *Blood* 2008; **111**: 945-953 [PMID: 17916743 DOI: 10.1182/blood-2007-07-103895]

297 **Ermann J**, Hoffmann P, Edinger M, Dutt S, Blankenberg FG, Higgins JP, Negrin RS, Fathman CG, Strober S. Only the CD62L+ subpopulation of CD4+CD25+ regulatory T cells protects from lethal acute GVHD. *Blood* 2005; **105**: 2220-2226 [PMID: 15546950 DOI: 10.1182/blood-2004-05-2044]

298 **Di Ianni M**, Falzetti F, Carotti A, Terenzi A, Castellino F, Bonifacio E, Del Papa B, Zei T, Ostini RI, Cecchini D, Aloisi T, Perruccio K, Ruggeri L, Balucani C, Pierini A, Sportoletti P, Aristei C, Falini B, Reisner Y, Velardi A, Aversa F, Martelli MF. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood* 2011; **117**: 3921-3928 [PMID: 21292771 DOI: 10.1182/blood-2010-10-311894]

299 **Brunstein CG**, Miller JS, Cao Q, McKenna DH, Hippen KL, Curtsinger J, Defor T, Levine BL, June CH, Rubinstein P, McGlave PB, Blazar BR, Wagner JE. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. *Blood* 2011; **117**: 1061-1070 [PMID: 20952687 DOI: 10.1182/blood-2010-07-293795]

300 **Raïch-Regué D**, Glancy M, Thomson AW. Regulatory dendritic cell therapy: from rodents to clinical application. *Immunol Lett* 2014; **161**: 216-221 [PMID: 24316407 DOI: 10.1016/j.imlet.2013.11.016]

301 **Steinman RM**, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol* 2003; **21**: 685-711 [PMID: 12615891 DOI: 10.1146/annurev.immunol.21.120601.141040]

302 **Divito SJ**, Wang Z, Shufesky WJ, Liu Q, Tkacheva OA, Montecalvo A, Erdos G, Larregina AT, Morelli AE. Endogenous dendritic cells mediate the effects of intravenously injected therapeutic immunosuppressive dendritic cells in transplantation. *Blood* 2010; **116**: 2694-2705 [PMID: 20576812 DOI: 10.1182/blood-2009-10-251058]

303 **Mackern-Oberti JP**, Vega F, Llanos C, Bueno SM, Kalergis AM. Targeting dendritic cell function during systemic autoimmunity to restore tolerance. *Int J Mol Sci* 2014; **15**: 16381-16417 [PMID: 25229821 DOI: 10.3390/ijms150916381]

304 **Lutz MB**, Suri RM, Niimi M, Ogilvie AL, Kukutsch NA, Rössner S, Schuler G, Austyn JM. Immature dendritic cells generated with low doses of GM-CSF in the absence of IL-4 are maturation resistant and prolong allograft survival in vivo. *Eur J Immunol* 2000; **30**: 1813-1822

[PMID: 10940870 DOI: 10.1002/1521-4141(200007)30: 7<1813: : AID-IMMU1813>3.0.CO; 2-8]

305 **Pêche H**, Trinité B, Martinet B, Cuturi MC. Prolongation of heart allograft survival by immature dendritic cells generated from recipient type bone marrow progenitors. *Am J Transplant* 2005; **5**: 255-267 [PMID: 15643985 DOI: 10.1111/j.1600-6143.2004.00683.x]

306 **Bériou G**, Pêche H, Guillonneau C, Merieau E, Cuturi MC. Donor-specific allograft tolerance by administration of recipient-derived immature dendritic cells and suboptimal immunosuppression. *Transplantation* 2005; **79**: 969-972 [PMID: 15849552]

307 **Sato K**, Yamashita N, Yamashita N, Baba M, Matsuyama T. Regulatory dendritic cells protect mice from murine acute graft-versus-host disease and leukemia relapse. *Immunity* 2003; **18**: 367-379 [PMID: 12648454]

308 **Hu J**, Wan Y. Tolerogenic dendritic cells and their potential applications. *Immunology* 2011; **132**: 307-314 [PMID: 21208205 DOI: 10.1111/j.1365-2567.2010.03396.x]

309 **Thomas DC**, Wong FS, Zaccone P, Green EA, Wållberg M. Protection of islet grafts through transforming growth factor-β-induced tolerogenic dendritic cells. *Diabetes* 2013; **62**: 3132-3142 [PMID: 23715623 DOI: 10.2337/db12-1740]

310 **Turnquist HR**, Raimondi G, Zahorchak AF, Fischer RT, Wang Z, Thomson AW. Rapamycin-conditioned dendritic cells are poor stimulators of allogeneic CD4+ T cells, but enrich for antigen-specific Foxp3+ T regulatory cells and promote organ transplant tolerance. *J Immunol* 2007; **178**: 7018-7031 [PMID: 17513751]

311 **Chesneau M**, Michel L, Degauque N, Brouard S. Regulatory B cells and tolerance in transplantation: from animal models to human. *Front Immunol* 2013; **4**: 497 [PMID: 24427159 DOI: 10.3389/fimmu.2013.00497]

312 **Evans JG**, Chavez-Rueda KA, Eddaoudi A, Meyer-Bahlburg A, Rawlings DJ, Ehrenstein MR, Mauri C. Novel suppressive function of transitional 2 B cells in experimental arthritis. *J Immunol* 2007; **178**: 7868-7878 [PMID: 17548625]

313 **Yanaba K**, Bouaziz JD, Haas KM, Poe JC, Fujimoto M, Tedder TF. A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. *Immunity* 2008; **28**: 639-650 [PMID: 18482568 DOI: 10.1016/j.immuni.2008.03.017]

314 **Lal G**, Nakayama Y, Sethi A, Singh AK, Burrell BE, Kulkarni N, Brinkman CC, Iwami D, Zhang T, Bromberg JS. Interleukin-10 From Marginal Zone Precursor B-Cell Subset Is Required for Costimulatory Blockade-Induced Transplantation Tolerance. *Transplantation* 2015; **99**: 1817-1828 [PMID: 25839706 DOI: 10.1097/TP.0000000000000718]

315 **Bouaziz JD**, Yanaba K, Tedder TF. Regulatory B cells as inhibitors of immune responses and inflammation. *Immunol Rev* 2008; **224**: 201-214 [PMID: 18759928 DOI: 10.1111/j.1600-065X.2008.00661.x]

316 **Matsushita T**, Horikawa M, Iwata Y, Tedder TF. Regulatory B cells (B10 cells) and regulatory T cells have independent roles in controlling experimental autoimmune encephalomyelitis initiation and late-phase immunopathogenesis. *J Immunol* 2010; **185**: 2240-2252 [PMID: 20624940 DOI: 10.4049/jimmunol.1001307]

317 **Carter NA**, Vasconcellos R, Rosser EC, Tulone C, Muñoz-Suano A, Kamanaka M, Ehrenstein MR, Flavell RA, Mauri C. Mice lacking endogenous IL-10-producing regulatory B cells develop exacerbated disease and present with an increased frequency of Th1/Th17 but a decrease in regulatory T cells. *J Immunol* 2011; **186**: 5569-5579 [PMID: 21464089 DOI: 10.4049/jimmunol.1100284]

318 **Pallier A**, Hillion S, Danger R, Giral M, Racapé M, Degauque N, Dugast E, Ashton-Chess J, Pettré S, Lozano JJ, Bataille R, Devys A, Cesbron-Gautier A, Braudeau C, Larrose C, Soulillou JP, Brouard S. Patients with drug-free long-term graft function display increased numbers of peripheral B cells with a memory and inhibitory phenotype. *Kidney Int* 2010; **78**: 503-513 [PMID: 20531452 DOI: 10.1038/ki.2010.162]

319 **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]

320 **Nouël A**, Ségalen I, Jamin C, Doucet L, Caillard S, Renaudineau Y, Pers JO, Le Meur Y, Hillion S. B cells display an abnormal distribution and an impaired suppressive function in patients with chronic antibody-mediated rejection. *Kidney Int* 2014; **85**: 590-599 [PMID: 24284517 DOI: 10.1038/ki.2013.457]

321 **Fehr T**, Haspot F, Mollov J, Chittenden M, Hogan T, Sykes M. Alloreactive CD8 T cell tolerance requires recipient B cells, dendritic cells, and MHC class II. *J Immunol* 2008; **181**: 165-173 [PMID: 18566381 DOI: 10.4049/jimmunol.181.1.165]

322 **Yan Y**, van der Putten K, Bowen DG, Painter DM, Kohar J, Sharland AF, McCaughan GW, Bishop GA. Postoperative administration of donor B cells induces rat kidney allograft acceptance: lack of association with Th2 cytokine expression in long-term accepted grafts. *Transplantation* 2002; **73**: 1123-1130 [PMID: 11965044 DOI: 10.1097/00007890-200204150-00020]

323 **Zhao G**, Moore DJ, Lee KM, Kim JI, Duff PE, O'Connor MR, Hirohashi T, Lei J, Yang M, Markmann JF, Deng S. An unexpected counter-regulatory role of IL-10 in B-lymphocyte-mediated transplantation tolerance. *Am J Transplant* 2010; **10**: 796-801 [PMID: 20199511 DOI: 10.1111/j.1600-6143.2010.03027.x]

324 **Moreau A**, Blair PA, Chai JG, Ratnasothy K, Stolarczyk E, Alhabbab R, Rackham CL, Jones PM, Smyth L, Elgueta R, Howard JK, Lechler RI, Lombardi G. Transitional-2 B cells acquire regulatory function during tolerance induction and contribute to allograft survival. *Eur J Immunol* 2015; **45**: 843-853 [PMID: 25408265 DOI: 10.1002/eji.201445082]

325 **Lee KM**, Stott RT, Zhao G, SooHoo J, Xiong W, Lian MM, Fitzgerald L, Shi S, Akrawi E, Lei J, Deng S, Yeh H, Markmann JF, Kim JI. TGF-β-producing regulatory B cells induce regulatory T cells and promote transplantation tolerance. *Eur J Immunol* 2014; **44**: 1728-1736 [PMID: 24700192 DOI: 10.1002/eji.201344062]

326 **Wang Y**, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. *Nat Immunol* 2014; **15**: 1009-1016 [PMID: 25329189 DOI: 10.1038/ni.3002]

327 **Liu H**, Liu S, Li Y, Wang X, Xue W, Ge G, Luo X. The role of SDF-1-CXCR4/CXCR7 axis in the therapeutic effects of hypoxia-preconditioned mesenchymal stem cells for renal ischemia/reperfusion injury. *PLoS One* 2012; **7**: e34608 [PMID: 22511954 DOI: 10.1371/journal.pone.0034608]

328 **Casiraghi F**, Azzollini N, Cassis P, Imberti B, Morigi M, Cugini D, Cavinato RA, Todeschini M, Solini S, Sonzogni A, Perico N, Remuzzi G, Noris M. Pretransplant infusion of mesenchymal stem cells prolongs the survival of a semiallogeneic heart transplant through the generation of regulatory T cells. *J Immunol* 2008; **181**: 3933-3946 [PMID: 18768848 DOI: 10.4049/jimmunol.181.6.3933]

329 **Ge W**, Jiang J, Arp J, Liu W, Garcia B, Wang H. Regulatory T-cell generation and kidney allograft tolerance induced by mesenchymal stem cells associated with indoleamine 2,3-dioxygenase expression. *Transplantation* 2010; **90**: 1312-1320 [PMID: 21042238 DOI: 10.1097/TP.0b013e3181fed001]

330 **Casiraghi F**, Azzollini N, Todeschini M, Cavinato RA, Cassis P, Solini S, Rota C, Morigi M, Introna M, Maranta R, Perico N, Remuzzi G, Noris M. Localization of mesenchymal stromal cells dictates their immune or proinflammatory effects in kidney transplantation. *Am J Transplant* 2012; **12**: 2373-2383 [PMID: 22642544 DOI: 10.1111/j.1600-6143.2012.04115.x]

331 **Coulson-Thomas VJ**, Gesteira TF, Hascall V, Kao W. Umbilical cord mesenchymal stem cells suppress host rejection: the role of the glycocalyx. *J Biol Chem* 2014; **289**: 23465-23481 [PMID: 24986866 DOI: 10.1074/jbc.M114.557447]

332 **Eggenhofer E**, Steinmann JF, Renner P, Slowik P, Piso P, Geissler EK, Schlitt HJ, Dahlke MH, Popp FC. Mesenchymal stem cells together with mycophenolate mofetil inhibit antigen presenting cell and T cell infiltration into allogeneic heart grafts. *Transpl Immunol* 2011; **24**: 157-163 [PMID: 21194567 DOI: 10.1016/j.trim.2010.12.002]

333 **Hara Y**, Stolk M, Ringe J, Dehne T, Ladhoff J, Kotsch K, Reutzel-Selke A, Reinke P, Volk HD, Seifert M. In vivo effect of bone marrow-derived mesenchymal stem cells in a rat kidney transplantation model with prolonged cold ischemia. *Transpl Int* 2011; **24**: 1112-1123 [PMID: 21880071 DOI: 10.1111/j.1432-2277.2011.01328.x]

334 **Kim YH**, Wee YM, Choi MY, Lim DG, Kim SC, Han DJ. Interleukin (IL)-10 induced by CD11b(+) cells and IL-10-activated regulatory T cells play a role in immune modulation of mesenchymal stem cells in rat islet allografts. *Mol Med* 2011; **17**: 697-708 [PMID: 21365122 DOI: 10.2119/molmed.2010.00098]

335 **Xu DM**, Yu XF, Zhang D, Zhang MX, Zhou JF, Tan PH, Ding YC. Mesenchymal stem cells differentially mediate regulatory T cells and conventional effector T cells to protect fully allogeneic islet grafts in mice. *Diabetologia* 2012; **55**: 1091-1102 [PMID: 22270222 DOI: 10.1007/s00125-011-2433-9]

336 **Mougiakakos D**, Jitschin R, Johansson CC, Okita R, Kiessling R, Le Blanc K. The impact of inflammatory licensing on heme oxygenase-1-mediated induction of regulatory T cells by human mesenchymal stem cells. *Blood* 2011; **117**: 4826-4835 [PMID: 21389316 DOI: 10.1182/blood-2010-12-324038]

337 **Li H**, Guo Z, Jiang X, Zhu H, Li X, Mao N. Mesenchymal stem cells alter migratory property of T and dendritic cells to delay the development of murine lethal acute graft-versus-host disease. *Stem Cells* 2008; **26**: 2531-2541 [PMID: 18635870 DOI: 10.1634/stemcells.2008-0146]

338 **Spaggiari GM**, Abdelrazik H, Becchetti F, Moretta L. MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2. *Blood* 2009; **113**: 6576-6583 [PMID: 19398717 DOI: 10.1182/blood-2009-02-203943]

339 **Zhang B**, Liu R, Shi D, Liu X, Chen Y, Dou X, Zhu X, Lu C, Liang W, Liao L, Zenke M, Zhao RC. Mesenchymal stem cells induce mature dendritic cells into a novel Jagged-2-dependent regulatory dendritic cell population. *Blood* 2009; **113**: 46-57 [PMID: 18832657 DOI: 10.1182/blood-2008-04-154138]

340 **Casiraghi F**, Remuzzi G, Perico N. Mesenchymal stromal cells to promote kidney transplantation tolerance. *Curr Opin Organ Transplant* 2014; **19**: 47-53 [PMID: 24257324 DOI: 10.1097/MOT.0000000000000035]

341 **Perico N**, Casiraghi F, Introna M, Gotti E, Todeschini M, Cavinato RA, Capelli C, Rambaldi A, Cassis P, Rizzo P, Cortinovis M, Marasà M, Golay J, Noris M, Remuzzi G. Autologous mesenchymal stromal cells and kidney transplantation: a pilot study of safety and clinical feasibility. *Clin J Am Soc Nephrol* 2011; **6**: 412-422 [PMID: 20930086 DOI: 10.2215/CJN.04950610]

342 **Tan J**, Wu W, Xu X, Liao L, Zheng F, Messinger S, Sun X, Chen J, Yang S, Cai J, Gao X, Pileggi A, Ricordi C. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. *JAMA* 2012; **307**: 1169-1177 [PMID: 22436957 DOI: 10.1001/jama.2012.316]

343 **Mold JE**, Michaëlsson J, Burt TD, Muench MO, Beckerman KP, Busch MP, Lee TH, Nixon DF, McCune JM. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. *Science* 2008; **322**: 1562-1565 [PMID: 19056990 DOI: 10.1126/science.1164511]

344 **Burt TD**. Fetal regulatory T cells and peripheral immune tolerance in utero: implications for development and disease. *Am J Reprod Immunol* 2013; **69**: 346-358 [PMID: 23432802 DOI: 10.1111/aji.12083]

345 **Somerset DA**, Zheng Y, Kilby MD, Sansom DM, Drayson MT. Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. *Immunology* 2004; **112**: 38-43 [PMID: 15096182 DOI: 10.1111/j.1365-2567.2004.01869.x]

346 **Sasaki Y**, Sakai M, Miyazaki S, Higuma S, Shiozaki A, Saito S. Decidual and peripheral blood CD4+CD25+ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. *Mol Hum Reprod* 2004; **10**: 347-353 [PMID: 14997000 DOI: 10.1093/molehr/gah044]

347 **Winger EE**, Reed JL. Low circulating CD4(+) CD25(+) Foxp3(+) T regulatory cell levels predict miscarriage risk in newly pregnant women with a history of failure. *Am J Reprod Immunol* 2011; **66**: 320-328 [PMID: 21314851 DOI: 10.1111/j.1600-0897.2011.00992.x]

348 **Gammill HS**, Stephenson MD, Aydelotte TM, Nelson JL. Microchimerism in recurrent miscarriage. *Cell Mol Immunol* 2014; **11**: 589-594 [PMID: 25242272 DOI: 10.1038/cmi.2014.82]

349 **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]

350 **Burlingham WJ**, Grailer AP, Heisey DM, Claas FH, Norman D, Mohanakumar T, Brennan DC, de Fijter H, van Gelder T, Pirsch JD, Sollinger HW, Bean MA. The effect of tolerance to noninherited maternal HLA antigens on the survival of renal transplants from sibling donors. *N Engl J Med* 1998; **339**: 1657-1664 [PMID: 9834302 DOI: 10.1056/NEJM199812033392302]

351 **Andrassy J**, Kusaka S, Jankowska-Gan E, Torrealba JR, Haynes LD, Marthaler BR, Tam RC, Illigens BM, Anosova N, Benichou G, Burlingham WJ. Tolerance to noninherited maternal MHC antigens in mice. *J Immunol* 2003; **171**: 5554-5561 [PMID: 14607963 DOI: 10.4049/jimmunol.171.10.5554]

352 **Molitor-Dart ML**, Andrassy J, Kwun J, Kayaoglu HA, Roenneburg DA, Haynes LD, Torrealba JR, Bobadilla JL, Sollinger HW, Knechtle SJ, Burlingham WJ. Developmental exposure to noninherited maternal antigens induces CD4+ T regulatory cells: relevance to mechanism of heart allograft tolerance. *J Immunol* 2007; **179**: 6749-6761 [PMID: 17982065 DOI: 10.4049/jimmunol.179.10.6749]

P- Reviewer: Ni Y **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Transplant 2017 February 24; 7(1): 26-33

DOI: 10.5500/wjt.v7.i1.26

ISSN 2220-3230 (online)

MINIREVIEWS

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

Gerold Thölking, Hans Ulrich Gerth, Katharina Schuette-Nuetgen, Stefan Reuter

Gerold Thölking, Hans Ulrich Gerth, Katharina Schuette-Nuetgen, Stefan Reuter, Division of General Internal Medicine, Nephrology and Rheumatology, Department of Medicine D, University Hospital of Münster, 48149 Münster, Germany

Author contributions: Thölking G wrote the paper, analyzed the data and designed the study; Gerth HU collected the data and wrote the paper; Schuette-Nuetgen K collected the data and wrote the paper; Reuter S designed the study and wrote the paper.

Conflict-of-interest statement: Gerold Thölking, Hans Ulrich Gerth and Katharina Schuette-Nuetgen declare no conflict of interests for this article; Stefan Reuter declares that he has received travel support from Astellas and lecture fees from Chiesi.

Manuscript source: Invited manuscript

Correspondence to: Gerold Thölking, MD, Division of General Internal Medicine, Nephrology and Rheumatology, Department of Medicine D, University Hospital of Münster, Albert-Schweitzer-Campus 1, Gebäude A1, 48149 Münster, Germany. gerold.thoelking@ukmuenster.de
Telephone: +49-251-8348001
Fax: +49-251-8346979

Received: October 5, 2016
Peer-review started: October 7, 2016
First decision: November 11, 2016
Revised: November 22, 2016
Accepted: January 11, 2017
Article in press: January 14, 2017
Published online: February 24, 2017

Abstract

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

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

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

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

Thölking G, Gerth HU, Schuette-Nuetgen K, Reuter S. Influence of tacrolimus metabolism rate on renal function after solid organ transplantation. *World J Transplant* 2017; 7(1): 26-33 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i1/26.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i1.26

INTRODUCTION

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

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

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

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

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

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

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

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

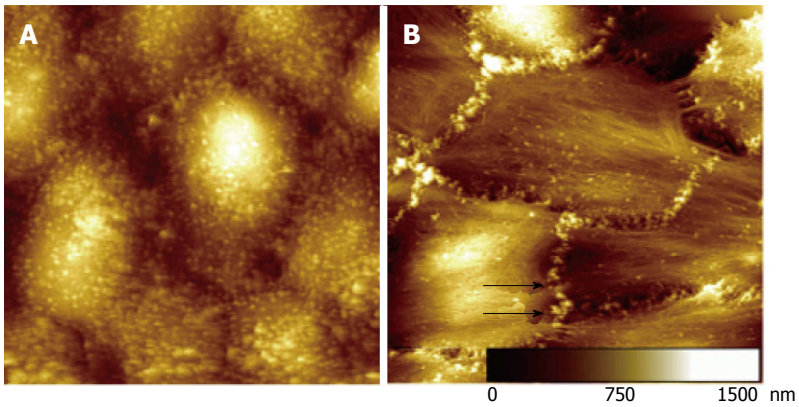


Figure 1 Morphological changes of cells undergoing epithelial to mesenchymal transformation. Images from atomic force microscopy of glutardialdehyde fixed cells in fluid (highest sample areas are represented in white). A: Typically, epithelial tubular cells (NRK-52E), (50 μm)^2, appear with numerous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

HISTOLOGICAL MANIFESTATION OF CNI-NEPHROTOXICITY

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

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

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

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

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

INFLUENCES ON TACROLIMUS METABOLISM

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

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

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

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

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

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

CLINICAL IMPACT OF TAC METABOLISM RATE

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

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

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

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

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

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

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

SUMMARY

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

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

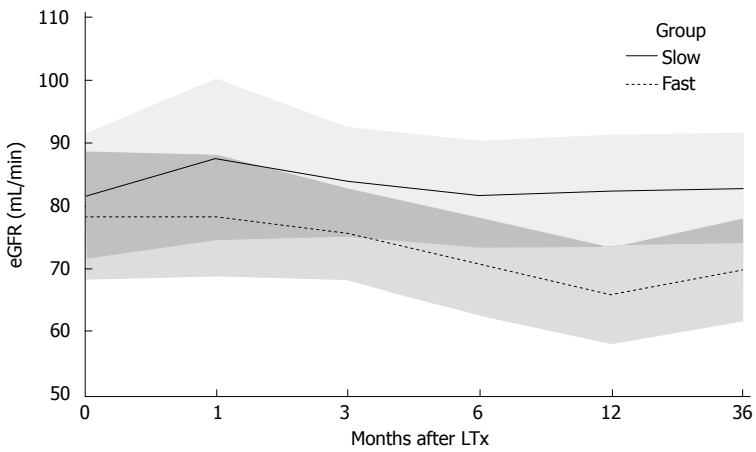


Figure 2 Estimated renal function measured by estimated glomerular filtration rate (Cockcroft-Gault eGFR, mL/min) after liver transplantation. There was no noticeable difference between fast and slow tacrolimus metabolizers at liver transplantation at 1 mo or 3 mo after liver transplantation. After 6,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

Table 1 Medication doses and blood trough concentrations

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

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

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

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

CONCLUSION

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

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

REFERENCES

1 **Nankivell BJ**, PNg CH, OConnell PJ, Chapman JR. Calcineurin Inhibitor Nephrotoxicity Through the Lens of Longitudinal Histology: Comparison of Cyclosporine and Tacrolimus Eras. *Transplantation* 2016; **100**: 1723-1731 [PMID: 27306529 DOI: 10.1097/TP.0000000000001243]

2 **Ekberg H**, Bernasconi C, Tedesco-Silva H, Vítko S, Hugo C, Demirbas A, Acevedo RR, Grinyó J, Frei U, Vanrenterghem Y, Daloze P, Halloran P. Calcineurin inhibitor minimization in the Symphony study: observational results 3 years after transplantation. *Am J Transplant* 2009; **9**: 1876-1885 [PMID: 19563339 DOI: 10.1111/j.1600-6143.2009.02726.x]

3 **Sterneck M**, Kaiser GM, Heyne N, Richter N, Rauchfuss F, Pascher A, Schemmer P, Fischer L, Klein CG, Nadalin S, Lehner F, Settmacher U, Gotthardt D, Loss M, Ladenburger S, Wimmer P, Dworak M, Schlitt HJ. Long-term follow-up of five yr shows superior renal function with everolimus plus early calcineurin inhibitor withdrawal in the PROTECT randomized liver transplantation study. *Clin Transplant* 2016; **30**: 741-748 [PMID: 27160359 DOI: 10.1111/ctr.12744]

4 **Gaber AO**, Alloway RR, Bodziak K, Kaplan B, Bunnapradist S. Conversion from twice-daily tacrolimus capsules to once-daily extended-release tacrolimus (LCPT): a phase 2 trial of stable renal transplant recipients. *Transplantation* 2013; **96**: 191-197 [PMID: 23715050 DOI: 10.1097/TP.0b013e3182962cc1]

5 **Beckebaum S**, Iacob S, Sweid D, Sotiropoulos GC, Saner F, Kaiser G, Radtke A, Klein CG, Erim Y, de Geest S, Paul A, Gerken G, Cicinnati VR. Efficacy, safety, and immunosuppressant adherence in stable

liver transplant patients converted from a twice-daily tacrolimus-based regimen to once-daily tacrolimus extended-release formulation. *Transpl Int* 2011; **24**: 666-675 [PMID: 21466596 DOI: 10.1111/j.1432-2277.2011.01254.x]

6 **Sommerer C**, Suwelack B, Dragun D, Schenker P, Hauser IA, Nashan B, Thaiss F. Design and rationale of the ATHENA study--A 12-month, multicentre, prospective study evaluating the outcomes of a de novo everolimus-based regimen in combination with reduced cyclosporine or tacrolimus versus a standard regimen in kidney transplant patients: study protocol for a randomised controlled trial. *Trials* 2016; **17**: 92 [PMID: 26888217 DOI: 10.1186/s13063-016-1220-9]

7 **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; ELITE-Symphony Study. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562-2575 [PMID: 18094377 DOI: 10.1056/NEJMoa067411]

8 **Webster AC**, Woodroffe RC, Taylor RS, Chapman JR, Craig JC. Tacrolimus versus ciclosporin as primary immunosuppression for kidney transplant recipients: meta-analysis and meta-regression of randomised trial data. *BMJ* 2005; **331**: 810 [PMID: 16157605 DOI: 10.1136/bmj.38569.471007.AE]

9 **Kidney Disease: Improving Global Outcomes Transplant Work G**. 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]

10 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Liver transplantation. *J Hepatol* 2016; **64**: 433-485 [PMID: 26597456 DOI: 10.1016/j.jhep.2015.10.006]

11 **McAlister VC**, Haddad E, Renouf E, Malthaner RA, Kjaer MS, Gluud LL. Cyclosporin versus tacrolimus as primary immunosuppressant after liver transplantation: a meta-analysis. *Am J Transplant* 2006; **6**: 1578-1585 [PMID: 16827858 DOI: 10.1111/j.1600-6143.2006.01360.x]

12 **O'Grady JG**, Hardy P, Burroughs AK, Elbourne D; UK and Ireland Liver Transplant Study Group. Randomized controlled trial of tacrolimus versus microemulsified cyclosporin (TMC) in liver transplantation: poststudy surveillance to 3 years. *Am J Transplant* 2007; **7**: 137-141 [PMID: 17109723 DOI: 10.1111/j.1600-6143.2006.01576.x]

13 **Hüsing A**, Schmidt M, Beckebaum S, Cicinnati VR, Koch R, Thölking G, Stella J, Heinzow H, Schmidt HH, Kabar I. Long-Term Renal Function in Liver Transplant Recipients After Conversion From Calcineurin Inhibitors to mTOR Inhibitors. *Ann Transplant* 2015; **20**: 707-713 [PMID: 26608590 DOI: 10.12659/AOT.895320]

14 **Fischer L**, Saliba F, Kaiser GM, De Carlis L, Metselaar HJ, De Simone P, Duvoux C, Nevens F, Fung JJ, Dong G, Rauer B, Junge G; Junge G; H2304 Study Group. Three-year Outcomes in De Novo Liver Transplant Patients Receiving Everolimus With Reduced Tacrolimus: Follow-Up Results From a Randomized, Multicenter Study. *Transplantation* 2015; **99**: 1455-1462 [PMID: 26151607 DOI: 10.1097/TP.0000000000000555]

15 **De Simone P**, Nevens F, De Carlis L, Metselaar HJ, Beckebaum S, Saliba F, Jonas S, Sudan D, Fung J, Fischer L, Duvoux C, Chavin KD, Koneru B, Huang MA, Chapman WC, Foltys D, Witte S, Jiang H, Hexham JM, Junge G. Everolimus with reduced tacrolimus improves renal function in de novo liver transplant recipients: a randomized controlled trial. *Am J Transplant* 2012; **12**: 3008-3020 [PMID: 22882750 DOI: 10.1111/j.1600-6143.2012.04212.x]

16 **Gruessner AC**, Gruessner RW. Long-term outcome after pancreas transplantation: a registry analysis. *Curr Opin Organ Transplant* 2016; **21**: 377-385 [PMID: 27258580 DOI: 10.1097/MOT.0000000000000331]

17 **Sageshima J**, Ciancio G, Chen L, Dohi T, El-Hinnawi A, Paloyo S, Misawa R, Ekwenna O, Yatawatta A, Burke GW. Everolimus with low-dose tacrolimus in simultaneous pancreas and kidney transplantation. *Clin Transplant* 2014; **28**: 797-801 [PMID: 24779669 DOI: 10.1111/ctr.12381]

18 **Gullestad L**, Eiskjaer H, Gustafsson F, Riise GC, Karason K, Dellgren G, Rådegran G, Hansson L, Gude E, Bjørtuft Ø, Jansson K, Schultz HH, Solbu D, Iversen M. Long-term outcomes of thoracic transplant recipients following conversion to everolimus with reduced calcineurin inhibitor in a multicenter, open-label, randomized trial. *Transpl Int* 2016; **29**: 819-829 [PMID: 27067532 DOI: 10.1111/tri.12783]

19 **Kaczmarek I**, Zaruba MM, Beiras-Fernandez A, Reimann R, Nickel T, Grinninger C, Sadoni S, Hagl C, Meiser B. Tacrolimus with mycophenolate mofetil or sirolimus compared with calcineurin inhibitor-free immunosuppression (sirolimus/mycophenolate mofetil) after heart transplantation: 5-year results. *J Heart Lung Transplant* 2013; **32**: 277-284 [PMID: 23415313 DOI: 10.1016/j.healun.2012.11.028]

20 **Neurohr C**, Huppmann P, Zimmermann G, Leuchte H, Baumgartner R, Hatz R, Frey L, Uberfuhr P, Bittmann I, Behr J, Reichart B; Munich Lung Transplant Group. Tacrolimus and mycophenolate mofetil as first line immunosuppression after lung transplantation. *Transpl Int* 2009; **22**: 635-643 [PMID: 19207186 DOI: 10.1111/j.1432-2277.2009.00843.x]

21 **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]

22 **Scheffert JL**, Raza K. Immunosuppression in lung transplantation. *J Thorac Dis* 2014; **6**: 1039-1053 [PMID: 25132971 DOI: 10.3978/j.issn.2072-1439.2014.04.23]

23 **Aliabadi A**, Cochrane AB, Zuckermann AO. Current strategies and future trends in immunosuppression after heart transplantation. *Curr Opin Organ Transplant* 2012; **17**: 540-545 [PMID: 22941325 DOI: 10.1097/MOT.0b013e328358000c]

24 **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]

25 **Kandula P**, Fridell J, Taber TE, Sharfuddin A, Yaqub MS, Phillips CL, Chen J, Mujtaba M. Impact of tacrolimus-sirolimus maintenance immunosuppression on proteinuria and kidney function in pancreas transplant alone recipients. *Transplantation* 2012; **94**: 940-946 [PMID: 23037007 DOI: 10.1097/TP.0b013e3182696a13]

26 **Tsuchiya T**, Ishida H, Tanabe T, Shimizu T, Honda K, Omoto K, Tanabe K. Comparison of pharmacokinetics and pathology for low-dose tacrolimus once-daily and twice-daily in living kidney transplantation: prospective trial in once-daily versus twice-daily tacrolimus. *Transplantation* 2013; **96**: 198-204 [PMID: 23792649 DOI: 10.1097/TP.0b013e318296c9d5]

27 **Klein IH**, Abrahams AC, van Ede T, Oey PL, Ligtenberg G, Blankestijn PJ. Differential effects of acute and sustained cyclosporine and tacrolimus on sympathetic nerve activity. *J Hypertens* 2010; **28**: 1928-1934 [PMID: 20577127 DOI: 10.1097/HJH.0b013e32833c20eb]

28 **Zuber M**, Donnerer J. Effect of FK506 on neurotransmitter content and expression of GAP-43 in neurotoxin-lesioned peripheral sensory and sympathetic neurons. *Pharmacology* 2002; **66**: 44-50 [PMID: 12169765]

29 **Singh L**, Singh G, Sharma A, Sinha A, Bagga A, Dinda AK. A comparative study on renal biopsy before and after long-term calcineurin inhibitors therapy: an insight for pathogenesis of its toxicity. *Hum Pathol* 2015; **46**: 34-39 [PMID: 25449629 DOI: 10.1016/j.humpath.2014.09.003]

30 **Gossmann J**, Radounikli A, Bernemann A, Schellinski O, Raab HP, Bickeböller R, Scheuermann EH. Pathophysiology of cyclosporine-induced nephrotoxicity in humans: a role for nitric oxide? *Kidney Blood Press Res* 2001; **24**: 111-115 [PMID: 11435743]

31 **Yamada K**, Sugisaki Y, Suzuki S, Akimoto M, Amemiya H, Yamanaka N. New morphological changes induced by FK506 in a short period in the rat kidney and the effect of superoxide dismutase and OKY-046 on THEM: the relationship of FK506 nephrotoxicity to lipid peroxidation and change in production of thromboxane A2 in the kidney. *Transpl Int* 1992; **5** Suppl 1: S564-S567 [PMID: 14621878]

32 **Raina A**, Horn ET, Benza RL. The pathophysiology of endothelin in complications after solid organ transplantation: a potential novel therapeutic role for endothelin receptor antagonists. *Transplantation* 2012; **94**: 885-893 [PMID: 23037008 DOI: 10.1097/TP.0b013e31825f0fbe]

33 **Bennett J**, Cassidy H, Slattery C, Ryan MP, McMorrow T. Tacrolimus Modulates TGF-β Signaling to Induce Epithelial-Mesenchymal Transition in Human Renal Proximal Tubule Epithelial Cells. *J Clin Med* 2016; **5**: pii: E50 [PMID: 27128949 DOI: 10.3390/jcm5050050]

34 **Bhowmick NA**, Ghiassi M, Bakin A, Aakre M, Lundquist CA, Engel ME, Arteaga CL, Moses HL. Transforming growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. *Mol Biol Cell* 2001; **12**: 27-36 [PMID: 11160820 DOI: 10.1091/mbc.12.1.27]

35 **Thoelking G**, Reiss B, Wegener J, Oberleithner H, Pavenstaedt H, Riethmuller C. Nanotopography follows force in TGF-beta1 stimulated epithelium. *Nanotechnology* 2010; **21**: 265102 [PMID: 20522928 DOI: 10.1088/0957-4484/21/26/265102]

36 **Koesters R**, Kaissling B, Lehir M, Picard N, Theilig F, Gebhardt R, Glick AB, Hähnel B, Hosser H, Gröne HJ, Kriz W. Tubular overexpression of transforming growth factor-beta1 induces autophagy and fibrosis but not mesenchymal transition of renal epithelial cells. *Am J Pathol* 2010; **177**: 632-643 [PMID: 20616344 DOI: 10.2353/ajpath.2010.091012]

37 **Rivelli RF**, Gonçalves RT, Leite M, Santos MA, Delgado AG, Cardoso LR, Takiya CM. Early withdrawal of calcineurin inhibitor from a sirolimus-based immunosuppression stabilizes fibrosis and the transforming growth factor-β signalling pathway in kidney transplant. *Nephrology* (Carlton) 2015; **20**: 168-176 [PMID: 25404086 DOI: 10.1111/nep.12368]

38 **Budde K**, Lehner F, Sommerer C, Reinke P, Arns W, Eisenberger U, Wüthrich RP, Mühlfeld A, Heller K, Porstner M, Veit J, Paulus EM, Witzke O; ZEUS Study Investigators. Five-year outcomes in kidney transplant patients converted from cyclosporine to everolimus: the randomized ZEUS study. *Am J Transplant* 2015; **15**: 119-128 [PMID: 25521535 DOI: 10.1111/ajt.12952]

39 **Kuypers DR**, de Loor H, Naesens M, Coopmans T, de Jonge H. Combined effects of CYP3A5*1, POR*28, and CYP3A4*22 single nucleotide polymorphisms on early concentration-controlled tacrolimus exposure in de-novo renal recipients. *Pharmacogenet Genomics* 2014; **24**: 597-606 [PMID: 25322286 DOI: 10.1097/FPC.0000000000000095]

40 **Stratta P**, Quaglia M, Cena T, Antoniotti R, Fenoglio R, Menegotto A, Ferrante D, Genazzani A, Terrazzino S, Magnani C. The interactions of age, sex, body mass index, genetics, and steroid weight-based doses on tacrolimus dosing requirement after adult kidney transplantation. *Eur J Clin Pharmacol* 2012; **68**: 671-680 [PMID: 22101623 DOI: 10.1007/s00228-011-1150-0]

41 **de Jonge H**, Vanhove T, de Loor H, Verbeke K, Kuypers DR. Progressive decline in tacrolimus clearance after renal transplantation is partially explained by decreasing CYP3A4 activity and increasing haematocrit. *Br J Clin Pharmacol* 2015; **80**: 548-559 [PMID: 26114223 DOI: 10.1111/bcp.12703]

42 **Gijsen V**, Mital S, van Schaik RH, Soldin OP, Soldin SJ, van der Heiden IP, Nulman I, Koren G, de Wildt SN. Age and CYP3A5 genotype affect tacrolimus dosing requirements after transplant in pediatric heart recipients. *J Heart Lung Transplant* 2011; **30**: 1352-1359 [PMID: 21930396 DOI: 10.1016/j.healun.2011.08.001]

43 **Anglicheau D**, Flamant M, Schlageter MH, Martinez F, Cassinat B, Beaune P, Legendre C, Thervet E. Pharmacokinetic interaction between corticosteroids and tacrolimus after renal transplantation. *Nephrol Dial Transplant* 2003; **18**: 2409-2414 [PMID: 14551375 DOI: 10.1093/ndt/gfg381]

44 **Kuypers DR**. Influence of interactions between immunosuppressive drugs on therapeutic drug monitoring. *Ann Transplant* 2008; **13**: 11-18 [PMID: 18806728]

45 **Uesugi M**, Masuda S, Katsura T, Oike F, Takada Y, Inui K. Effect of intestinal CYP3A5 on postoperative tacrolimus trough levels in living-donor liver transplant recipients. *Pharmacogenet Genomics* 2006; **16**: 119-127 [PMID: 16424824]

46 **Venkataramanan R**, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, McMichael J, Lever J, Burckart G, Starzl T. Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet* 1995; **29**: 404-430 [PMID: 8787947 DOI: 10.2165/00003088-199529060-00003]

47 **Sato K**, Amada N, Sato T, Miura S, Ohashi Y, Sekiguchi S, Satomi S, Okazaki H. Severe elevations of FK506 blood concentration due to diarrhea in renal transplant recipients. *Clin Transplant* 2004; **18**: 585-590 [PMID: 15344965 DOI: 10.1111/j.1399-0012.2004.00232.x]

48 **Lampen A**, Christians U, Gonschior AK, Bader A, Hackbarth I, von Engelhardt W, Sewing KF. Metabolism of the macrolide immunosuppressant, tacrolimus, by the pig gut mucosa in the Ussing chamber. *Br J Pharmacol* 1996; **117**: 1730-1734 [PMID: 8732283]

49 **Tavira B**, Coto E, Díaz-Corte C, Ortega F, Arias M, Torres A, Díaz JM, Selgas R, López-Larrea C, Campistol JM, Alvarez V; REDINREN Pharmacogenetics group. Pharmacogenetics of tacrolimus after renal transplantation: analysis of polymorphisms in genes encoding 16 drug metabolizing enzymes. *Clin Chem Lab Med* 2011; **49**: 825-833 [PMID: 21480817 DOI: 10.1515/CCLM.2011.143]

50 **Kuehl P**, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS, Schuetz E. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 2001; **27**: 383-391 [PMID: 11279519 DOI: 10.1038/86882]

51 **Masuda S**, Inui K. An up-date review on individualized dosage adjustment of calcineurin inhibitors in organ transplant patients. *Pharmacol Ther* 2006; **112**: 184-198 [PMID: 16759707 DOI: 10.1016/j.pharmthera.2006.04.006]

52 **Gijsen VM**, Madadi P, Dube MP, Hesselink DA, Koren G, de Wildt SN. Tacrolimus-induced nephrotoxicity and genetic variability: a review. *Ann Transplant* 2012; **17**: 111-121 [PMID: 22743729 DOI: 10.12659/AOT.883229]

53 **Kuypers DR**, de Jonge H, Naesens M, Lerut E, Verbeke K, Vanrenterghem Y. CYP3A5 and CYP3A4 but not MDR1 single-nucleotide polymorphisms determine long-term tacrolimus disposition and drug-related nephrotoxicity in renal recipients. *Clin Pharmacol Ther* 2007; **82**: 711-725 [PMID: 17495880 DOI: 10.1038/sj.clpt.6100216]

54 **Hesselink DA**, van Schaik RH, van Agteren M, de Fijter JW, Hartmann A, Zeier M, Budde K, Kuypers DR, Pisarski P, Le Meur Y, Mamelok RD, van Gelder T. CYP3A5 genotype is not associated with a higher risk of acute rejection in tacrolimus-treated renal transplant recipients. *Pharmacogenet Genomics* 2008; **18**: 339-348 [PMID: 18334918 DOI: 10.1097/FPC.0b013e3282f75f88]

55 **Shi WL**, Tang HL, Zhai SD. Effects of the CYP3A4*1B Genetic Polymorphism on the Pharmacokinetics of Tacrolimus in Adult Renal Transplant Recipients: A Meta-Analysis. *PLoS One* 2015; **10**: e0127995 [PMID: 26039043 DOI: 10.1371/journal.pone.0127995]

56 **Joy MS**, Hogan SL, Thompson BD, Finn WF, Nickeleit V. Cytochrome P450 3A5 expression in the kidneys of patients with calcineurin inhibitor nephrotoxicity. *Nephrol Dial Transplant* 2007; **22**: 1963-1968 [PMID: 17395652 DOI: 10.1093/ndt/gfm133]

57 **Metalidis C**, Lerut E, Naesens M, Kuypers DR. Expression of CYP3A5 and P-glycoprotein in renal allografts with histological signs of calcineurin inhibitor nephrotoxicity. *Transplantation* 2011; **91**: 1098-1102 [PMID: 21544031 DOI: 10.1097/TP.0b013e3182177502]

58 **Kim IW**, Noh H, Ji E, Han N, Hong SH, Ha J, Burckart GJ, Oh JM. Identification of factors affecting tacrolimus level and 5-year clinical outcome in kidney transplant patients. *Basic Clin Pharmacol Toxicol* 2012; **111**: 217-223 [PMID: 22469198 DOI: 10.1111/j.1742-7843.2012.00892.x]

59 **Thölking G**, Siats L, Fortmann C, Koch R, Hüsing A, Cicinnati VR, Gerth HU, Wolters HH, Anthoni C, Pavenstädt H, Suwelack B, Schmidt HH, Kabar I. Tacrolimus Concentration/Dose Ratio is Associated with Renal Function After Liver Transplantation. *Ann Transplant* 2016; **21**: 167-179 [PMID: 27003330 DOI: 10.12659/AOT.895898]

60 **Thölking G**, Fortmann C, Koch R, Gerth HU, Pabst D, Pavenstädt H, Kabar I, Hüsing A, Wolters H, Reuter S, Suwelack B. The tacrolimus metabolism rate influences renal function after kidney transplantation. *PLoS One* 2014; **9**: e111128 [PMID: 25340655 DOI: 10.1371/journal.pone.0111128]

61 **Ji E**, Choi L, Suh KS, Cho JY, Han N, Oh JM. Combinational effect of intestinal and hepatic CYP3A5 genotypes on tacrolimus

pharmacokinetics in recipients of living donor liver transplantation. *Transplantation* 2012; **94**: 866-872 [PMID: 22992768 DOI: 10.1097/TP.0b013e318263700a]

62 **Thölking G**, Schmidt C, Koch R, Schuette-Nuetgen K, Pabst D, Wolters H, Kabar I, Hüsing A, Pavenstädt H, Reuter S, Suwelack B. Influence of tacrolimus metabolism rate on BKV infection after kidney transplantation. *Sci Rep* 2016; **6**: 32273 [PMID: 27573493 DOI: 10.1038/srep32273]

63 **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]

64 **Genvigir FD**, Salgado PC, Felipe CR, Luo EY, Alves C, Cerda A, Tedesco-Silva H, Medina-Pestana JO, Oliveira N, Rodrigues AC, Doi SQ, Hirata MH, Hirata RD. Influence of the CYP3A4/5 genetic score and ABCB1 polymorphisms on tacrolimus exposure and renal function in Brazilian kidney transplant patients. *Pharmacogenet Genomics* 2016; **26**: 462-472 [PMID: 27434656 DOI: 10.1097/FPC.0000000000000237]

65 **Rojas L**, Neumann I, Herrero MJ, Bosó V, Reig J, Poveda JL, Megías J, Bea S, Aliño SF. Effect of CYP3A5*3 on kidney transplant recipients treated with tacrolimus: a systematic review and meta-analysis of observational studies. *Pharmacogenomics J* 2015; **15**: 38-48 [PMID: 25201288 DOI: 10.1038/tpj.2014.38]

66 **Chen JS**, Li LS, Cheng DR, Ji SM, Sun QQ, Cheng Z, Wen JQ, Sha GZ, Liu ZH. Effect of CYP3A5 genotype on renal allograft recipients treated with tacrolimus. *Transplant Proc* 2009; **41**: 1557-1561 [PMID: 19545678 DOI: 10.1016/j.transproceed.2009.01.097]

67 **Quteineh L**, Verstuyft C, Furlan V, Durrbach A, Letierce A, Ferlicot S, Taburet AM, Charpentier B, Becquemont L. Influence of CYP3A5 genetic polymorphism on tacrolimus daily dose requirements and acute rejection in renal graft recipients. *Basic Clin Pharmacol Toxicol* 2008; **103**: 546-552 [PMID: 19067682 DOI: 10.1111/j.1742-7843.2008.00327.x]

68 **Naesens M**, Lerut E, de Jonge H, Van Damme B, Vanrenterghem Y, Kuypers DR. Donor age and renal P-glycoprotein expression associate with chronic histological damage in renal allografts. *J Am Soc Nephrol* 2009; **20**: 2468-2480 [PMID: 19762492 DOI: 10.1681/ASN.2009020192]

69 **Laskow DA**, Vincenti F, Neylan JF, Mendez R, Matas AJ. An open-label, concentration-ranging trial of FK506 in primary kidney transplantation: a report of the United States Multicenter FK506 Kidney Transplant Group. *Transplantation* 1996; **62**: 900-905 [PMID: 8878381]

70 **Dharnidharka VR**, Cherikh WS, Abbott KC. An OPTN analysis of national registry data on treatment of BK virus allograft nephropathy in the United States. *Transplantation* 2009; **87**: 1019-1026 [PMID: 19352121 DOI: 10.1097/TP.0b013e31819cc383]

71 **Vincenti F**, Rostaing L, Grinyo J, Rice K, Steinberg S, Gaite L, Moal MC, Mondragon-Ramirez GA, Kothari J, Polinsky MS, Meier-Kriesche HU, Munier S, Larsen CP. Belatacept and Long-Term Outcomes in Kidney Transplantation. *N Engl J Med* 2016; **374**: 333-343 [PMID: 26816011 DOI: 10.1056/NEJMoa1506027]

P- Reviewer: Friedman EA, Kita K, Nechifor G, Trkulja V
S- Editor: Song XX **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Transplant 2017 February 24; 7(1): 34-42

DOI: 10.5500/wjt.v7.i1.34

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

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

Michelle Willicombe, Anna Rizzello, Dawn Goodall, Vassilios Papalois, Adam G McLean, David Taube

Michelle Willicombe, Anna Rizzello, Dawn Goodall, Vassilios Papalois, Adam G McLean, David Taube, Imperial College Kidney and Transplant Centre, Hammersmith Hospital, London W12 0HS, United Kingdom

Author contributions: All the authors contributed to the manuscript.

Institutional review board statement: This study was approved by the Imperial College Renal and Transplant Centre, Transplant Clinical and Research Group.

Informed consent statement: No individual informed consent was required for this study.

Conflict-of-interest statement: The authors declare no conflicts of interest regarding this manuscript.

Data sharing statement: No data were created no data are available.

Manuscript source: Invited manuscript

Correspondence to: Michelle Willicombe, MA, MRCP, MD, Imperial College Kidney and Transplant Centre, Hammersmith Hospital, South Wharf Road, London W12 0HS, United Kingdom. michelle.willicombe@imperial.nhs.uk
Telephone: +44-020-33135165
Fax: +44-020-33135169

Received: August 8, 2016
Peer-review started: August 10, 2016
First decision: September 12, 2016
Revised: October 27, 2016
Accepted: December 7, 2016
Article in press: December 9, 2016
Published online: February 24, 2017

Abstract

AIM

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

METHODS

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

RESULTS

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

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

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

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

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

INTRODUCTION

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

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

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

MATERIALS AND METHODS

Patients

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

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

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

Statistical analysis

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

Table 1 Patient demographics

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

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

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

RESULTS

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

Patient survival

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

Allograft outcomes

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

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

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

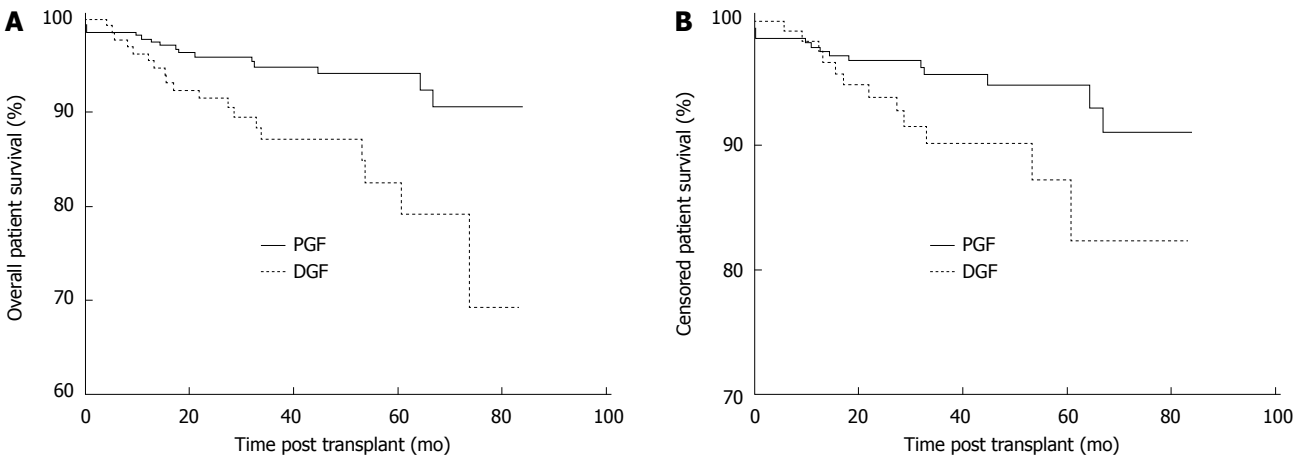


Figure 1 Patient survival in patients with delayed graft function. The 1, 3 and 5 year patient survival post-transplant: A: Overall patient survival: 96.3%, 87.2% and 82.5% in the DGF group and 97.9%, 95.0% and 94.2% in the PGF group, $P < 0.01$; B: Patient survival censored at the time of allograft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

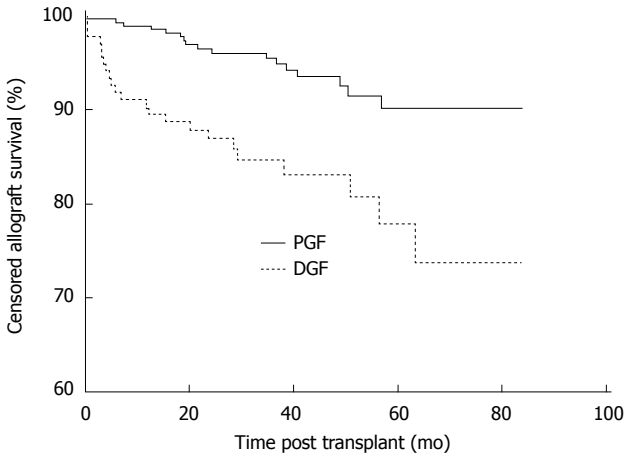


Figure 2 Censored allograft survival. Censored allograft survival in the DGF group was 90.3%, 84.7% and 77.9% at 1, 3 and 5 years compared with 99.0%, 95.5% and 90.2% in the PGF group, $P < 0.001$. DGF: Delayed graft function; PGF: Primary graft function.

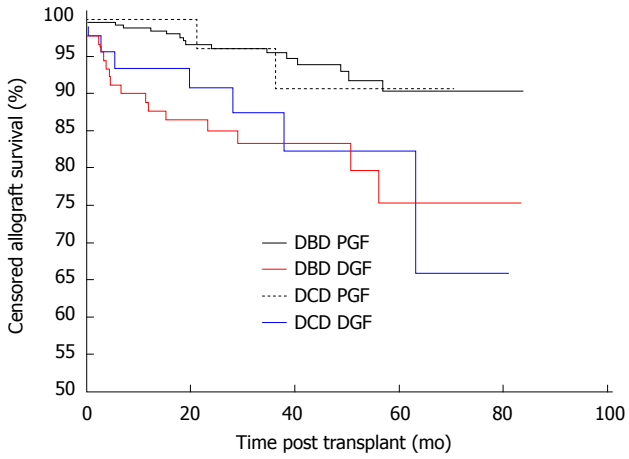


Figure 3 Allograft survival by donor type and delayed graft function. Allograft survival in the DBD and DCD donors with PGF was significantly higher than the recipients of DBD and DCD kidneys with DGF, with an allograft survival of 90.3%, 90.7%, 75.3% and 65.8% respectively, $P = 0.0016$. DGF: Delayed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

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

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

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

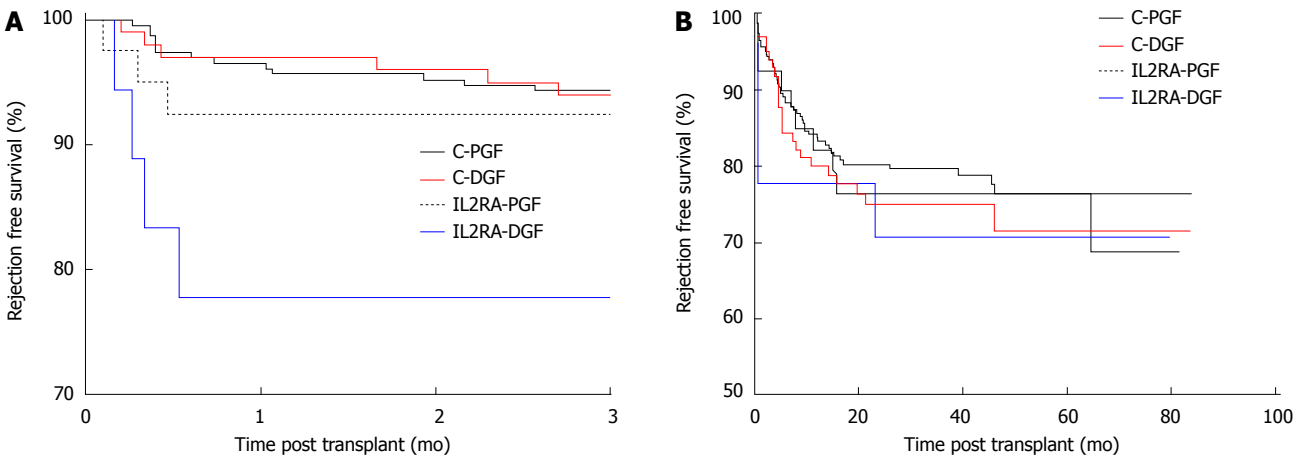


Figure 4 Three month and overall rejection free survival by induction agent and delayed graft function. Rejection free survival, censored for DSA+ in patients with alemtuzumab induction and PGF (C-PGF), alemtuzumab induction and DGF (C-DGF), IL2RA induction and PGF (IL2RA-PGF) and IL2RA induction and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

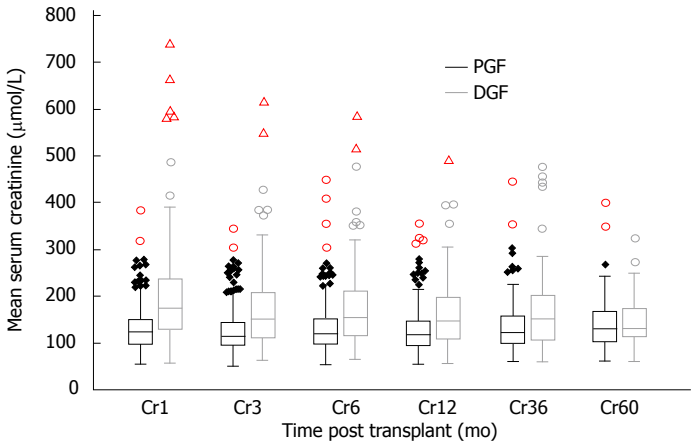


Figure 5 Allograft function according to delayed graft function or primary graft function. Mean serum creatinine was 203.4 ± 120.0, 172.3 ± 86.6, 161.9 ± 74.9, 167.2 ± 86.1 and 149.6 ± 59.4 μmol/L at 1, 6, 12, 36, 60 mo post-transplant in the DGF group compared with 132.4 ± 48.6, 133.8 ± 56.7, 127.8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

mo and 0.70 at 60 mo.

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

Risk factors associated with the development of DGF

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

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

Table 2 Independent risk factors for delayed graft function

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

CIT: Cold ischaemic time; DCD: Circulatory death.

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

DISCUSSION

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

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

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

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

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

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

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

COMMENTS

Background

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

Research frontiers

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

Innovations and breakthroughs

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

Applications

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

Terminology

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

Peer-review

The paper is very exciting and instructive article.

REFERENCES

1 **Yarlagadda SG**, Coca SG, Formica RN, Poggio ED, Parikh CR. Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. *Nephrol Dial Transplant* 2009; **24**: 1039-1047 [PMID: 19103734 DOI: 10.1093/ndt/gfn667]

2 **Grosso G**, Corona D, Mistretta A, Zerbo D, Sinagra N, Giaquinta A, Cimino S, Ekser B, Giuffrida G, Leonardi A, Gula R, Veroux P, Veroux M. Delayed graft function and long-term outcome in kidney transplantation. *Transplant Proc* 2012; **44**: 1879-1883 [PMID: 22974861 DOI: 10.1016/j.transproceed.2012.06.044]

3 **Narayanan R**, Cardella CJ, Cattran DC, Cole EH, Tinckam KJ, Schiff J, Kim SJ. Delayed graft function and the risk of death with graft function in living donor kidney transplant recipients. *Am J Kidney Dis* 2010; **56**: 961-970 [PMID: 20870331 DOI: 10.1053/j.ajkd.2010.06.024]

4 **Siedlecki A**, Irish W, Brennan DC. Delayed graft function in the kidney transplant. *Am J Transplant* 2011; **11**: 2279-2296 [PMID: 21929642 DOI: 10.1111/j.1600-6143.2011.03754.x]

5 **Hirt-Minkowski P**, Amico P, Hönger G, Praehauser C, Steiger J, Koller MT, Gürke L, Mayr M, Schaub S. Delayed graft function is not associated with an increased incidence of renal allograft rejection. *Clin Transplant* 2012; **26**: E624-E633 [PMID: 23106785 DOI: 10.1111/ctr.12041]

6 **Ojo AO**, Wolfe RA, Held PJ, Port FK, Schmouder RL. Delayed graft function: risk factors and implications for renal allograft survival. *Transplantation* 1997; **63**: 968-974 [PMID: 9112349]

7 OPTN SRTR Annual Data Report 2011. 2013-06-13. Available from: URL: http//srtr.transplant.hrsa.gov

8 **Irish WD**, Ilsley JN, Schnitzler MA, Feng S, Brennan DC. A risk prediction model for delayed graft function in the current era of deceased donor renal transplantation. *Am J Transplant* 2010; **10**: 2279-2286 [PMID: 20883559 DOI: 10.1111/j.1600-6143.2010.03179.x]

9 **Irish WD**, McCollum DA, Tesi RJ, Owen AB, Brennan DC, Bailly JE, Schnitzler MA. Nomogram for predicting the likelihood of delayed graft function in adult cadaveric renal transplant recipients. *J Am Soc*

Nephrol 2003; **14**: 2967-2974 [PMID: 14569108]
10 **Watson CJ**, Wells AC, Roberts RJ, Akoh JA, Friend PJ, Akyol M, Calder FR, Allen JE, Jones MN, Collett D, Bradley JA. Cold machine perfusion versus static cold storage of kidneys donated after cardiac death: a UK multicenter randomized controlled trial. *Am J Transplant* 2010; **10**: 1991-1999 [PMID: 20883534 DOI: 10.1111/j.1600-6143.2010.03165.x]
11 **Lam VW**, Laurence JM, Richardson AJ, Pleass HC, Allen RD. Hypothermic machine perfusion in deceased donor kidney transplantation: a systematic review. *J Surg Res* 2013; **180**: 176-182 [PMID: 23211958 DOI: 10.1016/j.jss.2012.10.055]
12 **Moers C**, Smits JM, Maathuis MH, Treckmann J, van Gelder F, Napieralski BP, van Kasterop-Kutz M, van der Heide JJ, Squifflet JP, van Heurn E, Kirste GR, Rahmel A, Leuvenink HG, Paul A, Pirenne J, Ploeg RJ. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med* 2009; **360**: 7-19 [PMID: 19118301 DOI: 10.1056/NEJMoa0802289]
13 **Jochmans I**, Moers C, Smits JM, Leuvenink HG, Treckmann J, Paul A, Rahmel A, Squifflet JP, van Heurn E, Monbaliu D, Ploeg RJ, Pirenne J. Machine perfusion versus cold storage for the preservation of kidneys donated after cardiac death: a multicenter, randomized, controlled trial. *Ann Surg* 2010; **252**: 756-764 [PMID: 21037431 DOI: 10.1097/SLA.0b013e3181ffc256]
14 **Deng R**, Gu G, Wang D, Tai Q, Wu L, Ju W, Zhu X, Guo Z, He X. Machine perfusion versus cold storage of kidneys derived from donation after cardiac death: a meta-analysis. *PLoS One* 2013; **8**: e56368 [PMID: 23536758 DOI: 10.1371/journal.pone.0056368]
15 **O'Callaghan JM**, Morgan RD, Knight SR, Morris PJ. Systematic review and meta-analysis of hypothermic machine perfusion versus static cold storage of kidney allografts on transplant outcomes. *Br J Surg* 2013; **100**: 991-1001 [PMID: 23754643 DOI: 10.1002/bjs.9169]
16 An investigation into the treatment of the donor kidney to see if this improves the recovery of the kidney after transplantation. Available from: URL: http// www.isrctn.com/ISRCTN49958194
17 **Dompé Farmaceutici S.p.A**. Reparixin in prevention of delayed graft dysfunction after kidney transplantation. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: http//www.clinicaltrials.gov/ct2/show/NCT00248040
18 **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]
19 **Kosieradzki M**, Rowiński W. Ischemia/reperfusion injury in kidney transplantation: mechanisms and prevention. *Transplant Proc* 2008; **40**: 3279-3288 [PMID: 19100373 DOI: 10.1016/j.transproceed.2008.10.004]
20 **Knechtle SJ**, Fernandez LA, Pirsch JD, Becker BN, Chin LT, Becker YT, Odorico JS, D'alessandro AM, Sollinger HW. Campath-1H in renal transplantation: The University of Wisconsin experience. *Surgery* 2004; **136**: 754-760 [PMID: 15467659 DOI: 10.1016/j.surg.2004.06.015]
21 **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]
22 **Brennan DC**, Daller JA, Lake KD, Cibrik D, Del Castillo D. Rabbit antithymocyte globulin versus basiliximab in renal transplantation. *N Engl J Med* 2006; **355**: 1967-1977 [PMID: 17093248 DOI: 10.1056/NEJMoa060068]
23 **Patel SJ**, Duhart BT, Krauss AG, Moore LW, Egidi MF, Amiri HS, Gaber LW, Gaber AO. Risk factors and consequences of delayed graft function in deceased donor renal transplant patients receiving antithymocyte globulin induction. *Transplantation* 2008; **86**: 313-320 [PMID: 18645496 DOI: 10.1097/TP.0b013e31817ef190]
24 **Mourad G**, Morelon E, Noël C, Glotz D, Lebranchu Y. The role of Thymoglobulin induction in kidney transplantation: an update. *Clin Transplant* 2012; **26**: E450-E464 [PMID: 23061755 DOI: 10.1111/ctr.12021]
25 **Woodle ES**, Alloway RR, Hanaway MJ, Buell JF, Thomas M, Roy-Chaudhury P, Trofe J. Early corticosteroid withdrawal under modern immunosuppression in renal transplantation: multivariate analysis of risk factors for acute rejection. *Transplant Proc* 2005; **37**: 798-799 [PMID: 15848535 DOI: 10.1016/j.transproceed.2004.12.074]
26 **Hanaway MJ**, Woodle ES, Mulgaonkar S, Peddi VR, Kaufman DB, First MR, Croy R, Holman J. Alemtuzumab induction in renal transplantation. *N Engl J Med* 2011; **364**: 1909-1919 [PMID: 21591943 DOI: 10.1056/NEJMoa1009546]
27 **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]
28 **Borrows R**, Chan K, Loucaidou M, Lawrence C, Van Tromp J, Cairns T, Griffith M, Hakim N, McLean A, Palmer A, Papalois V, Taube D. Five years of steroid sparing in renal transplantation with tacrolimus and mycophenolate mofetil. *Transplantation* 2006; **81**: 125-128 [PMID: 16421488]
29 **Chan K**, Taube D, Roufosse C, Cook T, Brookes P, Goodall D, Galliford J, Cairns T, Dorling A, Duncan N, Hakim N, Palmer A, Papalois V, Warrens AN, Willicombe M, McLean AG. Kidney transplantation with minimized maintenance: alemtuzumab induction with tacrolimus monotherapy--an open label, randomized trial. *Transplantation* 2011; **92**: 774-780 [PMID: 21836540 DOI: 10.1097/TP.0b013e31822ca7ca]
30 **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]
31 **Tyson M**, Castle E, Andrews P, Heilman R, Mekeel K, Moss A, Mulligan D, Reddy K. Early graft function after laparoscopically procured living donor kidney transplantation. *J Urol* 2010; **184**: 1434-1439 [PMID: 20727548 DOI: 10.1016/j.juro.2010.06.013]
32 **Liu Y**, Zhou P, Han M, Xue CB, Hu XP, Li C. Basiliximab or antithymocyte globulin for induction therapy in kidney transplantation: a meta-analysis. *Transplant Proc* 2010; **42**: 1667-1670 [PMID: 20620496 DOI: 10.1016/j.transproceed.2010.02.088]
33 **Farney AC**, Doares W, Rogers J, Singh R, Hartmann E, Hart L, Ashcraft E, Reeves-Daniels A, Gautreaux M, Iskandar SS, Moore P, Adams PL, Stratta RJ. A randomized trial of alemtuzumab versus antithymocyte globulin induction in renal and pancreas transplantation. *Transplantation* 2009; **88**: 810-819 [PMID: 19920781 DOI: 10.1097/TP.0b013e3181b4acfb]
34 **Ciancio G**, Burke GW, Gaynor JJ, Carreno MR, Cirocco RE, Mathew JM, Mattiazzi A, Cordovilla T, Roth D, Kupin W, Rosen A, Esquenazi V, Tzakis AG, Miller J. A randomized trial of three renal transplant induction antibodies: early comparison of tacrolimus, mycophenolate mofetil, and steroid dosing, and newer immune-monitoring. *Transplantation* 2005; **80**: 457-465 [PMID: 16123718]
35 **Ciancio G**, Burke GW, Gaynor JJ, Roth D, Kupin W, Rosen A, Cordovilla T, Tueros L, Herrada E, Miller J. A randomized trial of thymoglobulin vs. alemtuzumab (with lower dose maintenance immunosuppression) vs. daclizumab in renal transplantation at 24 months of follow-up. *Clin Transplant* 2008; **22**: 200-210 [PMID: 18339140 DOI: 10.1111/j.1399-0012.2007.00774.x]
36 **Parekh J**, Bostrom A, Feng S. Diabetes mellitus: a risk factor for delayed graft function after deceased donor kidney transplantation. *Am J Transplant* 2010; **10**: 298-303 [PMID: 20055796 DOI: 10.1111/j.1600-6143.2009.02936.x]
37 **Parekh J**, Roll GR, Feng S, Niemann CU, Hirose R. Peri-operative hyperglycemia is associated with delayed graft function in deceased donor renal transplantation. *Clin Transplant* 2013; **27**: E424-E430 [PMID: 23808826 DOI: 10.1111/ctr.12174]
38 **Giblin L**, O'Kelly P, Little D, Hickey D, Donohue J, Walshe JJ, Spencer S, Conlon PJ. A comparison of long-term graft survival

rates between the first and second donor kidney transplanted--the effect of a longer cold ischaemic time for the second kidney. *Am J Transplant* 2005; **5**: 1071-1075 [PMID: 15816888 DOI: 10.1111/j.1600-6143.2005.00798.x]

39 **Opelz G**, Döhler B. Multicenter analysis of kidney preservation. *Transplantation* 2007; **83**: 247-253 [PMID: 17297393 DOI: 10.1097/01.tp.0000251781.36117.27]

40 **van der Vliet JA**, Warlé MC, Cheung CL, Teerenstra S, Hoitsma AJ. Influence of prolonged cold ischemia in renal transplantation. *Clin Transplant* 2011; **25**: E612-E616 [PMID: 21919965 DOI: 10.1111/j.1399-0012.2011.01510.x]

41 **Taylor MJ**, Baicu SC. Current state of hypothermic machine perfusion preservation of organs: The clinical perspective. *Cryobiology* 2010; **60**: S20-S35 [PMID: 19857479 DOI: 10.1016/j.cryobiol.2009.10.006]

42 **Kienzl-Wagner K**, Schneiderbauer S, Bösmüller C, Schneeberger S, Pratschke J, Ollinger R. Nighttime procedures are not associated with adverse outcomes in kidney transplantation. *Transpl Int* 2013; **26**: 879-885 [PMID: 23773175 DOI: 10.1111/tri.12125]

P- Reviewer: Sert I, Vlachopanos G, Xu H **S- Editor**: Qiu S
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Transplant 2017 February 24; 7(1): 43-48

DOI: 10.5500/wjt.v7.i1.43

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Effectiveness and versatility of biological prosthesis in transplanted patients

Giovanni Vennarecci, Gianluca Mascianà, Edoardo De Werra, Giovanni Battista Levi Sandri, Daniele Ferraro, Mirco Burocchi, Giovanni Tortorelli, Nicola Guglielmo, Giuseppe Maria Ettorre

Giovanni Vennarecci, Gianluca Mascianà, Edoardo De Werra, Giovanni Battista Levi Sandri, Daniele Ferraro, Mirco Burocchi, Giovanni Tortorelli, Nicola Guglielmo, Giuseppe Maria Ettorre, Division of Surgical Oncology and Liver Transplantation, San Camillo Hospital, POIT San Camillo-INMI Lazzaro Spallanzani, 00149 Rome, Italy

Author contributions: All the authors have contributed to this paper.

Institutional review board statement: The study was reviewed and approved for publication by our Institutional Reviewer.

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

Conflict-of-interest statement: All the authors declare they have no conflict of interest related to the manuscript.

Manuscript source: Invited manuscript

Correspondence to: Giovanni Vennarecci, MD, Division of Surgical Oncology and Liver Transplantation, San Camillo Hospital, POIT San Camillo-INMI Lazzaro Spallanzani, Cir.ne Gianicolense N° 187, 00149 Rome,
Italy. gvennarecci@scamilloforlanini.rm.it
Telephone: +39-6-58704816
Fax: +39-6-58704719

Received: July 9, 2016
Peer-review started: July 12, 2016
First decision: September 9, 2016
Revised: October 9, 2016
Accepted: November 27, 2016
Article in press: November 29, 2016
Published online: February 24, 2017

Abstract

AIM

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

METHODS

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

RESULTS

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

CONCLUSION

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

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

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

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

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

INTRODUCTION

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

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

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

MATERIALS AND METHODS

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

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

RESULTS

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

Table 1 Patient characteristics

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

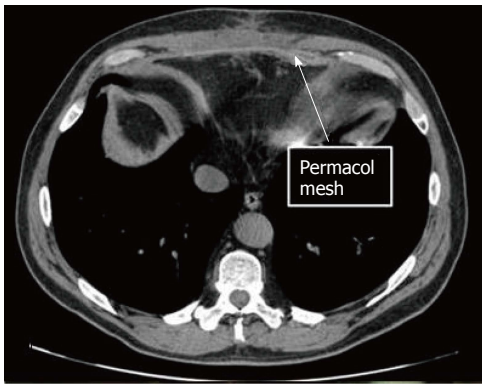


Figure 1 Computed tomography scan at 6 mo after abdominal wall repair. Arrow: Biological prosthesis.

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

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

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

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

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

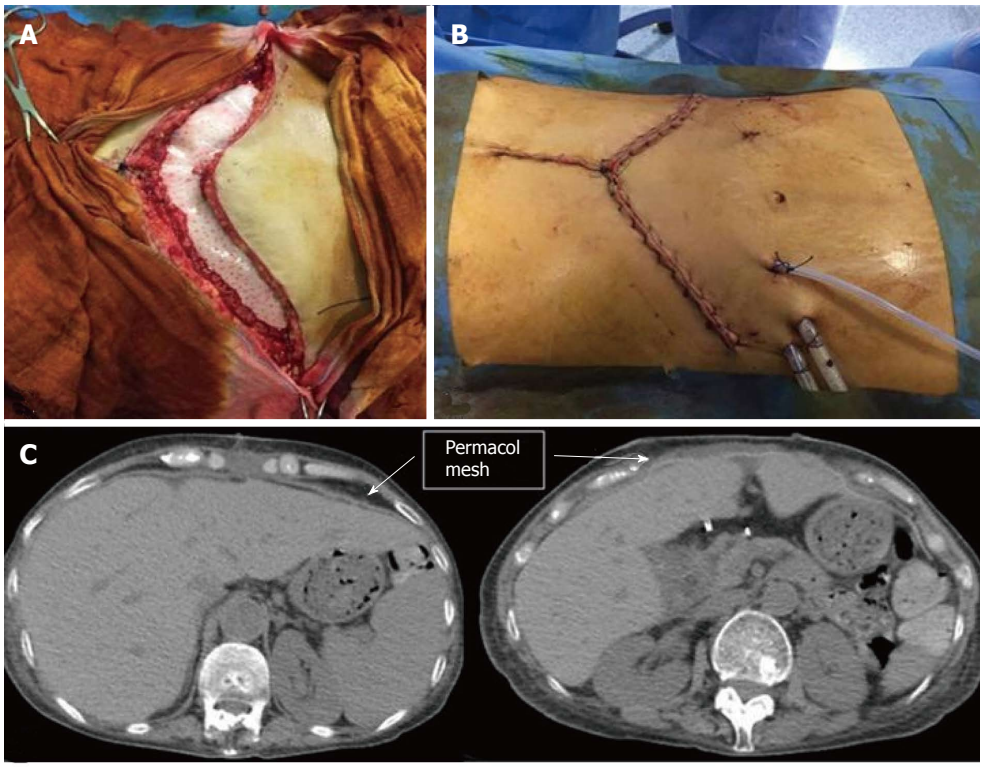


Figure 2 In order to prevent the onset of compartment syndrome, a temporary wound closure with Bogota Bag was performed. A: Implantation of Permacol™ mesh; B: Skin closure after Permacol™ mesh implantation; C: Computed tomography scan at 3 mo after abdominal wall repair (arrow: Biological prosthesis).

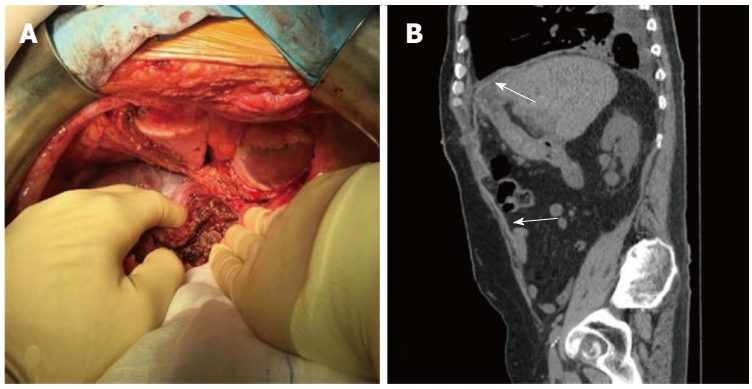


Figure 3 The abdominal exploration showed a neoplasm of left lobe liver graft with infiltration of the diaphragm which extended to the pleura and pericardium. A: Left liver lobectomy of the graft with resection of the diaphragm "*en bloc*" with adjacent portion of right pleura and pericardium; B: Computed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

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

DISCUSSION

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

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

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

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

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

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

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

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

COMMENTS

Background

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

Research frontiers

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

Innovations and breakthroughs

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

Applications

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

Terminology

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

Peer-review

It is a well-written paper.

REFERENCES

1 **Mazzucchi E**, Nahas WC, Antonopoulos I, Ianhez LE, Arap S. Incisional hernia and its repair with polypropylene mesh in renal transplant recipients. *J Urol* 2001; **166**: 816-819 [PMID: 11490225]

2 **Knight RJ**, Villa M, Laskey R, Benavides C, Schoenberg L, Welsh M, Kerman RH, Podder H, Van Buren CT, Katz SM, Kahan BD. Risk factors for impaired wound healing in sirolimus-treated renal transplant recipients. *Clin Transplant* 2007; **21**: 460-465 [PMID: 17645704 DOI: 10.1111/j.1399-0012.2007.00668.x]

3 **Piazzese E**, Montalti R, Beltempo P, Bertelli R, Puviani L, Pacilè V, Nardo B, Cavallari A. Incidence, predisposing factors, and results of surgical treatment of incisional hernia after orthotopic liver transplantation. *Transplant Proc* 2004; **36**: 3097-3098 [PMID: 15686704 DOI: 10.1016/j.transproceed.2004.10.047]

4 **Gastaca M**, Valdivieso A, Ruiz P, de Urbina JO. Reducing the incidence of incisional hernia after liver transplantation. *Transpl Int* 2010; **23**: 559-560 [PMID: 19906033 DOI: 10.1111/j.1432-2277.2009.00992.x]

5 **Piros L**, Máthé Z, Földes K, Langer RM. Incisional hernia after simultaneous pancreas kidney tranplantation: a single-center experience from Budapest. *Transplant Proc* 2011; **43**: 1303-1305 [PMID: 21620116 DOI: 10.1016/j.transproceed.2011.03.090]

6 **Hanish SI**, Petersen RP, Collins BH, Tuttle-Newhall J, Marroquin CE, Kuo PC, Butterly DW, Smith SR, Desai DM. Obesity predicts increased overall complications following pancreas transplantation. *Transplant Proc* 2005; **37**: 3564-3566 [PMID: 16298662 DOI: 10.1016/j.transproceed.2005.09.068]

7 **Abdelfatah MM**, Rostambeigi N, Podgaetz E, Sarr MG. Long-term outcomes (& gt; 5-year follow-up) with porcine acellular dermal matrix (Permacol) in incisional hernias at risk for infection. *Hernia* 2015; **19**: 135-140 [PMID: 24129420 DOI: 10.1007/s10029-013-1165-9]

8 **Beale EW**, Hoxworth RE, Livingston EH, Trussler AP. The role of biologic mesh in abdominal wall reconstruction: a systematic review

of the current literature. *Am J Surg* 2012; **204**: 510-517 [PMID: 23010617 DOI: 10.1016/j.amjsurg.2012.03.009]

9 **Vennarecci G**, Guglielmo N, Pelle F, Felli E, Ettorre GM. The use of Permacol™ surgical implant for subxiphoid incisional hernia repair in cardiac transplant patients. *Int J Surg* 2015; **21**: 68-69 [PMID: 26209583 DOI: 10.1016/j.ijsu.2015.07.641]

10 **Smith CT**, Katz MG, Foley D, Welch B, Leverson GE, Funk LM, Greenberg JA. Incidence and risk factors of incisional hernia formation following abdominal organ transplantation. *Surg Endosc* 2015; **29**: 398-404 [PMID: 25125093 DOI: 10.1007/s00464-014-3682-8]

11 **Chang EI**, Galvez MG, Padilla BE, Freise CE, Foster RD, Hoffman WY. Ten-year retrospective analysis of incisional herniorrhaphy following renal transplantation. *Arch Surg* 2011; **146**: 21-25 [PMID: 21242441 DOI: 10.1001/archsurg.2010.305]

12 **Lo Monte AI**, Damiano G, Maione C, Gioviale MC, Lombardo C, Buscemi G, Romano M. Use of intraperitoneal ePTFE Gore dual-mesh plus in a giant incisional hernia after kidney transplantation: a case report. *Transplant Proc* 2009; **41**: 1398-1401 [PMID: 19460570 DOI: 10.1016/j.transproceed.2009.02.060]

13 **Smart NJ**, Marshall M, Daniels IR. Biological meshes: a review of their use in abdominal wall hernia repairs. *Surgeon* 2012; **10**: 159-171 [PMID: 22436406 DOI: 10.1016/j.surge.2012.02.006]

14 **Bellows CF**, Smith A, Malsbury J, Helton WS. Repair of incisional hernias with biological prosthesis: a systematic review of current evidence. *Am J Surg* 2013; **205**: 85-101 [PMID: 22867726 DOI: 10.1016/j.amjsurg.2012.02.019]

15 **Tiengo C**, Giatsidis G, Azzena B. Fascia lata allografts as biological mesh in abdominal wall repair: preliminary outcomes from a retrospective case series. *Plast Reconstr Surg* 2013; **132**: 631e-639e [PMID: 24076711 DOI: 10.1097/PRS.0b013e31829fbe6f]

16 **Slater NJ**, van der Kolk M, Hendriks T, van Goor H, Bleichrodt RP. Biologic grafts for ventral hernia repair: a systematic review. *Am J Surg* 2013; **205**: 220-230 [PMID: 23200988 DOI: 10.1016/j.amjsurg.2012.05.028]

17 **Pentlow A**, Smart NJ, Richards SK, Inward CD, Morgan JD. The use of porcine dermal collagen implants in assisting abdominal wall closure of pediatric renal transplant recipients with donor size discrepancy. *Pediatr Transplant* 2008; **12**: 20-23 [PMID: 18086240 DOI: 10.1111/j.1399-3046.2007.00824.x]

18 **Kaleya RN**. Evaluation of implant/host tissue interactions following intraperitoneal implantation of porcine dermal collagen prosthesis in the rat. *Hernia* 2005; **9**: 269-276 [PMID: 16136391 DOI: 10.1007/s10029-005-0003-0]

19 **Brewer MB**, Rada EM, Milburn ML, Goldberg NH, Singh DP, Cooper M, Silverman RP. Human acellular dermal matrix for ventral hernia repair reduces morbidity in transplant patients. *Hernia* 2011; **15**: 141-145 [PMID: 21072551 DOI: 10.1007/s10029-010-0748-y]

20 **Schaffellner S**, Sereinigg M, Wagner D, Jakoby E, Kniepeiss D, Stiegler P, Haybäck J, Müller H. Ventral incisional hernia (VIH) repair after liver transplantation (OLT) with a biological mesh: experience in 3 cases. *Z Gastroenterol* 2016; **54**: 421-425 [PMID: 27171332 DOI: 10.1055/s-0042-103249]

21 **Iacco A**, Adeyemo A, Riggs T, Janczyk R. Single institutional experience using biological mesh for abdominal wall reconstruction. *Am J Surg* 2014; **208**: 480-484 [PMID: 24462172 DOI: 10.1016/j.amjsurg.2013.09.020]

22 **Patel KM**, Nahabedian MY, Albino F, Bhanot P. The use of porcine acellular dermal matrix in a bridge technique for complex abdominal wall reconstruction: an outcome analysis. *Am J Surg* 2013; **205**: 209-212 [PMID: 23195145 DOI: 10.1016/j.amjsurg.2012.05.031]

23 **Breuing K**, Butler CE, Ferzoco S, Franz M, Hultman CS, Kilbridge JF, Rosen M, Silverman RP, Vargo D. Incisional ventral hernias: review of the literature and recommendations regarding the grading and technique of repair. *Surgery* 2010; **148**: 544-558 [PMID: 20304452 DOI: 10.1016/j.surg.2010.01.008]

24 **Cuomo R**, Nisi G, Grimaldi L, Brandi C, Sisti A, D'Aniello C. Immunosuppression and Abdominal Wall Defects: Use of Autologous Dermis. *In Vivo* 2015; **29**: 753-755 [PMID: 26546532]

P- Reviewer: Darecchio A, Demirag A, Feretis M, Galun D
S- Editor: Qiu S **L- Editor**: Filipodia **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Transplant 2017 February 24; 7(1): 49-56

DOI: 10.5500/wjt.v7.i1.49

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

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

Nikolaos-Andreas Anastasopoulos, Evangelia Dounousi, Evangelos Papachristou, Charalampos Pappas, Eleni Leontaridou, Eirini Savvidaki, Dimitrios Goumenos, Michael Mitsis

Nikolaos-Andreas Anastasopoulos, Evangelia Dounousi, Michael Mitsis, Department of Medicine, School of Health Sciences, University of Ioannina, 45110 Ioannina, Greece

Nikolaos-Andreas Anastasopoulos, Evangelia Dounousi, Charalampos Pappas, Michael Mitsis, Renal Transplant Unit, University Hospital of Ioannina, 45500 Ioannina, Greece

Charalampos Pappas, Department of Nephrology, University Hospital of Ioannina, 45500 Ioannina, Greece

Evangelos Papachristou, Eleni Leontaridou, Eirini Savvidaki, Dimitrios Goumenos, School of Health Science, University of Patras, 26504 Patras, Greece

Evangelos Papachristou, Eleni Leontaridou, Eirini Savvidaki, Dimitrios Goumenos, Department of Nephrology and Transplantation, Patras University Hospital, 26504 Patras, Greece

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

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

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

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

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

Manuscript source: Invited manuscript

Correspondence to: Evangelia Dounousi, MD, PhD, Department of Medicine, School of Health Sciences, University of Ioannina, Stavrou Niarchou Avenue, 45110 Ioannina, Greece. evangeldou@gmail.com
Telephone: +30-26-51099653
Fax: +30-26-51099890

Received: August 25, 2016
Peer-review started: August 26, 2016
First decision: October 20, 2016
Revised: December 14, 2016
Accepted: January 2, 2017
Article in press: January 4, 2017
Published online: February 24, 2017

Abstract

AIM

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

METHODS

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

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

RESULTS

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

CONCLUSION

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

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

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

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

INTRODUCTION

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

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

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

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

MATERIALS AND METHODS

Patient characteristics

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

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

MACE definition and risk calculation

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

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

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

Statistical analysis

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

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

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

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

RESULTS

Characteristics of RTRs

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

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

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

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

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

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

MACE risk factors and calculator validation

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

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

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

DISCUSSION

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

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

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

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

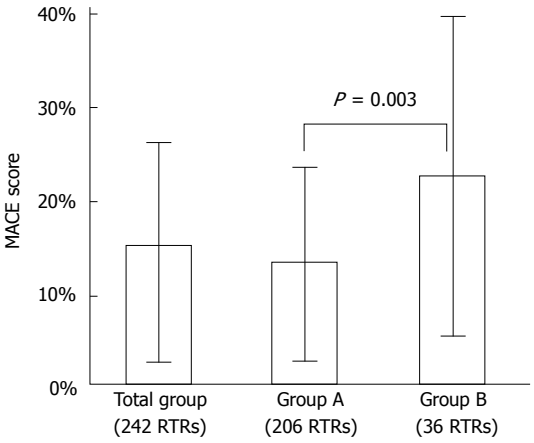


Figure 1 Calculated major advance cardiac event risk score in the 242 renal transplant recipients and in the two groups. MACE score for all the RTRs, group A, defined as RTRs without MACE and group B, defined as RTRs with MACE, is respectively 14.6% ± 12.5%, 13.3% ± 11.1% and 22.3% ± 17.1%. MACE: Major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

population.

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

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

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

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

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

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

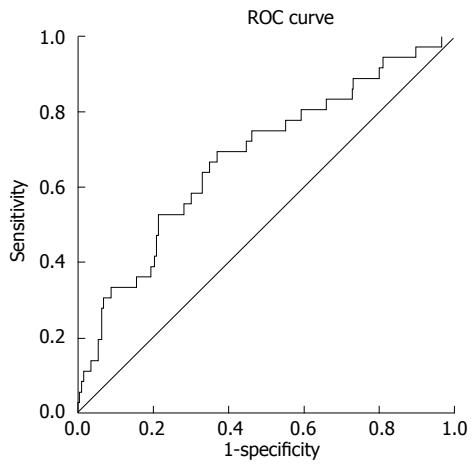


Figure 2 Discrimination. Receiver operating characteristics for major adverse cardiac event in the cohort of RTRs. Area under the curve is 0.68 (95%CI: 0.58-0.78). RTRs: Renal transplant recipients; ROC: Receiver operator curve.

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

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

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

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

patients.

ACKNOWLEDGMENTS

We would like to thank Mr Vasilis Koutlas and Ms Eirini Tzalavra, Transplant Coordinators of the Renal Transplant Unit of University Hospital of Ioannina, for helping with the data collection. We also would like to thank Ms Eufrosuni Mplathra, for helping with the collection and record of data.

COMMENTS

Background

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

Research frontiers

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

Innovations and breakthroughs

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

Applications

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

Terminology

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

Peer-review

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

REFERENCES

1 **Morales JM**, Marcén R, del Castillo D, Andres A, Gonzalez-Molina M, Oppenheimer F, Serón D, Gil-Vernet S, Lampreave I, Gainza FJ, Valdés F, Cabello M, Anaya F, Escuin F, Arias M, Pallardó L, Bustamante J. Risk factors for graft loss and mortality after renal transplantation according to recipient age: a prospective multicentre study. *Nephrol Dial Transplant* 2012; **27** Suppl 4: iv39-iv46 [PMID: 23258810 DOI: 10.1093/ndt/gfs544]

2 **Ma MK**, Lim WH, Craig JC, Russ GR, Chapman JR, Wong G. Mortality among Younger and Older Recipients of Kidney Transplants from Expanded Criteria Donors Compared with Standard Criteria Donors. *Clin J Am Soc Nephrol* 2016; **11**: 128-136 [PMID: 26681136 DOI: 10.2215/CJN.03760415]

3 **Foley RN**, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis* 1998; **32**: S112-S119 [PMID: 9820470 DOI: 10.1053/ajkd.1998.v32.pm9820470]

4 **Ojo AO**, Hanson JA, Wolfe RA, Leichtman AB, Agodoa LY, Port FK. Long-term survival in renal transplant recipients with graft function. *Kidney Int* 2000; **57**: 307-313 [PMID: 10620213 DOI: 10.1046/j.1523-1755.2000.00816.x]

5 **Dimény EM**. Cardiovascular disease after renal transplantation. *Kidney Int Suppl* 2002; **(80)**: 78-84 [PMID: 11982818 DOI: 10.1046/j.1523-1755.61.s80.14.x]

6 **Dounousi E**, Mitsis M, Naka KK, Pappas C, Lakkas L, Harisis C, Pappas K, Koutlas V, Tzalavra I, Spanos G, Michalis LK, Siamopoulos KC. Differences in cardiac structure assessed by echocardiography between renal transplant recipients and chronic kidney disease patients. *Transplant Proc* 2014; **46**: 3194-3198 [PMID: 25420857 DOI: 10.1016/j.transproceed.2014.10.034]

7 **Vaidya OU**, House JA, Coggins TR, Patil H, Vaidya A, Awad A, Main ML. Effect of renal transplantation for chronic renal disease on left ventricular mass. *Am J Cardiol* 2012; **110**: 254-257 [PMID: 22483386 DOI: 10.1016/j.amjcard.2012.02.067]

8 **Jardine AG**, Fellström B, Logan JO, Cole E, Nyberg G, Grönhagen-Riska C, Madsen S, Neumayer HH, Maes B, Ambühl P, Olsson AG, Pedersen T, Holdaas H. Cardiovascular risk and renal transplantation: post hoc analyses of the Assessment of Lescol in Renal Transplantation (ALERT) Study. *Am J Kidney Dis* 2005; **46**: 529-536 [PMID: 16129216 DOI: 10.1053/j.ajkd.2005.05.014]

9 **Silver SA**, Huang M, Nash MM, Prasad GV. Framingham risk score and novel cardiovascular risk factors underpredict major adverse cardiac events in kidney transplant recipients. *Transplantation* 2011; **92**: 183-189 [PMID: 21558986 DOI: 10.1097/TP.0b013e31821f303f]

10 **Israni AK**, Snyder JJ, Skeans MA, Peng Y, Maclean JR, Weinhandl ED, Kasiske BL. Predicting coronary heart disease after kidney transplantation: Patient Outcomes in Renal Transplantation (PORT) Study. *Am J Transplant* 2010; **10**: 338-353 [PMID: 20415903 DOI: 10.1111/j.1600-6143.2009.02949.x]

11 **Soveri I**, Holme I, Holdaas H, Budde K, Jardine AG, Fellström B. A cardiovascular risk calculator for renal transplant recipients. *Transplantation* 2012; **94**: 57-62 [PMID: 22683851 DOI: 10.1097/TP.0b013e3182516cdc]

12 **Soveri I**, Snyder J, Holdaas H, Holme I, Jardine AG, L'Italien GJ, Fellström B. The external validation of the cardiovascular risk equation for renal transplant recipients: applications to BENEFIT and BENEFIT-EXT trials. *Transplantation* 2013; **95**: 142-147 [PMID: 23192156 DOI: 10.1097/TP.0b013e31827722c9]

13 **Levey AS**, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006; **145**: 247-254 [PMID: 16908915 DOI: 10.7326/0003-4819-145-4-200608150-00004]

14 **Kasiske BL**, Guijarro C, Massy ZA, Wiederkehr MR, Ma JZ. Cardiovascular disease after renal transplantation. *J Am Soc Nephrol* 1996; **7**: 158-165 [PMID: 8808124]

15 **Jardine AG**, Gaston RS, Fellstrom BC, Holdaas H. Prevention of cardiovascular disease in adult recipients of kidney transplants. *Lancet* 2011; **378**: 1419-1427 [PMID: 22000138 DOI: 10.1016/S0140-6736(11)61334-2]

16 **Bostom AG**, Carpenter MA, Kusek JW, Levey AS, Hunsicker L, Pfeffer MA, Selhub J, Jacques PF, Cole E, Gravens-Mueller L, House AA, Kew C, McKenney JL, Pacheco-Silva A, Pesavento T, Pirsch J, Smith S, Solomon S, Weir M. Homocysteine-lowering and cardiovascular disease outcomes in kidney transplant recipients: primary results from the Folic Acid for Vascular Outcome Reduction in Transplantation trial. *Circulation* 2011; **123**: 1763-1770 [PMID: 21482964 DOI: 10.1161/CIRCULATIONAHA.110.000588]

17 **Rigatto C**. Anemia, renal transplantation, and the anemia paradox. *Semin Nephrol* 2006; **26**: 307-312 [PMID: 16949469 DOI: 10.1016/j.semnephrol.2006.05.007]

18 **Stoumpos S**, Jardine AG, Mark PB. Cardiovascular morbidity and mortality after kidney transplantation. *Transpl Int* 2015; **28**: 10-21 [PMID: 25081992 DOI: 10.1111/tri.12413]

19 **Kiberd B**, Panek R. Cardiovascular outcomes in the outpatient kidney transplant clinic: the Framingham risk score revisited. *Clin J Am Soc Nephrol* 2008; **3**: 822-828 [PMID: 18322053 DOI: 10.2215/CJN.00030108]

P- Reviewer: Amiya E, Friedman EA, Yong D, Yorioka N
S- Editor: Gong XM **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Transplant 2017 February 24; 7(1): 57-63

DOI: 10.5500/wjt.v7.i1.57

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Study

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

Paula Frassinetti Castelo Branco Camurça Fernandes, Reed André Siqueira, Evelyne Santana Girão, Rainne André Siqueira, Márcia Uchoa Mota, Leyla Castelo Branco Fernandes Marques, Silvana Cristina Albuquerque Andrade, Wilson Mendes Barroso, Sônia Leite Silva, Bruno Gomes Rodrigues dos Santos, Claúdia Maria Costa de Oliveira

Paula Frassinetti Castelo Branco Camurça Fernandes, Evelyne Santana Girão, Márcia Uchoa Mota, Leyla Castelo Branco Fernandes Marques, Silvana Cristina Albuquerque Andrade, Wilson Mendes Barroso, Sônia Leite Silva, Bruno Gomes Rodrigues dos Santos, Claúdia Maria Costa de Oliveira, Kidney Transplant Unit, Hospital Universitário Walter Cantídio, Universidade Federal do Ceará, Fortaleza, CE 60430-370, Brazil

Paula Frassinetti Castelo Branco Camurça Fernandes, Wilson Mendes Barroso, Department of Nephrology of Universidade Estadual do Ceará, Fortaleza, CE 60430-370, Brazil

Reed André Siqueira, Rainne André Siqueira, Universidade Estadual do Ceará, Fortaleza, CE 60430-370, Brazil

Author contributions: Fernandes PFCBC, Girão ES and de Oliveira CMC study conception and design; Siqueira RA, Siqueira RA, Mota MU, Marques LCBF, Andrade SCA, Barroso WM and Silva SL contributed to acquisition of data; Fernandes PFCBC, Girão ES, Siqueira RA, Siqueira RA and de Oliveira CMC contributed to analysis and interpretation of data; Siqueira RA, Siqueira RA, Girão ES, Fernandes PFCBC, Rodrigues dos Santos BG and de Oliveira CMC contributed to drafting of manuscript; Fernandes PFCBC, Girão ES, Rodrigues dos Santos BG and de Oliveira CMC contributed to critical revision.

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

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

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

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Bruno Gomes Rodrigues dos Santos, MD, Kidney Transplant Unit, Universitário Walter Cantídio, Hospital Universidade Federal do Ceará, Rua Capitão Francisco Pedro, 1290 - Rodolfo Teófilo, Fortaleza, CE 60430-370, Brazil. bgomesantos@hotmail.com
Telephone: +55-85-999466469

Received: July 29, 2016
Peer-review started: July 31, 2016
First decision: September 2, 2016
Revised: October 21, 2016
Accepted: January 11, 2017
Article in press: January 13, 2017
Published online: February 24, 2017

Abstract

AIM

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

METHODS

We retrospectively evaluated 11 renal transplant recipients

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

RESULTS

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

CONCLUSION

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

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

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

Fernandes PFCBC, Siqueira RA, Girão ES, Siqueira RA, Mota MU, Marques LCBF, Andrade SCA, Barroso WM, Silva SL, Rodrigues dos Santos BG, de Oliveira CMC. Dengue in renal transplant recipients: Clinical course and impact on renal function. *World J Transplant* 2017; 7(1): 57-63 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i1/57.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i1.57

INTRODUCTION

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

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

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

MATERIALS AND METHODS

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

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

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

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

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

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

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

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

RESULTS

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

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

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

COMMENTS

Background

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

Research frontiers

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

Innovations and breakthroughs

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

Applications

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

Terminology

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

Peer-review

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

REFERENCES

1 **Chen LH**, Wilson ME. Transmission of dengue virus without a mosquito vector: nosocomial mucocutaneous transmission and other routes of transmission. *Clin Infect Dis* 2004; **39**: e56-e60 [PMID: 15472803 DOI: 10.1086/423807]

2 **Tan FL**, Loh DL, Prabhakaran K, Tambyah PA, Yap HK. Dengue haemorrhagic fever after living donor renal transplantation. *Nephrol*

Dial Transplant 2005; **20**: 447-448 [PMID: 15673696 DOI: 10.1093/ndt/gfh601]

3 **Midgley CM**, Bajwa-Joseph M, Vasanawathana S, Limpitikul W, Wills B, Flanagan A, Waiyaiya E, Tran HB, Cowper AE, Chotiyarnwong P, Grimes JM, Yoksan S, Malasit P, Simmons CP, Mongkolsapaya J, Screaton GR. An in-depth analysis of original antigenic sin in dengue virus infection. *J Virol* 2011; **85**: 410-421 [PMID: 20980526 DOI: 10.1128/JVI.01826-10]

4 **Secretaria de Vigilância em Saúde - Ministério da Saúde**. Boletim Epidemiológico 2016. Vol 47, No 2. ISSN: 2358-9450

5 **Associação Brasileira de Transplantes de Órgãos (ABTO)**. Registro Brasileiro de Transplantes Veículo Oficial da Associação Brasileira de Transplante de Órgãos. Ano XXI N4: 9

6 **Garcia JH**, Rocha TD, Viana CF, Gonçalves BP, Girão ES, Vasconcelos JB, Coelho GR, Schreen D, Costa PE, Brasil IR. Dengue shock syndrome in a liver transplant recipient. *Transplantation* 2006; **82**: 850-851 [PMID: 17006337 DOI: 10.1097/01.tp.0000235151.60237.fe]

7 **Prasad N**, Bhadauria D, Sharma RK, Gupta A, Kaul A, Srivastava A. Dengue virus infection in renal allograft recipients: a case series during 2010 outbreak. *Transpl Infect Dis* 2012; **14**: 163-168 [PMID: 22212524 DOI: 10.1111/j.1399-3062.2011.00699.x]

8 **Nasim A**, Anis S, Baqi S, Akhtar SF, Baig-Ansari N. Clinical presentation and outcome of dengue viral infection in live-related renal transplant recipients in Karachi, Pakistan. *Transpl Infect Dis* 2013; **15**: 516-525 [PMID: 23890225 DOI: 10.1111/tid.12114]

9 **Azevedo LS**, Carvalho DB, Matuck T, Alvarenga MF, Morgado L, Magalhães I, Ianhez LE, Boulos M, David-Neto E. Dengue in renal transplant patients: a retrospective analysis. *Transplantation* 2007; **84**: 792-794 [PMID: 17893614 DOI: 10.1097/01.tp.0000280547.91617.25]

10 **Costa SD**, da Silva GB, Jacinto CN, Martiniano LV, Amaral YS, Paes FJ, De Mattos Brito Oliveira Sales ML, de Matos Esmeraldo R, De Francesco Daher E. Dengue Fever Among Renal Transplant Recipients: A Series of 10 Cases in a Tropical Country. *Am J Trop Med Hyg* 2015; **93**: 394-396 [PMID: 26033028 DOI: 10.4269/ajtmh.15-0038]

11 **World Health Organization**. Dengue Haemorrhagic Fever: diagnosis, treatment, prevention and control. 2nd ed. Geneva: WHO, 1997

12 **Brasil**. Ministério da Saúde. Secretaria de Vigilância em Saúde. Diretoria Técnica de Gestão, 2011. Dengue: diagnostico e manejo clínico- adulto e criança. 4st ed. Brasília, DF: Ministério da Saúde

13 **World Health Organization**. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. Geneva, Switzerland: WHO, 2009

14 **Renaud CJ**, Manjit K, Pary S. Dengue has a benign presentation in renal transplant patients: a case series. *Nephrology* (Carlton) 2007; **12**: 305-307 [PMID: 17498128 DOI: 10.1111/j.1440-1797.2007.00785.x]

15 **Teixeira Mda G**, Barreto ML, Costa Mda C, Ferreira LD, Vasconcelos PF, Cairncross S. Dynamics of dengue virus circulation: a silent epidemic in a complex urban area. *Trop Med Int Health* 2002; **7**: 757-762 [PMID: 12225506]

16 **Wilder-Smith A**, Schwartz E. Dengue in travelers. *N Engl J Med* 2005; **353**: 924-932 [PMID: 16135837 DOI: 10.1056/NEJMra041927]

17 **Beatty ME**, Clark GG. Prevention of specific infectious diseases. In: Cualteros. c. i. Dengue fever 2008. USA: CDC Traveler's Health, 2008

18 **Farrar J**, Focks D, Gubler D, Barrera R, Guzman MG, Simmons C, Kalayanarooj S, Lum L, McCall PJ, Lloyd L, Horstick O, Dayal-Drager R, Nathan MB, Kroeger A. Towards a global dengue research agenda. *Trop Med Int Health* 2007; **12**: 695-699 [PMID: 17550466 DOI: 10.1111/j.1365-3156.2007.01838.x]

P- Reviewer: Cantarovich F, Du C, Ramsay MA **S- Editor**: Kong JX
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Transplant 2017 February 24; 7(1): 64-69

DOI: 10.5500/wjt.v7.i1.64

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Study

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

Vivek B Kute, Himanshu V Patel, Pankaj R Shah, Pranjal R Modi, Veena R Shah, Sayyed J Rizvi, Bipin C Pal, Priya S Shah, Pavan S Wakhare, Saiprasad G Shinde, Vijay A Ghodela, Umesh T Varyani, Minaxi H Patel, Varsha B Trivedi, Hargovind L Trivedi

Vivek B Kute, Himanshu V Patel, Pankaj R Shah, Priya S Shah, Pavan S Wakhare, Saiprasad G Shinde, Vijay A Ghodela, Umesh T Varyani, Hargovind L Trivedi, Department of Nephrology and Clinical Transplantation, Institute of Kidney Diseases and Research Center, Dr HL Trivedi Institute of Transplantation Sciences (IKDRC-ITS), Ahmedabad 380016, India

Pranjal R Modi, Sayyed J Rizvi, Bipin C Pal, Department of Urology and transplantation, IKDRC-ITS, Ahmedabad 380016, India

Veena R Shah, Department of Anesthesia, IKDRC-ITS, Ahmedabad 380016, India

Minaxi H Patel, Varsha B Trivedi, Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, IKDRC-ITS, Ahmedabad 380016, India

Author contributions: All authors contributed to this paper.

Institutional review board statement: The study was reviewed and approved by the Science and Research Office of IKDRC-ITS, Ahmedabad 380016 (India).

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Dr. Vivek B Kute, MBBS, MD, FCPS, DM Nephrology (Gold Medalist), FASN, Associate Professor, Department of Nephrology and Clinical Transplantation, Institute of Kidney Diseases and Research Center, Dr HL Trivedi Institute of Transplantation Sciences (IKDRC-ITS), Civil Hospital Campus, Asarwa, Ahmedabad 380016, India. drvivekkute@rediffmail.com
Telephone: +91-90-99927543

Received: October 31, 2016
Peer-review started: November 2, 2016
First decision: November 14, 2016
Revised: November 18, 2016
Accepted: December 7, 2016
Article in press: December 9, 2016
Published online: February 24, 2017

Abstract

AIM

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

METHODS

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

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

RESULTS

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

CONCLUSION

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

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

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

Kute VB, Patel HV, Shah PR, Modi PR, Shah VR, Rizvi SJ, Pal BC, Shah PS, Wakhare PS, Shinde SG, Ghodela VA, Varyani UT, Patel MH, Trivedi VB, Trivedi HL. International kidney paired donation transplantations to increase kidney transplant of O group and highly sensitized patient: First report from India. *World J Transplant* 2017; 7(1): 64-69 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i1/64.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i1.64

INTRODUCTION

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

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

MATERIALS AND METHODS

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

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

Table 1 Human leukocyte antigen data of patient and donor

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

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

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

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

Immunosuppression

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

RESULTS

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

DISCUSSION

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

The ethical challenges

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

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

Table 2 Demographics and outcome of two way kidney exchange

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

KPD: Kidney paired donation.

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

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

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

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

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

Global kidney exchange

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

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

COMMENTS

Background

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

Research frontiers

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

Innovations and breakthroughs

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

Applications

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

Terminology

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

Peer-review

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

REFERENCES

1 **Jha V**, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, Saran R, Wang AY, Yang CW. Chronic kidney disease: global dimension and perspectives. *Lancet* 2013; **382**: 260-272 [PMID: 23727169 DOI: 10.1016/S0140-6736(13)60687-X]

2 **Rajapurkar MM**, John GT, Kirpalani AL, Abraham G, Agarwal SK, Almeida AF, Gang S, Gupta A, Modi G, Pahari D, Pisharody R, Prakash J, Raman A, Rana DS, Sharma RK, Sahoo RN, Sakhuja V, Tatapudi RR, Jha V. What do we know about chronic kidney disease in India: first report of the Indian CKD registry. *BMC Nephrol* 2012; **13**: 10 [PMID: 22390203 DOI: 10.1186/1471-2369-13-10]

3 **Kute VB**, Patel HV, Shah PR, Vanikar AV, Trivedi HL. National kidney paired donation programme in India: Challenges, solution, future direction. *Nephrology* (Carlton) 2015; **20**: 442 [PMID: 25900390 DOI: 10.1111/nep.12408]

4 **Kute VB**, Shah PS, Vanikar AV, Gumber MR, Patel HV, Engineer DP, Shah PR, Modi PR, Shah VR, Rizvi SJ, Trivedi HL. Increasing access to renal transplantation in India through our single-center kidney paired donation program: a model for the developing world to prevent commercial transplantation. *Transpl Int* 2014; **27**: 1015-1021 [PMID: 24947741 DOI: 10.1111/tri.12373]

5 **Kute VB**, Vanikar AV, Shah PR, Gumber MR, Patel HV, Engineer DP, Modi PR, Rizvi SJ, Shah VR, Modi MP, Kanodia KV, Trivedi HL. Ten kidney paired donation transplantation on World Kidney Day 2013: raising awareness and time to take action to increase donor pool. *Ren Fail* 2013; **35**: 1269-1272 [PMID: 23937166 DOI: 10.3109/0886022

X.2013.823997]
6 **Ferrari P**, Weimar W, Johnson RJ, Lim WH, Tinckam KJ. Kidney paired donation: principles, protocols and programs. *Nephrol Dial Transplant* 2015; **30**: 1276-1285 [PMID: 25294848 DOI: 10.1093/ndt/gfu309]
7 **Cantwell L**, Woodroffe C, Holdsworth R, Ferrari P. Four years of experience with the Australian kidney paired donation programme. *Nephrology* (Carlton) 2015; **20**: 124-131 [PMID: 25408125 DOI: 10.1111/nep.12369]
8 **Malik S**, Cole E. Foundations and principles of the Canadian living donor paired exchange program. *Can J Kidney Health Dis* 2014; **1**: 6 [PMID: 25780601 DOI: 10.1186/2054-3581-1-6]
9 **Johnson RJ**, Allen JE, Fuggle SV, Bradley JA, Rudge C. Early experience of paired living kidney donation in the United kingdom. *Transplantation* 2008; **86**: 1672-1677 [PMID: 19104403 DOI: 10.1097/TP.0b013e3181901a3d]
10 **Segev DL**, Kucirka LM, Gentry SE, Montgomery RA. Utilization and outcomes of kidney paired donation in the United States. *Transplantation* 2008; **86**: 502-510 [PMID: 18724216 DOI: 10.1097/TP.0b013e3181812f85]
11 **Roodnat JI**, van de Wetering J, Claas FH, Ijzermans J, Weimar W. Persistently low transplantation rate of ABO blood type O and highly sensitised patients despite alternative transplantation programs. *Transpl Int* 2012; **25**: 987-993 [PMID: 22775425 DOI: 10.1111/j.1432-2277.2012.01526.x]
12 **Connolly JS**, Terasaki PI, Veale JL. Kidney paired donation--the next step. *N Engl J Med* 2011; **365**: 868-869 [PMID: 21879922 DOI: 10.1056/NEJMc1106996]
13 **Kute VB**, Vanikar AV, Shah PR, Gumber MR, Patel HV, Modi PR, Trivedi HL. Facilitators to national kidney paired donation program. *Transpl Int* 2013; **26**: e38-e39 [PMID: 23437957 DOI: 10.1111/tri.12078]
14 **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]
15 **Tuncer M**, Tekin S, Yuksel Y, Yücetin L, Dosemeci L, Sengul A, Demirbaş A. First International Paired Exchange Kidney Transplantations of Turkey. *Transplant Proc* 2015; **47**: 1294-1295 [PMID: 26093701 DOI: 10.1016/j.transproceed.2015.04.011]
16 **Weiss J**, Kocher M, Immer FF. International collaboration and organ exchange in Switzerland. *J Thorac Dis* 2015; **7**: 543-548 [PMID: 25922737 DOI: 10.3978/j.issn.2072-1439.2014.12.44]
17 **Segev DL**, Veale JL, Berger JC, Hiller JM, Hanto RL, Leeser DB, Geffner SR, Shenoy S, Bry WI, Katznelson S, Melcher ML, Rees MA, Samara EN, Israni AK, Cooper M, Montgomery RJ, Malinzak L, Whiting J, Baran D, Tchervenkov JI, Roberts JP, Rogers J, Axelrod DA, Simpkins CE, Montgomery RA. Transporting live donor kidneys for kidney paired donation: initial national results. *Am J Transplant* 2011; **11**: 356-360 [PMID: 21272238 DOI: 10.1111/j.1600-6143.2010.03386.x]
18 **Simpkins CE**, Montgomery RA, Hawxby AM, Locke JE, Gentry SE, Warren DS, Segev DL. Cold ischemia time and allograft outcomes in live donor renal transplantation: is live donor organ transport feasible? *Am J Transplant* 2007; **7**: 99-107 [PMID: 17227561 DOI: 10.1111/j.1600-6143.2006.01597.x]
19 **Allen R**, Pleass H, Clayton PA, Woodroffe C, Ferrari P. Outcomes of kidney paired donation transplants in relation to shipping and cold ischaemia time. *Transpl Int* 2016; **29**: 425-431 [PMID: 26576040 DOI: 10.1111/tri.12719]
20 2016 American Transplant Congress Abstracts. *Am J Transplant* 2016; **16** Suppl 3: 5-798 [PMID: 27273422 DOI: 10.1111/ajt.13895]
21 **Rees MA**, Dunn TB, Kuhr CS, Marsh CL, Rogers J, Rees SE, Cicero A, Reece LJ, Roth AE, Ekwenna O, Fumo DE, Krawiec KD, Kopke JE, Jain S, Tan M, Paloyo SR. Kidney Exchange to Overcome Financial Barriers to Kidney Transplantation. *Am J Transplant* 2016 [DOI: 10.1111/ajt.14106]

P- Reviewer: Friedman EA, Tarantino G **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Transplant 2017 February 24; 7(1): 70-80

DOI: 10.5500/wjt.v7.i1.70

ISSN 2220-3230 (online)

SYSTEMATIC REVIEWS

Lobar lung transplantation from deceased donors: A systematic review

Michael Eberlein, Robert M Reed, Mayy Chahla, Servet Bolukbas, Amy Blevins, Dirk Van Raemdonck, Alessia Stanzi, Ilhan Inci, Silvana Marasco, Norihisa Shigemura, Clemens Aigner, Tobias Deuse

Michael Eberlein, Division of Pulmonary, Critical Care and Occupational Medicine, University of Iowa Hospitals and Clinics, Iowa City, IA 52242, United States

Michael Eberlein, Mayy Chahla, Department of Medicine, University of Iowa Hospitals and Clinics, University of Iowa Hospitals and Clinics, Iowa City, IA 52242, United States

Robert M Reed, Division of Pulmonary and Critical Care Medicine, University of Maryland, Baltimore, MD 21201, United States

Servet Bolukbas, Department of Thoracic Surgery, Helios Klinikum Wuppertal - University Hospital Witten/Herdecke, 42283 Wuppertal, Germany

Amy Blevins, Hardin Library for the Health Sciences, University of Iowa Hospitals and Clinics, Iowa City, IA 52242, United States

Dirk Van Raemdonck, Alessia Stanzi, Department of Thoracic Surgery and Lung Transplant Unit, University Hospitals Leuven, B-3000 Leuven, Belgium

Ilhan Inci, Department of Thoracic Surgery, Zurich University Hospital, 8091 Zurich, Switzerland

Silvana Marasco, Cardiothoracic Surgery Unit, The Alfred Hospital, Melbourne, VIC 3004, Australia

Norihisa Shigemura, Department of Cardiothoracic Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA 15213, United States

Clemens Aigner, Department of Thoracic Surgery, Medical University of Vienna, 1090 Vienna, Austria

Clemens Aigner, Department of Thoracic Surgery and Surgical Endoscopy Ruhrlandklinik, University Clinic Essen Tueschener Weg 40, 45239 Essen, Germany

Tobias Deuse, Department of Cardiovascular Surgery, University Heart Center Hamburg, 20246 Hamburg, Germany

Author contributions: Eberlein M, Reed RM and Chahla M contributed to conception and design; Eberlein M and Blevins A contributed to design of search strategy; Eberlein M and Chahla M contributed to study selection; Eberlein M and Deuse T contributed to writing of the manuscript; Eberlein M, Reed RM, Chahla M, Bolukbas S, Blevins A, Van Raemdonck D, Stanzi A, Inci I, Marasco S, Shigemura N, Aigner C and Deuse T contributed to revision of the manuscript.

Conflict-of-interest statement: The authors of this manuscript have no conflicts of interest to disclose.

Data sharing statement: Not applicable.

Manuscript source: Invited manuscript

Correspondence to: Michael Eberlein, MD, PhD, Division of Pulmonary, Critical Care and Occupational Medicine, University of Iowa Hospitals and Clinics, 200 Hawkins Drive, C 33 GH, Iowa City, IA 52242, United States. michael-eberlein@uiowa.edu
Telephone: +1-319-3561265
Fax: +1-319-3536406

Received: May 30, 2016
Peer-review started: June 3, 2016
First decision: July 5, 2016
Revised: December 2, 2016
Accepted: December 27, 2016
Article in press: December 29, 2016
Published online: February 24, 2017

Abstract

AIM

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

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

METHODS

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

RESULTS

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

CONCLUSION

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

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

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

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

INTRODUCTION

Lung transplantation (LTx) is an established therapy for appropriately selected patients suffering from end-stage lung disease. Since the implementation of the Lung Allocation Scoring (LAS) system, characteristics of candidates on the wait list have changed to include a sicker group of patients with a greater proportion of restrictive lung diseases (LAS diagnoses group D)[1,2]. As a consequence, wait-list mortality rates are again rising despite higher wait-list transplant rates compared to the pre-LAS era[3]. Potential LTx-recipients with short stature and small thoracic cavities have longer waiting times on the LTx list, as donor lungs considered to be size-appropriate are particularly limited[3,4]. This often affects patients with cystic fibrosis and pulmonary fibrosis[4]. In both groups, LTx can become an urgent issue when significant disease exacerbations occur, and in this setting in particular patients are at high risk for wait list mortality. Higher acuity at the time of LTx is in turn associated with decreased survival[5].

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

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

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

MATERIALS AND METHODS

Data sources

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

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

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

Study selection criteria

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

Study quality assessment

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

Data extraction

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

Assessment of donor to recipient size matching

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

Definitions of primary and secondary outcomes

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

Statistical analysis

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

RESULTS

Search results

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

Study range and characteristics

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

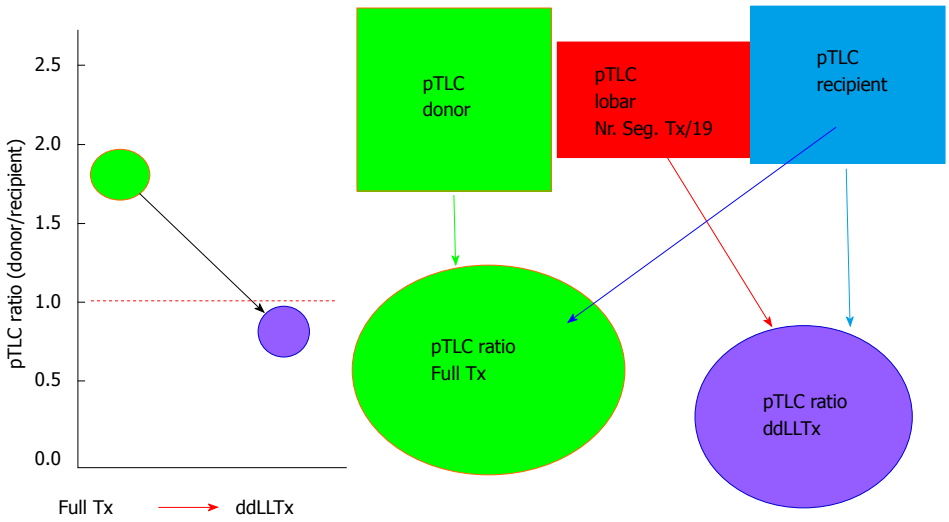


Figure 1 The parameter(s) used for the size matching were extracted for each study. For all studies that did not report recipient pTLC (pTLCrecipient), full donor pTLC (pTLCdonorFull) and donor pTLC after lobar resection (pTLCdonorLobar) the study authors were contacted and asked to provide: Recipient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

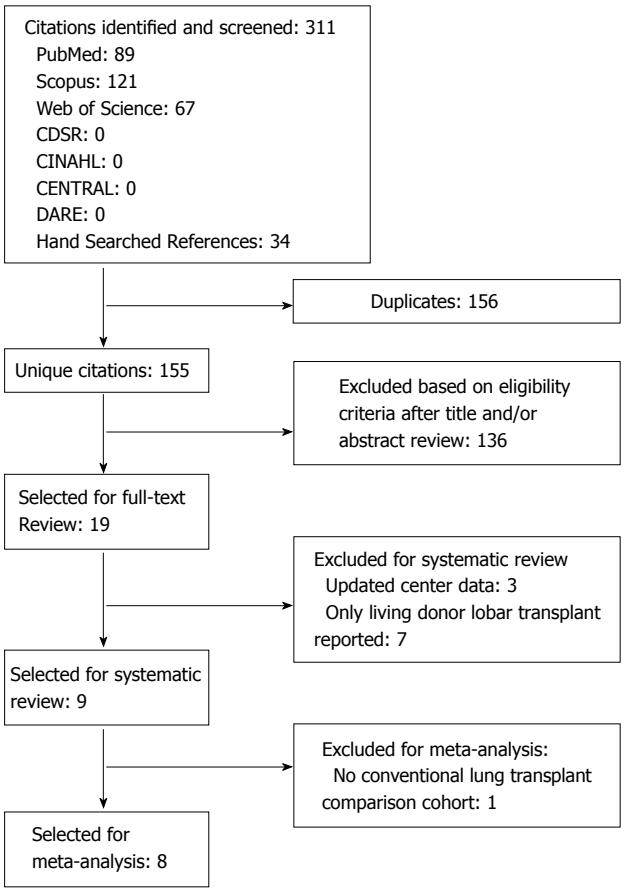


Figure 2 PRISMA diagram detailing study selection.

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

Indication for transplant and acuity

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

Donor to recipient size matching

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

Table 1 Study characteristics

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

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

Table 2 Size matching parameters and characteristics

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

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

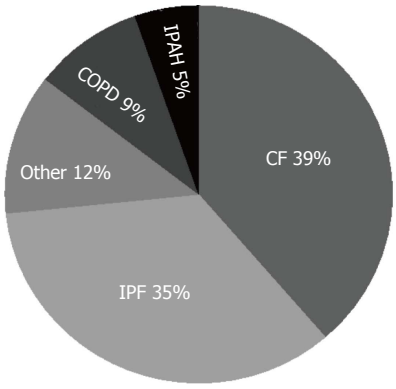


Figure 3 Pie chart of transplant indications. IPAH: Idiopathic pulmonary arterial hypertension; COPD: Chronic obstructive pulmonary disease;IPF: Idiopathic pulmonary fibrosis; CF: Cystic fibrosis.

(Figure 5).

Primary outcome: One year survival

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

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

Secondary outcomes

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

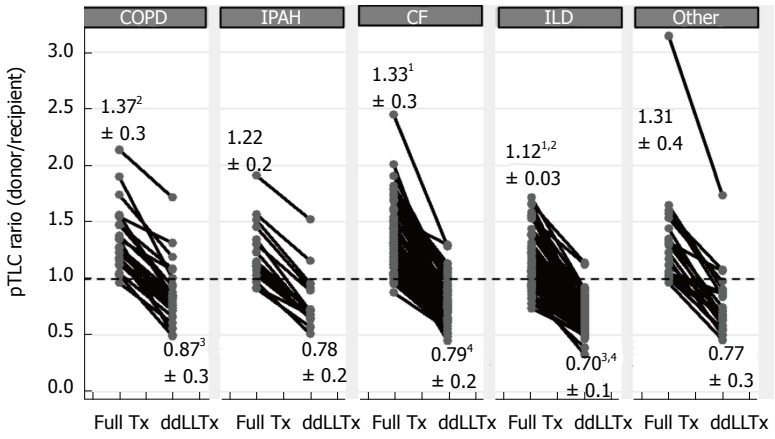


Figure 4 Donor to recipient size matching based on the donor to recipient predicted total lung capacity ratio, stratified by transplant indication. The predicted total lung capacity (pTLC) ratio that would have occurred using the entire donor lungs was calculated as $pTLCratio_{Full}$ = $pTLCdonor_{Full}$/pTLCrecipient.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

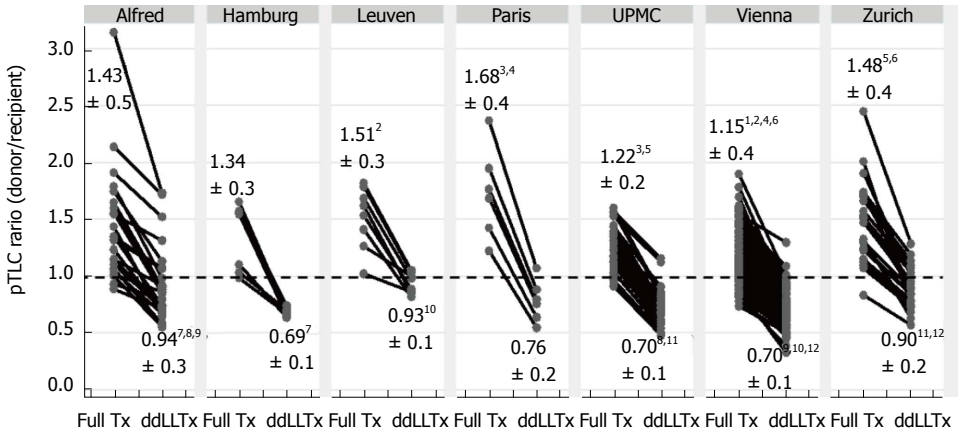


Figure 5 Donor to recipient size matching based on the donor to recipient predicted total lung capacity ratio, stratified by transplant center. See figure legend 3 for further details. [1,2,3,4,5,6]Indicate a significant difference in pTLCratioFull (one-way-anova *P*-value < 0.05) of pairwise comparisons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

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

DISCUSSION

The technique of deceased donor lobar lung trans-

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

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

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

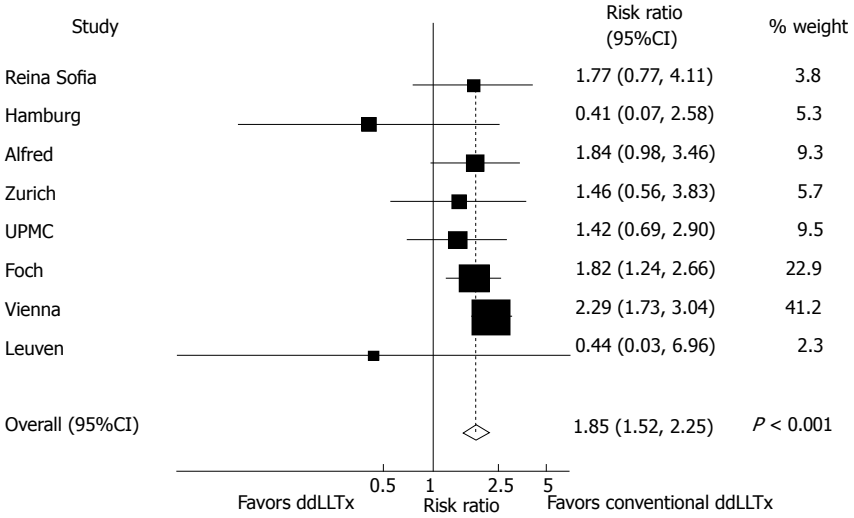


Figure 6 Forest plot for pooled analysis of 1 year survival comparing deceased donor lobar lung transplantation to conventional lung transplant. Vertical line is the "no difference" point in 1 year mortality between dLLTx and CLTx cohorts. Horizontal lines are 95%CI. ■ = Relative Risk (RR) and the size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

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

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

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

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

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

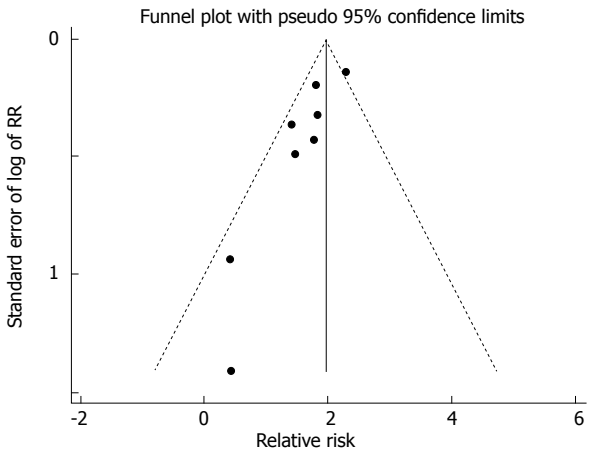


Figure 7 Funnel plot for assessment of publication bias in 1 year mortality results.

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

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

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

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

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

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

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

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

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

ACKNOWLEDGMENTS

Alison Beer participated in developing the literature search strategy and in registering the meta-analysis at PROSPERO International prospective register of systematic reviews (PROSPERO 2014:CRD42014004308). Robert M Reed is funded in part by the Flight Attendant Medical Research Institute (FAMRI).

COMMENTS

Background

Lung transplantation (LTx) is an established therapy for appropriately selected patients suffering from end-stage lung disease. Potential LTx-recipients with short stature and small thoracic cavities have longer waiting times on the LTx list, as donor lungs considered to be size-appropriate are particularly limited. Deceased-donor-lobar lung transplantation (ddLLTx) is an important and so far underutilized surgical option for lung transplant candidates with small chest cavities. The size matching decision for ddLLTx is complex and based on varying parameters.

Research frontiers

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

Innovations and breakthroughs

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

Applications

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

Terminology

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

Peer-review

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

REFERENCES

1 **Eberlein M**, Garrity ER, Orens JB. Lung allocation in the United States. *Clin Chest Med* 2011; **32**: 213-222 [PMID: 21511084 DOI: 10.1016/j.ccm.2011.02.004]

2 **Maxwell BG**, Mooney JJ, Lee PH, Levitt JE, Chhatwani L, Nicolls MR, Zamora MR, Valentine V, Weill D, Dhillon GS. Increased resource use in lung transplant admissions in the lung allocation score era. *Am J Respir Crit Care Med* 2015; **191**: 302-308 [PMID: 25517213 DOI: 10.1164/rccm.201408-1562OC]

3 **Aigner C**, Winkler G, Jaksch P, Ankersmit J, Marta G, Taghavi S, Wisser W, Klepetko W. Size-reduced lung transplantation: an advanced operative strategy to alleviate donor organ shortage. *Transplant Proc* 2004; **36**: 2801-2805 [PMID: 15621153 DOI: 10.1016/j.transproceed.2004.09.066]

4 **Keeshan BC**, Rossano JW, Beck N, Hammond R, Kreindler J, Spray TL, Fuller S, Goldfarb S. Lung transplant waitlist mortality: height as a predictor of poor outcomes. *Pediatr Transplant* 2015; **19**: 294-300 [PMID: 25406495 DOI: 10.1111/petr.12390]

5 **Merlo CA**, Weiss ES, Orens JB, Borja MC, Diener-West M, Conte JV, Shah AS. Impact of U.S. Lung Allocation Score on survival after lung transplantation. *J Heart Lung Transplant* 2009; **28**: 769-775 [PMID: 19632571 DOI: 10.1016/j.healun.2009.04.024]

6 **Couetil JP**, Tolan MJ, Loulmet DF, Guinvarch A, Chevalier PG, Achkar A, Birmbaum P, Carpentier AF. Pulmonary bipartitioning and lobar transplantation: a new approach to donor organ shortage. *J Thorac Cardiovasc Surg* 1997; **113**: 529-537 [PMID: 9081098 DOI: 10.1016/S0022-5223(97)70366-0]

7 **Deuse T**, Sill B, von Samson P, Yildirim Y, Kugler C, Oldigs M, Klose H, Meierling S, Rabe KF, Reichenspurner H. Surgical technique of lower lobe lung transplantation. *Ann Thorac Surg* 2011; **92**: e39-e42 [PMID: 21801900 DOI: 10.1016/j.athoracsur.2011.04.014]

8 **Bisson A**, Bonnette P, el Kadi NB, Leroy M, Colchen A. Bilateral pulmonary lobe transplantation: left lower and right middle and lower lobes. *Ann Thorac Surg* 1994; **57**: 219-221 [PMID: 8279898 DOI: 10.1016/0003-4975(94)90405-7]

9 **Inci I**, Benden C, Kestenholz P, Hillinger S, Schneiter D, Ganter M, Bechir M, Grünenfelder J, Weder W. Simultaneous bilateral lobar lung transplantation: one donor serves two recipients. *Ann Thorac Surg* 2013; **96**: e69-e71 [PMID: 23992734 DOI: 10.1016/j.athoracsur.2013.02.062]

10 **Espinosa D**, Algar FJ, Moreno P, Illana J, Alvarez A, Cerezo F, Baamonde C, Santos F, Vaquero JM, Redel J, Salvatierra A. Experience of the Reina Sofia hospital in lobar lung transplantation. *Transplant Proc* 2010; **42**: 3214-3216 [PMID: 20970656 DOI: 10.1016/j.transproceed.2010.05.048]

11 **Marasco SF**, Than S, Keating D, Westall G, Whitford H, Snell G, Gooi J, Williams T, Pick A, Zimmet A, Lee GA. Cadaveric lobar lung transplantation: technical aspects. *Ann Thorac Surg* 2012; **93**: 1836-1842 [PMID: 22551845 DOI: 10.1016/j.athoracsur.2012.03.051]

12 **Inci I**, Schuurmans MM, Kestenholz P, Schneiter D, Hillinger S, Opitz I, Boehler A, Weder W. Long-term outcomes of bilateral lobar lung transplantation. *Eur J Cardiothorac Surg* 2013; **43**: 1220-1225 [PMID: 23091227 DOI: 10.1093/ejcts/ezs541]

13 **Shigemura N**, D'Cunha J, Bhama JK, Shiose A, Abou El Ela A, Hackmann A, Zaldonis D, Toyoda Y, Pilewski JM, Luketich JD, Bermudez CA. Lobar lung transplantation: a relevant surgical option in the current era of lung allocation score. *Ann Thorac Surg* 2013; **96**: 451-456 [PMID: 23773735 DOI: 10.1016/j.athoracsur.2013.04.030]

14 **Mitilian D**, Sage E, Puyo P, Bonnette P, Parquin F, Stern M, Fischler M, Chapelier A. Techniques and results of lobar lung transplantations. *Eur J Cardiothorac Surg* 2014; **45**: 365-369 [PMID: 23900745 DOI: 10.1093/ejcts/ezt353]

15 **Slama A**, Ghanim B, Klikovits T, Scheed A, Hoda MA, Hoetzenecker K, Jaksch P, Matilla J, Taghavi S, Klepetko W, Aigner C. Lobar lung transplantation--is it comparable with standard lung transplantation? *Transpl Int* 2014; **27**: 909-916 [PMID: 24810771 DOI: 10.1111/tri.12348]

16 **Stanzi A**, Decaluwe H, Coosemans W, De Leyn P, Nafteux P, Van Veer H, Dupont L, Verleden GM, Van Raemdonck D. Lobar lung transplantation from deceased donors: a valid option for small-sized patients with cystic fibrosis. *Transplant Proc* 2014; **46**: 3154-3159 [PMID: 25420847 DOI: 10.1016/j.transproceed.2014.09.168]

17 **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]

18 **Mason DP**, Batizy LH, Wu J, Nowicki ER, Murthy SC, McNeill AM, Budev MM, Mehta AC, Pettersson GB, Blackstone EH. Matching donor to recipient in lung transplantation: How much does size matter? *J Thorac Cardiovasc Surg* 2009; **137**: 1234-40.e1 [PMID: 19379997 DOI: 10.1016/j.jtcvs2008.10.024]

19 **Eberlein M**, Permutt S, Brown RH, Brooker A, Chahla MF, Bolukbas S, Nathan SD, Pearse DB, Orens JB, Brower RG. Supranormal expiratory airflow after bilateral lung transplantation is associated with improved survival. *Am J Respir Crit Care Med* 2011; **183**: 79-87 [PMID: 20693376 DOI: 10.1164/rccm.201004-0593OC]

20 **Eberlein M**, Arnaoutakis GJ, Yarmus L, Feller-Kopman D, Dezube R, Chahla MF, Bolukbas S, Reed RM, Klesney-Tait J, Parekh KR, Merlo CA, Shah AS, Orens JB, Brower RG. The effect of lung size mismatch on complications and resource utilization after bilateral lung transplantation. *J Heart Lung Transplant* 2012; **31**: 492-500 [PMID: 22325691 DOI: 10.1016/j.healun.2011.12.009]

21 **Eberlein M**, Permutt S, Chahla MF, Bolukbas S, Nathan SD, Shlobin OA, Shelhamer JH, Reed RM, Pearse DB, Orens JB, Brower RG. Lung size mismatch in bilateral lung transplantation is associated with allograft function and bronchiolitis obliterans syndrome. *Chest* 2012; **141**: 451-460 [PMID: 21799025 DOI: 10.1378/chest.11-0767]

22 **Eberlein M**, Reed RM, Permutt S, Chahla MF, Bolukbas S, Nathan SD, Iacono A, Pearse DB, Fessler HE, Shah AS, Orens JB, Brower RG. Parameters of donor-recipient size mismatch and survival after bilateral lung transplantation. *J Heart Lung Transplant* 2012; **31**: 1207-1213.e7 [PMID: 22036314 DOI: 10.1016/j.healun.2011.07.015]

23 **Dezube R**, Arnaoutakis GJ, Reed RM, Bolukbas S, Shah AS, Orens JB, Brower RG, Eberlein M. The effect of lung-size mismatch on mechanical ventilation tidal volumes after bilateral lung transplantation. *Interact Cardiovasc Thorac Surg* 2013; **16**: 275-281 [PMID: 23243035 DOI: 10.1093/icvts/ivs493]

24 **Eberlein M**, Bolukbas S, Pena T, Reed RM. eComment. Lung size mismatch and graft dysfunction immediately after reperfusion. *Interact Cardiovasc Thorac Surg* 2016; **22**: 320 [PMID: 26874005 DOI: 10.1093/icvts/ivw026]

25 **Eberlein M**, Diehl E, Bolukbas S, Merlo CA, Reed RM. An oversized allograft is associated with improved survival after lung transplantation for idiopathic pulmonary arterial hypertension. *J Heart Lung Transplant* 2013; **32**: 1172-1178 [PMID: 23876630 DOI: 10.1016/j.healun.2013.06.011]

26 **Eberlein M**, Reed RM, Bolukbas S, Parekh KR, Arnaoutakis GJ, Orens JB, Brower RG, Shah AS, Hunsicker L, Merlo CA. Lung size mismatch and survival after single and bilateral lung transplantation. *Ann Thorac Surg* 2013; **96**: 457-463 [PMID: 23809729 DOI: 10.1016/j.athoracsur.2013.04.064]

27 **Eberlein M**, Reed RM, Maidaa M, Bolukbas S, Arnaoutakis GJ, Orens JB, Brower RG, Merlo CA, Hunsicker LG. Donor-recipient size matching and survival after lung transplantation. A cohort study. *Ann Am Thorac Soc* 2013; **10**: 418-425 [PMID: 23988005 DOI: 10.1513/AnnalsATS.201301-008OC]

28 **Reed RM**, Eberlein M. Sizing strategies in heart and lung transplantation: you cannot manage what you do not measure. *Future Cardiol* 2014; **10**: 303-306 [PMID: 24976463 DOI: 10.2217/fca.14.17]

29 **Loizzi D**, Aigner C, Jaksch P, Scheed A, Mora B, Sollitto F, Klepetko W. A scale for decision making between whole lung transplantation or lobar transplantation. *Eur J Cardiothorac Surg* 2010; **37**: 1122-1125 [PMID: 20045347 DOI: 10.1016/j.ejcts.2009.11.032]

30 **Force SD**. Invited Commentary. *Ann Thorac Surg* 2015; **100**: 2057-2058 [PMID: 26652515 DOI: 10.1016/j.athoracsur.2015.06.006]

31 **Eberlein M**, Bolukbas S, Reed RM. Bilateral lobar lung transplantation and size mismatch by pTLC-ratio. *Eur J Cardiothorac Surg* 2013; **44**: 394-395 [PMID: 23345179 DOI: 10.1093/ejcts/ezt004]

32 **Reed RM**, Eberlein M. Sizing considerations in lobar lung transplantation. *Transpl Int* 2014; **27**: e132-e133 [PMID: 24979677 DOI: 10.1111/tri.12391]

33 **Eberlein M**, Reed RM. Donor to recipient sizing in thoracic organ transplantation. *World J Transplant* 2016; **6**: 155-164 [PMID: 27011913 DOI: 10.5500/wjt.v6.i1.155]

34 **Hong JC**, Yersiz H, Busuttil RW. Where are we today in split liver transplantation? *Curr Opin Organ Transplant* 2011; **16**: 269-273 [PMID: 21467935 DOI: 10.1097/MOT.0b013e328346572e]

35 **Weder W**, Inci I, Korom S, Kestenholz PB, Hillinger S, Eich C, Irani S, Lardinois D. Airway complications after lung transplantation: risk factors, prevention and outcome. *Eur J Cardiothorac Surg* 2009; **35**: 293-298; discussion 298 [PMID: 19004637 DOI: 10.1016/j.ejcts.2008.09.035]

36 **Taher H**, Reed RM, Eberlein M. Characterization of Donor to Recipient Size Matching in Lung Transplantation. *Austin J Pulm Respir Med* 2014; **1**: 1014

37 **Christie JD**, Bellamy S, Ware LB, Lederer D, Hadjiliadis D, Lee J, Robinson N, Localio AR, Wille K, Lama V, Palmer S, Orens J, Weinacker A, Crespo M, Demissie E, Kimmel SE, Kawut SM. Construct validity of the definition of primary graft dysfunction after lung transplantation. *J Heart Lung Transplant* 2010; **29**: 1231-1239 [PMID: 20655249 DOI: 10.1016/j.healun.2010.05.013]

38 **Eberlein M**, Reed RM, Bolukbas S, Diamond JM, Wille KM, Orens JB, Brower RG, Christie JD. Lung size mismatch and primary graft dysfunction after bilateral lung transplantation. *J Heart Lung Transplant* 2015; **34**: 233-240 [PMID: 25447586 DOI: 10.1016/j.healun.2014.09.030]

39 **Horai T**, Shigemura N, Gries C, Pilewski J, Bhama JK, Bermudez CA, Zaldonis D, Toyoda Y. Lung transplantation for patients with high lung allocation score: single-center experience. *Ann Thorac Surg* 2012; **93**: 1592-1597; discussion 1597 [PMID: 22192755 DOI: 10.1016/j.athoracsur.2011.09.045]

40 **Sill B**, Oelschner C, Oldigs M, Deuse T. Elective Lobar Lung Transplantation - A Single Center Experience. *Thorac cardiovasc Surg* 2015; **63**: ePP42 [DOI: 10.1055/s-0035-1544538]

41 **Wilkens H**, Weingard B, Lo Mauro A, Schena E, Pedotti A, Sybrecht GW, Aliverti A. Breathing pattern and chest wall volumes during exercise in patients with cystic fibrosis, pulmonary fibrosis and COPD before and after lung transplantation. *Thorax* 2010; **65**: 808-814 [PMID: 20805177 DOI: 10.1136/thx.2009.131409]

42 **Konheim JA**, Kon ZN, Pasrija C, Luo Q, Sanchez PG, Garcia JP, Griffith BP, Jeudy J. Predictive equations for lung volumes from computed tomography for size matching in pulmonary transplantation. *J Thorac Cardiovasc Surg* 2016; **151**: 1163-1169.e1 [PMID: 26725712 DOI: 10.1016/j.jtcvs2015.10.051]

P- Reviewer: Belliato M, Nosotti M **S- Editor**: Kong JX
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Transplant 2017 February 24; 7(1): 81-87

DOI: 10.5500/wjt.v7.i1.81

ISSN 2220-3230 (online)

META-ANALYSIS

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

Wisit Cheungpasitporn, Charat Thongprayoon, Michael A Mao, Shennen A Mao, Matthew R D'Costa, Wonngarm Kittanamongkolchai, Kianoush B Kashani

Wisit Cheungpasitporn, Charat Thongprayoon, Michael A Mao, Shennen A Mao, Matthew R D'Costa, Wonngarm Kittanamongkolchai, Kianoush B Kashani, Division of Nephrology and Hypertension, Department of Medicine, Mayo Clinic, Rochester, MI 55905, United States

Author contributions: Cheungpasitporn W and Thongprayoon C contributed equally to this work; Cheungpasitporn W and Thongprayoon C performed the search, analysis, and interpretation of data, analysis of data, and final approval of the version to be published; Mao MA, Mao SA, D'Costa MR and Kittanamongkolchai W performed critical revising of the intellectual content and final approval of the version to be published; Kashani KB performed concept and design, critical revising of the intellectual content, and final approval of the version to be published.

Conflict-of-interest statement: All authors report no conflicts-of-interest.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Kianoush B Kashani, MD, Assistant Professor, Division of Nephrology and Hypertension, Department of Medicine, Mayo Clinic, 200 First Street SW, Rochester, MI 55905, United States. kashani.kianoush@mayo.edu
Telephone: +1-507-2667093
Fax: +1-507-2667891

Received: August 25, 2016
Peer-review started: August 26, 2016
First decision: September 27, 2016
Revised: November 12, 2016
Accepted: December 27, 2016
Article in press: December 29, 2016
Published online: February 24, 2017

Abstract

AIM

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

METHODS

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

RESULTS

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

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

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

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

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

INTRODUCTION

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

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

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

MATERIALS AND METHODS

Cheungpasitporn W and Thongprayoon C individually examined published studies and conference abstracts indexed in MEDLINE, EMBASE, and the Cochrane Database from the inception of the databases through July 2016. The search strategy used is detailed in the supplementary material (Supplementary material 1). Further pertinent studies were retrieved by conducting a manual search using references from the articles that were identified from the search strategy noted above. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews and meta-analyses[23] and previously published guidelines[24,25].

The inclusion criteria were as follows: (1) randomized controlled trials or observational studies; (2) patient population age ⩾ 18 years old; and (3) and additional data on kidney transplant recipients were provided. The search was limited to English-language studies. Both published studies and conference abstracts were incorporated. Study eligibility was independently determined by the two investigators mentioned above. Differing decisions were settled by joint agreement.

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

Statistical analysis

MetaXL software (EpiGear International Pty Ltd)[26] was utilized for data analysis. The incidence rates (IRs) and 95%CIs of adverse effects were reported using a DerSimonian-Laird random-effects model[27]. A random-effects model was implemented due to the high likelihood of inter-study variances. The Cochran Q test was completed to assess statistical heterogeneity. The I^2 statistic was added to evaluate the degree of variation across studies related to heterogeneity instead of chance. An I^2 of 0%-25% represents insignificant heterogeneity, 26%-50% low heterogeneity, 51%-75% moderate heterogeneity and > 75% high heterogeneity[28]. Bias funnel plots to assess for publication were used[29].

RESULTS

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

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

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

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

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

CIAKI definition

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

incidence of CIAKI in kidney transplant recipients

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

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

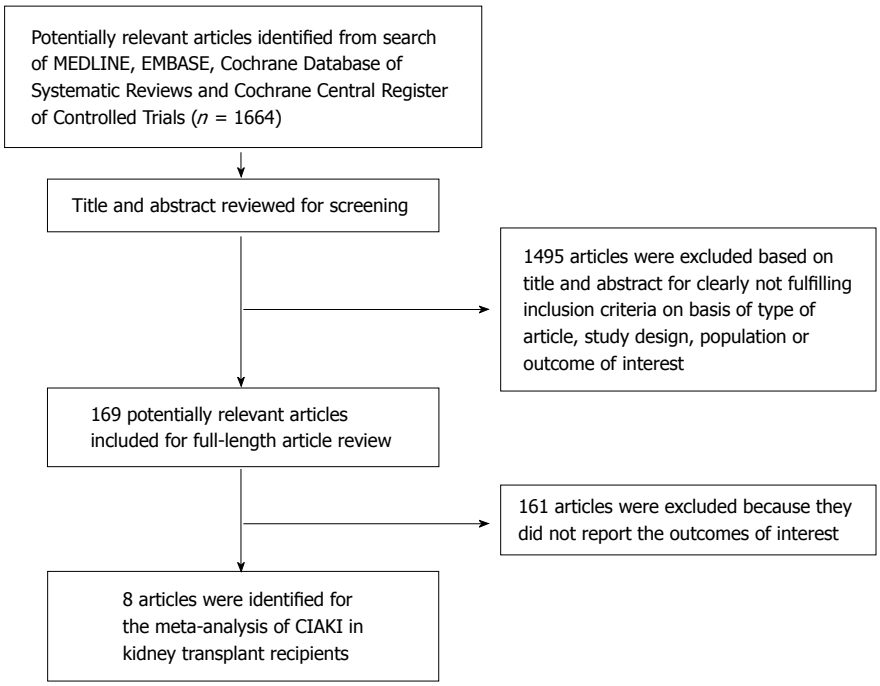


Figure 1 Search strategy. CIAKI: Contrast-induced acute kidney injury.

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

Types of procedure or intervention with contrast media

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

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

Risk factors and prevention measures for CIAKI

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

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

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

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

Although there were reported cases of severe CIAKI

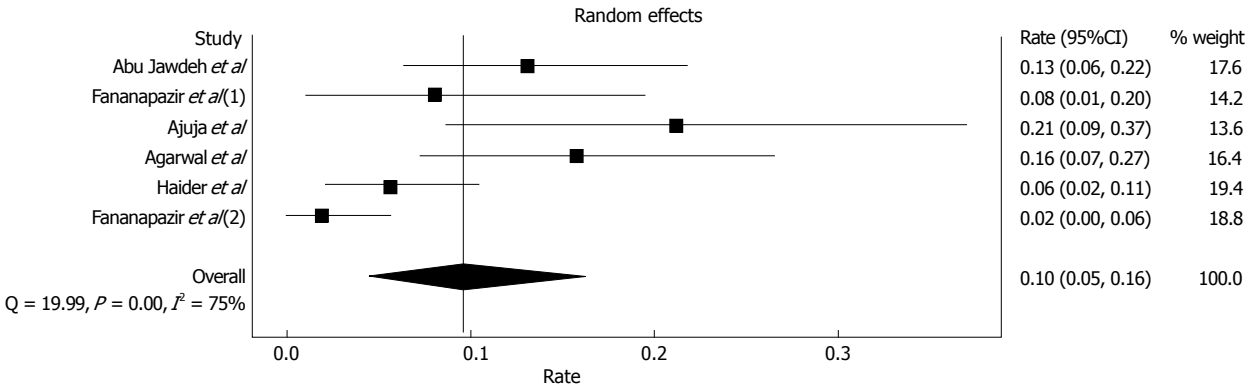


Figure 2 Forest plot of incidence of contrast-induced acute kidney injury in kidney transplant populations.

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

Evaluation for publication bias

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

DISCUSSION

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

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

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

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

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

transplant recipients.

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

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

COMMENTS

Background

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

Research frontiers

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

Innovations and breakthroughs

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

Applications

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

Peer-review

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

REFERENCES

1 **Marenzi G**, Lauri G, Assanelli E, Campodonico J, De Metrio M, Marana I, Grazi M, Veglia F, Bartorelli AL. Contrast-induced nephropathy in patients undergoing primary angioplasty for acute myocardial infarction. *J Am Coll Cardiol* 2004; **44**: 1780-1785 [PMID: 15519007 DOI: 10.1016/j.jacc.2004.07.043]

2 **Solomon RJ**, Mehran R, Natarajan MK, Doucet S, Katholi RE, Staniloae CS, Sharma SK, Labinaz M, Gelormini JL, Barrett BJ. Contrast-induced nephropathy and long-term adverse events: cause and effect? *Clin J Am Soc Nephrol* 2009; **4**: 1162-1169 [PMID: 19556381 DOI: 10.2215/cjn.00550109]

3 **Cheungpasitporn W**, Thongprayoon C, Brabec BA, Edmonds PJ, O'Corragain OA, Erickson SB. Oral hydration for prevention of contrast-induced acute kidney injury in elective radiological procedures: a systematic review and meta-analysis of randomized controlled trials. *N Am J Med Sci* 2014; **6**: 618-624 [PMID: 25599049 DOI: 10.4103/1947-2714.147977]

4 **Thongprayoon C**, Cheungpasitporn W, Podboy AJ, Gillaspie EA, Greason KL, Kashani KB. The effects of contrast media volume on acute kidney injury after transcatheter aortic valve replacement: a systematic review and meta-analysis. *J Evid Based Med* 2016; Epub ahead of print [PMID: 27314627 DOI: 10.1111/jebm.12208]

5 **Cheungpasitporn W**, Thongprayoon C, Kashani K. Transcatheter Aortic Valve Replacement: a Kidney's Perspective. *J Renal Inj Prev* 2016; **5**: 1-7 [PMID: 27069960 DOI: 10.15171/jrip.2016.01]

6 **Thamcharoen N**, Thongprayoon C, Edmonds PJ, Cheungpasitporn W. Periprocedural Nebivolol for the Prevention of Contrast-Induced Acute Kidney Injury: A Systematic Review and Meta-analysis. *N Am J Med Sci* 2015; **7**: 446-451 [PMID: 26713290 DOI: 10.4103/1947-2714.168670]

7 **Cheungpasitporn W**, Thongprayoon C, Kittanamongkolchai W, Edmonds PJ, O'Corragain OA, Srivali N, Ungprasert P, Erickson SB. Periprocedural effects of statins on the incidence of contrast-induced acute kidney injury: a systematic review and meta-analysis of randomized controlled trials. *Ren Fail* 2015; **37**: 664-671 [PMID: 25703707 DOI: 10.3109/0886022x.2015.1010939]

8 **Briguori C**, Airoldi F, D'Andrea D, Bonizzoni E, Morici N, Focaccio A, Michev I, Montorfano M, Carlino M, Cosgrave J, Ricciardelli B, Colombo A. Renal Insufficiency Following Contrast Media Administration Trial (REMEDIAL): a randomized comparison of 3 preventive strategies. *Circulation* 2007; **115**: 1211-1217 [PMID: 17309916 DOI: 10.1161/circulationaha.106.687152]

9 **Marenzi G**, Assanelli E, Marana I, Lauri G, Campodonico J, Grazi M, De Metrio M, Galli S, Fabbiocchi F, Montorsi P, Veglia F, Bartorelli AL. N-acetylcysteine and contrast-induced nephropathy in primary angioplasty. *N Engl J Med* 2006; **354**: 2773-2782 [PMID: 16807414 DOI: 10.1056/NEJMoa054209]

10 **Tepel M**, van der Giet M, Schwarzfeld C, Laufer U, Liermann D, Zidek W. Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. *N Engl J Med* 2000; **343**: 180-184 [PMID: 10900277 DOI: 10.1056/nejm200007203430304]

11 **Mitchell AM**, Jones AE, Tumlin JA, Kline JA. Incidence of contrast-induced nephropathy after contrast-enhanced computed tomography in the outpatient setting. *Clin J Am Soc Nephrol* 2010; **5**: 4-9 [PMID: 19965528 DOI: 10.2215/cjn.05200709]

12 **Huber W**, Eckel F, Hennig M, Rosenbrock H, Wacker A, Saur

D, Sennefelder A, Hennico R, Schenk C, Meining A, Schmelz R, Fritsch R, Weiss W, Hamar P, Heemann U, Schmid RM. Prophylaxis of contrast material-induced nephropathy in patients in intensive care: acetylcysteine, theophylline, or both? A randomized study. *Radiology* 2006; **239**: 793-804 [PMID: 16714461 DOI: 10.1148/radiol.2393041456]

13 **Feldkamp T**, Kribben A. Contrast media induced nephropathy: definition, incidence, outcome, pathophysiology, risk factors and prevention. *Minerva Med* 2008; **99**: 177-196 [PMID: 18431326]

14 **Katzberg RW**, Haller C. Contrast-induced nephrotoxicity: clinical landscape. *Kidney Int Suppl* 2006; **(100)**: S3-S7 [PMID: 16612398 DOI: 10.1038/sj.ki.5000366]

15 **Becker CR**, Davidson C, Lameire N, McCullough PA, Stacul F, Tumlin J, Adam A. High-risk situations and procedures. *Am J Cardiol* 2006; **98**: 37K-41K [PMID: 16949379 DOI: 10.1016/j.amjcard.2006.01.025]

16 **Cheungpasitporn W**, Thongprayoon C, Mao MA, Kittanamongkolchai W, Sathick IJ, Erickson SB. The Effect of Renin-angiotensin System Inhibitors on Kidney Allograft Survival: A Systematic Review and Meta-analysis. *N Am J Med Sci* 2016; **8**: 291-296 [PMID: 27583237 DOI: 10.4103/1947-2714.187141]

17 **Karthikeyan V**, Karpinski J, Nair RC, Knoll G. The burden of chronic kidney disease in renal transplant recipients. *Am J Transplant* 2004; **4**: 262-269 [PMID: 14974949]

18 **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]

19 **Fananapazir G**, Troppmann C, Corwin MT, Nikpour AM, Naderi S, Lamba R. Incidences of acute kidney injury, dialysis, and graft loss following intravenous administration of low-osmolality iodinated contrast in patients with kidney transplants. *Abdom Radiol* (NY) 2016; **41**: 2182-2186 [PMID: 27377897 DOI: 10.1007/s00261-016-0827-3]

20 **Morales JM**, Andres A, Rengel M, Rodicio JL. Influence of cyclosporin, tacrolimus and rapamycin on renal function and arterial hypertension after renal transplantation. *Nephrol Dial Transplant* 2001; **16** Suppl 1: 121-124 [PMID: 11369839]

21 Radiology ACo. Manual on Contrast Media. Version 10.1. 2015. Reston (VA): ACR Committee on Drugs and Contrast Media of the ACR Commission on Quality and Safety, 2015

22 **Elicker BM**, Cypel YS, Weinreb JC. IV contrast administration for CT: a survey of practices for the screening and prevention of contrast nephropathy. *AJR Am J Roentgenol* 2006; **186**: 1651-1658 [PMID: 16714655 DOI: 10.2214/ajr.05.0407]

23 **Moher D**, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 2009; **339**: b2535 [PMID: 19622551 DOI: 10.1136/bmj.b2535]

24 **Stroup DF**, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008-2012 [PMID: 10789670]

25 STROBE statement--checklist of items that should be included in reports of observational studies (STROBE initiative). *Int J Public Health* 2008; **53**: 3-4 [PMID: 18522360]

26 **Barendregt J**, Doi S. MetaXL User Guide: Version 1.0. Wilston, Australia: EpiGear International Pty Ltd, 2010

27 **DerSimonian R**, Kacker R. Random-effects model for meta-analysis of clinical trials: an update. *Contemp Clin Trials* 2007; **28**: 105-114 [PMID: 16807131]

28 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]

29 **Easterbrook PJ**, Berlin JA, Gopalan R, Matthews DR. Publication bias in clinical research. *Lancet* 1991; **337**: 867-872 [PMID: 1672966]

30 **Light JA**, Perloff LJ, Etheredge EE, Hill G, Spees EK. Adverse effects of meglumine diatrizoate on renal function in the early post-transplant period. *Transplantation* 1975; **20**: 404-409 [PMID: 1108314]

31 **Peters C**, Delmonico FL, Cosimi AB, Rubin RH, Tolkoff-Rubin N, Baker G, Russell PS. Risks versus benefits of contrast medium exposure in renal allograft recipients. *Surg Gynecol Obstet* 1983; **156**: 467-472 [PMID: 6340225]

32 **Ahuja TS**, Niaz N, Agraharkar M. Contrast-induced nephrotoxicity in renal allograft recipients. *Clin Nephrol* 2000; **54**: 11-14 [PMID: 10939751]

33 **Agrawal V**, Swami A, Kosuri R, Alsabbagh M, Agarwal M, Samarapungavan D, Rocher LL, McCullough PA. Contrast-induced acute kidney injury in renal transplant recipients after cardiac catheterization. *Clin Nephrol* 2009; **71**: 687-696 [PMID: 19473638]

34 **Haider M**, Yessayan L, Venkat KK, Goggins M, Patel A, Karthikeyan V. Incidence of contrast-induced nephropathy in kidney transplant recipients. *Transplant Proc* 2015; **47**: 379-383 [PMID: 25769577 DOI: 10.1016/j.transproceed.2015.01.008]

35 **Fananapazir G**, Troppmann C, Corwin MT, Bent CK, Vu CT, Lamba R. Incidence of Contrast-Induced Nephropathy After Renal Graft Catheter Arteriography Using Iodine-Based Contrast Medium. *AJR Am J Roentgenol* 2016; **206**: 783-786 [PMID: 26866337 DOI: 10.2214/ajr.15.15501]

36 **Abu Jawdeh B**, Sharma Y, Katipally S, Leonard A, Alloway R, Woodle E, Thakar C. Incidence and risk factors of contrast-induced nephropathy in renal allograft recipients. Proceedings of the American Journal of Transplantation Conference: 15th American Transplant Congress, ATC; 2015

37 **Lameire N**, Kellum JA. Contrast-induced acute kidney injury and renal support for acute kidney injury: a KDIGO summary (Part 2). *Crit Care* 2013; **17**: 205 [PMID: 23394215 DOI: 10.1186/cc11455]

38 **Moreau JF**, Kreis H, Barbanel Cl, Michel JR. [Effects of iodine contrast medias on the function of transplanted kidneys]. *Nouv Presse Med* 1975; **4**: 2643-2646 [PMID: 1105419]

39 **Katzberg RW**, Lamba R. Contrast-induced nephropathy after intravenous administration: fact or fiction? *Radiol Clin North Am* 2009; **47**: 789-800, v [PMID: 19744594 DOI: 10.1016/j.rcl.2009.06.002]

40 **Pirat B**, Müderrisoglu H, Korkmaz ME, Ozin B. Characteristics of coronary heart disease in renal transplant recipients. *Transplant Proc* 2004; **36**: 152-155 [PMID: 15013330 DOI: 10.1016/j.transproceed.2003.11.028]

41 **Ferguson ER**, Hudson SL, Diethelm AG, Pacifico AD, Dean LS, Holman WL. Outcome after myocardial revascularization and renal transplantation: a 25-year single-institution experience. *Ann Surg* 1999; **230**: 232-241 [PMID: 10450738]

42 **Cheungpasitporn W**, Thongprayoon C, Srivali N, O'Corragain OA, Edmonds PJ, Ungprasert P, Kittanamongkolchai W, Erickson SB. Preoperative renin-angiotensin system inhibitors use linked to reduced acute kidney injury: a systematic review and meta-analysis. *Nephrol Dial Transplant* 2015; **30**: 978-988 [PMID: 25800881 DOI: 10.1093/ndt/gfv023]

43 **McDonald RJ**, McDonald JS, Bida JP, Carter RE, Fleming CJ, Misra S, Williamson EE, Kallmes DF. Intravenous contrast material-induced nephropathy: causal or coincident phenomenon? *Radiology* 2013; **267**: 106-118 [PMID: 23360742 DOI: 10.1148/radiol.12121823]

44 **McDonald JS**, Katzberg RW, McDonald RJ, Williamson EE, Kallmes DF. Is the Presence of a Solitary Kidney an Independent Risk Factor for Acute Kidney Injury after Contrast-enhanced CT? *Radiology* 2016; **278**: 74-81 [PMID: 26523492 DOI: 10.1148/radiol.2015142676]

P- Reviewer: Hilmi I, Sureshkumar KK **S- Editor**: Kong JX
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Transplant 2017 February 24; 7(1): 88-93

DOI: 10.5500/wjt.v7.i1.88

ISSN 2220-3230 (online)

CASE REPORT

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

Kazuhiro Takahashi, Rohini Prashar, Krishna G Putchakayala, William J Kane, Jason E Denny, Dean Y Kim, Lauren E Malinzak

Kazuhiro Takahashi, Krishna G Putchakayala, William J Kane, Jason E Denny, Dean Y Kim, Lauren E Malinzak, Transplant and Hepatobiliary Surgery, Henry Ford Hospital, Detroit, MI 48202, United States

Rohini Prashar, Nephrology and Internal Medicine, Henry Ford Hospital, Detroit, MI 48202, United States

Author contributions: Takahashi K, Prashar R and Malinzak LE designed the report; Takahashi K, Putchakayala KG and Kane WJ collected the data; Takahashi K and Malinzak LE wrote the paper; Denny JE and Kim DY performed critical revisions of the paper.

Institutional review board statement: The case report was exempt from the Institutional Review Board standards at Henry Ford Hospital in Detroit.

Informed consent statement: The patient involved in this study gave written consent, authorizing use and disclosure of his protected health information.

Conflict-of-interest statement: None of the authors has conflicts of interests to declare.

Manuscript source: Unsolicited manuscript

Correspondence to: Lauren E Malinzak, MD, Transplant and Hepatobiliary Surgery, Henry Ford Hospital, 2790 West Grand Boulevard, Detroit, MI 48202, United States. lmalinz1@hfhs.org
Telephone: +1-313-9162941
Fax: +1-313-9164353

Received: October 30, 2016
Peer-review started: November 3, 2016
First decision: December 1, 2016
Revised: December 19, 2016
Accepted: January 11, 2017
Article in press: January 12, 2017
Published online: February 24, 2017

Abstract

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

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

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

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

INTRODUCTION

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

CASE REPORT

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

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

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

DISCUSSION

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

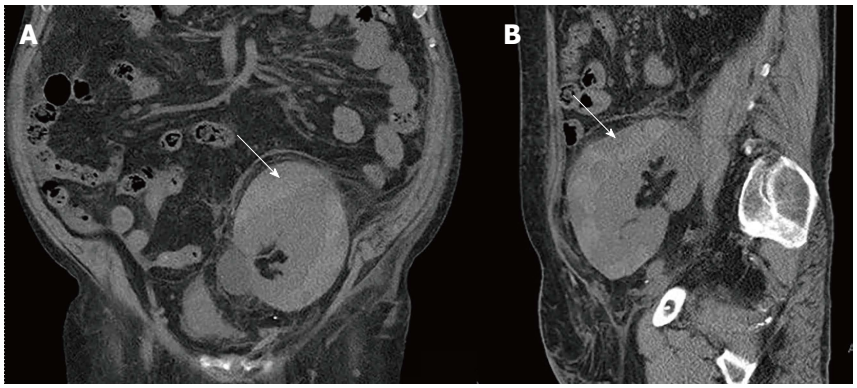


Figure 1 Computed tomography without intravenous contrast of the transplanted kidney. A: Coronal view; B: Sagittal view. A subscapular hematoma 12 cm × 2.5 cm in size was compressing the transplanted kidney (arrows).

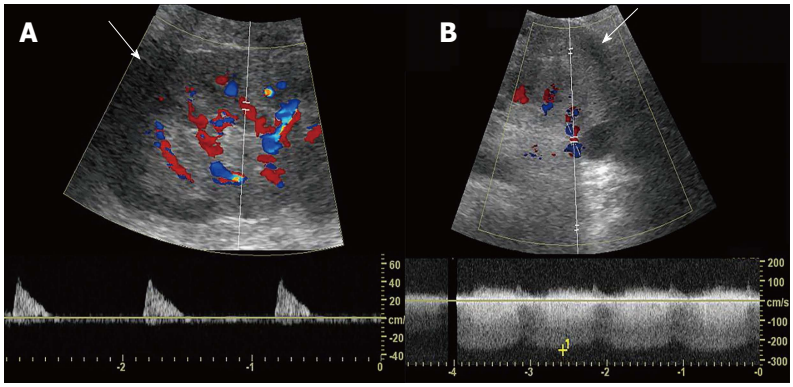


Figure 2 Presence of peri-allograft hematoma and Doppler ultrasound findings. A: Transplant arterial flow. Peri-allograft hypoechoic area (arrows) with absent diastolic flow in the arcuate arteries; B: Transplant venous flow. The transplant renal vein was patent.

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

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

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

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

Table 1 Acute Page kidney after kidney transplantation

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

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

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

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

COMMENTS

Case characteristics

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

Clinical diagnosis

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

Differential diagnosis

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

Laboratory diagnosis

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

Imaging

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

Pathological diagnosis

The transplant kidney biopsy showed no evidence of rejection.

Treatment

Emergent laparotomy for decompression of the hematoma.

Related reports

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

Term explanation

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

Experiences and lessons

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

Peer-review

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

REFERENCES

1 **Haydar A**, Bakri RS, Prime M, Goldsmith DJ. Page kidney--a review of the literature. *J Nephrol* 2003; **16**: 329-333 [PMID: 12832730]

2 **Aragona F**, Artibani W, Calabrò A, Villi G, Cisternino A, Ostardo E. Page kidney: a curable form of arterial hypertension. Case report and review of the literature. *Urol Int* 1991; **46**: 203-207 [PMID: 2053233 DOI: 10.1159/000282134]

3 **Dopson SJ**, Jayakumar S, Velez JC. Page kidney as a rare cause of hypertension: case report and review of the literature. *Am J Kidney Dis* 2009; **54**: 334-339 [PMID: 19167799 DOI: 10.1053/j.ajkd.2008.11.014]

4 **Butt FK**, Seawright AH, Kokko KE, Hawxby AM. An unusual presentation of a Page kidney 24 days after transplantation: case report. *Transplant Proc* 2010; **42**: 4291-4294 [PMID: 21168685 DOI: 10.1016/j.transproceed.2010.09.042]

5 **Goyal M**, Zukerberg B, Ozgen P, Graves M, Scheff A. Large subcapsular hematoma in transplant kidney seen on renal scan. *Clin Nucl Med* 1996; **21**: 345-346 [PMID: 8925634]

6 **Sedigh O**, Lasaponara F, Dalmasso E, Gai M, Hayashi Y, Bosio A, Pasquale G, Lillaz B, Biancone L, Frea B. Subcapsular Hematoma Causing Anuria After Renal Graft Trauma. *Exp Clin Transplant* 2015; Epub ahead of print [PMID: 26496471 DOI: 10.6002/ect.2015.0073]

7 **Salgado OJ**, Vidal AM, Semprun P, Garcia R. Conservative management of an extensive renal graft subcapsular hematoma arising during living donor nephrectomy. Role of Doppler sonographic posttransplant follow-up. *J Clin Ultrasound* 2010; **38**: 164-167 [PMID: 19856428 DOI: 10.1002/jcu.20644]

8 **Çiftçi S**, Stuart Wolf J. Laparoscopic treatment of Page kidney: a report of two cases and review of the literature. *Turk J Urol* 2013; **39**: 126-130 [PMID: 26328095 DOI: 10.5152/tud.2013.024]

9 **Sterns RH**, Rabinowitz R, Segal AJ, Spitzer RM. 'Page kidney'. Hypertension caused by chronic subcapsular hematoma. *Arch Intern Med* 1985; **145**: 169-171 [PMID: 3970635 DOI: 10.1001/archinte.1985.00360010215042]

10 **Kliewer MA**, Carroll BA. Ultrasound case of the day. Page kidney phenomenon in a transplanted kidney after biopsy. *Radiographics* 1991; **11**: 336-337 [PMID: 2028069 DOI: 10.1148/radiographics.11.2.2028069]

11 **Cromie WJ**, Jordan MH, Leapman SB. Pseudorejection: the Page kidney phenomenon in renal allografts. *J Urol* 1976; **116**: 658-659 [PMID: 789921]

12 **Ay N**, Beyazıt Ü, Alp V, Duymus R, Sevük U, Anıl M, Danış R. Rupture of a Subcapsular Hematoma After Kidney Transplant: Case Report. *Exp Clin Transplant* 2015; Epub ahead of print [PMID: 26496378 DOI: 10.6002/ect.2014.0270]

13 **Adjei-Gyamfi Y**, Koffman G, Amies T, Easty M, Marks SD, McHugh K. Reversible acute anuric kidney injury after surgical evacuation of perinephric hematomas as a complication of renal transplant biopsies. *Pediatr Transplant* 2014; **18**: E262-E265 [PMID: 25316156 DOI: 10.1111/petr.12375]

14 **Hamidian Jahromi A**, Fronek J, Kessaris N, Bydawell G, Patel U, MacPhee IA. Acute page kidney complicating kidney transplant artery stenting: presentation of a case and novel management. *Iran J Kidney Dis* 2013; **7**: 352-355 [PMID: 24072145]

15 **Gandhi V**, Khosravi M, Burns A. Page kidney in a 17-year-old renal allograft. *BMJ Case Rep* 2012; **2012**: [PMID: 23220837 DOI: 10.1136/bcr-2012-007653]

16 **Maurya KK**, Bhat HS, Mathew G, Kumar G. Page kidney following renal allograft biopsy - early recognition and treatment. *Saudi J Kidney Dis Transpl* 2011; **22**: 1012-1013 [PMID: 21912035]

17 **Okechukwu O**, Reddy S, Guleria S. A page in transplantation. *Saudi J Kidney Dis Transpl* 2011; **22**: 796-798 [PMID: 21743233]

18 **Posadas MA**, Yang V, Ho B, Omer M, Batlle D. Acute renal failure and severe hypertension from a page kidney post-transplant biopsy. *ScientificWorldJournal* 2010; **10**: 1539-1542 [PMID: 20694451 DOI: 10.1100/tsw.2010.150]

19 **Kamar N**, Sallusto F, Rostaing L. Acute Page kidney after a kidney allograft biopsy: successful outcome from observation and medical

treatment. *Transplantation* 2009; **87**: 453-454 [PMID: 19202455 DOI: 10.1097/TP.0b013e31819576fb]

20 **Caldés S**, Fernández A, Rivera M, Merino JL, González R, Amezquita Y, Marcén R, Burgos FJ, Ortuño J. A Page kidney case report with diastolic flow reversion in Doppler ultrasonography. *Transplantation* 2009; **87**: 303-304 [PMID: 19155989 DOI: 10.1097/TP.0b013e3181938a8f]

21 **Chung J**, Caumartin Y, Warren J, Luke PP. Acute Page kidney following renal allograft biopsy: a complication requiring early recognition and treatment. *Am J Transplant* 2008; **8**: 1323-1328 [PMID: 18444936 DOI: 10.1111/j.1600-6143.2008.02215.x]

22 **Heffernan E**, Zwirewich C, Harris A, Nguan C. Page kidney after renal allograft biopsy: sonographic findings. *J Clin Ultrasound* 2009; **37**: 226-229 [PMID: 18386812 DOI: 10.1002/jcu.20465]

23 **Patel TV**, Goes N. Page kidney. *Kidney Int* 2007; **72**: 1562 [PMID: 18046425 DOI: 10.1038/sj.ki.5002580]

24 **Gibney EM**, Edelstein CL, Wiseman AC, Bak T. Page kidney causing reversible acute renal failure: an unusual complication of transplant biopsy. *Transplantation* 2005; **80**: 285-286 [PMID: 16041280 DOI: 10.1097/01.TP.0000165097.42243.3F]

25 **Rea R**, Anderson K, Mitchell D, Harper S, Williams T. Subcapsular haematoma: a cause of post biopsy oliguria in renal allografts. *Nephrol Dial Transplant* 2000; **15**: 1104-1105 [PMID: 10862671 DOI: 10.1093/ndt/15.7.1104]

26 **Machida J**, Kitani K, Inadome A, Wada Y, Kawabata K, Yoshida M, Ueda S. Subcapsular hematoma and hypertension following percutaneous needle biopsy of a transplanted kidney. *Int J Urol* 1996; **3**: 228-230 [PMID: 8776622 DOI: 10.1111/j.1442-2042.1996.tb00521.x]

27 **Nguyen BD**, Nghiem DD, Adatepe MH. Page kidney phenomenon in allograft transplant. *Clin Nucl Med* 1994; **19**: 361-363 [PMID: 8004877 DOI: 10.1097/00003072-199404000-00022]

28 **Dempsey J**, Gavant ML, Cowles SJ, Gaber AO. Acute Page kidney phenomenon: a cause of reversible renal allograft failure. *South Med J* 1993; **86**: 574-577 [PMID: 8488410 DOI: 10.1097/00007611-199305000-00019]

29 **Ben Hamida F**, Westeel PF, Achard JM, Filloux V, Tribout B, Bouzernidj M, Petit J, Fournier A. Favorable outcome under simple heparin therapy of recurrent anuria due to graft renal vein thrombosis and subcapsular hematoma. *Transplant Proc* 1993; **25**: 2341-2342 [PMID: 8516921]

30 **Figueroa TE**, Frentz GD. Anuria secondary to percutaneous needle biopsy of a transplant kidney: a case report. *J Urol* 1988; **140**: 355-356 [PMID: 3294445]

31 **Yussim A**, Shmuely D, Levy J, Servadio C, Shapira Z. Page kidney phenomenon in kidney allograft following peritransplant lymphocele. *Urology* 1988; **31**: 512-514 [PMID: 3287745 DOI: 10.1016/0090-4295(88)90219-1]

P- Reviewer: Markic D, Niu CY, Scharman EJ **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Transplant 2017 February 24; 7(1): 94-97

DOI: 10.5500/wjt.v7.i1.94

ISSN 2220-3230 (online)

CASE REPORT

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

Fatih Ozdemir, Koray Kutluturk, Bora Barut, Perviz Abbasov, Ramazan Kutlu, Cuneyt Kayaalp, Sezai Yılmaz

Fatih Ozdemir, Koray Kutluturk, Bora Barut, Cuneyt Kayaalp, Sezai Yılmaz, Department of Surgery, Liver Transplantation Institute of Inonu University, 44280 Malatya, Turkey

Perviz Abbasov, Department of Surgery, Azerbaijan Medical School, Baku, AZ 1000, Azerbaijan

Ramazan Kutlu, Department of Radiology, Liver Transplantation Institute of Inonu University, 44280 Malatya, Turkey

Author contributions: Ozdemir F designed and wrote the paper; Kutluturk K, Barut B and Abbasov P collected the patient's data; Kutlu R interpreted the radiological imagination; Kayaalp C and Yılmaz S reviewed the paper.

Institutional review board statement: This case report was exempt from the Institutional Review Board standards at Inonu University, Malatya, Turkey.

Informed consent statement: The patient involved in this study gave his written informed consent authorizing use and disclosure of his protected health information.

Conflict-of-interest statement: All the authors have no conflicts of interests to declare.

Manuscript source: Unsolicited manuscript

Correspondence to: Dr. Fatih Ozdemir, Department of Surgery, Liver Transplantation Institute of Inonu University, Elazig Road 15th km., 44280 Malatya, Turkey. fatihup@hotmail.com
Telephone: +90-533-5475078

Received: October 1, 2016
Peer-review started: October 11, 2016
First decision: November 10, 2016
Revised: December 30, 2016
Accepted: January 16, 2017
Article in press: January 18, 2017
Published online: February 24, 2017

Abstract

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

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

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

managed our patient.

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

INTRODUCTION

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

CASE REPORT

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

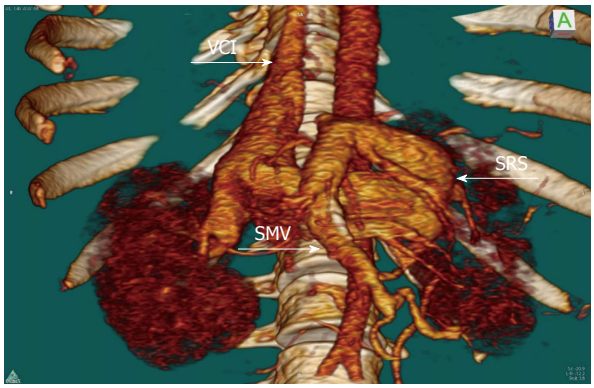


Figure 1 Active splenorenal shunt draining from the splenic vein into the left renal vein. VCI: Vena cava inferior; SRS: Splenorenal shunt; SMV: Superior mesenteric vein.

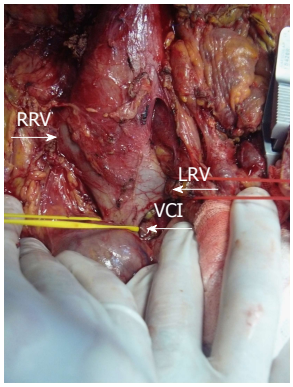


Figure 2 Anterior part of the infrahepatic vena cava was explored and dissected down to expose the bifurcation of the left renal vein. LRV: Left renal vein; VCI: Vena cava inferior; RRV: Right renal vein.

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

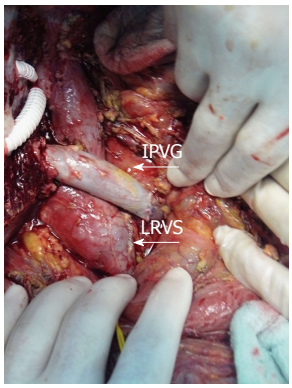


Figure 3 Right renal vein between left renal vein and graft portal vein with interposition vein graft. IPVG: Interposition vein graft; LRVS: Left renal vein stump.

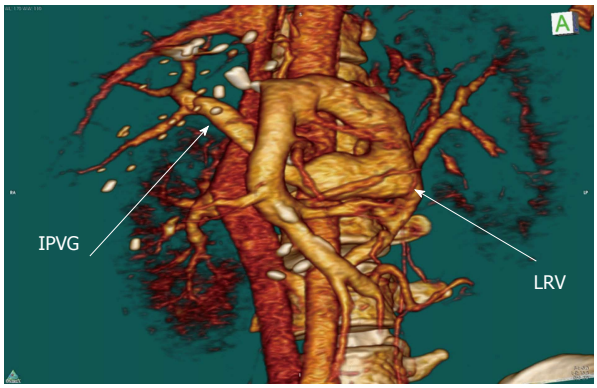


Figure 4 Computerized tomography scans visualize the patency of the right renal vein. IPVG: Interposition vein graft; LRV: Left renal vein.

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

DISCUSSION

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

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

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

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

COMMENTS

Case characteristics

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

stage liver disease.

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

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

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

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

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

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

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

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

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

REFERENCES

1 **Hirashita T**, Ohta M, Kai S, Masuda T, Eguchi H, Iwashita Y, Ogawa T, Kitano S. Implications of portal vein thrombosis after splenectomy for patients with idiopathic portal hypertension. *Surg Today* 2011; **41**: 1475-1480 [PMID: 21969148 DOI: 10.1007/s00595-010-4523-6]

2 **Tsochatzis EA**, Senzolo M, Germani G, Gatt A, Burroughs AK. Systematic review: portal vein thrombosis in cirrhosis. *Aliment Pharmacol Ther* 2010; **31**: 366-374 [PMID: 19863496 DOI: 10.1111/j.1365-2036.2009.04182.x]

3 **Gayowski TJ**, Marino IR, Doyle HR, Echeverri L, Mieles L, Todo S, Wagener M, Singh N, Yu VL, Fung JJ, Starzl TE. A high incidence of native portal vein thrombosis in veterans undergoing liver transplantation. *J Surg Res* 1996; **60**: 333-338 [PMID: 8598664]

4 **Egawa H**, Tanaka K, Kasahara M, Takada Y, Oike F, Ogawa K, Sakamoto S, Kozaki K, Taira K, Ito T. Single center experience of 39 patients with preoperative portal vein thrombosis among 404 adult living donor liver transplantations. *Liver Transpl* 2006; **12**: 1512-1518 [PMID: 17004256]

5 **Kato T**, Levi DM, DeFaria W, Nishida S, Tzakis AG. Liver transplantation with renoportal anastomosis after distal splenorenal shunt. *Arch Surg* 2000; **135**: 1401-1404 [PMID: 11115340]

6 **Sheil AG**, Stephen MS, Chui AK, Ling J, Bookallil MJ. A liver transplantation technique in a patient with a thrombosed portal vein and a functioning renal-lieno shunt. *Clin Transplant* 1997; **11**: 71-73 [PMID: 9067699]

7 **Yerdel MA**, Gunson B, Mirza D, Karayalçin K, Olliff S, Buckels J, Mayer D, McMaster P, Pirenne J. Portal vein thrombosis in adults undergoing liver transplantation: risk factors, screening, management, and outcome. *Transplantation* 2000; **69**: 1873-1881 [PMID: 10830225]

8 **Molmenti EP**, Roodhouse TW, Molmenti H, Jaiswal K, Jung G, Marubashi S, Sanchez EQ, Gogel B, Levy MF, Goldstein RM, Fasola CG, Elliott EE, Bursac N, Mulligan D, Gonwa TA, Klintmalm GB. Thrombendvenectomy for organized portal vein thrombosis at the time of liver transplantation. *Ann Surg* 2002; **235**: 292-296 [PMID: 11807371]

9 **Dumortier J**, Czyglik O, Poncet G, Blanchet MC, Boucaud C, Henry L, Boillot O. Eversion thrombectomy for portal vein thrombosis during liver transplantation. *Am J Transplant* 2002; **2**: 934-938 [PMID: 12482145]

10 **Bertelli R**, Nardo B, Montalti R, Beltempo P, Puviani L, Cavallari A. Liver transplantation in recipients with portal vein thrombosis: experience of a single transplant center. *Transplant Proc* 2005; **37**: 1119-1121 [PMID: 15848641]

11 **Figueras J**, Torras J, Rafecas A, Fabregat J, Ramos E, Moreno G, Lama C, Parés D, Jaurrieta E. Extra-anatomic venous graft for portal vein thrombosis in liver transplantation. *Transpl Int* 1997; **10**: 407-408 [PMID: 9287411]

12 **Bonnet S**, Sauvanet A, Bruno O, Sommacale D, Francoz C, Dondero F, Durand F, Belghiti J. Long-term survival after portal vein arterialization for portal vein thrombosis in orthotopic liver transplantation. *Gastroenterol Clin Biol* 2010; **34**: 23-28 [PMID: 19643558 DOI: 10.1016/j.gcb.2009.05.013]

13 **Francoz C**, Valla D, Durand F. Portal vein thrombosis, cirrhosis, and liver transplantation. *J Hepatol* 2012; **57**: 203-212 [PMID: 22446690 DOI: 10.1016/j.jhep.2011.12.034]

14 **Paskonis M**, Jurgaitis J, Mehrabi A, Kashfi A, Fonouni H, Strupas K, Büchler MW, Kraus TW. Surgical strategies for liver transplantation in the case of portal vein thrombosis--current role of cavoportal hemitransposition and renoportal anastomosis. *Clin Transplant* 2006; **20**: 551-562 [PMID: 16968480]

15 **Marubashi S**, Dono K, Nagano H, Gotoh K, Takahashi H, Hashimoto K, Miyamoto A, Takeda Y, Umeshita K, Kato T, Monden M. Living-donor liver transplantation with renoportal anastomosis for patients with large spontaneous splenorenal shunts. *Transplantation* 2005; **80**: 1671-1675 [PMID: 16378059]

16 **Moon DB**, Lee SG, Ahn CS, Ha TY, Park GC, Yu YD. Side-to-end renoportal anastomosis using an externally stented polytetrafluoroethylene vascular graft for a patient with a phlebosclerotic portal vein and a large spontaneous splenorenal shunt. *J Am Coll Surg* 2011; **212**: e7-e11 [PMID: 21356484 DOI: 10.1016/j.jamcollsurg.2010.12.013]

17 **Ikegami T**, Shimada M, Imura S, Arakawa Y, Nii A, Morine Y, Kanemura H. Current concept of small-for-size grafts in living donor liver transplantation. *Surg Today* 2008; **38**: 971-982 [PMID: 18958553 DOI: 10.1007/s00595-008-3771-1]

18 **Lee SG**, Moon DB, Ahn CS, Kim KH, Hwang S, Park KM, Ha TY, Ko GY, Sung KB, Song GW, Jung DH, Moon KM, Kim BS, Cho YP. Ligation of left renal vein for large spontaneous splenorenal shunt to prevent portal flow steal in adult living donor liver transplantation. *Transpl Int* 2007; **20**: 45-50 [PMID: 17181652]

P- Reviewer: Abdelaziz O, Fulop T, Qin JM **S- Editor**: Song XX
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Transplant 2017 February 24; 7(1): 98-102

DOI: 10.5500/wjt.v7.i1.98

ISSN 2220-3230 (online)

CASE REPORT

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

Ross Apostolov, Khashayar Asadi, Julie Lokan, Ning Kam, Adam Testro

Ross Apostolov, Khashayar Asadi, Julie Lokan, Ning Kam, Adam Testro, Australian Intestinal Transplant Service, Austin Health, Heidelberg, VIC 3084, Australia

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

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

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

Conflict-of-interest statement: All authors have no conflicts of interest to declare.

Manuscript source: Invited manuscript

Correspondence to: Adam Testro, MBBS, FRACP, PhD, Head of Intestinal Rehabilitation and Transplantation, Australian Intestinal Transplant Service, Austin Health, 145 Studley Road, Heidelberg, VIC 3084, Australia. adam.testro@austin.org.au
Telephone: +61-3-94965353
Fax: +61-3-94963487

Received: November 26, 2016
Peer-review started: November 29, 2016
First decision: December 15, 2016
Revised: December 21, 2016
Accepted: January 11, 2017
Article in press: January 13, 2017
Published online: February 24, 2017

Abstract

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

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

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

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

INTRODUCTION

Mycophenolate mofetil (MMF) acts by inhibiting inosine-5′-monophosphate dehydrogenase, leading to decreased purine synthesis in T and B lymphocytes. This inhibits lymphocyte proliferation and as a result suppresses cell mediated immunity and antibody formation, which are important factors in acute graft rejection[1].

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

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

CASE REPORT

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

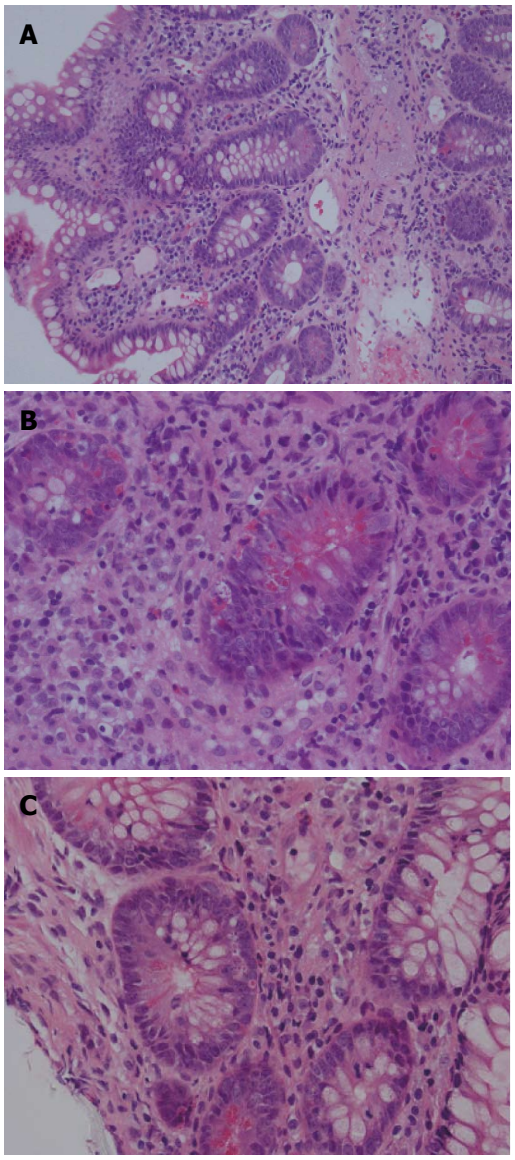


Figure 1 Small bowel allograft biopsy - day 13. A: Increased lamina propria inflammatory infiltrate, including activated cells, regenerative basophilia of crypt epithelium and increased epithelial apoptosis; B: High power view increased crypt apoptosis and rejection type inflammatory infiltrate within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

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

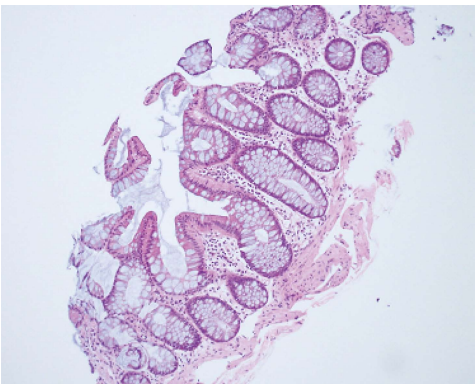


Figure 2 Native colonic biopsy - day 13. Unremarkable mucosa with preserved surface and crypt architecture with no significant inflammation and no crypt apoptosis.

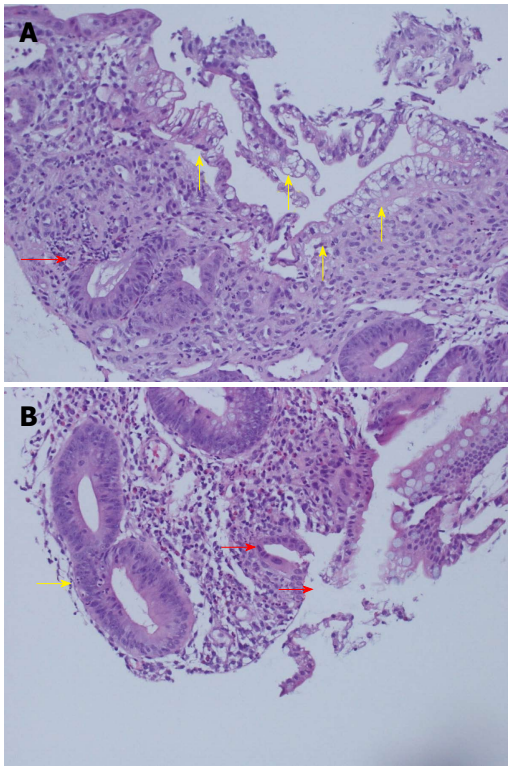


Figure 3 Small bowel allograft biopsy - day 23. A: Mucosal erosion with marked surface enterocyte degeneration and cytoplasmic vacuolation, sloughing (yellow arrows), inflamed granulation-like tissue within the lamina propria, prominent crypt injury (red arrow) and focal drop out; B: Cryptitis with increased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

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

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

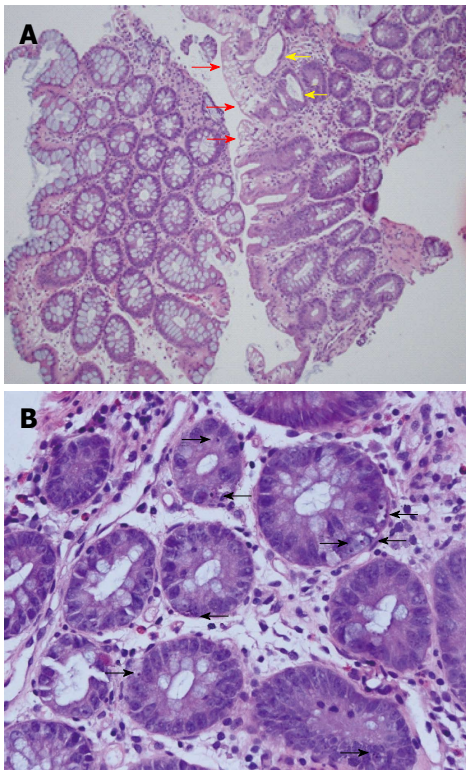


Figure 4 Native colonic biopsy - day 23. A: Striking focal surface epithelial vacuolation/degeneration (red arrows), associated with crypt epithelial injury, crypt withering and goblet cell reduction (yellow arrows); B: High power view - basal crypts with mucin reduction, increased basophilia and several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

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

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

Further endoscopy and biopsy 4 d later (post-operative

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

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

DISCUSSION

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

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

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

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

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

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

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

COMMENTS

Case characteristics

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

Clinical diagnosis

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

Differential diagnosis

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

Imaging diagnosis

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

Pathological diagnosis

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

Treatment

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

Related reports

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

Term explanation

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

Experiences and lessons

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

Peer-review

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

REFERENCES

1 **Allison AC**, Eugui EM. Mechanisms of action of mycophenolate mofetil in preventing acute and chronic allograft rejection. *Transplantation* 2005; **80**: S181-S190 [PMID: 16251851 DOI: 10.1097/01.tp.0000186390.10150.66]

2 **Sudan D**. The current state of intestine transplantation: indications, techniques, outcomes and challenges. *Am J Transplant* 2014; **14**: 1976-1984 [PMID: 25307033 DOI: 10.1111/ajt.12812]

3 **Abu-Elmagd KM**, Costa G, Bond GJ, Soltys K, Sindhi R, Wu T, Koritsky DA, Schuster B, Martin L, Cruz RJ, Murase N, Zeevi A, Irish W, Ayyash MO, Matarese L, Humar A, Mazariegos G. Five hundred intestinal and multivisceral transplantations at a single center: major advances with new challenges. *Ann Surg* 2009; **250**: 567-581 [PMID: 19730240 DOI: 10.1097/SLA.0b013e3181b67725]

4 **Vianna RM**, Mangus RS, Tector AJ. Current status of small bowel and multivisceral transplantation. *Adv Surg* 2008; **42**: 129-150 [PMID: 18953814 DOI: 10.1016/j.yasu.2008.03.008]

5 **Tzakis AG**, Weppler D, Khan MF, Koutouby R, Romero R, Viciana AL, Raskin J, Nery JR, Thompson J. Mycophenolate mofetil as primary and rescue therapy in intestinal transplantation. *Transplant Proc* 1998; **30**: 2677-2679 [PMID: 9745545 DOI: 10.1016/s0041-1345(98)00786-6]

6 **Nguyen T**, Park JY, Scudiere JR, Montgomery E. Mycophenolic acid (cellcept and myofortic) induced injury of the upper GI tract. *Am J Surg Pathol* 2009; **33**: 1355-1363 [PMID: 19542873 DOI: 10.1097/PAS.0b013e3181a755bd]

7 **Parfitt JR**, Jayakumar S, Driman DK. Mycophenolate mofetil-related gastrointestinal mucosal injury: variable injury patterns, including graft-versus-host disease-like changes. *Am J Surg Pathol* 2008; **32**: 1367-1372 [PMID: 18763324 DOI: 10.1097/pas.0b013e31816bf3fe]

8 **Al-Absi AI**, Cooke CR, Wall BM, Sylvestre P, Ismail MK, Mya M. Patterns of injury in mycophenolate mofetil-related colitis. *Transplant Proc* 2010; **42**: 3591-3593 [PMID: 21094821 DOI: 10.1016/j.transproceed.2010.08.066]

9 **Calmet FH**, Yarur AJ, Pukazhendhi G, Ahmad J, Bhamidimarri KR. Endoscopic and histological features of mycophenolate mofetil colitis in patients after solid organ transplantation. *Ann Gastroenterol* 2015; **28**: 366-373 [PMID: 26126799 DOI: 10.1097/00007890-199904150-01021]

10 **Behling KC**, Foster DM, Edmonston TB, Witkiewicz AK. Graft-versus-Host Disease-Like Pattern in Mycophenolate Mofetil Related Colon Mucosal Injury: Role of FISH in Establishing the Diagnosis. *Case Rep Gastroenterol* 2009; **3**: 418-423 [PMID: 21103265 DOI: 10.1159/000260903]

11 **Papadimitriou JC**, Cangro CB, Lustberg A, Khaled A, Nogueira J, Wiland A, Ramos E, Klassen DK, Drachenberg CB. Histologic features of mycophenolate mofetil-related colitis: a graft-versus-host disease-like pattern. *Int J Surg Pathol* 2003; **11**: 295-302 [PMID: 14615824 DOI: 10.1177/106689690301100406]

12 **Liapis G**, Boletis J, Skalioti C, Bamias G, Tsimaratou K, Patsouris E, Delladetsima I. Histological spectrum of mycophenolate mofetil-related colitis: association with apoptosis. *Histopathology* 2013; **63**: 649-658 [PMID: 24025088 DOI: 10.1111/his.12222]

13 **Selbst MK**, Ahrens WA, Robert ME, Friedman A, Proctor DD, Jain D. Spectrum of histologic changes in colonic biopsies in patients treated with mycophenolate mofetil. *Mod Pathol* 2009; **22**: 737-743 [PMID: 19329937]

14 **O'Keefe SJ**, El Hajj II, Wu T, Martin D, Mohammed K, Abu-Elmagd K. Endoscopic evaluation of small intestine transplant grafts. *Transplantation* 2012; **94**: 757-762 [PMID: 22955230 DOI: 10.1097/TP.0b013e31825f4410]

15 **Lauro A**, Bagni A, Zanfi C, Pellegrini S, Dazzi A, Del Gaudio M, Ravaioli M, Di Simone M, Ramacciato G, Pironi L, Pinna AD. Mortality after steroid-resistant acute cellular rejection and chronic rejection episodes in adult intestinal transplants: report from a single center in induction/preconditioning era. *Transplant Proc* 2013; **45**: 2032-2033 [PMID: 23769102 DOI: 10.1016/j.transproceed.2012.09.124]

16 **Delacruz V**, Weppler D, Island E, Gonzalez M, Tryphonopoulos P, Moon J, Smith L, Tzakis A, Ruiz P. Mycophenolate mofetil-related gastrointestinal mucosal injury in multivisceral transplantation. *Transplant Proc* 2010; **42**: 82-84 [PMID: 20172286 DOI: 10.1016/j.transproceed.2009.12.027]

P- Reviewer: Akamatsu N, Kayaalp C, Ramos E **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

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

ISSN 2220-3230 (online)

World Journal of *Transplantation*

World J Transplant 2017 April 24; 7(2): 103-160

Published by Baishideng Publishing Group Inc

WJT World Journal of Transplantation

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

Editorial Board

2016-2019

The *World Journal of Transplantation* Editorial Board consists of 361 members, representing a team of worldwide experts in transplantation. They are from 43 countries, including Argentina (1), Australia (7), Austria (3), Belgium (4), Brazil (7), Bulgaria (1), Canada (13), China (32), Cuba (1), Czech Republic (1), Denmark (1), Finland (1), France (4), Georgia (1), Germany (14), Greece (5), Hungary (2), India (7), Iran (7), Israel (4), Italy (33), Japan (18), Jordan (1), Macedonia (1), Mexico (2), Morocco (1), Netherlands (4), Nigeria (1), Norway (1), Pakistan (1), Poland (2), Qatar (1), Saudi Arabia (3), Singapore (1), South Korea (16), Spain (9), Sweden (1), Switzerland (3), Thailand (2), Tunisia (1), Turkey (6), United Kingdom (17), and United States (120).

WJT World Journal of Transplantation

Contents

Bimonthly Volume 7 Number 2 April 24, 2017

Contents

World Journal of Transplantation
Volume 7 Number 2 April 24, 2017

ABOUT COVER

Editorial Board Member of *World Journal of Transplantation*, Frieder Keller, MD, Doctor, Nephrology Division, Medical Department Innere 1, University Hospital, D-89070 Ulm, Germany

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, PubMed Central.

FLYLEAF

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Huan-Liang Wu*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Fang-Fang Ji*
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL
World Journal of Transplantation

ISSN
ISSN 2220-3230 (online)

LAUNCH DATE
December 24, 2011

FREQUENCY
Bimonthly

EDITOR-IN-CHIEF
Maurizio Salvadori, MD, Professor, Renal Unit, Careggi University Hospital, Florence 50139, Italy

EDITORIAL BOARD MEMBERS
All editorial board members resources online at http://www.wjgnet.com/2220-3230/editorialboard.htm

EDITORIAL OFFICE
Xiu-Xia Song, Director

World Journal of Transplantation
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
April 24, 2017

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 April 24; 7(2): 103-116

DOI: 10.5500/wjt.v7.i2.103

ISSN 2220-3230 (online)

REVIEW

Role of gastroesophageal reflux disease in lung transplantation

Kelly E Hathorn, Walter W Chan, Wai-Kit Lo

Kelly E Hathorn, Division of Internal Medicine, Brigham and Women's Hospital, Boston, MA 02115, United States

Walter W Chan, Wai-Kit Lo, Division of Gastroenterology, Hepatology and Endoscopy, Brigham and Women's Hospital, Boston, MA 02115, United States

Walter W Chan, Wai-Kit Lo, Harvard Medical School, Boston, MA 02115, United States

Author contributions: All authors contributed to this manuscript.

Conflict-of-interest statement: The authors do not report any conflict of interests and have no financial disclosures relevant to the subjects of the manuscript.

Manuscript source: Invited manuscript

Correspondence to: Walter W Chan, MD, MPH, Division of Gastroenterology, Hepatology and Endoscopy, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, United States. wwchan@partners.org
Telephone: +1-617-7326389
Fax: +1-617-5250338

Received: August 25, 2016
Peer-review started: August 26, 2016
First decision: October 20, 2016
Revised: January 15, 2017
Accepted: February 8, 2017
Article in press: February 13, 2017
Published online: April 24, 2017

Abstract

Lung transplantation is one of the highest risk solid organ transplant modalities. Recent studies have demonstrated a relationship between gastroesophageal reflux disease (GERD) and lung transplant outcomes, including acute and chronic rejection. The aim of this review is to discuss the pathophysiology, evaluation, and management of GERD in lung transplantation, as informed by the most recent publications in the field. The pathophysiology of reflux-induced lung injury includes the effects of aspiration and local immunomodulation in the development of pulmonary decline and histologic rejection, as reflective of allograft injury. Modalities of reflux and esophageal assessment, including ambulatory pH testing, impedance, and esophageal manometry, are discussed, as well as timing of these evaluations relative to transplantation. Finally, antireflux treatments are reviewed, including medical acid suppression and surgical fundoplication, as well as the safety, efficacy, and timing of such treatments relative to transplantation. Our review of the data supports an association between GERD and allograft injury, encouraging a strategy of early diagnosis and aggressive reflux management in lung transplant recipients to improve transplant outcomes. Further studies are needed to explore additional objective measures of reflux and aspiration, better compare medical and surgical antireflux treatment options, extend follow-up times to capture longer-term clinical outcomes, and investigate newer interventions including minimally invasive surgery and advanced endoscopic techniques.

Key words: Lung transplant; Reflux; Aspiration; Rejection; Bronchiolitis obliterans syndrome; Fundoplication

Core tip: Gastroesophageal reflux disease (GERD) has been associated with increased morbidity in lung transplant patients through a proposed pathway of reflux, aspiration, immunomodulation, and allograft injury, culminating in functional decline and rejection. This paper reviews the mechanisms of GERD-induced

injury, describes outcome measures important in post-transplant assessment, and discusses the timing and modalities of diagnostic evaluation and management, including medical and surgical antireflux treatment, in optimizing post-transplant outcomes. A greater awareness of the harmful effects of GERD in the lung transplant population is important in the early diagnosis and management of such patients to minimize allograft injury and improve outcomes.

Hathorn KE, Chan WW, Lo WK. Role of gastroesophageal reflux disease in lung transplantation. *World J Transplant* 2017; 7(2): 103-116 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i2/103.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i2.103

INTRODUCTION

Lung transplantation has proven to be an effective therapeutic option for the treatment of different end-stage pulmonary disorders, improving the quality of life and extending survival[1] for the recipients. Since the first human lung transplant in 1963[2], we have seen improvements in surgical technique, lung preservation, immunosuppression, and the treatment of ischemic reperfusion injury and infection. However, it remains one of the highest risk solid-organ transplant modalities, with 5-year survival rates of 53%[3], compared to 75% for heart transplantation[4], and 71% for liver transplantation[5].

Over time, transplanted lungs may become susceptible to injury manifesting as acute or chronic rejection, diagnosed clinically and histologically using established guidelines of the International Society of Heart and Lung Transplantation (ISHLT)[6]. Acute rejection is an early manifestation of allograft injury occurring usually within the first year after transplantation, impacting up to 55% of patients[7,8], and includes acute cellular rejection (grade A rejection), and lymphocytic bronchiolitis (grade B rejection). Both are independently associated with later development of chronic rejection[7-9].

Chronic rejection traditionally encompassed the spectrum of bronchiolitis obliterans (BO) and bronchiolitis obliterans syndrome (BOS). Bronchiolitis obliterans is a type of progressive airway obstruction occurring as a result of macrophage and myofibroblast infiltration, which induces fibrous obliteration and scar formation[10-12]. The diagnosis is made histologically, requiring surgical biopsy which can be invasive, and may present additional challenges given the patchy involvement of disease[10,13]. Therefore, the clinical correlate of BOS is often applied. BOS was originally defined as a persistent drop in forced expiratory volume in 1 s (FEV1) by 20% in the absence of other identifiable causes[14]. However, given the significance of BOS in predicting poor long-term outcomes, the criteria were adjusted to include an early BOS stage (BOS 0-p) in which an FEV1 of 81%-90% and/or a drop in mid-expiratory flow rate (FEF 25-75) may alert physicians to a need for closer functional monitoring and in-depth assessment[15]. BOS has a variable course, with some patients experiencing rapid decline in lung function, while others develop a slower and more gradual loss of function[16]. Regardless of the speed of progression, BOS remains one of the greatest impediments to long-term survival after lung transplantation, as it ultimately affects up to 80% of transplant recipients by five years[17-19], and most transplant deaths beyond the first year occur directly or indirectly as a result of BOS[7,14].

Recently, a new restrictive form of chronic rejection has been described, termed restrictive allograft syndrome (RAS). RAS manifests as progressive, restrictive physiology with an appearance of increasing fibrosis on imaging studies[20,21], and is defined as a persistent decline in total lung capacity alongside a decline in FEV1[22]. RAS is histologically characterized by diffuse alveolar damage and extensive fibrosis in the alveolar interstitium, visceral pleura, and interlobular septa, and may also contain scattered obliterative bronchiolitis lesions[21-24]. Recent research using immunofluorescence labeling for α-smooth muscle actin has demonstrated massive infiltration of myofibroblasts in the peripheral lung tissue of RAS patients; whereas in BOS, myofibroblasts were observed predominantly in the small airway obliterative bronchiolitis lesions and not in the peripheral lung[21], affording a potential method to differentiate the two types of chronic allograft rejection.

As a consequence of these findings, a new descriptor of the effects of chronic rejection, termed chronic lung allograft dysfunction (CLAD), has been created to cover obstructive, restrictive, and all other manifestations of chronic rejection, including those as yet undetermined, with resulting clinical decline[25]. This review will focus on the chronic rejection syndromes of BO and BOS, which have been studied more extensively in the setting of gastroesophageal reflux disease (GERD).

Immune-mediated lung injury, including cellular and humoral rejection, has been recognized as the leading cause of BOS[7,26-28] and chronic rejection; however, non-immune mechanisms, such as infection, ischemic reperfusion injury, brain death, chronic aspiration, and GERD may also contribute[14,15,19,26,29-32]. GERD, in particular, has been identified as a potential risk factor for both early allograft injury[27], including acute rejection and lymphocytic bronchiolitis, and chronic airway rejection associated with BOS[28,29]. Although no clear causal link has yet been demonstrated, many studies have proposed that GERD is a risk factor in the development of BOS through silent aspiration of stomach contents, leading to direct airway injury and/or upregulation of the inflammatory response in the lung[29,33-38]. Given the significant commonality between GERD and chronic respiratory diseases, the high prevalence of GERD in the lung transplant population[33,39-41], and the more rapid progression to BOS in transplant recipients with objective evidence

of aspiration[34,40,42,43], many groups have begun investigating the impact of diagnosis and treatment of reflux on pulmonary outcomes in this population.

GERD AND LUNG DISEASE: SIGNIFICANCE OF THE PROBLEM

Population-based studies have demonstrated that as many as 11% of Americans experience typical symptoms of reflux daily, and 33% experience symptoms during a 72-h period[44]. It is well known that there may be a higher prevalence of GERD in patients with end-stage lung disease[33,34,45-48]. For example, D'Ovidio *et al*[47] described a 63% (49 of 78 patients) prevalence of gastroesophageal reflux-related symptoms in end-stage lung disease, 38% with documented significant acid reflux on objective testing, which was often asymptomatic[47,49]. Additionally, in patients with idiopathic pulmonary fibrosis (IPF), GERD has been shown to have increased prevalence in comparison to other chronic lung diseases[46,50,51]. Gavini *et al*[52] demonstrated that patients with IPF undergoing pre-lung transplant evaluation have a significantly higher prevalence of abnormal reflux compared to those with COPD, after controlling for potential confounders such as underlying disease severity. Savarino *et al*[53] demonstrated that IPF patients had a higher total reflux episodes and total proximal reflux episodes compared to both non-IPF chronic lung disease patients and healthy volunteers. These findings support the theory that GERD may increase microaspiration episodes, resulting in activation of an inflammatory cascade in lung tissue, which over time, induces fibrotic changes that characterize IPF[42,54,55].

In addition to its higher prevalence in patients with underlying lung disease prior to transplantation, numerous studies have also documented that GERD is increased following transplantation. Young *et al*[56] have shown that the incidence of GERD rose from 35% pre-transplant to 65% post-transplant in their cohort of patients. Similarly, other groups have demonstrated a prevalence of reflux as high as 51-69% in patients after transplant[33,48]. D'Ovidio *et al*[57] have investigated the prevalence of reflux at 3- and 12-mo post-transplant, and found that it increased from 32% to 53%, suggesting that transplantation may itself induce worsened reflux[56,57]. Fisichella *et al*[58] have demonstrated that distal and proximal reflux were more prevalent in patients with bilateral lung transplant or re-transplant, and less prevalent in patients after unilateral transplant, regardless of the cause of their lung disease, suggesting not only the importance of screening for reflux in the post-transplant population, but also the necessity for higher vigilance in patients following double lung transplantation. Various factors have been implicated, including intraoperative vagal nerve damage, loss of cough reflex, impaired mucociliary clearance, and development of gastroparesis as a side effect of calcineurin inhibitors, steroids, mycophenolate mofetil, and other post-transplant immunosuppression treatments[16,39,56,57,59-70].

BACKGROUND AND PATHOPHYSIOLOGY

The association between reflux and rejection post-lung transplant has been investigated in both animal and human studies (Table 1). Stovold *et al*[35] demonstrated that in rats, exposure of the lung allograft to gastric juice leads to high grade acute rejection, which is characterized by monocyte infiltration, fibrosis, and lung destruction. Aspiration has also been shown to increase allograft CD8+ T cells, which are involved in acute rejection[71], and chronic aspiration has been associated with bronchiolitis obliterans[72]. Meltzer *et al*[73] demonstrated similar results in a miniature swine study where chronic aspiration was associated with increased shedding of allograft alloantigens and increased activity of the indirect alloimmune response, which may contribute to fibrosis, obliterative bronchiolitis, and infection.

The central belief is that BOS is a chronic inflammatory and fibrotic process of the small airways, marked by recurrent injury, remodeling, and repair, ultimately resulting in allograft failure typified by obliterative fibrosis[74,75]. Multiple studies supporting this claim have shown that aspiration of gastroduodenal contents is linked to immunomodulation, including increased local levels of IL-1α, IL-1B, IL-6, IL-10, TNF-α, TNF-β[72], increased alveolar neutrophils[37,76,77], increased IL-8[37,76], increased IL-15, IL-17, basic-FGF, TNF-α, and MPO and reduced alpha-1-antitrypsin[42], augmented indirect allorecognition[73], and reduced levels of surfactant proteins SP-A and SP-D[57].

Additionally, numerous studies have investigated the specific role of bile acids and pepsin in the association between reflux and BOS. Bile acids and pepsin, used as markers of aspiration and reflux, have been demonstrated in bronchoalveolar (BAL) fluid of post-lung transplant patients[35,37,57,78,79]. Bile aspiration is cytotoxic, disrupts cellular membranes, and damages type II pneumocytes[80], which are responsible for surfactant protein and phospholipid production and homeostasis[37,57,81,82]. D'Ovidio *et al*[37] investigated 120 post-transplant patients, and found that 20 (17%) had high concentrations of bile acids in BAL. They also noted an association between the presence of bile acids and decreased surfactant proteins and phospholipids, suggesting that aspiration of bile acids may have impaired the innate immunity of the allograft[37]. Importantly, they demonstrated that the highest concentrations of bile acids were found in 70% of patients with early onset (< 1 year post-transplant) and most severe manifestation of BOS, suggesting a temporal and dose-related relationship[37,57]. Blondeau *et al*[78] found that 50% of the lung transplant patients in their study demonstrated elevated levels of bile acids, and 70% of

Table 1 Papers summarizing effects of gastroesophageal reflux disease on transplant outcomes

Ref.	Population	Definition GERD and/or aspiration	Outcomes evaluated	Adjunctive therapy
King *et al*[29], 2009	59 pts. Post-LTx	Abnormal acid and non-acid reflux on esophageal impedance monitoring	Effect of reflux on time to development of BOS *via* hazard ratio	
Hadjiliadis *et al*[33], 2003	43 pts. Post-LTx, survived > 6 mo, and underwent pH and manometry testing	Abnormal acid exposure time on 24-h pH testing	Effect of reflux on FEV1 (*via* Pearson correlation coefficient for time of study, *via* multivariable linear regression to assess overall effect)	PPI d/c'ed > 5 d prior to testing, H2 blockers and pro-motility agents > 1 d prior to testing
Stovold *et al*[35], 2007	36 asymptomatic pts. Post-LTx *vs* 4 healthy volunteers *vs* 17 patients with chronic cough	Increased levels of pepsin in BALF	Presence of pepsin, association between level of pepsin and acute rejection	30 LTx patients on antireflux therapy
Blondeau *et al*[36], 2009	24 pts. Post-LTx	Abnormal reflux on 24-h impedance-pH testing, bile acids in BALF	Relationship between acid exposure, volume exposure, or reflux events and bile acids in BALF	PPI d/c'ed 1 wk prior to testing
D'Ovidio *et al*[37], 2005	120 pts. Post-LTx	Increased levels of bile acids in BALF	Relationship between increased levels of bile acids, IL-8, neutrophils on development of BOS	
Benden *et al*[41], 2005	10 pts. Post-LTx	Abnormal reflux on 24-h pH testing	Prevalence of GERD in population	
Fisichella *et al*[42], 2013	105 pts. Post-LTx with 257 BALF samples	24-h pH testing and DeMeester score calculation, Increased levels of pepsin in BALF	Association between aspiration and patterns of dysregulation of immune mediator concentrations and BOS	PPI d/c'ed 2 wk prior to testing, H2 blocker d/c'ed 3 d prior to testing
Young *et al*[56], 2003	23 pts. evaluated pre- and post-LTx	Total, upright, and supine acid exposure time on 24-h pH testing, esophageal manometry, gastric-emptying study	Paired comparison between pre-transplant and post-transplant results (paired *t* test)	Acid suppression and gastric motility meds discontinued before testing
D'Ovidio *et al*[57], 2006	70 pts. Post-LTx	Esophageal manometry, 24-h pH-testing (DeMeester score calculation, Castell's method) and gastric emptying study; BALF analysis	Actuarial freedom from BOS, impact of aspiration on pulmonary surfactant collectin proteins	PPI d/c'ed 7 d prior, H2-blockers d/c'ed 2 d prior
Fisichella *et al*[58], 2012	61 pts. Post-LTx	Esophageal impedance-manometry, 24-h pH testing (DeMeester score calculation), EGD, barium swallow, gastric emptying study	Relationship between prevalence and extent of GERD and type of transplant (unilateral *vs* bilateral *vs* retransplant)	PPI d/c'ed 14 d prior to pH testing, H2 blockers stopped 3 d prior to pH testing
Fisichella *et al*[74], 2012	8 pts. Post-LARS and LTx in whom BALF had been collected	Esophageal 24-h impedance-pH testing (DeMeester score calculation), gastric emptying study	Comparison of BALF concentrations of leukocytes, immune mediators, and pepsin pre- and post-LARS and post-LTx	PPI d/c'ed 14 d prior to pH testing, H2 blockers stopped 3 d prior to pH testing
Blondeau *et al*[78], 2008	45 pts. Post-LTx off PPI, 18 pts. Post-LTx on PPI	Esophageal 24-h impedance-pH catheter, BALF analysis for pepsin and bile acids	Association between the prevalence and type of reflux and gastric aspiration in pts. with and without BOS	Antacids and promotility agents d/c'ed > 14 d prior to testing *vs* remained on for testing
Griffin *et al*[45], 2013	18 pts. Post-LTx	RSI, esophageal manometry and 24-h impedance-pH monitoring, BALF analysis	Quantification of reflux, aspiration, and allograft injury immediately post-operatively	Testing performed on PPI
Davis *et al*[84], 2013	100 pts Post-LTx with 252 BALF samples	BALF pepsin concentration, esophageal manometry, esophageal 24-h pH catheter (DeMeester score calculation), gastric emptying study	Association between concentration of pepsin in BALF and results of esophageal function testing, barium swallow and gastric emptying to identify risk factors for GERD	PPI d/c'ed 14 d prior to pH testing, H2 blockers d/c'ed 3 d prior to pH testing
Hartwig *et al*[71], 2006	7 models of rat lung transplantation	Weekly injection of gastric contents for 4-8 wk	Degree of pulmonary allograft dysfunction reflective of chronic aspiration	N/A
Li *et al*[72], 2008	9 models of rat lung transplantation	Weekly injection of gastric contents for 8 wk	Association between chronic aspiration and development of OB	N/A
Meltzer *et al*[73], 2008	3 models of swine lung transplantation	Daily injection of gastric contents for 50 d	Effect on chronic aspiration on the direct and indirect pathways of allorecognition	N/A

BALF: Bronchoalveolar lavage fluid; BOS: Bronchiolitis obliterans syndrome; OB: Obliterative bronchiolitis; RSI: Reflux severity index; GERD: Gastroesophageal reflux disease; N/A: Not available.

those with BOS had elevated bile acids, compared to 31% without BOS, indicating that bile acid may be a specific marker for allograft injury.

Pepsin is a proteolytic enzyme, active at acidic pH, which is increasingly reported as a marker of inflammation in asthma, COPD, bronchiectasis, CF, and following cardiothoracic surgery[83]. Numerous studies have documented increased levels of pepsin in BAL of patients following lung-transplantation[35,78,79,84]. In a small study by Ward *et al*[79], pepsin was present in the BAL of all lung allografts, while not detected in the control group. In a later follow-up study of 36 post-transplant patients, 4 normal volunteers, and 1 patient with unexplained chronic cough, it was shown that pepsin levels were significantly higher in the transplant cohort; among these patients, pepsin levels were highest in those with acute rejection, a risk factor for the progression to BOS[85,86]. Stovold *et al*[35] also demonstrated consistently elevated levels of pepsin in the BAL fluid of lung transplant patients, again with the highest levels in association with acute rejection. Davis *et al*[84] have even specifically compared patients with IPF to those with alpha-1-antitrypsin deficiency, cystic fibrosis, or COPD, and have found that patients with IPF had higher pepsin concentrations and greater frequency of acute rejection than those with other diseases. Interestingly, despite higher pepsin concentrations and rates of acute rejection, IPF patients did not have a significantly greater incidence of BOS compared with other indications for lung transplantation[84], though the short follow-up time was a significant limitation that likely reduced development of the BOS outcome.

Furthermore, as previously mentioned, both acute cellular rejection[7-9] and lymphocytic bronchiolitis[9] are independently associated with bronchiolitis obliterans. Acute cellular rejection may represent an earlier endpoint in the model of chronic lung injury, supporting the relationship between early allograft injury and eventual development of BOS. Lymphocytic bronchiolitis not only represents an independent risk factor for bronchiolitis obliterans[9], but also has been associated with the occurrence and severity of acute cellular rejection[10]. While no causal relationship between lymphocytic bronchiolitis and BOS has been identified, a prior study has documented the presence of lymphocytic infiltration and esophageal inflammation in association with GERD in the upper gastrointestinal tract, which improves with acid suppression therapy[87]. Therefore, GERD and aspiration may play a role in early development of both lymphocytic bronchiolitis and acute cellular rejection, which in turn, independently predict onset of BOS[7-9].

EVALUATION AND DIAGNOSIS

There is mounting evidence that patients with reflux have a higher risk of poor outcomes post-transplant. For example, King *et al*[29] have demonstrated that increased reflux is associated with BOS, even after controlling for the graft ischemic time, type of surgery, recipient age, underlying pathology, CMV mismatch, or HLA mismatches, concluding that reflux is a prevalent and modifiable risk factor[29]. Hadjiliadis *et al*[33] have even demonstrated a negative correlation between measurements of FEV1 and pH test results in a post-transplant population. These and other studies highlight the importance of identifying patients at risk for allograft injury relating to GERD. Typical GI symptoms, such as heartburn and regurgitation symptoms, have not been predictive of respiratory symptoms attributed to GERD, and are an unreliable correlate between reflux and airway disease[16,29,47,49-51,88-92]. Sweet *et al*[49] have demonstrated that in patients with IPF, 67% had pathologic reflux, which frequently extended into the proximal esophagus, and that heartburn symptoms were unreliable means of patient detection, demonstrating sensitivity of 65% and specificity of 71%. This again emphasizes the importance of screening transplant candidates for GERD to identifying those at increased risk of poor outcomes.

In the past, gastric transit studies[62], esophagoscopy[93], and radiologic swallow studies[93] were used as tenuous proxies for reflux. Recently, a variety of more sophisticated techniques have been utilized to characterize reflux in the lung transplant population, including 24-h ambulatory pH monitoring, multichannel intraluminal impedance and pH (MII-pH) testing, and bronchoscopy with BAL evaluation. Collection of exhaled breath condensate for pH and other chemical assays has been used with limited accuracy and poor availability, and is primarily a research tool[87-89]. While ambulatory pH testing is the most universally advocated, the optimal testing modality remains undefined.

Ambulatory pH testing has the longest history of use in the assessment of transplant patients. Hadjiliadis *et al*[33] used 24-h pH monitoring to demonstrate that 69.8% of patients in their post-transplant group had abnormal total acid exposure times, and that there was an inverse correlation between total or upright acid reflux and FEV1 at the time of the ambulatory pH study. Similarly, Young *et al*[56] have also used pH monitoring to demonstrate that 65% of their patients had abnormal acid exposure times post-transplant. However, ambulatory pH monitoring has had variable sensitivity for reflux detection in this population, ranging from 50%-80%[41,84,90]. One possible reason for this limitation may be that the test underestimates the amount and frequency of reflux, as it is not capable of detecting nonacidic or bolus reflux. Other modalities for evaluation of acid reflux, such as BRAVO capsule-based pH monitoring (Given Imaging, Yoqneam, Israel)[94] have not been assessed in the transplant population, but may offer few benefits over catheter-based testing as it requires endoscopic evaluation prior to placement.

To better assess potential contributions from nonacid and bolus reflux, impedance testing was developed to sensitively detect the presence of liquid bolus, its direction of movement, and the proximal extent of reflux, independent of pH[29,95,96]. Through this minimally

invasive outpatient procedure, patients at risk of reflux and aspiration can be identified[29]. In one study, impedance detected 96% of reflux events compared with 28% detected by ambulatory pH study alone[97], highlighting that a significant portion of reflux events may be nonacidic or weakly acidic events not detectable by pH testing, but still potentially contributing to the pathophysiology of post-transplant reflux-induced allograft injury. Similarly, our group has demonstrated that impedance data, specifically the additional information regarding nonacid reflux, offers statistically significant advantages over their corresponding pH-only parameters in predicting lung transplant outcomes[98]. It is our general belief that impedance is being under-utilized, and our data suggests a role for more routine use of impedance as a standard part of pre-transplant evaluation[98].

Although not specifically for reflux assessment, use of high resolution esophageal manometry (HREM) is also growing in the transplant population. Practically, HREM may help identify the lower esophageal sphincter to guide proper placement of the pH catheter. Additionally, esophageal motility disorders may present primarily with GERD symptoms and can impact GERD severity, including connective tissue diseases, so HREM may be helpful in the diagnosis of secondary reflux. Esophageal dysmotility may also impact candidacy for surgical antireflux treatment. Further studies are required to assess the relationship between HREM measures of esophageal function and pulmonary outcomes.

Oelschlager *et al*[89] have demonstrated that in 518 patients, the combination of symptoms, esophageal manometry, and ambulatory pH monitoring was insufficient to accurately identify reflux as the cause of aspiration. While this included only standard ambulatory pH monitoring rather than MII-pH, it raises the possibility that additional tests may be required to more directly assess reflux severity. Some groups have proposed that BAL fluid analysis may contribute additional information in the evaluation of these patients. For example, BAL may be used to quantify pepsin and bile acids as markers of aspiration, which have been associated with progression to BOS[75,79,99-101]. However, bronchoscopy sampling is relatively expensive, more invasive than other techniques, and time consuming[29]. Additionally, because only a single sample is taken at a moment in time[29,39], without standardization of results or a full understanding of temporal changes in bile acid or pepsin concentrations, this test may be exquisitely sensitive to provider technique[39]. In short, clinical feasibility remains a challenge.

In addition to poor consensus on the optimal mode of reflux testing among lung transplant candidates[98], there is no standard for timing of testing. Our group favors routine pre-transplant impedance testing, as we have previously shown that prolonged bolus clearance, increased total distal reflux episodes, and increased total proximal reflux episodes on pre-transplant MII-pH were associated with decreased time to early allograft injury after lung transplantation[102]. Researchers from Duke University have suggested the following approach based on available data, and previous experience at their center: Prior to transplant, all patients undergo esophageal manometry, 24-h ambulatory pH or MII-pH study (off anti-secretory therapy), and upper GI series[13]. However, not all groups have adopted this pre-transplant assessment approach, especially given the tenuous pulmonary status of some transplant candidates. It does seem, however, that if evaluation were to be performed post-transplant, the importance of early assessment should not be ignored. As mentioned previously in this review, there are several processes during and after transplant surgery that may result in worsening of reflux, and thus, it is imperative to screen for reflux in the early post-transplant period if not before. Griffin *et al*[45] recommended that all patients should be routinely assessed within 1 mo post-transplant given the high prevalence of reflux and aspiration in the immediate post-transplant period, despite use of proton-pump inhibitor (PPI). Additionally, as our group has demonstrated the benefits of timely antireflux surgery in improving transplant outcomes[103], earlier reflux assessment may be essential to guide management.

TREATMENT

Medical treatment of reflux consists of the conventional pharmacologic methods of histamine-2 receptor blockers and PPIs, and prokinetic agents to enhance esophageal and gastric clearance. These agents may ameliorate symptoms, diminish the acid component of gastric refluxate, and promote bolus clearance. Additionally, recent publications have suggested that antireflux therapies may prolong survival and decrease the incidence of acute disease exacerbation in patients with IPF (Table 2)[53,104-109]. Blondeau *et al*[78] demonstrated that PPI use did reduce acid exposure in lung transplant patients, but had minimal effect on pepsin as a surrogate marker of aspiration. Unfortunately, additional literature on the effects of medical acid suppression in the lung transplant population is sparse. Azithromycin has been used as a therapy for BOS with some success, possibly relating to its mild pro-kinetic effects, although the full mechanism of action is not clearly defined[32,110,111]. Mertens *et al*[112] used impedance and BAL testing to evaluate the effect of azithromycin on reflux and gastric aspiration parameters, and found that patients on azithromycin had significantly less reflux, including decreased number of reflux events, fewer proximal reflux episodes, and decreased esophageal acid exposure. In addition, bile acid levels in the BAL were significantly reduced after azithromycin treatment[112]. However, given the unclear mode of action and concern for antibiotic overuse, routine application of azithromycin has not been recommended.

While the aforementioned pharmacologic therapies may ameliorate symptoms, diminish the acid

Table 2 Papers on the effect of pharmacologic reflux treatment on transplant outcome

Ref.	*n*	Population	Treatment type	Adjunctive treatments	Outcomes assessed
Yates *et al*[32], 2005	20	Post-LTx with diagnosis of BOS (*n* = 18) or potential BOS (*n* = 2)	AZI 250 mg QOD from time of BOS diagnosis to time of manuscript writing (mean 6.25 mo)	Immunosuppressive regimen, no additional antireflux agents specified	Effect on FEV1
Verleden *et al*[110], 2004	8	Post-LTx with significant decrease in their FEV1 attributed to BOS	AZI 250 mg qd × 5 d then 250 mg po QOD	Immunosuppressive regimen, no additional antireflux agents specified	Effect on FEV1
Verleden *et al*[111], 2006	14	Post-LTx with BOS	AZI 250 mg po qd × 5 d then AZI 250 mg po 3 × /wk × 3 mo	Immunosuppressive regimen, no additional antireflux agents specified	Reduction in airway neutrophilia and IL-8 mRNA, effect on FEV1
Mertens *et al*[112], 2009	12	Post-LTx on AZI with pH monitoring	AZI 250 mg PO 3 ×/wk	Immunosuppressive regimen, held antireflux treatments × 1 wk prior to testing	Effect on impedance-pH monitoring, gastric aspiration *via* BAL analysis
Blondeau *et al*[78], 2008	18	Post-LTx on PPI *vs* off PPI at time of testing (secondary cohort)	Omeprazole 20 mg PO BID	Immunosuppressive regimen	Prevalence of reflux on objective testing, effect on aspiration in BAL

n: Patients in the study in the treatment arm; BOS: Bronchiolitis obliterans syndrome; LTx: Lung transplant; AZI: Azithromycin; QOD: Every other day; FEV1: Forced expiratory volume in 1 s; BID: Twice a day.

component of gastric refluxate, and improve clearance, the underlying mechanism provoking reflux often persists[29,39,78,113-116]. For example, Patti *et al*[114] demonstrated that while acid-reducing medications alter the pH of the refluxate, clinical symptoms may recur, suggesting persistence of pathology in spite of medical antireflux therapy, and that surgery may provide more definitive treatment of reflux and aspiration regardless of pH. Blondeau *et al*[78] demonstrated that 71% of lung transplant recipients taking PPIs had increased non-acid reflux, and that PPI use did not reduce the number of reflux events, non-acid reflux exposure, proximal reflux extent, or markers of aspiration on BAL.

Consequently, many groups are now turning to antireflux surgery as a more definitive approach to reflux management and for prevention of further complications. Previous studies have shown that antireflux surgery is a safe procedure in this patient population[34,40,75,117-122], and is associated with improved survival and stabilization of lung function (Table 3)[29,33,34,40,43,75,117,118,123-125]. For example, Robertson *et al*[75] demonstrated that post-lung transplant antireflux surgery resulted in no deaths or serious post-operative complications in all 16 patients undergoing surgery, although one patient required minor surgical revision for dysphagia. Fisichella *et al*[119] similarly demonstrated that post-lung transplant patients had perioperative morbidity and mortality rates similar to those of transplant-free controls undergoing laparoscopic antireflux surgery. However, these and other studies have been limited by single-center experiences and small patient numbers. Subsequently, Kilic *et al*[17] performed a study using the all-payer database in the United States to evaluate nationwide outcomes of antireflux surgery in transplant recipients *vs* transplant-free controls, confirming similar outcomes in both groups. The post-lung transplant group did not demonstrate an increased risk of respiratory complications, although they did have a longer median hospital stay, higher resource utilization, and higher median cost of inpatient care[17]. In congruence with these results, O'Halloran *et al*[121] demonstrated that while lung transplant patients in their study also required longer hospital stay and had higher rates of readmission compared to controls, no differences were detected with regard to operative time, estimated blood loss, or peri-operative complications. Furthermore, no intra- or peri-operative deaths were seen, and both transplant and control groups reported symptom resolution following surgery.

Additional studies have focused on the efficacy of antireflux surgical management with regard to transplant outcomes such as pulmonary function and allograft rejection. Halsey *et al*[124] published a case report on a post-transplant patient with progressive allograft dysfunction, associated with a significant decline in FEV1 and FVC, despite twice-daily use of PPI. Their patient underwent impedance testing, which demonstrated ongoing non-acid reflux, and proceeded to laparoscopic Nissen fundoplication. Post-operatively, the patient improved symptomatically and spirometry results returned to baseline[124]. Hoppo *et al*[16] demonstrated that antireflux surgery either improved or prolonged native lung or allograft function during the pre- or post-lung transplant period, respectively. One year after antireflux surgery, significant improvement in FEV1 was detected in 91% of the post-lung transplant patients ($P < 0.01$) and 85% of the pre-lung transplant patients ($P = 0.02$)[16]. Additionally, all patients in this study were using anti-secretory medications, which lends further credence to the observation that acid suppression alone may not be sufficient to prevent reflux in every case[16]. Hartwig *et al*[126] have similarly demonstrated that early fundoplication was associated with preservation of lung function, and Lau *et al*[118] reported that 67% of lung transplant recipients actually had improvement in

Table 3 Papers of surgical antireflux procedures and lung transplant outcomes

Ref.	*n*	Population undergoing surgery	Type of surgical intervention (Type Nissen: *n*)	Outcomes assessed
Davis *et al*[32], 2003	43	Post-LTx with abnormal pH study (*n* = 39), severe reflux with normal manometry (*n* = 2), repetitive aspiration events leading to retransplant (*n* = 1) or pneumonia (*n* = 1)	Laparoscopic: 36 Open: 3 Partial Toupet: 4	In-hospital or 30-d mortality, FEV1 pre- and post-procedure
Cantu *et al*[40], 2004	74	Post-LTx with abnormal pH studies	Laparoscopic: 71 Open: 5 Partial Toupet: 4 Other: 5[1]	In-hospital or 30 d mortality, freedom from BOS in early *vs* late fundoplication groups
Robertson *et al*[75], 2012	16	Post-LTx undergoing antireflux surgery	Laparoscopic: 16	Effect on quality of life, peri-operative mortality and complications, reduction in deterioration of lung function
Linden *et al*[117], 2006	19	Pre-LTx IPF with h/o reflux, symptoms, and severe reflux on pH and manometry testing	Laparoscopic: 19	Peri-operative complications, post-operative lung function
Lau *et al*[118], 2002	18	Post-LTx with documented GERD	Laparoscopic: 13 Open: 1 Partial Toupet: 4	Length of hospital stay, post-operative lung function, morbidity and mortality
Fisichella *et al*[119], 2011	29	Post-LTx with GERD dx on symptoms, BAL, or decreased lung function; with abnormal pH monitoring	Laparoscopic: 27	30-d morbidity and mortality, hospital readmissions
Fisichella *et al*[43], 2011	19	Post-LTx with GERD symptoms, aspiration on BAL, or unexplained decrease in lung function	Partial Toupet: 2 Laparoscopic: 19	decreased aspiration as defined by the presence of pepsin in the BALF
Fisichella *et al*[74], 2012	8	Post-LTx patients with GERD and evidence of reflux on ambulatory pH monitoring	Laparoscopic: 8	Quantification and comparison of pulm leukocyte differential and concentration of inflammatory mediators in BAL, freedom from BOS, effect on FEV1, and survival
Burton *et al*[120], 2009	21	Post-LTx with reflux confirmed on EGD, pH testing, or BALF	Laparoscopic: 5 Partial Toupet: 16	Patient satisfaction, symptom changes and side effects, effect on lung function, BMI, rate progression to BOS
O'Halloran *et al*[121], 2004	28	Post-LTx with reflux on pH testing and manometry	Laparoscopic: 28	Perioperative complications, length of stay, readmission rate, effect on lung function
Gasper *et al*[122], 2008	35	Pre-LTx in 15 patients, Post-LTx in 20 patients with GERD or delayed gastric emptying study	Laparoscopic: 27 Partial Toupet: 5 Other: 3[2]	Length of stay, perioperative complications pre- or post-LTx
Kilic *et al*[17], 2013	401	Post-LTx who pursued elective antireflux procedure	Laparoscopic: 338[3] Open: 23	Inpatient mortality, length of stay, perioperative complications, hospital costs
Hoppo *et al*[16], 2011	43	Pre-LTx in 19 patients, Post-LTx in 24 patients with documented symptoms or signs of GERD on EGD, barium, manometry, pH or impedance testing; or declining lung function	Laparoscopic: 24 Other: 17[4]	Effect on lung function, number cases of pneumonia and acute rejection episodes
Hartwig *et al*[126], 2011	157	Post-LTx with abnormal acid contact times before or early after transplantation	Laparoscopic: 157[3]	Effect on lung function
Lo *et al*[103], 2016	48	Pre-LTx or Post-LTx patients with persistent symptoms on maximal PPI and with objective evidence of reflux on pH testing	Laparoscopic = 48	Time to early allograft injury in pre-LTx *vs* early *vs* late post-LTx groups
Patti *et al*[114], 2000	39	Pt with GERD and respiratory symptoms on H2 agents *vs* PPI *vs* pro-kinetic agents, ± bronchodilators (*n* = 3) and bronchodilators/prednisone (*n* = 4)	Laparoscopic = 39	Outcome of surgery on GERD-induced respiratory symptoms

[1]Three cases Belsey-Mark IVs, 1 Toupet and 1 Nissen at OSH (without further information); [2]Two cases had pyloroplasty without fundoplication, 1 case had hypotension at induction and was discharged without operation; [3]Does not specify full Nissen *vs* partial toupet, only laparoscopic *vs* open approach; [4]Seventeen cases underwent laparoscopic Dor procedure. *n*: Study patients in the fundoplication group specifically; LTx: Lung transplant; BALF: Bronchoalveolar lavage fluid; BOS: Bronchiolitis obliterans syndrome; GERD: Gastroesophageal reflux disease; BMI: Body mass index; EGD: esophagogastroduodenoscopy.

their pulmonary function following antireflux surgery. Interestingly, Fisichella *et al*[119] investigated changes in BAL fluid analysis four weeks after antireflux surgery, and showed that in 8 lung transplant recipients, the percentages of neutrophils and lymphocytes in the BAL fluid were reduced, the concentration of myeloperoxide

and IL-1b tended to decrease, and the percentage of macrophages was increased. While this was a limited study given its small sample size, the findings suggest that antireflux surgery may restore the physiologic balance of pulmonary leukocyte populations with ensuing reduction in pro-inflammatory mediators[119]. Additionally, this same group detected decreased pepsin levels in transplant recipients with reflux that underwent antireflux surgery, compared to those that did not receive surgery. Both groups had higher pepsin levels compared against controls, whose levels were undetectable[43]. Notably, subjects with increased pepsin levels were noted to have more acute rejection episodes and faster progression to BOS[43], further underscoring the relevance and necessity of reflux and aspiration management in this patient population.

One important consideration surrounding antireflux surgery in this population is the appropriate timing of the procedure, not just before or after transplant, but also how soon after transplant would be of greatest benefit. Several groups argue that antireflux surgery should be considered in the pre-transplant period[50,117,122]. Linden *et al*[117] focused specifically on IPF patients, and demonstrated no perioperative complications or decrease in lung function over the 15-mo average follow-up. Importantly, patients treated with antireflux surgery had stable oxygen requirements, while control patients with IPF on the waiting list had a statistically significant deterioration[117]. Thus, in spite of theoretical risks in the setting of pre-transplant pulmonary compromise, the absence of serious complications in clinical practice led to the conclusions that pre-transplant antireflux surgery is safe, may ameliorate the progression of underlying disease while awaiting transplant, and provide early protection from reflux and aspiration upon transplantation[117]. Other groups similarly note that pre-transplant surgery may be performed safely, but acknowledge the high-risk nature of these patients given their limited pulmonary reserve. To accommodate these risks, the decision to operate should be made individually, based on objective measures of pulmonary function[16], and under the guidance of an experienced surgical team[122].

In patients that are unable to tolerate pre-transplant antireflux surgery, the timing of surgery post-transplant may be of great importance. Cantu *et al*[40] demonstrated that early fundoplication within 90 d of transplantation resulted in greater freedom from BOS and improved survival compared to later fundoplication, with post-transplant reflux incidence of 76%. Importantly, both BOS and survival were improved in the early post-transplant antireflux surgery group, compared to those with later surgery as well as those with reflux but without surgical intervention. Our group has similarly demonstrated the importance of early intervention. In a retrospective cohort study of 48 patients, we detected a significant increase in early allograft injury in late post-transplant antireflux surgery patients (mean time from transplant 1.8 years) compared to pre-transplant (mean time 3.5 years prior to transplant) and early post-transplant (mean time from transplant 118 d) antireflux surgical groups[103]. The surgeries were well tolerated in the pre- and early post-transplant groups. One death was reported in the late post-transplant group in a patient that had already developed BOS. The trend in this study supports the pathophysiologic model in which antireflux surgery reduces microaspiration events, as suggested by prior studies[16,34,74], and it is our speculation that the earlier antireflux surgery is performed, the greater the protection against reflux and aspiration events, which lowers the risk of pulmonary decline[103]. Interestingly, our study also highlights the lack of additional benefit to providing antireflux surgery pre-transplant compared to within 6 mo post-transplantation. Given the potentially elevated risks of pre-transplant surgery in this population, it may be reasonable to wait for the early post-transplant period to reduce peri-operative risks. Finally, although antireflux surgery performed concurrently with lung transplantation has been reported anecdotally, it has not been extensively studied and is not available at our institution. Over time, with the development of new and less invasive antireflux technologies such as the LYNX magnetic reflux management system (Torax, Shoreview, MN, USA), concurrent surgical antireflux management alongside transplantation may come under greater consideration.

CONCLUSION

This review has highlighted an abundance of research regarding the role of reflux in the pathophysiology of allograft injury following lung transplantation, along with options for diagnosis and management. Nevertheless, unanswered questions remain, and additional studies are needed to clarify the optimal modality and timing for reflux evaluation and management in these patients. As King *et al*[29] have previously discussed, there remains frustratingly no clear causal relationship between reflux and the development of BOS. Additionally, the absence of a gold standard to diagnose GERD, and the difficulties of defining and describing reflux severity continue to limit accuracy in patient stratification, given potential contributions from acid reflux, non-acid or bolus reflux, and aspiration[29]. Future studies should explore different objective measurements of reflux and aspiration parameters, better compare medical and surgical antireflux treatment options, extend follow-up times to capture longer-term clinical outcomes such as RAS or CLAD, and investigate newer antireflux interventions including minimally invasive surgery and advanced endoscopic techniques. However, it is clear that a definite association exists between reflux and lung disease, which represents a tangible and significant target to improve outcomes in the lung transplant population.

REFERENCES

1 **Trulock EP**, Christie JD, Edwards LB, Boucek MM, Aurora P, Taylor DO, Dobbels F, Rahmel AO, Keck BM, Hertz MI. Registry of the International Society for Heart and Lung Transplantation: twenty-fourth official adult lung and heart-lung transplantation report-2007. *J Heart Lung Transplant* 2007; **26**: 782-795 [PMID: 17692782 DOI: 10.1016/j.healun.2007.06.003]

2 **Blumenstock DA**, Lewis C. The first transplantation of the lung in a human revisited. *Ann Thorac Surg* 1993; **56**: 1423-1424; discussion 1424-1425 [PMID: 8267457]

3 **Christie JD**, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Dobbels F, Kirk R, Rahmel AO, Stehlik J, Hertz MI. The Registry of the International Society for Heart and Lung Transplantation: 29th adult lung and heart-lung transplant report-2012. *J Heart Lung Transplant* 2012; **31**: 1073-1086 [PMID: 22975097 DOI: 10.1016/j.healun.2012.08.004]

4 **Keck BM**, Bennett LE, Fiol BS, Dally OP, Novick RJ, Hosenpud JD. Worldwide thoracic organ transplantation: a report from the UNOS/ISHLT International Registry for Thoracic Organ Transplantation. *Clin Transpl* 1996; 31-45 [PMID: 9286557]

5 **Goh A**. An analysis of liver transplant survival rates from the UNOS registry. *Clin Transpl* 2008; 19-34 [PMID: 19711511]

6 **Meyer KC**, Raghu G, Verleden GM, Corris PA, Aurora P, Wilson KC, Brozek J, Glanville AR. An international ISHLT/ATS/ERS clinical practice guideline: diagnosis and management of bronchiolitis obliterans syndrome. *Eur Respir J* 2014; **44**: 1479-1503 [PMID: 25359357 DOI: 10.1183/09031936.00107514]

7 **Martinu T**, Chen DF, Palmer SM. Acute rejection and humoral sensitization in lung transplant recipients. *Proc Am Thorac Soc* 2009; **6**: 54-65 [PMID: 19131531 DOI: 10.1513/pats.200808-080GO]

8 **Girgis RE**, Tu I, Berry GJ, Reichenspurner H, Valentine VG, Conte JV, Ting A, Johnstone I, Miller J, Robbins RC, Reitz BA, Theodore J. Risk factors for the development of obliterative bronchiolitis after lung transplantation. *J Heart Lung Transplant* 1996; **15**: 1200-1208 [PMID: 8981205]

9 **Glanville AR**, Aboyoun CL, Havryk A, Plit M, Rainer S, Malouf MA. Severity of lymphocytic bronchiolitis predicts long-term outcome after lung transplantation. *Am J Respir Crit Care Med* 2008; **177**: 1033-1040 [PMID: 18263803 DOI: 10.1164/rccm.200706-951OC]

10 **Yousem SA**, Berry GJ, Cagle PT, Chamberlain D, Husain AN, Hruban RH, Marchevsky A, Ohori NP, Ritter J, Stewart S, Tazelaar HD. Revision of the 1990 working formulation for the classification of pulmonary allograft rejection: Lung Rejection Study Group. *J Heart Lung Transplant* 1996; **15**: 1-15 [PMID: 8820078]

11 **Grossman EJ**, Shilling RA. Bronchiolitis obliterans in lung transplantation: the good, the bad, and the future. *Transl Res* 2009; **153**: 153-165 [PMID: 19304274 DOI: 10.1016/j.trsl.2009.01.005]

12 **Stewart S**. Pathology of lung transplantation. *Semin Diagn Pathol* 1992; **9**: 210-219 [PMID: 1523359]

13 **Castor JM**, Wood RK, Muir AJ, Palmer SM, Shimpi RA. Gastroesophageal reflux and altered motility in lung transplant rejection. *Neurogastroenterol Motil* 2010; **22**: 841-850 [PMID: 20507544 DOI: 10.1111/j.1365-2982.2010.01522.x]

14 **Sharples LD**, McNeil K, Stewart S, Wallwork J. Risk factors for bronchiolitis obliterans: a systematic review of recent publications. *J Heart Lung Transplant* 2002; **21**: 271-281 [PMID: 11834356]

15 **Estenne M**, Maurer JR, Boehler A, Egan JJ, Frost A, Hertz M, Mallory GB, Snell GI, Yousem S. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. *J Heart Lung Transplant* 2002; **21**: 297-310 [PMID: 11897517]

16 **Hoppo T**, Jarido V, Pennathur A, Morrell M, Crespo M, Shigemura N, Bermudez C, Hunter JG, Toyoda Y, Pilewski J, Luketich JD, Jobe BA. Antireflux surgery preserves lung function in patients with gastroesophageal reflux disease and end-stage lung disease before and after lung transplantation. *Arch Surg* 2011; **146**: 1041-1047 [PMID: 21931001 DOI: 10.1001/archsurg.2011.216]

17 **Kilic A**, Shah AS, Merlo CA, Gourin CG, Lidor AO. Early outcomes of antireflux surgery for United States lung transplant recipients. *Surg Endosc* 2013; **27**: 1754-1760 [PMID: 23271270 DOI: 10.1007/s00464-012-2674-9]

18 **Heng D**, Sharples LD, McNeil K, Stewart S, Wreghitt T, Wallwork J. Bronchiolitis obliterans syndrome: incidence, natural history, prognosis, and risk factors. *J Heart Lung Transplant* 1998; **17**: 1255-1263 [PMID: 9883768]

19 **Valentine VG**, Robbins RC, Berry GJ, Patel HR, Reichenspurner H, Reitz BA, Theodore J. Actuarial survival of heart-lung and bilateral sequential lung transplant recipients with obliterative bronchiolitis. *J Heart Lung Transplant* 1996; **15**: 371-383 [PMID: 8732596]

20 **Husain AN**, Garrity ER. Lung Transplantation: The State of the Airways. *Arch Pathol Lab Med* 2016; **140**: 241-244 [PMID: 26927718 DOI: 10.5858/arpa.2015-0295-SA]

21 **Sato M**, Waddell TK, Wagnetz U, Roberts HC, Hwang DM, Haroon A, Wagnetz D, Chaparro C, Singer LG, Hutcheon MA, Keshavjee S. Restrictive allograft syndrome (RAS): a novel form of chronic lung allograft dysfunction. *J Heart Lung Transplant* 2011; **30**: 735-742 [PMID: 21419659 DOI: 10.1016/j.healun.2011.01.712]

22 **Traxler D**, Schweiger T, Schwarz S, Schuster MM, Jaksch P, Lang G, Birner P, Klepetko W, Ankersmit HJ, Hoetzenecker K. The Lymphatic Phenotype of Lung Allografts in Patients With Bronchiolitis Obliterans Syndrome and Restrictive Allograft Syndrome. *Transplantation* 2017; **101**: 310-315 [PMID: 27163544 DOI: 10.1097/TP.0000000000001263]

23 **Verleden SE**, Ruttens D, Vandermeulen E, Bellon H, Van Raemdonck DE, Dupont LJ, Vanaudenaerde BM, Verleden G, Vos R. Restrictive chronic lung allograft dysfunction: Where are we now? *J Heart Lung Transplant* 2015; **34**: 625-630 [PMID: 25577564 DOI: 10.1016/j.healun.2014.11.007]

24 **Sato M**. Chronic lung allograft dysfunction after lung transplantation: the moving target. *Gen Thorac Cardiovasc Surg* 2013; **61**: 67-78 [PMID: 23138970 DOI: 10.1007/s11748-012-0167-3]

25 **Verleden GM**, Raghu G, Meyer KC, Glanville AR, Corris P. A new classification system for chronic lung allograft dysfunction. *J Heart Lung Transplant* 2014; **33**: 127-133 [PMID: 24374027 DOI: 10.1016/j.healun.2013.10.022]

26 **Nicod LP**. Mechanisms of airway obliteration after lung transplantation. *Proc Am Thorac Soc* 2006; **3**: 444-449 [PMID: 16799090 DOI: 10.1513/pats.200601-007AW]

27 **Burton CM**, Iversen M, Carlsen J, Mortensen J, Andersen CB, Steinbrüchel D, Scheike T. Acute cellular rejection is a risk factor for bronchiolitis obliterans syndrome independent of post-transplant baseline FEV1. *J Heart Lung Transplant* 2009; **28**: 888-893 [PMID: 19716040 DOI: 10.1016/j.healun.2009.04.022]

28 **Hachem RR**, Khalifah AP, Chakinala MM, Yusen RD, Aloush AA, Mohanakumar T, Patterson GA, Trulock EP, Walter MJ. The significance of a single episode of minimal acute rejection after lung transplantation. *Transplantation* 2005; **80**: 1406-1413 [PMID: 16340783]

29 **King BJ**, Iyer H, Leidi AA, Carby MR. Gastroesophageal reflux in bronchiolitis obliterans syndrome: a new perspective. *J Heart Lung Transplant* 2009; **28**: 870-875 [PMID: 19716037 DOI: 10.1016/j.healun.2009.05.040]

30 **Hadjiliadis D**, Davis RD, Palmer SM. Is transplant operation important in determining posttransplant risk of bronchiolitis obliterans syndrome in lung transplant recipients? *Chest* 2002; **122**: 1168-1175 [PMID: 12377838]

31 **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]

32 **Yates B**, Murphy DM, Forrest IA, Ward C, Rutherford RM, Fisher AJ, Lordan JL, Dark JH, Corris PA. Azithromycin reverses airflow obstruction in established bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med* 2005; **172**: 772-775 [PMID: 15976371 DOI: 10.1164/rccm.200411-1537OC]

33 **Hadjiliadis D**, Duane Davis R, Steele MP, Messier RH, Lau CL, Eubanks SS, Palmer SM. Gastroesophageal reflux disease in lung transplant recipients. *Clin Transplant* 2003; **17**: 363-368 [PMID:

12868994]
34 **Davis RD**, Lau CL, Eubanks S, Messier RH, Hadjiliadis D, Steele MP, Palmer SM. Improved lung allograft function after fundoplication in patients with gastroesophageal reflux disease undergoing lung transplantation. *J Thorac Cardiovasc Surg* 2003; **125**: 533-542 [PMID: 12658195 DOI: 10.1067/mtc.2003.166]
35 **Stovold R**, Forrest IA, Corris PA, Murphy DM, Smith JA, Decalmer S, Johnson GE, Dark JH, Pearson JP, Ward C. Pepsin, a biomarker of gastric aspiration in lung allografts: a putative association with rejection. *Am J Respir Crit Care Med* 2007; **175**: 1298-1303 [PMID: 17413126 DOI: 10.1164/rccm.200610-1485OC]
36 **Blondeau K**, Mertens V, Vanaudenaerde BA, Verleden GM, Van Raemdonck DE, Sifrim D, Dupont LJ. Nocturnal weakly acidic reflux promotes aspiration of bile acids in lung transplant recipients. *J Heart Lung Transplant* 2009; **28**: 141-148 [PMID: 19201339 DOI: 10.1016/j.healun.2008.11.906]
37 **D'Ovidio F**, Mura M, Tsang M, Waddell TK, Hutcheon MA, Singer LG, Hadjiliadis D, Chaparro C, Gutierrez C, Pierre A, Darling G, Liu M, Keshavjee S. Bile acid aspiration and the development of bronchiolitis obliterans after lung transplantation. *J Thorac Cardiovasc Surg* 2005; **129**: 1144-1152 [PMID: 15867792 DOI: 10.1016/j.jtcvs.2004.10.035]
38 **Krishnan A**, Chow S, Thomas P, Malouf M, Glanville A, Yates D. 221: exhaled breath condensate pepsin: A new noninvasive marker of GERD after lung transplantation. *J Heart Lung Transplant* 2007; **26**: S139 [DOI: 10.1016/j.healun.2006.11.239]
39 **Davis CS**, Gagermeier J, Dilling D, Alex C, Lowery E, Kovacs EJ, Love RB, Fisichella PM. A review of the potential applications and controversies of non-invasive testing for biomarkers of aspiration in the lung transplant population. *Clin Transplant* 2010; **24**: E54-E61 [PMID: 20331688 DOI: 10.1111/j.1399-0012.2010.01243.x]
40 **Cantu E**, Appel JZ, Hartwig MG, Woreta H, Green C, Messier R, Palmer SM, Davis RD. J. Maxwell Chamberlain Memorial Paper. Early fundoplication prevents chronic allograft dysfunction in patients with gastroesophageal reflux disease. *Ann Thorac Surg* 2004; **78**: 1142-1151; discussion 1142-1151 [PMID: 15464462 DOI: 10.1016/j.athoracsur.2004.04.044]
41 **Benden C**, Aurora P, Curry J, Whitmore P, Priestley L, Elliott MJ. High prevalence of gastroesophageal reflux in children after lung transplantation. *Pediatr Pulmonol* 2005; **40**: 68-71 [PMID: 15880421 DOI: 10.1002/ppul.20234]
42 **Fisichella PM**, Davis CS, Lowery E, Ramirez L, Gamelli RL, Kovacs EJ. Aspiration, localized pulmonary inflammation, and predictors of early-onset bronchiolitis obliterans syndrome after lung transplantation. *J Am Coll Surg* 2013; **217**: 90-100; discussion 100-101 [PMID: 23628225 DOI: 10.1016/j.jamcollsurg.2013.03.008]
43 **Fisichella PM**, Davis CS, Lundberg PW, Lowery E, Burnham EL, Alex CG, Ramirez L, Pelletiere K, Love RB, Kuo PC, Kovacs EJ. The protective role of laparoscopic antireflux surgery against aspiration of pepsin after lung transplantation. *Surgery* 2011; **150**: 598-606 [PMID: 22000170 DOI: 10.1016/j.surg.2011.07.053]
44 **Hunt RH**. Importance of pH control in the management of GERD. *Arch Intern Med* 1999; **159**: 649-657 [PMID: 10218743]
45 **Griffin SM**, Robertson AG, Bredenoord AJ, Brownlee IA, Stovold R, Brodlie M, Forrest I, Dark JH, Pearson JP, Ward C. Aspiration and allograft injury secondary to gastroesophageal reflux occur in the immediate post-lung transplantation period (prospective clinical trial). *Ann Surg* 2013; **258**: 705-711; discussion 711-712 [PMID: 24121257 DOI: 10.1097/SLA.0b013e3182a6589b]
46 **Tobin RW**, Pope CE, Pellegrini CA, Emond MJ, Sillery J, Raghu G. Increased prevalence of gastroesophageal reflux in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1998; **158**: 1804-1808 [PMID: 9847271 DOI: 10.1164/ajrccm.158.6.9804105]
47 **D'Ovidio F**, Singer LG, Hadjiliadis D, Pierre A, Waddell TK, de Perrot M, Hutcheon M, Miller L, Darling G, Keshavjee S. Prevalence of gastroesophageal reflux in end-stage lung disease candidates for lung transplant. *Ann Thorac Surg* 2005; **80**: 1254-1260 [PMID: 16181849 DOI: 10.1016/j.athoracsur.2005.03.106]
48 **Davis CS**, Shankaran V, Kovacs EJ, Gagermeier J, Dilling D, Alex CG, Love RB, Sinacore J, Fisichella PM. Gastroesophageal reflux disease after lung transplantation: pathophysiology and implications for treatment. *Surgery* 2010; **148**: 737-744; discussion 744-745 [PMID: 20727564 DOI: 10.1016/j.surg.2010.07.011]
49 **Sweet MP**, Herbella FA, Leard L, Hoopes C, Golden J, Hays S, Patti MG. The prevalence of distal and proximal gastroesophageal reflux in patients awaiting lung transplantation. *Ann Surg* 2006; **244**: 491-497 [PMID: 16998357 DOI: 10.1097/01.sla.0000237757.49687.03]
50 **Sweet MP**, Patti MG, Leard LE, Golden JA, Hays SR, Hoopes C, Theodore PR. Gastroesophageal reflux in patients with idiopathic pulmonary fibrosis referred for lung transplantation. *J Thorac Cardiovasc Surg* 2007; **133**: 1078-1084 [PMID: 17382656 DOI: 10.1016/j.jtcvs.2006.09.085]
51 **Raghu G**, Freudenberger TD, Yang S, Curtis JR, Spada C, Hayes J, Sillery JK, Pope CE, Pellegrini CA. High prevalence of abnormal acid gastro-oesophageal reflux in idiopathic pulmonary fibrosis. *Eur Respir J* 2006; **27**: 136-142 [PMID: 16387946 DOI: 10.118/09031936.06.0037005]
52 **Gavini S**, Finn RT, Lo WK, Goldberg HJ, Burakoff R, Feldman N, Chan WW. Idiopathic pulmonary fibrosis is associated with increased impedance measures of reflux compared to non-fibrotic disease among pre-lung transplant patients. *Neurogastroenterol Motil* 2015; **27**: 1326-1332 [PMID: 26176338 DOI: 10.111/nmo.12627]
53 **Savarino E**, Carbone R, Marabotto E, Furnari M, Sconfienza L, Ghio M, Zentilin P, Savarino V. Gastro-oesophageal reflux and gastric aspiration in idiopathic pulmonary fibrosis patients. *Eur Respir J* 2013; **42**: 1322-1331 [PMID: 23471347 DOI: 10.1183/09031936.00101212]
54 **Perng DW**, Chang KT, Su KC, Wu YC, Wu MT, Hsu WH, Tsai CM, Lee YC. Exposure of airway epithelium to bile acids associated with gastroesophageal reflux symptoms: a relation to transforming growth factor-beta1 production and fibroblast proliferation. *Chest* 2007; **132**: 1548-1556 [PMID: 17908704 DOI: 10.1378/chest.07-1373]
55 **Appel JZ**, Lee SM, Hartwig MG, Li B, Hsieh CC, Cantu E, Yoon Y, Lin SS, Parker W, Davis RD. Characterization of the innate immune response to chronic aspiration in a novel rodent model. *Respir Res* 2007; **8**: 87 [PMID: 18042282 DOI: 10.1186/1465-9921-8-87]
56 **Young LR**, Hadjiliadis D, Davis RD, Palmer SM. Lung transplantation exacerbates gastroesophageal reflux disease. *Chest* 2003; **124**: 1689-1693 [PMID: 14605036]
57 **D'Ovidio F**, Mura M, Ridsdale R, Takahashi H, Waddell TK, Hutcheon M, Hadjiliadis D, Singer LG, Pierre A, Chaparro C, Gutierrez C, Miller L, Darling G, Liu M, Post M, Keshavjee S. The effect of reflux and bile acid aspiration on the lung allograft and its surfactant and innate immunity molecules SP-A and SP-D. *Am J Transplant* 2006; **6**: 1930-1938 [PMID: 16889547 DOI: 10.1111/j.1600-6143.2006.01357.x]
58 **Fisichella PM**, Davis CS, Shankaran V, Gagermeier J, Dilling D, Alex CG, Kovacs EJ, Joehl RJ, Love RB. The prevalence and extent of gastroesophageal reflux disease correlates to the type of lung transplantation. *Surg Laparosc Endosc Percutan Tech* 2012; **22**: 46-51 [PMID: 22318059 DOI: 10.1097/SLE.0b013e31824017d4]
59 **Robertson AG**, Ward C, Pearson JP, Corris PA, Dark JH, Griffin SM. Lung transplantation, gastroesophageal reflux, and fundoplication. *Ann Thorac Surg* 2010; **89**: 653-660 [PMID: 20103377 DOI: 10.1016/j.athoracsur.2009.09.001]
60 **Berkowitz N**, Schulman LL, McGregor C, Markowitz D. Gastroparesis after lung transplantation. Potential role in postoperative respiratory complications. *Chest* 1995; **108**: 1602-1607 [PMID: 7497768]
61 **Duarte AG**, Terminella L, Smith JT, Myers AC, Campbell G, Lick S. Restoration of cough reflex in lung transplant recipients. *Chest* 2008; **134**: 310-316 [PMID: 18339778 DOI: 10.1378/chest.07-2934]
62 **Reid KR**, McKenzie FN, Menkis AH, Novick RJ, Pflugfelder PW, Kostuk WJ, Ahmad D. Importance of chronic aspiration in recipients of heart-lung transplants. *Lancet* 1990; **336**: 206-208 [PMID: 1973771]

63 **Au J**, Hawkins T, Venables C, Morritt G, Scott CD, Gascoigne AD, Corris PA, Hilton CJ, Dark JH. Upper gastrointestinal dysmotility in heart-lung transplant recipients. *Ann Thorac Surg* 1993; **55**: 94-97 [PMID: 8417718]

64 **Bodet-Milin C**, Querellou S, Oudoux A, Haloun A, Horeau-Llanglard D, Carlier T, Bizais Y, Couturier O. Delayed gastric emptying scintigraphy in cystic fibrosis patients before and after lung transplantation. *J Heart Lung Transplant* 2006; **25**: 1077-1083 [PMID: 16962469 DOI: 10.1016/j.healun.2006.04.013]

65 **Suen HC**, Hendrix H, Patterson GA. Physiologic consequences of pneumonectomy. Consequences on the esophageal function. *Chest Surg Clin N Am* 1999; **9**: 475-483, xiii [PMID: 10365277]

66 **Kopec SE**, Irwin RS, Umali-Torres CB, Balikian JP, Conlan AA. The postpneumonectomy state. *Chest* 1998; **114**: 1158-1184 [PMID: 9792592]

67 **Veale D**, Glasper PN, Gascoigne A, Dark JH, Gibson GJ, Corris PA. Ciliary beat frequency in transplanted lungs. *Thorax* 1993; **48**: 629-631 [PMID: 8346493]

68 **Kirk AJ**, Colquhoun IW, Corris PA, Hilton CJ, Dark JH. Impaired gastrointestinal motility in pulmonary transplantation. *Lancet* 1990; **336**: 752 [PMID: 1975923]

69 **Herve P**, Silbert D, Cerrina J, Simonneau G, Dartevelle P. Impairment of bronchial mucociliary clearance in long-term survivors of heart/lung and double-lung transplantation. The Paris-Sud Lung Transplant Group. *Chest* 1993; **103**: 59-63 [PMID: 8380268]

70 **Sodhi SS**, Guo JP, Maurer AH, O'Brien G, Srinivasan R, Parkman HP. Gastroparesis after combined heart and lung transplantation. *J Clin Gastroenterol* 2002; **34**: 34-39 [PMID: 11743243]

71 **Hartwig MG**, Appel JZ, Li B, Hsieh CC, Yoon YH, Lin SS, Irish W, Parker W, Davis RD. Chronic aspiration of gastric fluid accelerates pulmonary allograft dysfunction in a rat model of lung transplantation. *J Thorac Cardiovasc Surg* 2006; **131**: 209-217 [PMID: 16399314 DOI: 10.1016/j.jtcvs.2005.06.054]

72 **Li B**, Hartwig MG, Appel JZ, Bush EL, Balsara KR, Holzknecht ZE, Collins BH, Howell DN, Parker W, Lin SS, Davis RD. Chronic aspiration of gastric fluid induces the development of obliterative bronchiolitis in rat lung transplants. *Am J Transplant* 2008; **8**: 1614-1621 [PMID: 18557728 DOI: 10.111/j.1600-6143.2008.02298.x]

73 **Meltzer AJ**, Weiss MJ, Veillette GR, Sahara H, Ng CY, Cochrane ME, Houser SL, Sachs DH, Rosengard BR, Madsen JC, Wain JC, Allan JS. Repetitive gastric aspiration leads to augmented indirect allorecognition after lung transplantation in miniature swine. *Transplantation* 2008; **86**: 1824-1829 [PMID: 19104429 DOI: 10.1097/TP.0b013e318190afe6]

74 **Fisichella PM**, Davis CS, Lowery E, Pittman M, Gagermeier J, Love RB, Kovacs EJ. Pulmonary immune changes early after laparoscopic antireflux surgery in lung transplant patients with gastroesophageal reflux disease. *J Surg Res* 2012; **177**: e65-e73 [PMID: 22537841 DOI: 10.1016/j.jss.2012.03.066]

75 **Robertson AG**, Krishnan A, Ward C, Pearson JP, Small T, Corris PA, Dark JH, Karat D, Shenfine J, Griffin SM. Anti-reflux surgery in lung transplant recipients: outcomes and effects on quality of life. *Eur Respir J* 2012; **39**: 691-697 [PMID: 21778169 DOI: 10.1183/09031936.00061811]

76 **Vos R**, Blondeau K, Vanaudenaerde BM, Mertens V, Van Raemdonck DE, Sifrim D, Dupont LJ, Verleden GM. Airway colonization and gastric aspiration after lung transplantation: do birds of a feather flock together? *J Heart Lung Transplant* 2008; **27**: 843-849 [PMID: 18656796 DOI: 10.1016/j.healun.2008.05.022]

77 **Reynaud-Gaubert M**, Thomas P, Gregoire R, Badier M, Cau P, Sampol J, Giudicelli R, Fuentes P. Clinical utility of bronchoalveolar lavage cell phenotype analyses in the postoperative monitoring of lung transplant recipients. *Eur J Cardiothorac Surg* 2002; **21**: 60-66 [PMID: 11788258]

78 **Blondeau K**, Mertens V, Vanaudenaerde BA, Verleden GM, Van Raemdonck DE, Sifrim D, Dupont LJ. Gastro-oesophageal reflux and gastric aspiration in lung transplant patients with or without chronic rejection. *Eur Respir J* 2008; **31**: 707-713 [PMID: 18057058 DOI: 10.1183/09031936.00064807]

79 **Ward C**, Forrest IA, Brownlee IA, Johnson GE, Murphy DM, Pearson JP, Dark JH, Corris PA. Pepsin like activity in bronchoalveolar lavage fluid is suggestive of gastric aspiration in lung allografts. *Thorax* 2005; **60**: 872-874 [PMID: 16055614 DOI: 10.1163/thx.2004.036426]

80 **Oelberg DG**, Downey SA, Flynn MM. Bile salt-induced intracellular Ca++ accumulation in type II pneumocytes. *Lung* 1990; **168**: 297-308 [PMID: 2126319]

81 **Aaronson KD**, Slaughter MS, Miller LW, McGee EC, Cotts WG, Acker MA, Jessup ML, Gregoric ID, Loyalka P, Frazier OH, Jeevanandam V, Anderson AS, Kormos RL, Teuteberg JJ, Levy WC, Naftel DC, Bittman RM, Pagani FD, Hathaway DR, Boyce SW. Use of an intrapericardial, continuous-flow, centrifugal pump in patients awaiting heart transplantation. *Circulation* 2012; **125**: 3191-3200 [PMID: 22619284 DOI: 10.1161/CIRCULATIONAHA.111.058412]

82 **D'Ovidio F**, Keshavjee S. Gastroesophageal reflux and lung transplantation. *Dis Esophagus* 2006; **19**: 315-320 [PMID: 16984525 DOI: 10.1111/J.1442-2050.2006.00603.x]

83 **Ward C**, Walters EH, Zheng L, Whitford H, Williams TJ, Snell GI. Increased soluble CD14 in bronchoalveolar lavage fluid of stable lung transplant recipients. *Eur Respir J* 2002; **19**: 472-478 [PMID: 11936525]

84 **Davis CS**, Mendez BM, Flint DV, Pelletiere K, Lowery E, Ramirez L, Love RB, Kovacs EJ, Fisichella PM. Pepsin concentrations are elevated in the bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis after lung transplantation. *J Surg Res* 2013; **185**: e101-e108 [PMID: 23845868 DOI: 10.1016/j.jss.2013.06.011]

85 **Kroshus TJ**, Kshettry VR, Savik K, John R, Hertz MI, Bolman RM. Risk factors for the development of bronchiolitis obliterans syndrome after lung transplantation. *J Thorac Cardiovasc Surg* 1997; **114**: 195-202 [PMID: 9270635 DOI: 10.1016/S0022-5223(97)70144-2]

86 **Egan JJ**. Obliterative bronchiolitis after lung transplantation: a repetitive multiple injury airway disease. *Am J Respir Crit Care Med* 2004; **170**: 931-932 [PMID: 15504816 DOI: 10.1164/rccm.2408010]

87 **Cohen S**, Saxena A, Waljee AK, Piraka C, Purdy J, Appelman H, McKenna B, Elmunzer BJ, Singal AG. Lymphocytic esophagitis: a diagnosis of increasing frequency. *J Clin Gastroenterol* 2012; **46**: 828-832 [PMID: 22751335 DOI: 10.1097/MCG.0b013e3182500de8]

88 **Patti MG**, Debas HT, Pellegrini CA. Clinical and functional characterization of high gastroesophageal reflux. *Am J Surg* 1993; **165**: 163-166; discussion 166-168 [PMID: 8418693]

89 **Oelschlager BK**, Chang L, Pope CE, Pellegrini CA. Typical GERD symptoms and esophageal pH monitoring are not enough to diagnose pharyngeal reflux. *J Surg Res* 2005; **128**: 55-60 [PMID: 16115493 DOI: 10.1016/j.jss.2005.02.021]

90 **Tomonaga T**, Awad ZT, Filipi CJ, Hinder RA, Selima M, Tercero F, Marsh RE, Shiino Y, Welch R. Symptom predictability of reflux-induced respiratory disease. *Dig Dis Sci* 2002; **47**: 9-14 [PMID: 11837739]

91 **Koufman JA**. The otolaryngologic manifestations of gastroesophageal reflux disease (GERD): a clinical investigation of 225 patients using ambulatory 24-hour pH monitoring and an experimental investigation of the role of acid and pepsin in the development of laryngeal injury. *Laryngoscope* 1991; **101**: 1-78 [PMID: 1895864]

92 **Irwin RS**. Chronic cough due to gastroesophageal reflux disease: ACCP evidence-based clinical practice guidelines. *Chest* 2006; **129**: 80S-94S [PMID: 16428697]

93 Macha M, Molina E, Monteiro Gul. e. 225: gastroesophageal reflux does not impact survival after lung transplantation. *J Heart Lung Transplant* 2007; **26**: S140 [DOI: 10.1016/j.healun.2006.11.243]

94 **Chotiprashidi P**, Liu J, Carpenter S, Chuttani R, DiSario J, Hussain N, Somogyi L, Petersen BT. ASGE Technology Status Evaluation Report: wireless esophageal pH monitoring system. *Gastrointest Endosc* 2005; **62**: 485-487 [PMID: 16185957 DOI: 10.1016/j.gie.2005.07.007]

95 **Bredenoord AJ**, Smout AJ. Esophageal motility testing: impedance-based transit measurement and high-resolution manometry. *Gastroenterol Clin North Am* 2008; **37**: 775-791, vii [PMID: 19028317 DOI: 10.1016/j.gtc.2008.09.010]

96 **Bredenoord AJ**, Tutuian R, Smout AJ, Castell DO. Technology review: Esophageal impedance monitoring. *Am J Gastroenterol* 2007; **102**: 187-194 [PMID: 17100961 DOI: 10.1111/j.1572-0241.2006.00966.x]

97 **Wise JL**, Murray JA. Utilising multichannel intraluminal impedance for diagnosing GERD: a review. *Dis Esophagus* 2007; **20**: 83-88 [PMID: 17439589 DOI: 10.1111/j.1442-2050.2007.00654.x]

98 **Lo WK**, Burakoff R, Goldberg HJ, Feldman N, Chan WW. Pre-lung transplant measures of reflux on impedance are superior to pH testing alone in predicting early allograft injury. *World J Gastroenterol* 2015; **21**: 9111-9117 [PMID: 26290637 DOI: 10.3748/wjg.v21.i30.9111]

99 **Tasker A**, Dettmar PW, Panetti M, Koufman JA, P Birchall J, Pearson JP. Is gastric reflux a cause of otitis media with effusion in children? *Laryngoscope* 2002; **112**: 1930-1934 [PMID: 12439157 DOI: 10.1097/00005537-200211000-0004]

100 **Tasker A**, Dettmar PW, Panetti M, Koufman JA, Birchall JP, Pearson JP. Reflux of gastric juice and glue ear in children. *Lancet* 2002; **359**: 493 [PMID: 11853797 DOI: 10.1016/S0140-6736(02)07665-1]

101 **Ufberg JW**, Bushra JS, Patel D, Wong E, Karras DJ, Kueppers F. A new pepsin assay to detect pulmonary aspiration of gastric contents among newly intubated patients. *Am J Emerg Med* 2004; **22**: 612-614 [PMID: 15666273]

102 **Lo WK**, Burakoff R, Goldberg HJ, Feldman N, Chan WW. Pre-transplant impedance measures of reflux are associated with early allograft injury after lung transplantation. *J Heart Lung Transplant* 2015; **34**: 26-35 [PMID: 25444368 DOI: 10.1016/j.healun.2014.09.005]

103 **Lo WK**, Goldberg HJ, Wee J, Fisichella PM, Chan WW. Both Pre-Transplant and Early Post-Transplant Antireflux Surgery Prevent Development of Early Allograft Injury After Lung Transplantation. *J Gastrointest Surg* 2016; **20**: 111-118; discussion 118 [PMID: 26493975 DOI: 10.1007/s11605-015-2983-0]

104 **Lee JS**, Collard HR, Anstrom KJ, Martinez FJ, Noth I, Roberts RS, Yow E, Raghu G. Anti-acid treatment and disease progression in idiopathic pulmonary fibrosis: an analysis of data from three randomised controlled trials. *Lancet Respir Med* 2013; **1**: 369-376 [PMID: 24429201 DOI: 10.1016/S2213-2600(13)70105-X]

105 **Raghu G**, Meyer KC. Silent gastro-oesophageal reflux and microaspiration in IPF: mounting evidence for anti-reflux therapy? *Eur Respir J* 2012; **39**: 242-245 [PMID: 22298612 DOI: 10.118/09031936.00211311]

106 **Lee JS**, Ryu JH, Elicker BM, Lydell CP, Jones KD, Wolters PJ, King TE, Collard HR. Gastroesophageal reflux therapy is associated with longer survival in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011; **184**: 1390-1394 [PMID: 21700909 DOI: 10.1164/rccm.201101-0138OC]

107 **Allaix ME**, Fisichella PM, Noth I, Herbella FA, Borraez Segura B, Patti MG. Idiopathic pulmonary fibrosis and gastroesophageal reflux. Implications for treatment. *J Gastrointest Surg* 2014; **18**: 100-114; discussion 100-114 [PMID: 24002768 DOI: 10.1007/s11605-013-2333-z]

108 **Hoppo T**, Komatsu Y, Jobe BA. Gastroesophageal reflux disease and patterns of reflux in patients with idiopathic pulmonary fibrosis using hypopharyngeal multichannel intraluminal impedance. *Dis Esophagus* 2014; **27**: 530-537 [PMID: 23107023 DOI: 10.1111/j.1442-2050.2012.01446.x]

109 **Lozo Vukovac E**, Lozo M, Mise K, Gudelj I, Puljiz Ž, Jurcev-Savicevic A, Bradaric A, Kokeza J, Mise J. Bronchoalveolar pH and inflammatory biomarkers in newly diagnosed IPF and GERD patients: a case-control study. *Med Sci Monit* 2014; **20**: 255-261 [PMID: 24535066 DOI: 10.12659/MSM.889800]

110 **Verleden GM**, Dupont LJ. Azithromycin therapy for patients with bronchiolitis obliterans syndrome after lung transplantation. *Transplantation* 2004; **77**: 1465-1467 [PMID: 15167610]

111 **Verleden GM**, Vanaudenaerde BM, Dupont LJ, Van Raemdonck DE. Azithromycin reduces airway neutrophilia and interleukin-8 in patients with bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med* 2006; **174**: 566-570 [PMID: 16741151 DOI: 10.1164/rccm.200601-071OC]

112 **Mertens V**, Blondeau K, Pauwels A, Farre R, Vanaudenaerde B, Vos R, Verleden G, Van Raemdonck DE, Dupont LJ, Sifrim D. Azithromycin reduces gastroesophageal reflux and aspiration in lung transplant recipients. *Dig Dis Sci* 2009; **54**: 972-979 [PMID: 19241165 DOI: 10.1007/s10620-009-0725-4]

113 **Tamhankar AP**, Peters JH, Portale G, Hsieh CC, Hagen JA, Bremner CG, DeMeester TR. Omeprazole does not reduce gastroesophageal reflux: new insights using multichannel intraluminal impedance technology. *J Gastrointest Surg* 2004; **8**: 890-897; discussion 897-898 [PMID: 15531244 DOI: 10.1016/j.gassur.2004.08.001]

114 **Patti MG**, Arcerito M, Tamburini A, Diener U, Feo CV, Safadi B, Fisichella P, Way LW. Effect of laparoscopic fundoplication on gastroesophageal reflux disease-induced respiratory symptoms. *J Gastrointest Surg* 2000; **4**: 143-149 [PMID: 10675237]

115 **Vela MF**, Camacho-Lobato L, Srinivasan R, Tutuian R, Katz PO, Castell DO. Simultaneous intraesophageal impedance and pH measurement of acid and nonacid gastroesophageal reflux: effect of omeprazole. *Gastroenterology* 2001; **120**: 1599-1606 [PMID: 11375942]

116 **Tack J**, Koek G, Demedts I, Sifrim D, Janssens J. Gastroesophageal reflux disease poorly responsive to single-dose proton pump inhibitors in patients without Barrett's esophagus: acid reflux, bile reflux, or both? *Am J Gastroenterol* 2004; **99**: 981-988 [PMID: 15180713 DOI: 10.111/j.1572-0241.2004.04171.x]

117 **Linden PA**, Gilbert RJ, Yeap BY, Boyle K, Deykin A, Jaklitsch MT, Sugarbaker DJ, Bueno R. Laparoscopic fundoplication in patients with end-stage lung disease awaiting transplantation. *J Thorac Cardiovasc Surg* 2006; **131**: 438-446 [PMID: 16434276 DOI: 10.1016/j.jtcvs.2005.10.014]

118 **Lau CL**, Palmer SM, Howell DN, McMahon R, Hadjiliadis D, Gaca J, Pappas TN, Davis RD, Eubanks S. Laparoscopic antireflux surgery in the lung transplant population. *Surg Endosc* 2002; **16**: 1674-1678 [PMID: 12140642 DOI: 10.1007/s00464-001-8251-2]

119 **Fisichella PM**, Davis CS, Gagermeier J, Dilling D, Alex CG, Dorfmeister JA, Kovacs EJ, Love RB, Gamelli RL. Laparoscopic antireflux surgery for gastroesophageal reflux disease after lung transplantation. *J Surg Res* 2011; **170**: e279-e286 [PMID: 21816422 DOI: 10.1016/j.jss.2011.05.038]

120 **Burton PR**, Button B, Brown W, Lee M, Roberts S, Hassen S, Bailey M, Smith A, Snell G. Medium-term outcome of fundoplication after lung transplantation. *Dis Esophagus* 2009; **22**: 642-648 [PMID: 19515194 DOI: 10.1111/j.1442-2050.2009.00980.x]

121 **O'Halloran EK**, Reynolds JD, Lau CL, Manson RJ, Davis RD, Palmer SM, Pappas TN, Clary EM, Eubanks WS. Laparoscopic Nissen fundoplication for treating reflux in lung transplant recipients. *J Gastrointest Surg* 2004; **8**: 132-137 [PMID: 14746846]

122 **Gasper WJ**, Sweet MP, Hoopes C, Leard LE, Kleinhenz ME, Hays SR, Golden JA, Patti MG. Antireflux surgery for patients with end-stage lung disease before and after lung transplantation. *Surg Endosc* 2008; **22**: 495-500 [PMID: 17704875 DOI: 10.1007/s00464-007-9494-3]

123 **Robertson AG**, Griffin SM, Murphy DM, Pearson JP, Forrest IA, Dark JH, Corris PA, Ward C. Targeting allograft injury and inflammation in the management of post-lung transplant bronchiolitis obliterans syndrome. *Am J Transplant* 2009; **9**: 1272-1278 [PMID: 19459806 DOI: 10.1111/j.1600-7143.2009.02648.x]

124 **Halsey KD**, Wald A, Meyer KC, Torrealba JR, Gaumnitz EA. Non-acidic supraesophageal reflux associated with diffuse alveolar damage and allograft dysfunction after lung transplantation: a case report. *J Heart Lung Transplant* 2008; **27**: 564-567 [PMID: 18442725 DOI: 10.1016/j.healun.2008.01.020]

125 **Palmer SM**, Miralles AP, Howell DN, Brazer SR, Tapson VF, Davis RD. Gastroesophageal reflux as a reversible cause of allograft dysfunction after lung transplantation. *Chest* 2000; **118**: 1214-1217 [PMID: 11035701]

126 **Hartwig MG**, Anderson DJ, Onaitis MW, Reddy S, Snyder LD, Lin

SS, Davis RD. Fundoplication after lung transplantation prevents the allograft dysfunction associated with reflux. *Ann Thorac Surg* 2011; **92**: 462-468; discussion; 468-469 [PMID: 21801907 DOI: 10.1016/j.athoracsur.2011.04.035]

P- Reviewer: Belliato M, Herbella FAM, Nosotti M
S- Editor: Kong JX **L- Editor**: A **E- Editor**: Wu HL

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 April 24; 7(2): 117-128

DOI: 10.5500/wjt.v7.i2.117

ISSN 2220-3230 (online)

REVIEW

Intra-islet endothelial cell and β-cell crosstalk: Implication for islet cell transplantation

Siddharth Narayanan, Gopalakrishnan Loganathan, Maheswaran Dhanasekaran, William Tucker, Ankit Patel, Venugopal Subhashree, SriPrakash Mokshagundam, Michael G Hughes, Stuart K Williams, Appakalai N Balamurugan

Siddharth Narayanan, Gopalakrishnan Loganathan, Maheswaran Dhanasekaran, William Tucker, Ankit Patel, SriPrakash Mokshagundam, Michael G Hughes, Stuart K Williams, Appakalai N Balamurugan, Clinical Islet Cell Laboratory, Center for Cellular Transplantation, Cardiovascular Innovation Institute, Department of Surgery, University of Louisville, Louisville, KY 40202, United States

Venugopal Subhashree, School of Biosciences and Technology, VIT University, Vellore 632014, India

Author contributions: All authors contributed to this paper.

Conflict-of-interest statement: None declared.

Manuscript source: Invited manuscript

Correspondence to: Appakalai N Balamurugan, PhD, Clinical Islet Cell Laboratory, Center for Cellular Transplantation, Cardiovascular Innovation Institute, Department of Surgery, University of Louisville, 302 E Muhammad Ali Blvd., Louisville, KY 40202, United States. bala.appakalai@louisville.edu
Telephone: +1-502-7947070
Fax: +1-502-8540558

Received: January 4, 2017
Peer-review started: January 7, 2017
First decision: February 17, 2017
Revised: February 28, 2017
Accepted: March 23, 2017
Article in press: March 24, 2017

Published online: April 24, 2017

Abstract

The intra-islet microvasculature is a critical interface between the blood and islet endocrine cells governing a number of cellular and pathophysiological processes associated with the pancreatic tissue. A growing body of evidence indicates a strong functional and physical interdependency of β-cells with endothelial cells (ECs), the building blocks of islet microvasculature. Intra-islet ECs, actively regulate vascular permeability and appear to play a role in fine-tuning blood glucose sensing and regulation. These cells also tend to behave as "guardians", controlling the expression and movement of a number of important immune mediators, thereby strongly contributing to the physiology of islets. This review will focus on the molecular signalling and crosstalk between the intra-islet ECs and β-cells and how their relationship can be a potential target for intervention strategies in islet pathology and islet transplantation.

Key words: Islets; Endothelial cells; Islet cell transplantation; Beta-cells; Microvasculature; Paracrine signalling

Core tip: This review article summarizes recent developments in the cross-talk relationship between intra-islet endothelial cells and beta cells. The molecules involved in the signalling pathways can be potential targets for therapeutic strategies and islet transplantation.

Narayanan S, Loganathan G, Dhanasekaran M, Tucker W, Patel A, Subhashree V, Mokshagundam S, Hughes MG, Williams SK, Balamurugan AN. Intra-islet endothelial cell and β-cell crosstalk: Implication for islet cell transplantation. *World J Transplant* 2017; 7(2): 117-128 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i2/117.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i2.117

INTRODUCTION

Pancreatic islets represent endocrine "island" cell clusters, embedded and scattered throughout the pancreas within large amounts of exocrine acinar tissue[1]. Islets are perfused by a dense, specialized microcirculation and receive 10% of the pancreatic blood flow despite comprising only 1%-2% of the overall tissue mass[2]. Most islets are irregularly shaped spheroids with a size distribution ranging from 50-500 μm, each composed of 800-3000 individual cells. The islet microcirculation is characterized by pre islet arterioles that rapidly arcade to a dense population of capillaries[3].

The cellular components of the islet include β-cells, other endocrine cells, as well as endothelium, perivascular, and support cells such as pericytes[4-9]. The cellular composition of islets is not uniform across species. Rodent and rabbit islets are primarily composed of a β-cell core with other cell types in the periphery whereas human and primate islets exhibit endocrine cell types intermingled with each other[4,10,11]. Beta cells, the central regulator of glucose homeostasis, are the largest cellular component of islets in most species[9,10].

Studies using vascular corrosion casts have demonstrated that 1-3 arterioles feed larger islets[12]. The capillary network within islets is about five times denser in comparison with exocrine tissue[3]. The capillary wall is composed of a permeable layer of ECs and contains ten times more fenestrae than ECs present in the exocrine pancreas[13,14]. The islet endothelial fenestra are highly specialized and contain a diaphragm that regulates solute transport[15,16]. Typically, a microvessel consists of ECs arranged into a tube formation wrapped by one or more layers of perivascular cells. Vascular ECs represent a major cell type present in islets and these cells are organized into a highly regulated and morphologically unique microcirculation. In culture, islet ECs express the classic endothelial markers such as von Willebrand factor, CD31, CD105, CD146, uptake of acetylated LDL, expression of leucocyte adhesion molecules, contain Weibel-Palade bodies in the cytoplasm, and form tight junctions[17,18]. Other markers expressed within islet ECs include α-1 antitrypsin, a major proteinase inhibitor[17,19,20]; nephrin, a highly specific barrier protein[16]; platelet-activating factor receptor[21], and genes expressing angiogenic (vascular endothelial growth factor, VEGF) and angiostatic (endostatin, pigment epithelial-derived factor) molecules[22].

Islet ECs have a significant relationship with islet function. For example, islets grafts, when co-transplanted[23] with ECs in diabetes induced rats or coated[24] with ECs in diabetes induced mice, have better engraftment capacity and improved islet function. Donor islet ECs, immediately after transplantation, participate in neovascularization by increasing β-cell survival[25] and promote both pancreatic stem cell proliferation and islet regeneration after β-cell injury[26]. Research performed over the last two decades has evaluated the link between islets and the ECs, demonstrating how the molecular interplay between these two cell types can regulate many critical physiological processes associated with the islet.

THE SIGNALS FROM β-CELL TO ECS

In vitro studies demonstrate that conditioned medium derived from cultured rat islets induces liver and islet-derived EC proliferation and migration[27], suggesting presence and secretion of paracrine pro-angiogenic factors (Figure 1) which promote islet vascularization[28]. As a major soluble β-cell secreted product, insulin promotes β-cell survival. In addition, insulin causes the upregulation of endothelial nitric oxide synthase in ECs promoting intra-islet blood flow[29]. Post-natal beta mass is dynamic and can increase in function and mass to compensate for additional physiological requirements[30].

VEGFs

The family of VEGF ligands and their receptors are critical as they regulate a number of developmental processes and play major roles in wound healing and vessel homeostasis in adult organisms[31,32]. VEGF secretion is stimulated by tumor, hypoxia, low pH and many other factors. Beta-cells secrete large amounts of VEGF-A early in development and throughout adult life[33]. The VEGF binds to its receptor (VEGFR) located on the blood vessel ECs, which activates multiple signalling cascades eventually resulting in the production of enzymes and other specific molecules required for EC growth and proliferation. Other activation effects include mobilization of endothelial progenitor cells from bone marrow, increased vascular permeability and tissue factor induction[34]. The VEGF family comprises seven secreted glycoproteins that are designated VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental growth factor and VEGF-F[35-37]. VEGF family members interact with three main receptors, VEGFR-1 (FLt-1), VEGFR-2 (KDR in humans and Flk-1 in mice) and VEGFR-3 (Flt4), all tyrosine kinase receptors and members of the PGDF receptor family. VEGFR-2 appears to be the main receptor responsible for mediating the proangiogenic effects of VEGF-A[35,38,39]. The consequence of this specific ligand-receptor interaction facilitates EC proliferation *via* the PKC-Ras pathway (by inducing MAPK/ERK pathways)[40,41]; promotes cytoskeletal reorganization and cell migration *via* p38 and focal adhesion kinase

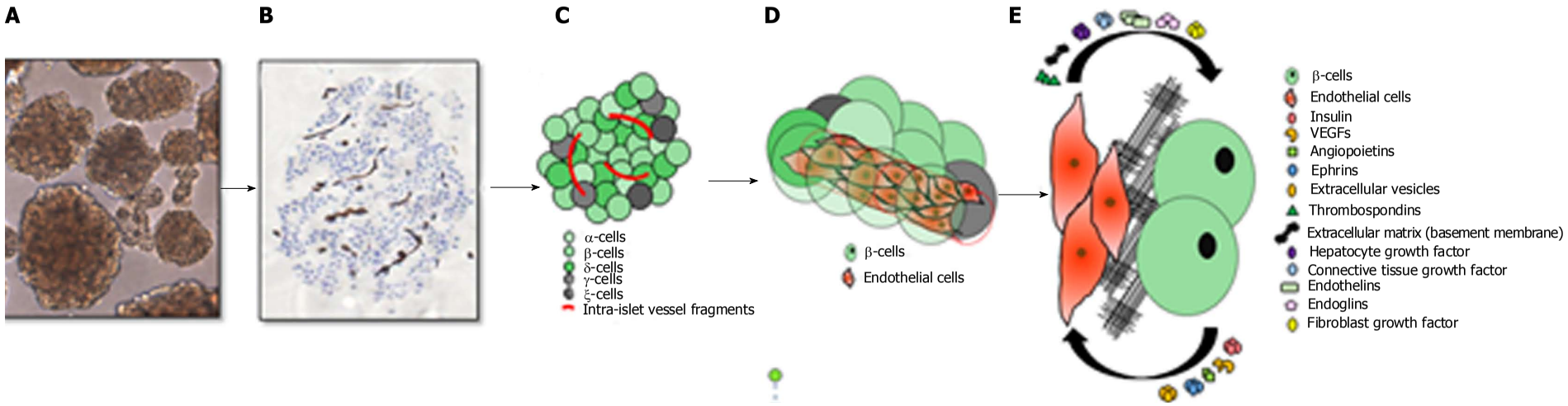


Figure 1 A model demonstrating the intra-islet endothelial cell and β-cell crosstalk. A: An image of freshly isolated human islets; B: Immunohistochemical staining of an islet demonstrating intra-islet vessels stained with CD31 (brown); C: Schematic representation of different cells within an islet along with intra-islet vessel fragments; D: A three dimensional (3D) depiction of islet cells and how these surround the intra-islet vessels, which are a group of endothelial cells arranged into a tube like structure; E: A model demonstrating a cross-talk relationship between endothelial cells and β-cells mediated by various endocrine factors/molecules. VEGFs, angiopoietins, insulin, cell surface molecules including ephrins mainly produced by the β-cell, are important factors for endothelial cell proliferation. Endothelium-derived factors such as hepatocyte growth factor, thrombospondins, basement membrane components (laminins, collagens) improve β-cell survival and promote insulin transcription and secretion. Other EC-derived factors include fibroblast growth factor and the vasoconstrictive endothelin-1. VEGF: Vascular endothelial growth factor; EC: Endothelial cell.

activation[42]; and supports EC cell survival and migration by activating the PI3K/Akt/PKB pathway[43,44].

VEGF-A is known to utilize the VEGFR-2 receptor on ECs[45], with the receptor highly expressed in intra-islet capillaries[46]. VEGF likely stimulates EC growth in neonatal pancreas; increased levels of VEGF-A correspond with islet growth in pregnant rats[47]. VEGF-A signaling is also essential in maintaining vascular beds in adult islets, this was validated using VEGF receptor antagonists[48]. VEGF-A expression is further upregulated in islets by hypoxia and glucose[49,50] and is important for the establishment of native intra-islet vasculature[51], maintenance of β-cell mass[52], and the revascularization of islets following transplantation[53].

Angiopoietins

Apart from VEGF-A, other known factors such as those within Ang/Tie family are known to contribute towards the survival and integrity of blood vessels[33,54,55]. These angiogenic factors consist of ligands Ang-1, Ang-2 and Ang-4 (its mouse orthologue, Ang-3) and the tyrosine kinase receptors Tie-1 and Tie-2. Ang-1 is expressed mainly by the perivascular cells and β-cells in mouse and human islets[33], and its agonist Tie-2 is expressed by the ECs. Ang-1 activates the pI3k/Akt pathway and prevents cytokine mediated apoptosis in ECs[56]. Moreover, β-cell specific overexpression of Ang-1 or Ang-2 only slightly impairs insulin secretion and glucose tolerance together with marginal altered vascularization, islet mass and morphology[57]. Reports also suggest that Ang-1/Tie-2 signaling promotes cell-cell contacts and contact to extracellular matrix (ECM)[58,59]. Ang-2 however, expressed by ECs, classically antagonizes Tie-2 signaling[60] and plays key roles in angiogenesis and inflammation.

Ephrins

Ephrin ligands and their tyrosine kinase receptors are involved in various aspects of cell communication[61,62]. Each ephrin ligand together with its specific receptor (Eph) is categorised either into the A or B subclass. Most EphA receptors bind to ephrin-A ligand, while most EphB receptors bind to ephrin-B ligands[63]. Transcriptome analyses

suggest that Eph-ephrin interaction between exocrine and endocrine cells contributes to pancreatic function[64]. Ephrin-A and its receptor EphA play a role in β-cell to β-cell communication; specifically, ephrin subtype A5 is required for glucose stimulated insulin secretion and the EphA-ephrin-A mediated interaction between β-cells is bidirectional[65]. The blood vessel ECs within pancreatic islets express Eph subtype A4 receptors[66] but how these ligands and receptors play a role between EC and β-cell crosstalk is subject to investigation.

Extracellular vesicles

Recent reports establish extracellular vesicles (EVs) as a novel player in cell-to-cell communication[67,68] and have been characterized both in human islets[69] and in experimental models of human islet xenotransplantation in SCID mice[70]. Studies exploring the functional contribution of β-cell EVs on islet ECs demonstrate that islet-derived EVs have the capacity to affect the surrounding ECs, which are then able to internalize the islet EVs in a dose dependent manner[69]. Furthermore, internalization of islet EVs results in transfer of multiple RNAs, including insulin mRNA and various microRNAs. Uptake of islet EVs conferred endothelial cell resistance to apoptosis and up-regulated expression of numerous proangiogenic factors[69]. In a different study, endothelial progenitor cell EVs, when internalized by islet α-, β- and ECs resulted in improved glucose-stimulated proliferation and angiogenesis[70].

THE ENDOCRINE EFFECT OF ISLET ECS ON β-CELLS

Islet ECs, apart from their pivotal role in angiogenesis, also possess endocrine function. They produce multiple factors (Figure 1) that govern proliferation, survival, and gene expression, which contribute to the physiology and function of the β-cell[71-75].

Basement membrane

ECM proteins provide biochemical cues interpreted by cell surface receptors and initiate signalling cascades controlling morphogenesis, cell survival, proliferation, differentiation, and stem cell state[76-78]. Islets are surrounded by a peri-islet basement membrane (BM) and an associated interstitial matrix containing multiple components such as collagen, laminin, fibronectin, perlecans, nidogens, and heparin sulphate[79,80]. Beta-cells depend on intra-islet ECs to synthesize their ECM components[75]. It has been reported that collagen IV, secreted by islet endothelium, can potentiate insulin secretion *via* interaction with its receptor integrin $\alpha_1\beta_1$ on β-cells[81] similar to other BM components such as laminins and fibronectin which have been reported to act as endothelial signals promoting insulin gene expression and proliferation in β-cells[75,82]. Interaction of collagen IV with its receptors also contributes to β-cell differentiation, maturation, and survival[83-85]. Other BM components such as fibronectin and heparin sulfate also play roles in β-cell migration, growth, differentiation and survival[1,86-88].

Connective tissue growth factor

The β-cell proliferative factor, connective tissue growth factor (CTGF/CCN2), is a member of the CCN family of secreted ECM-associated proteins[89,90] and is expressed in ECs during development[90,91]. It induces expression of platelet derived growth factor B (PDGF-B) in ECs, required for pericyte recruitment and retention[91]. CTGF promotes β-cell regeneration[92], proliferation[93], and modulates the response to high glucose[94]. Its inactivation results in defects in islet cell lineage allocation and β-cell proliferation during embryogenesis[95].

Hepatocyte growth factor

Islet ECs release the hepatocyte growth factor (HGF)[13] which induces β-cell proliferation and differentiation in embryonic and postnatal pancreas[47,75,95-98]. HGF plays a positive role in β-cell mitogenesis, differentiation, glucose sensing, and transplant survival[99,100]. *In vitro*, VEGF-A and insulin are islet-derived factors that induce the HGF secretion within purified islet ECs. *In vivo*, utilizing of pregnant rat pancreas, where a high physiological proliferation of β cells occur, resulted in a prominent expression of HGF, coinciding with the peak of β-cell proliferation[74].

Thrombospondins

Thrombospondins are matricellular glycoproteins that participate in a regulating cell proliferation, migration, and apoptosis, and have been implicated in angiogenesis, tumour invasion, and metastasis[101,102]. Thrombospondin-1 (TSP-1) is almost exclusively expressed by the intra-islet endothelium[71,103,104] and is not downregulated by hypoxia[105]. TSP-1 is mainly known for its antiangiogenic properties[106] but also may alter the morphology of pancreatic islets and function as a major activator of transforming growth factor TGFβ-1[107]. Animals deficient of this glycoprotein are characterized by hypervascular islets[107] and the EC-derived TSP-1 is important to maintain β-cell function postnatally[71].

Endothelins

Endothelin is a vasoconstrictive protein. Endothelin-1 (ET-1) predominantly is found to have strong effects on native islet blood vessels[108] while ET-1 and ET-3 may directly stimulate β-cell insulin secretion and release[73,109]. The gene expression of ET-1 in both ECs and islet endocrine cells is regulated by hypoxia[110,111]. Insulin can also stimulate the expression and secretion of ET-1 from bovine ECs[112] and endogenous insulin can regulate circulating ET-1 concentrations in humans[113]. ET-1 also upregulates the expression of the *FOXO1* gene

(encoding a transcription factor) on ECs contributing to its survival[114].

Endoglin

Endoglin (Eng) is a homodimeric transmembrane glycol protein within the TGF-β superfamily and is expressed by vascular ECs[115-118]. Studies have identified two distinct Eng positive cell types within human and mouse islets: The ECs and the mesenchymal stromal cells[119]. EC-specific endoglin expression in islets is sensitive to VEGF playing partial roles in driving islet vascular development[120].

IMPLICATIONS OF β-CELL AND ENDOTHELIAL CROSSTALK ON ISLET TRANSPLANTATION

Islet transplantation and revascularization

The human islet isolation technique completely severs the islet vasculature[121,122]. During the enzymatic digestion step, islets undergo a number of cellular assaults such as ischemia, mechanical stress, loss of basement proteins, and partial disruption of intra-islet ECs[123-125] resulting in a substantial loss of viability before transplantation. Other than being devoid of ECs to support rapid revascularization, cytotoxic damage and cell death account for a loss of up to 80% of transplanted islets[126,127]. Rapid and adequate revascularization is critical for survival and function of transplanted islets[121,128,129]. Transplanted islet grafts initially have a significant reduction in vascular supply and low oxygen tension in comparison to normal islets[130-132]. The return of islet function depends on re-establishment of new vessels within islet grafts to derive blood flow from the host vascular system[123,133]. Islet engraftment is a slow process, while the islet blood flow re-establishment requires about two weeks, vessel maturation is likely to take a much longer period. Using immunosuppressive drugs such as rapamycin further affect this process by exerting antiangiogenic activities on mouse and human islet endothelium[134].

Though transplanted islets are considered avascular, freshly isolated islets retain angiogenic capacity as they contain intra-islet ECs. These cells can be triggered by various inducers such as VEGF to form vessels *via* angiogenic sprouting[33,135,136]. Revascularization is an important process for adequate engraftment of islets. Prevascularizing islets prior to transplantation could potentially improve islet survivability and function by aiding islet-to-host inosculation[25]. The intra-islet vasculature can also act as a barrier against infiltrating insults of autoreactive cells in type 1 diabetes (T1D) thereby implicating ECs as an important target in type 2 diabetes (T2D)[137-139].

Studies involving cell and tissue engineering approaches have considered factors such as pancreatic islet size-dependency[140], use of stem cells[141-144], creating engineered vascular beds and hydrogels[145-147], endothelial progenitor cell derived microvesicles[70], and repurposed biological scaffolds[148] to improve islet revascularization potential. The angiogenic capacity of islet ECs has been previously determined[136]. A number of factors which may potentially improve islet transplantation involve ECs. For example, vascular ECs of the embryonic aorta induce the development of endocrine cells from pancreatic epithelium in mice[149,150] and the overexpression of VEGF-A in transplanted mouse islets improves insulin secretion and blood glucose regulation in recipient mice[33,53]. Identifying novel factors and understanding nature of mechanisms that underlie bidirectional communication between β-cells and ECs should be of immense relevance for improved human islet transplantation or preventing pancreas associated diseases such as pancreatitis and diabetes.

ECs and β-cell crosstalk: Islet pathophysiology, current perspectives and future directions

Evaluation of factors contributing to mechanisms responsible for regulating the interaction between β-cells and intra-islet ECs would broaden our understanding of pancreatic tissue function, growth, and disease. In this context, VEGF-A has been the most well studied molecule[51,53]; however, reports have suggested the detrimental effects of VEGF on islets. Continued β-cell overexpression of VEGF-A impairs islet morphology and function by eliciting an inflammatory response[57,151]. Elevated levels of serum VEGF, Ang-2, and soluble Tie-2 have also been associated with T2D and vascular dysfunction[152-154]. Achieving an optimal VEGF-A dose to potentiate islet vascularization is subject to further investigation. The HGF production is increased during pregnancy in adult rats[74] and helps balance high glucose levels in diabetes induced mice[155]. *HGF* gene therapy has been suggested as a potential approach for improving islet transplantation rates and treatment of diabetes[156,157].

The dense pancreatic vasculature along with its associated ECM plays a key role in the physiology and disease associated with pancreatic islets. The islet is an ideal "tissue" model because of its heterogeneous cell population embedded within the ECM. Understanding the nature of how these cells communicate with each other and with their underlying BM is crucial for normal islet physiology and pathology. The β-cells rely on intra-islet ECs to synthesise their ECM components[75]. This dependency may potentially be compromised in chronic inflammatory pancreatic diseases such as chronic pancreatitis which is characterized by a number of alterations within ECM formation and composition resulting in destruction of acinar and islet cells, and subsequent replacement by connective tissue[158,159]. This connective tissue appears to result from an increased deposition and disorganization of the ECM proteins including collagens, fibronectins, and laminins[160-163]. Moreover, reports also suggest that one of the most

enriched groups of over-expressed proteins in pancreatitis (mild and severe) and pancreatic ductal adenocarcinoma include those involved in the ECM structure and organization[164,165]. In addition, glycoproteins, especially those with N-linked glycosylation sites, are significantly enriched among the over-expressed proteins in mild and chronic pancreatitis[164]. Collagen, proteoglycans, and other ECM specialized glycoproteins such as fibrillin, fibronectin, and laminin, all part of the peri-islet BM, contain various degrees of glycosylation[166].

The connection between ECs and β-cells has been previously evaluated[28,51,57,167,168], particularly where different approaches have been utilized to increase β-cell mass and thereby insulin production. New factors have also been identified which may potentially contribute in further understanding islet cell communication and function. For example, R-spondins-1, an intestinal growth factor containing a thrombospondin domain, has been identified as a novel β-cell growth factor and insulin secretagogue[169]. It has potential to enhance β-cell growth and function in patients with T2D, and enhance of β-cell mass[170]. Connexins, ephrins, and cadherins, members of the transmembrane family of proteins are expressed in pancreatic islets. The major β-cell connexin is Cx36[171], Cx43, and Cx45 are specifically expressed on intra-islet ECs[172] whereas Cx30.2, recently identified, is expressed at cell-cell junctions in both cell types[173].

A number of studies have demonstrated that ECs play a very critical role within the islet microenvironment. A dysfunctional intra-islet vascular endothelium may contribute to the severity or progression of pancreatic disease etiologies. A deeper knowledge of islet endothelial phenotype and function will help identify specific targets and strategies for T1D prevention and successful outcomes for islet transplantation. Identifying and validating the potential therapeutic benefits of novel factors which either maintain the integrity of EC and β-cell communication or reinstate and balance the disrupted crosstalk is likely to benefit patients with diabetes and other pancreatic disorders.

ACKNOWLEDGMENTS

The authors thank the Jewish Heritage Fund for Excellence for providing generous support to our program. The authors sincerely thank Kentucky Organ Donor Affiliates (KODA) for the supply of human pancreases. Special thanks to Leigh Kleinert and Brian Gettler for their assistance.

REFERENCES

1 **Stendahl JC**, Kaufman DB, Stupp SI. Extracellular matrix in pancreatic islets: relevance to scaffold design and transplantation. *Cell Transplant* 2009; **18**: 1-12 [PMID: 19476204 DOI: 10.3727/096368909788237195]

2 **Chandra R**, Liddle RA. Neural and hormonal regulation of pancreatic secretion. *Curr Opin Gastroenterol* 2009; **25**: 441-446 [PMID: 19535978 DOI: 10.1097/MOG.0b013e32832e9c41]

3 **Henderson JR**, Moss MC. A morphometric study of the endocrine and exocrine capillaries of the pancreas. *Q J Exp Physiol* 1985; **70**: 347-356 [PMID: 3898188 DOI: 10.1113/expphysiol.1985.sp002920]

4 **Brissova M**, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, Harlan DM, Powers AC. Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. *J Histochem Cytochem* 2005; **53**: 1087-1097 [PMID: 15923354 DOI: 10.1369/jhc.5C6684.2005]

5 **Khandekar N**, Berning BA, Sainsbury A, Lin S. The role of pancreatic polypeptide in the regulation of energy homeostasis. *Mol Cell Endocrinol* 2015; **418** Pt 1: 33-41 [PMID: 26123585 DOI: 10.1016/j.mce.2015.06.028]

6 **Yada T**, Damdindorj B, Rita RS, Kurashina T, Ando A, Taguchi M, Koizumi M, Sone H, Nakata M, Kakei M, Dezaki K. Ghrelin signalling in β-cells regulates insulin secretion and blood glucose. *Diabetes Obes Metab* 2014; **16** Suppl 1: 111-117 [PMID: 25200304 DOI: 10.1111/dom.12344]

7 **DiGruccio MR**, Mawla AM, Donaldson CJ, Noguchi GM, Vaughan J, Cowing-Zitron C, van der Meulen T, Huising MO. Comprehensive alpha, beta and delta cell transcriptomes reveal that ghrelin selectively activates delta cells and promotes somatostatin release from pancreatic islets. *Mol Metab* 2016; **5**: 449-458 [PMID: 27408771 DOI: 10.1016/j.molmet.2016.04.007]

8 **Braun M**. The somatostatin receptor in human pancreatic β-cells. *Vitam Horm* 2014; **95**: 165-193 [PMID: 24559918 DOI: 10.1016/B978-0-12-800174-5.00007-7]

9 **Brereton MF**, Vergari E, Zhang Q, Clark A. Alpha-, Delta- and PP-cells: Are They the Architectural Cornerstones of Islet Structure and Co-ordination? *J Histochem Cytochem* 2015; **63**: 575-591 [PMID: 26216135 DOI: 10.1369/0022155415583535]

10 **Cabrera O**, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A. The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci USA* 2006; **103**: 2334-2339 [PMID: 16461897 DOI: 10.1073/pnas.0510790103]

11 **Röder PV**, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. *Exp Mol Med* 2016; **48**: e219 [PMID: 26964835 DOI: 10.1038/emm.2016.6]

12 **Bonner-Weir S**, Orci L. New perspectives on the microvasculature of the islets of Langerhans in the rat. *Diabetes* 1982; **31**: 883-889 [PMID: 6759221 DOI: 10.2337/diabetes.31.10.883]

13 **Olsson R**, Carlsson PO. The pancreatic islet endothelial cell: emerging roles in islet function and disease. *Int J Biochem Cell Biol* 2006; **38**: 710-714 [PMID: 16607697 DOI: 10.1016/j.biocel.2005.06.021]

14 **Cao Z**, Wang X. The endocrine role between β cells and intra-islet endothelial cells. *Endocr J* 2014; **61**: 647-654 [PMID: 24681780 DOI: 10.1507/endocrj.EJ14-0045]

15 **Hart TK**, Pino RM. Pseudoislet vascularization. Induction of diaphragm-fenestrated endothelia from the hepatic sinusoids. *Lab Invest* 1986; **54**: 304-313 [PMID: 2419665]

16 **Zanone MM**, Favaro E, Doublier S, Lozanoska-Ochser B, Deregibus MC, Greening J, Huang GC, Klein N, Cavallo Perin P, Peakman M, Camussi G. Expression of nephrin by human pancreatic islet endothelial cells. *Diabetologia* 2005; **48**: 1789-1797 [PMID: 16010520 DOI: 10.1007/s00125-005-1865-5]

17 **Lou J**, Triponez F, Oberholzer J, Wang H, Yu D, Buhler L, Cretin N, Mentha G, Wollheim CB, Morel P. Expression of alpha-1 proteinase inhibitor in human islet microvascular endothelial cells. *Diabetes* 1999; **48**: 1773-1778 [PMID: 10480607 DOI: 10.2337/diabetes.48.9.1773]

18 **Favaro E**, Bottelli A, Lozanoska-Ochser B, Ferioli E, Huang GC, Klein N, Chiaravalli A, Perin PC, Camussi G, Peakman M, Conaldi PG, Zanone MM. Primary and immortalised human pancreatic islet endothelial cells: phenotypic and immunological characterisation. *Diabetologia* 2005; **48**: 2552-2562 [PMID: 16292462 DOI:

10.1007/s00125-005-0008-3]
19 **Lewis EC**. Expanding the clinical indications for α(1)-antitrypsin therapy. *Mol Med* 2012; **18**: 957-970 [PMID: 22634722 DOI: 10.2119/molmed.2011.00196]
20 **Papaccio G**, Pedullà M, Ammendola E, Todaro M. Cytokine regulatory effects on alpha-1 proteinase inhibitor expression in NOD mouse islet endothelial cells. *J Cell Biochem* 2002; **85**: 123-130 [PMID: 11891856 DOI: 10.1002/jcb.10114]
21 **Flickinger BD**, Olson MS. Localization of the platelet-activating factor receptor to rat pancreatic microvascular endothelial cells. *Am J Pathol* 1999; **154**: 1353-1358 [PMID: 10329588 DOI: 10.1016/S0002-9440(10)65389-8]
22 **Mattsson G**, Danielsson A, Kriz V, Carlsson PO, Jansson L. Endothelial cells in endogenous and transplanted pancreatic islets: differences in the expression of angiogenic peptides and receptors. *Pancreatology* 2006; **6**: 86-95 [PMID: 16327285 DOI: 10.1159/000090027]
23 **Pan X**, Xue W, Li Y, Feng X, Tian X, Ding C. Islet graft survival and function: concomitant culture and transplantation with vascular endothelial cells in diabetic rats. *Transplantation* 2011; **92**: 1208-1214 [PMID: 22067310 DOI: 10.1097/TP.0b013e3182356ca7]
24 **Barba-Gutierrez DA**, Daneri-Navarro A, Villagomez-Mendez JJ, Kanamune J, Robles-Murillo AK, Sanchez-Enriquez S, Villafan-Bernal JR, Rivas-Carrillo JD. Facilitated Engraftment of Isolated Islets Coated With Expanded Vascular Endothelial Cells for Islet Transplantation. *Transplant Proc* 2016; **48**: 669-672 [PMID: 27110026 DOI: 10.1016/j.transproceed.2016.02.036]
25 **Nyqvist D**, Speier S, Rodriguez-Diaz R, Molano RD, Lipovsek S, Rupnik M, Dicker A, Ilegems E, Zahr-Akrawi E, Molina J, Lopez-Cabeza M, Villate S, Abdulreda MH, Ricordi C, Caicedo A, Pileggi A, Berggren PO. Donor islet endothelial cells in pancreatic islet revascularization. *Diabetes* 2011; **60**: 2571-2577 [PMID: 21873551 DOI: 10.2337/db10-1711]
26 **Rivas-Carrillo SD**, Kanamune J, Iwanaga Y, Uemoto S, Daneri-Navarro A, Rivas-Carrillo JD. Endothelial cells promote pancreatic stem cell activation during islet regeneration in mice. *Transplant Proc* 2011; **43**: 3209-3211 [PMID: 22099759 DOI: 10.1016/j.transproceed.2011.09.082]
27 **Johansson A**, Lau J, Sandberg M, Borg LA, Magnusson PU, Carlsson PO. Endothelial cell signalling supports pancreatic beta cell function in the rat. *Diabetologia* 2009; **52**: 2385-2394 [PMID: 19669728 DOI: 10.1007/s00125-009-1485-6]
28 **Watada H**. Role of VEGF-A in pancreatic beta cells. *Endocr J* 2010; **57**: 185-191 [PMID: 20179357 DOI: 10.1507/endocrj.K09E-035]
29 **Kuboki K**, Jiang ZY, Takahara N, Ha SW, Igarashi M, Yamauchi T, Feener EP, Herbert TP, Rhodes CJ, King GL. Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo : a specific vascular action of insulin. *Circulation* 2000; **101**: 676-681 [PMID: 10673261 DOI: 10.1161/01.CIR.101.6.676]
30 **Yuan Q**, Chen L, Liu C, Xu K, Mao X, Liu C. Postnatal pancreatic islet β cell function and insulin sensitivity at different stages of lifetime in rats born with intrauterine growth retardation. *PLoS One* 2011; **6**: e25167 [PMID: 22022381 DOI: 10.1371/journal.pone.0025167]
31 **Ferrara N**. VEGF and the quest for tumour angiogenesis factors. *Nat Rev Cancer* 2002; **2**: 795-803 [PMID: 12360282 DOI: 10.1038/nrc909]
32 **Patel-Hett S**, D'Amore PA. Signal transduction in vasculogenesis and developmental angiogenesis. *Int J Dev Biol* 2011; **55**: 353-363 [PMID: 21732275 DOI: 10.1387/ijdb.103213sp]
33 **Brissova M**, Shostak A, Shiota M, Wiebe PO, Poffenberger G, Kantz J, Chen Z, Carr C, Jerome WG, Chen J, Baldwin HS, Nicholson W, Bader DM, Jetton T, Gannon M, Powers AC. Pancreatic islet production of vascular endothelial growth factor--a is essential for islet vascularization, revascularization, and function. *Diabetes* 2006; **55**: 2974-2985 [PMID: 17065333 DOI: 10.2337/db06-0690]
34 **Hoeben A**, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA. Vascular endothelial growth factor and angiogenesis. *Pharmacol Rev* 2004; **56**: 549-580 [PMID: 15602010 DOI: 10.1124/pr.56.4.3]
35 **Ferrara N**, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003; **9**: 669-676 [PMID: 12778165 DOI: 10.1038/nm0603-669]
36 **Houck KA**, Ferrara N, Winer J, Cachianes G, Li B, Leung DW. The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. *Mol Endocrinol* 1991; **5**: 1806-1814 [PMID: 1791831 DOI: 10.1210/mend-5-12-1806]
37 **Suto K**, Yamazaki Y, Morita T, Mizuno H. Crystal structures of novel vascular endothelial growth factors (VEGF) from snake venoms: insight into selective VEGF binding to kinase insert domain-containing receptor but not to fms-like tyrosine kinase-1. *J Biol Chem* 2005; **280**: 2126-2131 [PMID: 15542594 DOI: 10.1074/jbc.M411395200]
38 **Dvorak HF**. Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. *J Clin Oncol* 2002; **20**: 4368-4380 [PMID: 12409337 DOI: 10.1200/JCO.2002.10.088]
39 **Cross MJ**, Dixelius J, Matsumoto T, Claesson-Welsh L. VEGF-receptor signal transduction. *Trends Biochem Sci* 2003; **28**: 488-494 [PMID: 13678960 DOI: 10.1016/S0968-0004(03)00193-2]
40 **Mazure NM**, Chen EY, Laderoute KR, Giaccia AJ. Induction of vascular endothelial growth factor by hypoxia is modulated by a phosphatidylinositol 3-kinase/Akt signaling pathway in Ha-ras-transformed cells through a hypoxia inducible factor-1 transcriptional element. *Blood* 1997; **90**: 3322-3331 [PMID: 9345014]
41 **Arsham AM**, Plas DR, Thompson CB, Simon MC. Phosphatidylinositol 3-kinase/Akt signaling is neither required for hypoxic stabilization of HIF-1 alpha nor sufficient for HIF-1-dependent target gene transcription. *J Biol Chem* 2002; **277**: 15162-15170 [PMID: 11859074 DOI: 10.1074/jbc.M111162200]
42 **Rousseau S**, Houle F, Landry J, Huot J. p38 MAP kinase activation by vascular endothelial growth factor mediates actin reorganization and cell migration in human endothelial cells. *Oncogene* 1997; **15**: 2169-2177 [PMID: 9393975 DOI: 10.1038/sj.onc.1201380]
43 **Fulton D**, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, Sessa WC. Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. *Nature* 1999; **399**: 597-601 [PMID: 10376602 DOI: 10.1038/21218]
44 **Gerber HP**, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, Ferrara N. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. *J Biol Chem* 1998; **273**: 30336-30343 [PMID: 9804796 DOI: 10.1074/jbc.273.46.30336]
45 **Eichmann A**, Simons M. VEGF signaling inside vascular endothelial cells and beyond. *Curr Opin Cell Biol* 2012; **24**: 188-193 [PMID: 22366328 DOI: 10.1016/j.ceb.2012.02.002]
46 **Kostromina E**, Wang X, Han W. Altered islet morphology but normal islet secretory function in vitro in a mouse model with microvascular alterations in the pancreas. *PLoS One* 2013; **8**: e71277 [PMID: 23923060 DOI: 10.1371/journal.pone.0071277]
47 **Johansson M**, Andersson A, Carlsson PO, Jansson L. Perinatal development of the pancreatic islet microvasculature in rats. *J Anat* 2006; **208**: 191-196 [PMID: 16441563 DOI: 10.1111/j.1469-7580.2006.00520.x]
48 **Kamba T**, Tam BY, Hashizume H, Haskell A, Sennino B, Mancuso MR, Norberg SM, O'Brien SM, Davis RB, Gowen LC, Anderson KD, Thurston G, Joho S, Springer ML, Kuo CJ, McDonald DM. VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature. *Am J Physiol Heart Circ Physiol* 2006; **290**:

H560-H576 [PMID: 16172168 DOI: 10.1152/ajpheart.00133.2005]
49 **Vasir B**, Aiello LP, Yoon KH, Quickel RR, Bonner-Weir S, Weir GC. Hypoxia induces vascular endothelial growth factor gene and protein expression in cultured rat islet cells. *Diabetes* 1998; **47**: 1894-1903 [PMID: 9836521 DOI: 10.2337/diabetes.47.12.1894]
50 **Adams RH**, Alitalo K. Molecular regulation of angiogenesis and lymphangiogenesis. *Nat Rev Mol Cell Biol* 2007; **8**: 464-478 [PMID: 17522591 DOI: 10.1038/nrm2183]
51 **Reinert RB**, Brissova M, Shostak A, Pan FC, Poffenberger G, Cai Q, Hundemer GL, Kantz J, Thompson CS, Dai C, McGuinness OP, Powers AC. Vascular endothelial growth factor-a and islet vascularization are necessary in developing, but not adult, pancreatic islets. *Diabetes* 2013; **62**: 4154-4164 [PMID: 23884891 DOI: 10.2337/db13-0071]
52 **Xiao X**, Guo P, Chen Z, El-Gohary Y, Wiersch J, Gaffar I, Prasadan K, Shiota C, Gittes GK. Hypoglycemia reduces vascular endothelial growth factor A production by pancreatic beta cells as a regulator of beta cell mass. *J Biol Chem* 2013; **288**: 8636-8646 [PMID: 23378532 DOI: 10.1074/jbc.M112.422949]
53 **Zhang N**, Richter A, Suriawinata J, Harbaran S, Altomonte J, Cong L, Zhang H, Song K, Meseck M, Bromberg J, Dong H. Elevated vascular endothelial growth factor production in islets improves islet graft vascularization. *Diabetes* 2004; **53**: 963-970 [PMID: 15047611 DOI: 10.2337/diabetes.53.4.963]
54 **Jabs N**, Franklin I, Brenner MB, Gromada J, Ferrara N, Wollheim CB, Lammert E. Reduced insulin secretion and content in VEGF-a deficient mouse pancreatic islets. *Exp Clin Endocrinol Diabetes* 2008; **116** Suppl 1: S46-S49 [PMID: 18777454 DOI: 10.1055/s-2008-1081486]
55 **Lammert E**, Gu G, McLaughlin M, Brown D, Brekken R, Murtaugh LC, Gerber HP, Ferrara N, Melton DA. Role of VEGF-A in vascularization of pancreatic islets. *Curr Biol* 2003; **13**: 1070-1074 [PMID: 12814555 DOI: 10.1016/S0960-9822(03)00378-6]
56 **Su D**, Zhang N, He J, Qu S, Slusher S, Bottino R, Bertera S, Bromberg J, Dong HH. Angiopoietin-1 production in islets improves islet engraftment and protects islets from cytokine-induced apoptosis. *Diabetes* 2007; **56**: 2274-2283 [PMID: 17596403 DOI: 10.2337/db07-0371]
57 **Cai Q**, Brissova M, Reinert RB, Pan FC, Brahmachary P, Jeansson M, Shostak A, Radhika A, Poffenberger G, Quaggin SE, Jerome WG, Dumont DJ, Powers AC. Enhanced expression of VEGF-A in β cells increases endothelial cell number but impairs islet morphogenesis and β cell proliferation. *Dev Biol* 2012; **367**: 40-54 [PMID: 22546694 DOI: 10.1016/j.ydbio.2012.04.022]
58 **Scharpfenecker M**, Fiedler U, Reiss Y, Augustin HG. The Tie-2 ligand angiopoietin-2 destabilizes quiescent endothelium through an internal autocrine loop mechanism. *J Cell Sci* 2005; **118**: 771-780 [PMID: 15687104 DOI: 10.1242/jcs.01653]
59 **Fukuhara S**, Sako K, Minami T, Noda K, Kim HZ, Kodama T, Shibuya M, Takakura N, Koh GY, Mochizuki N. Differential function of Tie2 at cell-cell contacts and cell-substratum contacts regulated by angiopoietin-1. *Nat Cell Biol* 2008; **10**: 513-526 [PMID: 18425120 DOI: 10.1038/ncb1714]
60 **Maisonpierre PC**, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, Daly TJ, Davis S, Sato TN, Yancopoulos GD. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science* 1997; **277**: 55-60 [PMID: 9204896 DOI: 10.1126/science.277.5322.55]
61 **Himanen JP**, Nikolov DB. Eph signaling: a structural view. *Trends Neurosci* 2003; **26**: 46-51 [PMID: 12495863 DOI: 10.1016/S0166-2236(02)00005-X]
62 **Pasquale EB**. Eph receptor signalling casts a wide net on cell behaviour. *Nat Rev Mol Cell Biol* 2005; **6**: 462-475 [PMID: 15928710 DOI: 10.1038/nrm1662]
63 **Gale NW**, Holland SJ, Valenzuela DM, Flenniken A, Pan L, Ryan TE, Henkemeyer M, Strebhardt K, Hirai H, Wilkinson DG, Pawson T, Davis S, Yancopoulos GD. Eph receptors and ligands comprise two major specificity subclasses and are reciprocally compartmentalized during embryogenesis. *Neuron* 1996; **17**: 9-19 [PMID: 8755474 DOI: 10.1016/S0896-6273(00)80276-7]
64 **Dorrell C**, Schug J, Lin CF, Canaday PS, Fox AJ, Smirnova O, Bonnah R, Streeter PR, Stoeckert CJ, Kaestner KH, Grompe M. Transcriptomes of the major human pancreatic cell types. *Diabetologia* 2011; **54**: 2832-2844 [PMID: 21882062 DOI: 10.1007/s00125-011-2283-5]
65 **Konstantinova I**, Nikolova G, Ohara-Imaizumi M, Meda P, Kucera T, Zarbalis K, Wurst W, Nagamatsu S, Lammert E. EphA-Ephrin-A-mediated beta cell communication regulates insulin secretion from pancreatic islets. *Cell* 2007; **129**: 359-370 [PMID: 17448994 DOI: 10.1016/j.cell.2007.02.044]
66 **Yao VJ**, Ozawa MG, Trepel M, Arap W, McDonald DM, Pasqualini R. Targeting pancreatic islets with phage display assisted by laser pressure catapult microdissection. *Am J Pathol* 2005; **166**: 625-636 [PMID: 15681844 DOI: 10.1016/S0002-9440(10)62283-3]
67 **Camussi G**, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. *Kidney Int* 2010; **78**: 838-848 [PMID: 20703216 DOI: 10.1038/ki.2010.278]
68 **Ratajczak J**, Wysoczynski M, Hayek F, Janowska-Wieczorek A, Ratajczak MZ. Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. *Leukemia* 2006; **20**: 1487-1495 [PMID: 16791265 DOI: 10.1038/sj.leu.2404296]
69 **Figliolini F**, Cantaluppi V, De Lena M, Beltramo S, Romagnoli R, Salizzoni M, Melzi R, Nano R, Piemonti L, Tetta C, Biancone L, Camussi G. Isolation, characterization and potential role in beta cell-endothelium cross-talk of extracellular vesicles released from human pancreatic islets. *PLoS One* 2014; **9**: e102521 [PMID: 25028931 DOI: 10.1371/journal.pone.0102521]
70 **Cantaluppi V**, Biancone L, Figliolini F, Beltramo S, Medica D, Deregibus MC, Galimi F, Romagnoli R, Salizzoni M, Tetta C, Segoloni GP, Camussi G. Microvesicles derived from endothelial progenitor cells enhance neoangiogenesis of human pancreatic islets. *Cell Transplant* 2012; **21**: 1305-1320 [PMID: 22455973 DOI: 10.3727/096368911X627534]
71 **Olerud J**, Mokhtari D, Johansson M, Christoffersson G, Lawler J, Welsh N, Carlsson PO. Thrombospondin-1: an islet endothelial cell signal of importance for β-cell function. *Diabetes* 2011; **60**: 1946-1954 [PMID: 21617177 DOI: 10.2337/db10-0277]
72 **Lin HM**, Lee JH, Yadav H, Kamaraju AK, Liu E, Zhigang D, Vieira A, Kim SJ, Collins H, Matschinsky F, Harlan DM, Roberts AB, Rane SG. Transforming growth factor-beta/Smad3 signaling regulates insulin gene transcription and pancreatic islet beta-cell function. *J Biol Chem* 2009; **284**: 12246-12257 [PMID: 19265200 DOI: 10.1074/jbc.M805379200]
73 **Gregersen S**, Thomsen JL, Brock B, Hermansen K. Endothelin-1 stimulates insulin secretion by direct action on the islets of Langerhans in mice. *Diabetologia* 1996; **39**: 1030-1035 [PMID: 8877285 DOI: 10.1007/BF00400650]
74 **Johansson M**, Mattsson G, Andersson A, Jansson L, Carlsson PO. Islet endothelial cells and pancreatic beta-cell proliferation: studies in vitro and during pregnancy in adult rats. *Endocrinology* 2006; **147**: 2315-2324 [PMID: 16439446 DOI: 10.1210/en.2005-0997]
75 **Nikolova G**, Jabs N, Konstantinova I, Domogatskaya A, Tryggvason K, Sorokin L, Fässler R, Gu G, Gerber HP, Ferrara N, Melton DA, Lammert E. The vascular basement membrane: a niche for insulin gene expression and Beta cell proliferation. *Dev Cell* 2006; **10**: 397-405 [PMID: 16516842 DOI: 10.1016/j.devcel.2006.01.015]
76 **Loganathan R**, Rongish BJ, Smith CM, Filla MB, Czirok A, Bénazéraf B, Little CD. Extracellular matrix motion and early morphogenesis. *Development* 2016; **143**: 2056-2065 [PMID: 27302396 DOI: 10.1242/dev.127886]
77 **Rozario T**, DeSimone DW. The extracellular matrix in development and morphogenesis: a dynamic view. *Dev Biol* 2010; **341**: 126-140 [PMID: 19854168 DOI: 10.1016/j.ydbio.2009.10.026]

78 **Hynes RO**. The evolution of metazoan extracellular matrix. *J Cell Biol* 2012; **196**: 671-679 [PMID: 22431747 DOI: 10.1083/jcb.201109041]

79 **Myllyharju J**. Extracellular matrix and developing growth plate. *Curr Osteoporos Rep* 2014; **12**: 439-445 [PMID: 25212565 DOI: 10.1007/s11914-014-0232-1]

80 **Irving-Rodgers HF**, Ziolkowski AF, Parish CR, Sado Y, Ninomiya Y, Simeonovic CJ, Rodgers RJ. Molecular composition of the peri-islet basement membrane in NOD mice: a barrier against destructive insulitis. *Diabetologia* 2008; **51**: 1680-1688 [PMID: 18633594 DOI: 10.1007/s00125-008-1085-x]

81 **Kaido T**, Yebra M, Cirulli V, Montgomery AM. Regulation of human beta-cell adhesion, motility, and insulin secretion by collagen IV and its receptor alpha1beta1. *J Biol Chem* 2004; **279**: 53762-53769 [PMID: 15485856 DOI: 10.1074/jbc.M411202200]

82 **Nikolova G**, Strilic B, Lammert E. The vascular niche and its basement membrane. *Trends Cell Biol* 2007; **17**: 19-25 [PMID: 17129728 DOI: 10.1016/j.tcb.2006.11.005]

83 **Krishnamurthy M**, Li J, Fellows GF, Rosenberg L, Goodyer CG, Wang R. Integrin {alpha}3, but not {beta}1, regulates islet cell survival and function via PI3K/Akt signaling pathways. *Endocrinology* 2011; **152**: 424-435 [PMID: 21177833 DOI: 10.1210/en.2010-0877]

84 **Riopel M**, Krishnamurthy M, Li J, Liu S, Leask A, Wang R. Conditional β1-integrin-deficient mice display impaired pancreatic β cell function. *J Pathol* 2011; **224**: 45-55 [PMID: 21381031 DOI: 10.1002/path.2849]

85 **Riopel MM**, Li J, Liu S, Leask A, Wang R. β1 integrin-extracellular matrix interactions are essential for maintaining exocrine pancreas architecture and function. *Lab Invest* 2013; **93**: 31-40 [PMID: 23069938 DOI: 10.1038/labinvest.2012.147]

86 **Choong FJ**, Freeman C, Parish CR, Simeonovic CJ. Islet heparan sulfate but not heparan sulfate proteoglycan core protein is lost during islet isolation and undergoes recovery post-islet transplantation. *Am J Transplant* 2015; **15**: 2851-2864 [PMID: 26104150 DOI: 10.1111/ajt.13366]

87 **Ziolkowski AF**, Popp SK, Freeman C, Parish CR, Simeonovic CJ. Heparan sulfate and heparanase play key roles in mouse β cell survival and autoimmune diabetes. *J Clin Invest* 2012; **122**: 132-141 [PMID: 22182841 DOI: 10.1172/JCI46177]

88 **Hamamoto Y**, Fujimoto S, Inada A, Takehiro M, Nabe K, Shimono D, Kajikawa M, Fujita J, Yamada Y, Seino Y. Beneficial effect of pretreatment of islets with fibronectin on glucose tolerance after islet transplantation. *Horm Metab Res* 2003; **35**: 460-465 [PMID: 12953162 DOI: 10.1055/s-2003-41802]

89 **Katsube K**, Sakamoto K, Tamamura Y, Yamaguchi A. Role of CCN, a vertebrate specific gene family, in development. *Dev Growth Differ* 2009; **51**: 55-67 [PMID: 19128405 DOI: 10.1111/j.1440-169X.2009.01077.x]

90 **Hall-Glenn F**, Lyons KM. Roles for CCN2 in normal physiological processes. *Cell Mol Life Sci* 2011; **68**: 3209-3217 [PMID: 21858450 DOI: 10.1007/s00018-011-0782-7]

91 **Hall-Glenn F**, De Young RA, Huang BL, van Handel B, Hofmann JJ, Chen TT, Choi A, Ong JR, Benya PD, Mikkola H, Iruela-Arispe ML, Lyons KM. CCN2/connective tissue growth factor is essential for pericyte adhesion and endothelial basement membrane formation during angiogenesis. *PLoS One* 2012; **7**: e30562 [PMID: 22363445 DOI: 10.1371/journal.pone.0030562]

92 **Riley KG**, Pasek RC, Maulis MF, Peek J, Thorel F, Brigstock DR, Herrera PL, Gannon M. Connective tissue growth factor modulates adult β-cell maturity and proliferation to promote β-cell regeneration in mice. *Diabetes* 2015; **64**: 1284-1298 [PMID: 25392241 DOI: 10.2337/db14-1195]

93 **Guney MA**, Petersen CP, Boustani A, Duncan MR, Gunasekaran U, Menon R, Warfield C, Grotendorst GR, Means AL, Economides AN, Gannon M. Connective tissue growth factor acts within both endothelial cells and beta cells to promote proliferation of developing beta cells. *Proc Natl Acad Sci USA* 2011; **108**: 15242-15247 [PMID: 21876171 DOI: 10.1073/pnas.1100072108]

94 **James LR**, Le C, Doherty H, Kim HS, Maeda N. Connective tissue growth factor (CTGF) expression modulates response to high glucose. *PLoS One* 2013; **8**: e70441 [PMID: 23950936 DOI: 10.1371/journal.pone.0070441]

95 **Crawford LA**, Guney MA, Oh YA, Deyoung RA, Valenzuela DM, Murphy AJ, Yancopoulos GD, Lyons KM, Brigstock DR, Economides A, Gannon M. Connective tissue growth factor (CTGF) inactivation leads to defects in islet cell lineage allocation and beta-cell proliferation during embryogenesis. *Mol Endocrinol* 2009; **23**: 324-336 [PMID: 19131512 DOI: 10.1210/me.2008-0045]

96 **Jiang FX**, Cram DS, DeAizpurua HJ, Harrison LC. Laminin-1 promotes differentiation of fetal mouse pancreatic beta-cells. *Diabetes* 1999; **48**: 722-730 [PMID: 10102687 DOI: 10.2337/diabetes.48.4.722]

97 **Golocheikine A**, Tiriveedhi V, Angaswamy N, Benshoff N, Sabarinathan R, Mohanakumar T. Cooperative signaling for angiogenesis and neovascularization by VEGF and HGF following islet transplantation. *Transplantation* 2010; **90**: 725-731 [PMID: 20714284 DOI: 10.1097/TP.0b013e3181ef8a63]

98 **Lai Y**, Schneider D, Kidszun A, Hauck-Schmalenberger I, Breier G, Brandhorst D, Brandhorst H, Iken M, Brendel MD, Bretzel RG, Linn T. Vascular endothelial growth factor increases functional beta-cell mass by improvement of angiogenesis of isolated human and murine pancreatic islets. *Transplantation* 2005; **79**: 1530-1536 [PMID: 15940042 DOI: 10.1097/01.TP.0000163506.40189.65]

99 **Garcia-Ocaña A**, Takane KK, Syed MA, Philbrick WM, Vasavada RC, Stewart AF. Hepatocyte growth factor overexpression in the islet of transgenic mice increases beta cell proliferation, enhances islet mass, and induces mild hypoglycemia. *J Biol Chem* 2000; **275**: 1226-1232 [PMID: 10625667 DOI: 10.1074/jbc.275.2.1226]

100 **García-Ocaña A**, Vasavada RC, Cebrian A, Reddy V, Takane KK, López-Talavera JC, Stewart AF. Transgenic overexpression of hepatocyte growth factor in the beta-cell markedly improves islet function and islet transplant outcomes in mice. *Diabetes* 2001; **50**: 2752-2762 [PMID: 11723058 DOI: 10.2337/diabetes.50.12.2752]

101 **Qian X**, Rothman VL, Nicosia RF, Tuszynski GP. Expression of thrombospondin-1 in human pancreatic adenocarcinomas: role in matrix metalloproteinase-9 production. *Pathol Oncol Res* 2001; **7**: 251-259 [PMID: 11882904 DOI: 10.1007/BF03032381]

102 **Chen H**, Herndon ME, Lawler J. The cell biology of thrombospondin-1. *Matrix Biol* 2000; **19**: 597-614 [PMID: 11102749 DOI: 10.1016/S0945-053X(00)00107-4]

103 **Dubois S**, Madec AM, Mesnier A, Armanet M, Chikh K, Berney T, Thivolet Ch. Glucose inhibits angiogenesis of isolated human pancreatic islets. *J Mol Endocrinol* 2010; **45**: 99-105 [PMID: 20505014 DOI: 10.1677/JME-10-0020]

104 **Olerud J**, Johansson M, Lawler J, Welsh N, Carlsson PO. Improved vascular engraftment and graft function after inhibition of the angiostatic factor thrombospondin-1 in mouse pancreatic islets. *Diabetes* 2008; **57**: 1870-1877 [PMID: 18420490 DOI: 10.2337/db07-0724]

105 **Tillmar L**, Welsh N. In vitro cultured rat islets express genes that both prevent and promote angiogenesis. *JOP* 2004; **5**: 81-91 [PMID: 15007189]

106 **Jiménez B**, Volpert OV, Crawford SE, Febbraio M, Silverstein RL, Bouck N. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. *Nat Med* 2000; **6**: 41-48 [PMID: 10613822 DOI: 10.1038/71517]

107 **Crawford SE**, Stellmach V, Murphy-Ullrich JE, Ribeiro SM, Lawler J, Hynes RO, Boivin GP, Bouck N. Thrombospondin-1 is a major activator of TGF-beta1 in vivo. *Cell* 1998; **93**: 1159-1170 [PMID: 9657149 DOI: 10.1016/S0092-8674(00)81460-9]

108 **Lai EY**, Persson AE, Bodin B, Källskog O, Andersson A, Pettersson U, Hansell P, Jansson L. Endothelin-1 and pancreatic islet vasculature: studies in vivo and on isolated, vascularly perfused pancreatic islets. *Am J Physiol Endocrinol Metab* 2007; **292**: E1616-E1623 [PMID: 17284574 DOI: 10.1152/

ajpendo.00640.2006]
109 **De Carlo E**, Milanesi A, Martini C, Maffei P, Sicolo N, Scandellari C. Endothelin-1 and endothelin-3 stimulate insulin release by isolated rat pancreatic islets. *J Endocrinol Invest* 2000; **23**: 240-245 [PMID: 10853710 DOI: 10.1007/BF03343715]
110 **Kugelmeier P**, Nett PC, Züllig R, Lehmann R, Weber M, Moritz W. Expression and hypoxic regulation of the endothelin system in endocrine cells of human and rat pancreatic islets. *JOP* 2008; **9**: 133-149 [PMID: 18326921]
111 **Kourembanas S**, Marsden PA, McQuillan LP, Faller DV. Hypoxia induces endothelin gene expression and secretion in cultured human endothelium. *J Clin Invest* 1991; **88**: 1054-1057 [PMID: 1885767 DOI: 10.1172/JCI115367]
112 **Hu RM**, Levin ER, Pedram A, Frank HJ. Insulin stimulates production and secretion of endothelin from bovine endothelial cells. *Diabetes* 1993; **42**: 351-358 [PMID: 8425673 DOI: 10.2337/diab.42.2.351]
113 **Ferri C**, Bellini C, Desideri G, De Mattia G, Santucci A. Endogenous insulin modulates circulating endothelin-1 concentrations in humans. *Diabetes Care* 1996; **19**: 504-506 [PMID: 8732718 DOI: 10.2337/diacare.19.5.504]
114 **Cifarelli V**, Lee S, Kim DH, Zhang T, Kamagate A, Slusher S, Bertera S, Luppi P, Trucco M, Dong HH. FOXO1 mediates the autocrine effect of endothelin-1 on endothelial cell survival. *Mol Endocrinol* 2012; **26**: 1213-1224 [PMID: 22570335 DOI: 10.1210/me.2011-1276]
115 **Cheifetz S**, Bellón T, Calés C, Vera S, Bernabeu C, Massagué J, Letarte M. Endoglin is a component of the transforming growth factor-beta receptor system in human endothelial cells. *J Biol Chem* 1992; **267**: 19027-19030 [PMID: 1326540]
116 **Barbara NP**, Wrana JL, Letarte M. Endoglin is an accessory protein that interacts with the signaling receptor complex of multiple members of the transforming growth factor-beta superfamily. *J Biol Chem* 1999; **274**: 584-594 [PMID: 9872992 DOI: 10.1074/jbc.274.2.584]
117 **Fonsatti E**, Sigalotti L, Arslan P, Altomonte M, Maio M. Emerging role of endoglin (CD105) as a marker of angiogenesis with clinical potential in human malignancies. *Curr Cancer Drug Targets* 2003; **3**: 427-432 [PMID: 14683500 DOI: 10.2174/1568009033481741]
118 **Fonsatti E**, Nicolay HJ, Altomonte M, Covre A, Maio M. Targeting cancer vasculature via endoglin/CD105: a novel antibody-based diagnostic and therapeutic strategy in solid tumours. *Cardiovasc Res* 2010; **86**: 12-19 [PMID: 19812043 DOI: 10.1093/cvr/cvp332]
119 **Alva JA**, Zovein AC, Monvoisin A, Murphy T, Salazar A, Harvey NL, Carmeliet P, Iruela-Arispe ML. VE-Cadherin-Cre-recombinase transgenic mouse: a tool for lineage analysis and gene deletion in endothelial cells. *Dev Dyn* 2006; **235**: 759-767 [PMID: 16450386 DOI: 10.1002/dvdy.20643]
120 **Clarkin CE**, Mahmoud M, Liu B, Sobamowo EO, King A, Arthur H, Jones PM, Wheeler-Jones CP. Modulation of endoglin expression in islets of langerhans by VEGF reveals a novel regulator of islet endothelial cell function. *BMC Res Notes* 2016; **9**: 362 [PMID: 27456002 DOI: 10.1186/s13104-016-2142-z]
121 **Bellacen K**, Kalay N, Ozeri E, Shahaf G, Lewis EC. Revascularization of pancreatic islet allografts is enhanced by α-1-antitrypsin under anti-inflammatory conditions. *Cell Transplant* 2013; **22**: 2119-2133 [PMID: 23050776 DOI: 10.3727/096368912X657701]
122 **Zhao M**, Choudhary P, Srinivasan P, Tang H, Heaton N, Fung M, Barthel A, Bornstein SR, Amiel SA, Huang GC. Modification of human islet preparation: an effective approach to improve graft outcome after islet transplantation? *Horm Metab Res* 2015; **47**: 72-77 [PMID: 25372780]
123 **Jansson L**, Carlsson PO. Graft vascular function after transplantation of pancreatic islets. *Diabetologia* 2002; **45**: 749-763 [PMID: 12107718 DOI: 10.1007/s00125-002-0827-4]
124 **Konstantinova I**, Lammert E. Microvascular development: learning from pancreatic islets. *Bioessays* 2004; **26**: 1069-1075 [PMID: 15382139 DOI: 10.1002/bies.20105]
125 **Lukinius A**, Jansson L, Korsgren O. Ultrastructural evidence for blood microvessels devoid of an endothelial cell lining in transplanted pancreatic islets. *Am J Pathol* 1995; **146**: 429-435 [PMID: 7531955]
126 **Emamaullee JA**, Shapiro AM. Factors influencing the loss of beta-cell mass in islet transplantation. *Cell Transplant* 2007; **16**: 1-8 [PMID: 17436849 DOI: 10.3727/000000007783464461]
127 **Davalli AM**, Ogawa Y, Ricordi C, Scharp DW, Bonner-Weir S, Weir GC. A selective decrease in the beta cell mass of human islets transplanted into diabetic nude mice. *Transplantation* 1995; **59**: 817-820 [PMID: 7701574 DOI: 10.1097/00007890-199503000-00003]
128 **Zeng W**, Gouw AS, van den Heuvel MC, Zwiers PJ, Zondervan PE, Poppema S, Zhang N, Platteel I, de Jong KP, Molema G. The angiogenic makeup of human hepatocellular carcinoma does not favor vascular endothelial growth factor/angiopoietin-driven sprouting neovascularization. *Hepatology* 2008; **48**: 1517-1527 [PMID: 18924243 DOI: 10.1002/hep.22490]
129 **Jiang X**, Abiatari I, Kong B, Erkan M, De Oliveira T, Giese NA, Michalski CW, Friess H, Kleeff J. Pancreatic islet and stellate cells are the main sources of endocrine gland-derived vascular endothelial growth factor/prokineticin-1 in pancreatic cancer. *Pancreatology* 2009; **9**: 165-172 [PMID: 19077468 DOI: 10.1159/000178888]
130 **Mattsson G**, Jansson L, Carlsson PO. Decreased vascular density in mouse pancreatic islets after transplantation. *Diabetes* 2002; **51**: 1362-1366 [PMID: 11978631 DOI: 10.2337/diabetes.51.5.1362]
131 **Carlsson PO**, Palm F, Andersson A, Liss P. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes* 2001; **50**: 489-495 [PMID: 11246867 DOI: 10.2337/diabetes.50.3.489]
132 **Lau J**, Carlsson PO. Low revascularization of human islets when experimentally transplanted into the liver. *Transplantation* 2009; **87**: 322-325 [PMID: 19202435 DOI: 10.1097/TP.0b013e3181943b3d]
133 **Menger MD**, Yamauchi J, Vollmar B. Revascularization and microcirculation of freely grafted islets of Langerhans. *World J Surg* 2001; **25**: 509-515 [PMID: 11344405 DOI: 10.1007/s002680020345]
134 **Cantaluppi V**, Biancone L, Romanazzi GM, Figliolini F, Beltramo S, Ninniri MS, Galimi F, Romagnoli R, Franchello A, Salizzoni M, Perin PC, Ricordi C, Segoloni GP, Camussi G. Antiangiogenic and immunomodulatory effects of rapamycin on islet endothelium: relevance for islet transplantation. *Am J Transplant* 2006; **6**: 2601-2611 [PMID: 16981910 DOI: 10.1111/j.1600-6143.2006.01534.x]
135 **Nyqvist D**, Köhler M, Wahlstedt H, Berggren PO. Donor islet endothelial cells participate in formation of functional vessels within pancreatic islet grafts. *Diabetes* 2005; **54**: 2287-2293 [PMID: 16046293 DOI: 10.2337/diabetes.54.8.2287]
136 **Linn T**, Schneider K, Hammes HP, Preissner KT, Brandhorst H, Morgenstern E, Kiefer F, Bretzel RG. Angiogenic capacity of endothelial cells in islets of Langerhans. *FASEB J* 2003; **17**: 881-883 [PMID: 12670881 DOI: 10.1096/fj.02-0615fje]
137 **Enghofer M**, Bojunga J, Ludwig R, Oldenburg A, Bernd A, Usadel KH, Kusterer K. Lymphocyte transfer in streptozotocin-induced diabetes: adhesion of donor cells to islet endothelium. *Am J Physiol* 1998; **274**: E928-E935 [PMID: 9612252]
138 **Heller B**, Wang ZQ, Wagner EF, Radons J, Bürkle A, Fehsel K, Burkart V, Kolb H. Inactivation of the poly(ADP-ribose) polymerase gene affects oxygen radical and nitric oxide toxicity in islet cells. *J Biol Chem* 1995; **270**: 11176-11180 [PMID: 7744749 DOI: 10.1074/jbc.270.19.11176]
139 **Steiner L**, Kröncke K, Fehsel K, Kolb-Bachofen V. Endothelial cells as cytotoxic effector cells: cytokine-activated rat islet endothelial cells lyse syngeneic islet cells via nitric oxide. *Diabetologia* 1997; **40**: 150-155 [PMID: 9049474 DOI: 10.1007/s001250050656]
140 **Kampf C**, Mattsson G, Carlsson PO. Size-dependent revascularization of transplanted pancreatic islets. *Cell Transplant* 2006; **15**: 205-209 [PMID: 16719055 DOI: 10.3727/000000006783982124]
141 **Johansson U**, Rasmusson I, Niclou SP, Forslund N, Gustavsson L, Nilsson B, Korsgren O, Magnusson PU. Formation of composite

endothelial cell-mesenchymal stem cell islets: a novel approach to promote islet revascularization. *Diabetes* 2008; **57**: 2393-2401 [PMID: 18519803 DOI: 10.2337/db07-0981]

142 **Ilgun H**, Kim JW, Luo L. Adult Stem Cells and Diabetes Therapy. *J Stem Cell Res Transplant* 2015; **2**: pii: 1020 [PMID: 27123495]

143 **Rackham CL**, Dhadda PK, Le Lay AM, King AJ, Jones PM. Preculturing Islets With Adipose-Derived Mesenchymal Stromal Cells Is an Effective Strategy for Improving Transplantation Efficiency at the Clinically Preferred Intraportal Site. *Cell Med* 2014; **7**: 37-47 [PMID: 26858891 DOI: 10.3727/215517914X680047]

144 **Fransson M**, Brännström J, Duprez I, Essand M, Le Blanc K, Korsgren O, Magnusson PU. Mesenchymal stromal cells support endothelial cell interactions in an intramuscular islet transplantation model. *Regen Med Res* 2015; **3**: 1 [PMID: 26430512 DOI: 10.1186/s40340-015-0010-9]

145 **Kaufman-Francis K**, Koffler J, Weinberg N, Dor Y, Levenberg S. Engineered vascular beds provide key signals to pancreatic hormone-producing cells. *PLoS One* 2012; **7**: e40741 [PMID: 22808248 DOI: 10.1371/journal.pone.0040741]

146 **Phelps EA**, Templeman KL, Thulé PM, García AJ. Engineered VEGF-releasing PEG-MAL hydrogel for pancreatic islet vascularization. *Drug Deliv Transl Res* 2015; **5**: 125-136 [PMID: 25787738 DOI: 10.1007/s13346-013-0142-2]

147 **Amer LD**, Mahoney MJ, Bryant SJ. Tissue engineering approaches to cell-based type 1 diabetes therapy. *Tissue Eng Part B Rev* 2014; **20**: 455-467 [PMID: 24417705 DOI: 10.1089/ten.teb.2013.0462]

148 **Willenberg BJ**, Oca-Cossio J, Cai Y, Brown AR, Clapp WL, Abrahamson DR, Terada N, Ellison GW, Mathews CE, Batich CD, Ross EA. Repurposed biological scaffolds: kidney to pancreas. *Organogenesis* 2015; **11**: 47-57 [PMID: 26252820 DOI: 10.1080/15476278.2015.1067354]

149 **Lammert E**, Cleaver O, Melton D. Induction of pancreatic differentiation by signals from blood vessels. *Science* 2001; **294**: 564-567 [PMID: 11577200 DOI: 10.1126/science.1064344]

150 **Yoshitomi H**, Zaret KS. Endothelial cell interactions initiate dorsal pancreas development by selectively inducing the transcription factor Ptf1a. *Development* 2004; **131**: 807-817 [PMID: 14736742 DOI: 10.1242/dev.00960]

151 **Agudo J**, Ayuso E, Jimenez V, Casellas A, Mallol C, Salavert A, Tafuro S, Obach M, Ruzo A, Moya M, Pujol A, Bosch F. Vascular endothelial growth factor-mediated islet hypervascularization and inflammation contribute to progressive reduction of β-cell mass. *Diabetes* 2012; **61**: 2851-2861 [PMID: 22961079 DOI: 10.2337/db12-0134]

152 **Rasul S**, Reiter MH, Ilhan A, Lampichler K, Wagner L, Kautzky-Willer A. Circulating angiopoietin-2 and soluble Tie-2 in type 2 diabetes mellitus: a cross-sectional study. *Cardiovasc Diabetol* 2011; **10**: 55 [PMID: 21699724 DOI: 10.1186/1475-2840-10-55]

153 **Lim HS**, Blann AD, Chong AY, Freestone B, Lip GY. Plasma vascular endothelial growth factor, angiopoietin-1, and angiopoietin-2 in diabetes: implications for cardiovascular risk and effects of multifactorial intervention. *Diabetes Care* 2004; **27**: 2918-2924 [PMID: 15562207 DOI: 10.2337/diacare.27.12.2918]

154 **Campochiaro PA**, Peters KG. Targeting Tie2 for Treatment of Diabetic Retinopathy and Diabetic Macular Edema. *Curr Diab Rep* 2016; **16**: 126 [PMID: 27778249 DOI: 10.1007/s11892-016-0816-5]

155 **Nakano M**, Yasunami Y, Maki T, Kodama S, Ikehara Y, Nakamura T, Tanaka M, Ikeda S. Hepatocyte growth factor is essential for amelioration of hyperglycemia in streptozotocin-induced diabetic mice receiving a marginal mass of intrahepatic islet grafts. *Transplantation* 2000; **69**: 214-221 [PMID: 10670629 DOI: 10.1097/00007890-200001270-00004]

156 **Lopez-Talavera JC**, Garcia-Ocaña A, Sipula I, Takane KK, Cozar-Castellano I, Stewart AF. Hepatocyte growth factor gene therapy for pancreatic islets in diabetes: reducing the minimal islet transplant mass required in a glucocorticoid-free rat model of allogeneic portal vein islet transplantation. *Endocrinology* 2004; **145**: 467-474 [PMID: 14551233 DOI: 10.1210/en.2003-1070]

157 **Rao P**, Cozar-Castellano I, Roccisana J, Vasavada RC, Garcia-Ocaña A. Hepatocyte growth factor gene therapy for islet transplantation. *Expert Opin Biol Ther* 2004; **4**: 507-518 [PMID: 15102600 DOI: 10.1517/14712598.4.4.507]

158 **Park WG**. Clinical chronic pancreatitis. *Curr Opin Gastroenterol* 2016 Jun 22; Epub ahead of print [PMID: 27341355 DOI: 10.1097/MOG.0000000000000293]

159 **DiMagno MJ**, DiMagno EP. Chronic pancreatitis. *Curr Opin Gastroenterol* 2011; **27**: 452-459 [PMID: 21844753 DOI: 10.1097/MOG.0b013e328349e333]

160 **Ellenrieder V**, Schneiderhan W, Bachem M, Adler G. Fibrogenesis in the pancreas. *Rocz Akad Med Bialymst* 2004; **49**: 40-46 [PMID: 15631312]

161 **Imamura T**, Iguchi H, Manabe T, Ohshio G, Yoshimura T, Wang ZH, Suwa H, Ishigami S, Imamura M. Quantitative analysis of collagen and collagen subtypes I, III, and V in human pancreatic cancer, tumor-associated chronic pancreatitis, and alcoholic chronic pancreatitis. *Pancreas* 1995; **11**: 357-364 [PMID: 8532652 DOI: 10.1097/00006676-199511000-00007]

162 **Shields MA**, Dangi-Garimella S, Redig AJ, Munshi HG. Biochemical role of the collagen-rich tumour microenvironment in pancreatic cancer progression. *Biochem J* 2012; **441**: 541-552 [PMID: 22187935 DOI: 10.1042/BJ20111240]

163 **Buchholz M**, Biebl A, Neesse A, Wagner M, Iwamura T, Leder G, Adler G, Gress TM. SERPINE2 (protease nexin I) promotes extracellular matrix production and local invasion of pancreatic tumors in vivo. *Cancer Res* 2003; **63**: 4945-4951 [PMID: 12941819]

164 **Pan S**, Chen R, Stevens T, Bronner MP, May D, Tamura Y, McIntosh MW, Brentnall TA. Proteomics portrait of archival lesions of chronic pancreatitis. *PLoS One* 2011; **6**: e27574 [PMID: 22132114 DOI: 10.1371/journal.pone.0027574]

165 **Paulo JA**, Kadiyala V, Brizard S, Banks PA, Steen H, Conwell DL. A proteomic comparison of formalin-fixed paraffin-embedded pancreatic tissue from autoimmune pancreatitis, chronic pancreatitis, and pancreatic cancer. *JOP* 2013; **14**: 405-414 [PMID: 23846938]

166 **Järveläinen H**, Sainio A, Koulu M, Wight TN, Penttinen R. Extracellular matrix molecules: potential targets in pharmacotherapy. *Pharmacol Rev* 2009; **61**: 198-223 [PMID: 19549927 DOI: 10.1124/pr.109.001289]

167 **Virtanen I**, Banerjee M, Palgi J, Korsgren O, Lukinius A, Thornell LE, Kikkawa Y, Sekiguchi K, Hukkanen M, Konttinen YT, Otonkoski T. Blood vessels of human islets of Langerhans are surrounded by a double basement membrane. *Diabetologia* 2008; **51**: 1181-1191 [PMID: 18438639 DOI: 10.1007/s00125-008-0997-9]

168 **Brissova M**, Shostak A, Fligner CL, Revetta FL, Washington MK, Powers AC, Hull RL. Human Islets Have Fewer Blood Vessels than Mouse Islets and the Density of Islet Vascular Structures Is Increased in Type 2 Diabetes. *J Histochem Cytochem* 2015; **63**: 637-645 [PMID: 26216139 DOI: 10.1369/0022155415573324]

169 **Wong VS**, Yeung A, Schultz W, Brubaker PL. R-spondin-1 is a novel beta-cell growth factor and insulin secretagogue. *J Biol Chem* 2010; **285**: 21292-21302 [PMID: 20442404 DOI: 10.1074/jbc.M110.129874]

170 **Chahal JK**, Wong VS, Chaboissier MC, Brubaker PL. R-spondin1 deficiency enhances β-Cell neogenesis in a murine model of diabetes. *Pancreas* 2014; **43**: 93-102 [PMID: 24263108 DOI: 10.1097/MPA.0b013e3182a70bfb]

171 **Benninger RK**, Head WS, Zhang M, Satin LS, Piston DW. Gap junctions and other mechanisms of cell-cell communication regulate basal insulin secretion in the pancreatic islet. *J Physiol* 2011; **589**: 5453-5466 [PMID: 21930600 DOI: 10.1113/jphysiol.2011.218909]

172 **Theis M**, Mas C, Döring B, Degen J, Brink C, Caille D, Charollais A, Krüger O, Plum A, Nepote V, Herrera P, Meda P, Willecke K. Replacement by a lacZ reporter gene assigns mouse connexin36, 45 and 43 to distinct cell types in pancreatic islets. *Exp Cell Res* 2004; **294**: 18-29 [PMID: 14980497 DOI: 10.1016/j.yexcr.2003.09.031]

173 **Coronel-Cruz C**, Hernández-Tellez B, López-Vancell R, López-

Vidal Y, Berumen J, Castell A, Pérez-Armendariz EM. Connexin 30.2 is expressed in mouse pancreatic beta cells. *Biochem Biophys Res Commun* 2013; **438**: 772-777 [PMID: 23831630 DOI: 10.1016/j.bbrc.2013.06.100]

P- Reviewer: Fujino Y, Kruel CRP, Perse M, Sumi S, Wang CX
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Wu HL

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 April 24; 7(2): 129-133

DOI: 10.5500/wjt.v7.i2.129

ISSN 2220-3230 (online)

MINIREVIEWS

Smoking in Renal Transplantation; Facts Beyond Myth

Ahmed Aref, Ajay Sharma, Ahmed Halawa

Ahmed Aref, Department of Nephrology, Sur hospital, Sur 411, Sultanate of Oman

Ahmed Aref, Ajay Sharma, Ahmed Halawa, Faculty of Health and Science, Institute of Learning and Teaching, University of Liverpool, Liverpool L69 3GB, United Kingdom

Ajay Sharma, Royal Liverpool University Hospital, Liverpool L7 8XP, United Kingdom

Ahmed Halawa, Sheffield Teaching Hospital, University of Sheffield, Sheffield S5 7AU, United Kingdom

Author contributions: Aref A contributes by designing the work, data collection, writing the manuscript; Sharma A reviewed and edited the manuscript; Halawa A contributes by choosing the topic of our work, reviewing and final editing of the manuscript.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other co-authors contributed their efforts in this manuscript.

Manuscript source: Unsolicited manuscript

Correspondence to: Dr. Ahmed Halawa, Consultant Transplant Surgeon, Sheffield Teaching Hospital, University of Sheffield, Herries Road, Sheffield S5 7AU, United Kingdom. ahmed.halawa@sth.nhs.uk
Telephone: +44-77-87542128
Fax: +44-11-42714604

Received: September 30, 2016
Peer-review started: October 10, 2016
First decision: December 1, 2016
Revised: December 18, 2016
Accepted: February 28, 2017
Article in press: March 2, 2017

Published online: April 24, 2017

Abstract

Smoking is one of the preventable leading causes of death worldwide. Most of the studies focused on the association between smoking and cardiovascular disease, pulmonary diseases, malignancy and death. However, the direct effect of smoking on the renal system was undermind. There are emerging evidence correlating tobacco use with pathological changes in the normal kidneys. The effect is more obvious on the renal allograft most probably due to the chronic immune suppression status and the metabolic effect of the drugs. Several studies have documented a deleterious effect of smoking on the renal transplant recipients. Smoking was associated with lowering patient and graft survival. Smoking cessation proved to improve graft survival and to a lesser extent recipient survival. Even receiving a renal transplant from a smoker donor increases the risk of death for the recipient and carries a poorer graft survival compared to non-smoking donors. Most of the studies investigating the effect of smoking were based on self-reporting questioners, which may be misleading due to poor recall or the desire to give socially acceptable answers. This made the need of a reliable biomarker of ultimate importance. Cotinine was proposed as a promising biomarker that may help to provide objective evidence regarding the status of smoking and the dose of nicotine exposure, yet there are still some limitations of its use. The aim of this work is to review the current evidence to improve our understanding of this critical topic. Indeed, this will help to guide better-designed studies in the future.

Key words: Smoking; Kidney donor; Kidney recipient; Renal transplantation

Core tip: There are several studies addressing the effect

of smoking on different body systems, yet, there are only few exploring the effect of smoking on the outcome of renal transplantation. Our present article summarizes all the available data published over the past 2 decades for better understanding of this topic and may also guide future studies.

Aref A, Sharma A, Halawa A. Smoking in Renal Transplantation; Facts Beyond Myth. *World J Transplant* 2017; 7(2): 129-133 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i2/129.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i2.129

INTRODUCTION

Smoking is a challenging health care problem; it has a well-established correlation with many serious medical conditions like cardiovascular diseases, pulmonary diseases, malignancy and death[1]. Cigarette smoking assumes to have a role in atherosclerosis, endothelial dysfunction, progression of vascular disease progression of proteinuria, as it contains large amounts of free radicals[2]. This makes smoking a significant renal risk factor, with considerable consequences on health care budget[3].

The effect of smoking is aggravated in renal transplant recipients due to the effect of immune suppression medications on carcinogenesis, in addition to the effect of chronic kidney disease itself on cardiovascular risk and mortality[1].

Despite the extensive smoking-related research, yet studies that investigated this phenomenon in the transplant populations are relatively few, and most of them are retrospective, poorly randomised or small sample size[2].

EFFECTS OF SMOKING ON THE KIDNEY

The hazards of smoking were investigated thoroughly in association with cardiovascular disease, lung disease and oncogenesis. However, the effect of smoking on healthy kidney and progression of primary kidney diseases did not attract great attention[3]. Indeed, many studies confirmed the role played by smoking in the progression of many intrinsic renal diseases (*e.g.,* diabetic nephropathy, IgA nephropathy and autosomal dominant polycystic kidney disease)[3].

Ritz *et al*[4] studied the effect of smoking on healthy normotensive volunteers. They reported a significant increase in arginine vasopressin levels (from 1.27 ± 0.72 to 19.9 ± 27.2 pg/mL) and serum epinephrine (from 37 ± 13 to 140 ± 129 pg/mL). There was an increase in renal vascular resistance by 11% and a decrease in the glomerular filtration rate (GFR) by 15%. They assumed these effects are secondary to nicotine itself as these findings were reproduced by using nicotine containing gum[4].

Pinto-Sietsma *et al*[5] performed a leading cross-sectional study on 7476 participants to evaluate the effect of smoking on the development of albuminuria and abnormal kidney functions in non-diabetic population. They documented the presence of a dose-dependent association between smoking and development of both microalbuminuria and renal impairment in this screening. These findings were less obvious or absent in former smokers[5].

RECIPIENT SMOKING AND TRANSPLANTATION OUTCOME

Smoking is strongly correlated to some of the potentially fatal outcomes, and there is some evidence that these complications are aggravated in solid organ transplant recipients[6].

Smoking is a well-known risk factor for cardiovascular disease. Ponticelli *et al*[7] have addressed the role of cardiovascular disease as the leading cause of death in renal transplant recipient. The development of de novo cardiovascular insult in the first year post-transplant was associated with pre-existing cardiovascular disease, older age, pre-transplant hypertension, smoking and duration of dialysis[7].

The second leading cause of death post-transplantation was malignancy[2,7] with a clear association between smoking and increased risk for certain types of malignancy[1].

The effect of smoking on renal transplant recipients was investigated in relatively few studies, and most of them are retrospective. Table 1 summarises the results of most of these studies[1,8-20].

It worth to mentioning that Zitt *et al*[16] had a unique approach by studying the relation between smoking and renal biopsy findings of 76 kidney transplant recipients. Current smokers had an increase in the severity of vascular intimal fibrous thickening (P = 0.004). While the degree of chronic sclerosing nephropathy (P = 0.05) and arteriolar hyalinosis (P < 0.001) were associated with the duration of time post-transplantation[16].

Most of these studies have revealed a clear benefit of smoking cessation on graft survival, but the effect on patient survival is less clear possibly reflecting the permanent atherosclerotic effect on the vascular system[20].

EFFECT OF SMOKING HABIT OF KIDNEY DONOR ON THE OUTCOME OF TRANSPLANTATION

It may be logic that the recipient smoking will affect his own survival, but surprisingly, even the donor smoking will affect the recipient survival years after transplantation[21,22].

Lin *et al*[21] have analysed data from the United Network for Organ Sharing from 1994 to 1999, and

Table 1 The impact of smoking on kidney transplant recipient

Ref.	Year	Study design	No. of cases		Results	Conclusion
			Total	smokers		
Arend *et al*[8]	1997	Retrospective analysis	916	394	RR 2.2 of mortality after the first year of transplantation (95%CI)	The risk of mortality after the first year was higher in older patients, men, diabetics, hypertensive and smokers
Cosio *et al*[9]	1999	Retrospective analysis	523	147	Patient survival shorter in smokers by Cox regression (P = 0.0005), univariate and multivariate analysis (P = 0.0004)	History of smoking correlates with decreased patient survival, the effect of smoking on transplant recipient is quantitatively similar to the effect of diabetes
Kasiske *et al*[10]	2000	Retrospective analysis	1334	330	RR 1.3 of graft loss with smoking more than 25 pack/yr at transplantation (95%CI) and increase the risk of death (RR = 1.42, 95%CI)	The effect of smoking dissipates after five years from quitting
Doyle *et al*[11]	2000	Retrospective analysis	206	155	RR 8.1 for graft loss (P < 0.001) and RR 7.9 for mortality (P < 0.001)	Tobacco use was associated with worse patient and graft survival compared to those who never smoked or those who quit smoking at least two months before transplantation
Matas *et al*[12]	2001	Retrospective analysis	2540	Not mentioned	Pre-transplant smoking has RR 2.1 for graft loss	Pre-transplant smoking, peripheral vascular disease or dialysis more than one year were all associated with worse long-term outcome
Sung *et al*[13]	2001	Retrospective analysis	645	156	RR 2.3 for graft loss, graft survival in smokers *vs* non-smokers were (84% *vs* 88%) at 1 yr, (65% *vs* 78%) at 5 yr and (48% *vs* 62%) at 10 yr follow up (P = 0.007)	Smoking significantly affects graft survival, an effect that is not explained by increases in rejection or patient death. Smoking cessation has beneficial effect on graft survival
Yavuz *et al*[14]	2004	Retrospective analysis	226	97	There was no significant relation between pre-transplant smoking and graft loss (P = 0.129), or mortality (P = 0.138)	They suspected that the non-significant effect of smoking might be attributed to the limited number of cases included
Kheradmand *et al*[15]	2005	Retrospective analysis	199	41	Pre-transplant smoking was associated with reduced overall graft survival (P = 0.01)	Smoking contributes to graft loss but has no significant relation with rejection episodes
Zitt *et al*[16]	2007	Retrospective analysis	279	62	Smokers had higher serum creatinine levels. Transplant biopsy was indicated more often in smokers compared to non-smokers (39% *vs* 24%, P = 0.02)	Smoking was associated with vascular fibrous intimal thickening in transplanted kidneys so that it may have a role in the development of chronic allograft nephropathy and graft loss
Gombos *et al*[17]	2010	cross-sectional study	402	102	In spite that kidney functions in smokers were not affected after one month of transplantation, yet, there was significant lower kidney function in smokers after three years (P < 0.05). This correlates with the intensity of smoking (P < 0.05)	Smoking is common following kidney transplantation in Hungary, and this may be a risk of a poor long-term outcome
Nogueira *et al*[18]	2010	Retrospective analysis	997	329	Patient and graft survival were worse in smokers (AHR for patient survival was 1.6, 95%CI, P = 0.02, and graft survival AHR 1.47, 95%CI, P = 0.01). Glomerular filtration rate after one year was lower in smokers	History of smoking will negatively affect patient and graft survival. Also, it increases the risk of early rejection
Hurst *et al*[19]	2011	Retrospective analysis	41705	5832	New onset smokers have increased risk of graft failure (AHR = 1.46, P < 0.001) and death (AHR = 2.32, P < 00.1) compared with never smokers	New onset smoking post-transplant associated with lower patient and graft survival
Agarwal *et al*[20]	2011	Prospective observational study	604	133	Current smokers have increased risk of graft failure compared to recipients who never smoke (HR = 3.3, P = 0.002). While past smokers had an almost similar risk of graft failure compared to non-smokers (HR = 1.1, P = 0.7) On the other hand, current and past smokers were at higher risk of mortality compared to non-smoker recipients (HR = 2.1, 95%CI: 1.1-3.8, P = 0.016, and HR = 2.4, 95%CI: 1.4-4.0, P = 0.001, respectively)	Current smoking is a risk factor for graft failure and mortality Despite the finding that smoking cessation may not alter the risk of mortality, but at least it will improve the graft survival

Opelz *et al*[1]	2016	Retrospective analysis	46548	15086	Patients who quit smoking before transplantation had clear benefits regarding patient and graft survival when compared to those who continues to smoke {all-cause graft failure (HR 1.1 *vs* 1.5, $P < 0.001$), all-cause mortality (HR 1.1 *vs* 1.6, $P < 0.001$) and death with functioning graft due to malignancy (HR 1.4 *vs* 2.6, $P = 0.001$)} However, they still have a higher risk for graft loss, malignancy and death compared to those who never smoke before	Smoking cessation before transplantation improve patient and graft survival. There is also a substantial reduction in certain types of malignancy compared to those who continued to smoke (lower incidence of respiratory, urinary tract, female genital organs, lips and oral cavity tumours)

AHR: Adjusted hazard ratio.

they declared that smoking habit of the donor has mild, yet statistically significant effect on recipient survival (HR = 1.06, $P < 0.05$), and graft survival (HR = 1.05, $P < 0.05$).

Underwood *et al*[22] studied a retrospective analysis of 602 kidney transplant recipients and their living donors. The effect of donor smoking on graft survival was statistically insignificant (HR = 1.19, $P = 0.515$), unlike the recipient smoking which proved to be significant (HR = 1.74, $P = 0.05$). However, the recipient survival was negatively correlated to donor smoking (HR = 1.93, 95%CI: 1.27-2.94, $P = 0.002$) and recipient smoking (HR = 1.74, 95%CI: 1.01-3.00, $P = 0.048$)[22].

Heldt *et al*[23] evaluated GFR of 100 living donors and their recipients, the recipients of smoking donors had lower calculated GFR (37.0 mL/min per 1.73 m^2 *vs* 53.0 mL/min per 1.73 m^2; $P < 0.001$) at a mean follow-up of 38 mo.

SMOKING BIOMARKER AND RENAL TRANSPLANTATION

Smoking exposure and analysis of dose of smoking depends on self-reporting in most of the studies[24], which we strongly believe it lacks accuracy. A proper estimation of the risks associated with tobacco use depends on accurate measurement of exposure, which may be difficult in certain population such as pregnant women and parents of young children, where smoking considered socially unaccepted[24]. Some patients may not recall the number of cigarettes accurately (digit bias)[25], and finally the tobacco dose differs between individuals due to the difference between cigarettes as well as the difference in inhaling habits (passive smoking)[25]. All these factors made the development of a valid and accurate biomarker for tobacco smoking of ultimate importance.

Cotinine is the major metabolite of nicotine. It has a relatively constant level due its long half-life (16 h *vs* 2-3 h for nicotine), which can be measured in plasma or urine. For these reasons, cotinine is considered a promising biomarker of smoking exposure[25].

Hellemons *et al*[25] studied 603 renal transplant recipients for a mean follow-up of 6.9 years. The aim was to investigate the relation of self-reporting and cotinine exposure in transplant population and to evaluate the use of cotinine as an alternative for self-report[25]. They concluded that active smoking had a negative impact on patient and graft survival, while former smokers had increased the risk of mortality but not graft failure. They documented that cotinine measurement (especially plasma cotinine) provides a valid alternative to self-reported smoking exposure, and it may even be preferred over self-reporting in epidemiological studies[25].

The use of cotinine also has its limitations. Cotinine level is a reflection of smoking over the past few days, and this may be misleading if the patient is smoking occasionally (like in weekends) or if the patient was smoking less due to a period of illness. The second limitation lies in its inability to differentiate between never-smoking and former-smoking[25]. Differentiating never-smoking from former-smoking is clinically relevant as former-smoking was proved to be associated with increasing risk of recipient mortality[20,25].

We believe that the combination of cotinine measurement and self-reporting of smoking exposure will be the most reliable approach in evaluating renal transplant population.

CONCLUSION

Smoking remains a major modifiable health care challenge; it is the leading cause of variable morbidities and mortality. The use of smoking biomarkers proved to be reliable in evaluation and quantification of smoking exposure in the transplant population. Donor smoking and recipient former smoking proved to have a negative impact on survival. Transplant community should pay more attention to donor and recipient smoking cessation programs.

REFERENCES

1 **Opelz G**, Döhler B. Influence of Current and Previous Smoking on Cancer and Mortality After Kidney Transplantation. *Transplantation* 2016; **100**: 227-232 [PMID: 26102616 DOI: 10.1097/TP.0000000000000804]

2 **Nourbala MH**, Nemati E, Rostami Z, Einollahi B. Impact of cigarette smoking on kidney transplant recipients: a systematic review. *Iran J Kidney Dis* 2011; **5**: 141-148 [PMID: 21525572]

3 **Orth SR**, Ogata H, Ritz E. Smoking and the kidney. *Nephrol Dial Transplant* 2000; **15**: 1509-1511 [PMID: 11007813 DOI: 10.1093/ndt/15.10.1509]

4 **Ritz E**, Benck U, Franek E, Keller C, Seyfarth M, Clorius J. Effects of smoking on renal hemodynamics in healthy volunteers and in patients with glomerular disease. *J Am Soc Nephrol* 1998; **9**: 1798-1804 [PMID: 9773780]

5 **Pinto-Sietsma SJ**, Mulder J, Janssen WM, Hillege HL, de Zeeuw D, de Jong PE. Smoking is related to albuminuria and abnormal renal function in nondiabetic persons. *Ann Intern Med* 2000; **133**: 585-591 [PMID: 11033585 DOI: 10.7326/0003-4819-133-8-200010170-00008]

6 **Corbett C**, Armstrong MJ, Neuberger J. Tobacco smoking and solid organ transplantation. *Transplantation* 2012; **94**: 979-987 [PMID: 23169222 DOI: 10.1097/TP.0b013e318263ad5b]

7 **Ponticelli C**, Villa M, Cesana B, Montagnino G, Tarantino A. Risk factors for late kidney allograft failure. *Kidney Int* 2002; **62**: 1848-1854 [PMID: 12371988 DOI: 10.1046/j.1523-1755.2002.00612.x]

8 **Arend SM**, Mallat MJ, Westendorp RJ, van der Woude FJ, van Es LA. Patient survival after renal transplantation; more than 25 years follow-up. *Nephrol Dial Transplant* 1997; **12**: 1672-1679 [PMID: 9269647 DOI: 10.1093/ndt/12.8.1672]

9 **Cosio FG**, Falkenhain ME, Pesavento TE, Yim S, Alamir A, Henry ML, Ferguson RM. Patient survival after renal transplantation: II. The impact of smoking. *Clin Transplant* 1999; **13**: 336-341 [PMID: 10485376 DOI: 10.1034/j.1399-0012.1999.130410.x]

10 **Kasiske BL**, Klinger D. Cigarette smoking in renal transplant recipients. *J Am Soc Nephrol* 2000; **11**: 753-759 [PMID: 10752535]

11 **Doyle SE**, Matas AJ, Gillingham K, Rosenberg ME. Predicting clinical outcome in the elderly renal transplant recipient. *Kidney Int* 2000; **57**: 2144-2150 [PMID: 10792636 DOI: 10.1046/j.1523-1755.2000.00066.x]

12 **Matas AJ**, Payne WD, Sutherland DE, Humar A, Gruessner RW, Kandaswamy R, Dunn DL, Gillingham KJ, Najarian JS. 2,500 living donor kidney transplants: a single-center experience. *Ann Surg* 2001; **234**: 149-164 [PMID: 11505060 DOI: 10.1097/00000658-200108000-00004]

13 **Sung RS**, Althoen M, Howell TA, Ojo AO, Merion RM. Excess risk of renal allograft loss associated with cigarette smoking. *Transplantation* 2001; **71**: 1752-1757 [PMID: 11455254 DOI: 10.1097/00007890-200106270-00009]

14 **Yavuz A**, Tuncer M, Gürkan A, Demirbaş A, Süleymanlar G, Ersoy F, Yakupoğlu G. Cigarette smoking in renal transplant recipients. *Transplant Proc* 2004; **36**: 108-110 [PMID: 15013315 DOI: 10.1016/j.transproceed.2003.11.051]

15 **Kheradmand A**, Shahbazian H. The role of pretransplant smoking on allograft survival in kidney recipients. *Urol J* 2005; **2**: 36-39 [PMID: 17629894]

16 **Zitt N**, Kollerits B, Neyer U, Mark W, Heininger D, Mayer G, Kronenberg F, Lhotta K. Cigarette smoking and chronic allograft nephropathy. *Nephrol Dial Transplant* 2007; **22**: 3034-3039 [PMID: 17517794 DOI: 10.1093/ndt/gfm275]

17 **Gombos P**, Langer RM, Korbely R, Varga M, Kaposi A, Dinya E, Müller V. Smoking following renal transplantation in Hungary and its possible deleterious effect on renal graft function. *Transplant Proc* 2010; **42**: 2357-2359 [PMID: 20692480 DOI: 10.1016/j.transproceed.2010.05.143]

18 **Nogueira JM**, Haririan A, Jacobs SC, Cooper M, Weir MR. Cigarette smoking, kidney function, and mortality after live donor kidney transplant. *Am J Kidney Dis* 2010; **55**: 907-915 [PMID: 20176427 DOI: 10.1053/j.ajkd.2009.10.058]

19 **Hurst FP**, Altieri M, Patel PP, Jindal TR, Guy SR, Sidawy AN, Agodoa LY, Abbott KC, Jindal RM. Effect of smoking on kidney transplant outcomes: analysis of the United States Renal Data System. *Transplantation* 2011; **92**: 1101-1107 [PMID: 21956202 DOI: 10.1097/TP.0b013e3182336095]

20 **Agarwal PK**, Hellemons ME, Zelle DM, van Ree RM, van den Born J, van der Heide JJ, Gans RO, van Son WJ, Navis G, Bakker SJ. Smoking is a risk factor for graft failure and mortality after renal transplantation. *Am J Nephrol* 2011; **34**: 26-31 [PMID: 21659736 DOI: 10.1159/000328903]

21 **Lin SJ**, Koford JK, Baird BC, Hurdle JF, Krikov S, Habib AN, Goldfarb-Rumyantzev AS. Effect of donors' intravenous drug use, cigarette smoking, and alcohol dependence on kidney transplant outcome. *Transplantation* 2005; **80**: 482-486 [PMID: 16123722 DOI: 10.1097/01.tp.0000168154.14458.28]

22 **Underwood PW**, Sheetz KH, Cron DC, Terjimanian MN, Englesbe MJ, Waits SA. Cigarette smoking in living kidney donors: donor and recipient outcomes. *Clin Transplant* 2014; **28**: 419-422 [PMID: 24617506 DOI: 10.1111/ctr.12330]

23 **Heldt J**, Torrey R, Han D, Baron P, Tenggardjaja C, McLarty J, Lindler T, Baldwin DD. Donor Smoking Negatively Affects Donor and Recipient Renal Function following Living Donor Nephrectomy. *Adv Urol* 2011; **2011**: 929263 [PMID: 21912540 DOI: 10.1155/2011/929263]

24 **Florescu A**, Ferrence R, Einarson T, Selby P, Soldin O, Koren G. Methods for quantification of exposure to cigarette smoking and environmental tobacco smoke: focus on developmental toxicology. *Ther Drug Monit* 2009; **31**: 14-30 [PMID: 19125149 DOI: 10.1097/FTD.0b013e3181957a3b]

25 **Hellemons ME**, Sanders JS, Seelen MA, Gans RO, Muller Kobold AC, van Son WJ, Postmus D, Navis GJ, Bakker SJ. Assessment of Cotinine Reveals a Dose-Dependent Effect of Smoking Exposure on Long-term Outcomes After Renal Transplantation. *Transplantation* 2015; **99**: 1926-1932 [PMID: 25710609 DOI: 10.1097/TP.0000000000000636]

P- Reviewer: Botha P, Salvatore SP, Yueh CY **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Wu HL

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 April 24; 7(2): 134-143

DOI: 10.5500/wjt.v7.i2.134

ISSN 2220-3230 (online)

MINIREVIEWS

Past, present and future of kidney paired donation transplantation in India

Vivek B Kute, Himanshu V Patel, Pankaj R Shah, Pranjal R Modi, Veena R Shah, Sayyed J Rizvi, Bipin C Pal, Manisha P Modi, Priya S Shah, Umesh T Varyani, Pavan S Wakhare, Saiprasad G Shinde, Vijay A Ghodela, Minaxi H Patel, Varsha B Trivedi, Hargovind L Trivedi

Vivek B Kute, Himanshu V Patel, Pankaj R Shah, Priya S Shah, Umesh T Varyani, Pavan S Wakhare, Saiprasad G Shinde, Vijay A Ghodela, Hargovind L Trivedi, Department of Nephrology and Clinical Transplantation, Institute of Kidney Diseases and Research Centre, Dr HL Trivedi Institute of Transplantation Sciences (IKDRC-ITS), Ahmedabad 380016, India

Pranjal R Modi, Sayyed J Rizvi, Bipin C Pal, Department of Urology and transplantation, IKDRC-ITS, Ahmedabad 380016, India

Veena R Shah, Manisha P Modi, Department of Anaesthesia, IKDRC-ITS, Ahmedabad 380016, India

Minaxi H Patel, Varsha B Trivedi, Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, IKDRC-ITS, Ahmedabad 380016, India

Author contributions: All authors contributed to the acquisition of data, writing, and revision of this manuscript.

Conflict-of-interest statement: All the authors have no conflicts of interests to declare.

Manuscript source: Invited manuscript

Correspondence to: Dr. Vivek B Kute, MBBS, MD, FCPS, DM Nephrology (Gold Medalist), FASN, Associate Professor, Department of Nephrology and Clinical Transplantation, Institute of Kidney Diseases and Research Centre, Dr HL Trivedi Institute of Transplantation Sciences, B-130, Triveni, Ph-1, Ahmedabad 380016, India. drvivekkute@rediffmail.com

Telephone: +91-90-99927543

Received: November 1, 2016
Peer-review started: November 4, 2016
First decision: November 30, 2016
Revised: December 11, 2016
Accepted: January 2, 2017
Article in press: January 4, 2017
Published online: April 24, 2017

Abstract

One third of healthy willing living kidney donors are rejected due to ABO blood group incompatibility and donor specific antibody. This increases pre-transplant dialysis duration leading to increased morbidity and mortality on the kidney transplantation waiting list. Over the last decade kidney paired donation is most rapidly increased source of living kidney donors. In a kidney transplantation program dominated by living donor kidney transplantation, kidney paired donation is a legal and valid alternative strategy to increase living donor kidney transplantation. This is more useful in countries with limited resources where ABO incompatible kidney transplantation or desensitization protocol is not feasible because of costs/infectious complications and deceased donor kidney transplantation is in initial stages. The matching allocation, ABO blood type imbalance, reciprocity, simultaneity, geography were the limitation for the expansion of kidney paired donation. Here we describe different successful ways to increase living donor kidney transplantation through kidney paired donation. Compatible pairs, domino chain, combination of kidney paired donation with desensitization or ABO incompatible transplantation, international kidney paired donation, non-simultaneous, extended, altruistic donor chain and list exchange are different ways to expand the donor pool.

In absence of national kidney paired donation program, a dedicated kidney paired donation team will increase access to living donor kidney transplantation in individual centres with team work. Use of social networking sites to expand donor pool, HLA based national kidney paired donation program will increase quality and quantity of kidney paired donation transplantation. Transplant centres should remove the barriers to a broader implementation of multicentre, national kidney paired donation program to further optimize potential of kidney paired donation to increase transplantation of O group and sensitized patients. This review assists in the development of similar programs in other developing countries.

Key words: Living donor kidney transplantation; Kidney paired donation; Renal replacement therapy; Developing country

Core tip: Over the last decade kidney paired donation is most rapidly increased source of living kidney donors. Here we describe different successful ways to increase living donor kidney transplantation through kidney paired donation. Compatible pairs, domino chain, combination of kidney paired donation with desensitization or ABO incompatible transplantation, international kidney paired donation, non-simultaneous, extended, altruistic donor chain and list exchange are different ways to expand the donor pool. Transplant centres should remove the barriers to a broader implementation of multicentre, national kidney paired donation program to further optimize potential of kidney paired donation to increase transplantation of O group and sensitized patients.

Kute VB, Patel HV, Shah PR, Modi PR, Shah VR, Rizvi SJ, Pal BC, Modi MP, Shah PS, Varyani UT, Wakhare PS, Shinde SG, Ghodela VA, Patel MH, Trivedi VB, Trivedi HL. Past, present and future of kidney paired donation transplantation in India. *World J Transplant* 2017; 7(2): 134-143 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i2/134.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i2.134

INTRODUCTION

Low insurance coverage, poor public health system leading to out of pocket health expenditure and unavailability of adequate trained doctors and staff are problems of renal replacement therapies in developing country. Living donor kidney transplants have a greater long-term graft survival rate than deceased donor kidney transplantation (primarily from brain-dead donors). Kidney paired donation has all advantages of living donor kidney transplantation (similar patient survival, graft survival and outcome). Successful kidney paired donation program requires healthy mixture of enthusiasm, mathematical modeling, patience and team work. Learning curves, need of infrastructural support, additional cost are not required in kidney paired donation. It can be done at center of their choice under their primary nephrologist. Worldwide kidney paired donation has increased access to living donor kidney transplantation in national and single centre programs in the last decade[1-10]. Here we describe different successful ways to increase living donor kidney transplantation through kidney paired donation[11-13]. This review assists in the development of similar programs in other developing countries.

CONVENTIONAL BALANCED KIDNEY PAIRED DONATION

The pair 1 (A patient and B donor) exchanges kidney with pair 2 (B patient and A donor) and both the pairs are benefitted resulting in two ABO compatible kidney transplantation. Kidney paired donation initially started in Dutch program as closed loop of 2-way kidney exchange. It can be arranged as 3-way, 4-way and n-way exchanges. Two way single centre kidney paired donation program increases waiting time to find suitable donor in kidney exchange program. It has less match rate and has limited scope to increase transplant rate. The 3-way exchange increases match rate from 54% to 66% in one simulation study[11]. Dutch program reported that 3 way exchange is the most optimum length of kidney paired donation to achieve good match rate and to carry out simultaneous kidney transplantation especially for newly starting single centre kidney paired donation programs[7-10]. The longer chains do not lead to significantly more kidney transplantation. Multiple simultaneous kidney transplantation surgeries increase logistic burden on the transplant team, and requires stringent and careful transplant coordination.

UNCONVENTIONAL KIDNEY PAIRED DONATION WITH USE OF COMPATIBLE PAIRS

The ABO incompatible pair 1 (O patient and non-O donor) exchange kidney with ABO compatible but ABO non-identical pair 2 (non-O patient and O donor). This is also known as altruistically unbalanced paired donation. The compatible pairs can be offered benefit by better HLA matched donor or younger donor. Transplant surgery should not be delayed for the compatible pair to find better matched donor especially in developing countries where the morbidity and mortality on long term maintenance dialysis is high. Bingaman *et al*[12,13] reported increase in match and transplant rate with use of compatible pairs. The compatible pairs increase the match rate for incompatible pairs (28.2% to 64.5% for single-centre program, 37.4% to 75.4% for national program). Legal, logistical, and governmental controversies, lack of awareness and counselling have limited the growth kidney paired donation with compatible pairs. KPD transplantation can be offered to non-HLA identical compatible pairs with

donors over 45 years to get better (HLA or younger donor) matched donor[12,13].

Over the last three decades the short term graft survival is improved but long term graft survival and outcome is similar with use of modern potent immunosuppression. The age of ESRD patient in developing countries like India is younger than developed countries. The leading cause of morbidity and mortality after kidney transplantation in India is Infection. Better HLA matched kidney transplantation for the compatible pairs will result in better long term outcome and need of re-transplantation which is common cause of sensitization. Commercial interest should be carefully ruled out in such kind of exchange with careful selection. Basu *et al*[14] reported the need of large donor pool (multicentre or national kidney paired donation program) to find better HLA matched donor. The willingness of ABO compatible pairs to participate in kidney paired donation should be evaluated in more studies to increase the long term graft survival[15,16].

Multiple studies have demonstrated HLA-matched transplant had higher rates of survival, a lower incidence of rejection, and a lower risk of graft loss due to immune injury[17]. The Collaborative Transplant Study, the United Kingdom Transplant and Euro-transplant data showed that DR matching having a much greater effect than that of B or A. In India majority of living donors are females and most of them are spousal donors. If all spousal donors above 45 years of age even though ABO compatible (especially blood group O donors) are included in national kidney paired donation program, it will increase the number of transplants of O group and sensitized recipients[12,13].

NON-DIRECTED ANONYMOUS DONORS

Non-directed anonymous donors (Good Samaritan or altruistic donors) are donors who want to donate a kidney, but do not have an intended recipient. Non-directed anonymous donors from the general population can initiate the kidney paired donation chain to increase transplant rate for O group and sensitized patients in kidney paired donation[18-21]. One of the key to the success of Canadian kidney paired donation program is non-directed anonymous donors chains, where non-directed anonymous donors facilitated transplants in 61% of all incompatible kidney paired donation pairs[4]. There should be legal permission for non-directed anonymous donors as per organ act of the country. Transplantation of Human Organs Act (THOA), India did not permit non-directed anonymous donors transplants.

USE OF KIDNEY PAIRED DONATION TO INCREASE ACCESS TO LIVING DONOR KIDNEY TRANSPLANTATION FOR SENSITIZED PATIENTS

Kidney paired donation in the presence of low-level donor specific antibody can be performed in carefully selected highly sensitized patients with minimal to no desensitization. The patients should be aware of possible poor long term outcomes with low level donor specific antibody and negative flow cross-match due to the impact of memory responses[22]. The use of ABO incompatible pairs also increases match rate for highly sensitized patients. Kidney paired donation combined with desensitization protocol can be performed with donor of low immunological risk in absence of other better option for the carefully selected highly sensitized patients. This strategy is used in Johns Hopkins Hospital[23,24]. The Global kidney exchange will increase the living donor kidney transplantation opportunity for sensitized and O group patients by direct benefit of increase in donor pool and benefit from differences in heterogeneity of blood types distribution in the population, antigens and antibodies profile. It will also improve the quality and quantity of transplant.

DOMINO PAIRED DONATION

Kidney exchange transplants can be increased by 20% with domino paired donation[25]. In one South Korean centre, 179 living donor kidney transplantations were performed, with 70 domino chains initiated by an altruistic living non-directed donor. The patient and graft survival rates at 1-year and 5-year were 97.2% and 90.8%, and 98.3% and 87.7%, respectively. Multi-centre domino kidney paired donation increases access to living donor kidney transplantation, with similar outcome to conventional kidney paired donation[26].

KIDNEY PAIRED DONATION COMBINED WITH ABO-INCOMPATIBLE TRANSPLANTATION

Patient donor pair with high ABO titres [for examples pair 1: patient 1 (O group) and donor 1 (A group) with anti-A isoagglutinin titer $\geqslant$ 512; pair 2: patient 2 (O group) and donor 2 (B group) with anti-B isoagglutinin titre $\geqslant$ 512] exchange kidney to get donor with low ABO titres [pair 1: patient 1 (O group) and donor 2 (B group) with anti-B *isoagglutinin* titer $\leqslant$ 64; pair 2: patient 2 (O group) and donor1 (A group) with anti-A isoagglutinin titre $\leqslant$ 64]. This will minimizes cost, deceases need of immunosuppression and improve long term outcome of ABO incompatible kidney transplantation and increases match rate for the sensitized patients. ABO-incompatible transplantation in the absence of donor specific antibody with low baseline ABO titre $\leqslant$ 1:64 has good outcome[27,28]. The cut-off value of high ABO antibody titre may vary as per experience of the transplant unit. This strategy is used effectively in the various national kidney paired donation program (Australian > United Kingdom > Canada)[28].

INTERNATIONAL KIDNEY PAIRED DONATION

The single centre kidney paired donation program which

is commonly practiced in India has inherent limitations to expand the donor pool. Garonzik-Wang *et al*[29] reported international kidney exchange between the United States and Canada in a 10-way domino chain kidney transplantation between September 2009 to July 2010. The success was attributed to close geography reducing kidney transport time, close collaboration, similar language and philosophical understandings between the Canada and the United States transplant team. Three international living donation kidney transplantation from kidney exchange program between May 2013, and March 2014 were reported in Turkey where national kidney paired donation program increased living donation kidney transplantation by 5%[30]. The international organ exchange from deceased donors substantially contributed (7.2% of deceased donor kidney transplantation) to the Swiss transplant activity during the period 2009-2013[31]. Each state, region and all the developing countries needs a more robust, organised kidney sharing scheme and efforts should be made to establish a national/regional pool of kidney sharing registry as is the case with the European, North American and other developed countries. Local/regional/national kidney sharing options should be fully explored prior to embarking on international kidney sharing. Global registry of incompatible pairs from diverse population of patient-donor pairs is expected to yield transplant to these pairs.

LIST EXCHANGE AND INDIA

In a living donor list exchange program, the living donor in ABO or HLA incompatible pair donate kidney to the deceased donor kidney transplantation waitlist patient and in return the incompatible patient get top priority on the deceased donor kidney transplantation waitlist. Melcher *et al*[32] reported utilization of deceased donor kidneys to initiate living donor kidney transplantation chains. Ross *et al*[33] reported to restrict list paired exchanges to A, B, AB blood group and sensitized patient donor pair excluding O group patients. The deceased donor kidney transplantation waiting time is prolonged for O group patients with use of list exchange. Single centre kidney paired donation program in Ahmedabad India, demonstrated that deceased donor - living donor list exchange is not required for A and B blood group patient donor pair as they can be readily transplanted in living donor kidney paired donation within reasonable waiting time[34]. The graft half-life of deceased donor and living donor kidney is 13.8 and 21.6 years respectively[35]. This shows that including non-O blood group (A and B group) patient donor pair in list exchange will be unfair as the intended patient will receive a deceased donor kidney rather than a living donor kidney. Patient donor pairs were more willing to participate in living donor kidney paired donation as compared to deceased donor -living donor exchange program. The major reason for this was their intended recipient received kidney from a living donor as compared to deceased donor and intended recipients would get transplants at the same time. Similar findings were also reported by Waterman *et al*[36]. This could be the reason for the significant increase in living donor kidney paired donation program compared to living donor –deceased donor list exchange in the last decade all over the world. The older, diabetic and highly sensitized patients could get benefit from accepting deceased donor kidney of lower quality as compared to living donor kidney early after end stage renal disease, whereas younger, A and B group patients benefit from receiving higher quality living donor kidney even with longer dialysis exposure[37].

In India, allocation of deceased donor kidney is done according to waiting time and not by HLA matching. There is no provision of list exchange in Transplantation of Human Organs October 2013, India. For deceased donor-living donor list exchange program, deceased donor wait list should be transparent with uniform enrolment rules for patients. Deceased donor should be standard criteria donor with uniform donor acceptance policy and definitely should not be the expanded criteria donor. Cold ischemia time should be minimized to improve long term outcome. Donor associated infections should be carefully ruled out. The quality of the kidney should be confirmed by frozen section biopsy whenever required. Every attempt should be made to improve the quality of organ to improve the long term survival. More studies are required to address this issue to balance principal of utility and justice of kidney transplantation.

ALLOCATION ALGORITHMS IN KIDNEY PAIRED DONATION

The virtual cross-matching is used effectively for donor allocation by the various national kidney paired donation program. The manual allocation can be performed by transplant team member with bonus points to sensitised patient, difficult to match patient (O group patient and non - O donor), retransplantation, donor age similarity, dialysis time, HLA match and waiting time[38,39].

KEY ELEMENTS OF FOUR NATIONAL KIDNEY PAIRED DONATION REGISTRIES

Dedicated central support staff, multi-way and domino exchanges, frequency of match cycles every 3-4 mo, donor allocation algorithm with the virtual cross-match, accepts ABO incompatible donor matching (Australia and United Kingdom program), Donor travel (The Netherlands and Canada) or organ transport (Australia and United Kingdom program), and good HLA laboratories support are the key components of four national kidney paired donation registries. The match and transplant rates from two-way and three-way exchanges are not dependent on donor pool size at the time of allocation. Dutch kidney paired donation program reported that the success of a living donor kidney exchange program depends on good co-ordination between the participating transplant centres, common protocol for the selection of donor and patient,

Table 1 Outcome of single center kidney paired donation program India[40-44]

	Pahwa *et al*[41]	Waigankar *et al*[40]	Jha *et al*[42]	Kute *et al*[43]	Kute *et al*[44]
Duration	2006-2011	2008-2011	2010-2013	2000-2012	2013
Patients (*n*)	44	14	26	70	56
2-way exchange	22	7	13	35	25
Follow up	3 yr	12-18 mo	20 mo (median)	2.72 yr (mean)	1 yr
Graft survival	100%	100%	92.30%	81%	97.50%
Patient survival	97.70%	100%	96.16%	90%	94.60%
Acute rejection	-	14.20%	11.50%	14.20%	16%
Reason for joining kidney paired donation (*n*)					
ABO incompatible	40	8	26	56	52
Sensitized	4	0	0	14	4

Table 2 Advantages and disadvantages of single *vs* multicentre kidney paired donation transplant

	Singe center	Multicenter
Donor pool	Less	More
Donor transport	Not required	Required
Shipping of kidneys	Not required	Required
Surgical team skills	Same	Different
Surgical team requirement	More	Less
Cold ischemia time	Less	More
Hospital atmosphere	Familiar	Unfamiliar
Follow up	Same center	Difficult follow up
Administrative cost	Less	More

supervision by an independent allocation organization and a central HLA and tissue typing laboratory responsible for the cross-matches. The protocol consisted of four different steps the registration procedure for participants, allocation - and matching criteria, cross-match procedure in the central national reference laboratory and surgical and follow-up procedures.

OUTCOME OF SINGLE CENTER KIDNEY PAIRED DONATION PROGRAM IN INDIA

Between January 2000 and July 2016, 3616 living donor kidney transplantation and 561 deceased donor kidney transplantation were performed at Institute of Kidney Diseases and Research Centre, Dr HL Trivedi Institute of Transplantation Sciences, Ahmedabad, India with 300 of them (8.3%) using kidney paired donation. Kidney paired donation contributed to 56 kidney paired donation transplantations in 2013 and 2014 leading to increase living donor kidney transplantation by 15.8% and 18.1% respectively[40-59]. Seventy seven kidney paired donation increased the living donor kidney transplantation rate by 25% in one year in 2015. Our centre in Ahmedabad India has used different forms of kidney exchanges including 2-way, 3-way, 4-way, 6-way kidney exchange, use of compatible patient donor pairs, kidney exchange with desensitization, non-simultaneous kidney exchange and international kidney exchange[51-57] (Table 1).

Advantages and disadvantages of single *vs* multicentre kidney paired donation transplant are given in Table 2. In absence of computer allocation system and national kidney paired donation program, the single center can start manual allocation of 2-way or 3-way exchange of ABO incompatible pairs. Matching at the single-centre kidney paired donation program would eliminate the need for co-ordination between different transplant centres, common standard protocols between centres for medical selection of donor-recipient pair; privacy and legal concerns. The virtual cross matching can be used in case of cross match positive pairs. Multicenter or national kidney paired donation program can increase match rate for difficult to match patients like O group and cross match positive donor-recipient pair.

The single centre study showed that outcome (patient survival, graft survival, and rejection rate) of living related donor kidney transplantation (n = 190) is similar to kidney paired donation (n = 34) at 2 years follow up[47]. The use of carefully selected older living donor and patient-donor age difference has no significant impact on long term graft survival in living donor kidney transplantation (n = 49). This is useful in single centre kidney paired donation program with limited donor pool.

POTENTIAL AND SUSTAINABILITY OF A SINGLE-CENTRE KIDNEY PAIRED DONATION PROGRAM

Methodist San Antonio kidney paired donation program reported outcome of 134 kidney paired donation transplants (117 incompatible pairs and 17 compatible pairs) performed over a 3-year period (November 2007 to February 2011)[12,13]. There was significant increase

in access to living donor kidney transplantation with kidney paired donation over the 3 years in Methodist San Antonio kidney paired donation program (11%, 27%, 35%). These data also validate impact of single centre kidney paired donation program. Key elements of the Methodist San Antonio kidney paired donation program were computer allocation, storage of blood specimens for future cross-match testing with consent of patient-donor pairs, A1 and A2 subtype of all blood type A donors and use of more compatible pairs. All patients had negative cross match at the time of transplant, prospective counselling of all patient-donor pairs regarding kidney paired donation, comprehensive immunological assessment with donor specific antibody and HLA testing of all patient-donor pairs, combination of kidney paired donation with desensitization for highly sensitized patients were the strategies implemented by single centre program like San Antonio. It has increased access to kidney paired donation transplantation for traditionally disadvantaged cohorts of patients (female recipients (61%) and previous transplant (32%).

Key to success of the single centre kidney paired donation program in India[40,41] are formation of registry to maintain database of incompatible pairs, awareness and mandatory counselling about advantages of living donor kidney paired donation program, expert transplant coordinator, dedicated HLA laboratory, patient-mentorship program to increase awareness about kidney paired donation, dedicated transplant team for evaluating donors and recipients and supporting the patients to overcome a variety of logistical barriers, dedicated transplant team to run the living donation kidney transplantation program, use of compatible pairs and active participation of patients. Medical profession, government and politicians willingness and support is required for the expansion of kidney exchange in India. In a high volume living donor kidney transplantation program all A and B blood group donor recipient pairs without sensitization can be transplanted with kidney paired donation within reasonable waiting time even with manual allocation without using the computer allocation[40,41].

MATCH RATES BY PATIENT-DONOR PAIR CHARACTERISTICS TO DECIDE ABOUT KIDNEY PAIRED DONATION *VS* DESENSITIZATION

Panel reactive antibodies indicate the ability to match in kidney paired donation. Donor specific antibody indicates ability to desensitize. Panel reactive antibodies and donor specific antibody in combination help to predict which modality (kidney paired donation, desensitization or a combination of both) increases early access to cost effective living donation kidney transplantation with best long term outcome. Donor-recipient pair who are easy in kidney paired donation and desensitization [low panel reactive antibodies, low-strength donor specific antibody (narrow sensitization), O donor] should be tried in kidney paired donation first for the few months and if no match is found in kidney paired donation should undergo desensitization therapy with written informed consent of the pairs. Donor-recipient pair who are easy to match in kidney paired donation and hard to desensitize [low panel reactive antibodies, high-strength donor specific antibody (highly sensitized), O donor] should wait in kidney paired donation. Donor-recipient pair who are hard to match in kidney paired donation and easy to match in desensitization [high panel reactive antibodies, low-strength donor specific antibody (narrow sensitization), non-O donor (specially AB), O recipient] should first look in kidney paired donation pool but probably not worth waiting for the long time and if no match found in kidney paired donation within few months should undergo desensitization therapy with written informed consent of the pairs. Donor-recipient pair who are hard to match in kidney paired donation and hard to desensitize [high panel reactive antibodies, high-strength donor specific antibody (highly sensitized), non-O donor (specially AB), O recipient] may not benefit by single modality of kidney paired donation or desensitization therapy. They should be considered for the combination of the kidney paired donation and desensitization therapy to find a "better" donor. Risk associated with HLA incompatible higher than that associated with ABO incompatible. Kidney paired donation should be preferred over the desensitization therapy. Patients who are hard-to-desensitize (high-strength donor specific antibody) should wait for a match in kidney paired donation, unless they are also hard-to-match (high panel reactive antibodies).

Kidney paired donation limitations and expansions

The expansion of kidney paired donation can be achieved if all the limiting factors are properly solved.

Coercion

The potential kidney donor can deny for donation due to medical reasons like ABO incompatible or cross match positive. Kidney paired donation can increases pressure on the donors for donation. The care should therefore be taken that kidney donor is motivated for the donation and there is no pressure on the donor for the indirect donation.

Anonymity: Kidney paired donation initially started as an anonymous transplantation. The advantage of anonymity is that transplantation team will save the time of organising meetings between the different donor-recipient pair. There will be no extra psychological pressure or conflicts between the two pairs when the results of the two transplantations are not equal especially in the simultaneous single centre kidney transplantation. Donor-recipient pair will not withdraw from the kidney paired donation due to non-medical reasons like cast, *etc*., after meeting with the intended donor. A disadvantage of anonymity is that the donor will

not be informed about the functioning of the donated kidney. In fact formal meeting between the two donor-recipient pair increases the trust between donor-recipient pair and transplant team. They should be counselled that although kidneys are exchange of similar good quality, post-transplant outcome can be different in the two patients depending on the patient related factors like immunology. In the Indian scenario authorization committee take the meeting of the 2 donor-recipient pair together and evaluate about the consent to participate in kidney paired donation. Anonymity is very difficult to maintain in case of simultaneous transplant surgery in single centre kidney paired donation program.

DISTRIBUTION OF BLOOD GROUP TYPES IN INCOMPATIBLE DONORS AND PATIENTS

One of the limitations of kidney paired donation is imbalance between O donor and non-O recipients in the ABO blood group type distribution in general population and incompatible donor recipient pairs. In typical kidney paired donation pools, participation of donor recipients pairs with type O blood group recipients, and non-O blood group donors is more. The compatible pairs would greatly alleviate this imbalance and increases transplant rate for O group and sensitized patients.

Reciprocal match requirement

The kidney paired donation matches require reciprocal compatibility.

Simultaneous donor nephrectomy requirements

It is standard practice to consider simultaneous donor nephrectomy and transplant surgery in kidney paired donation. Majority of Indian transplant centres perform simultaneous two way kidney exchanges and long chains are not preferred due to limited transplant team (operating rooms and surgical staff) and infrastructure. More than 2-way exchanges and long chains can be performed with single centre non-simultaneous kidney paired donation or multi-centre simultaneous kidney paired donation. Multi-centre simultaneous kidney paired donation requires donor travel or transport of kidney. The long term graft survival is not significantly affected when cold ischemia time is short (< 8 h). Despite prolonged cold ischemia time for interstate exchanges, the Australian kidney exchange program preferred to transport donor kidneys rather than kidney donors[60]. However, there is no multi-centre kidney paired donation transplant practice in India. This requires uniform pre-transplant evaluation and acceptance criteria for living donors and fitness of patients among the participating transplant centres. Hospital atmosphere would be unfamiliar for the donor and donor-recipient pair may not trust on the transplant team in other hospital in case of multicentre simultaneous kidney paired donation. In India, only one report of multi-centre simultaneous kidney paired donation of 5 donor-recipient pairs has been reported[58]. Careful selection, written informed consent of pairs and permission from authorization committee is required in single centre non-simultaneous kidney transplantation. In non-simultaneous kidney transplantation, the long chain can break if donor reneges or recipient become medically unfit. Proper counselling of the pairs can avoid donor reneging and standard criteria deceased donor kidney can be allocated on priority in case of donor reneging. All the patients should remain medically fit for transplantation in non-simultaneous kidney transplantation.

Kidney paired donation for O group patients with non-O donor

Living donor kidney transplantation options for O group patients with non-O kidney donor and low ABO titer (< 1:64) are participation in kidney paired donation with compatible pair, international kidney paired donation, global kidney exchange, ABO incompatible kidney transplantation.

Kidney transplantation options for O group patients with non-O kidney donor and high ABO titer are participation in kidney paired donation with compatible pair, international kidney paired donation, global kidney exchange, kidney paired donation combined with ABO-incompatible transplantation, living-deceased donor kidney exchange and deceased donor kidney transplantation.

There is a need for Indian guidelines for incompatible pairs but there is ever more need to develop practice algorithms at least for this part of the world. This should focus on cost, long term patient/graft survival, availability of therapy and local resource limitations.

Legal barriers and new hope

Kidney paired donation is underutilized in India despite tremendous potential for the growth. It could be attributed to lack of national database about incompatible pairs, lack of awareness/counselling about kidney paired donation and administrative challenges (legal permission, *etc.*). This is new hope to overcome administrative challenges from different state authorization committee. In India, Transplantation of Human Organs Act 2011 gives legal permission for kidney paired donation[59]. When the donor-recipient pairs are from different geographic area and state of residence, it was mandatory to take legal permission from authorization committee from all the states rather than only from authorization committee from the state in which transplantation is proposed to be done. This increases waiting time in administrative legal permission. According to Transplantation of Human Organs Act 2013, cases of kidney paired donation from near relative from different states Governments can be approved by authorization committee of hospital in which kidney transplantation is proposed to be done. It will promote multicentre and national kidney paired donation program. The altruistic donors are not allowed for organ donation in kidney paired donation in India.

Global kidney exchange[61,62]

There is financial barrier to kidney transplantation in developing world due to poverty and lack of national health insurance. Poor patient (A blood group patient and O blood group donor) could not undergo kidney transplantation despite having healthy, willing, compatible living kidney donor. The barrier to kidney transplantation in developed world is immunological (O blood group patient and A blood group donor) rather than financial. In global kidney exchange, these two patient donor pairs in developing and developed world exchange kidney with each other to overcome the barriers for kidney transplantation. Global kidney exchange is cost effective even if the cost of both kidney transplantations including the immunosuppression is paid by the health insurance payer of the developed country. Legal and logistical problems should be carefully solved for successful implementation of this strategy. More studied are required to address willingness of patients, health care professionals to participate in global kidney exchange.

Regulated compensation for living kidney donation

Most United states voters view living kidney donation positively, and reported that they would be motivated toward organ donation if offered compensation for living kidney donation of $50000[63]. Certain compensation amounts or health insurance to donor/family members could motivate the public to donate without being perceived as an undue inducement. The direct payment of money and paid leaves are the most preferred forms of compensation. A program of government compensation of kidney donors would provide the following benefits[64,65]: (1) Cost effective as dialysis is more expensive than transplant; (2) Increase living donor kidney transplantation will be available for the poor and productivity of society will increase and a good deal for taxpayers also; and (3) This will decrease morbidity and mortality of long term dialysis and increase quality of life for transplanted patients. The recent study from India reported that live donors should be given incentives for donating their kidney[66]. More studied are required to address regulated compensation for living kidney donation.

CONCLUSION

An effective kidney paired donation program should be implemented in each transplantation centre. Kidney paired donation has all advantages of living donor kidney transplantation (similar patient, graft survival, cost and outcome) without long waiting time for deceased donor kidney transplantation. Successful kidney paired donation program requires healthy mixture of enthusiasm, mathematical modeling, patience and team work. Transplant centres should remove the barriers to a broader implementation of multicentre, national kidney paired donation program to further optimize potential of kidney paired donation to increase transplantation of O group and sensitized patients.

REFERENCES

1 **Hadaya K**, Fehr T, Rüsi B, Ferrari-Lacraz S, Jean V, Ferrari P. Kidney paired donation: a plea for a Swiss National Programme. *Swiss Med Wkly* 2015; **145**: w14083 [PMID: 25742633 DOI: 10.4414/smw.2015.14083]

2 **Cantwell L**, Woodroffe C, Holdsworth R, Ferrari P. Four years of experience with the Australian kidney paired donation programme. *Nephrology* (Carlton) 2015; **20**: 124-131 [PMID: 25408125 DOI: 10.1111/nep.12369]

3 **Cole EH**, Nickerson P, Campbell P, Yetzer K, Lahaie N, Zaltzman J, Gill JS. The Canadian kidney paired donation program: a national program to increase living donor transplantation. *Transplantation* 2015; **99**: 985-990 [PMID: 25340607 DOI: 10.1097/TP.0000000000000455]

4 **Malik S**, Cole E. Foundations and principles of the Canadian living donor paired exchange program. *Can J Kidney Health Dis* 2014; **1**: 6 [PMID: 25780601 DOI: 10.1186/2054-3581-1-6]

5 **Ferrari P**, Weimar W, Johnson RJ, Lim WH, Tinckam KJ. Kidney paired donation: principles, protocols and programs. *Nephrol Dial Transplant* 2015; **30**: 1276-1285 [PMID: 25294848 DOI: 10.1093/ndt/gfu309]

6 **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]

7 **de Klerk M**, Witvliet MD, Haase-Kromwijk BJ, Weimar W, Claas FH. A flexible national living donor kidney exchange program taking advantage of a central histocompatibility laboratory: the Dutch model. *Clin Transpl* 2008: 69-73 [PMID: 19711512]

8 **Johnson RJ**, Allen JE, Fuggle SV, Bradley JA, Rudge C. Early experience of paired living kidney donation in the United kingdom. *Transplantation* 2008; **86**: 1672-1677 [PMID: 19104403 DOI: 10.1097/TP.0b013e3181901a3d]

9 **Segev DL**, Kucirka LM, Gentry SE, Montgomery RA. Utilization and outcomes of kidney paired donation in the United States. *Transplantation* 2008; **86**: 502-510 [PMID: 18724216 DOI: 10.1097/TP.0b013e3181812f85]

10 **Keizer KM**, de Klerk M, Haase-Kromwijk BJ, Weimar W. The Dutch algorithm for allocation in living donor kidney exchange. *Transplant Proc* 2005; **37**: 589-591 [PMID: 15848466 DOI: 10.1016/j.transproceed.2004.12.096]

11 **Saidman SL**, Roth AE, Sönmez T, Unver MU, Delmonico FL. Increasing the opportunity of live kidney donation by matching for two- and three-way exchanges. *Transplantation* 2006; **81**: 773-782 [PMID: 16534482 DOI: 10.1097/01.tp.0000195775.77081.25]

12 **Bingaman AW**, Wright FH, Kapturczak M, Shen L, Vick S, Murphey CL. Single-center kidney paired donation: the Methodist San Antonio experience. *Am J Transplant* 2012; **12**: 2125-2132 [PMID: 22548839 DOI: 10.1111/j.1600-6143.2012.04070.x]

13 **Bingaman AW**, Wright FH, Murphey CL. Kidney paired donation in live-donor kidney transplantation. *N Engl J Med* 2010; **363**: 1091-1092 [PMID: 20825328 DOI: 10.1056/NEJMc1004959]

14 **Basu G**, Daniel D, Rajagopal A, Neelakantan N, John GT. A model for human leukocyte antigen-matched donor-swap transplantation in India. *Transplantation* 2008; **85**: 687-692 [PMID: 18337661 DOI: 10.1097/TP.0b013e318163827e]

15 **Kranenburg LW**, Zuidema W, Weimar W, Passchier J, Hilhorst M, de Klerk M, IJzermans JN, Busschbach JJ. One donor, two transplants: willingness to participate in altruistically unbalanced exchange donation. *Transpl Int* 2006; **19**: 995-999 [PMID: 17081229 DOI: 10.1111/j.1432-2277.2006.00378.x]

16 **Gentry SE**, Segev DL, Simmerling M, Montgomery RA. Expanding kidney paired donation through participation by compatible pairs. *Am J Transplant* 2007; **7**: 2361-2370 [PMID: 17845570 DOI: 10.1111/j.1600-6143.2007.01935.x]

17 **Takemoto SK**, Terasaki PI, Gjertson DW, Cecka JM. Twelve years' experience with national sharing of HLA-matched cadaveric kidneys for transplantation. *N Engl J Med* 2000; **343**: 1078-1084 [PMID: 11027742 DOI: 10.1056/NEJM200010123431504]

18 **Matas AJ**, Garvey CA, Jacobs CL, Kahn JP. Nondirected donation of kidneys from living donors. *N Engl J Med* 2000; **343**: 433-436 [PMID: 10933745 DOI: 10.1056/NEJM200008103430611]

19 **Bramstedt KA**, Dave S. The silence of Good Samaritan kidney donation in Australia: a survey of hospital websites. *Clin Transplant* 2013; **27**: E244-E248 [PMID: 23383858 DOI: 10.1111/ctr.12087]

20 **Rees MA**, Kopke JE, Pelletier RP, Segev DL, Rutter ME, Fabrega AJ, Rogers J, Pankewycz OG, Hiller J, Roth AE, Sandholm T, Unver MU, Montgomery RA. A nonsimultaneous, extended, altruistic-donor chain. *N Engl J Med* 2009; **360**: 1096-1101 [PMID: 19279341 DOI: 10.1056/NEJMoa0803645]

21 **Roodnat JI**, Zuidema W, van de Wetering J, de Klerk M, Erdman RA, Massey EK, Hilhorst MT, Ijzermans JN, Weimar W. Altruistic donor triggered domino-paired kidney donation for unsuccessful couples from the kidney-exchange program. *Am J Transplant* 2010; **10**: 821-827 [PMID: 20199504 DOI: 10.1111/j.1600-6143.2010.03034.x]

22 **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]

23 **Montgomery RA**, Lonze BE, Jackson AM. Using donor exchange paradigms with desensitization to enhance transplant rates among highly sensitized patients. *Curr Opin Organ Transplant* 2011; **16**: 439-443 [PMID: 21666478 DOI: 10.1097/MOT.0b013e32834897c1]

24 **Sharif A**, Zachary AA, Hiller J, Segev D, Alachkar N, Kraus ES, Desai NM, Dagher NN, Singer AL, Montgomery RA. Rescue kidney paired donation as emergency salvage for failed desensitization. *Transplantation* 2012; **93**: e27-e29 [PMID: 22450596 DOI: 10.1097/TP.0b013e318249b10e]

25 **Gentry SE**, Montgomery RA, Swihart BJ, Segev DL. The roles of dominos and nonsimultaneous chains in kidney paired donation. *Am J Transplant* 2009; **9**: 1330-1336 [PMID: 19656136 DOI: 10.1111/j.1600-6143.2009.02622.x]

26 **Lee YJ**, Lee SU, Chung SY, Cho BH, Kwak JY, Kang CM, Park JT, Han DJ, Kim DJ. Clinical outcomes of multicenter domino kidney paired donation. *Am J Transplant* 2009; **9**: 2424-2428 [PMID: 19624563 DOI: 10.1111/j.1600-6143.2009.02747.x]

27 **Tanabe K**. Japanese experience of ABO-incompatible living kidney transplantation. *Transplantation* 2007; **84**: S4-S7 [PMID: 18162988 DOI: 10.1097/01.tp.0000296008.08452.4c]

28 **Ferrari P**, Hughes PD, Cohney SJ, Woodroffe C, Fidler S, D'Orsogna L. ABO-incompatible matching significantly enhances transplant rates in kidney paired donation. *Transplantation* 2013; **96**: 821-826 [PMID: 23860086 DOI: 10.1097/TP.0b013e3182a01311]

29 **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]

30 **Tuncer M**, Tekin S, Yuksel Y, Yücetin L, Dosemeci L, Sengul A, Demirbaş A. First International Paired Exchange Kidney Transplantations of Turkey. *Transplant Proc* 2015; **47**: 1294-1295 [PMID: 26093701 DOI: 10.1016/j.transproceed.2015.04.011]

31 **Weiss J**, Kocher M, Immer FF. International collaboration and organ exchange in Switzerland. *J Thorac Dis* 2015; **7**: 543-548 [PMID: 25922737 DOI: 10.3978/j.issn.2072-1439.2014.12.44]

32 **Melcher ML**, Roberts JP, Leichtman AB, Roth AE, Rees MA. Utilization of Deceased Donor Kidneys to Initiate Living Donor Chains. *Am J Transplant* 2016; **16**: 1367-1370 [PMID: 26833680 DOI: 10.1111/ajt.13740]

33 **Ross LF**, Zenios S. Restricting living-donor-cadaver-donor exchanges to ensure that standard blood type O wait-list candidates benefit. *Transplantation* 2004; **78**: 641-646 [PMID: 15371662]

34 **Kute VB**, Patel HV, Shah PR, Modi PR, Shah VR, Trivedi HL. A Potential Solution to Make the Best Use of a Living Donor-Deceased Donor List Exchange. *Am J Transplant* 2016; Epub ahead of print [PMID: 27454322 DOI: 10.1111/ajt.13974]

35 **Hariharan S**, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med* 2000; **342**: 605-612 [PMID: 10699159 DOI: 10.1056/NEJM200003023420901]

36 **Waterman AD**, Schenk EA, Barrett AC, Waterman BM, Rodrigue JR, Woodle ES, Shenoy S, Jendrisak M, Schnitzler M. Incompatible kidney donor candidates' willingness to participate in donor-exchange and non-directed donation. *Am J Transplant* 2006; **6**: 1631-1638 [PMID: 16827864 DOI: 10.1111/j.1600-6143.2006.01350.x]

37 **Schold JD**, Meier-Kriesche HU. Which renal transplant candidates should accept marginal kidneys in exchange for a shorter waiting time on dialysis? *Clin J Am Soc Nephrol* 2006; **1**: 532-538 [PMID: 17699256 DOI: 10.2215/CJN.01130905]

38 **Glorie K**, Haase-Kromwijk B, van de Klundert J, Wagelmans A, Weimar W. Allocation and matching in kidney exchange programs. *Transpl Int* 2014; **27**: 333-343 [PMID: 24112284 DOI: 10.1111/tri.12202]

39 **Mierzejewska B**, Durlik M, Lisik W, Baum C, Schroder P, Kopke J, Rees M, Stepkowski S. Current approaches in national kidney paired donation programs. *Ann Transplant* 2013; **18**: 112-124 [PMID: 23792511 DOI: 10.12659/AOT.889096]

40 **Waigankar SS**, Kamat MH, Joshi S, Gandhi BV, Bahadur M, Deshpande RV. Living donor transplant options in end-stage renal disease patients with ABO incompatibility. *Indian J Urol* 2013; **29**: 114-118 [PMID: 23956512 DOI: 10.4103/0970-1591.114031]

41 **Pahwa M**, Saifee Y, Tyagi V, Chadha S, Jauhari H. Paired exchange kidney donation in India: a five-year single-center experience. *Int Urol Nephrol* 2012; **44**: 1101-1105 [PMID: 22415451 DOI: 10.1007/s11255-012-0155-7]

42 **Jha PK**, Sethi S, Bansal SB, Jain M, Sharma R, Phanish MK, Duggal R, Ahlawat R, Kher V. Paired kidney exchange transplantation: Maximizing the donor pool. *Indian J Nephrol* 2015; **25**: 349-354 [PMID: 26664210 DOI: 10.4103/0971-4065.150721]

43 **Kute VB**, Gumber MR, Patel HV, Shah PR, Vanikar AV, Modi PR, Shah VR, Patel MP, Trivedi HL. Outcome of kidney paired donation transplantation to increase donor pool and to prevent commercial transplantation: a single-center experience from a developing country. *Int Urol Nephrol* 2013; **45**: 1171-1178 [PMID: 23136032 DOI: 10.1007/s11255-012-0323-9]

44 **Kute VB**, Vanikar AV, Shah PR, Gumber MR, Patel HV, Engineer DP, Modi PR, Shah VR, Trivedi HL. Increasing access to kidney transplantation in countries with limited resources: the Indian experience with kidney paired donation. *Nephrology* (Carlton) 2014; **19**: 599-604 [PMID: 24995599 DOI: 10.1111/nep.12307]

45 **Kute VB**, Shah PS, Vanikar AV, Gumber MR, Patel HV, Engineer DP, Shah PR, Modi PR, Shah VR, Rizvi SJ, Trivedi HL. Increasing access to renal transplantation in India through our single-center kidney paired donation program: a model for the developing world to prevent commercial transplantation. *Transpl Int* 2014; **27**: 1015-1021 [PMID: 24947741 DOI: 10.1111/tri.12373]

46 **Kute VB**, Vanikar AV, Shah PR, Gumber MR, Patel HV, Engineer DP, Modi PR, Rizvi SJ, Shah VR, Modi MP, Kanodia KV, Trivedi HL. Ten kidney paired donation transplantation on World Kidney Day 2013: raising awareness and time to take action to increase donor pool. *Ren Fail* 2013; **35**: 1269-1272 [PMID: 23937166 DOI: 10.3109/0886022X.2013.823997]

47 **Kute VB**, Gumber MR, Vanikar AV, Shah PR, Patel HV, Engineer DP, Modi PR, Rizvi JS, Shah VR, Trivedi HL. Comparison of kidney paired donation transplantations with living related donor kidney transplantation: implications for national kidney paired donation program. *Ren Fail* 2013; **35**: 504-508 [PMID: 23473004 DOI: 10.3109/0886022X.2013.773914]

48 **Kute VB**, Vanikar AV, Shah PR, Gumber MR, Patel HV, Engineer DP, Modi PR, Shah VR, Trivedi HL. Does donor-recipient age difference matter in outcome of kidney transplantation? Implications for kidney paired donation. *Ren Fail* 2014; **36**: 378-383 [PMID: 24295219 DOI: 10.3109/0886022X.2013.862769]

49 **Kute VB**, Vanikar AV, Shah PR, Gumber MR, Patel HV, Modi PR, Trivedi HL. Facilitators to national kidney paired donation program. *Transpl Int* 2013; **26**: e38-e39 [PMID: 23437957 DOI: 10.1111/tri.12078]

50 **Kute VB**, Gumber MR, Dhananjay KL, Vanikar AV, Yadav DK, Patel MP, Patel HV, Shah PR, Trivedi HL. Living donor exchange programs

in renal transplantation: a paradigm ready for broad implementation. *Int Urol Nephrol* 2013; **45**: 597-599 [PMID: 22684762 DOI: 10.1007/s11255-012-0204-2]

51 **Kute VB**, Gumber MR, Shah PR, Patel HV, Vanikar AV, Modi PR, Shah VR, Trivedi HL. Successful three-way kidney paired donation transplantation: The first Indian report. *Indian J Nephrol* 2014; **24**: 45-47 [PMID: 24574632 DOI: 10.4103/0971-4065.125094]

52 **Kute VB**, Patel HV, Shah PR, Modi PR, Shah VR, Rizvi SJ, Pal BC, Modi MP, Shah PS, Varyani UT, Wakhare PS, Shinde SG, Ghodela VA, Patel MH, Trivedi VB, Trivedi HL. Increasing access to kidney transplantation for sensitized recipient through three-way kidney paired donation with desensitization: The first Indian report. *World J Clin Cases* 2016; **4**: 351-355 [PMID: 27803919 DOI: 10.12998/wjcc.v4.i10.351]

53 **Kute VB**, Vanikar AV, Gumber MR, Shah PR, Patel HV, Engineer DP, Balwani MR, Gautam RS, Gera DN, Modi PR, Shah VR, Trivedi HL. Successful three-way kidney paired donation with compatible pairs to increase donor pool. *Ren Fail* 2014; **36**: 447-450 [PMID: 24344717 DOI: 10.3109/0886022X.2013.868294]

54 **Kute VB**, Patel HV, Shah PR, Vanikar AV, Trivedi HL. National kidney paired donation programme in India: Challenges, solution, future direction. *Nephrology* (Carlton) 2015; **20**: 442 [PMID: 25900390 DOI: 10.1111/nep.12408]

55 **Kute VB**, Patel HV, Varyani UT, Shah PR, Modi PR, Shah VR, Rizvi SJ, Pal BC, Shah PS, Wakhare PS, Ghodela VA, Shinde SG, Trivedi VB, Patel MH, Trivedi HL. Six end-stage renal disease patients benefited from first non-simultaneous single center 6-way kidney exchange transplantation in India. *World J Nephrol* 2016; **5**: 531-537 [PMID: 27872835 DOI: 10.5527/wjn.v5.i6.531]

56 **Kute VB**, Patel HV, Shah PR, Modi PR, Shah VR, Rizvi SJ, Pal BC, Shah PS, Wakhare PS, Shinde SG, Ghodela VA, Varyani UT, Patel MH, Trivedi VB, Trivedi HL. International kidney paired donation transplantations to increase kidney transplant of o group and highly sensitized patient: First report from India. *World J Transplant* 2016; **7**: 64-69 [PMID: 28280697 DOI: 10.5500/wjt.v7.i1.64]

57 **Kute VB**, Vanikar AV Patel HV, Shah PR, Gumber MR, Engineer DP, Trivedi HL. Combining kidney paired donation with desensitization increases renal transplantation rate in highly sensitized patients. *Indian J Transplant* 2013; **7**: 109-111 [DOI: 10.1016/j.ijt.2013.05.002]

58 **Travasso C**. Five patients benefit from India's first "domino" kidney swap. *BMJ* 2013; **347**: f4260 [PMID: 23820530 DOI: 10.1136/bmj.f4260]

59 **Agarwal SK**, Srivastava RK, Gupta S, Tripathi S. Evolution of the Transplantation of Human Organ Act and law in India. *Transplantation* 2012; **94**: 110-113 [PMID: 22728294 DOI: 10.1097/TP.0b013e31825ace15]

60 **Allen R**, Pleass H, Clayton PA, Woodroffe C, Ferrari P. Outcomes of kidney paired donation transplants in relation to shipping and cold ischaemia time. *Transpl Int* 2016; **29**: 425-431 [PMID: 26576040 DOI: 10.1111/tri.12719]

61 2016 American Transplant Congress Abstracts. *Am J Transplant* 2016; **16** Suppl 3: 5-798 [PMID: 27273422 DOI: 10.1111/ajt.13895]

62 **Rees MA**, Dunn TB, Kuhr CS, Marsh CL, Rogers J, Rees SE, Cicero A, Reece LJ, Roth AE, Ekwenna O, Fumo DE, Krawiec KD, Kopke JE, Jain S, Tan M, Paloyo SR. Kidney Exchange to Overcome Financial Barriers to Kidney Transplantation. *Am J Transplant* 2017; **17**: 782-790 [PMID: 27992110 DOI: 10.1111/ajt.14106]

63 **Peters TG**, Fisher JS, Gish RG, Howard RJ. Views of US Voters on Compensating Living Kidney Donors. *JAMA Surg* 2016; **151**: 710-716 [PMID: 27007405 DOI: 10.1001/jamasurg.2016.0065]

64 **Held PJ**, McCormick F, Ojo A, Roberts JP. A Cost-Benefit Analysis of Government Compensation of Kidney Donors. *Am J Transplant* 2016; **16**: 877-885 [PMID: 26474298 DOI: 10.1111/ajt.13490]

65 **Gordon EJ**, Patel CH, Sohn MW, Hippen B, Sherman LA. Does financial compensation for living kidney donation change willingness to donate? *Am J Transplant* 2015; **15**: 265-273 [PMID: 25425398 DOI: 10.1111/ajt.13004]

66 **Almeida N**, Almeida R F, Almeida K, Almeida A. Attitude of medical professionals regarding controversial issues in kidney donation/transplantation. *Indian J Nephrol* 2016; **26**: 393-377 [DOI: 10.4103/0971-4065.176147]

P- Reviewer: Friedman EA, Keller F, Watanabe T **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 April 24; 7(2): 144-151

DOI: 10.5500/wjt.v7.i2.144

ISSN 2220-3230 (online)

META-ANALYSIS

Systemic meta-analysis assessing the short term applicability of early conversion to mammalian target of rapamycin inhibitors in kidney transplant

Jayant Kumar, Isabella Reccia, Tomokazu Kusano, Bridson M Julie, Ajay Sharma, Ahmed Halawa

Jayant Kumar, Isabella Reccia, Tomokazu Kusano, Department of Cancer and Surgery, Imperial College, London W120HS, United Kingdom

Bridson M Julie, Ajay Sharma, Ahmed Halawa, Faculty of Health and Sciences, Institute of Learning and Teaching, University of Liverpool, Liverpool L693BX, United Kingdom

Ajay Sharma, Department of Surgery, Royal Liverpool University Hospital, Liverpool L78XP, United Kingdom

Ahmed Halawa, Department of Surgery, Sheffield Teaching Hospitals, Sheffield S102JF, United Kingdom

Author contributions: Kumar J and Halawa A designed study; Kumar J and Reccia I collected data; Kumar J, Reccia I, Kusano T and Julie BM analyzed data; Kumar J, Sharma A and Halawa A wrote paper.

Conflict-of-interest statement: None of the contributing authors have any conflict of interest, including specific financial interests or relationships and affiliations relevant to the subject matter or materials discussed in the manuscript.

Data sharing statement: The manuscript summarizes data as have been reported in published literature to date. There were no new patients studied, and no new data compiled. No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Jayant Kumar, MD, MS, Department of Cancer and Surgery, Imperial College, Du Cane Road, London W120HS, United Kingdom. jkumar@ic.ac.uk
Telephone: +44-74-59934454

Received: November 22, 2016
Peer-review started: November 24, 2016
First decision: January 16, 2017
Revised: February 6, 2017
Accepted: February 28, 2017
Article in press: March 2, 2017
Published online: April 24, 2017

Abstract

AIM

To consolidate the present evidence of effectiveness in renal functioning and graft survival following early introduction of mammalian target of rapamycin (mTOR) inhibitors with or without calcineurin inhibitors (CNIs) in renal transplant recipients.

METHODS

We analysed the current literature following PROSPERO approval describing the role of immunosuppressive agent, mTOR inhibitors as an alternative to CNI within six months of renal transplant by searching the PubMed, EMBASE, Cochrane, Crossref, and Scopus using MeSH terms.

RESULTS

Six articles of early withdrawal of CNI and introduction of mTOR-inhibitors within six months of renal transplantation were sought. Glomerular filtration rate (GFR) and serum creatinine were significantly better in mTOR inhibitor group with equivalent survival at 12 mo, even though Biopsy Proven Acute rejection was significantly higher in mTOR-inhibitor group.

CONCLUSION

The evidence reviewed in this meta-analysis suggests

that early introduction mTOR-inhibitors substantial CNI minimization. The mTOR inhibitors such as everolimus and sirolimus, due to their complementary mechanism of action and favourable nephrotoxicity profile; better glomerular filtration, lower serum creatinine with equivalent survival. Having said that, due to the higher rejection rate, may influence the use of these regimens to patients with moderate to high immunological risk patients.

Key words: Adverse events; Calcineurin inhibitors; Graft failure; Kidney transplantation; Mammalian target of rapamycin inhibitors

Core tip: Early calcineurin inhibitor withdrawal seems to be more pragmatic approach as it bestows better renal functioning in the low immunological risk renal transplant recipients.

Kumar J, Reccia I, Kusano T, Julie BM, Sharma A, Halawa A. Systemic meta-analysis assessing the short term applicability of early conversion to mammalian target of rapamycin inhibitors in kidney transplant. *World J Transplant* 2017; 7(2): 144-151 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i2/144.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i2.144

INTRODUCTION

Inventions in medical science enhance life which has been realized in the concept of kidney transplantation and add significant amount of productive years to the patients of chronic kidney disease[1]. The calcineurin inhibitors (CNIs), cyclosporine A (CsA) and tacrolimus (Tac) were instituted in clinical practice in 1980's. and established themselves as an effective immunosuppressive agent with more than 90% one-year graft survival whilst maintaining a rejection rate of less than 20%[2]. Anyhow, the superlative results of short-term allograft survival have not been maintained for long that could be because of slow, steady decline in renal functioning as, eGFR reduced to below 50% in a span of ten years[3]. Studies have reported chronic allograft nephropathy as the most common cause of late graft loss in 40% kidney transplant patients, whilst the mortality incidence with delayed functioning graft (DFG) was reported in 43% cases. The cardiovascular diseases and malignancies are considered as the most important causes of DFG in transplant patients[4].

The CNI induced nephrotoxicity is considered as an important cause of long-term graft failure in 96.8% of allograft biopsies by virtue of increased production of vasoconstrictors, such as thromboxane and endothelin, together with decreasing the turn-out of vasodilators, such as nitric oxide, prostaglandin E2, and prostacyclin[5,6]. Nankivell *et al*[7] (2004) outlined that more than 50% of kidney allograft biopsies unveiled attestation of chronic CNI toxicity following ten years transplant as 79.2%-100% exhibit histological alterations as tubular atrophy, nodular arteriolar hyalinosis, tubular vacuolization, luminal narrowing, interstitial fibrosis, focal or global segmental sclerosis and micro-calcifications. Surprisingly, the reward of minimal early acute rejection has not been translated into any long term benefits. In addition, CNIs have been associated with development of various cardiovascular risk factors such as hyperlipidemia, hypertension, and new onset diabetes mellitus after transplantation[8,9].

However, the biggest challenge with immunosuppression therapy is to maintain the balance of immunosuppression need in order to avert any rejection episode whilst keeping the check on the toxicities. The recent introduction of better and more efficient non-nephrotoxic immunosuppressive agents such as the mammalian target of rapamycin (mTOR) inhibitors, sirolimus (SRL) and everolimus (EVR), with mechanism of action similar to that of CNIs, forms the basis of use of these drugs[10,11].

CNIs as Tacrolimus (Tac) and Cyclosporin A (CsA) attach with the intracellular proteins called FKBP and immunophilins to form complex which blocks the effect of calcineurin which normally potentiates the intracellular processes associated with the activation of T-lymphocytes. This causes decreased production of interleukin-2 and inhibit the proliferation of T-cells[12,13].

In the similar manner mTOR inhibitors as SRL and EVR form a complex with FKBP to reduces T-cell activation by blocking growth-factor-mediated cell proliferation in the response to an alloantigen[14-17]. The distinct immunological properties with and limited nephrotoxic potential of mTOR-inhibitors have prevailed clinicians to use them as a surrogate to CNIs in renal transplantation[18-21].

The main aim of this review is to focus on the short term benefit early conversion to mTOR-inhibitors with or without CNI in renal transplant recipients in terms of graft functioning and graft survival.

MATERIALS AND METHODS

This meta-analysis was performed following registration in PROSPERO an international database of prospectively registered systematic reviews (CRD42017054458). An extensive search of all the published literature on the role of early conversion to mTOR inhibitors as an alternative to CNI has been made on National Library of Medicine Database (PubMed), EMBASE, Cochrane, Crossref, and Scopus databases on 30th August 2016. The search covered the period 2001 (the year of the first reported early CsA withdrawal with sirolimus in the literature) to September 30th, 2016[22]. The following medical subject headings (MeSH) terms: "Adverse events", "calcineurin inhibitors", "cyclosporin",

Table 1 Criteria for the inclusion of early mammalian target of rapamycin inhibitor conversion studies

Study design	Prospective cohort design with a well-defined study population
Study group	Post renal transplant
Conversion time	Period of 2 wk to 6 mo post-transplant
Study size	> 30 patients
Length of follow-up	Any
Source	Peer-reviewed journals
Language	English
Outcome measure	Patient safety, exposure-response relationships, adverse events, and graft functioning and long-term survival

"everolimus", "graft rejection", "graft survival", "kidney transplantation", "mTOR inhibitors", "sirolimus", "tacrolimus" were searched.

Study selection methodology

The original English literature articles published between 2001-September 2016 were included. Only studies which systematically and quantitatively assessed the graft functioning and graft survival of more than or equal to 12 mo following early conversion to mTORI with or without CNI in different randomised clinical studies were analysed. All kind of comparative studies, retrospective and prospective were included. We have excluded publications as editorials, reviews and letters (Table 1).

Data extraction

Two separate physician reviewers Kumar J, Reccia I reviewed all the articles. Disagreements were resolved through discussion, whilst in scenarios were consensus could not be achieved were resolved by a third author (Ahmed Halawa). We have analysed all papers with empirical studies using a standardised quality assessment tool and pre-specified inclusion and exclusion criteria. The present meta-analysis was performed using the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines and registered in PROSPERO an international database of prospectively registered systematic reviews (Figure 1).

Statistical analysis

The QUADAS-II (quality assessment of diagnostic accuracy studies-II) based analysis was done to assess the internal validity of pre-specified inclusion and exclusion criteria of the various studies. QUADAS-2 is an evidence-based bias assessment tool to evaluate the quality of diagnostic accuracy studies in a systematic review.

A total of six peer-reviewed multi-institutional studies were included in the present meta-analysis. We reviewed each study comprehensively, and data were extracted for the outcomes such as patient safety, exposure-response relationships, adverse events, and various shortcomings or weaknesses to improve the graft functioning and long-term survival (Table 2).

Review Manager (RevMan) Version 5.3 was used to analyse continuous and dichotomous trial data when at least two trials reported. Odds ratios (OR) for dichotomous outcomes, mean difference (MD) for continuous outcomes including a 95%CI, heterogeneity between the trials was measured using the statistic with > 30% considered as significant. The random effects model was used in cases of significant heterogeneity by visualizing the forest plot of involved trials.

RESULTS

The initial search yielded a total of 112 manuscripts. After careful evaluation, 98 articles were excluded on basis period of introduction was not within six months of transplantation. Eventually, a total of six articles matched the previously described inclusion criteria, *i.e.*, ZEUS trial (2011), CENTRAL trial (2012), CONCEPT trial (2009), SMART trial (2010), Spare the Nephron trial (2010)[23-27] and Heilman *et al*[28] (2011) (Table 2). The comprehensive data of all these studies summarizing the renal functioning, Biopsy Proven Acute rejection (BPAR), survival and adverse events were included in Table 3, below we have further analyzed these studies in the time frame of 12 mo following transplantation.

Renal function

The 12 mo estimated renal function (eGFR) was significantly better in the mTOR inhibitor group compared to CNI group (six trials, 1257 patients, mean difference 5.24 mL/min per 1.73 m^2, 95%CI: 2.18 to 8.29, P = 0.00, I^2 = 70%) (Figure 2). Similarly, the measured serum creatinine was significantly lower in the mTOR inhibitors groups at 12 mo (six trials, 1256 patients, mean difference = -11.59 μmol/L, 95%CI: -20.08 to -3.09, P < 0.00, I^2 = 73%) (Figure 3).

BPAR

The incidence of BPAR was significantly higher in mTORs groups compared to CNIs groups (six trials, 1265 patients, OR = 2.11, 95%CI: 1.43 to 3.11, P = 0.00, I^2 = 3%) at 12 mo (Figure 4).

Graft survival and adverse events

At 12 mo, the rates of graft survival were comparable for mTOR inhibitor group and the CNI groups (Table 3). There was no significant difference in the incidence of serious adverse events/infection between the mTOR inhibitors and CNI groups in majority of studies.

DISCUSSION

The initiation of mTOR-inhibitors in early post-transplant period is one of the arduous decision taken by clinicians as it should be done following the period of the heightened immunological risk is over, but no evidence of CNI related toxicity evolved[29,30]. Various

Table 2 Summary of Different Early Conversion Clinical Trials

Ref.	Study design	Time of conversion	Group 1	Group 2
Everolimus				
Budde *et al*[23], 2011 (ZEUS Study)	Multicentre, Prospective, Randomized Study (n = 300), 12 mo	4.5th month	EVR (C0, 6-10 ng/mL) Induction: Basiliximab (n = 155)	CsA (C0, 120-180 ng/mL till 4.5-6 mo then decreased to 100-150 ng/mL) Induction: Basiliximab (n = 145)
Mjörnstedt *et al*[24], 2012 (CENTRAL trial)	Multicentre, Prospective, Randomized Study, (n = 269), 12 mo	7th week	EVR (C0, 6-10 ng/mL) + MMF (1.4 g/d till 2 wk then decreased to 1.08 g/d) + S (n = 92)	Low CsA (C0, 75-200 ng/mL till 2 wk then decreased to 50-150 ng/mL) + MMF (1.4 g/d) + S (n = 90)
Sirolimus				
Lebranchu *et al*[25], 2009 (CONCEPT Study)	Multicentre Prospective, Randomized Study, (n = 193), 12 mo	3rd month	SRL (C0, 8-15 ng/mL till 39 wk then decreased to 5-10 ng/mL) + MMF + S (Induction: Daclizumab) (n = 95)	CsA (C0, 500-800 ng/mL) + MMF + S (Induction: Daclizumab) (n = 97)
Guba *et al*[26], 2010 (SMART Trial)	Multicentre Prospective, Randomized Study, (n = 140), 12 mo	10-24th day	SRL (C0, 8-12 ng/mL then decreased to 5-10 ng/mL) + MMF (1.5 g/d) + S (Induction: ATG) (n = 69)	CsA (C0, 150-200 ng/mL then decreased to 100-150 ng/mL) + MMF (2 g/d) + S (Induction: ATG) (n = 71)
Weir *et al*[27], 2010 (Spare the Nephron Trial)	Multicentre, Prospective, Randomized Study, (n = 299), 12 mo	Within 115 d	MMF + SRL (n = 148)	MMF + CNI (n = 151)
Heilman *et al*[28], 2011	Multicentre Prospective, Randomized Study, (n = 122), 12 mo	1 mo	SRL (C0, 9.8 ± 3.6 ng/mL) + MMF + S (Induction: Basiliximab) (n = 62)	TAC (C0, 6.9 ± 4.6 ng/mL) + MMF + S (Induction: Basiliximab) (n = 60)

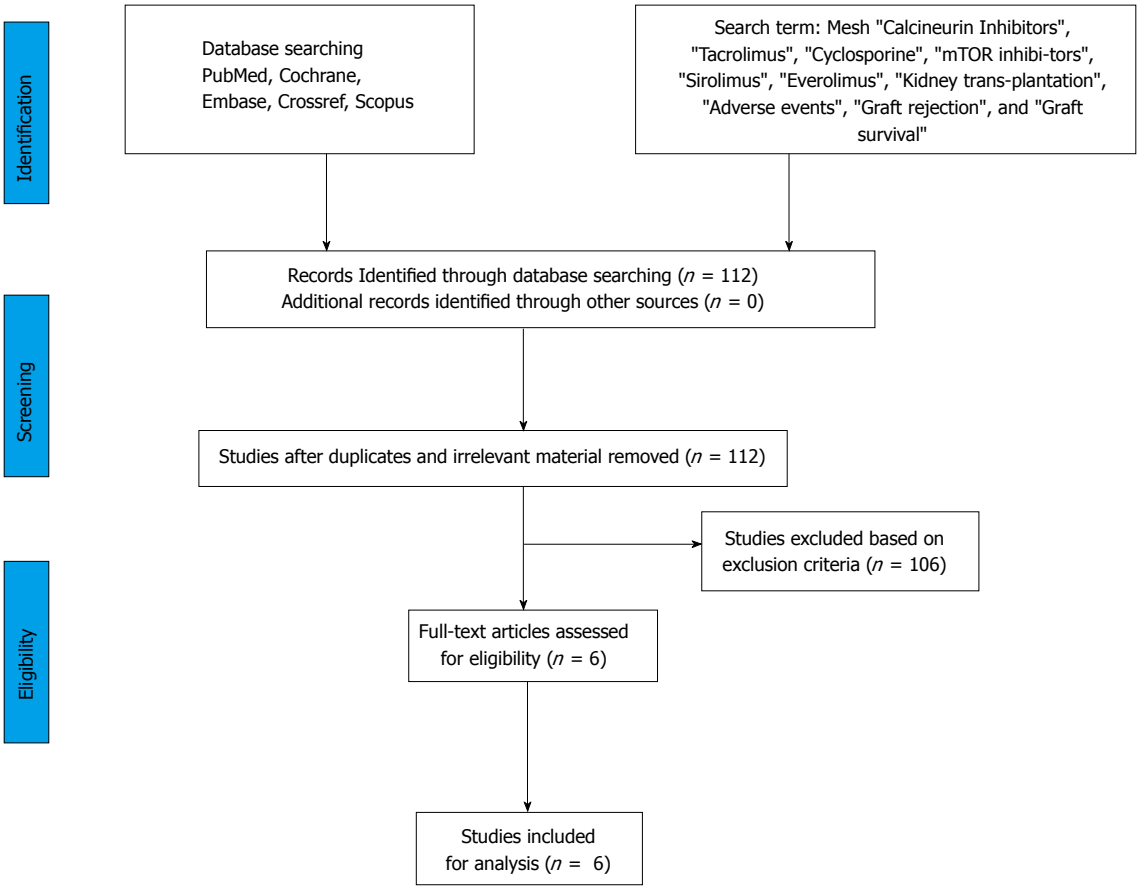


Figure 1 Search strategy and study selection used in this systematic review as per PRISMA protocol.

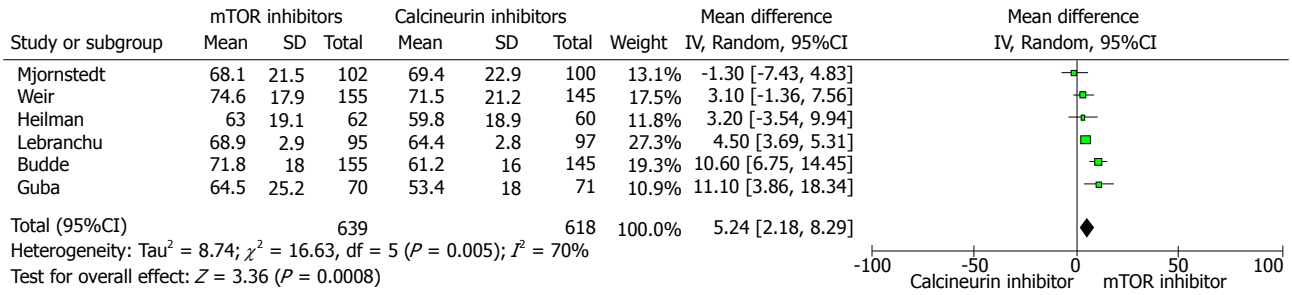


Figure 2 Forest plot represents the glomerular filtration rate at 12 mo in kidney transplant recipients when treated with mammalian target of rapamycin inhibitor or calcineurin inhibitor therapy. Squares represent size effects of studies, comparing the weight of the study in the meta-analysis. The diamond summary effect shows significant favour towards mTOR inhibitors. 95%CIs represented in horizontal bars. mTOR: Mammalian target of rapamycin.

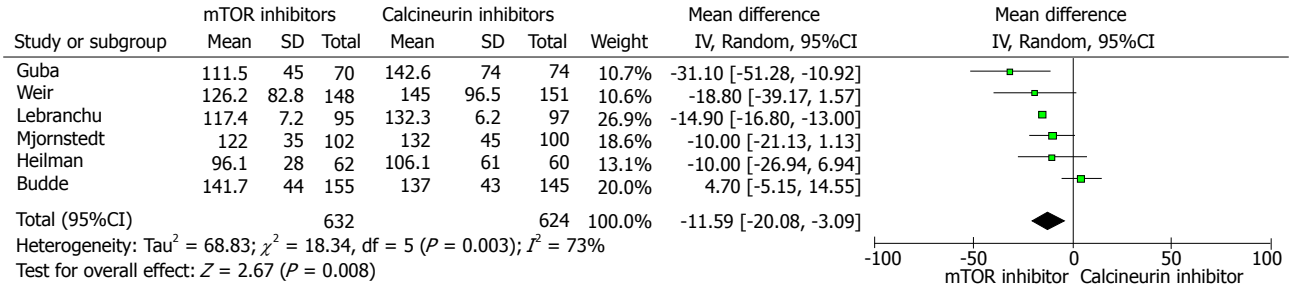


Figure 3 Forest plot represents the serum creatinine at 12 mo in kidney transplant recipients when treated with mammalian target of rapamycin inhibitor or calcineurin inhibitor therapy. Squares represent size effects of studies, comparing the weight of the study in the meta-analysis. The diamond shows summary effect towards mTOR inhibitors with 95%CIs represented in horizontal bars. mTOR: Mammalian target of rapamycin.

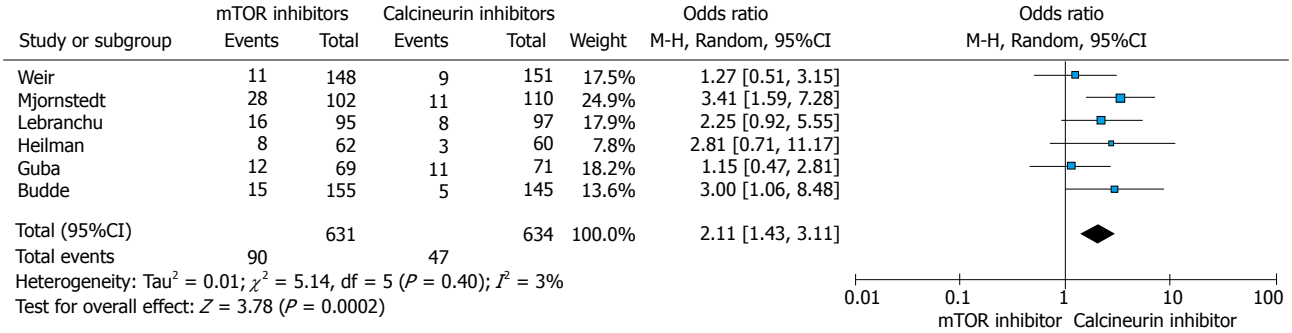


Figure 4 Forest plot represents the biopsy proven acute rejection at 12 mo in kidney transplant recipients when treated with mammalian target of rapamycin inhibitor or calcineurin inhibitor therapy. Squares represent size effects of studies, comparing the weight of the study in the meta-analysis. The meta-analysis significantly favours CNI, with 95%CIs represented in horizontal bars. CNI: Calcineurin inhibitor.

CNI free or reduced dosing regimens have been tried to minimize nephrotoxic adverse effect. The peril of increased risk of rejection with the denovo use of CNI free protocols, has been pared down with the early introduction mTOR inhibitors. Howbeit, data regarding optimal transmutation time to mTOR inhibitor based immunosuppression is not clear. Though, the present literatures support the notion of early conversion to mTOR inhibitors within the six months of transplant whereas the reward of conversion after month 6 is not that encouraging. The major hindrance in the expected outcome following late conversion might be because the CNI related nephrotoxicity has already settled in[23,25].

In the present rationale, mTOR inhibitors should be introduced within a period of 2 wk to 6 mo, *i.e.*, following the period of increased risk for rejection and wound infection has been over.

In a ZEUS study, which was multicenter randomised trial done by Budde *et al*[23] (2011) considered early conversion from CsA to everolimus at 4.5 mo after renal transplantation. Two hundred and sixty-nine patients were randomised into two groups the first group received everolimus with MMF, while another group was maintained on gradually tapered lower dose of CsA with MMF. The group has reported a statistically significant improvement in renal functioning, *i.e.*, eGFR for the everolimus group (71.8 ± 18 mL/min *vs* 61.2 ± 16 mL/min; P = 0.000), at 12 mo while, BPAR was a higher in the everolimus group (13.9% *vs* 7.5%, P = 0.09). Nevertheless, they heralded no difference in terms of graft and patient survival[23].

In a CENTRAL trial by Mjörnstedt *et al*[24] (2012)

Table 3 Summary of outcomes in Different Early Conversion Clinical Trials

Ref.	Renal function (Gp1 *vs* Gp 2)	BPAR (Gp1 *vs* Gp 2)	Adverse event (Gp1 *vs* Gp 2)	Remarks
Everolimus				
Budde *et al*[23], 2011, (ZEUS Study)	12 mo Sr. Cr: 141.7 ± 44 μmol/L *vs* 137.0 ± 43 μmol/L (*P* = NS) eGFR: 71.8 ± 18 mL/min *vs* 61.2 ± 16 mL/min (*P* = 0.000)	9.7% *vs* 3.4% (*P* = 0.03)	SAE/Infection: 61% *vs* 59% (*P* = NS) UTI: 57.0% *vs* 53% (*P* = NS) Diarrhoea: 36% *vs* 27% (*P* = NS) HPL: 14% *vs* 10% (*P* = NS)	Graft survival: 100% *vs* 100% (*P* = NS) Patient survival 100% *vs* 99% (*P* = NS)
Mjornstedt *et al*[24], 2012 (CENTRAL trial)	12 mo Sr. Cr: 122.0 ± 35 μmol/L *vs* 132.0 ± 45 μmol/L (*P* = NS) eGFR: 68.1 ± 21.5 mL/min *vs* 69.4 ± 22.9 mL/min (*P* = NS)	27.5% *vs* 11.0% (*P* = 0.004)	SAE/Infection: 53.9% *vs* 38.0% (*P* = 0.025) CMV infection: 8.8% *vs* 13.0% (*P* = NS) Edema: 29.4% *vs* 21.0% (*P* = NS) Anaemia: 16.7% *vs* 6.0% (*P* = 0.02) HPL: 12.7% *vs* 9.0% (*P* = NS) Proteinuria: 4.9% *vs* 0% (*P* = 0.06) Acne: 12.7% *vs* 2.0% (*P* = 0.006) Mouth Ulceration: 12.7% *vs* 2.0 % (*P* = 0.001)	Graft survival: 100% *vs* 100% (*P* = NS) Patient survival 98% *vs* 98% (*P* = NS)
Sirolimus				
Lebranchu *et al*[25], 2009 (CONCEPT Study)	12 mo: Sr. Cr: 117.4 μmol/L *vs* 132.3 μmol/L (*P* < 0.001) eGFR: 68.9 mL/min *vs* 64.4 mL/min (*P* = 0.017)	16.8% *vs* 8.2% (*P* = NS)	Peripheral Edema: 28.1% *vs* 22.6% (*P* = NS) SAE/infection: 60% *vs* 44% (*P* = 0.025) Diarrhoea: 30.2% *vs* 9.2% (*P* < 0.001) Dyslipidemia: 5.20% *vs* 4.12% (*P* = NS) Proteinuria: 9.3% *vs* 3.09 % (*P* = NS) NODAT: 3.1% *vs* 2.06% (*P* = NS) Apthous Stomatitis: 45.8% *vs* 5.15% (*P* < 0.001)	Graft Survival: 99% (*P* = NS) Patient Survival 97% (*P* = NS)
Guba *et al*[26], 2010, (SMART Trial)	12 mo: Sr Cr: 111.5 ± 45 mg/dL *vs* 142.6 ± 74 mg/dL (*P* = 0.004) eGFR: 64.5 ± 25.2 mL/min *vs* 53.4 ± 18.0 mL/min (*P* = 0.001)	17.4% *vs* 15.5% (*P* = NS)	Wound Healing Disorder: 10.1% *vs* 11.3%, (*P* = NS) Infection: 52.2% *vs* 60.6% (*P* = NS) CMV: 7.3% *vs* 28.2% (*P* < 0.001) HPL: 20.3% *vs* 7.0% (*P* = 0.02) Diarrhoea: 13.0% *vs* 9.9% (*P* = NS) Lymphocele: 27.5% *vs* 23.9% (*P* = NS)	Graft Survival: 99% *vs* 97% (*P* = NS) Patient Survival 99% *vs* 99% (*P* = NS)
Weir *et al*[27], 2010 (Spare the Nephron Trial)	12 mo Sr. Cr: 126.2 ± 82.8 μmol/L *vs* 145.0 ± 96.5 μmol/L (*P* = NS) eGFR: 74.6 ± 17.9 mL/min *vs* 71.5 ± 21.2 mL/min (*P* = 0.06)	7.4% *vs* 6.0% (*P* = NS)	Infection: 16.2% *vs* 18.3% (*P* = NS) HPL: 24.3% *vs* 10.5% (*P* = 0.000) CMV: 4.7% *vs* 9.2% (*P* = NS) Polyoma virus: 2% *vs* 4% (*P* = NS) Diarrhoea: 29.7% *vs* 9.8% (*P* = 0.001) Malignancy: 4.7% *vs* 6.5% (*P* = NS)	Graft Survival: 98% *vs* 97.4% (*P* = NS) Patient Survival 100% *vs* 98% (*P* = NS)
Heilman *et al*[28], 2011	12 mo Sr. Cr: 96.1 ± 28 μmol/L *vs* 106.1 ± 61 μmol/L (*P* = NS) eGFR: 63.0 ± 19.1 mL/min *vs* 59.8 ± 18.9 mL/min (*P* = NS)	13% *vs* 5% (*P* = NS)	CMV: 13% *vs* 13% (*P* = NS) Polyoma virus: 2% *vs* 4% (*P* = NS)	NA

eGFR: Estimated renal function; NA: Not Available; Not Significant; NODAT: New-onset diabetes after transplantation; CMV: Acute cytomegalovirus.

they studied the effect of early conversion from CsA to everolimus in the seventh week of the post-transplant. About two hundred and two patients who were randomised to receive intervention group everolimus (C0, 3-8 ng/mL) and were compared with CsA (C0, 75-200 ng/mL for two weeks then reduced, further maintained at 50-150 ng/mL) with oral steroids and MMF. They didn't report significant improvement in GFR in everolimus group (68.1 ± 21.5 mL/min *vs* 69.4 ± 22.9 mL/min, *P* = NS) at 12 mo, although serum creatinine was lower in mTOR inhibitor group (122.0 ± 35 μmol/L *vs* 132.0 ± 45 μmol/L, *P* = NS).

Though the reported incidence of BPAR was significantly higher in EVR group than in CsA group (27.5% *vs* 11.0%, *P* = 0.004), the survival outcomes were similar at 12 mo. The reported side effects as proteinuria, anaemia, hyperlipidemia, acne and mouth ulceration were significantly more frequent in the everolimus group[24].

In the CONCEPT study 2009 by Lebranchu *et al*[25], instituted Sirolimus by replacing CsA in the third month of the post-transplantation. Their literature listed significantly better eGFR (68.9 mL/min *vs* 64.4 mL/min) and significantly lower serum creatinine (117.4 μmol/L *vs* 132.3 μmol/L, *P* < 0.001) in the sirolimus group at 12 mo. The detailed BPAR was similar for entire period of observation. The side effects such as diarrhoea, SAE, aphthous stomatitis, proteinuria and new onset diabetes mellitus were either significantly higher or higher in the sirolimus group[25].

Guba *et al*[26] (2010) carried out a multicenter randomised SMART trial, to explore the effects of very early conversion to sirolimus from CsA only 10 to 24 d after the renal transplantation. They randomised one hundred and forty-one patients were into two groups to confer sirolimus with MMF and steroid, on the other hand the second group was maintained on gradually tapered lower dose of CsA with MMF and steroid. They

reported statistically significant improvement in renal functioning, eGFR (64.5 ± 25.2 mL/min *vs* 53.4 ± 18 mL/min; P = 0.001) with significantly reduced serum creatinine (111.5 ± 45 μmol/L *vs* 142.6 ± 74 μmol/L, P = 0.004) for the sirolimus group at 12 mo. The detailed incidence of BPAR (17.4% *vs* 15.5%, P = NS) was similar in both groups, likewise, the graft and patient survival were quite similar. In addition, the recipients in the sirolimus group reported a significantly higher number of adverse effects such as acne, hyperlipidemia and lower number CMV viremia withal the incidence of BPAR was similar in both groups (20.2% *vs* 19.7%, P = NS)[26].

In Spare the Nephron Trial, Weir *et al*[27] (2010) randomized 299 kidney transplant recipients into two groups following 115 d of the transplant. The first group received sirolimus with MMF while the second group was maintained on CNI and MMF. They reported significant improvement in renal function in terms of higher eGFR (74.6 ± 17.9 mL/min *vs* 71.5 ± 21.2 mL/min; P = 0.06) and lower serum creatinine (126.2 ± 82.8 μmol/L *vs* 145.0 ± 96.5 μmol/L, P = NS) in the sirolimus group. They delineated the likewise patient and graft survival in both groups. However, patients in the sirolimus group reported a significantly higher number of adverse effects as hyperlipidemia and diarrhoea[27].

In the 2011 study by Heilman *et al*[28], sirolimus introduced in the first month of the renal transplant. They have given the account of significant improvement in eGFR (63.0 ± 19.1 mL/min *vs* 59.8 ± 18.9 mL/min; P = NS) and set out lower serum creatinine in the sirolimus group at 12 mo while the reported BPAR was likewise in both groups[28].

Publication bias is an important point to consider in a meta-analysis because all the researches which take place are not published. Studies with a significant result are more likely to be published. Studies with a significant result are more likely to be placed in a higher impact journal compared to the studies with null results. Moreover, well controlled and properly carried out studies are less likely to achieve significance.

In general, early CNI withdrawal in the wake of mTOR inhibitor based regimen institution seems a more empirical and constructive approach towards immunosuppressive management of renal transplant recipients. Natheless, taking into account of the high rejection rate contemplated in these studies, it will be a judicious decision of not to proffer this therapy to patients with moderate to high immunological risk though additional studies with long duration of follow-up are demanded to confirm present conjecture[29-33].

Despite the fact that the data on the Tac minimization strategies are limited, the present evidence suggest that treatment with mTOR-inhibitors allows early and substantial CNI minimization and provides better renal functioning at the end of first year of transplantation. Thus, it is not judicious to extend these regimens to patients with moderate to high immunological risk. However, further trials directed towards different ethnicity and geography are needed to determine further evidence.

COMMENTS

Background

The aim of this review is to assess the one-year effectiveness of the early introduction of mammalian target of rapamycin (mTOR) inhibitors with or without calcineurin inhibitors (CNIs) within six months of renal transplantation.

Research frontiers

The current literature was reviewed to assess the role of immunosuppressive agent, mTOR inhibitors as an alternative to CNI within six months of renal transplant in terms of better renal functioning and survival by assessing glomerular filtration rate (GFR), serum creatinine, Biopsy Proven Acute Rejection (BPAR) and survival.

Innovations and breakthroughs

The major advantages were observed regarding better renal functioning, GFR and serum creatinine were better in mTOR inhibitor group at 12 mo. BPAR was significantly higher in the mTOR-inhibitor group though survival was comparable.

Application

In general, early CNI withdrawal seems to be a more empirical and constructive approach as it provides better renal functioning in the low immunological risk transplant recipients.

Peer-review

This study is a systemic review and meta-analysis of the effect on renal function and graft survival following early conversion of CNI to mTOR inhibitors with or without CNI after kidney transplantation. The authors initially selected 112 manuscripts, and of them, only 6 papers were useful for meta-analysis. They conclude that introduction of mTOR-inhibitors allows early and substantial CNI minimization.

REFERENCES

1 **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]

2 **Knops N**, Levtchenko E, van den Heuvel B, Kuypers D. From gut to kidney: transporting and metabolizing calcineurin-inhibitors in solid organ transplantation. *Int J Pharm* 2013; **452**: 14-35 [PMID: 23711732 DOI: 10.1016/j.ijpharm.2013.05.033]

3 **Diekmann F**, Andrés A, Oppenheimer F. mTOR inhibitor-associated proteinuria in kidney transplant recipients. *Transplant Rev* (Orlando) 2012; **26**: 27-29 [PMID: 22137729 DOI: 10.1016/j.trre.2011.10.003]

4 **Krieger NR**, Becker BN, Heisey DM, Voss BJ, D'Alessandro AM, Becker YT, Odorico JS, Kalayoglu M, Pirsch JD, Sollinger HW, Knechtle SJ. Chronic allograft nephropathy uniformly affects recipients of cadaveric, nonidentical living-related, and living-unrelated grafts. *Transplantation* 2003; **75**: 1677-1682 [PMID: 12777855 DOI: 10.1097/01.TP.0000063830.60937.06]

5 **Cornell LD**, Colvin RB. Chronic allograft nephropathy. *Curr Opin Nephrol Hypertens* 2005; **14**: 229-234 [PMID: 15821415]

6 **Li C**, Yang CW. The pathogenesis and treatment of chronic allograft nephropathy. *Nat Rev Nephrol* 2009; **5**: 513-519 [PMID: 19636333 DOI: 10.1038/nrneph.2009.113]

7 **Nankivell BJ**, Borrows RJ, Fung CL, O'Connell PJ, Chapman JR, Allen RD. Delta analysis of posttransplantation tubulointerstitial damage. *Transplantation* 2004; **78**: 434-441 [PMID: 15316373]

8 **Flechner SM**, Goldfarb D, Solez K, Modlin CS, Mastroianni B, Savas K, Babineau D, Kurian S, Salomon D, Novick AC, Cook DJ. Kidney transplantation with sirolimus and mycophenolate mofetil-based immunosuppression: 5-year results of a randomized

prospective trial compared to calcineurin inhibitor drugs. *Transplantation* 2007; **83**: 883-892 [PMID: 17460558 DOI: 10.1097/01.tp.0000258586.52777.4c]

9 **Flechner SM**. Sirolimus in kidney transplantation indications and practical guidelines: de novo sirolimus-based therapy without calcineurin inhibitors. *Transplantation* 2009; **87**: S1-S6 [PMID: 19384179 DOI: 10.1097/TP.0b013e3181a059a1]

10 **Augustine JJ**, Knauss TC, Schulak JA, Bodziak KA, Siegel C, Hricik DE. Comparative effects of sirolimus and mycophenolate mofetil on erythropoiesis in kidney transplant patients. *Am J Transplant* 2004; **4**: 2001-2006 [PMID: 15575902 DOI: 10.1111/j.1600-6143.2004.00612.x]

11 **Hernández D**, Martínez D, Gutiérrez E, López V, Gutiérrez C, García P, Cobelo C, Cabello M, Burgos D, Sola E, González-Molina M. Clinical evidence on the use of anti-mTOR drugs in renal transplantation. *Nefrologia* 2011; **31**: 27-34 [PMID: 21270910 DOI: 10.3265/Nefrologia.pre2010.Jul.10512]

12 **Gullestad L**, Iversen M, Mortensen SA, Eiskjaer H, Riise GC, Mared L, Bjørtuft O, Ekmehag B, Jansson K, Simonsen S, Gude E, Rundqvist B, Fagertun HE, Solbu D, Bergh CH. Everolimus with reduced calcineurin inhibitor in thoracic transplant recipients with renal dysfunction: a multicenter, randomized trial. *Transplantation* 2010; **89**: 864-872 [PMID: 20061999 DOI: 10.1097/TP.0b013e3181cbac2d]

13 **Serre JE**, Michonneau D, Bachy E, Noël LH, Dubois V, Suberbielle C, Kreis H, Legendre C, Mamzer-Bruneel MF, Morelon E, Thaunat O. Maintaining calcineurin inhibition after the diagnosis of post-transplant lymphoproliferative disorder improves renal graft survival. *Kidney Int* 2014; **85**: 182-190 [PMID: 23802193 DOI: 10.1038/ki.2013.253]

14 **Zaza G**, Tomei P, Ria P, Granata S, Boschiero L, Lupo A. Systemic and nonrenal adverse effects occurring in renal transplant patients treated with mTOR inhibitors. *Clin Dev Immunol* 2013; **2013**: 403280 [PMID: 24151517 DOI: 10.1155/2013/403280]

15 **Dowling RJ**, Topisirovic I, Fonseca BD, Sonenberg N. Dissecting the role of mTOR: lessons from mTOR inhibitors. *Biochim Biophys Acta* 2010; **1804**: 433-439 [PMID: 20005306 DOI: 10.1016/j.bbapap.2009.12.001]

16 **Nashan B**. Induction therapy and mTOR inhibition: minimizing calcineurin inhibitor exposure in de novo renal transplant patients. *Clin Transplant* 2013; **27** Suppl 25: 16-29 [PMID: 23909498 DOI: 10.1111/ctr.12156]

17 **Gonzalez-Vilchez F**, Vazquez de Prada JA, Paniagua MJ, Gomez-Bueno M, Arizon JM, Almenar L, Roig E, Delgado J, Lambert JL, Perez-Villa F, Sanz-Julve ML, Crespo-Leiro M, Segovia J, Lopez-Granados A, Martinez-Dolz L, Mirabet S, Escribano P, Diaz-Molina B, Farrero M, Blasco T. Use of mTOR inhibitors in chronic heart transplant recipients with renal failure: calcineurin-inhibitors conversion or minimization? *Int J Cardiol* 2014; **171**: 15-23 [PMID: 24309084 DOI: 10.1016/j.ijcard.2013.11.036]

18 **Filler G**. Calcineurin inhibitors in pediatric renal transplant recipients. *Paediatr Drugs* 2007; **9**: 165-174 [PMID: 17523697 DOI: 10.2165/00148581-200709030-00005]

19 **Rostaing L**, Kamar N. mTOR inhibitor/proliferation signal inhibitors: entering or leaving the field? *J Nephrol* 2010; **23**: 133-142 [PMID: 20155724]

20 **Ganschow R**, Pape L, Sturm E, Bauer J, Melter M, Gerner P, Höcker B, Ahlenstiel T, Kemper M, Brinkert F, Sachse MM, Tönshoff B. Growing experience with mTOR inhibitors in pediatric solid organ transplantation. *Pediatr Transplant* 2013; **17**: 694-706 [PMID: 24004351 DOI: 10.1111/petr.12147]

21 **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]

22 **Pescovitz MD**, Govani M. Sirolimus and mycophenolate mofetil for calcineurin-free immunosuppression in renal transplant recipients. *Am J Kidney Dis* 2001; **38**: S16-S21 [PMID: 11583940]

23 **Budde K**, Becker T, Arns W, Sommerer C, Reinke P, Eisenberger U, Kramer S, Fischer W, Gschaidmeier H, Pietruck F. Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. *Lancet* 2011; **377**: 837-847 [PMID: 21334736 DOI: 10.1016/S0140-6736(10)62318-5]

24 **Mjörnstedt L**, Sørensen SS, von Zur Mühlen B, Jespersen B, Hansen JM, Bistrup C, Andersson H, Gustafsson B, Undset LH, Fagertun H, Solbu D, Holdaas H. Improved renal function after early conversion from a calcineurin inhibitor to everolimus: a randomized trial in kidney transplantation. *Am J Transplant* 2012; **12**: 2744-2753 [PMID: 22812414 DOI: 10.1111/j.1600-6143.2012.04162.x]

25 **Lebranchu Y**, Thierry A, Toupance O, Westeel PF, Etienne I, Thervet E, Moulin B, Frouget T, Le Meur Y, Glotz D, Heng AE, Onno C, Buchler M, Girardot-Seguin S, Hurault de Ligny B. Efficacy on renal function of early conversion from cyclosporine to sirolimus 3 months after renal transplantation: concept study. *Am J Transplant* 2009; **9**: 1115-1123 [PMID: 19422337 DOI: 10.1111/j.1600-6143.2009.02615.x]

26 **Guba M**, Pratschke J, Hugo C, Krämer BK, Nohr-Westphal C, Brockmann J, Andrassy J, Reinke P, Pressmar K, Hakenberg O, Fischereder M, Pascher A, Illner WD, Banas B, Jauch KW. Renal function, efficacy, and safety of sirolimus and mycophenolate mofetil after short-term calcineurin inhibitor-based quadruple therapy in de novo renal transplant patients: one-year analysis of a randomized multicenter trial. *Transplantation* 2010; **90**: 175-183 [PMID: 20463641]

27 **Weir MR**, Mulgaonkar S, Chan L, Shidban H, Waid TH, Preston D, Kalil RN, Pearson TC. Mycophenolate mofetil-based immunosuppression with sirolimus in renal transplantation: a randomized, controlled Spare-the-Nephron trial. *Kidney Int* 2011; **79**: 897-907 [PMID: 21191361 DOI: 10.1038/ki.2010.492]

28 **Heilman RL**, Younan K, Wadei HM, Mai ML, Reddy KS, Chakkera HA, Gonwa TA. Results of a prospective randomized trial of sirolimus conversion in kidney transplant recipients on early corticosteroid withdrawal. *Transplantation* 2011; **92**: 767-773 [PMID: 21775930 DOI: 10.1097/TP.0b013e31822805d7]

29 **Silva HT**, Felipe CR, Garcia VD, Neto ED, Filho MA, Contieri FL, de Carvalho DD, Pestana JO. Planned randomized conversion from tacrolimus to sirolimus-based immunosuppressive regimen in de novo kidney transplant recipients. *Am J Transplant* 2013; **13**: 3155-3163 [PMID: 24266969 DOI: 10.1111/ajt.12481]

30 **Groetzner J**, Kaczmarek I, Schulz U, Stegemann E, Kaiser K, Wittwer T, Schirmer J, Voss M, Strauch J, Wahlers T, Sohn HY, Wagner F, Tenderich G, Stempfle HU, Mueller-Ehmsen J, Schmid C, Vogeser M, Koch KC, Reichenspurner H, Daebritz S, Meiser B, Reichart B. Mycophenolate and sirolimus as calcineurin inhibitor-free immunosuppression improves renal function better than calcineurin inhibitor-reduction in late cardiac transplant recipients with chronic renal failure.*Transplantation* 2009; **87**: 726-733 [PMID: 19295318 DOI: 10.1097/TP.0b013e3181963371]

31 **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]

32 **Kaczmarek I**, Zaruba MM, Beiras-Fernandez A, Reimann R, Nickel T, Grinninger C, Sadoni S, Hagl C, Meiser B. Tacrolimus with mycophenolate mofetil or sirolimus compared with calcineurin inhibitor-free immunosuppression (sirolimus/mycophenolate mofetil) after heart transplantation: 5-year results. *J Heart Lung Transplant* 2013; **32**: 277-284 [PMID: 23415313 DOI: 10.1016/j.healun.2012.11.028]

33 **Gatault P**, Lebranchu Y. Conversion to mTOR-inhibitor-based immunosuppression: which patients and when? *Transplant Res* 2013; **2**: S3 [PMID: 24565231 DOI: 10.1186/2047-1440-2-S1-S3]

P- Reviewer: Novosel MK, Okumura K **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Wu HL

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 April 24; 7(2): 152-160

DOI: 10.5500/wjt.v7.i2.152

ISSN 2220-3230 (online)

META-ANALYSIS

Living related and living unrelated kidney transplantations: A systematic review and meta-analysis

Nasser Simforoosh, Hamidreza Shemshaki, Mohammad Nadjafi-Semnani, Mehdi Sotoudeh

Nasser Simforoosh, Hamidreza Shemshaki, Mohammad Nadjafi-Semnani, Mehdi Sotoudeh, Department of Urology, Shahid Labbafi Nejad Hospital, Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, Tehran 198396-3113, Iran

Author contributions: Simforoosh N, Shemshaki H, Nadjafi-Semnani M and Sotoudeh M acquisition of data, analysis and interpretation of data, drafting the article and final approval; Sotoudeh M interpretation of data, revising the article and final approval.

Conflict-of-interest statement: The authors deny any conflict of interest.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Nasser Simforoosh, Professor, Department of Urology, Shahid Labbafi Nejad Hospital, Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, 9th Boostan Avenue, Pasdaran Avenue, Tehran 198396-3113, Iran. simforoosh@iurtc.org.ir
Telephone: +98-21-22588018
Fax: +98-21-22588016

Received: November 21, 2016
Peer-review started: November 23, 2016
First decision: January 16, 2017
Revised: February 20, 2017
Accepted: April 6, 2017
Article in press: April 10, 2017
Published online: April 24, 2017

Abstract

AIM

To compare the outcomes between related and unrelated kidney transplantations.

METHODS

Literature searches were performed following the Cochrane guidelines. We conducted a systematic review and a meta-analysis, which included 12 trials that investigated outcomes including the long-term (ten years), mid-term (one to five years), and short-term (one year) graft survival rate as well as the acute rejection rate. Meta-analyses were performed using fixed and random-effects models, which included tests for publication bias and heterogeneity.

RESULTS

No difference in graft survival rate was detected in patients who underwent living related kidney transplantations compared to unrelated (P = 0.44) transplantations after ten years. There were no significant differences between the graft survival rate in living related and unrelated kidney transplantations after a short- and mid-term follow-up (P = 0.35, P = 0.46). There were no significant differences between the acute rejection rate in living related and unrelated kidney transplantations (P = 0.06).

CONCLUSION

The long, mid and short term follow-up of living related and unrelated kidney transplantation showed no significant difference in graft survival rate. Also, acute rejection rate was not significantly different between groups.

Key words: Transplantation; Living related; Living unrelated; Graft survival rate

Core tip: The long, mid and short term follow-up of living related and unrelated kidney transplantation showed no significant difference in graft survival rate. Also, acute rejection rate was not significantly different between groups.

Simforoosh N, Shemshaki H, Nadjafi-Semnani M, Sotoudeh M. Living related and living unrelated kidney transplantations: A systematic review and meta-analysis. *World J Transplant* 2017; 7(2): 152-160 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i2/152.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i2.152

INTRODUCTION

Renal failure is a disease with a high rate of morbidity and mortality. By the end of 2001, with the help of dialysis and renal transplantations, approximately 1479000 people were kept alive. This number increased to 1783000 by the end of 2004[1]. Nowadays, renal transplantation has become the optimal treatment for patients with end-stage renal disease[2]. The recipients of renal transplant had a higher quality of life and a greater survival rate in comparison to patients who underwent dialysis. Due to these results, the demand of renal transplantations has increased over time, but the gap between supply and demand has widened. Consequently, the number of patients who are on the renal transplant waiting list for deceased-donor transplantation has increased and thousands of patients have died while waiting for their renal transplantation. This has made it necessary to search for alternatives.

During the past two decades, several approaches have been adopted to increase living related organ donations, but living unrelated donors remain an underutilized source. The result of living unrelated transplantations was widely disputed. While the Brazilian[3], Iranian[4,5], and Egyptian[6] experiences resulted in excellent outcomes that were superior to those in cadavers and were comparable to living related-donor transplantations, there were contradictory reports in several studies[7,8]. To our knowledge, there was no systematic review and meta-analysis that evaluated outcomes in patients who underwent living related *vs* unrelated kidney transplantations. This systematic review and meta-analysis was designed to compare the outcomes including the long-, mid- and short-term graft survival rate, and the acute rejection rate between related and unrelated kidney transplantations.

MATERIALS AND METHODS

Literature search

The review was conducted in accordance with the guidelines described in the Cochrane handbook for the systematic review and meta-analysis of interventions.

Eligibility criteria and study characteristics

The criteria for studies included the following: (1) the patients considered had undergone living related or unrelated kidney transplantations; (2) the study involved the comparison of the outcomes in patients whom underwent kidney transplantation from related *vs* unrelated kidney donations; and (3) the primary outcome was long-term (ten years) graft survival rate, while the secondary outcomes were short-term (one year) and mid-term (one to five years) graft survival rate and acute rejection rate.

Both English language studies and non-English language studies were included in the meta-analysis.

Study identification and data abstraction

Two independent reviewers completed a systematic computerized search of online databases, including PubMed, Ovid, MEDLINE, EMBASE, the Cochrane Controlled Trials Register, HealthSTAR, CINAHL, Google, and Google Scholar to locate studies exploring the evaluation outcomes of patients who underwent kidney transplantation from living related *vs* unrelated kidney donations published in any language throughout March 2016. The keywords used for the search included kidney transplant, related, unrelated, and living. Thereafter, a search on MEDLINE was refined to clinical trials. We also searched the Cochrane Database of Systematic Reviews, the Cochrane Central Register of Controlled Trials, Clinical Trials (www.clinicaltrials.gov), Centre watch (www.centerwatch.com), Trials Central (www.trialscentral.org/ClinicalTrials.aspx), and the United Kingdom National Research Register (www.nrr.nhs.uk).

After reviewing the titles of these studies, we retrieved the abstracts that were appropriate for use in our study. We independently reviewed these abstracts and chose those studies that were potentially relevant to our work. We reviewed the bibliographies of all of the studies that were included to identify any additional studies which required inclusion. A data-extraction form was designed and agreed upon by the authors. Initially, two authors independently extracted the data, which were later reviewed jointly to reach an agreement on its accuracy. The data that were collected from all the manuscripts included the following fields: Number of patients, mean follow-up, recipient mean age, recipient sex, Immunosuppression regimen, the short-term, mid-term and long-term survival rate and the acute rejection rate, mean serum creatinine at 1 year and final follow-up, and post-transplant infectious complications. Disagreements were resolved by consensus or consultation with senior authors (Table 1). The authors of individual trials were contacted directly to provide additional information when necessary. We analysed the quality of studies with a questionnaire and only the studies that had a score greater than eight were included in our study (Table 2). In cases where the full text or data were not accessible, we tried to contact the authors in order to have them provided.

Table 1 Study design

Patients	Patients underwent kidney transplantation
Literature search	Keyword search in PubMed, Google scholar and Scopus
Databases	Pubmed, Ovid, MEDLINE, EMBASE, the Cochrane Controlled Trials Register, HealthSTAR, CINAHL, Google, and Google Scholar
Limits	Only comprehensive articles without time limit Humans In English
Keywords	Kidney transplantation Renal transplant Related Unrelated
Eligibility criteria	Article in Full-text (no abstracts) Unique publication (no duplicate articles) Reported each of the interested outcomes (graft survival rate, and acute rejection rate) Original report as determined from reading the abstract or if necessary the full text Outcome reported in a usable form (each surgical approach was reported as a separate cohort, no additional confounding treatments, no missing or unreliable data; could not have > 10% difference in values between text and tables Reported on surgical approaches of interest
Exclusion criteria	Duplicate patient population, where some or all of the same patients were included in a different study reporting on the same parameters (prevents double counting) Early case experience (prevents bias toward approaches with more experienced surgeons)
Data abstraction	Articles needed to report which contain each of outcome of interest to be included in the analysis Data were abstracted by two individuals into a custom database table including list of variables. 50% of articles were abstracted by one reviewer and other 50% with other one. The data for 50% of the articles was double-entered by a second individual, and any discrepancies were resolved through repeated review and discussion prior to data analysis All primary outcomes were then double-checked and any discrepancies resolved Variables in four types were abstracted from each study: Those necessary to determine inclusion and exclusion criteria, surgical approach, baseline patient characteristics, and clinical outcomes All studies were reviewed by two independent reviewers using the total QASs (Table 3) to assess the methodological quality of the studies that were included. Although the QASs were reported for each study, they were not used to weight the studies in the meta-analysis
Primary outcomes	Graft survival rate
Secondary outcomes	Acute rejection rate
Controls for Publication bias	Performed a funnel plot analysis

QASs: Quality assessments.

Table 2 Quality assessment items and possible scores

Was the assigned treatment adequately concealed prior to allocation?
- 2 = method did not allow disclosure of assignment
- 1 = small but possible chance of disclosure of assignment or unclear
- 0 = quasi-randomized or open list/tables

Were the outcomes of participants who withdrew described and included in the analysis (intention-to-treat)?
- 2 = withdrawals well described and accounted for in analysis
- 1 = withdrawals described and analysis not possible
- 0 = no mention, inadequate mention, or obvious differences and no adjustment

Were the outcome assessors blinded to treatment status?
- 2 = effective action taken to blind assessors
- 1 = small or moderate chance of unblinding of assessors
- 0 = not mentioned or not possible

Were the treatment and control groups comparable at entry? (likely confounders may be age, partial or total rupture, activity level, acute or chronic injury)
- 2 = good comparability of groups, or confounding adjusted for in analysis
- 1 = confounding small; mentioned but not adjusted for
- 0 = large potential for confounding, or not discussed

Were the participants blind to assignment status after allocation?
- 2 = effective action taken to blind participants
- 1 = small or moderate chance of unblinding of participants
- 0 = not possible, or not mentioned (unless double-blind), or possible but not done

Were the treatment providers blind to assignment status?
- 2 = effective action taken to blind treatment providers
- 1 = small or moderate chance of unblinding of treatment providers
- 0 = not possible, or not mentioned (unless double-blind), or possible but not done

Were care programes, other than the trial options, identical?
- 2 = care programes clearly identical
- 1 = clear but trivial differences
- 0 = not mentioned or clear and important differences in care programes

Were the inclusion and exclusion criteria clearly defined?
- 2 = clearly defined
- 1 = inadequately defined
- 0 = not defined

Were the interventions clearly defined?
- 2 = clearly defined interventions are applied with a standardized protocol
- 1 = clearly defined interventions are applied but the application protocol is not standardized
- 0 = intervention and/or application protocol are poorly or not defined

Were the outcome measures used clearly defined? (by outcome)
- 2 = clearly defined
- 1 = inadequately defined
- 0 = not defined

Were diagnostic tests used in outcome assessment clinically useful? (by outcome)
- 2 = optimal
- 1 = adequate
- 0 = not defined, not adequate

Was the surveillance active, and of clinically appropriate duration?
- 2 = active surveillance and appropriate duration
- 1 = active surveillance, but inadequate duration
- 0 = surveillance not active or not defined

Statistical analysis

The Review Manager Database (RevManversion 5.0, The Cochrane Collaboration 2008) was used to analyse the selected studies. Continuous data for each arm of a particular study were expressed as mean and standard deviation. Dichotomous data were expressed as proportions or risks, with the treatment effect reported as a relative risk with 95%CI.

The data were analysed for the outcomes that were of interest to us. The risk ratio (RR) was defined as the number of patients with a successful graft survival rate. The RR referred to the multiplication of the rate of graft

surveillance that occurred with the use of related and unrelated kidney transplantations. The heterogeneity between the studies was assessed using the χ^2 test and the I^2 statistic. The latter is a measure of the percentage of variation in data that results from heterogeneity as opposed to chance. A P value of < 0.1 and an I^2 value > 50% were considered suggestive of statistical heterogeneity, prompting a random effects modelling estimate. Conversely, a non-significant chi-squared test result (a P value $\geqslant$ 0.1 and an I^2 value $\leqslant$ 50%) only suggested that there was no evidence of heterogeneity; it did not necessarily imply that homogeneity existed because there may have been insufficient power to detect heterogeneity. The Mantel-Haenszel (M-H) method was used to combine the studies. If their significant heterogeneity were indicated (P < 0.1 and I^2 > 50%), a random-effect model was used; if not, a fixed-effect model was used. In addition, funnel plots were constructed for the outcomes to assess publication bias, *i.e.*, the tendency not to publish studies with negative results; the more asymmetric the funnel plot is, the more potential bias there is. The statistical significance was set at P < 0.05.

RESULTS

Study selection

Using our search terms, 376 references were identified. The first search of studies exploring the evaluation of the outcomes of patients yielded the following results: PubMed (n = 11590), Ovid (n = 24), EMBASE (n = 3300), the Cochrane Controlled Trials Register (n = 9719), and Google Scholar (n = 1430). Out of these, we included 12 studies after applying our eligibility criteria to their titles and/or abstracts, excluding duplicates (Figure 1).

The eligible trials included 12 relevant comparisons (Table 3) involving 9954 participants. We could not assess the differences in the outcomes between post-operative infections, post-operative hypertension, diabetes, and post-operative creatinine due to the lack of data.

Study presentation

Cortesini *et al*[9] evaluated 527 kidney allografts from living donors. Of these, 302 living donors were first-degree relatives of the recipient and shared one haplotype (living related donor) and 172 were unrelated. They showed actuarial graft survival rates in the living related and living unrelated groups, which were 91% and 87% in 1 year, 77% and 79% in 5 years, and 66% and 69% in 9 years. In conclusion, they reported that kidney transplantation between unrelated donors and recipients might be a valid alternative in view of the cadaver organ shortage, its success as a procedure and its potential to provide the "gift of life" to both the patient and the family.

Voiculescu *et al*[10] evaluated 62 out of 112 potential living donors for types of rejections, complications, and kidney functions. Of them, 38 cases were living related and 24 cases were living unrelated. They showed that acute rejection rate was similar in both groups (52.2% *vs* 54.2%); however, there were more complications of infection in the living related group (66.7% *vs* 36.4%) and a trend showing more surgical complications in living related transplantations (28.9% *vs* 8.3%). They concluded that the results for the living unrelated group are equivalent to the living related transplantation group. They determined that careful selection of donors and recipients is a prerequisite for success.

Kizilisik *et al*[11] evaluated 109 living donor kidney transplants. Seventy-eight percent of living donors were from living related donors and 22% were from living unrelated donors. The resultant one- and three-year patient survival rates were 97.6% and 93.2%, with 1- and 3-year graft survival rates of 93.2% and 88.3%, respectively. Among the patients of Kizilisik *et al*[11], there were 6 delayed graft functions (5.5%), 16 acute cellular rejections (10%), and 10 chronic rejections (9%). They suggested that living donors represent a valuable source because of the limited number of cadaveric kidneys available for transplant and stated that the use of living-unrelated donors has produced an additional supply of organs.

Park *et al*[12] evaluated 77 living-donor renal transplants (41 were living unrelated and 36 were living related transplants). They reported that 11 recipients lost their grafts (6 from living unrelated and 5 from living related); most of these losses were due to chronic rejection (n = 7). Overall 3-, 5- and 10-year graft survival rates in live donors were 92.8%, 86.6% and 76.9%, respectively; for the living unrelated, the graft survival at 3-, 5- and 10-years was 91.9%, 88.5% and 74.7% *vs* 94%, 84% and 78.8% for the living related transplants. They concluded that acute rejection episodes markedly decreased long-term graft survival in live donor renal transplants, the use of living related transplants provides graft survival comparable with living related transplants, and proper management of acute rejection is essential for long-term graft survival.

Wolters *et al*[13] evaluated 95 living donor transplantations (69% related, 31% unrelated). They showed that at a mean follow-up of 35 mo, 94.7% of grafts were functioning. Three grafts were lost due to acute (in related transplants) or chronic (in unrelated transplants) rejection or due to multi-organ failures. They concluded that although HLA mismatching was significantly different between related and unrelated donors, no difference in the outcome was observed.

Simforoosh *et al*[14], between 1984 and 2004, evaluated 2155 kidney transplantations; out of this, 374 were from living related donors and 1760 were from unrelated donors. The resultant 1-, 3-, 5-, 10- and 15-year graft survival rates among the related group were 91.6%, 81.7%, 76.4%, 64.4% and 48.4%; and for unrelated group, these rates were 91.5%, 86.7%, 81.4%, 68.2%

Table 3 The characteristics of included study which reported related *vs* unrelated living kidney transplantation outcomes

Ref.	Number	Mean follow up (mo)	Recipient mean age (yr)	Recipient sex M/F	Immunosuppression regimen	One year graft survival rate	five years graft survival rate	10 yr graft survival rate	Acute rejection rate	Mean serum Cr at 1 yr	Mean serum Cr at final follow up	Post-transplant infectious complications
Cortesini *et al*[9], 2002	302 *vs* 172	42	32.8 ± 7.3 *vs* 44 ± 9.9	215/87 *vs* 133/39	Cyclosporine	275 (91) *vs* 150 (87)	232 (77) *vs* 136 (79)	199 (66) *vs* 118 (69)	N/D	1.9 ± 0.8 *vs* 2.0 ± 0.8	2.0 ± 0.8 *vs* 1.9 ± 0.8	N/D
Simforoosh *et al*[5], 2016	411 *vs* 3305	N/D	27.6 ± 10.1 *vs* 35.6 ± 15.6	270/138 *vs* 2164/1136	Cyclosporine	89% *vs* 90%	288 (70.2) *vs* 2697 (81.6)	225 (54.9) *vs* 2350 (71.1)	N/D	N/D	N/D	N/D
Voiculescu *et al*[10], 2003	38 *vs* 24	19.6 ± 15.4	37.7 ± 12.1 *vs* 53.6 ± 7.8	26/12 *vs* 14/10	Steroids, cyclosporine, mycophenolate mofetil	36 (94.8) *vs* 24 (100)	N/D	N/D	20 (52.5) *vs* 13 (54.2)	N/D	1.76 ± 0.6 *vs* 1.62 ± 0.5	25 (66.7) *vs* 9 (36.4)
Ahmad *et al*[15], 2008	261 *vs* 61	45	28 ± 16 *vs* 48 ± 12	N/D	Cyclosporine	247 (94.8) *vs* 60 (98.4)	N/D	N/D	107 (41) *vs* 21 (35)	N/D	N/D	N/D
Kizilisik *et al*[11], 2004	85 *vs* 24	36	N/D	N/D	Cyclosporine, azathioprine, steroid, tacrolimus, mycofenolatemofetil	81 (95) *vs* 23 (95.8)	75(88.3) *vs* 21 (87.5)	N/D	11(13) *vs* 5 (20)	N/D	N/D	7 (8.3) *vs* 8 (3.5)
Park *et al*[12], 2004	36 *vs* 41	N/D	33.6 *vs* 38.3	21/15 *vs* 28/13	Cyclosporine, steroid and mycophenolatemofetil	N/D	30 (84) *vs* 36 (88.5)	28 (78.8) *vs* 41 (74.7)	11 (30) *vs* 13 (31)	N/D	N/D	N/D
Wolters *et al*[13], 2005	66 *vs* 29	35	31 ± 12.5 *vs* 51 ± 8.5	41/25 *vs* 23/6	Cyclosporine/MMF/ prednisone *vs* MMF/prednisone	N/D	62 (94.7) *vs* 23 (94.7)	N/D	6 (9) *vs* 5 (17.2)	N/D	N/D	N/D
Simforoosh *et al*[14], 2006	374 *vs* 1760	45.68 ± 46.80	28.97 ± 9.58 *vs* 33.46 ± 14.61	N/D	Cyclosporine, azathioprine, and prednisone	342 (91.6) *vs* 1610 (91.5)	286 (76.4) *vs* 1432 (81.4)	241 (64.4) *vs* 1200 (68.2)	N/D	N/D	N/D	N/D
Ishikawa *et al*[16], 2012	66 *vs* 44	12	36.1 ± 12.4 *vs* 55.0 ± 8.8	29/15 *vs* 38/28	Plasmaphresis, tacro, celecpt, Basiliximab, rituximab, methyl prednisolone, cyclosporine, deoxypergualin	65 (98.5) *vs* 43 (97.7)	N/D	N/D	16 (24.2) *vs* 14 (31.8)	N/D	N/D	N/D
Santori *et al*[17], 2012	111 *vs* 24	128.17 ± 86.64 *vs* 103.53 ± 86.85	26.94 ± 13.51 *vs* 50.04 ± 8.86	78/33 *vs* 18/6	Cyclosporine, tacro, steroids, celecept	N/D	N/D	71 (63.8) *vs* 21 (87.8)	N/D	N/D	N/D	N/D
Matter *et al*[18], 2016	2075 *vs* 410	7.72 ± 6.15	28.8 ± 9.8 *vs* 34.8 ± 11.1	1554/521 *vs* 297/113	Steroid-Azathioprine or MMF	2012 (97) *vs* 389 (95)	1784 (86) *vs* 340 (83)	1660 (67) *vs* 270 (66)	71 (3.4) *vs* 26 (6.3)	1.38 ± 0.69 *vs* 1.35 ± 0.61	1.71 ± 1.04 *vs* 1.59 ± 0.89	N/D
Ali *et al*[19]	92 *vs* 143	5	N/D	N/D	Methyl prednisolone, Cyclosporine or tacrolimus MMF	90 (97) *vs* 141 (98.6)	80 (86) *vs* 125 (87.4)	N/D	N/D	N/D	N/D	N/D

Data is presented as *n* (%) and Mean ± SD. N/D: Not determined; MMF: Mycophenolatemofetil.

and 53.2%, respectively. Patient survivals for 1-, 3-, 5-, 10- and 15-years in the living related group were 94.6%, 91.9%, 83%, 79.5% and 73.9%; and in the unrelated group, these were 93.6%, 91.7%, 89.3%, 84% and 76.4%, respectively. They concluded that the results of living unrelated kidney transplantation upon long-term follow-up in a large number of cases was as effective as living related kidney transplantation.

Ahmad *et al*[15] retrospectively analysed the outcome of 322 living-donor renal transplants (related donors: 261; unrelated donors = 61). They reported that 33 grafts failed: 30 in the living related (11%) and 3 in the unrelated donor group (5%). Acute rejections occurred in 41% of recipients in the living related group

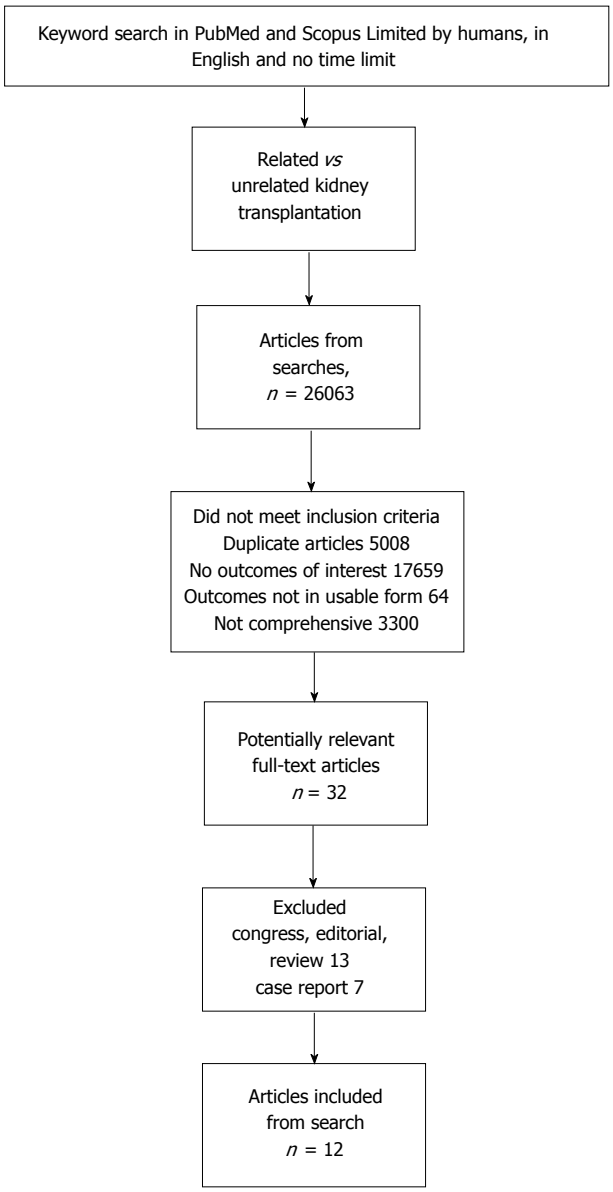


Figure 1 Study selection.

and 35% of recipients in the unrelated group. One- and 3-year patient survival for the living related and unrelated group was 98.7% and 96.3% and 97.7% and 95%, respectively. One- and 3-year graft survival was equivalent at 94.8% and 92.3% for the living related, and at 98.4% and 93.7% for the living unrelated group, respectively. They concluded that the outcome of living related donors and living unrelated donors is comparable in terms of patient and graft survival, acute rejection rate, and the estimated GFR despite the differences in demographics, HLA matching, and re-transplants of recipients.

Ishikawa *et al*[16] evaluated 112 cases of living kidney transplantations including 46 (41%) unrelated donors and 66 cases of received kidneys from living related donors. They showed that the incidences of an acute rejection episode were 31.8% and 24.2% in the living unrelated and the related groups, respectively. They demonstrated that living transplantation from an unrelated group was equivalent to related ones.

Santori *et al*[17] evaluated 135 procedures using living donors (living related: 111; living unrelated: 24). They reported no significant difference in patient survival after stratifying for donor type (living related: 93.9%; unrelated donors: 95.8%) or in graft survival after stratifying for donor type (related: 63.8%; unrelated: 87.8%). After entering donor type as an independent variable in a univariate Cox regression, they showed no significance for either recipient or graft survival. They suggested that living unrelated donor utilization should be encouraged in kidney transplantation programmes.

Simforoosh *et al*[5] evaluated 3,716 kidney transplantations (411 related donors and 3305 unrelated donors). They showed that donor age was the only statistically significant predictor of graft survival rate (hazard ratio = 1.021; 95%CI: 1.012-1.031). Patient survival and graft survival was similar in transplantations from living unrelated and related donors. They concluded that transplants from LURDs might be proposed as an acceptable management for patients with end stage renal disease.

Matter *et al*[18] from March 1976 to December 2013, divided the patients into two groups: (1) 2075 kidney transplant recipients (1554 or 74.9% male and 521 or 25.1% female) for whom the donors were living related; (2) 410 kidney transplant recipients (297 or 72.4% male and 113 or 27.6% female) for whom the donors were living unrelated. They showed the percentages of patients with acute vascular rejection were significantly higher in the unrelated group, while percentages of patients with no rejection were significantly higher in the related group, but there were no significant differences regarding patient and graft survivals between both groups.

Ali *et al*[19] evaluated 250 kidney transplantations (92 related donors, 143 unrelated donors and 15 spouse). They showed the one-year graft survival for related and unrelated donor transplants was 98.9% and 91.8%, respectively. Graft survival was lower (82.9%) in recipients with acute rejection episodes. The patient survival at one-year was 94%. The three year graft and patient survival was 91% and 90%, respectively, and five-year survival for grafts and patients was 87.1% and 88%, respectively.

Meta-analysis

Long term (ten year) graft survival rate: We conducted random effect meta-analyses (Figure 2) because the results from the studies which reported ten years graft survival rate after living related and unrelated renal transplantation showed significant heterogeneity (P = 0.001). No significant difference in graft survival rate was detected after ten years in patients who underwent living related kidney transplantations in comparison to

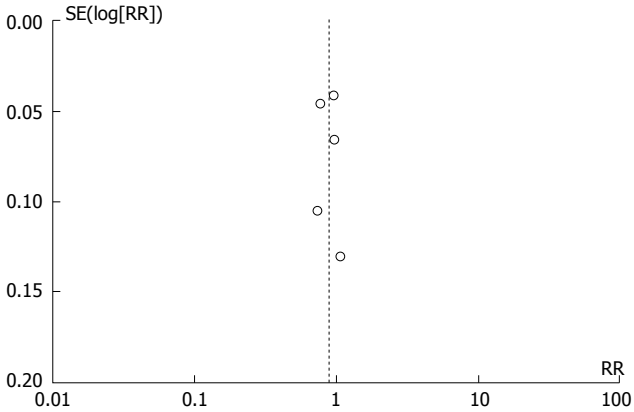


Figure 2 Significant heterogeneity in long term follow up between living related and unrelated kidney transplantation in funnel plot. RR: Risk ratio.

those who underwent unrelated kidney transplantations (P = 0.44) (Figure 3).

Mid-term (one to five year) graft survival rate: We conducted random effect meta-analyses because the results from studies reporting 1-5 years graft survival rate after living related and unrelated renal transplantation showed significant heterogeneity (P = 0.002). There were no significant differences between graft survival rate in living related and unrelated kidney transplantations after mid-term follow-ups (P = 0.46) (Figure 3).

Short-term (one year) graft survival rate: We conducted fixed effect meta-analyses because the results from the studies reporting one year graft survival rate after living related and unrelated renal transplantations showed no significant heterogeneity (P = 0.11). There were no significant differences between the graft survival rate in living related and unrelated kidney transplantations after a one year follow-up (P = 0.35) (Figure 3).

Acute rejection rate: We conducted fixed effect meta-analyses because the results from the studies reporting acute rejection rate after living related and unrelated renal transplantations showed no significant heterogeneity (P = 0.17). There were no significant differences between the acute rejection rate in living related and unrelated kidney transplantations (P = 0.06) (Figure 3).

DISCUSSION

This systematic meta-analysis showed that no significant difference existed in graft survival rate between living related and unrelated kidney transplantations in short, mid and long-term follow-ups.

In comparison to dialysis, transplantation has lengthened the patient's survival and improved their quality of life; in the medical field, it has broadened knowledge; to sponsors, it has provided a cost-effective solution for a never-ending problem. On the other hand, the shortcoming of transplantation is the unavailability of enough donors. This led to scientists using living unrelated kidney transplantations as an available source, but there were strong controversies in this respect. A detailed analysis suggests that the difference was related to a "centre effect". The inferior outcomes of living unrelated-donor transplantations were caused by the low standards of medical care in commercial transplantation programmes, the infections transmitted between the donor organs or patient non-compliance. After correcting these factors[20,21], the reports have shown no significant difference in graft outcomes when compared with living related transplantations. Our results support the finding that showed no significant difference between living related and unrelated kidney graft survival rates after mid-term and short-term follow-ups.

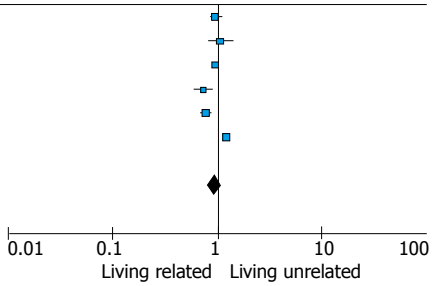
This systematic review and meta-analysis showed that the long-term graft survival rate has not a significant difference between the living related and the living unrelated groups. In our previous report[5], we evaluated the recipients of kidney transplants for 25 years and a comparable survival rate was found between the two groups. Park *et al*[12] reported the graft survival rates at 3, 5 and 10 years as 91.9%, 88.5% and 74.7% for the LURD *vs* 94%, 84% and 78.8% for the LRD transplants, with no significant difference. In contrast to our findings, previous studies showed no significant difference in long-term graft surveillance between the two groups[5,9,14]. This might be because of significant heterogeneity between the studies. As the funnel plot described, there is significant heterogeneity between the studies; therefore, in the future, more studies with a high quality of methodology are warranted.

While unrelated kidney transplantations are not widely accepted, the concern for transplantations continues to revolve around the issue of inadequate material benefits for potential donors[22]. The only model that resolved this issue was the model used in Iran. This model is organized by a non-profit organization known as the "Dialysis and Transplant Patients' Association (DATPA)"[23]. The DATPA's task is to assign appropriate donors for certain recipients and to offer medicolegal coverage. Donors receive a form of compensation from the government and the DATPA, and in addition, they are granted free life-long health insurance, and often, a "rewarding gift from the recipient"[23]. This model has been very successful over the past two decades in Iran, nearly eradicating the names on the transplant waiting list and gracefully providing a second chance at life for patients with ESRD; this model comprises over 75% of the total kidney transplant activity in Iran.

As a limitation, because of the lack of data, we could not evaluate the difference in HLA mismatches between the studies. Nevertheless, previous studies have reported equivalent short-, medium- and long-term outcomes of transplantation in LURD series in comparison to LRDs.

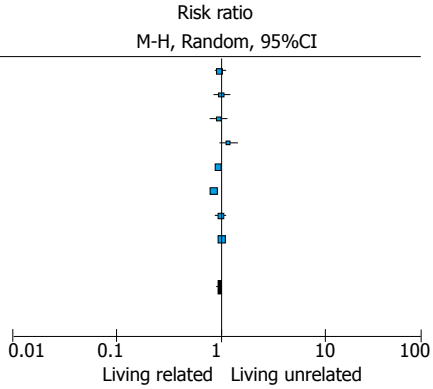
Ten years graft survival rate	Living related		living unrelated			Risk ratio	
Study or subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95%CI	Year
Cortesini 2002	199	302	118	172	17.1%	0.96 [0.84, 1.09]	2002
Park 2004	28	36	30	41	13.4%	1.06 [0.82, 1.37]	2004
Simforoosh 2006	241	374	1200	1760	18.2%	0.95 [0.87, 1.03]	2006
Santori 2012	71	111	21	24	14.9%	0.73 [0.60, 0.90]	2012
Simforoosh 2015	225	411	2350	3305	18.0%	0.77 [0.70, 0.84]	2015
Matter 2016	1660	2075	270	410	18.4%	1.21 [1.13, 1.31]	2016
Total (95%CI)		3309		5712	100.0%	0.94 [0.79, 1.11]	
Toatl events	2424		3989				

Heterogeneity: Tau^2 = 0.04; χ^2 = 70.08, df = 5 (P < 0.00001); I^2 = 93%
Test for overall effect: Z = 0.77 (P = 0.44)



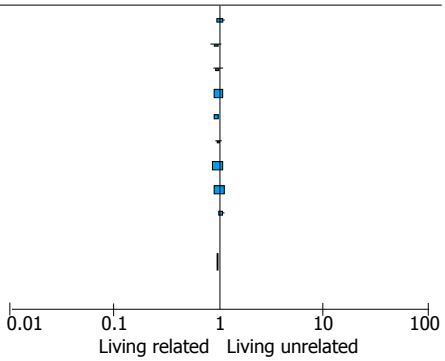
Five years graft survival rate	Living related		Living unrelated			Risk ratio	
Study or subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95%CI	Year
Cortesini 2002	232	302	136	172	13.4%	0.97 [0.88, 1.07]	2002
Kizilisik 2004	75	85	21	24	8.4%	1.01 [0.85, 1.20]	2004
Park 2004	30	36	36	41	7.5%	0.95 [0.79, 1.14]	2004
Wolters 2005	62	66	23	29	7.1%	1.18 [0.97, 1.44]	2005
Simforoosh 2006	286	374	1432	1760	16.6%	0.94 [0.88, 1.00]	2006
Simforoosh 2015	288	411	2697	3305	16.2%	0.86 [0.80, 0.92]	2015
Ali 2016	80	92	125	143	13.2%	0.99 [0.90, 1.10]	2016
Matter 2016	1784	2075	340	410	17.6%	1.04 [0.99, 1.09]	2016
Total (95%CI)		3441		5884	100.0%	0.98 [0.91, 1.04]	
Toatl events	2837		4810				

Heterogeneity: Tau^2 = 0.01; χ^2 = 28.14, df = 7 (P = 0.0002); I^2 = 75%
Test for overall effect: Z = 0.74 (P = 0.46)



One year graft survival rate	Living related		Living unrelated			Risk ratio	
Study or subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95%CI	Year
Cortesini 2002	275	302	150	172	8.0%	1.04 [0.98, 1.12]	2002
Voiculescu 2003	36	38	24	24	1.3%	1.95 [0.86, 1.05]	2003
Kizilisik 2004	81	85	23	24	1.5%	0.99 [0.90, 1.09]	2004
Simforoosh 2006	342	374	1610	1760	23.7%	1.00 [0.97, 1.03]	2006
Nadeem 2008	247	261	60	61	4.1%	0.96 [0.92, 1.00]	2008
Ishikawa 2012	65	66	43	44	2.2%	1.01 [0.95, 1.06]	2012
Simforoosh 2015	366	411	2974	3305	27.6%	0.99 [0.95, 1.03]	2015
Matter 2016	2012	2075	389	410	27.3%	1.02 [1.00, 1.05]	2016
Ali 2016	90	92	131	143	4.3%	1.07 [1.01, 1.13]	2016
Total (95%CI)		3704		5943	100.0%	1.01 [0.99, 1.02]	
Toatl events	3514		5404				

Heterogeneity: χ^2 = 12.99, df = 8 (P = 0.11); I^2 = 38%
Test for overall effect: Z = 0.93 (P = 0.35)



Acute rejection rate	Living related		Living unrelated			Risk ratio	
Study or subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95%CI	Year
Voiculescu 2003	20	38	13	24	11.6%	0.97 [0.60, 1.56]	2003
Park 2004	11	36	13	41	8.9%	0.96 [0.49, 1.88]	2004
Kizilisik 2004	11	85	5	24	5.7%	1.62 [0.24, 1.61]	2004
Wolters 2005	6	66	5	29	5.1%	1.53 [0.17, 1.59]	2005
Nadeem 2008	107	261	21	61	24.8%	1.19 [0.82, 1.73]	2008
Ishikawa 2012	16	66	14	44	12.3%	0.76 [0.42, 1.40]	2012
Matter 2016	71	2075	26	410	31.7%	0.54 [0.35, 0.83]	2016
Total (95%CI)		2627		633	100.0%	1.82 [0.67, 1.01]	
Toatl events	242		97				

Heterogeneity: χ^2 = 9.03, df = 6 (P = 0.17); I^2 = 34%
Test for overall effect: Z = 1.89 (P = 0.06)

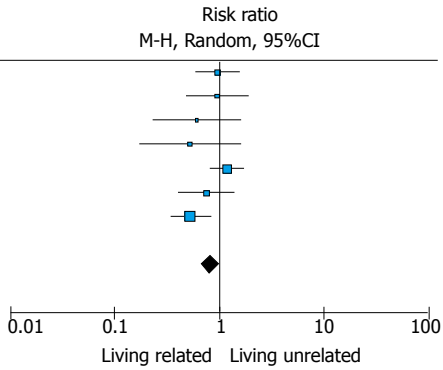


Figure 3 Comparing long, mid and short term graft survival rate and acute rejection rate between living related and unrelated kidney transplantations.

In conclusion, the long, mid and short-term follow-up of living related and unrelated kidney transplantation showed no significant difference in graft survival rate. Also, acute rejection rate was not significantly different between groups. We suggest that the Iranian model is a fair compromise because it avoids the rampant transplant commercialism.

COMMENTS

Background

The number of patients who are on the renal transplant waiting list for deceased-

donor transplantation has increased and thousands of patients have died while waiting for renal transplantation. Despite this, no systematic review and meta-analysis has been performed yet.

Research frontiers

Nowadays the outcomes of living related *vs* unrelated kidney transplantation are debatable. Worldwide research is directed towards the use of living unrelated kidney transplantation as a potential source.

Innovations and breakthroughs

In the present study, the authors investigated the outcomes of two kinds of sources in kidney transplantation by pooling results from different centres. This is the first report of a meta-analysis comparing these sources in receipts.

Applications

The present report provides an understanding of living unrelated kidney transplantation as an excellent source.

Peer-review

In this manuscript authors performed a meta-analysis to compare related and unrelated living donor kidney transplant outcome. Results indicate comparable outcome of kidney transplant from living unrelated *vs* related donors in the short, mid and long term follow up.

REFERENCES

1 **Moeller S**, Gioberge S, Brown G. ESRD patients in 2001: global overview of patients, treatment modalities and development trends. *Nephrol Dial Transplant* 2002; **17**: 2071-2076 [PMID: 12454213 DOI: 10.1093/ndt/17.12.2071]

2 **Schnuelle P**, Lorenz D, Trede M, Van Der Woude FJ. Impact of renal cadaveric transplantation on survival in end-stage renal failure: evidence for reduced mortality risk compared with hemodialysis during long-term follow-up. *J Am Soc Nephrol* 1998; **9**: 2135-2141 [PMID: 9808102]

3 **Sesso R**, Josephson MA, ANção MS, Draibe SA, Sigulem D. A retrospective study of kidney transplant recipients from living unrelated donors. *J Am Soc Nephrol* 1998; **9**: 684-691 [PMID: 9580370]

4 **Ghods AJ**. Renal transplantation in Iran. *Nephrol Dial Transplant* 2002; **17**: 222-228 [PMID: 11812870 DOI: 10.1093/ndt/17.2.222]

5 **Simforoosh N**, Basiri A, Tabibi A, Javanmard B, Kashi AH, Soltani MH, Obeid K. Living Unrelated Versus Related Kidney Transplantation: A 25-Year Experience with 3716 Cases. *Urol J* 2016; **13**: 2546-2551 [PMID: 26945660]

6 **Barsoum RS**. The Egyptian transplant experience. *Transplant Proc* 1992; **24**: 2417-2420 [PMID: 1465813]

7 **Sever MS**, Kazancioğlu R, Yildiz A, Türkmen A, Ecder T, Kayacan SM, Celik V, Sahin S, Aydin AE, Eldegez U, Ark E. Outcome of living unrelated (commercial) renal transplantation. *Kidney Int* 2001; **60**: 1477-1483 [PMID: 11576362 DOI: 10.1046/j.1523-1755.2001.00951.x]

8 **Jha V**, Chugh KS. The case against a regulated system of living kidney sales. *Nat Clin Pract Nephrol* 2006; **2**: 466-467 [PMID: 16941033 DOI: 10.1038/ncpneph0268]

9 **Cortesini R**, Pretagostini R, Bruzzone P, Alfani D. Living unrelated kidney transplantation. *World J Surg* 2002; **26**: 238-242 [PMID: 11865354 DOI: 10.1007/s00268-001-0211-4]

10 **Voiculescu A**, Ivens K, Hetzel GR, Hollenbeck M, Sandmann W, Grabitz K, Balzer K, Schneider F, Grabensee B. Kidney transplantation from related and unrelated living donors in a single German centre. *Nephrol Dial Transplant* 2003; **18**: 418-425 [PMID: 12543901]

11 **Kizilisik AT**, Ray JM, Nylander WA, Langone AJ, Helderman JH, Shaffer D. Living donor kidney transplantation in a Veterans Administration medical center. *Am J Surg* 2004; **188**: 611-613 [PMID: 15546581 DOI: 10.1016/j.amjsurg.2004.07.022]

12 **Park YH**, Min SK, Lee JN, Lee HH, Jung WK, Lee JS, Lee JH, Lee YD. Risk factors on graft survival of living donor kidney transplantation. *Transplant Proc* 2004; **36**: 2023-2025 [PMID: 15518732 DOI: 10.1016/j.transproceed.2004.09.009]

13 **Wolters HH**, Heidenreich S, Dame C, Brockmann JG, Senninger N, Krieglstein CF. Living donor kidney transplantation: impact of differentiated immunosuppressive regimen. *Transplant Proc* 2005; **37**: 1616-1617 [PMID: 15866688 DOI: 10.1016/j.transproceed.2004.09.022]

14 **Simforoosh N**, Basiri A, Fattahi MR, Einollahi B, Firouzan A, Pour-Reza-Gholi F, Nafar M, Farrokhi F. Living unrelated versus living related kidney transplantation: 20 years' experience with 2155 cases. *Transplant Proc* 2006; **38**: 422-425 [PMID: 16549137 DOI: 10.1016/j.transproceed.2006.01.012]

15 **Ahmad N**, Ahmed K, Khan MS, Calder F, Mamode N, Taylor J, Koffman G. Living-unrelated donor renal transplantation: an alternative to living-related donor transplantation? *Ann R Coll Surg Engl* 2008; **90**: 247-250 [PMID: 18430342 DOI: 10.1308/003588408X261636]

16 **Ishikawa N**, Yagisawa T, Kimura T, Sakuma Y, Fujiwara T, Nukui A, Yashi M. Kidney transplantation of living unrelated and ABO-incompatible donor-recipient combinations. *Transplant Proc* 2013; **45**: 1242-1244 [PMID: 23622668 DOI: 10.1016/j.transproceed.2013.02.028]

17 **Santori G**, Barocci S, Fontana I, Bertocchi M, Tagliamacco A, Biticchi R, Valente U, Nocera A. Kidney transplantation from living donors genetically related or unrelated to the recipients: a single-center analysis. *Transplant Proc* 2012; **44**: 1892-1896 [PMID: 22974864 DOI: 10.1016/j.transproceed.2012.05.061]

18 **Matter YE**, Nagib AM, Lotfy OE, Alsayed AM, Donia AF, Refaie AF, Akl AI, Abbas MH, Abuelmagd MM, Shaeashaa HA, Shokeir AA. Impact of Donor Source on the Outcome of Live Donor Kidney Transplantation: A Single Center Experience. *Nephrourol Mon* 2016; **8**: e34770 [PMID: 27570751 DOI: 10.5812/numonthly.34770]

19 **Ali AA**, Al-Saedi AJ, Al-Mudhaffer AJ, Al-Taee KH. Five years renal transplantation data: Single-center experience from Iraq. *Saudi J Kidney Dis Transpl* 2016; **27**: 341-347 [PMID: 26997389 DOI: 10.4103/1319-2442.178559]

20 **Terasaki PI**, Cecka JM, Gjertson DW, Takemoto S. High survival rates of kidney transplants from spousal and living unrelated donors. *N Engl J Med* 1995; **333**: 333-336 [PMID: 7609748 DOI: 10.1056/NEJM199508103330601]

21 **Gheith O**, Sabry A, El-Baset SA, Hassan N, Sheashaa H, Bahgat S, El-Shahawy el-M. Study of the effect of donor source on graft and patient survival in pediatric renal transplant recipients. *Pediatr Nephrol* 2008; **23**: 2075-2079 [PMID: 18446383 DOI: 10.1007/s00467-008-0760-y]

22 **Delmonico FL**. The development of the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. *Nephrol Dial Transplant* 2008; **23**: 3381-3382 [PMID: 18922862 DOI: 10.1093/ndt/gfn552]

23 **Ghods AJ**, Savaj S. Iranian model of paid and regulated living-unrelated kidney donation. *Clin J Am Soc Nephrol* 2006; **1**: 1136-1145 [PMID: 17699338 DOI: 10.2215/CJN.00700206]

P- Reviewer: Friedman EA, Gheith O, Piancatelli D, Shrestha BM
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Wu HL

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.f6publishing.com

ISSN 2220-3230 (online)

World Journal of *Transplantation*

World J Transplant 2017 June 24; 7(3): 161-221

Published by Baishideng Publishing Group Inc

WJT World Journal of Transplantation

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

Editorial Board

2016-2019

The *World Journal of Transplantation* Editorial Board consists of 361 members, representing a team of worldwide experts in transplantation. They are from 43 countries, including Argentina (1), Australia (7), Austria (3), Belgium (4), Brazil (7), Bulgaria (1), Canada (13), China (32), Cuba (1), Czech Republic (1), Denmark (1), Finland (1), France (4), Georgia (1), Germany (14), Greece (5), Hungary (2), India (7), Iran (7), Israel (4), Italy (33), Japan (18), Jordan (1), Macedonia (1), Mexico (2), Morocco (1), Netherlands (4), Nigeria (1), Norway (1), Pakistan (1), Poland (2), Qatar (1), Saudi Arabia (3), Singapore (1), South Korea (16), Spain (9), Sweden (1), Switzerland (3), Thailand (2), Tunisia (1), Turkey (6), United Kingdom (17), and United States (120).

WJT World Journal of Transplantation

Contents

Bimonthly Volume 7 Number 3 June 24, 2017

Contents

World Journal of Transplantation
Volume 7 Number 3 June 24, 2017

ABOUT COVER

Editorial Board Member of *World Journal of Transplantation*, Alfred Konigsrainer, MD, Director, Head, Professor, Department of Surgery and Transplantation, University Hospital Tuebingen Germany, 72076 Tuebingen, Germany

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, PubMed Central.

FLYLEAF

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Ya-Jing Lu*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Jin-Xin Kong*
Proofing Editorial Office Director: *Ze-Mao Gong*

NAME OF JOURNAL
World Journal of Transplantation

ISSN
ISSN 2220-3230 (online)

LAUNCH DATE
December 24, 2011

FREQUENCY
Bimonthly

EDITOR-IN-CHIEF
Maurizio Salvadori, MD, Professor, Renal Unit, Careggi University Hospital, Florence 50139, Italy

EDITORIAL BOARD MEMBERS
All editorial board members resources online at http://www.wjgnet.com/2220-3230/editorialboard.htm

EDITORIAL OFFICE
Xiu-Xia Song, Director

World Journal of Transplantation
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
June 24, 2017

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 June 24; 7(3): 161-178

DOI: 10.5500/wjt.v7.i3.161

ISSN 2220-3230 (online)

REVIEW

Biomarkers in renal transplantation: An updated review

Maurizio Salvadori, Aris Tsalouchos

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

Aris Tsalouchos, Division of Nephrology, Careggi University Hospital, 50134 Florence, Italy

Author contributions: Salvadori M and Tsalouchos A equally contributed to the manuscript.

Conflict-of-interest statement: Maurizio Salvadori and Aris Tsalouchos do not have any conflict of interest in relation to the manuscript, as in the attached form.

Manuscript source: Invited manuscript

Correspondence to: Maurizio Salvadori, MD, Renal Unit, 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: February 7, 2017
Peer-review started: February 12, 2017
First decision: March 29, 2017
Revised: April 7, 2017
Accepted: April 18, 2017
Article in press: April 19, 2017
Published online: June 24, 2017

Abstract

Genomics, proteomics and molecular biology lead to tremendous advances in all fields of medical sciences. Among these the finding of biomarkers as non invasive indicators of biologic processes represents a useful tool in the field of transplantation. In addition to define the principal characteristics of the biomarkers, this review will examine the biomarker usefulness in the different clinical phases following renal transplantation. Biomarkers of ischemia-reperfusion injury and of delayed graft function are extremely important for an early diagnosis of these complications and for optimizing the treatment. Biomarkers predicting or diagnosing acute rejection either cell-mediated or antibody-mediated allow a risk stratification of the recipient, a prompt diagnosis in an early phase when the histology is still unremarkable. The kidney solid organ response test detects renal transplant recipients at high risk for acute rejection with a very high sensitivity and is also able to make diagnosis of subclinical acute rejection. Other biomarkers are able to detect chronic allograft dysfunction in an early phase and to differentiate the true chronic rejection from other forms of chronic allograft nephropathies no immune related. Finally biomarkers recently discovered identify patients tolerant or almost tolerant. This fact allows to safely reduce or withdrawn the immunosuppressive therapy.

Key words: Renal transplantation; Biomarkers; Genomic; Proteomics; Transplant outcome; Molecular signatures

Core tip: The uses of biomarkers as a non invasive tool instead of renal biopsy in diagnosing transplant renal complications are entering the clinical practice. Progress in genomics, proteomics and all the "omics" fields has allowed the finding of robust, predictive and useful biomarkers. They are modifying our window on transplantation and are allowing us to predict the renal injury earlier because the pathologic process is evident at molecular level before its histological or clinical manifestations. The future is exciting because new international researches and trials are ongoing in this field.

Salvadori M, Tsalouchos A. Biomarkers in renal transplantation:

An updated review. *World J Transplant* 2017; 7(3): 161-178
Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i3/161.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i3.161

INTRODUCTION

Kidney transplantation represents the optimal therapeutic tool for patients affected by end-stage renal disease (ESRD). Improvements in immunosuppressive therapy have resulted in a decrease in acute rejections (AR) and have significantly increased graft short-term half life[1]. However, late kidney graft loss remains a major problem and challenge in kidney transplantation[2]. To date, renal function after transplantation is primarily evaluated by serum creatinine measurement and core renal biopsy. The latter is considered the gold standard in transplant evaluation. Nonetheless, both approaches have several drawbacks. Serum creatinine levels increase late in injury and are non-specific for the type of injury. Additionally, the serum level of creatinine is not able to predict or evaluate the progression of chronic injury and as a consequence is not specific or predictive. Similarly, renal core biopsy cannot be used to monitor the progression of injury because it is invasive and cannot be performed serially. Additionally, there are problems and possible biases in evaluating the specimen and the procedure is not completely free of complications. Moreover, the predictive power of renal core biopsy is poor. In fact, in the National Institutes of Health (NIH) clinical trial "Steroid-Free *vs* Steroids-based Immunosuppression in pediatric renal transplantation" (SNSO1) protocol, renal biopsies were unable to measure "hidden" tissue injury in clinically stable patients[3,4]. In addition, using protocol biopsies, Naesens *et al*[5] reported that examination of tissue at the molecular level is able to reveal abnormalities in innate and adoptive immune responses long before those abnormalities appear at the histological level. Clearly, the development of noninvasive reliable and predictive biomarkers for early diagnosis and monitoring of any clinical condition after kidney transplantation is essential for tailored and individualized treatment[6-8].

In studying the entire transplantation process, biological markers may be used throughout all phases, starting from the donor and donor kidney retrieval. In this phase, biomarkers may be useful for predicting short-term outcomes, and the incidence and severity of delayed graft function (DGF).

The most studied and used biomarkers are those related to the diagnosis and the identification of different aspects of subacute and acute kidney rejection. In addition, biomarkers able to differentiate true chronic rejection (CR), which is immunologically mediated, from the so-called "chronic allograft dysfunction" (CAD), are important because the treatments are different. Indeed, recently, mining the human urine proteome for monitoring renal transplant injury, Sigdel *et al*[9] found urinary peptides specific for AR, urinary peptides specific for chronic allograft nephropathy (CAN) and urinary peptides specific for BK virus nephropathy (BKVN).

Finally, relevant markers are those associated with tolerance, as these markers might allow for decreasing immunosuppressive treatment, withdrawing or discontinuing any immunosuppressant and monitoring the effects of such measures.

In this review, we describe the principal characteristics of current biomarkers, their power and limitation, the principal sources and their relevance in different clinical settings post renal transplantation.

RESEARCH METHODOLOGY

For this review, we have analyzed the available papers on biomarkers in renal transplantation. A literature search was performed using PubMed (NCBI/NIH) with the search words renal transplantation, biomarkers, genomic, proteomics, transplant outcome, molecular signatures. Firstly, papers published in the last three years were examined, then we proceeded in a backward way and also studies published previously have been included. Studies currently under way were searched for in "clinical trial.gov" and the European EUDRACT register. Only randomized clinical trials (RCTs) active and enrolling patients have been selected.

DEFINITION AND PRINCIPAL CHARACTERISTICS OF THE BIOLOGICAL MARKERS

In addition to clinical markers and pathological markers, monitoring of the outcome of a clinical process may be performed using biological markers (biomarkers). A NIH working group recommended the following terms and definitions[10]: A biomarker is a characteristic that is objectively measured and evaluated as an indicator of a normal biological process, pathogenic process or pharmacological response to a therapeutic intervention.

Principal applications of biomarkers are as follows: (1) diagnosis or identification of patients affected by a disease or an abnormal condition; (2) staging of the severity or extent of a disease; (3) prognosis of a disease; and (4) prediction and monitoring of a clinical response to an intervention.

Table 1 clarifies both the definition and the principal characteristics of the biomarkers and the technologies involved[11]. A variety of innovative technologies, ranging from genomics, proteomics, peptidomics, antibodyomics, microbiomics and metabolomics, among others, all referred to as "omics", have emerged in medical fields, to generate new biomarkers[12] .

Genomics refers to the study of the genome, and epigenomics is the study of the complete set of epigenetic modifications of the genetic materials of a cell. Transcriptomics is the study of the set of all messenger

Table 1 Definition and principal characteristics of biomarkers

Biomarker	A characteristic objectively measured as an indicator of a biological process or a response to a pharmacological intervention
Proteomics	The systematic analysis of proteins for their identity, quantity and function
Genomics	The study of the genome for estimating the risk for an individual to develop a disease
Transcriptomics	The study of expression patterns of all gene transcript
Metabolomics	The quantitative analysis of all the metabolites of a specific biological sample

Table 2 Biomarker candidates in the context of ischemia reperfusion injury and delayed graft function

Symbol	Gene description	Cytoband
ACTA2	Actin, alpha 2, smooth muscle, aorta	*10q23.31*
UMOD	Uromodulin	*16p12.3*
LGALS3	Lectin, galactoside-binding, soluble, 3	*14q22.3*
SAT1	Spermidine/spermine N1-acetyltransferase 1	*Xp22.11*
HAVCR1	Hepatitis A virus cellular receptor 1	*5q33.3*
CXCL1	Chemokine (C-X-C motif) ligand 1	*4q13.3*
ANXA2	Annexin A2	*15q22.2*
S100A6	S100 calcium binding protein A6	*1q21.3*
CYR61	Cysteine rich angiogenic inducer 61	*1p22.3*
S100B	S100 calcium binding protein B	*21q22.3*
AMBP	Alpha-1-microglobulin/bikunin precursor	*9q32*
LCN2	Lipocalin 2	*9q34.11*
C3	Complement component 3	*19p13.3*
FABP1	Fatty acid binding protein 1, liver	*2p11.2*
ATF3	Activating transcription factor 3	*1q32.3*
NTN1	Netrin 1	*17p13.1*
ENG	Endoglin	*9q34.11*
GUCY2G	Guanylate cyclase 2G	*10q25.2*
BID	BH3 interacting domain death agonist	*22q11.21*
BCL2	B-Cell CLL/lymphoma 2	*18q21.33*
BAX	BCL2 associated X protein	*19q13.33*
PTGS2	Prostaglandin-endoperoxide synthase 2	*1q31.1*
ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif 1	*21q21.3*
CDKN1A	Cyclin dependent kinase inhibitor 1A	*6p21.2*

RNA molecules in a population of cells, whereas proteomics is the systematic analysis of proteins with regard to their identity, quantity and function. Metabolomics is the study of all chemical processes involving metabolites.

Overall, the principal characteristics, challenges and limitations of the biomarkers applied in renal transplantation are as follows: (1) Sensitivity, specificity, positive and negative predictive values and receiver operating characteristics curves (ROC) of biomarkers are essential for assessing their clinical utility; (2) noninvasive candidate biomarkers principally include mRNA transcripts, lymphocyte phenotype markers, chemokines, microRNA (miRNA) and donor-specific antibodies; (3) robust validation studies and assay standardization are needed to identify new biomarkers; and (4) biomarker validations is challenging because of interindividual variations as well as interlaboratory and interplatform variability[13-15].

The main sources of biomarkers in renal transplantation are serum, urine, peripheral blood lymphocytes and tissue.

BIOMARKERS OF ISCHEMIA-REPERFUSION SYNDROME AND DGF

Ischemia reperfusion injury (IRI) is an unavoidable step occurring after kidney transplantation and may influence both short-term and long-term graft outcomes. Clinically, IRI may be associated with delayed DGF, graft rejection, CR and CAD[16]. The degree of IRI is related to several factors that may occur in the donor, during organ storage and in the recipient[17]. The increasing use of extended criteria donors and the use of organs recovered from non-heart-beating donors (NHBDs) represent an increased risk of severe IRI. Clearly, understanding the factors that potentially lead to severe IRI allow for stratifying the risk to the recipients and for a prompt diagnosis of IRI, enabling the adoption of possible therapeutic measures of prevention and treatment. Identification of biomarkers for IRI may assist in this effort.

Table 2 report a number of biomarkers candidates within the context of IRI and DGF. Such biomarkers have been studied pre or post-transplantation[18].

Pre-transplant biomarkers for IRI and DGF

A number of molecules expressing tubular or vascular damage in the donor organ are associated with the incidence and severity of IRI. In turn, the severity of IRI conditions the incidence of DGF[19,20] and graft survival is strictly related to the incidence of DGF[21].

Proteomic studies: Holmen *et al*[22] documented the predictive value of urinary neutrophil gelatinase-associated lipocalin (uNGAL) levels for prolonged DGF. This finding has been confirmed by a study of Reese *et al*[23]. A predictive value of donor uNGAL, urinary kidney injury molecule 1 (uKIM-1) and urinary fatty acid protein binding protein (u-FABP) for DGF was recently documented by a study of Koo *et al*[24].

Other studies documented the association of recipient pretransplant levels of different cytokines as the soluble interleukin 6 receptor (sIL-6R)[25] and the low soluble gp130 with post-transplant DGF.

Recently, Nguyen *et al*[26] measuring tumour necrosis factor receptor 2 (TNFR-2) expressed on circulating T reg cells documented that recipient peripheral blood T reg is a pre-transplant predictor of DGF.

Genomic studies: Several studies have investigated the pre-transplant up-regulation of genes possibly associated with IRI and DGF. One of the main limitations in identifying these molecules as a real marker of inflammation and a potential therapeutic target is the lack of causal proof.

In two different studies Schwartz *et al*[27,28] documented that the expression of tubular epithelial cell adhesion molecules was predictive of post-transplant DGF and, similarly, that the lack of up regulation of anti apoptotic genes as B cell lymphoma 2 (*Bcl-2*) and B cell lymphoma extralarge (*Bcl-xl*) in donor kidneys was associated with DGF. More recently, Kaminska *et al*[29] studying the pre-transplant intragraft expression of 29 genes, found that lipocalin-2 (*LCN)* or *NGAL* were related to DGF.

Hauser *et al*[30] and Kainz *et al*[31] studied the expression of 48 genes associated with DGF in pretransplant biopsies and found an up-regulation of genes related to complement and to metabolic and immune pathways. More recently McGuinnes *et al*[32] found that an elevated expression of cyclin-dependent kinase inhibitor 2A (CDKN2A*)* correlated with high DGF incidence.

A recent trial was conducted (ISRCTN78828338) to verify whether steroid pretreatment of the deceased organ donor was able to reduce the incidence of IRI and DGF.

Genomic analysis showed suppressed inflammation and immune response in kidney biopsies from deceased donors who received corticosteroids. Among the proteins encoded by these identified genes, steroids significantly reduced FK506-binding protein 5 (*FKBP5*), ring finger protein 186 (*RNF186*), TSC22 domain family member 3 (*TSC22D3*), Phospholambam (*PLN*), Solute carrier family 25, member 45 (*SLC25A45*), Small G protein signaling modulator 3 (*SGM3*) and Sushi domain-containing protein 3 (*SUSD3*). However, two studies related to the trial[33,34] concluded that such inflammation suppression did not reduce the incidence or duration of post-transplant DGF in allograft recipients; taken together, the studies documented that steroid pretreatment of organ donors did not improve outcomes after kidney or liver transplantation.

Post-transplant biomarkers for IRI and DGF

Proteomic and genomic studies: Liangos *et al*[35] conducted a study on patients affected by DGF and documented an association between KIM 1 levels and disease severity.

Several studies have examined the utility of determining serum or urinary levels of NGAL in predicting DGF after renal transplantation.

Experimental and clinical models have documented that urinary biomarkers such as uNGAL, uKIM-1, uIL-18 and u-FABP are specific for acute kidney injury (AKI) and/or IRI[36,37]. Several recipient urinary biomarkers are also reported to be related to graft dysfunction[38-42].

More recently, two studies documented that urinary clusterin and IL-18 allow predicting DGF within 4 h after transplantation[43]. Similarly, NGAL reflects the entity of renal impairment, representing a useful biomarker and an independent risk factor not only for DGF but also for long-term graft dysfunction[44].

A study by Hall *et al*[45,46] showed by multivariate analysis that elevated urinary levels of NGAL or IL-18 were able to predict DGF, with a ROC of 0.82.

Other studies[47,48] documented that high urinary levels of NGAL soon after transplantation are found in patients with AKI, in particular when AKI is due to AR. In a more recent meta-analysis involving 16500 critically ill patients or following cardiac surgery, elevated plasma or urinary levels of NGAL were associated with AKI but not related to rejection[49]. Finally, in a recent review[50], high urinary or serum NGAL levels were found to serve as a predictor of DGF and were associated with reduced graft function at 1 year.

To date several studies have investigated the role of miRNAs as biomarkers of DGF. miRNAs, short endogenous non-coding RNAs that inhibit gene expression, play a fundamental role in DNA and protein biosynthesis. Some studies found that miRNAs contribute to both the induction and progression of chronic kidney disease (CKD)[51]. miRNAs also represent novel therapeutic targets for CKD and for various complications after renal transplantation[52]. A role in the pathogenesis of post-transplant DGF was found for 2 miRNAs: miR-182-5p and mi-21-3p[53]. The same author found high levels of secretory leukocyte peptidase inhibitor (SLPI) in serum and urine proteome of patients affected by AKI post-transplantation as well as a novel miRNA, miR-182-5p[53].

In summary, miRNAs have a potential role as new biomarkers in all phases of kidney transplantation, even though most of the studies concerning IRI thus far have been conducted on mice[54].

Overall the use of biomarkers, though relevant, has several limitations in the field of IRI. First most studies have been conducted on mice, and their translation to humans is questionable. Second, a proof of cause is lacking, and the only study performed with regard to reducing markers of inflammation failed to report a reduction in IRI incidence and severity. Third, a gold standard for comparison, such as renal biopsy, is lacking.

BIOMARKERS FOR ACUTE REJECTION

For acute rejection also pretransplant biomarkers have been described.

Pre-transplant biomarkers for acute rejection

The most investigated pre-transplant serum biomarker has been the soluble form of CD30 (sCD30). sCD30 is a glycoprotein expressed on human $CD4^+CD8^+$ T cells that secretes Th2-type cytokines[55]. sCD30 reflects those recipients who will generate an alloimmune response against a grafted kidney. Weimer *et al*[56] documented that sCD30 was a predictor of a poor graft outcome. Other studies highlighted that more often such poor outcome was related to a higher incidence of AR[57-61].

Other studies[62,63] found that recipients with increased levels of C-X-C motif chemokine ligand 10 (CXCL10), an interferon induced chemokine associated with Th1 immune response have higher incidence transplant failure due to a higher AR incidence. Similar findings have been reported for C-X-C motif chemokine ligand 9 (CXCL9)[64].

Using systematic application of interferon-gamma (IFN-gamma) enzyme linked immunospot (ELISPOT) assay, different studies documented that the pretransplant frequency of donor specific IFN-gamma-producing cells correlates with AR among recipients of cadaveric kidney allograft[65-68].

Post-transplant biomarkers for acute rejection

Based on the studies of Naesens *et al*[5] and Sigdel *et al*[9], including genomic and proteomic studies, there are two important points concerning acute and CR, both from genomic and proteomic studies. First, genomic studies have confirmed that smoldering tissue immune activation increases over-time after transplantation and drives progressive CAN independently from AR episodes. Second, the same genomic studies reported that molecular injury in CAN and AR is similar. There is a "so-called" threshold effect for AR, and in the clinical phase of AR, the molecular injury is the same as that found in CAN, though at a higher level. These results were confirmed by urinary proteomic studies. It is therefore important to determine a sensitive and robust biomarker for differentiating AR from other forms of CAD.

Several unbiased plasma and urine proteomic studies have revealed molecules associated with AR. Cohen Freue *et al*[69] found that 7 proteins were up-regulated in the plasma of patients with acute rejection, including connectin (TTN), lipopolysaccharide-binding protein (LBP), peptidase inhibitor 16 (PI16), complement factor D (CFD), mannose-binding lectin (MBL2), recombinant SERPINA10 protein (SERPINA 10) and beta 2 microglobulin (B2M). Using urine samples, Sigdel[70] found proteins related to major histocompatibility complex (MHC) antigens and the complement cascade. Proteins such as uromodulin, serpin peptidase inhibitor, clade F member 1 (SERPINF1) and CD44 were further validated by enzyme-linked immunosorbent assay (ELISA) and Wu *et al*[71] reported 66 proteins in plasma associated with AR, including nuclear factor kappa B (NF-κB), signal transducer and activator of transcription 1 (STAT1) and STAT3. In addition, Loftheim *et al*[72] reported growth-related proteins as Insulin-like growth factor-binding protein (IGFBP7), Vasorin, epidermal growth factor (EGF) and Galactin-3 binding protein (Gal-3BP) to be up-regulated in urine during AR.

Finally, in a recent study, Sigdel *et al*[73] identified and validated by ELISA three urine proteins: Fibrinogen beta (FGB), fibrinogen gamma (FGG) and HLADRB1 during AR. Proteins related to BKVN and CAN were also identified in the same study. All these studies are listed in Table 3.

Other selected studies of biomarkers specific for AR were recently reported by Lo *et al*[7]. Granzyme B (GZMB), perforin (PRF1) and Fas Ligand (FASLG) mRNA are elevated in peripheral blood and tissue[74]. GZMB and PRF1 mRNA are also elevated in the urine of patients with AR[75]. By investigating mRNAs in urinary cells, elevated levels of gene signature of tumor necrosis factor (TNF) receptor superfamily member 4 (*TNFRSF4),* TNF ligand superfamily member 4 (*TNFSF4),* and programmed cell death protein 1 (*PDCD1*) were found in another study[76]. The multicenter CTOT 04 trial reported a urinary three- gene signature of 18S ribosomal RNA of CD3ε mRNA, interferon inducible protein 10 (CXCL10) mRNA and 18S rRNA in patients with biopsy-confirmed acute cellular rejection[77]. CTOT-01 study[78] also revealed elevated levels of urinary CXCL9 mRNA as the best predictor of AR and the authors of this study[78] concluded that low urinary CXCL9 could be used as a biomarker to identify transplant recipients at low risk for immunological events[79]. The findings of the CTOT-01 study represent important news in the field of biomarkers and immunological events in transplantation. Nonetheless, the following open questions remain: (1) whether urinary CXCL9 can be used to decrease indication rates for performing renal biopsy; (2) whether CXCL9 is an adequate tool to distinguish between rejection and injury not immunologically related; and (3) whether the absence of urinary CXCL9 might help to identify the subset of patients whose immunosuppression may be reduced without risks. In a Canadian study[80], the urinary CXCR3 chemokine receptor was shown to be the most promising candidate for detecting subclinical inflammation. This receptor decreases after successful treatment and has a predictive value for detecting subsequent CAN.

In a recent review of urine proteomics[81] , several urine biomarkers were correlated with allograft injury, including CXCL9, CXCL10, C-C motif chemokine ligand 2 (CCL2), NGAL, IL-18, cystatin C, KIM1, T-cell immunoglobulin and mucine domains-containing protein 3 (TIM3). The review also highlighted the aforementioned findings of the CTOT-01 study[78]. In a very recent study[82], four new proteins were found to be related to AR: Alpha-1-antitrypsin (A1AT), alpha 2 antiplasmin (A2AP), serum amyloid A (SAA) and apolipoprotein CIII (APOC3).

miRNAs play critical roles in the modulation of innate and adaptive immune responses. Sui *et al*[83] found 20

Table 3 Unbiased proteomic studies for acute rejection

Ref.	Biomarker candidate	Sample type	Sample numbers	Outcome
Freue *et al*[69]	TTN, LBP, CFD, MBL2, SERPINA10, AFM, KNG1, LCAT, SHBG	Plasma	32	AR
Sigdel *et al*[70]	UMOD, PEDF, CD44	Urine	60	AR
Wu *et al*[71]	NF-κB, STAT1, STAT3 and 63 other proteins	Plasma	13	AR
Loftheim *et al*[72]	IGFBP7, VASN, EGF, LG3BP	Urine	12	AR
Sigdel *et al*[73]	HLA-DRB1, FGB, FGA, KRT14, HIST1H4B, ACTB, KRT7, DPP4	Urine	154	AR

AR: Acute rejection; TTN: Titin; LBP: Lipid binding protein; MBL2: Mannose binding lectin 2; SERPINA 10: Protein Z-dependent protease inhibitor; AFM: Atomic force microscopy; KNG1: Kininogen1 protein; LCAT: Lecithin–cholesterol acyltransferase; SHBG: Sex hormon binding protein; UMOD: Uromodulin; PEDF: Pigment epithelium derived factor; NFκB: Nuclear factor kappa B; STAT1: Signal transducer and activator of transcription 1; STAT3: Signal transducer and activator of transcription 3; IGFBP7: Insulin like growth factor binding protein 7; VASN: Vasorin; EGF: Epidermal growth factor; LG3BP: Galectin-3-binding protein; FGB: Fibrinogen beta chain precursor; FGA: Fibrinogen alpha chain precursor; KRT14: Keratin14; HIST1H4B: Histone cluster 1 H4 family member b; ACTB: Actin beta; KRT7: Keratin 7; DPP4: Dipeptidil-peptidasi 4.

Table 4 Selected promising molecules and pathways evaluated as biomarkers in acute rejection[7]

Biomarker	Sample (assay method)	Patients/ samples	Rejection/no rejection	Sensitivity/ specificity (%)	PPV/NPV(%)	AUC
Granzyme B, perforin and FasL[74]	PBL (PCR)	25/31	11/20	100/95	100/95	NA
FOXP3[88]	PBL, urine (PCR)	65/78	20/58	94-100/ 95/100	94-100/ 95/100	0.95-1.00
Granzyme B, perforin[75]	Urine (PCR)	85/151	24/127	79-83/77-83	NA	NA
OX40, OX40L, PD-1 and FOXP3[76]	Urine (PCR)	46/46	21/25	95/92	NA	0.98
CD3ε,CXCL10, 18S rRNA[77]	Urine (PCR)	485/4300	43/1,70	79/78 (71/72)	NA	0.85 (0.74)
TIM-3[81]	PBL, urine (PCR)	115/160	65/95	87-100/95-100	87-100/93-100	0.96-1.00
CXCL9, CXCL10[78]	Urine (multiplex bead assay)	156/156	25/131	80-86/76-80	NA	0.83-0.87
CXCL9 mRNA and protein[79]	PBL, urine (PCR, ELISA, SELDI-TOF-MS	280/2770	37/113	66.7-85.2/ 79.6/80.7	61.5/67.6/83-92	0.78-0.85
miR-142-5p miR-155 miR-223[83]	Biopsy sample (PCR)	32/33	12/21	92-100/90-95	NA	0.96-0.99
miR-210[85]	Urine (PCR)	81/88	68/20	52/74	NA	0.7
IFNγ-producing memory T cells[89]	PBL (ELISPOT)	23/23	12/10	80/83	NA	0.8

All the studies include a validation set. PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under the curve; PBL: Peripheral blood lymphocytes; PCR: Polymerase chain reaction; NA: Not available; PD-1: Programmed death 1; CXCL10: Interferon-inducible cytokine IP-10; 18S rRNA: 18S ribosomal RNA; TIM-3: T-cell immunoglobulin and mucin-domain containing-3; CXCL9: C-X-C motif chemokine 9; ELISA: Enzyme-linked immunosorbent assay; SELDI-TOF-MS: Surface-enhanced laser desorption/ionization time-of-flight MS; miRNA: microRNA; IFNγ: Interferon gamma; ELISPOT: Enzyme-linked immunoSpot.

miRNAs in AR samples, 8 of which were up-regulated and 12 down-regulated. These findings were confirmed in another study by Anglicheau *et al*[84]. Lorenzen *et al*[85] demonstrated a specific role for urinary miR-210, decreasing during AR but normalizing after successful treatment.

Studies of miRNA in peripheral blood cells (PBCs) are also emerging. For example, Betts *et al*[86] in a small study found miR-223 and miRNA 10a to be significantly reduced during AR. In another study Grigoryev *et al*[87] found that inhibition of miR-155 and miR-221 is associated with T cell proliferation, whereas miR-142-3p is associated with tolerant kidney allograft recipients.

Other studies have documented that the level of forkhead box P3 (FOXP3) mRNA in urinary cells is higher in patients with biopsy-confirmed AR[88]. In the same study, the association between low FOXP3 mRNA and high serum creatinine predicted a poor allograft outcome.

T lymphocytes are also being studied as markers of AR. ELISPOT is the best tool for evaluating T lymphocyte phenotypes, and more reliable results are obtained by studies detecting the quantity of IFNγ-producing T cells after stimulation with donor antigens[89]. The Reprogramming the Immune System for Establishment of Tolerance (RISET) consortium has also demonstrated the value of the IFNγ assay[90]. All these studies are reported in Table 4.

Finally, donor-derived cell-free DNA (ddcfDNA) may be detected in the recipient's blood and urine[91]. Indeed, García Moreira *et al*[92] documented an increase in ddcfDNA during AR.

However, the specificity of this finding is questionable because Sigdel *et al*[93] found that ddcfDNA in urine was also present in AR and in BKVN. Additionally, urinary ddcfDNA may be present in cases of pyelonephritis[94]. Thus, the usefulness of ddcfDNA in detecting AR remains questionable.

Genomic studies for acute rejection: With the evolution of array technologies, new insight is surfacing and

Table 5 Seventeen genes involved in the study kidney solid organ response test

Symbol	Gene name	Cytoband
Genes derived from the NIH SNSO1 study		
DUSP1	Dual-specificity phosphatase 1	*5q35.1*
NAMPT	Nicotinamide phosphoribosyltransferase	*7q22.3*
PSEN1	Presenilin 1	*14q24.2*
MAPK9	Mitogen-activated protein kinase 9	*5q35.3*
NKTR	Natural killer cell triggering receptor	*3p22.1*
CFLAR	CASP8 and FADD like apoptosis regulator gene	*2q33.1*
IFNGR1	Ligand binding chain of the gamma interferon receptor gene	*6q23.3*
ITGAX	Integrin alphaXchain protein encoding gene	*16p11.2*
RNF130	Ring finger motif encoding gene	*5q35.3*
RYBP	RING1 and YY1 binding protein encoding gene	*3p13*
Genes added to improve the accuracy of kSORT		
CEACAM4	Carcinoembryonic antigen related cell adhesion molecule 4	*19q13.2*
EPOR	Erythropoietin receptor encoding gene	*19p13.2*
GZMK	Granzyme K encoding gene	*5q11.2*
RARA	Retinoic acid receptor encoding gene	*17q21.2*
RHEB	Ras homolog enriched in brain encoding gene	*7q36.1*
RXRA	Retinoic X receptor alpha encoding gene	*9q34.2*
SLC25A37	Solute carrier family 25 number 37 encoding gene	*8p21.2*

The 17 gene set was selected in 143 samples for acute rejection classification and predicted AR up to 3 mo prior to detection by the current gold standard (biopsy). kSORT: Kidney solid organ response test; SNSO1: Steroid-Free *vs* Steroid-Based Immunosuppression in Pediatric Renal (Kidney) Transplantation.

genomic studies are being applied to detect AR[95].

In the CTOT-04 study, Suthanthiran *et al*[77] found an AR diagnostic three gene signature: CD3ε, IP-10 and 185r RNAs[78].

Flechner *et al*[96] in a small study reported that several genes in peripheral blood lymphocytes (PBLs) and in kidney biopsies are able to characterize patients with AR. These genes are related to immune inflammation, transcription factors, cell growth and DNA metabolism.

The NIH SNSO1 randomized study collected human blood and graft biopsies from 367 patients from 12 United States pediatric transplant programs. The genes revealed by microarray were subsequently validated by quantitative polymerase chain reaction (qPCR). A five-gene set [dual specifity phosphatase 1 (*DUSP1*), nicotinamide phosphoribosyltransferase (*PBEF1*), presenil 1 gene (*PSEN1*), mitogen-activated protein kinase 9 gene (*MAPK9*) and natural killer cell-triggering receptor gene (*NKTR*)] was able to identify patients affected by AR with high accuracy (ROC AUC = 0.955), though the addition of five other genes known to be involved in AR did not improve the accuracy[97,98]. Kurian *et al*[99] reported 200 genes possibly related to AR, with ROC values ranging from 76% to 95%. However, the number of patients enrolled was rather small, and the findings need to be verified.

The assessment of AR in renal transplantation (the AART study) involved 436 adult/pediatric renal transplant patients from eight transplant centers in the United States, Spain and Mexico, and the kidney solid organ response test (kSORT) was used to detect renal transplant patients at high risk for AR in the AART study[100]. A 43 rejection-gene set related to AR was identified by genome microarray analysis of biopsies and blood from patients enrolled in the study[97,101].

Ten of these genes were also found in the NIH SNSO1 study[97]. Utilizing different statistical methods for improve accuracy in diagnosing AR, seven additional genes were added in the kSORT study. All these genes are shown in Table 5.

The kSORT results using a 17-gene set had very high sensitivity (AUC = 0.944), and these results were validated in several ways, such as in adult *vs* pediatric recipients, in samples collected from different sites and in samples across different ages and settings.

Overall, kSORT performance was similar among different cohorts (training set, validation set, cross-validation set (Table 6).

kSORT was also able to predict subclinical acute rejection (scAR) alone or in combination with the IFNγ ELISPOT. In the evaluation of subclinical acute rejection prediction study (ESCAPE)[102], both techniques were applied in renal transplant patients with protocol biopsies at 6 mo. The kSORT assay documented high accuracy in predicting both sub clinical antibody-mediated rejection (scABMR) and sub clinical T cell-mediated rejection (scTCMR). ELISPOT was also predictive for scTCMR but less specific in diagnosing scABMR. The predictive probabilities for diagnosing both scABMR and scTCMR were higher when combining the assays, with an AUC > 0.85.

A different approach for identifying acute rejection genes is to employ meta-analysis of eight independent datasets from four different organs (heart, kidney, liver and lung allograft), and a common rejection module (CRM) consisting of 11 genes significantly over-expressed in AR was thus identified[103]. These genes are presented in Table 7.

Table 6 Performance of kidney solid organ response test in the acute rejection in renal transplantation AART143, AART124, and AART100 cohorts

	kSORT predictions					
	AART143 (training set)		AART124 (validation set)		AART100 (cross-validation set)	
	AR	No AR	AR	No AR	AR	No AR
Real results						3
AR	39	8	21	2	36	43
No AR	9	87	1	100	3	
Sensitivity (95%CI)	82.98% (69.19%-92.35%)		91.30% (71.96%-98.38%)		92.31% (79.13%-98.38%)	
Specificity (95%CI)	90.63% (82.95%-95.62%)		99.01% (94.61%-99.97%)		93.48% (82.1%-96.63%)	
PPV (95%CI)	81.25% (68.06%-89.81%)		95.46% (78.20%-99.19%)		93.21% (79.68%-97.35%)	
NPV (95%CI)	91.58% (84.25%-95.67%)		98.04% (93.13%-99.46%)		93.48% (82.45%-97.76%)	
AUC (95%CI)	0.94 (0.91-0.98)		0.95 (0.88-1.00)		0.92 (0.86-0.98)	

kSORT: Kidney solid organ response test; AART: Assessment of acute rejection in renal transplantation; AR: Acute rejection; PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under the curve.

Table 7 Eleven genes overexpressed in the common rejection module[103]

Symbol	Gene name	Cytoband
BASP1	Brain abundant membrane attached signal protein 1	*5p15.1*
CD6	CD6 molecule	*11q12.2*
CXCL10	C-X-C Motif chemokine ligand 10	*4q21.1*
CXCL9	C-X-C Motif chemokine ligand 9	*4q21.1*
INPP5D	Inositol polyphosphate-5-phosphatase D	*2q37.1*
ISG20	Interferon stimulated exonuclease gene 20	*15q26.1*
LCK	LCK protooncogene, SRC family tyrosine kinase	*1p35.2*
NKG7	Natural killer cell granule protein 7	*19q13.41*
PSMB9	Proteasome subunit beta 9	*6p21.32*
RUNX3	Runt related transcription factor 3	*1p36.11*
TAP1	Transporter 1, ATP binding cassette subfamily B member	*6p21.32*

These genes were overexpressed in acute rejection across all transplanted organs and could diagnose acute rejection with high specificity and sensitivity.

In a study on the kidney, the 11-gene qPCR CRM score (tCRM) was found to be significantly increased in AR, with the greatest significance for CXCL9 and CXCL10[104]. Additionally, the tCRM score correlated with the extent of AR lesions and was predictive of CAD. In the already mentioned paper by Li *et al*[97], 8 genes were found by qPCR to be overexpressed in AR (*CFLAR*, P = 0.0016; *DUSP1*, P = 0.0013; *IFNGR1*, P = 0.0062; *ITGAX*, P = 0.0011; *PBEF1*, P = 0.00008; *PSEN1*, P = 0.00007; *RNF130*, P = 0.0459; and *RYBP*, P = 0012) and 2 genes were underexpressed (*MAPK9*, P = 0.0006; *NKTR*, P = 0016).

More recently[105], PCR measurement of the above gene set was evaluated in the urine of transplanted patients with acute allograft dysfunction; only 5/11 genes were highly significant at the time of rejection, and in a validation cohort, the urine common rejection module (uCRM) score for AR had an AUC of 0.961. However, in another study, the uCRM score was found to be elevated in other kidney injuries, such as acute tubular necrosis (ATN) and BKVN.

In summary, the suspicion of AR in kidney transplantation may be assessed by both proteomic and genomic biomarkers. Principal limitations appear to be the specificity of the biomarkers, as many of them are common with CAN and other forms of chronic nephropathies such as the related condition BKVN.

In the last years, genomic analyses are becoming more specific, and relevant progress has been made by kSORT applied to AART study. Unifying databases derived from studies on acute rejection of other organs such as the liver, lung and heart have allowed for realization of a common rejection module from which new genes specific for kidney rejection can be found.

BIOMARKERS FOR CAD

The term CAD has replaced the term CAN because the latter has been used too broadly, preventing identification of true CR and other aetiologies of chronic dysfunction, such as drugs and viruses, not related to immunological causes. Two main concerns are associated with the identification of non-invasive biomarkers of CAD. First several proteomic and genomic studies[7,9] have found that the molecular mechanisms responsible for acute and CR may be extremely similar and that differentiation should be principally based on the so-called "threshold effect". As a consequence, identification of biomarkers

Table 8 Analysis of pooled urine proteins collected from patients with acute rejection, BK virus nephropathy, and chronic allograft nephropathy when compared to STA urine with the criteria of > 1.5 fold change of each transplant injury phenotype (acute rejection, BK virus nephropathy, and chronic allograft nephropathy), compared to STA pooled urine and with a *P*-value of ≤ 0.05[131]

Increased in AR	Increased in BKVN	Increased in CAN
HLA-DRB1, FGB, FGA, FGG, KRT14, HIST1H4B, KRT7, DPP4	KRT18, SUMO2, STMN1, CFHR2, KRT8, KRT19, RPL18, KRT75, FAM3C, HIST1H2BA	CALR, FAM151A, SERPINA2P, FAM3C, DAG1, KITLG, LUM, FABP4, AGT, LRG1

AR: Acute rejection; BKVN: BK virus nephropathy; CAN: Chronic allograft nephropathy; FGB: Fibrinogen beta chain; FGA: Fibrinogen alpha chain; FGG: Fibrinogen gamma chain; KRT14: Keratin 14; HIST1H4B: Histone cluster 1 H4 family member b; KRT7: Keratin 7; DPP4: Dipeptidyl peptidase 4; KRT18: Keratin 18; SUMO2: Small ubiquitin-like modifier 2; STMN1: Stathmin1; CFHR2: Complement factor H related 2; KRT8: Keratin 8; KRT19: Keratin 19; RPL18: Ribosomal protein L18; KRT75: Keratin 75; FAM3C: Family with sequence similarity 3 member C; HIST1H2BA: Histone cluster 1 H2B family member a; CALR: Calreticulin; FAM151A: Family with sequence similarity 151 member A; SERPINA2P: Serpin family A member 2; FAM3C: Family with sequence similarity 3 member C; DAG1: Dystroglycan 1; KITLG: KIT ligand; LUM: Lumican; FABP4: Fatty acid binding protein 4; AGT: Angiotensinogen; LRG1: Leucine rich alpha-2-glycoprotein 1.

responsible for CAD should be performed with extreme caution and with careful dosing of the suspected molecules. Second, the causes of CAD may be quite different, and the aim of these studies should also take into account differentiation of the molecules or genes responsible for different aetiologies.

Non-invasive biomarkers of CAD are essentially based on proteomics and genomics.

Proteomic studies for CAD

In a review published in 2012, Bohra *et al*[11] discussed the main proteomic and metabolomic studies aimed at identifying biomarkers of CAD. Additionally, Johnston *et al*[106] reported β2 microglobulin as a urinary biomarker for CAD. In a large study by Kurian *et al*[107], 302 proteins in peripheral blood were identified as responsible for mild CAD and 509 for severe CAD, and Quintana *et al*[108] found uromodulin and kininogen in urine to be useful biomarkers for CAD. Based on a two-dimensional differential gel electrophoresis of urine, Bañon Maneus *et al*[109] found 21 proteins associated with CAD, including A1AT, α-1 β glycoprotein (A1BG), angiotensinogen (AGT), anti-TNF alpha antibody light chain, β2 microglobulin (B2M), brevin, heparan sulfate proteoglycan (HSPG), leucine-rich α 2-glycoprotein 1 (LRG1) and transferrin.

In a more recent study, Nakorchevsky *et al*[110] in a large-scale proteogenomic analysis of tissue biopsies found more than 1000 proteins associated with mild to-severe CAD.

Jahnukainen *et al*[111] in a proteomic analysis of urine in kidney transplant patients with BKVN applied surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) analysis to distinguish protein profile characteristics of BKVN but were unable to identify different proteins. More recently, Sigdel *et al*[73] found BKVN selective proteins to be associated with contractile fibers, with gene expression regulation, with glycolysis and with response to viruses. In this study the top 10 most significant urine proteins for AR, BKVN and CAN are shown (Table 8).

Recent studies on calcineurin inhibitor toxicity documented altered expression of 38 proteins *in vitro* after incubation with cyclosporine (CyA)[112], and in a clinical setting, urine N-acetylβ-D-glucosaminidase (NAG) was found to be specific for CyA-related toxicity[113].

The discovery and use of mRNAs has shed new light on CAD and on the unique form of CAD called interstitial fibrosis/tubular atrophy (IF/TA).

One recent study reported the miRNA characteristics of patients affected by IF/TA[114], in particular five miRNAs (miR142-3p, miR-32, miR204, miR-107 and miR-211) were differentially expressed in tissue biopsy samples. These miRNAs were further confirmed in the urine of patients affected by CAD. In a follow-up study by the same group[115], a selected panel of miRNAs, miR99a, miR-140-3p, mi 200b and miR-200, monitored at different time points after transplantation were found to be differentially expressed in urine according to graft outcome and useful markers in graft monitoring. In a recent study, Zununi Vahed *et al*[116] observed that urinary miRNAs exibit different behaviors in patients affected by IF/TA according to whether they received a living or cadaveric donor kidney.

In another recent study on renal biopsies of patients affected by IF/TA, miR-142-5p and miR-142-3p were significantly up-regulated, whereas miR-211 was significantly down-regulated[117]. As the same results were observed in PBCs from the same patients, the authors suggested that PBCs might be used in a non-invasive approach for monitoring kidney graft function.

Finally, evaluating miRNA profiles in transplanted patients, Iwasaki *et al*[118] found that miR-486-5p was significantly over-expressed in these patients who produced donor-specific antibodies (DSA) and exhibited biopsy-proven chronic antibody-mediated rejection (CAMR).

Genomic studies for CAD

Mas *et al*[119] used microarrays to evaluate renal tissue from patients affected by CAD with IF/TA and found up-regulation of genes related to fibrosis, extracellular matrix deposition and the immune response, as provided in Table 9. Markers of genes such as transforming growth factor beta (TGF-β), epidermal growth factor receptor (EGFR), and AGT were similarly found to be elevated in

Table 9 Genes higher (fold change higher than 6.00) expressed in renal tissue of patients affected by interstitial fibrosis/tubular atrophy[119]

Symbol	Gene name	Cytoband
IGHA1	Immunoglobulin heavy constant alpha 1	*14q32.33*
IGHG1	Immunoglobulin heavy constant gamma 1	*14q32.33*
CCR2	Chemokine C-C motif receptor 2	*3p21.31*
DFFB	DNA fragmentation factor 40 Da beta subunit	*1p36.32*
CD44	CD44 antigen	*11p13*
IFNA1	Interferon alpha 1	*9p21.3*
GZMK	Granzyme K	*5q11.2*
MMP9	Matrix metallopeptidase 9	*20q13.12*
TNFRSF17	Tumor necrosis factor receptor superfamily, member 17	*16p13.13*
CXCR4	Chemokine C-X-C motif receptor 4	*2q22.1*

urine samples.

In the multicenter CTOT-04 trial, in addition to validating the three-gene signature of CD3ε mRNA, CXCL10-mRNA and 18S rRNA, which is predictive of acute rejection, Lee *et al*[120], examined urinary mRNA by PCR and reported a 4-gene signature of mRNAs for vimentin, NKCC2, E-cadherin and 18S rRNA that was diagnostic of IF/TA.

The above-mentioned tCRM[104] is a computational gene expression score for predicting immune injury in renal allograft. A subset of 7 genes [CD6 molecule (*CD6*), inositol polyphosphate-5-phosphatase D (*INPP5D*), interferon-stimulated exonuclease hene 20 (*ISG20*), natural killer cell granule protein 7 (*NKG7*), proteasome subunit beta 9 (*PSMB9*), runt-related transcription factor 3 (*RUNX3*) and transporter 1, ATP-binding cassette subfamily B member (*TAP1*)] had higher predictive value for patients developing IF/TA over time.

A relevant international study of Genomics of Chronic Allograft Rejection (GoCAR) (Clinical Trials.gov NCT 00611702)[121] aimed to identify genes that correlate with chronic allograft dysfunction index (CADI) scores at 12 mo in patients with a normal biopsy at three months.

A set of 13 genes showed independent predictive value for the development of fibrosis (Table 10). This gene set also has a predictive value higher than that of clinical and pathological variables.

A new approach of the Mount Sinai group[122] is to utilize genomics to identify therapeutic agents for IF/TA. Based on an 85-gene signature from IF/TA molecular datasets in Gene Expression Omnibus and using a computational repurposing analysis, two new drugs, in addition to well-known azathioprine already used for AR and pulmonary fibrosis, appear to be promising: Kamferol, which attenuates TGF-β1, and Esculetin, which inhibits the Wnt/β catenin pathway. Both drugs were effective and safe in preclinical models.

BIOMARKERS TO PREDICT AND MONITOR TOLERANCE

No more than 100 cases of clinical operational tolerance (COT) have been reported in renal transplantation[123].

A number of consortia have been realized in an attempt to find valid tolerance signatures. The more important consortia are reported in Table 11[124,125].

Thirty-nine genes have been found to be up-regulated in COTs in different sites, in different patient cohorts and using different microarrays; 24 of these genes (69%) are B cell related, with CD79b and prepronociceptin (PNOC) being the more highly expressed[126-128]. Additionally, Danger *et al*[129] documented up-regulation of miR-142-3p in B cells of COT patients.

T reg cells ($CD4^+$, $CD25^+$, Fox $P3^+$) have been extensively studied in operational tolerance, though their role in COT remains unclear[128,130]. A role for natural killer (NK) cells in COTs has also been postulated[128].

In another relevant study, Roedder *et al*[131] highlighted that tolerance biomarkers are dependent on the age of the recipient and may differ according the organ transplanted and that there is a need for further validation studies. The same authors identified different biomarkers according to age and the organ transplanted.

Genomic studies for tolerance

A study on gene expression in peripheral B cells showed an up-regulation of membrane-spanning 4-domains A1 (*MS4A1*) (*CD20*), T-cell leukemia/lymphoma 1A (*TCL1A*), CD79b molecule, immunoglobulin-associated beta (*CD79B*), tolerance-associated gene 1 (*TOAG1*) and Forkhead Box P3 (*FOXP3*) genes. *TOAG1* was also up-regulated intragrafts[132].

In a recent study, a group from Northwestern University in Chicago found an important role for Treg cells. Indeed, in their study on COTs patients *vs* non-tolerant patients, the number of circulating Treg cells was significantly time-dependently higher in tolerant patients[133]. Additionally, in the same study, a role for a different 357 gene signatures of tolerance was found (Table 12).

A principal approach for identifying genes actually involved in COTs derives from comparison of tolerant patients *vs* those with immunosuppression; immunosuppressive treatment in the latter group might influence and generate bias in the gene expression signature. To overcome the problem, a multicenter study[134] reviewed a cohort of 246 kidney transplant recipients (232 with

Table 10 Thirteen genes associated with chronic allograft dysfunction identified by biopsy transcriptome expression[121]

Symbol	Gene description	Cytoband	CADI 12 mo correlation	*P* value
CHCHD10	Coiled-coil-helix-coiled- coil helix domain containing 10	*22q11.23*	0.404	2.85×10^{-5}
KLHL13	Kelch-like family member 13	*Xq23-q24*	0.369	1.49×10^{-4}
FJX1	Four jointed box 1	*11p13*	0.367	1.60×10^{-4}
MET	Met proto-oncogene	*7q31*	0.352	3.01×10^{-4}
SERINC5	Serine incorporator 5	*5q14.1*	0.318	0.0012
RNF149	Ring finger protein 149	*2q11.2*	0.28	0.0046
SPRY4	Sprouty homolog 4	*5q31.3*	0.27	0.0062
TGIF1	TGF-β induced factor homeobox 1	*18p11.3*	0.244	0.0140
KAAG1	Kidney associated antigen 1	*6p22.1*	0.24	0.0154
ST5	Suppressor of tumorigenicity 5	*11p15*	0.232	0.0197
WNT9A	Wingless-type MMTV integration site family member 9A	*1q42*	0.212	0.0332
ASB15	Ankirin repeat and SOCS box-containing 15	*7q31.31*	-263	0.0079
RXRA	Retinoid X receptor alpha	*9q34.3*	-0.3	0.0023

CADI: Chronic allograft dysfunction index.

Table 11 International research consortia in rejection/tolerance

Acronym	Description	Year
ITN	Immune tolerance network	Since 2002
IOC	Indices of tolerance	2003-2007
RISET	Reprogramming the immune system for establishment of tolerance	2005-2010
GAMBIT Study	Genetic analysis and monitoring of biomarkers of immunological tolerance	2010
The One Study	A unified approach to evaluating cellular immunotherapy in solid organ transplantation	2011
Bio-DRIM	Personalized minimization or immunosuppression after solid organ transplantation by biomarker driven stratification of patients to improve the long-term outcome and health-economic data of transplantation	2012
BIOMARGIN	Biomarkers of renal graft injuries in kidney allograft recipients	2013

GAMBIT: Genetic Analysis and Monitoring of Biomarkers of Immunological Tolerance.

immunosuppression, 14 tolerant) using the Genetic Analysis and Monitoring of Biomarkers of Immunological Tolerance method, and the investigators were able to identify a nine gene immunosuppression-independent gene signature (Table 13).

Recently, 21 genes involved in tolerance were identified at the University of California San Francisco (UCSF), in the program kidney spontaneous operational tolerance test (kSPOT). These investigators studied 348 HLA-mismatched renal transplant patients and identified 21 genes involved in COT. These 21 TOL genes were validated, and independent qPCR for the 21 genes was preformed. Additionally, the authors were able to refine and validate a three-gene assay [Kruppel-Like Factor 6 (*KLF6*), Basonuclin 2 (*BNC2*), and Cytochrome P450 Family 1 Subfamily B Member 1 (*CYP1B1*)] to detect the state of operational tolerance, with an AUC 0.95[135]. Interestingly, *BNC2* and *CYP1B1* are both related to tolerance in kidney and liver transplantation[136,137].

In conclusion, a number of studies have searched for a "tolerance signature". However, such an endeavour is difficult because of the small number of COT patients. The search for biomarkers is principally useful for identifying tolerant patients. Among the different studies, that of Newell *et al*[127], which was aimed at finding a gene expression profile for tolerant patients, and the microarray analysis of Sagoo *et al*[128] stand out in this field.

In addition, the reclassification of transplant patients according to immune risk threshold may be achieved using the cited kSORT, tCRM, uCRM and kSPOT. This might help in determining which recipients would benefit from withdrawal or minimization of immunosuppression.

FUTURE PERSPECTIVES

Several prospective research programs and clinical trials are ongoing using already-known biomarkers or are searching for new ones.

Biomarker-driven personalized immunosuppression (BIO-DrIM) is a European Consortium aimed at the Methodical and Clinical Validation of Biomarkers for guiding immunosuppression[138]. The programs of the Consortium include: (1) The targeting and partial weaning of immunosuppression in long-term liver and kidney transplant patients; and (2) biomarker analysis and data management.

The biomarker platforms of BIODrIM are as follows: (1) An ELISPOT platform for detecting donor-reactive memory/effector T cells[139]; (2) a real-time RT-PCR platform to identify molecular tolerance signatures[140]; and (3) a multiparameter flowcytometry platform to characterize circulating immune cell subsets[141].

The BIODrIM consortium is designing two clinical trials in solid organ transplantation using biomarkers for decision making.

Table 12 Immune/inflammatory molecules among the 357 gene signatures of tolerance

Categories	Diseases or functions annotation	Molecules	No. of molecules
Cell-to-cell signaling and interaction, cellular function and maintenance, hematological system development and function, inflammatory response	Phagocytosis of leukocyte cell lines	FGR, MRC1, TLR4	3
Cell-to-cell signaling and interaction, hematological system development and function, immune cell trafficking, inflammatory response, tissue development	Binding of neutrophils	FGR, LSP1, TLR4	3
Antimicrobial response, inflammatory response	Antibacterial response	CARD9, FGR, LYST, NLRC4, TLR4	5
Cell-to-cell signaling and interaction, hematological system development and function, inflammatory response	Binding of professional phagocytic cells	FGR, LSP1, NOTCH2, TLR4	4
Inflammatory response	Immune response of cells	CARD9, CLEC7A, ETS2, FGR, MRC1, SCARF1, MYO7A, TLR4	8
Antimicrobial response, inflammatory response	Antimicrobial response	CARD9, CLEC7A, FGR, LYST, NLRC4, TLR4	6
Inflammatory response	Innate immune response	CARD9, CLEC7A, TLR4, TRIM59	4
Cellular function and maintenance, inflammatory response	Phagocytosis	CLEC7A, ETS2, FGR, MRC1, MYO7A, TLR4, TPCN2	7
Cell-to-cell signaling and interaction, cellular growth and proliferation, hematological system development and function, inflammatory response	Stimulation of phagocytes	IL4R, TLR4	2
Antimicrobial response, humoral immune response, inflammatory response	Antifungal response	CARD9, CLEC7A	2
Cell-to-cell signaling and interaction, cellular function and maintenance, inflammatory response	Phagocytosis of cells	CLEC7A, ETS2, FGR, MRC1,MYO7A, TLR4	6

These genes potentially predict those patients that can be successfully weaned off immunosuppression[133]. FGR: Tyrosine-protein kinase Fgr; MRC1: Mannose receptor, C type 1; TLR4: Toll-like receptor 4; FGR: Tyrosine-protein kinase Fgr; LSP1: Lymphocyte-specific protein 1; CARD9: Caspase recruitment domain family member 9; LYST: Lysosomal-trafficking regulator; NLRC4: NLR family CARD domain-containing protein 4; NOTCH2: Neurogenic locus notch homolog protein 2; CLEC7A: C-type lectin domain family 7 member A; ETS2: Protein C-ets-2; SCARF1: Scavenger receptor class F member 1; MYO7A: Unconventional myosin-VIIa; TRIM59: Tripartite motif-containing protein 59; TPCN2: Two pore calcium channel protein 2; IL4R: Interleukin 4 receptor.

Table 13 Immunosuppression-independent gene signatures predicting tolerance[134]

Symbol	Gene name	Molecular function	Biological processes
ATXN3 ↓	Ataxin 3	Ubiquitin-specific protease activity	Protein metabolism
BCLA1 ↓	BCL2-related protein A1	Receptor signaling complex scaffold activity	Apoptosis
EEF1A1 ↓	Eukaryotic translation elongation factor 1 alpha 1	Transcription regulator activity	Regulation of cell cycle
GEMIN7 ↑	Gem associated protein 9	Ribonucleoprotein	Regulation of nucleobase, nucleosides, nucleotide and nucleic acid metabolism
IGLC1 ↑	Immunoglobulin lambda constant 1	Antigen binding	Immune response
MS4A4A ↑	Membrane-spanning 4-domains, subfamily A, member 4A	- - -	- - -
NFκBIA ↑	Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, alpha	Transcription regulator activity	Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism
RAB40C ↑	RAB40C, member of RAS oncogene family	GTPase activity	Cell communication, signal transduction
TNFAIP3 ↓	Tumor necrosis factor, alpha-induced protein 3	Transcription regulator activity	Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism

↓Immunosuppression-free gene expression downregulated in tolerant patients; ↑Immunosuppression-free gene expression upregulated in tolerant patients; BCL2: B-cell lymphoma 2.

The trial LIST[138] will apply molecular signatures to guide immunosuppression in liver transplant patients.

The kidney transplant trial design of BIODrIM is Cellimin, a prospective multicenter randomized trial utilizing IFNγ ELISPOT to stratify kidney transplant recipients into high/low responders. Only low-responder patients will be randomized to receive either standard immunosuppression or low-dose immunosuppression. The trial will evaluate the donor specific cellular alloresponse for immunosuppression minimization (EudraCT-Number: 2013-005041-37)[142].

Another European research program is "Biomarkers of Renal Graft Injuries in kidney allograft recipients" (BIOMARGIN)[143], which has the aims to: (1) select and validate blood or urine biomarkers at different-omics levels related to allograft lesions; and (2) select

and validate biomarkers as early predictors of CAD. The research will allow for selecting the best candidate biomarkers and biomarker signatures. In addition, the work will evaluate the sensitivity, selectivity, false positive value and false negative value of biomarkers. Finally, one goal of the study is to select biomarker signature predictors of three-year graft outcomes.

By using the aforementioned biomarkers of kSORT, the TITRATE trial has the aim of testing immunosuppression Threshold in Renal Allografts to improve the estimated glomerular filtration rate (eGFR). Overall, the main outcomes of the trial are the rate and severity of acute rejection and the CADI score at one year based on protocol biopsy. Evaluation of eGFR is also a principal endpoint. The study is ongoing in Mexico and at UCSF[144].

Another Clinical Trial, NIH UO1 trial TASK, employs the biomarkers of kSORT, uCRM, and tCRM. The TASK trial has the aim of evaluating Treg adoptive therapy for subclinical inflammation in kidney transplantation by comparing the results of three patients' cohorts according to surrogate markers of the immune response[145].

The Precision Medicine Offers Belatacept Monotherapy study[146] is being conducted at four centers in the United States, Spain, France and Mexico. The trial has the aim of determining the safety and feasibility of converting kidney transplant recipients to Belatacept monotherapy. In addition, the trial has the goal of evaluating the percentage of patients who can be converted to a Belatacept regimen of once every 8 wk. The patients enrolled in the trial will have a quiescent immunologic profile evaluated by kSORT, uCRM and tCRM. Only those with elevated kSPOT will be tested for the once every 8-wk administration.

The epithelial-to-mesenchymal transition (EMT) is a process in which fibrosis is generated due to the transformation from the epithelial to mesenchymal phenotype. The process is induced and facilitated by several molecular signatures, among which TGF beta, EGF, insulin like growth factor 2 and fibroblast growth factor 2 (FGF2) are prominent[147]. An interesting ongoing trial is Prediction of Chronic Allograft Nephropathy (Prefigur)[148]. By using non-invasive biomarkers and evaluating urinary cells in the first year post-transplantation, the investigators are developing a non-invasive approach for predicting fibrosis as a substitute of allograft biopsy, *via* longitudinal assessment of the mRNA expression level of genes implicated in EMT fibrogenesis.

REFERENCES

1 **Hariharan S**, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med* 2000; **342**: 605-612 [PMID: 10699159 DOI: 10.1056/NEJM200003023420901]

2 **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]

3 **Naesens M**, Salvatierra O, Benfield M, Ettenger RB, Dharnidharka V, Harmon W, Mathias R, Sarwal MM; SNS01-NIH-CCTPT Multicenter Trial. 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]

4 **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]

5 **Naesens M**, Khatri P, Li L, Sigdel TK, Vitalone MJ, Chen R, Butte AJ, Salvatierra O, Sarwal MM. Progressive histological damage in renal allografts is associated with expression of innate and adaptive immunity genes. *Kidney Int* 2011; **80**: 1364-1376 [PMID: 21881554 DOI: 10.1038/ki.2011.245]

6 **Mas VR**, Mueller TF, Archer KJ, Maluf DG. Identifying biomarkers as diagnostic tools in kidney transplantation. *Expert Rev Mol Diagn* 2011; **11**: 183-196 [PMID: 21405969 DOI: 10.1586/erm.10.119]

7 **Lo DJ**, Kaplan B, Kirk AD. Biomarkers for kidney transplant rejection. *Nat Rev Nephrol* 2014; **10**: 215-225 [PMID: 24445740 DOI: 10.1038/nrneph.2013.281]

8 **Fehr T**, Cohen CD. Predicting an allograft's fate. *Kidney Int* 2011; **80**: 1254-1255 [PMID: 22126981 DOI: 10.1038/ki.2011.328]

9 **Sigdel TK**, Gao Y, He J, Wang A, Nicora CD, Fillmore TL, Shi T, Webb-Robertson BJ, Smith RD, Qian WJ, Salvatierra O, Camp DG, Sarwal MM. Mining the human urine proteome for monitoring renal transplant injury. *Kidney Int* 2016; **89**: 1244-1252 [PMID: 27165815 DOI: 10.1016/j.kint.2015.12.049]

10 **Biomarkers Definitions Working Group**. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001; **69**: 89-95 [PMID: 11240971 DOI: 10.1067/mcp.2001.113989]

11 **Bohra R**, Klepacki J, Klawitter J, Klawitter J, Thurman JM, Christians U. Proteomics and metabolomics in renal transplantation-quo vadis? *Transpl Int* 2013; **26**: 225-241 [PMID: 23350848 DOI: 10.1111/tri.12003]

12 **Bontha SV**, Maluf DG, Mueller TF, Mas VR. Systems Biology in Kidney Transplantation: The Application of Multi-Omics to a Complex Model. *Am J Transplant* 2017; **17**: 11-21 [PMID: 27214826 DOI: 10.1111/ajt.13881]

13 **Hockley SL**, Mathijs K, Staal YC, Brewer D, Giddings I, van Delft JH, Phillips DH. Interlaboratory and interplatform comparison of microarray gene expression analysis of HepG2 cells exposed to benzo(a)pyrene. *OMICS* 2009; **13**: 115-125 [PMID: 19245359 DOI: 10.1089/omi.2008.0060]

14 **Sato F**, Tsuchiya S, Terasawa K, Tsujimoto G. Intra-platform repeatability and inter-platform comparability of microRNA microarray technology. *PLoS One* 2009; **4**: e5540 [PMID: 19436744 DOI: 10.1371/journal.pone.0005540]

15 **Mao S**, Wang C, Dong G. Evaluation of inter-laboratory and cross-platform concordance of DNA microarrays through discriminating genes and classifier transferability. *J Bioinform Comput Biol* 2009; **7**: 157-173 [PMID: 19226665]

16 **Salvadori M**, Rosso G, Bertoni E. Update on ischemia-reperfusion injury in kidney transplantation: Pathogenesis and treatment. *World J Transplant* 2015; **5**: 52-67 [PMID: 26131407 DOI: 10.5500/wjt.v5.i2.52]

17 **Cheung KP**, Kasimsetty SG, McKay DB. Innate immunity in donor procurement. *Curr Opin Organ Transplant* 2013; **18**: 154-160 [PMID: 23313940 DOI: 10.1097/MOT.0b013e32835e2b0d]

18 **Mühlberger I**, Perco P, Fechete R, Mayer B, Oberbauer R. Biomarkers in renal transplantation ischemia reperfusion injury. *Transplantation* 2009; **88**: S14-S19 [PMID: 19667956 DOI: 10.1097/TP.0b013e3181af65b5]

19 **Mueller TF**, Solez K, Mas V. Assessment of kidney organ quality and prediction of outcome at time of transplantation. *Semin Immunopathol* 2011; **33**: 185-199 [PMID: 21274534 DOI: 10.1007/s00281-011-0248-x]

20 **UNOS/SRTR**. Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant

Recipients: Transplant Data 1997-2006. Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation, Rockville, MD 2007

21 **Ojo AO**, Wolfe RA, Held PJ, Port FK, Schmouder RL. Delayed graft function: risk factors and implications for renal allograft survival. *Transplantation* 1997; **63**: 968-974 [PMID: 9112349]

22 **Hollmen ME**, Kyllönen LE, Inkinen KA, Lalla ML, Merenmies J, Salmela KT. Deceased donor neutrophil gelatinase-associated lipocalin and delayed graft function after kidney transplantation: a prospective study. *Crit Care* 2011; **15**: R121 [PMID: 21545740 DOI: 10.1186/cc10220]

23 **Reese PP**, Hall IE, Weng FL, Schröppel B, Doshi MD, Hasz RD, Thiessen-Philbrook H, Ficek J, Rao V, Murray P, Lin H, Parikh CR. Associations between Deceased-Donor Urine Injury Biomarkers and Kidney Transplant Outcomes. *J Am Soc Nephrol* 2016; **27**: 1534-1543 [PMID: 26374609 DOI: 10.1681/ASN.2015040345]

24 **Koo TY**, Jeong JC, Lee Y, Ko KP, Lee KB, Lee S, Park SJ, Park JB, Han M, Lim HJ, Ahn C, Yang J. Pre-transplant Evaluation of Donor Urinary Biomarkers can Predict Reduced Graft Function After Deceased Donor Kidney Transplantation. *Medicine* (Baltimore) 2016; **95**: e3076 [PMID: 26986138 DOI: 10.1097/MD.0000000000003076]

25 **Sadeghi M**, Daniel V, Naujokat C, Mehrabi A, Opelz G. Association of high pretransplant sIL-6R plasma levels with acute tubular necrosis in kidney graft recipients. *Transplantation* 2006; **81**: 1716-1724 [PMID: 16794539 DOI: 10.1097/01.tp.0000226076.04938.98]

26 **Nguyen MT**, Fryml E, Sahakian SK, Liu S, Cantarovich M, Lipman M, Tchervenkov JI, Paraskevas S. Pretransplant Recipient Circulating CD4+CD127lo/- Tumor Necrosis Factor Receptor 2+ Regulatory T Cells: A Surrogate of Regulatory T Cell-Suppressive Function and Predictor of Delayed and Slow Graft Function After Kidney Transplantation. *Transplantation* 2016; **100**: 314-324 [PMID: 26425877 DOI: 10.1097/TP.0000000000000942]

27 **Schwarz C**, Regele H, Steininger R, Hansmann C, Mayer G, Oberbauer R. The contribution of adhesion molecule expression in donor kidney biopsies to early allograft dysfunction. *Transplantation* 2001; **71**: 1666-1670 [PMID: 11435980]

28 **Schwarz C**, Hauser P, Steininger R, Regele H, Heinze G, Mayer G, Oberbauer R. Failure of BCL-2 up-regulation in proximal tubular epithelial cells of donor kidney biopsy specimens is associated with apoptosis and delayed graft function. *Lab Invest* 2002; **82**: 941-948 [PMID: 12118096]

29 **Kamińska D**, Kościelska-Kasprzak K, Drulis-Fajdasz D, Hałoń A, Polak W, Chudoba P, Jańczak D, Mazanowska O, Patrzałek D, Klinger M. Kidney ischemic injury genes expressed after donor brain death are predictive for the outcome of kidney transplantation. *Transplant Proc* 2011; **43**: 2891-2894 [PMID: 21996181 DOI: 10.1016/j.transproceed.2011.08.062]

30 **Hauser P**, Schwarz C, Mitterbauer C, Regele HM, Mühlbacher F, Mayer G, Perco P, Mayer B, Meyer TW, Oberbauer R. Genome-wide gene-expression patterns of donor kidney biopsies distinguish primary allograft function. *Lab Invest* 2004; **84**: 353-361 [PMID: 14704720 DOI: 10.1038/labinvest.3700037]

31 **Kainz A**, Mitterbauer C, Hauser P, Schwarz C, Regele HM, Berlakovich G, Mayer G, Perco P, Mayer B, Meyer TW, Oberbauer R. Alterations in gene expression in cadaveric vs. live donor kidneys suggest impaired tubular counterbalance of oxidative stress at implantation. *Am J Transplant* 2004; **4**: 1595-1604 [PMID: 15367214 DOI: 10.1111/j.1600-6143.2004.00554.x]

32 **McGuinness D**, Leierer J, Shapter O, Mohammed S, Gingell-Littlejohn M, Kingsmore DB, Little AM, Kerschbaum J, Schneeberger S, Maglione M, Nadalin S, Wagner S, Königsrainer A, Aitken E, Whalen H, Clancy M, McConnachie A, Koppelstaetter C, Stevenson KS, Shiels PG. Identification of Molecular Markers of Delayed Graft Function Based on the Regulation of Biological Ageing. *PLoS One* 2016; **11**: e0146378 [PMID: 26734715 DOI: 10.1371/journal.pone.0146378]

33 **Kainz A**, Wilflingseder J, Mitterbauer C, Haller M, Burghuber C, Perco P, Langer RM, Heinze G, Oberbauer R. Steroid pretreatment of organ donors to prevent postischemic renal allograft failure: a randomized, controlled trial. *Ann Intern Med* 2010; **153**: 222-230 [PMID: 20713790 DOI: 10.7326/0003-4819-153-4-201008170-00003]

34 **Amatschek S**, Wilflingseder J, Pones M, Kainz A, Bodingbauer M, Mühlbacher F, Langer RM, Gerlei Z, Oberbauer R. The effect of steroid pretreatment of deceased organ donors on liver allograft function: a blinded randomized placebo-controlled trial. *J Hepatol* 2012; **56**: 1305-1309 [PMID: 22326464 DOI: 10.1016/j.jhep.2012.01.020]

35 **Liangos O**, Perianayagam MC, Vaidya VS, Han WK, Wald R, Tighiouart H, MacKinnon RW, Li L, Balakrishnan VS, Pereira BJ, Bonventre JV, Jaber BL. Urinary N-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. *J Am Soc Nephrol* 2007; **18**: 904-912 [PMID: 17267747 DOI: 10.1681/ASN.2006030221]

36 **Haase M**, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A, Haase-Fielitz A; NGAL Meta-analysis Investigator Group. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis* 2009; **54**: 1012-1024 [PMID: 19850388 DOI: 10.1053/j.ajkd.2009.07.020]

37 **Siew ED**, Ware LB, Ikizler TA. Biological markers of acute kidney injury. *J Am Soc Nephrol* 2011; **22**: 810-820 [PMID: 21493774 DOI: 10.1681/ASN.2010080796]

38 **Fonseca I**, Oliveira JC, Almeida M, Cruz M, Malho A, Martins LS, Dias L, Pedroso S, Santos J, Lobato L, Castro Henriques A, Mendonça D. Neutrophil gelatinase-associated lipocalin in kidney transplantation is an early marker of graft dysfunction and is associated with one-year renal function. *J Transplant* 2013; **2013**: 650123 [PMID: 24288591 DOI: 10.1155/2013/650123]

39 **Mishra J**, Ma Q, Kelly C, Mitsnefes M, Mori K, Barasch J, Devarajan P. Kidney NGAL is a novel early marker of acute injury following transplantation. *Pediatr Nephrol* 2006; **21**: 856-863 [PMID: 16528543 DOI: 10.1007/s00467-006-0055-0]

40 **Sureshkumar KK**, Marcus RJ. Urinary biomarkers as predictors of long-term allograft function after renal transplantation. *Transplantation* 2010; **90**: 688-689 [PMID: 20847632 DOI: 10.1097/TP.0b013e3181ebc0d6]

41 **Pajek J**, Škoberne A, Šosterič K, Adlešič B, Leskošek B, Bučar Pajek M, Osredkar J, Lindič J. Non-inferiority of creatinine excretion rate to urinary L-FABP and NGAL as predictors of early renal allograft function. *BMC Nephrol* 2014; **15**: 117 [PMID: 25027586 DOI: 10.1186/1471-2369-15-117]

42 **Malyszko J**, Koc-Zorawska E, Malyszko JS, Mysliwiec M. Kidney injury molecule-1 correlates with kidney function in renal allograft recipients. *Transplant Proc* 2010; **42**: 3957-3959 [PMID: 21168598 DOI: 10.1016/j.transproceed.2010.10.005]

43 **Pianta TJ**, Peake PW, Pickering JW, Kelleher M, Buckley NA, Endre ZH. Clusterin in kidney transplantation: novel biomarkers versus serum creatinine for early prediction of delayed graft function. *Transplantation* 2015; **99**: 171-179 [PMID: 25083615 DOI: 10.1097/TP.0000000000000256]

44 **Lacquaniti A**, Caccamo C, Salis P, Chirico V, Buemi A, Cernaro V, Noto A, Pettinato G, Santoro D, Bertani T, Buemi M, David A. Delayed graft function and chronic allograft nephropathy: diagnostic and prognostic role of neutrophil gelatinase-associated lipocalin. *Biomarkers* 2016; **21**: 371-378 [PMID: 26900638 DOI: 10.3109/1354750X.2016.1141991]

45 **Hall IE**, Yarlagadda SG, Coca SG, Wang Z, Doshi M, Devarajan P, Han WK, Marcus RJ, Parikh CR. IL-18 and urinary NGAL predict dialysis and graft recovery after kidney transplantation. *J Am Soc Nephrol* 2010; **21**: 189-197 [PMID: 19762491 DOI: 10.1681/ASN.2009030264]

46 **Hall IE**, Doshi MD, Poggio ED, Parikh CR. A comparison of alternative serum biomarkers with creatinine for predicting allograft function after kidney transplantation. *Transplantation* 2011; **91**: 48-56 [PMID: 21441853 DOI: 10.1097/TP.0b013e3181fc4b3a]

47 **Rostami Z**, Nikpoor M, Einollahi B. Urinary Neutrophil Gelatinase Associated Lipocalin (NGAL) for Early Diagnosis of Acute Kidney Injury in Renal Transplant Recipients. *Nephrourol Mon* 2013; **5**: 745-752 [PMID: 23841038 DOI: 10.5812/numonthly.9385]

48 **Heyne N**, Kemmner S, Schneider C, Nadalin S, Königsrainer A,

Häring HU. Urinary neutrophil gelatinase-associated lipocalin accurately detects acute allograft rejection among other causes of acute kidney injury in renal allograft recipients. *Transplantation* 2012; **93**: 1252-1257 [PMID: 22513480 DOI: 10.1097/TP.0b013e31824fd892]

49 **Haase-Fielitz A**, Haase M, Devarajan P. Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury: a critical evaluation of current status. *Ann Clin Biochem* 2014; **51**: 335-351 [PMID: 24518531 DOI: 10.1177/0004563214521795]

50 **Ramirez-Sandoval JC**, Herrington W, Morales-Buenrostro LE. Neutrophil gelatinase-associated lipocalin in kidney transplantation: A review. *Transplant Rev* (Orlando) 2015; **29**: 139-144 [PMID: 26071983 DOI: 10.1016/j.trre.2015.04.004]

51 **Wilflingseder J**, Reindl-Schwaighofer R, Sunzenauer J, Kainz A, Heinzel A, Mayer B, Oberbauer R. MicroRNAs in kidney transplantation. *Nephrol Dial Transplant* 2015; **30**: 910-917 [PMID: 25170095 DOI: 10.1093/ndt/gfu280]

52 **Trionfini P**, Benigni A, Remuzzi G. MicroRNAs in kidney physiology and disease. *Nat Rev Nephrol* 2015; **11**: 23-33 [PMID: 25385286 DOI: 10.1038/nrneph.2014.202]

53 **Wilflingseder J**, Sunzenauer J, Toronyi E, Heinzel A, Kainz A, Mayer B, Perco P, Telkes G, Langer RM, Oberbauer R. Molecular pathogenesis of post-transplant acute kidney injury: assessment of whole-genome mRNA and miRNA profiles. *PLoS One* 2014; **9**: e104164 [PMID: 25093671 DOI: 10.1371/journal.pone.0104164]

54 **Scian MJ**, Maluf DG, Mas VR. MiRNAs in kidney transplantation: potential role as new biomarkers. *Expert Rev Mol Diagn* 2013; **13**: 93-104 [PMID: 23256706 DOI: 10.1586/erm.12.131]

55 **Del Prete G**, De Carli M, Almerigogna F, Daniel CK, D'Elios MM, Zancuoghi G, Vinante F, Pizzolo G, Romagnani S. Preferential expression of CD30 by human CD4+ T cells producing Th2-type cytokines. *FASEB J* 1995; **9**: 81-86 [PMID: 7821763]

56 **Weimer R**, Zipperle S, Daniel V, Carl S, Staehler G, Opelz G. Pretransplant CD4 helper function and interleukin 10 response predict risk of acute kidney graft rejection. *Transplantation* 1996; **62**: 1606-1614 [PMID: 8970616]

57 **Rajakariar R**, Jivanji N, Varagunam M, Rafiq M, Gupta A, Sheaff M, Sinnott P, Yaqoob MM. High pre-transplant soluble CD30 levels are predictive of the grade of rejection. *Am J Transplant* 2005; **5**: 1922-1925 [PMID: 15996240 DOI: 10.1111/j.1600-6143.2005.00966.x]

58 **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 DOI: 10.1097/01.TP.0000152660.56055.53]

59 **Sengul S**, Keven K, Gormez U, Kutlay S, Erturk S, Erbay B. Identification of patients at risk of acute rejection by pretransplantation and posttransplantation monitoring of soluble CD30 levels in kidney transplantation. *Transplantation* 2006; **81**: 1216-1219 [PMID: 16641611 DOI: 10.1097/01.tp.0000203324.49969.30]

60 **Altermann W**, Schlaf G, Rothhoff A, Seliger B. High variation of individual soluble serum CD30 levels of pre-transplantation patients: sCD30 a feasible marker for prediction of kidney allograft rejection? *Nephrol Dial Transplant* 2007; **22**: 2795-2799 [PMID: 17616534 DOI: 10.1093/ndt/gfm397]

61 **Shooshtarizadeh T**, Mohammadali A, Ossareh S, Ataipour Y. Relation between pretransplant serum levels of soluble CD30 and acute rejection during the first 6 months after a kidney transplant. *Exp Clin Transplant* 2013; **11**: 229-233 [PMID: 23477385 DOI: 10.6002/ect.2012.0113]

62 **Rotondi M**, Rosati A, Buonamano A, Lasagni L, Lazzeri E, Pradella F, Fossombroni V, Cirami C, Liotta F, La Villa G, Serio M, Bertoni E, Salvadori M, Romagnani P. High pretransplant serum levels of CXCL10/IP-10 are related to increased risk of renal allograft failure. *Am J Transplant* 2004; **4**: 1466-1474 [PMID: 15307834 DOI: 10.1111/j.1600-6143.2004.00525.x]

63 **Lazzeri E**, Rotondi M, Mazzinghi B, Lasagni L, Buonamano A, Rosati A, Pradella F, Fossombroni V, La Villa G, Gacci M, Bertoni E, Serio M, Salvadori M, Romagnani P. High CXCL10 expression in rejected kidneys and predictive role of pretransplant serum CXCL10 for acute rejection and chronic allograft nephropathy. *Transplantation* 2005; **79**: 1215-1220 [PMID: 15880073 DOI: 10.1097/01.TP.0000160759.85080.2E]

64 **Rotondi M**, Netti GS, Lazzeri E, Stallone G, Bertoni E, Chiovato L, Grandaliano G, Gesualdo L, Salvadori M, Schena FP, Romagnani P, Serio M. High pretransplant serum levels of CXCL9 are associated with increased risk of acute rejection and graft failure in kidney graft recipients. *Transpl Int* 2010; **23**: 465-475 [PMID: 19929857 DOI: 10.1111/j.1432-2277.2009.01006.x]

65 **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]

66 **Bendjelloul F**, Desin TS, Shoker AS. Donor non-specific IFN-gamma production by primed alloreactive cells as a potential screening test to predict the alloimmune response. *Transpl Immunol* 2004; **12**: 167-176 [PMID: 14967315 DOI: 10.1016/j.trim.2003.08.003]

67 **Heeger PS**, Greenspan NS, Kuhlenschmidt S, Dejelo C, Hricik DE, Schulak JA, Tary-Lehmann M. Pretransplant frequency of donor-specific, IFN-gamma-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. *J Immunol* 1999; **163**: 2267-2275 [PMID: 10438971]

68 **Bellisola G**, Tridente G, Nacchia F, Fior F, Boschiero L. Monitoring of cellular immunity by interferon-gamma enzyme-linked immunosorbent spot assay in kidney allograft recipients: preliminary results of a longitudinal study. *Transplant Proc* 2006; **38**: 1014-1017 [PMID: 16757248 DOI: 10.1016/j.transproceed.2006.02.142]

69 **Freue GV**, Sasaki M, Meredith A, Günther OP, Bergman A, Takhar M, Mui A, Balshaw RF, Ng RT, Opushneva N, Hollander Z, Li G, Borchers CH, Wilson-McManus J, McManus BM, Keown PA, McMaster WR; Genome Canada Biomarkers in Transplantation Group. Proteomic signatures in plasma during early acute renal allograft rejection. *Mol Cell Proteomics* 2010; **9**: 1954-1967 [PMID: 20501940 DOI: 10.1074/mcp.M110.000554]

70 **Sigdel TK**, Kaushal A, Gritsenko M, Norbeck AD, Qian WJ, Xiao W, Camp DG, Smith RD, Sarwal MM. Shotgun proteomics identifies proteins specific for acute renal transplant rejection. *Proteomics Clin Appl* 2010; **4**: 32-47 [PMID: 20543976 DOI: 10.1002/prca.200900124]

71 **Wu D**, Zhu D, Xu M, Rong R, Tang Q, Wang X, Zhu T. Analysis of transcriptional factors and regulation networks in patients with acute renal allograft rejection. *J Proteome Res* 2011; **10**: 175-181 [PMID: 20812764 DOI: 10.1021/pr100473w]

72 **Loftheim H**, Midtvedt K, Hartmann A, Reisæter AV, Falck P, Holdaas H, Jenssen T, Reubsaet L, Asberg A. Urinary proteomic shotgun approach for identification of potential acute rejection biomarkers in renal transplant recipients. *Transplant Res* 2012; **1**: 9 [PMID: 23369437 DOI: 10.1186/2047-1440-1-9]

73 **Sigdel TK**, Salomonis N, Nicora CD, Ryu S, He J, Dinh V, Orton DJ, Moore RJ, Hsieh SC, Dai H, Thien-Vu M, Xiao W, Smith RD, Qian WJ, Camp DG, Sarwal MM. The identification of novel potential injury mechanisms and candidate biomarkers in renal allograft rejection by quantitative proteomics. *Mol Cell Proteomics* 2014; **13**: 621-631 [PMID: 24335474 DOI: 10.1074/mcp.M113.030577]

74 **Vasconcellos LM**, Schachter AD, Zheng XX, Vasconcellos LH, Shapiro M, Harmon WE, Strom TB. Cytotoxic lymphocyte gene expression in peripheral blood leukocytes correlates with rejecting renal allografts. *Transplantation* 1998; **66**: 562-566 [PMID: 9753332]

75 **Li B**, Hartono C, Ding R, Sharma VK, Ramaswamy R, Qian B, Serur D, Mouradian J, Schwartz JE, Suthanthiran M. Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. *N Engl J Med* 2001; **344**: 947-954 [PMID: 11274620 DOI: 10.1056/NEJM200103293441301]

76 **Afaneh C**, Muthukumar T, Lubetzky M, Ding R, Snopkowski C, Sharma VK, Seshan S, Dadhania D, Schwartz JE, Suthanthiran M. Urinary cell levels of mRNA for OX40, OX40L, PD-1, PD-L1, or PD-L2 and acute rejection of human renal allografts. *Transplantation*

2010; **90**: 1381-1387 [PMID: 21079547 DOI: 10.1097/TP.0b013e3181ffbadd]

77 **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; Clinical Trials in Organ Transplantation 04 (CTOT-04) Study Investigators. 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]

78 **Hricik DE**, Nickerson P, Formica RN, Poggio ED, Rush D, Newell KA, Goebel J, Gibson IW, Fairchild RL, Riggs M, Spain K, Ikle D, Bridges ND, Heeger PS; CTOT-01 consortium. Multicenter validation of urinary CXCL9 as a risk-stratifying biomarker for kidney transplant injury. *Am J Transplant* 2013; **13**: 2634-2644 [PMID: 23968332 DOI: 10.1111/ajt.12426]

79 **Srinivas TR**, Kaplan B. Urinary biomarkers and kidney transplant rejection: fine-tuning the radar. *Am J Transplant* 2013; **13**: 2519-2521 [PMID: 24007513 DOI: 10.1111/ajt.12427]

80 **Hirt-Minkowski P**, De Serres SA, Ho J. Developing renal allograft surveillance strategies - urinary biomarkers of cellular rejection. *Can J Kidney Health Dis* 2015; **2**: 28 [PMID: 26285614 DOI: 10.1186/s40697-015-0061-x]

81 **Kim SC**, Page EK, Knechtle SJ. Urine proteomics in kidney transplantation. *Transplant Rev* (Orlando) 2014; **28**: 15-20 [PMID: 24321302 DOI: 10.1016/j.trre.2013.10.004]

82 **Perez JD**, Sakata MM, Colucci JA, Spinelli GA, Felipe CR, Carvalho VM, Cardozo KH, Medina-Pestana JO, Tedesco-Silva H, Schor N, Casarini DE. Plasma proteomics for the assessment of acute renal transplant rejection. *Life Sci* 2016; **158**: 111-120 [PMID: 27393492 DOI: 10.1016/j.lfs.2016.06.029]

83 **Sui W**, Yang M, Li F, Chen H, Chen J, Ou M, Zhang Y, Lin H, Xue W, Dai Y. Serum microRNAs as new diagnostic biomarkers for pre- and post-kidney transplantation. *Transplant Proc* 2014; **46**: 3358-3362 [PMID: 25498051 DOI: 10.1016/j.transproceed.2014.08.050]

84 **Anglicheau D**, Sharma VK, Ding R, Hummel A, Snopkowski C, Dadhania D, Seshan SV, Suthanthiran M. MicroRNA expression profiles predictive of human renal allograft status. *Proc Natl Acad Sci USA* 2009; **106**: 5330-5335 [PMID: 19289845 DOI: 10.1073/pnas.0813121106]

85 **Lorenzen JM**, Volkmann I, Fiedler J, Schmidt M, Scheffner I, Haller H, Gwinner W, Thum T. Urinary miR-210 as a mediator of acute T-cell mediated rejection in renal allograft recipients. *Am J Transplant* 2011; **11**: 2221-2227 [PMID: 21812927 DOI: 10.1111/j.1600-6143.2011.03679.x]

86 **Betts G**, Shankar S, Sherston S, Friend P, Wood KJ. Examination of serum miRNA levels in kidney transplant recipients with acute rejection. *Transplantation* 2014; **97**: e28-e30 [PMID: 24531825 DOI: 10.1097/01.TP.0000441098.68212.de]

87 **Grigoryev YA**, Kurian SM, Hart T, Nakorchevsky AA, Chen C, Campbell D, Head SR, Yates JR, Salomon DR. MicroRNA regulation of molecular networks mapped by global microRNA, mRNA, and protein expression in activated T lymphocytes. *J Immunol* 2011; **187**: 2233-2243 [PMID: 21788445 DOI: 10.4049/jimmunol.1101233]

88 **Muthukumar T**, Dadhania D, Ding R, Snopkowski C, Naqvi R, Lee JB, Hartono C, Li B, Sharma VK, Seshan SV, Kapur S, Hancock WW, Schwartz JE, Suthanthiran M. Messenger RNA for FOXP3 in the urine of renal-allograft recipients. *N Engl J Med* 2005; **353**: 2342-2351 [PMID: 16319383 DOI: 10.1056/NEJMoa051907]

89 **Augustine JJ**, Hricik DE. T-cell immune monitoring by the ELISPOT assay for interferon gamma. *Clin Chim Acta* 2012; **413**: 1359-1363 [PMID: 22732764 DOI: 10.1016/j.cca.2012.03.006]

90 **Bestard O**, Crespo E, Stein M, Lúcia M, Roelen DL, de Vaal YJ, Hernandez-Fuentes MP, Chatenoud L, Wood KJ, Claas FH, Cruzado JM, Grinyó JM, Volk HD, Reinke P. Cross-validation of IFN-γ Elispot assay for measuring alloreactive memory/effector T cell responses in renal transplant recipients. *Am J Transplant* 2013; **13**: 1880-1890 [PMID: 23763435 DOI: 10.1111/ajt.12285]

91 **Gielis EM**, Ledeganck KJ, De Winter BY, Del Favero J, Bosmans JL, Claas FH, Abramowicz D, Eikmans M. Cell-Free DNA: An Upcoming Biomarker in Transplantation. *Am J Transplant* 2015; **15**: 2541-2551 [PMID: 26184824 DOI: 10.1111/ajt.13387]

92 **García Moreira V**, Prieto García B, Baltar Martín JM, Ortega Suárez F, Alvarez FV. Cell-free DNA as a noninvasive acute rejection marker in renal transplantation. *Clin Chem* 2009; **55**: 1958-1966 [PMID: 19729469 DOI: 10.1373/clinchem.2009.129072]

93 **Sigdel TK**, Vitalone MJ, Tran TQ, Dai H, Hsieh SC, Salvatierra O, Sarwal MM. A rapid noninvasive assay for the detection of renal transplant injury. *Transplantation* 2013; **96**: 97-101 [PMID: 23756769 DOI: 10.1097/TP.0b013e318295ee5a]

94 **Zhong XY**, Hahn D, Troeger C, Klemm A, Stein G, Thomson P, Holzgreve W, Hahn S. Cell-free DNA in urine: a marker for kidney graft rejection, but not for prenatal diagnosis? *Ann N Y Acad Sci* 2001; **945**: 250-257 [PMID: 11708487 DOI: 10.1111/j.1749-6632.2001.tb03893.x]

95 **Ong S**, Mannon RB. Genomic and proteomic fingerprints of acute rejection in peripheral blood and urine. *Transplant Rev* (Orlando) 2015; **29**: 60-67 [PMID: 25542607 DOI: 10.1016/j.trre.2014.12.003]

96 **Flechner SM**, Kurian SM, Head SR, Sharp SM, Whisenant TC, Zhang J, Chismar JD, Horvath S, Mondala T, Gilmartin T, Cook DJ, Kay SA, Walker JR, Salomon DR. Kidney transplant rejection and tissue injury by gene profiling of biopsies and peripheral blood lymphocytes. *Am J Transplant* 2004; **4**: 1475-1489 [PMID: 15307835 DOI: 10.1111/j.1600-6143.2004.00526.x]

97 **Li L**, Khatri P, Sigdel TK, Tran T, Ying L, Vitalone MJ, Chen A, Hsieh S, Dai H, Zhang M, Naesens M, Zarkhin V, Sansanwal P, Chen R, Mindrinos M, Xiao W, Benfield M, Ettenger RB, Dharnidharka V, Mathias R, Portale A, McDonald R, Harmon W, Kershaw D, Vehaskari VM, Kamil E, Baluarte HJ, Warady B, Davis R, Butte AJ, Salvatierra O, Sarwal MM. A peripheral blood diagnostic test for acute rejection in renal transplantation. *Am J Transplant* 2012; **12**: 2710-2718 [PMID: 23009139 DOI: 10.1111/j.1600-6143.2012.04253.x]

98 **Allison SJ**. Transplantation: Biomarkers in peripheral blood detect acute rejection. *Nat Rev Nephrol* 2012; **8**: 681 [PMID: 23070573 DOI: 10.1038/nrneph.2012.227]

99 **Kurian SM**, Williams AN, Gelbart T, Campbell D, Mondala TS, Head SR, Horvath S, Gaber L, Thompson R, Whisenant T, Lin W, Langfelder P, Robison EH, Schaffer RL, Fisher JS, Friedewald J, Flechner SM, Chan LK, Wiseman AC, Shidban H, Mendez R, Heilman R, Abecassis MM, Marsh CL, Salomon DR. Molecular classifiers for acute kidney transplant rejection in peripheral blood by whole genome gene expression profiling. *Am J Transplant* 2014; **14**: 1164-1172 [PMID: 24725967 DOI: 10.1111/ajt.12671]

100 **Roedder S**, Sigdel T, Salomonis N, Hsieh S, Dai H, Bestard O, Metes D, Zeevi A, Gritsch A, Cheeseman J, Macedo C, Peddy R, Medeiros M, Vincenti F, Asher N, Salvatierra O, Shapiro R, Kirk A, Reed EF, Sarwal MM. The kSORT assay to detect renal transplant patients at high risk for acute rejection: results of the multicenter AART study. *PLoS Med* 2014; **11**: e1001759 [PMID: 25386950 DOI: 10.1371/journal.pmed.1001759]

101 **Shen-Orr SS**, Tibshirani R, Khatri P, Bodian DL, Staedtler F, Perry NM, Hastie T, Sarwal MM, Davis MM, Butte AJ. Cell type-specific gene expression differences in complex tissues. *Nat Methods* 2010; **7**: 287-289 [PMID: 20208531 DOI: 10.1038/nmeth.1439]

102 **Crespo E**, Roedder S, Sigdel T, Hsieh SC, Luque S, Cruzado JM, Tran TQ, Grinyó JM, Sarwal MM, Bestard O. Molecular and Functional Noninvasive Immune Monitoring in the ESCAPE Study for Prediction of Subclinical Renal Allograft Rejection. *Transplantation* 2017; **101**: 1400-1409 [PMID: 27362314 DOI: 10.1097/TP.0000000000001287]

103 **Khatri P**, Roedder S, Kimura N, De Vusser K, Morgan AA, Gong Y, Fischbein MP, Robbins RC, Naesens M, Butte AJ, Sarwal MM. A common rejection module (CRM) for acute rejection across multiple organs identifies novel therapeutics for organ transplantation. *J Exp Med* 2013; **210**: 2205-2221 [PMID: 24127489 DOI: 10.1084/jem.20122709]

104 **Sigdel TK**, Bestard O, Tran TQ, Hsieh SC, Roedder S, Damm I, Vincenti F, Sarwal MM. A Computational Gene Expression Score for Predicting Immune Injury in Renal Allografts. *PLoS One* 2015; **10**: e0138133 [PMID: 26367000 DOI: 10.1371/journal.pone.0138133]

105 **Sigdel T**, Tran T, Bestard O, Vincenti F, Sarwal M. The Urine

Common Rejection Module (uCRM) Is a Sentinal Assay for Graft Rejection. [abstract]. *Am J Transplant* 2016; 16 (suppl 3)

106 **Johnston O**, Cassidy H, O'Connell S, O'Riordan A, Gallagher W, Maguire PB, Wynne K, Cagney G, Ryan MP, Conlon PJ, McMorrow T. Identification of β2-microglobulin as a urinary biomarker for chronic allograft nephropathy using proteomic methods. *Proteomics Clin Appl* 2011; **5**: 422-431 [PMID: 21751411 DOI: 10.1002/prca.201000160]

107 **Kurian SM**, Heilman R, Mondala TS, Nakorchevsky A, Hewel JA, Campbell D, Robison EH, Wang L, Lin W, Gaber L, Solez K, Shidban H, Mendez R, Schaffer RL, Fisher JS, Flechner SM, Head SR, Horvath S, Yates JR, Marsh CL, Salomon DR. Biomarkers for early and late stage chronic allograft nephropathy by proteogenomic profiling of peripheral blood. *PLoS One* 2009; **4**: e6212 [PMID: 19593431 DOI: 10.1371/journal.pone.0006212]

108 **Quintana LF**, Campistol JM, Alcolea MP, Bañon-Maneus E, Sol-González A, Cutillas PR. Application of label-free quantitative peptidomics for the identification of urinary biomarkers of kidney chronic allograft dysfunction. *Mol Cell Proteomics* 2009; **8**: 1658-1673 [PMID: 19357086 DOI: 10.1074/mcp.M900059-MCP200]

109 **Bañón-Maneus E**, Diekmann F, Carrascal M, Quintana LF, Moya-Rull D, Bescós M, Ramírez-Bajo MJ, Rovira J, Gutierrez-Dalmau A, Solé-González A, Abián J, Campistol JM. Two-dimensional difference gel electrophoresis urinary proteomic profile in the search of nonimmune chronic allograft dysfunction biomarkers. *Transplantation* 2010; **89**: 548-558 [PMID: 20134395 DOI: 10.1097/TP.0b013e3181c690e3]

110 **Nakorchevsky A**, Hewel JA, Kurian SM, Mondala TS, Campbell D, Head SR, Marsh CL, Yates JR, Salomon DR. Molecular mechanisms of chronic kidney transplant rejection via large-scale proteogenomic analysis of tissue biopsies. *J Am Soc Nephrol* 2010; **21**: 362-373 [PMID: 20093355 DOI: 10.1681/ASN.2009060628]

111 **Jahnukainen T**, Malehorn D, Sun M, Lyons-Weiler J, Bigbee W, Gupta G, Shapiro R, Randhawa PS, Pelikan R, Hauskrecht M, Vats A. Proteomic analysis of urine in kidney transplant patients with BK virus nephropathy. *J Am Soc Nephrol* 2006; **17**: 3248-3256 [PMID: 17035609 DOI: 10.1681/ASN.2006050437]

112 **Puigmulé M**, López-Hellin J, Suñé G, Tornavaca O, Camaño S, Tejedor A, Meseguer A. Differential proteomic analysis of cyclosporine A-induced toxicity in renal proximal tubule cells. *Nephrol Dial Transplant* 2009; **24**: 2672-2686 [PMID: 19369687 DOI: 10.1093/ndt/gfp149]

113 **Bone JM**, Amara AB, Shenkin A, Hammad A, Sells RA, Alexander JL, McArdle F, Rustom R. Calcineurin inhibitors and proximal renal tubular injury in renal transplant patients with proteinuria and chronic allograft nephropathy. *Transplantation* 2005; **79**: 119-122 [PMID: 15714179 DOI: 10.1097/01.TP.0000146843.23824.93]

114 **Scian MJ**, Maluf DG, David KG, Archer KJ, Suh JL, Wolen AR, Mba MU, Massey HD, King AL, Gehr T, Cotterell A, Posner M, Mas V. MicroRNA profiles in allograft tissues and paired urines associate with chronic allograft dysfunction with IF/TA. *Am J Transplant* 2011; **11**: 2110-2122 [PMID: 21794090 DOI: 10.1111/j.1600-6143.2011.03666.x]

115 **Maluf DG**, Dumur CI, Suh JL, Scian MJ, King AL, Cathro H, Lee JK, Gehrau RC, Brayman KL, Gallon L, Mas VR. The urine microRNA profile may help monitor post-transplant renal graft function. *Kidney Int* 2014; **85**: 439-449 [PMID: 24025639 DOI: 10.1038/ki.2013.338]

116 **Zununi Vahed S**, Omidi Y, Ardalan M, Samadi N. Dysregulation of urinary miR-21 and miR-200b associated with interstitial fibrosis and tubular atrophy (IFTA) in renal transplant recipients. *Clin Biochem* 2017; **50**: 32-39 [PMID: 27521993 DOI: 10.1016/j.clinbiochem.2016.08.007]

117 **Soltaninejad E**, Nicknam MH, Nafar M, Sharbafi MH, Keshavarz Shahbaz S, Barabadi M, Yekaninejad MS, Bahrami T, Ahmadpoor P, Amirzargar A. Altered Expression of MicroRNAs Following Chronic Allograft Dysfunction with Interstitial Fibrosis and Tubular Atrophy. *Iran J Allergy Asthma Immunol* 2015; **14**: 615-623 [PMID: 26725559]

118 **Iwasaki K**, Yamamoto T, Inanaga Y, Hiramitsu T, Miwa Y, Murotani K, Narumi S, Watarai Y, Katayama A, Uchida K, Kobayashi T. MiR-142-5p and miR-486-5p as biomarkers for early detection of chronic antibody-mediated rejection in kidney transplantation. *Biomarkers* 2017; **22**: 45-54 [PMID: 27323802 DOI: 10.1080/1354750X.2016.1204000]

119 **Mas V**, Maluf D, Archer K, Yanek K, Mas L, King A, Gibney E, Massey D, Cotterell A, Fisher R, Posner M. Establishing the molecular pathways involved in chronic allograft nephropathy for testing new noninvasive diagnostic markers. *Transplantation* 2007; **83**: 448-457 [PMID: 17318078 DOI: 10.1097/01.tp.0000251373.17997.9a]

120 **Lee JR**, Muthukumar T, Dadhania D, Ding R, Sharma VK, Schwartz JE, Suthanthiran M. Urinary cell mRNA profiles predictive of human kidney allograft status. *Immunol Rev* 2014; **258**: 218-240 [PMID: 24517436 DOI: 10.1111/imr.12159]

121 **O'Connell PJ**, Zhang W, Menon MC, Yi Z, Schröppel B, Gallon L, Luan Y, Rosales IA, Ge Y, Losic B, Xi C, Woytovich C, Keung KL, Wei C, Greene I, Overbey J, Bagiella E, Najafian N, Samaniego M, Djamali A, Alexander SI, Nankivell BJ, Chapman JR, Smith RN, Colvin R, Murphy B. Biopsy transcriptome expression profiling to identify kidney transplants at risk of chronic injury: a multicentre, prospective study. *Lancet* 2016; **388**: 983-993 [PMID: 27452608 DOI: 10.1016/S0140-6736(16)30826-1]

122 **Li L**, Greene I, Readhead B, Menon MC, Kidd BA, Uzilov AV, Wei C, Philippe N, Schroppel B, He JC, Chen R, Dudley JT, Murphy B. Novel Therapeutics Identification for Fibrosis in Renal Allograft Using Integrative Informatics Approach. *Sci Rep* 2017; **7**: 39487 [PMID: 28051114 DOI: 10.1038/srep39487]

123 **Orlando G**, Hematti P, Stratta RJ, Burke GW, Di Cocco P, Pisani F, Soker S, Wood K. Clinical operational tolerance after renal transplantation: current status and future challenges. *Ann Surg* 2010; **252**: 915-928 [PMID: 21107102 DOI: 10.1097/SLA.0b013e3181f3efb0]

124 **Mastoridis S**, Issa F, Wood KJ. Novel biomarkers and functional assays to monitor cell-therapy-induced tolerance in organ transplantation. *Curr Opin Organ Transplant* 2015; **20**: 64-71 [PMID: 25563993 DOI: 10.1097/MOT.0000000000000154]

125 **Viklicky O**, Hribova P, Brabcova I. Molecular markers of rejection and tolerance: lessons from clinical research. *Nephrol Dial Transplant* 2013; **28**: 2701-2708 [PMID: 23739154 DOI: 10.1093/ndt/gft102]

126 **Lozano JJ**, Pallier A, Martinez-Llordella M, Danger R, López M, Giral M, Londoño MC, Rimola A, Soulillou JP, Brouard S, Sánchez-Fueyo A. Comparison of transcriptional and blood cell-phenotypic markers between operationally tolerant liver and kidney recipients. *Am J Transplant* 2011; **11**: 1916-1926 [PMID: 21827613 DOI: 10.1111/j.1600-6143.2011.03638.x]

127 **Newell KA**, Asare A, Kirk AD, Gisler TD, Bourcier K, Suthanthiran M, Burlingham WJ, Marks WH, Sanz I, Lechler RI, Hernandez-Fuentes MP, Turka LA, Seyfert-Margolis VL; Immune Tolerance Network ST507 Study Group. Identification of a B cell signature associated with renal transplant tolerance in humans. *J Clin Invest* 2010; **120**: 1836-1847 [PMID: 20501946 DOI: 10.1172/JCI39933]

128 **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]

129 **Danger R**, Pallier A, Giral M, Martínez-Llordella M, Lozano JJ, Degauque N, Sanchez-Fueyo A, Soulillou JP, Brouard S. Upregulation of miR-142-3p in peripheral blood mononuclear cells of operationally tolerant patients with a renal transplant. *J Am Soc Nephrol* 2012; **23**: 597-606 [PMID: 22282590 DOI: 10.1681/ASN.2011060543]

130 **Haynes LD**, Jankowska-Gan E, Sheka A, Keller MR, Hernandez-Fuentes MP, Lechler RI, Seyfert-Margolis V, Turka LA, Newell KA, Burlingham WJ. Donor-specific indirect pathway analysis reveals a B-cell-independent signature which reflects outcomes in kidney transplant recipients. *Am J Transplant* 2012; **12**: 640-648 [PMID: 22151236 DOI: 10.1111/j.1600-6143.2011.03869.x]

131 **Roedder S**, Gao X, Sarwal MM. The pits and pearls in translating operational tolerance biomarkers into clinical practice. *Curr Opin*

Organ Transplant 2012; **17**: 655-662 [PMID: 23080065 DOI: 10.1097/MOT.0b013e32835a6f62]

132 **Viklicky O**, Krystufkova E, Brabcova I, Sekerkova A, Wohlfahrt P, Hribova P, Wohlfahrtova M, Sawitzki B, Slatinska J, Striz I, Volk HD, Reinke P. B-cell-related biomarkers of tolerance are up-regulated in rejection-free kidney transplant recipients. *Transplantation* 2013; **95**: 148-154 [PMID: 23222918 DOI: 10.1097/TP.0b013e3182789a24]

133 **Leventhal JR**, Mathew JM, Salomon DR, Kurian SM, Friedewald JJ, Gallon L, Konieczna I, Tambur AR, Charette J, Levitsky J, Jie C, Kanwar YS, Abecassis MM, Miller J. Nonchimeric HLA-Identical Renal Transplant Tolerance: Regulatory Immunophenotypic/Genomic Biomarkers. *Am J Transplant* 2016; **16**: 221-234 [PMID: 26227106 DOI: 10.1111/ajt.13416]

134 **Rebollo-Mesa I**, Nova-Lamperti E, Mobillo P, Runglall M, Christakoudi S, Norris S, Smallcombe N, Kamra Y, Hilton R, Bhandari S, Baker R, Berglund D, Carr S, Game D, Griffin S, Kalra PA, Lewis R, Mark PB, Marks S, Macphee I, McKane W, Mohaupt MG, Pararajasingam R, Kon SP, Serón D, Sinha MD, Tucker B, Viklický O, Lechler RI, Lord GM, Hernandez-Fuentes MP. Biomarkers of Tolerance in Kidney Transplantation: Are We Predicting Tolerance or Response to Immunosuppressive Treatment? *Am J Transplant* 2016; **16**: 3443-3457 [PMID: 27328267 DOI: 10.1111/ajt.13932]

135 **Roedder S**, Li L, Alonso MN, Hsieh SC, Vu MT, Dai H, Sigdel TK, Bostock I, Macedo C, Metes D, Zeevi A, Shapiro R, Salvatierra O, Scandling J, Alberu J, Engleman E, Sarwal MM. A Three-Gene Assay for Monitoring Immune Quiescence in Kidney Transplantation. *J Am Soc Nephrol* 2015; **26**: 2042-2053 [PMID: 25429124 DOI: 10.1681/ASN.2013111239]

136 **Brouard S**, Mansfield E, Braud C, Li L, Giral M, Hsieh SC, Baeten D, Zhang M, Ashton-Chess J, Braudeau C, Hsieh F, Dupont A, Pallier A, Moreau A, Louis S, Ruiz C, Salvatierra O, Soulillou JP, Sarwal M. Identification of a peripheral blood transcriptional biomarker panel associated with operational renal allograft tolerance. *Proc Natl Acad Sci USA* 2007; **104**: 15448-15453 [PMID: 17873064 DOI: 10.1073/pnas.0705834104]

137 **Bohne F**, Martínez-Llordella M, Lozano JJ, Miquel R, Benítez C, Londoño MC, Manzia TM, Angelico R, Swinkels DW, Tjalsma H, López M, Abraldes JG, Bonaccorsi-Riani E, Jaeckel E, Taubert R, Pirenne J, Rimola A, Tisone G, Sánchez-Fueyo A. Intra-graft expression of genes involved in iron homeostasis predicts the development of operational tolerance in human liver transplantation. *J Clin Invest* 2012; **122**: 368-382 [PMID: 22156196 DOI: 10.1172/JCI59411]

138 **Volk HD**, Banas B, Bemelman F, Bestard O, Brouard S, Cuturi C, Grinyo JM, Hernandez-Fuentes M, Koch M, Nashan Bjorn, Rebollo-Mesa I, Sanchez-Fueyo A, Sawitzki B, JM ten Merge I, Viklicky O, Wood K, Reinke P Strategy to achieve biomarker-driven immunosuppression after solid organ transplantation by an academic-industry partnership within the European BIO-DrIM consortium. *Advances in Precision Medicine* 2016; **1**: 34-47 [DOI: 10.18063/APM.2016.01.001]

139 **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 DOI: 10.1034/j.1600-6143.2003.00132.x]

140 **Hernandez-Fuentes MP**, Lechler RI. A 'biomarker signature' for tolerance in transplantation. *Nat Rev Nephrol* 2010; **6**: 606-613 [PMID: 20717098 DOI: 10.1038/nrneph.2010.112]

141 **Streitz M**, Miloud T, Kapinsky M, Reed MR, Magari R, Geissler EK, Hutchinson JA, Vogt K, Schlickeiser S, Kverneland AH, Meisel C, Volk HD, Sawitzki B. Standardization of whole blood immune phenotype monitoring for clinical trials: panels and methods from the ONE study. *Transplant Res* 2013; **2**: 17 [PMID: 24160259 DOI: 10.1186/2047-1440-2-17]

142 EudractCT-Number: 2013-005041-37. Available from: URL: https://www.clinicaltrialsregister.eu

143 **Anglicheau D**, Naesens M, Essig M, Gwinner W, Marquet P. Establishing Biomarkers in Transplant Medicine: A Critical Review of Current Approaches. *Transplantation* 2016; **100**: 2024-2038 [PMID: 27479159 DOI: 10.1097/TP.0000000000001321]

144 **Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran**. Testing Immunosuppression Threshold in Renal Allografts To Extend eGFR (TITRATE). In ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: https://clinicaltrials.gov/ct2/show/NCT02581436

145 **University of California**, San Francisco. Treg Adoptive Therapy for Subclinical Inflammation in Kidney Transplantation (TASK). In ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://Clinicaltrials.gov/ct2/show/ NCT02088931

146 **University of California**, San Francisco. Precision Medicine Offers Belatacept Monotherapy (PROBE). In ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://Clinicaltrials.gov/ct2/show/ NCT020939365

147 **Kalluri R**, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest* 2003; **112**: 1776-1784 [PMID: 14679171 DOI: 10.1172/JCI20530]

148 **Assistance Publique - Hôpitaux de Paris**. Prediction of Chronic Allograft Nephropathy (Prefigur). In ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: URL: https://Clinicaltrials.gov/ct2/show/ NCT01380847

P- Reviewer: Shrestha BM, Wang CX, Wu CC **S- Editor**: Song XX
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 June 24; 7(3): 179-192

DOI: 10.5500/wjt.v7.i3.179

ISSN 2220-3230 (online)

REVIEW

Historical perspective of cell transplantation in Parkinson's disease

Alejandra Boronat-García, Magdalena Guerra-Crespo, René Drucker-Colín

Alejandra Boronat-García, Magdalena Guerra-Crespo, René Drucker-Colín, Departamento de Neuropatología Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad de México 04910, México

Author contributions: Boronat-García A and Guerra-Crespo M contributed equally to this work; Boronat-García A, Guerra-Crespo M and Drucker-Colín R wrote the paper.

Supported by DGAPA-PAPIIT, No. IN207116; and CONACyT, No. 179927.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Manuscript source: Invited manuscript

Correspondence to: René Drucker-Colín, PhD, Departamento de Neuropatología Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, Ciudad de México 04910, México. drucker@unam.mx
Telephone: +52-5-6225732
Fax: +52-5-5500064

Received: January 20, 2017
Peer-review started: January 20, 2017
First decision: April 14, 2017
Revised: May 8, 2017
Accepted: May 12, 2017
Article in press: May 15, 2017
Published online: June 24, 2017

Abstract

Cell grafting has been considered a therapeutic approach for Parkinson's disease (PD) since the 1980s. The classical motor symptoms of PD are caused by the loss of dopaminergic neurons in the substantia nigra pars compacta, leading to a decrement in dopamine release in the striatum. Consequently, the therapy of cell-transplantation for PD consists in grafting dopamine-producing cells directly into the brain to reestablish dopamine levels. Different cell sources have been shown to induce functional benefits on both animal models of PD and human patients. However, the observed motor improvements are highly variable between individual subjects, and the sources of this variability are not fully understood. The purpose of this review is to provide a general overview of the pioneering studies done in animal models of PD that established the basis for the first clinical trials in humans, and compare these with the latest findings to identify the most relevant aspects that remain unanswered to date. The main focus of the discussions presented here will be on the mechanisms associated with the survival and functionality of the transplants. These include the role of the dopamine released by the grafts and the capacity of the grafted cells to extend fibers and to integrate into the motor circuit. The complete understanding of these aspects will require extensive research on basic aspects of molecular and cellular physiology, together with neuronal network function, in order to uncover the real potential of cell grafting for treating PD.

Key words: Parkinson's disease; Cell replacement; Animal models; Nigrostriatal pathway; Striatum; Dopaminergic loss

Core tip: The first studies on cell transplantation for

Parkinson's disease were published during the early 80s. Since then, it has been shown that different cell types induce functional benefits but with high variability among subjects. Here, we first provide a general overview of the field during its early years. Then, we discuss some factors associated with the functionality of the graft based on the latest findings, and highlight the importance of understanding basic aspects (*e.g.*, factors influencing graft integration) which ultimately could contribute to reducing the variability of the functional outcome-an important requirement for its application in the clinic.

Boronat-García A, Guerra-Crespo M, Drucker-Colín R. Historical perspective of cell transplantation in Parkinson's disease. *World J Transplant* 2017; 7(3): 179-192 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i3/179.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i3.179

INTRODUCTION

The transplantation of different tissues into the brain began as an experimental approach for understanding fundamental aspects of the development and function of the central nervous system. The first transplant in an animal model of Parkinson's disease (PD) was performed in 1979 with the objective of determining whether grafted dopamine-producing cells were able to reduce the motor alterations in the animal model[1,2]. These and other initial reports of graft tissue survival in the brain, and its beneficial effects on a PD animal model, contributed to the beginning of cell grafting era in PD, including both basic and clinical research approaches. Nearly 40 years after the first studies in this field, there is continuing interest in the development of cell-replacement therapies for treating PD, with a particular focus on the search for optimal cell-sources for grafting. The objective of this review is to perform a general description and a critical evaluation of our current understanding of the mechanisms underlying the success of cell-replacement therapy in animal models of PD. We will mainly focus on the mechanisms underlying the functionality of the grafts when evaluated using pharmacological tests, and on the comparison of the results obtained principally with fetal ventral mesencephalic cells (FVM) and embryonic stem cells (ESCs)-derived midbrain dopaminergic neurons. Ultimately, the purpose of this review is to provide a perspective of what has been gained relative to the prevailing knowledge during the starting point of this research area: Basically, that in order to provide a long-term benefit in PD motor symptoms, functional integration of the transplanted cells into the host brain circuit is essential.

EARLY YEARS OF CELL GRAFTING INTO THE BRAIN

The earliest known report of neural tissue transplantation into the brain was conducted by Thompson[3] in 1890. He published a brief description of the transient survival of grafted cat cortical tissue into the brain of a dog, in a work entitled "Successful brain grafting"[3]. In 1907, in another attempt to prove that brain grafting was possible, Del Conte[4] implanted fetal cortex tissue into an adult mammalian brain, showing similar results to those reported by Thompson. In 1909 Ranson provided evidence that suggested that postnatal nervous tissue, the cervical ganglion obtained from 1-wk-old rats, survived when grafted into the adult cortex[5]. Later, in 1917 Dunn found that rat neonatal cerebral cortex tissue transplanted into the adult rat brain was able to survive, grow, and even exhibited myelinated fibers[6]. Other studies were performed during the following years (*e.g.*, Ref[7,8]), which together with those described thus far, constitute the earliest antecedents for cell transplantation.

The functional consequences of brain transplants were not evaluated until 1979[1,2] using the 6-hydroxydopamine (6-OHDA) animal model of PD, which was developed 10 years before[9]. This model allows the selective destruction of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of only one hemisphere of a rat's brain[9]. The motor asymmetry observed in this toxin-based model is characterized by a turning behavior contralateral or ipsilateral to the side of the lesion, and is induced by the systemic administration of dopaminergic agonists (amphetamine or apomorphine) (Figure 1A and B)[9,10]. These experimental approaches allowed to test the functional consequences of cell transplantation by grafting dopamine-producing cells[1,2,11]. The general assumption was that, since motor asymmetry is a consequence of a decrement in dopamine in the striatum, then that asymmetry could be reversed by grafting dopaminergic cells, as long as they release dopamine in the host (Figure 1C and D).

CELL GRAFTING IN PD: THE PIONEERING STUDIES (1979-1990)

FVM grafts in pre-clinical studies

Cells derived from FVM tissue were the first type of cells used for brain grafting in the 6-OHDA rat model of PD[1,2] (for a timeline of pre-clinical studies see Figure 2). This tissue was selected because it contains dopaminergic neurons[12]. In 1979 and 1980, two independent studies confirmed that FVM cells were able to survive (from few to approximately 4000 surviving grafted cells observed 1-7 mo after transplantation), to extend projections into the host striatum after being grafted into the lateral ventricle (Figure 3)[1] or in a cavity at the surface of the striatum (Figure 3)[2], and to reduced circling behavior induced either by apomorphine[1] or amphetamine[2] by approximately 50%, when compared to measurements of motor asymmetry before transplantation. These results were encouraging as they were the first demonstration of a functional outcome induced by grafting exogenous cells in the brain.

The mechanism underlying the functional effects of the grafts was proposed to be the dopamine released

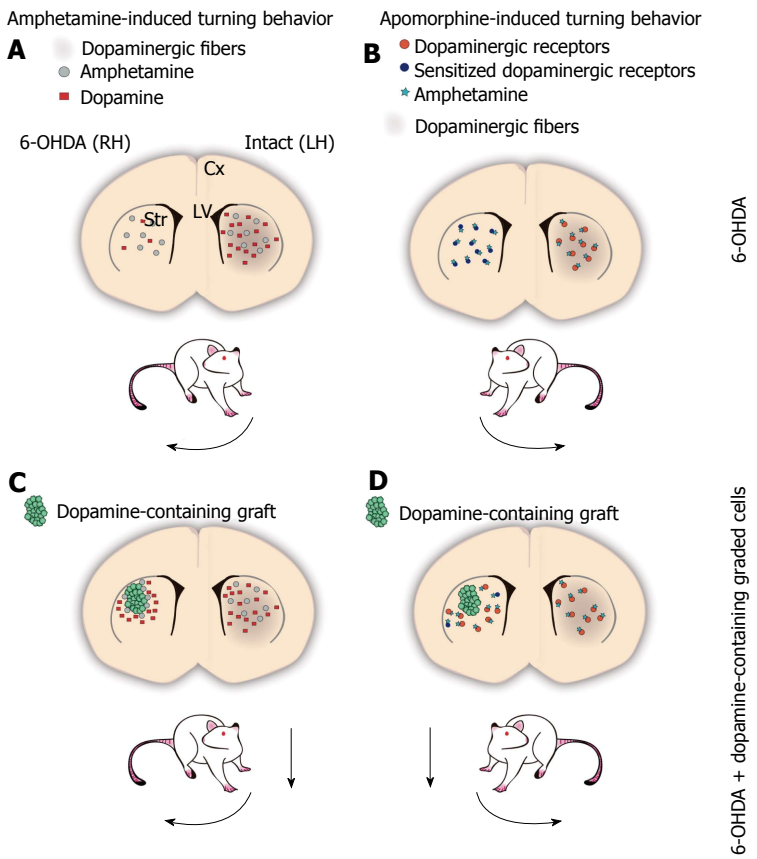


Figure 1 The 6-OHDA rat model of Parkinson's disease. A-D: Schemes of a coronal representation of the rat brain. Dopaminergic fibers are depicted with brown shadowing, which is lacking in the 6-OHDA-lesioned hemisphere; A: Amphetamine (grey circles) administration promotes the release of dopamine (red squares) from the intact dopaminergic terminals of the striatum, disproportionally increasing dopamine concentration in the non-lesioned side relative to the lesioned side, as the latter contains fewer (or none at all) dopaminergic terminals. The asymmetry in extracellular dopamine levels between both hemispheres induces the stereotypical behavior known as circling or turning behavior, ipsilateral to the lesioned side (curved arrow next to the rat); B: Apomorphine is a dopaminergic receptor agonist that can activate postsynaptic dopamine receptors in the striatum (orange circles). 6-OHDA-induced dopaminergic denervation in one hemisphere of the striatum, results in postsynaptic supersensitivity to dopamine in the lesioned side (sensitized dopamine receptors are represented as dark blue circles), such that apomorphine (teal stars) stimulation increases the activity in the lesioned side to a greater extent than in the non-lesioned side. The supersensitivity effect promotes that lesioned animals turn contralateral to the lesioned side after apomorphine administration (curved arrow); C: Amphetamine stimulates dopamine-containing cells (green circles) grafted into the denervated striatum increasing extracellular dopamine concentration in the lesioned side, which leads to a decrement in motor asymmetry (dashed arrow); D: Grafted cells that release dopamine decrease the supersensitivity effect on the lesioned hemisphere, normalizing the response to dopamine or agonists relative to the non-lesioned side. Thus, after apomorphine administration, grafted animals decrease their turn number (dashed arrow). Cx: Cortex; LH: Left hemisphere; LV: Lateral ventricles; RH: Right hemisphere; Str: Striatum.

from FVM cells (Figure 1C and D). However, the first studies found that some animals with surviving grafts did not exhibit any improvement in turning behavior. Several authors using either the same cell type[13-19] or a different cell source[11,20] have replicated these observations, which has not received a complete explanation to date. However, by that time, Björklund and Stenevi[2] proposed that fiber ingrowth from grafted cells into the striatum was, together with the release of dopamine, the determining factors for producing a reduction in circling behavior. Subsequently, a correlation between the reduction in amphetamine-induced turning behavior and the degree of fiber ingrowth was reported[21]. The observations on the variability in the motor improvement in grafted animals with surviving transplants was also found to correlate with the degree of dopaminergic lesion[1,22] and graft survival[23].

One year after the first reports of cell grafting in an animal model of PD, evidence confirmed that dopamine was present in the lesioned striatum of FVM grafted animals[14]. Dopamine tissue-content was found to correlate with the reduction of circling behavior induced by amphetamine. It was also found that a restoration of at least 3% of normal dopamine levels in the striatum was sufficient to reduce the motor asymmetry[24]. However, these observations only demonstrated that mesencephalic transplants contain the neurotransmitter, but not that they release it. In 1983, Freed *et al*[25] provided more direct evidence for the role of dopamine on motor improvement in the 6-OHDA model of PD. The authors suggested that the graft can release dopamine spontaneously on a tonic basis, reversing the supersensitivity effect caused by dopaminergic denervation by directly quantifying the binding of dopamine to its receptors using a dopamine-receptor binding assay. A few years later, Zetterström *et al*[26], conducted a study using an *in vivo* dialysis assay, where they corroborated that mesencephalic transplants release dopamine spontaneously, and after amphetamine administration. One-year later, the same group observed that dopamine release was higher in animals with more surviving grafted cells and more fiber ingrowth, reaching about 85% of normal dopamine levels under basal conditions[23].

In addition to the reduction in turn number induced either by amphetamine or apomorphine, FVM grafts were shown to reduce some aspects of spontaneous abnormal behaviors observed in the PD animal model, such as sensorimotor orientation deficits and asymmetric limb use[15,27]. However, other studies failed to replicate these results[28,29].

Nowadays, FVM-derived cells remain as one of the most promising sources for cell grafting[30], and much more information has been obtained by using this cell source compared with other cell types. However, a major problem related to the use of FVM tissue as a source for cell grafting was the ethical concern due to the use of fetal-derived tissue, which led to the search for alternative cell-sources.

Adrenal medulla grafts in pre-clinical studies: Dopamine vs neurotrophic effects

Chromaffin cells are neuroendocrine cells that synthesize and release catecholamines from the adrenal medulla (AM) into the bloodstream in response to sympathetic

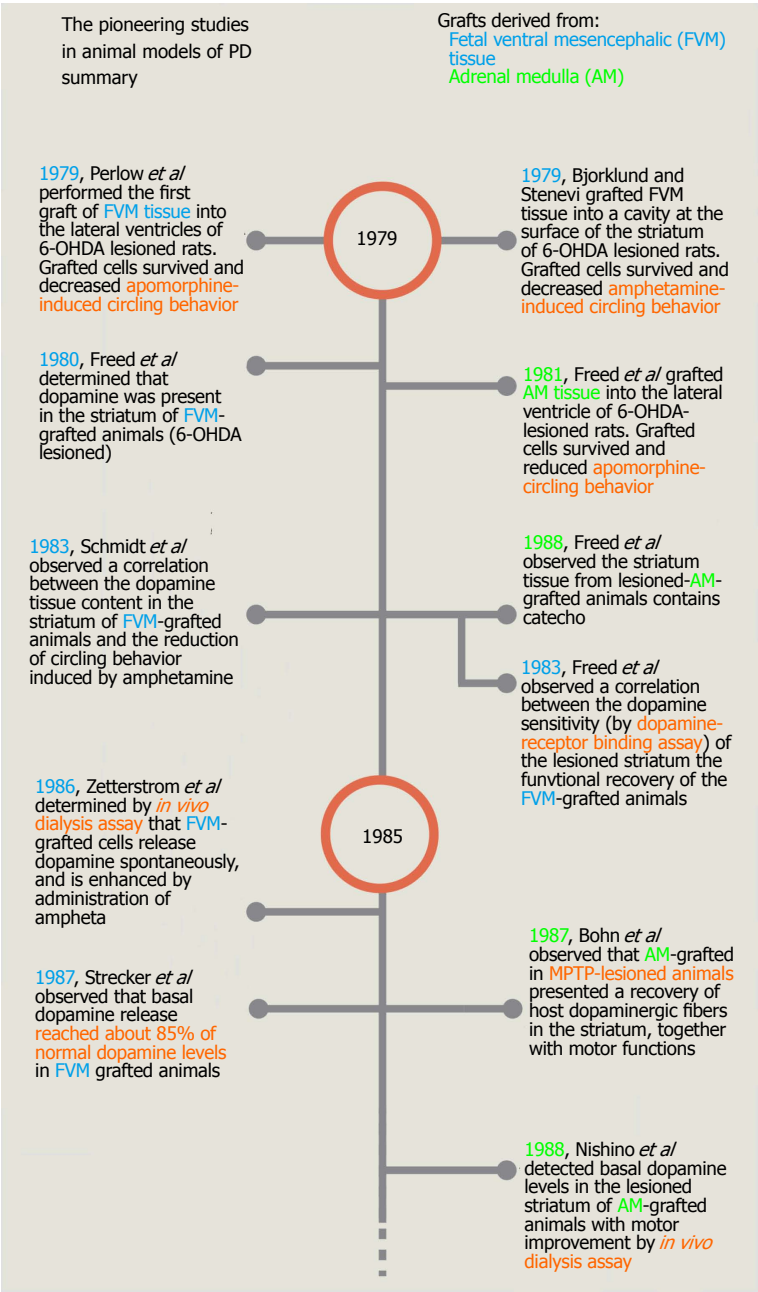


Figure 2 Timeline of the pioneering studies on cell transplantation in animal models of Parkinson's disease. This timeline shows only a few of the studies performed during the first 10 years of cell grafting in animal models of PD. Most of these studies were selected because they were the first published reports of either the use of a new animal model of PD, a site of grafting, a type of cell or a specific technique. PD: Parkinson's disease; FVM: Fetal ventral mesencephalic cells; AM: Adrenal medulla.

stimulation, triggering the fight-or-flight response. This cell source was chosen for use in cell replacement therapy mainly due to the capacity of chromaffin cells to produce dopamine (for review[31]). The first published report using AM tissue grafted in a PD animal model was conducted by Freed *et al*[11]. They demonstrated that AM grafted into the lateral ventricle of 6-OHDA-lesioned rats reduced apomorphine-circling behavior by 20%-50% relative to the initial values before grafting, and this effect lasted for at least 2 mo[11]. However, the cells extended only very few fibers into the host tissue and the mean number of surviving cells was approximately 1535 two months post-grafting[11]. In addition, the animal with the highest number of surviving grafted cells (approximately 4000) did not reduce its circling behavior.

During the eighties it was assumed that the mechanism of action of AM grafts was similar to FVM cells, consisting of the diffusion of high concentrations of dopamine spontaneously released by the graft[11]. Later, it was demonstrated that AM grafts contain high concentrations of adrenaline and noradrenaline, but low concentrations of dopamine[32], mirroring their native characteristics in the AM. When the release of these catecholamines by AM grafts was evaluated by *in vivo* dialysis assays, the authors of the study detected basal dopamine levels only in those animals with motor improvement. Surprisingly, the dopamine levels found were only 50% lower than normal values in the non-lesioned striatum, despite the low survival of grafted chromaffin cells (approximately 50-600 cells)[20]. However, other authors found that the results obtained from chromaffin cell grafts were highly variable and unpredictable in terms of survival and functional outcome, especially when grafts were placed into the striatum (intraparenchymal)[33].

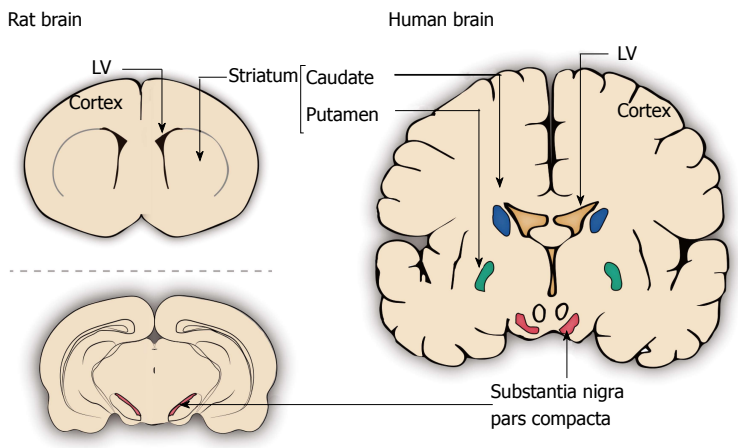


Figure 3 Schematic representation of different sites in the rat and human brains used for grafting in Parkinson's disease. The depicted grafting sites include the lateral ventricles (LV), the striatum (in rat) or caudate nucleus and putamen (in human) and the substantia nigra pars compacta. The above schemes are coronal sections of the rat striatum and human caudate (blue) and putamen (green) together with the substantia nigra pars compacta (red). The scheme below is a coronal section at the level of the rat substantia nigra pars compacta (red).

The discrepancies observed when AM-tissue was grafted in the 6-OHDA model of PD, together with results derived using a different model of PD, the 1-methyl-1,2,3,6-tetrahydropiridine (MPTP)[34], led the scientific community to suggest a different mechanism of action for chromaffin cell grafts: A neurotrophic effect. In this regard, different authors observed that MPTP-lesioned animals with chromaffin cell grafts presented an enhanced recovery of host dopaminergic fibers in the grafted striatum of mouse[35] and monkeys[36], together with a transient functional recovery[37]. These studies suggested a neuroprotective action of the chromaffin cells, which induced the reappearance of tyrosine hydroxylase (TH) immunoreactivity (THir) or the sprouting of surviving host fibers, leading to an increment of dopamine released by the endogenous cell (for review[31]). However, a direct comparison of AM grafts to FVM-derived cells in 6-OHDA lesioned rats demonstrated that AM grafts were less effective in terms of functionality and in their long-term survival, even when AM grafts were placed in the lateral ventricles[38], a site which was assumed to induce a better survival of AM grafts. Thus, despite some studies showing transitory and modest recovery of motor function, AM-derived cells were shown to induce variable and unpredictable results, probably derived from their different mechanism of action compared to FVM grafts.

Clinical studies: A brief description

Although this review is focused in studies using animal models of PD, it is also important to provide at least a general overview of the clinical trials that have been done using both cell sources described above (FVM- and AM-derived cells) (for a timeline of the pioneering studies see Figure 4). There are several extensive reviews aimed at describing critically and in a deeper way the results derived from clinical studies (*e.g.*, Ref[30]).

AM-derived cells were the first to be tested in human patients with PD, with similar results as those observed in animals: Variable and transitory restoration of some motor function[39-41]. Autologous chromaffin cells were first grafted in three different places: The caudate nucleus (Figure 3)[39], the putamen (Figure 3)[40], or in a cavity made at the interface between the caudate nucleus and the lateral ventricles (Figure 3)[41]. In the two first studies the patients showed only moderate recovery that did not last longer than a few months[39,40]. However, by placing the grafts in proximity to the lateral ventricles, other authors reported that one of their two patients showed motor improvements that persisted for at least 10 mo after the grafting procedure[41]. As a result, many clinical studies were done worldwide (*e.g.*, Ref[42-46]) despite the fact that the original articles only reported transitory and modest improvements. As described in a comprehensive review on the topic by Barker *et al*[30], the scientific community started to be concerned about the clinical trials that were taking place, due to the poor or absent functional outcome induced by the AM grafts, the frequent complications from the surgery (*e.g.*, psychiatric alterations), and the fact that post-mortem studies revealed a poor survival of the grafted cells. This led to the abandonment of the use of AM tissue for transplantation.

FVM tissue was the second cell source to be grafted in patients with PD. The grafted tissue was placed into the caudate nucleus[47], the putamen (*e.g.*, Ref[48,49]), both sites (*e.g.*, Ref[50]), in a cavity made at the interface between the caudate nucleus and the lateral ventricles[51] and even directly into the SNpc (Figure 3)[52]. Unfortunately, the results varied from clear benefits to poor or none, but there were also promising results showing improvements by [^{18}F]-DOPA uptake by positron emission tomography (PET) imaging[47].

One of the most controversial issues with these studies was the lack of control groups to discard a placebo effect. In 2001, Freed *et al*[53] performed the first double-blind study that included a placebo control group, in which some patients received FVM cell-grafts bilaterally implanted in the putamen, and observed a modest recovery compared with the sham group. Other double-blind studies were done during subsequent years with a similarly variable symptomatic outcome[54]. Another important issue that became apparent several years after the surgery was that some of the grafted patients started to develop dyskinesias (involuntary movements) as a side effect of the transplant (see[55] for review).

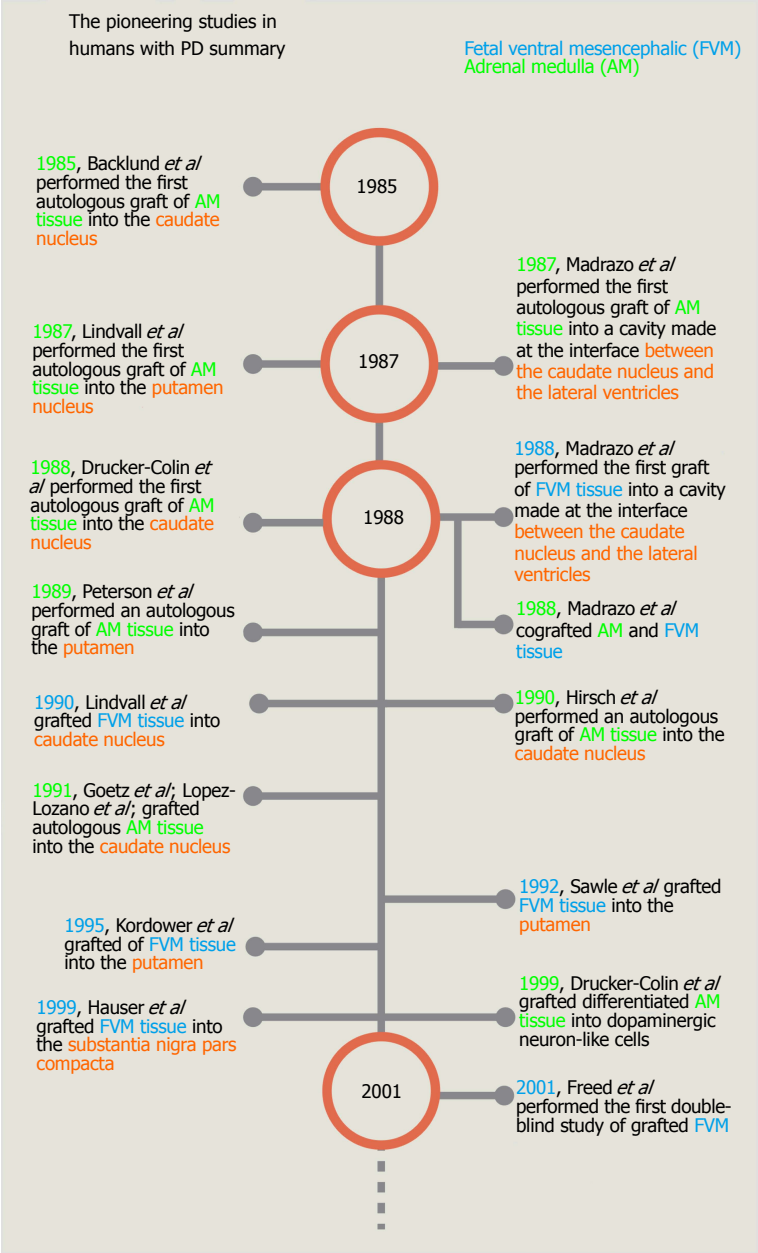


Figure 4 Timeline of the pioneering studies on cell transplantation in human patients with Parkinson's disease. This timeline shows only a few of the studies performed during the first 15 years of cell grafting in patients with PD. Most of them are the first published reports in which, a new site of grafting or a new type of cell were used. PD: Parkinson's disease.

Many clinical studies were subsequently done using FVM cells, chromaffin cells or other types of cell sources including retinal pigmented epithelial cells attached to microcarriers[56,57], adult neural stem cells[58] and autologous bone marrow-derived mesenchymal stem cells[59], all with similar results: Some patients showed moderate recovery, whereas others showed poor or no recovery at all (for review see[60,61], visit http://clinicaltrials.gov for clinical NIH-funded trials currently underway, Table 1). The highly variable results obtained even to date strongly argue that some of the key requirements for this type of therapeutic option to work are still unknown.

WHAT DO WE KNOW NOW?

The study of graft-associated mechanisms producing motor improvements in animal models of PD has been largely done using experimental paradigms with a strong bias towards the role of dopamine. However, actually we know that several additional factors also somehow influence the functional motor recovery. These include the degree of survival of the graft, the capacity of the graft to extend fibers into the host, the ability of these fibers to establish functional connections with host cells and the extrinsic factors that influence all the previously mentioned aspects. The next section of this review focuses on comparing the facts that we knew in the early years with the latest advances in the field. We will describe the results derived using two cell types, which have been widely demonstrated to possess the greatest capacity to survive and to decrease the circling behavior and improve other motor functions in animal models of PD: FVM-derived cells and ESC-derived dopaminergic neurons.

Table 1 Current clinical trials (2013-2016)

	Type of cells	Site of procedure	Age of patients	No. of patients	Control group(s)	Phase[2]	Current status and notes
The University of Texas Health Science Center, United States. NCT02611167[1]	Allogeneic bone marrow-derived mesenchymal stem cell	Delivered intravenously	45-70	20	No	I and II	Nov 2017. Starts on May 2016
ISCO-Florey. Cyto Therapeutics Pty Limited. Australia. NCT02452723[1]	Human parthenogenetic stem cells-derived neural stem cells	Striatum and SNpc	30-70	12	No	I and II	Approved from the TGA of Australia (received on December 2015)
University of Saskatchewan and Manitoba, Canada. NCT02538315[1]	Fetal dopaminergic grafts	NS	18 and older	30	NS	NS	Study type: Observational. Using [^{18}F]FDOPA PET/CT to monitor the effectiveness of grafts. Started on December 2015
University of Kentucky, United States. NCT01833364[1]	Autologous peripheral nerve	SNpc	40-75	16	No	NS	Started on 2015.
TRANSEURO, Europe. NCT01898390[a]	FVM Tissue	NS	30-68	40	Yes (no surgery)	I	No updates. Patients undergoing deep brain stimulation surgery Enrolling participants.
CHA University, South Korea. NCT01860794[1]	Mesencephalic neural precursor cells	NS	18-70	15	NS	I and II	No updates since December 2014 Started on 2013. No updates
Living cell technologies. Auckland City Hospital, New Zealand. NCT01734733[a]	NTCELL [immunoprotected (alginate-encapsulated) choroid plexus cells]	NS	40-70	NS	NS	I and II	Started on 2013. No updates

[1]Is the ClinicalTrials.gov identifier. For more information and other trials visit the website; [2]Clinical phases: I: Test a new treatment in a small group to evaluate its safety, dosage range and side effects; II: Treatment in a small group to see its effectiveness and to further, evaluate its safety. NS: No specified; TGA: Therapeutic Goods Administration.

Graft survival and the effects of grafting into the striatum and the SNpc

The survival of the grafted cells is modified by different factors including the age of the donor tissue, the graft composition and the location of the graft.

The relation between survival and functional recovery has been studied by different authors[17,19,23,62,63]. FVM tissue is usually obtained from 12.5-d-old mouse embryos or 14-d-old rat embryos. However, it has been observed that the survival of intra-striatal grafts of FVM-derived dopaminergic neurons is higher when 12-d-old rat embryos are used[64]. Interestingly, the increment in survival of grafted FVM cells (derived from rat embryos of 12 d *vs* 14 d) is not necessarily accompanied by an equivalent improvement in the functional outcome. This suggests that a critical number of cells is required for improvement, above which a higher survival does not contribute to further improvement[65]. Sauer *et al*[19] estimated that approximately 2000 surviving cells were necessary for complete recovery of turning behavior, whereas only 600 cells were necessary for a moderate level of recovery. It is important to note that, in that study, the improvement observed in four animals with 600-1500 surviving cells ranged from negligible to low[19]. In other reports, it has been observed that an even smaller number (100-200) of surviving TH^+ cells was sufficient to obtain a 50% reduction in turning behavior (*e.g.*, Ref[17,62]). More recently, using human FVM cells, it was observed that at least 657 TH^+ surviving cells were necessary to induce a significant reduction (50% relative to the initial circling behavior before grafting) in apomorphine- induced circling behavior[66]. Similarly, using human ESC-derived midbrain dopaminergic neurons, a complete recovery of amphetamine-induced circling behavior was achieved with approximately 986 TH^+ surviving cells[67]. Therefore, in general, the studies that have correlated survival of the grafted cells with behavioral improvement have, surprisingly, found that a very small number of cells are sufficient to produce a robust motor improvement.

An additional factor to be considered in the case of FVM-derived cells is that the age of the donor tissue in turn influences the composition of the grafted cells. The developing mesencephalon contains two major sub-populations of neurons: A9 and A10 neurons[12,68]. The A9 sub-population in particular corresponds to dopaminergic neurons that will form the SNpc, whereas the A10 neurons are dopaminergic neurons that form the ventral tegmental area. Each subtype differs in multiple characteristics, including their morphology, their protein-expression profile and their target areas in the brain (SNpc in the dorsal striatum and ventral tegmental area in the ventral striatum). Since FVM grafts contain a mix of these two sub-populations[69,70], researchers started to elucidate the role of each subtype on the functional outcome induced by the graft. A9 neurons were found to be critically important for a major functional recovery, due to these grafted-cells

innervating the regions of the striatum corresponding to the areas normally innervated by dopaminergic neurons from the SNpc[71].

Thus far, we have only discussed ectopic sites (*i.e.*, located outside the SNpc) for grafting as a therapeutic approach to reverse the motor alterations observed in PD. However, we have to consider that dopaminergic cells from the nigrostriatal pathway are part of a complex circuit that receives regulatory inputs from other structures (*e.g.*, SN pars reticulata). In agreement with this, it has been observed that intrastriatal grafts do not ameliorate all the symptoms associated with degeneration of the nigrostriatal pathway, since the proper function of the basal ganglia circuitry is far from being restored[16,28,65,72]. Current approaches on this front focus on the possibility of reconstructing the nigrostriatal pathway, by grafting cells into the SNpc (Figure 3) and directing their fibers to reestablish the lost dopaminergic circuitry in the striatum[73]. The first studies that attempted this procedure succeeded in demonstrating that FVM grafts survive when placed into the SNpc and that, in some cases, the neurons extended projections into the striatum and induced some reduction in drug-induced circling behavior[74-79]. However, the survival of FVM cells grafted into the SNpc was less prominent as compared to intra-striatal grafts[74,78,80].

Fiber ingrowth and dopamine release

The occurrence of fiber ingrowth from the graft into the host depends in part on the type of cell used. Intra-striatal grafts of FVM cells[67], ESC-derived dopaminergic neurons[67] and induced pluripotent stem cells (iPSC)-derived dopaminergic neurons[81] have been shown to extend fibers into the host striatum. It has been suggested that the extension of projections is important for mesencephalic grafts[13,16,21,23,27], although FVM-grafts have been shown to produce motor improvement without any detectable projections[1,82]. However, it is reasonable to consider that the greater the extension of the graft projections, the further the molecules they release can diffuse. In addition, with more and longer projections, the establishment of synaptic contacts between the host cells and the graft becomes more likely.

Certainly, an ideal scenario for intra-striatal grafts is one in which dopamine release and clearance are regulated by the necessities of the host circuit. Different authors have shown that FVM grafts release dopamine under basal conditions, and that the release can be enhanced by stimulation with amphetamine[18,26] or high extracellular potassium[83,84]. This has also been demonstrated for ESC-derived dopaminergic neurons[67,85]. Notably, these two types of cells have been shown to deliver sufficient dopamine into the striatum to restore its concentration to normal levels[67,85]. Interestingly, a recent study showed that grafts of FVM cells placed into the SNpc increased striatal dopamine levels to 77% compared to lesioned animals[86]. This study also observed extensive axonal growth from the grafted cells (confirmed by grafting cells from transgenic mice overexpressing green fluorescent protein, GFP) that reached the striatum, together with a significant behavioral recovery in the apomorphine-induced rotation of 94% relative to the initial rotation numbers before grafting[86]. Another study published the same year showed similar results[87], and demonstrated that over-expression of glial cell-derived neurotrophic factor (GDNF) enhanced survival and axonal growth from the grafted cells positioned in the SNpc. The authors also observed a reduction in turn number induced by amphetamine of approximately 75% relative to the initial values before grafting in GDNF-treated animals, which lasted for at least 12 wk[87]. In a more recent study, Grealish *et al*[67] demonstrated that human ESC-derived dopaminergic neurons (A9 and A10 phenotypes) can restore dopaminergic transmission in the transplanted striatum, as occupancy of D2/D3 receptors by dopamine measured using PET showed dopamine binding levels that were similar to the non-lesioned side. More importantly, the study demonstrated that human ESC-derived midbrain dopaminergic neurons grafted into the SNpc provided widespread innervation that extended more than 10 mm throughout the forebrain, with dense innervation in the striatum (A9 subtype), as well as nucleus accumbens, amygdala and frontal cortex (A10 subtype), which are normally innervated by endogenous dopaminergic fibers from the SNpc. In addition, they obtained similar results using human FVM, with an average axonal number of 2169 for the FVM cells and 2453 for the human ESC-derived cells[67]; although, the functional effects of the nigral grafts were not determined in this study. Taken together, these findings are encouraging, suggesting that the reconstruction of the dopaminergic pathway is a plausible approach. However, more research is necessary, to determine whether normal connectivity and physiology are established by the grafted cells into the SNpc. In this regard, it seems that the projections extended by the grafted cells are highly specific, as they connect exclusively to targets that are normally innervated by dopaminergic fibers from the SNpc (for a review on this topic see[73]).

Establishment of connections

A property of central importance for the grafted cells is their capacity to integrate into the host circuit by establishing functional synaptic connections with other cells. This feature marks a difference between grafted cells that function only as release-pumps for dopamine and trophic factors, and those that integrate into the circuit and respond to the physiological needs of the site.

Different sources of evidence support the idea that some types of grafted cells, especially FVM cells and human ESC derived-dopaminergic neurons, establish synapsis with the host cells[88-93]. Electrophysiological studies were initially difficult to perform, as no direct method existed for differentiating the graft from the host cells. Hence, in early electrophysiological studies the recorded cells were chosen blindly, and later identified by THir or by their electrophysiological properties[88,89].

These electrophysiological recordings showed that host striatal cells close to THir fiber projections of FVM cells decreased their firing rates to levels normally observed in a healthy striatum[89]. In contrast, cells located far from the graft or graft-projections presented altered firing rates[89]. Additionally, Freund *et al*[90] demonstrated by using electronic microscopy that FVM cell grafts establish synapses with the dendritic shafts and spines of the striatal neurons, including medium spiny neurons and giant cholinergic interneurons. However, they failed to track reciprocal afferent connections to the graft from the host striatum[90]. Evidence of synaptic connections, both from graft to host and from host to graft, was later observed by other authors using immunostaining for postsynaptic and presynaptic markers and electron microscopy[91]. These results confirmed that some FVM cell grafts have the capacity to integrate into the host circuit and induce changes in host cell firing rates. Concurrently, to identify electrically active afferent and efferent connections of the graft to the host cells, Arbuthnott *et al*[88] grafted FVM cells in the striatum and implanted stimulating electrodes under the grafts in the striatum but also in the frontal cortex, locus coeruleus or dorsal raphe nuclei of 6-OHDA-lesioned animals. They found that grafted cells fired action potentials after striatal stimulation in a similar manner as naïve SNpc dopaminergic neurons, but remarkably, only in those animals in which rotational behavior was compensated and had longer antidromic latencies[88]. They also observed that some grafted cells were activated after stimulation in the frontal cortex, locus coeruleus or raphe nuclei[88].

More direct evidence supporting electrical activity and connectivity of grafts has been recently obtained using FVM grafts derived from transgenic mice expressing GFP under the control of the TH gene promoter, and measuring their electrical activity with whole-cell patch clamp recordings[92]. They observed that a higher proportion of grafted cells in the lesioned striatum fired spontaneous action potentials than grafted cells in the non-lesioned striatum. However, the firing frequency was similar for both[92]. Furthermore, they measured lower frequency of inhibitory and excitatory postsynaptic currents in cells grafted into lesioned, as compared to non-lesioned, animals[92]. Based on these data, the authors suggested that dopamine levels in the striatum could modulate the activity of grafted cells by the activation of D_2 autoreceptors in FVM cells. Another possibility is that the grafts in non-lesioned animals received more GABAergic synaptic inputs[92].

The evidence presented thus far did not confirm that dopamine release was regulated by electrical activity, and that the release was responsible for the functional recovery observed in behavioral experiments. Interestingly, Dell'Anno *et al*[94] were able to control the electrical properties and neurotransmitter release of grafted reprogramed dopaminergic neurons by using designer receptors exclusively activated by designer drug technology. The authors demonstrated that the functional outcome is higher when the neural activity of the striatal-grafted cells is stimulated by clozapine-N-oxide (the pharmacologically inert molecule that activates the designed receptor expressed by the cells), achieving similar results to those observed using FVM tissue[94]. *In vitro*, stimulation of the reprogramed cells resulted also in an increment in neural activity (number of spikes per second) together with an increment of dopamine release[94].

Using a different approach to control the neuronal activity of the grafted cells in order to understand its relation to the functional outcome, Steinbeck *et al*[93] grafted differentiated mesencephalic dopaminergic neurons derived from human ESC that expressed the inhibitory light-activated chloride pump halorhodopsin (eNpHR3.0-EYEP, also known as HALO). After corroborating the functionality of the cells *in vitro*, they were grafted into the striatum of 6-OHDA lesioned immunodeficient mice. The authors observed that transplanted animals gradually decreased their amphetamine-induced turning behavior for a period of 4 mo[93]. Electrophysiological recordings on brain slices showed that the grafted cells produced action potentials that ceased after illumination (*i.e.*, activation of the HALO-mediated chloride conductance). It was also corroborated that grafted cells are able to modulate the activity of spiny medium neurons, and that they receive functional glutamatergic inputs from the host cells[93]. *In vivo* studies performed in freely moving grafted animals showed that the reduction of spontaneous rotations and sensorimotor deficits evaluated with the corridor test is dependent on graft activity, as optogenetic silencing of the cells reversed the recovery[93]. To test the dependence of recovery on dopamine release by grafted cells, the animals were injected with apomorphine before optogenetic silencing. The authors observed that after illumination the recovery of the behavior was still present, as host dopaminergic receptors were expected to be occupied by apomorphine. This study provides an appropriate strategy to interrogate the mechanisms underlying the functionality of grafted cells. In general, grafted cells have been proven to be able to integrate into the host tissue but more experiments are necessary for a complete understanding of their role in the population dynamics of the striatal circuit.

GENERAL DISCUSSION: LOOKING INTO THE FUTURE, BACK TO BASICS

After the studies by Perlow[1] and Björklund and Stenevi[2], several authors have replicated their results with the same type of cells as well as different dopamine-containing cells. As laid out in the preceding sections, there are several different cell sources that have demonstrated a capacity to survive and reverse motor alterations in animal models of PD. However, the clinical benefits of brain grafting in PD patients have not yielded the expected results. A look back in history indicates that some questions related to basic aspects of molecular and cellular physiology, as well as neuronal network function, remain unanswered.

One important issue is to identify the factors that determine whether a graft will induce motor recovery or not. Independently of the cell type used, the available evidence shows that, in animal models or human subjects, some graft recipients exhibit no recovery despite having equivalent levels of graft survival to the individuals that presented striking motor improvement. The reason for this variability is still unknown. Some results have shown that electrical activity of the grafted cells is a common feature of those animals with compensated behavior[88]. But how is this electrical activity or integration of the grafted cells achieved? The question remains unanswered. One possibility is that the host needs to have one or more individual-specific traits to provide a permissive microenvironment for the correct integration of the graft into the host tissue. These traits may involve molecular and cellular signaling pathways and communication between the endogenous and exogenous cells. What are these traits? Are there genetic or immunologic factors involved? Knowing the answer to these questions would allow clinicians to predict who can be a candidate for cell-replacement therapy, or even adjust the microenvironment of a host or the nature of the grafted cells to successfully treat all PD patients in an individualized manner. Current technology can be used for answering these questions. For example, current genome engineering technology such as CRISPR-Cas (see[95] for review) and TetR-, Cre- or Flp-mediated DNA recombination (see[96] for review) could allow us to delete, insert, reverse, silence or enhance the expression of different genes in order to elucidate the factors involved in the permissibility of the host. This technology would also contribute to understanding the mechanisms and molecules involved in the communication between the cells from the graft and those from the host. Additionally, regarding the influence of the microenvironment on the grafted cells, it has been shown that uncommitted ESC-derived cells grafted into different areas of the brain are capable of sensing the host site, and respond by modifying their survival and differentiation into a specific cell type[97].

Another important aspect is to understand the mechanisms related to the functionality of the graft. The unanswered questions in this regard are more related to systems-biology aspects concerning the consequences of the graft on the basal ganglia circuit. Further studies are necessary to determine the physiological consequences of grafting over the altered basal ganglia connections during natural behavior, as opposed to the use of pharmacological tools. By combining current approximations such as *in vivo* electrophysiological recordings or optogenetic activation and calcium imaging, it would be possible to determine whether grafts have differential effects on the activity of the direct and indirect pathways of the basal ganglia, and in general over the dynamics of the striatal microcircuit. These technologies have been used for the study of the normal function of the basal ganglia circuit and have also been applied to animal models of PD (*e.g.*, Ref[98-100]). Additionally, by coupling *in vivo* pharmacology experiments with optogenetics[101], we can understand more about the mechanisms underlying the functionality of the grafts in PD, as has been done recently[93].

Survival of grafted dopaminergic neurons remain as a limitation; only 1% to 20% of FVM-derived cells are able to survive in animal models of PD[102]. Different cellular stress responses occurring by the dissection of the cells and after the graft procedure are part responsible for the observed cell death[102]. The majority of the studies that follow graft survival and behavior in animal models focus on analyzing short and medium periods of time (*e.g.*, Ref[64,102]). However, despite the low survival of grafted cells, clinical trials have shown cases with significant motor improvements that last for varying time periods (*e.g.*, over some years to 20 years after grafting of human mesencephalic tissue[103]). Thus, as long as the underlying mechanisms related to the variability observed between subjects is comprehended, controlled and reduced, transplantation of dopaminergic-containing cells could be a potential treatment for motor symptoms in PD.

Finally, we have to remember that PD is a very complex disease that affects other systems in addition to the dopaminergic pathway[104]. Thus, the aim of cell replacement therapy in PD is merely symptomatic, and focused exclusively on the motor symptoms associated with the degeneration of the nigrostriatal pathway. An important concern related to the pathology *per se* is the fact that some PD-grafted patients have shown Lewy-body inclusions in the grafted cells[105]. Lewy-bodies are aggregates of normal, misfolded and truncated proteins and ubiquitin enzymes mainly composed of α-synuclein, and constitute the histological hallmark of PD (see[106] for review). This discovery is part of the evidence that supports the idea that PD spreads as a prion-like pathology (see[107] for review). Thus, it is probable that independently of the site of grafting, striatum or SNpc, the grafted cells will eventually develop the pathology. However, as Petit, Olsson and Brundin[108] have argued, the observation of Lewy-body inclusions does not necessarily invalidate the cell replacement therapy approach, based on the following arguments: Some patients have demonstrated motor improvements for up to 18 years; only a small proportion of grafted cells present Lewy-body inclusions; and finally we have to examine the cost-effectiveness relationship. Despite the logic of the arguments, on which we agree, we still have to remember that cell replacement therapy is not a cure for the disease, but rather a symptomatic relief. Thus, understanding the mechanisms related to the pathophysiology of PD is of fundamental importance if we wish to provide a more definitive strategy to face this disease (for review see[109]).

CONCLUSION

Important progress has been made since the first demonstration of a functional effect of dopaminergic-cell grafts in an animal model of PD. After the first decade of cell grafting in PD, it was clear that FVM-derived cells

were a better cell source for grafting in comparison to chromaffin cells derived from the AM. To date FVM-derived cells are considered as the most promising source for cell therapy in PD. After all these years of extensive efforts, it has been demonstrated that striatal FVM grafts survive, extend projections, release dopamine and more importantly, alleviate motor alterations in both animal models and in human subjects with Parkinson's disease. Cell integration is also important for achieving a positive functional outcome in other cell sources such as ESC-derived dopaminergic neurons. In addition, midbrain dopaminergic neuron grafts placed directly into the SNpc have also been shown to survive, to extend projections into the striatum, to increase striatal dopamine content, and to induce functional recovery. These observations are important and encouraging as they point to the possibility of reconstructing the nigrostriatal dopaminergic pathway.

ACKNOWLEDGMENTS

We thank Dr. Andres Jara-Oseguera (NIH-NINDS) for critical comments and his contribution to editing the manuscript. We thank to Dr. Fatuel Tecuapetla (IFC-UNAM) for his helpful discussions. We are also grateful to Alejandra Morato-Torres, Marcela Palomero-Rivero, Diana Millán-Aldaco and Ruth Rincón Heredia for their critical comments. Finally, we thank to Francisco Pérez-Eugenio and Ana María Escalante (computer facility) for their technical assistance.

REFERENCES

1 **Perlow MJ**, Freed WJ, Hoffer BJ, Seiger A, Olson L, Wyatt RJ. Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. *Science* 1979; **204**: 643-647 [PMID: 571147 DOI: 10.1126/science.571147]

2 **Björklund A**, Stenevi U. Reconstruction of the nigrostriatal dopamine pathway by intracerebral nigral transplants. *Brain Res* 1979; **177**: 555-560 [PMID: 574053 DOI: 10.1016/0006-8993(79)90472-4]

3 **Thompson V**. Successful brain grafting. *NY Med J* 1890; **51**: 701-702

4 **Del Conte G**. Einpflanzungen von embryonalem Gewebe ins Gehirn. *Beitr Pathol Anat Allg Pathol* 1907; **42**: 193-202

5 **Ranson SW**. Transplantation of the spinal ganglion into the brain. *Q Bull Northwest Univ Med Sch* 1909; **11**: 176-178

6 **Dunn E**. Primary and secondary findings in a series of attempts to transplant cerebral cortex in the albino rat. *J Comp Neurol* 1917; **27**: 565-582 [DOI: 10.1002/cne.900270403]

7 **Faldino G**. Sullo sviluppo dei tessuti embrionali omoplastici innestati nella camera anteriore dell'occhio del coniglio. *Arch Sci Biol* 1924; **5**: 328-346

8 **Willis R**. Experiments on the Intracerebral Implantation of Embryo Tissues in Rats. *Proc R Soc B Ser* 1935; **117**: 400-412 [DOI: 10.1098/rspb.1935.0036]

9 **Ungerstedt U**. 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *Eur J Pharmacol* 1968; **5**: 107-110 [PMID: 5718510 DOI: 10.1016/0014-2999(68)90164-7]

10 **Ungerstedt U**, Butcher LL, Butcher SG, Andén NE, Fuxe K. Direct chemical stimulation of dopaminergic mechanisms in the neostriatum of the rat. *Brain Res* 1969; **14**: 461-471 [PMID: 4893861 DOI: 10.1016/0006-8993(69)90122-X]

11 **Freed WJ**, Morihisa JM, Spoor E, Hoffer BJ, Olson L, Seiger A, Wyatt RJ. Transplanted adrenal chromaffin cells in rat brain reduce lesion-induced rotational behaviour. *Nature* 1981; **292**: 351-352 [PMID: 7254334 DOI: 10.1038/292351a0]

12 **Dahlstroem A**, Fuxe K. Evidence for the existence of monoamine-containing neurons in the central nervous system. i. demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol Scand Suppl* 1964; **SUPPL 232**: 1-55 [PMID: 14229500]

13 **Björklund A**, Dunnett SB, Stenevi U, Lewis ME, Iversen SD. Reinnervation of the denervated striatum by substantia nigra transplants: functional consequences as revealed by pharmacological and sensorimotor testing. *Brain Res* 1980; **199**: 307-333 [PMID: 7417786 DOI: 10.1016/0006-8993(80)90692-7]

14 **Freed WJ**, Perlow MJ, Karoum F, Seiger A, Olson L, Hoffer BJ, Wyatt RJ. Restoration of dopaminergic function by grafting of fetal rat substantia nigra to the caudate nucleus: long-term behavioral, biochemical, and histochemical studies. *Ann Neurol* 1980; **8**: 510-519 [PMID: 7436393 DOI: 10.1002/ana.410080508]

15 **Dunnett SB**, Björklund A, Schmidt RH, Stenevi U, Iversen SD. Intracerebral grafting of neuronal cell suspensions. IV. Behavioural recovery in rats with unilateral 6-OHDA lesions following implantation of nigral cell suspensions in different forebrain sites. *Acta Physiol Scand Suppl* 1983; **522**: 29-37 [PMID: 6426250]

16 **Herman JP**, Choulli K, Geffard M, Nadaud D, Taghzouti K, Le Moal M. Reinnervation of the nucleus accumbens and frontal cortex of the rat by dopaminergic grafts and effects on hoarding behavior. *Brain Res* 1986; **372**: 210-216 [PMID: 3011207 DOI: 10.1016/0006-8993(86)91127-3]

17 **Brundin P**, Isacson O, Björklund A. Monitoring of cell viability in suspensions of embryonic CNS tissue and its use as a criterion for intracerebral graft survival. *Brain Res* 1985; **331**: 251-259 [PMID: 3986568 DOI: 10.1016/0006-8993(85)91550-1]

18 **Brundin P**, Nilsson OG, Strecker RE, Lindvall O, Astedt B, Björklund A. Behavioural effects of human fetal dopamine neurons grafted in a rat model of Parkinson's disease. *Exp Brain Res* 1986; **65**: 235-240 [PMID: 3542544 DOI: 10.1007/BF00243848]

19 **Sauer H**, Brundin P. Effects of cool storage on survival and function of intrastriatal ventral mesencephalic grafts. *Restor Neurol Neurosci* 1991; **2**: 123-135 [PMID: 21551593 DOI: 10.3233/RNN-1991-2302]

20 **Nishino H**, Ono T, Shibata R, Kawamata S, Watanabe H, Shiosaka S, Tohyama M, Karadi Z. Adrenal medullary cells transmute into dopaminergic neurons in dopamine-depleted rat caudate and ameliorate motor disturbances. *Brain Res* 1988; **445**: 325-337 [PMID: 2897225 DOI: 10.1016/0006-8993(88)91195-X]

21 **Björklund A**, Schmidt RH, Stenevi U. Functional reinnervation of the neostriatum in the adult rat by use of intraparenchymal grafting of dissociated cell suspensions from the substantia nigra. *Cell Tissue Res* 1980; **212**: 39-45 [PMID: 6254660 DOI: 10.1007/BF00234031]

22 **Kirik D**, Winkler C, Björklund A. Growth and functional efficacy of intrastriatal nigral transplants depend on the extent of nigrostriatal degeneration. *J Neurosci* 2001; **21**: 2889-2896 [PMID: 11306640]

23 **Strecker RE**, Sharp T, Brundin P, Zetterström T, Ungerstedt U, Björklund A. Autoregulation of dopamine release and metabolism by intrastriatal nigral grafts as revealed by intracerebral dialysis. *Neuroscience* 1987; **22**: 169-178 [PMID: 2819773 DOI: 10.1016/0306-4522(87)90207-7]

24 **Schmidt RH**, Björklund A, Stenevi U, Dunnett SB, Gage FH. Intracerebral grafting of neuronal cell suspensions. III. Activity of intrastriatal nigral suspension implants as assessed by measurements of dopamine synthesis and metabolism. *Acta Physiol Scand Suppl* 1983; **522**: 19-28 [PMID: 6326471]

25 **Freed WJ**, Ko GN, Niehoff DL, Kuhar MJ, Hoffer BJ, Olson L, Cannon-Spoor HE, Morihisa JM, Wyatt RJ. Normalization of spiroperidol binding in the denervated rat striatum by homologous grafts of substantia nigra. *Science* 1983; **222**: 937-939 [PMID: 6635666 DOI: 10.1126/science.6635666]

26 **Zetterström T**, Brundin P, Gage FH, Sharp T, Isacson O, Dunnett SB, Ungerstedt U, Björklund A. In vivo measurement of spontaneous release and metabolism of dopamine from intrastriatal nigral grafts using intracerebral dialysis. *Brain Res* 1986; **362**: 344-349 [PMID: 2417666 DOI: 10.1016/0006-8993(86)90460-9]

27 **Dunnett SB**, Björklund A, Stenevi U, Iversen SD. Grafts of embryonic substantia nigra reinnervating the ventrolateral striatum ameliorate sensorimotor impairments and akinesia in rats with 6-OHDA lesions

of the nigrostriatal pathway. *Brain Res* 1981; **229**: 209-217 [PMID: 6796195 DOI: 10.1016/0006-8993(81)90759-9]

28 **Dunnett SB**, Whishaw IQ, Rogers DC, Jones GH. Dopamine-rich grafts ameliorate whole body motor asymmetry and sensory neglect but not independent limb use in rats with 6-hydroxydopamine lesions. *Brain Res* 1987; **415**: 63-78 [PMID: 3113665 DOI: 10.1016/0006-8993(87)90269-1]

29 **Montoya CP**, Astell S, Dunnett SB. Effects of nigral and striatal grafts on skilled forelimb use in the rat. *Prog Brain Res* 1990; **82**: 459-466 [PMID: 2127111 DOI: 10.1016/s0079-6123(08)62634-5]

30 **Barker RA**, Drouin-Ouellet J, Parmar M. Cell-based therapies for Parkinson disease-past insights and future potential. *Nat Rev Neurol* 2015; **11**: 492-503 [DOI: 10.1038/nrneurol.2015.123]

31 **Fernandez-Espejo E**, Armengol JA, Flores JA, Galan-Rodriguez B, Ramiro S. Cells of the sympathoadrenal lineage: biological properties as donor tissue for cell-replacement therapies for Parkinson's disease. *Brain Res Brain Res Rev* 2005; **49**: 343-354 [PMID: 16111561 DOI: 10.1016/j.brainresrev.2005.01.004]

32 **Freed WJ**, Karoum F, Spoor HE, Morihisa JM, Olson L, Wyatt RJ. Catecholamine content of intracerebral adrenal medulla grafts. *Brain Res* 1983; **269**: 184-189 [PMID: 6683581 DOI: 10.1016/0006-8993(83)90980-0]

33 **Date I**, Felten SY, Olschowka JA, Felten DL. Limited recovery of striatal dopaminergic fibers by adrenal medullary grafts in MPTP-treated aging mice. *Exp Neurol* 1990; **107**: 197-207 [PMID: 2307201 DOI: 10.1016/0014-4886(90)90136-G]

34 **Hallman H**, Lange J, Olson L, Strömberg I, Jonsson G. Neurochemical and histochemical characterization of neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on brain catecholamine neurones in the mouse. *J Neurochem* 1985; **44**: 117-127 [PMID: 3880581 DOI: 10.1111/j.1471-4159.1985.tb07120.x]

35 **Bohn MC**, Cupit L, Marciano F, Gash DM. Adrenal medulla grafts enhance recovery of striatal dopaminergic fibers. *Science* 1987; **237**: 913-916 [PMID: 2887034 DOI: 10.1126/science.2887034]

36 **Fiandaca MS**, Kordower JH, Hansen JT, Jiao SS, Gash DM. Adrenal medullary autografts into the basal ganglia of Cebus monkeys: injury-induced regeneration. *Exp Neurol* 1988; **102**: 76-91 [PMID: 2903068 DOI: 10.1016/0014-4886(88)90080-5]

37 **Bankiewicz KS**, Plunkett RJ, Kophin IJ, Jacobowitz DM, London WT, Oldfield EH. Transient behavioral recovery in hemiparkinsonian primates after adrenal medullary allografts. *Prog Brain Res* 1988; **78**: 543-549 [PMID: 3247447 DOI: 10.1016/s0079-6123(08)60329-5]

38 **Brown VJ**, Dunnett SB. Comparison of adrenal and foetal nigral grafts on drug-induced rotation in rats with 6-OHDA lesions. *Exp Brain Res* 1989; **78**: 214-218 [PMID: 2512178 DOI: 10.1007/BF00230701]

39 **Backlund EO**, Granberg PO, Hamberger B, Knutsson E, Mårtensson A, Sedvall G, Seiger A, Olson L. Transplantation of adrenal medullary tissue to striatum in parkinsonism. First clinical trials. *J Neurosurg* 1985; **62**: 169-173 [PMID: 2578558 DOI: 10.3171/jns.1985.62.2.0169]

40 **Lindvall O**, Backlund EO, Farde L, Sedvall G, Freedman R, Hoffer B, Nobin A, Seiger A, Olson L. Transplantation in Parkinson's disease: two cases of adrenal medullary grafts to the putamen. *Ann Neurol* 1987; **22**: 457-468 [PMID: 3435067 DOI: 10.1002/ana.410220403]

41 **Madrazo I**, Drucker-Colín R, Díaz V, Martínez-Mata J, Torres C, Becerril JJ. Open microsurgical autograft of adrenal medulla to the right caudate nucleus in two patients with intractable Parkinson's disease. *N Engl J Med* 1987; **316**: 831-834 [PMID: 3821826 DOI: 10.1056/NEJM198704023161402]

42 **Drucker-Colín R**, Madrazo I, Ostrosky-Solís F, Shkurovich M, Franco R, Torres C. Adrenal medullary tissue transplants in the caudate nucleus of Parkinson's patients. *Prog Brain Res* 1988; **78**: 567-574 [PMID: 3247451 DOI: 10.1016/S0079-6123(08)60332-5]

43 **Hirsch EC**, Duyckaerts C, Javoy-Agid F, Hauw JJ, Agid Y. Does adrenal graft enhance recovery of dopaminergic neurons in Parkinson's disease? *Ann Neurol* 1990; **27**: 676-682 [PMID: 2360804 DOI: 10.1002/ana.410270615]

44 **Goetz CG**, Stebbins GT, Klawans HL, Koller WC, Grossman RG, Bakay RA, Penn RD. United Parkinson Foundation Neurotransplantation Registry on adrenal medullary transplants: presurgical, and 1- and 2-year follow-up. *Neurology* 1991; **41**: 1719-1722 [PMID: 1944898 DOI: 10.1212/WNL.41.11.1719]

45 **Peterson DI**, Price ML, Small CS. Autopsy findings in a patient who had an adrenal-to-brain transplant for Parkinson's disease. *Neurology* 1989; **39**: 235-238 [PMID: 2915795 DOI: 10.1212/WNL.39.2.235]

46 **Drucker-Colín R**, Verdugo-Díaz L, Morgado-Valle C, Solís-Maldonado G, Ondarza R, Boll C, Miranda G, Wang GJ, Volkow N. Transplant of cultured neuron-like differentiated chromaffin cells in a Parkinson's disease patient. A preliminary report. *Arch Med Res* 1999; **30**: 33-39 [PMID: 10071423 DOI: 10.1016/S0188-0128(98)00007-4]

47 **Lindvall O**, Brundin P, Widner H, Rehncrona S, Gustavii B, Frackowiak R, Leenders KL, Sawle G, Rothwell JC, Marsden CD. Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science* 1990; **247**: 574-577 [PMID: 2105529 DOI: 10.1126/science.2105529]

48 **Sawle GV**, Bloomfield PM, Björklund A, Brooks DJ, Brundin P, Leenders KL, Lindvall O, Marsden CD, Rehncrona S, Widner H. Transplantation of fetal dopamine neurons in Parkinson's disease: PET [18F]6-L-fluorodopa studies in two patients with putaminal implants. *Ann Neurol* 1992; **31**: 166-173 [PMID: 1575455 DOI: 10.1002/ana.410310207]

49 **Kordower JH**, Freeman TB, Snow BJ, Vingerhoets FJ, Mufson EJ, Sanberg PR, Hauser RA, Smith DA, Nauert GM, Perl DP. Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. *N Engl J Med* 1995; **332**: 1118-1124 [PMID: 7700284 DOI: 10.1056/NEJM199504273321702]

50 **Gildenberg PL**, Pettigrew LC, Merrell R, Butler I, Conklin R, Katz J, DeFrance J. Transplantation of adrenal medullary tissue to caudate nucleus using stereotactic techniques. *Stereotact Funct Neurosurg* 1990; **54-55**: 268-271 [PMID: 2080340 DOI: 10.1159/000100221]

51 **Madrazo I**, León V, Torres C, Aguilera MC, Varela G, Alvarez F, Fraga A, Drucker-Colín R, Ostrosky F, Skurovich M. Transplantation of fetal substantia nigra and adrenal medulla to the caudate nucleus in two patients with Parkinson's disease. *N Engl J Med* 1988; **318**: 51 [PMID: 3336384 DOI: 10.1056/NEJM198801073180115]

52 **Hauser RA**, Freeman TB, Snow BJ, Nauert M, Gauger L, Kordower JH, Olanow CW. Long-term evaluation of bilateral fetal nigral transplantation in Parkinson disease. *Arch Neurol* 1999; **56**: 179-187 [PMID: 10025423 DOI: 10.1001/archneur.56.2.179]

53 **Freed CR**, Greene PE, Breeze RE, Tsai WY, DuMouchel W, Kao R, Dillon S, Winfield H, Culver S, Trojanowski JQ, Eidelberg D, Fahn S. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. *N Engl J Med* 2001; **344**: 710-719 [PMID: 11236774 DOI: 10.1056/NEJM200103083441002]

54 **Olanow CW**, Goetz CG, Kordower JH, Stoessl AJ, Sossi V, Brin MF, Shannon KM, Nauert GM, Perl DP, Godbold J, Freeman TB. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. *Ann Neurol* 2003; **54**: 403-414 [PMID: 12953276 DOI: 10.1002/ana.10720]

55 **Allan LE**, Petit GH, Brundin P. Cell transplantation in Parkinson's disease: problems and perspectives. *Curr Opin Neurol* 2010; **23**: 426-432 [PMID: 20489615 DOI: 10.1097/WCO.0b013e32833b1f62]

56 **Watts RL**, Raiser CD, Stover NP, Cornfeldt ML, Schweikert AW, Allen RC, Subramanian T, Doudet D, Honey CR, Bakay RA. Stereotaxic intrastriatal implantation of human retinal pigment epithelial (hRPE) cells attached to gelatin microcarriers: a potential new cell therapy for Parkinson's disease. *J Neural Transm Suppl* 2003; **(65)**: 215-227 [PMID: 12946059 DOI: 10.1007/978-3-7091-0643-3_14]

57 **Farag ES**, Vinters HV, Bronstein J. Pathologic findings in retinal pigment epithelial cell implantation for Parkinson disease. *Neurology* 2009; **73**: 1095-1102 [PMID: 19726750 DOI: 10.1212/WNL.0b013e3181bbff1c]

58 **Lévesque M**, Neuman T, Rezak M. Therapeutic microinjection of autologous adult human neural stem cells and differentiated neurons for Parkinson's disease: five-year post-operative outcome. *Open Stem Cell J* 2009; **1**: 20-29 [DOI: 10.2174/1876893800901010020]

59 **Venkataramana NK**, Kumar SK, Balaraju S, Radhakrishnan RC, Bansal A, Dixit A, Rao DK, Das M, Jan M, Gupta PK, Totey SM. Open-labeled study of unilateral autologous bone-marrow-derived

mesenchymal stem cell transplantation in Parkinson's disease. *Transl Res* 2010; **155**: 62-70 [PMID: 20129486 DOI: 10.1016/j.trsl.2009.07.006]

60 **Brundin P**, Barker RA, Parmar M. Neural grafting in Parkinson's disease Problems and possibilities. *Prog Brain Res* 2010; **184**: 265-294 [PMID: 20887880 DOI: 10.1016/S0079-6123(10)84014-2]

61 **Barker RA**, Barrett J, Mason SL, Björklund A. Fetal dopaminergic transplantation trials and the future of neural grafting in Parkinson's disease. *Lancet Neurol* 2013; **12**: 84-91 [PMID: 23237903 DOI: 10.1016/S1474-4422(12)70295-8]

62 **Brundin P**, Barbin G, Strecker RE, Isacson O, Prochiantz A, Björklund A. Survival and function of dissociated rat dopamine neurones grafted at different developmental stages or after being cultured in vitro. *Brain Res* 1988; **467**: 233-243 [PMID: 3378172 DOI: 10.1016/0165-3806(88)90027-2]

63 **Rioux L**, Gaudin DP, Bui LK, Grégoire L, DiPaolo T, Bédard PJ. Correlation of functional recovery after a 6-hydroxydopamine lesion with survival of grafted fetal neurons and release of dopamine in the striatum of the rat. *Neuroscience* 1991; **40**: 123-31 [DOI: 10.1016/0306-4522(91)90179-R]

64 **Torres EM**, Monville C, Gates MA, Bagga V, Dunnett SB. Improved survival of young donor age dopamine grafts in a rat model of Parkinson's disease. *Neuroscience* 2007; **146**: 1606-1617 [PMID: 17478050 DOI: 10.1016/j.neuroscience.2007.03.037]

65 **Torres EM**, Dowd E, Dunnett SB. Recovery of functional deficits following early donor age ventral mesencephalic grafts in a rat model of Parkinson's disease. *Neuroscience* 2008; **154**: 631-640 [PMID: 18468807 DOI: 10.1016/j.neuroscience.2008.03.048]

66 **Rath A**, Klein A, Papazoglou A, Pruszak J, Garcia J, Krause M, Maciaczyk J, Dunnett SB, Nikkhah G. Survival and functional restoration of human fetal ventral mesencephalon following transplantation in a rat model of Parkinson's disease. *Cell Transplant* 2013; **22**: 1281-1293 [PMID: 22963760 DOI: 10.3727/096368912X654984]

67 **Grealish S**, Diguet E, Kirkeby A, Mattsson B, Heuer A, Bramoulle Y, Van Camp N, Perrier AL, Hantraye P, Björklund A, Parmar M. Human ESC-derived dopamine neurons show similar preclinical efficacy and potency to fetal neurons when grafted in a rat model of Parkinson's disease. *Cell Stem Cell* 2014; **15**: 653-665 [PMID: 25517469 DOI: 10.1016/j.stem.2014.09.017]

68 **Björklund A**, Hökfelt T. Handbook of Chemical Neuroanatomy. Amsterdam: Elsevier, 1983: 560

69 **Mendez I**, Sanchez-Pernaute R, Cooper O, Viñuela A, Ferrari D, Björklund L, Dagher A, Isacson O. Cell type analysis of functional fetal dopamine cell suspension transplants in the striatum and substantia nigra of patients with Parkinson's disease. *Brain* 2005; **128**: 1498-1510 [PMID: 15872020 DOI: 10.1093/brain/awh510]

70 **Thompson L**, Barraud P, Andersson E, Kirik D, Björklund A. Identification of dopaminergic neurons of nigral and ventral tegmental area subtypes in grafts of fetal ventral mesencephalon based on cell morphology, protein expression, and efferent projections. *J Neurosci* 2005; **25**: 6467-6477 [PMID: 16000637 DOI: 10.1523/JNEUROSCI.1676-05.2005]

71 **Grealish S**, Jönsson ME, Li M, Kirik D, Björklund A, Thompson LH. The A9 dopamine neuron component in grafts of ventral mesencephalon is an important determinant for recovery of motor function in a rat model of Parkinson's disease. *Brain* 2010; **133**: 482-495 [PMID: 20123725 DOI: 10.1093/brain/awp328]

72 **Mandel RJ**, Brundin P, Björklund A. The Importance of Graft Placement and Task Complexity for Transplant-Induced Recovery of Simple and Complex Sensorimotor Deficits in Dopamine Denervated Rats. *Eur J Neurosci* 1990; **2**: 888-894 [PMID: 12106096 DOI: 10.1111/j.1460-9568.1990.tb00400.x]

73 **Gaillard A**, Jaber M. Rewiring the brain with cell transplantation in Parkinson's disease. *Trends Neurosci* 2011; **34**: 124-133 [PMID: 21316770 DOI: 10.1016/j.tins.2011.01.003]

74 **Nikkhah G**, Bentlage C, Cunningham MG, Björklund A. Intranigral fetal dopamine grafts induce behavioral compensation in the rat Parkinson model. *J Neurosci* 1994; **14**: 3449-3461 [PMID: 7911516]

75 **Brecknell JE**, Du JS, Muir E, Fidler PS, Hlavin ML, Dunnett SB, Fawcett JW. Bridge grafts of fibroblast growth factor-4-secreting schwannoma cells promote functional axonal regeneration in the nigrostriatal pathway of the adult rat. *Neuroscience* 1996; **74**: 775-784 [PMID: 8884773 DOI: 10.1016/0306-4522(96)00167-4]

76 **Wang Y**, Tien LT, Lapchak PA, Hoffer BJ. GDNF triggers fiber outgrowth of fetal ventral mesencephalic grafts from nigra to striatum in 6-OHDA-lesioned rats. *Cell Tissue Res* 1996; **286**: 225-233 [PMID: 8854891 DOI: 10.1007/s004410050691]

77 **Wilby MJ**, Sinclair SR, Muir EM, Zietlow R, Adcock KH, Horellou P, Rogers JH, Dunnett SB, Fawcett JW. A glial cell line-derived neurotrophic factor-secreting clone of the Schwann cell line SCTM41 enhances survival and fiber outgrowth from embryonic nigral neurons grafted to the striatum and to the lesioned substantia nigra. *J Neurosci* 1999; **19**: 2301-2312 [PMID: 10066280]

78 **Collier TJ**, Sortwell CE, Elsworth JD, Taylor JR, Roth RH, Sladek JR, Redmond DE. Embryonic ventral mesencephalic grafts to the substantia nigra of MPTP-treated monkeys: feasibility relevant to multiple-target grafting as a therapy for Parkinson's disease. *J Comp Neurol* 2002; **442**: 320-330 [PMID: 11793337 DOI: 10.1002/cne.10108]

79 **Dunnett SB**, Rogers DC, Richards SJ. Nigrostriatal reconstruction after 6-OHDA lesions in rats: combination of dopamine-rich nigral grafts and nigrostriatal "bridge" grafts. *Exp Brain Res* 1989; **75**: 523-535 [PMID: 2568267 DOI: 10.1007/BF00249903]

80 **Nikkhah G**, Cunningham MG, Jödicke A, Knappe U, Björklund A. Improved graft survival and striatal reinnervation by microtransplantation of fetal nigral cell suspensions in the rat Parkinson model. *Brain Res* 1994; **633**: 133-143 [PMID: 7907929 DOI: 10.1016/0006-8993(94)91532-6]

81 **Hallett PJ**, Deleidi M, Astradsson A, Smith GA, Cooper O, Osborn TM, Sundberg M, Moore MA, Perez-Torres E, Brownell AL, Schumacher JM, Spealman RD, Isacson O. Successful function of autologous iPSC-derived dopamine neurons following transplantation in a non-human primate model of Parkinson's disease. *Cell Stem Cell* 2015; **16**: 269-274 [PMID: 25732245 DOI: 10.1016/j.stem.2015.01.018]

82 **Mendoza-Ramírez JL**, Aguilar-Roblero R, Zainos-Rosales A, Drucker-Colín R. Fetal substantia nigra and adrenal medullary grafts placed contralateral to the nigrostriatal lesion side induce a decrease in turning behavior but not in dopamine receptor density. *Restor Neurol Neurosci* 1991; **3**: 177-185 [PMID: 21551635 DOI: 10.3233/RNN-1991-3402]

83 **Wang Y**, Wang SD, Lin SZ, Liu JC. Restoration of dopamine overflow and clearance from the 6-hydroxydopamine lesioned rat striatum reinnervated by fetal mesencephalic grafts. *J Pharmacol Exp Ther* 1994; **270**: 814-821 [PMID: 7915321]

84 **Wang Y**, Lin JC, Chiou AL, Liu JY, Liu JC, Zhou FC. Human ventromesencephalic grafts restore dopamine release and clearance in hemiparkinsonian rats. *Exp Neurol* 1995; **136**: 98-106 [PMID: 7498419 DOI: 10.1006/exnr.1995.1087]

85 **Kirkeby A**, Grealish S, Wolf DA, Nelander J, Wood J, Lundblad M, Lindvall O, Parmar M. Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. *Cell Rep* 2012; **1**: 703-714 [PMID: 22813745 DOI: 10.1016/j.celrep.2012.04.009]

86 **Gaillard A**, Decressac M, Frappé I, Fernagut PO, Prestoz L, Besnard S, Jaber M. Anatomical and functional reconstruction of the nigrostriatal pathway by intranigral transplants. *Neurobiol Dis* 2009; **35**: 477-488 [PMID: 19616502 DOI: 10.1016/j.nbd.2009.07.003]

87 **Thompson LH**, Grealish S, Kirik D, Björklund A. Reconstruction of the nigrostriatal dopamine pathway in the adult mouse brain. *Eur J Neurosci* 2009; **30**: 625-638 [PMID: 19674082 DOI: 10.1111/j.1460-9568.2009.06878.x]

88 **Arbuthnott G**, Dunnett S, MacLeod N. Electrophysiological properties of single units in dopamine-rich mesencephalic transplants in rat brain. *Neurosci Lett* 1985; **57**: 205-210 [PMID: 2993967 DOI: 10.1016/0304-3940(85)90064-3]

89 **Strömberg I**, Johnson S, Hoffer B, Olson L. Reinnervation of dopamine-denervated striatum by substantia nigra transplants: immunohistochemical and electrophysiological correlates. *Neuroscience* 1985; **14**: 981-990

[PMID: 2860618 DOI: 10.1016/0306-4522(85)90270-2]
90 **Freund TF**, Bolam JP, Björklund A, Stenevi U, Dunnett SB, Powell JF, Smith AD. Efferent synaptic connections of grafted dopaminergic neurons reinnervating the host neostriatum: a tyrosine hydroxylase immunocytochemical study. *J Neurosci* 1985; **5**: 603-616 [PMID: 2857778]
91 **Mahalik TJ**, Finger TE, Stromberg I, Olson L. Substantia nigra transplants into denervated striatum of the rat: ultrastructure of graft and host interconnections. *J Comp Neurol* 1985; **240**: 60-70 [PMID: 2865279 DOI: 10.1002/cne.902400105]
92 **Sørensen AT**, Thompson L, Kirik D, Björklund A, Lindvall O, Kokaia M. Functional properties and synaptic integration of genetically labelled dopaminergic neurons in intrastriatal grafts. *Eur J Neurosci* 2005; **21**: 2793-2799 [PMID: 15926926 DOI: 10.1111/j.1460-9568.2005.04116.x]
93 **Steinbeck JA**, Choi SJ, Mrejeru A, Ganat Y, Deisseroth K, Sulzer D, Mosharov EV, Studer L. Optogenetics enables functional analysis of human embryonic stem cell-derived grafts in a Parkinson's disease model. *Nat Biotechnol* 2015; **33**: 204-209 [PMID: 25580598 DOI: 10.1038/nbt.3124]
94 **Dell'Anno MT**, Caiazzo M, Leo D, Dvoretskova E, Medrihan L, Colasante G, Giannelli S, Theka I, Russo G, Mus L, Pezzoli G, Gainetdinov RR, Benfenati F, Taverna S, Dityatev A, Broccoli V. Remote control of induced dopaminergic neurons in parkinsonian rats. *J Clin Invest* 2014; **124**: 3215-3229 [PMID: 24937431 DOI: 10.1172/JCI74664]
95 **Sander JD**, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat Biotechnol* 2014; **32**: 347-355 [PMID: 24584096 DOI: 10.1038/nbt.2842]
96 **Lewandoski M**. Conditional control of gene expression in the mouse. *Nat Rev Genet* 2001; **2**: 743-755 [PMID: 11584291 DOI: 10.1038/35093537]
97 **Maya-Espinosa G**, Collazo-Navarrete O, Millán-Aldaco D, Palomero-Rivero M, Guerrero-Flores G, Drucker-Colín R, Covarrubias L, Guerra-Crespo M. Mouse embryonic stem cell-derived cells reveal niches that support neuronal differentiation in the adult rat brain. *Stem Cells* 2015; **33**: 491-502 [PMID: 25267362 DOI: 10.1002/stem.1856]
98 **Jáidar O**, Carrillo-Reid L, Hernández A, Drucker-Colín R, Bargas J, Hernández-Cruz A. Dynamics of the Parkinsonian striatal microcircuit: entrainment into a dominant network state. *J Neurosci* 2010; **30**: 11326-11336 [PMID: 20739553 DOI: 10.1523/JNEUROSCI.1380-10.2010]
99 **Kravitz AV**, Freeze BS, Parker PR, Kay K, Thwin MT, Deisseroth K, Kreitzer AC. Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature* 2010; **466**: 622-626 [PMID: 20613723 DOI: 10.1038/nature09159]
100 **Pérez-Ortega J**, Duhne M, Lara-González E, Plata V, Gasca D, Galarraga E, Hernández-Cruz A, Bargas J. Pathophysiological signatures of functional connectomics in parkinsonian and dyskinetic striatal microcircuits. *Neurobiol Dis* 2016; **91**: 347-361 [PMID: 26951948 DOI: 10.1016/j.nbd.2016.02.023]
101 **Jeong JW**, McCall JG, Shin G, Zhang Y, Al-Hasani R, Kim M, Li S, Sim JY, Jang KI, Shi Y, Hong DY, Liu Y, Schmitz GP, Xia L, He Z, Gamble P, Ray WZ, Huang Y, Bruchas MR, Rogers JA. Wireless Optofluidic Systems for Programmable In Vivo Pharmacology and Optogenetics. *Cell* 2015; **162**: 662-674 [PMID: 26189679 DOI: 10.1016/j.cell.2015.06.058]
102 **Brundin P**, Karlsson J, Emgård M, Schierle GS, Hansson O, Petersén A, Castilho RF. Improving the survival of grafted dopaminergic neurons: a review over current approaches. *Cell Transplant* 2000; **9**: 179-195 [PMID: 10811392]
103 **Kefalopoulou Z**, Politis M, Piccini P, Mencacci N, Bhatia K, Jahanshahi M, Widner H, Rehncrona S, Brundin P, Björklund A, Lindvall O, Limousin P, Quinn N, Foltynie T. Long-term clinical outcome of fetal cell transplantation for Parkinson disease: two case reports. *JAMA Neurol* 2014; **71**: 83-87 [PMID: 24217017 DOI: 10.1001/jamaneurol.2013.4749]
104 **Braak H**, Ghebremedhin E, Rüb U, Bratzke H, Del Tredici K. Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res* 2004; **318**: 121-134 [PMID: 15338272 DOI: 10.1007/s00441-004-0956-9]
105 **Kordower JH**, Chu Y, Hauser RA, Freeman TB, Olanow CW. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. *Nat Med* 2008; **14**: 504-506 [PMID: 18391962 DOI: 10.1038/nm1747]
106 **Surmeier DJ**. Calcium, ageing, and neuronal vulnerability in Parkinson's disease. *Lancet Neurol* 2007; **6**: 933-938 [PMID: 17884683 DOI: 10.1016/S1474-4422(07)70246-6]
107 **Chu Y**, Kordower JH. The prion hypothesis of Parkinson's disease. *Curr Neurol Neurosci Rep* 2015; **15**: 28 [PMID: 25868519 DOI: 10.1007/s11910-015-0549-x]
108 **Petit GH**, Olsson TT, Brundin P. The future of cell therapies and brain repair: Parkinson's disease leads the way. *Neuropathol Appl Neurobiol* 2014; **40**: 60-70 [PMID: 24372386 DOI: 10.1111/nan.12110]
109 **Obeso JA**, Rodriguez-Oroz MC, Goetz CG, Marin C, Kordower JH, Rodriguez M, Hirsch EC, Farrer M, Schapira AH, Halliday G. Missing pieces in the Parkinson's disease puzzle. *Nat Med* 2010; **16**: 653-661 [PMID: 20495568 DOI: 10.1038/nm.2165]

P- Reviewer: Scott Goebel W **S- Editor**: Song XX **L- Editor**: A
E- Editor: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 June 24; 7(3): 193-202

DOI: 10.5500/wjt.v7.i3.193

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Basic Study

Copper as an alternative antimicrobial coating for implants - An *in vitro* study

Claudia Bergemann, Sarah Zaatreh, Katharina Wegner, Kathleen Arndt, Andreas Podbielski, Rainer Bader, Cornelia Prinz, Ulrich Lembke, J Barbara Nebe

Claudia Bergemann, J Barbara Nebe, Department of Cell Biology, University Medical Center Rostock, 18057 Rostock, Germany

Sarah Zaatreh, Katharina Wegner, Rainer Bader, Biomechanics and Implant Technology Research Laboratory, University Medical Center Rostock, 18057 Rostock, Germany

Kathleen Arndt, Andreas Podbielski, Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Rostock, 18057 Rostock, Germany

Cornelia Prinz, Ulrich Lembke, DOT GmbH, 18059 Rostock, Germany

Author contributions: Bergemann C, Wegner K and Arndt K performed research study; Lembke U contributed material samples; Bergemann C, Wegner K and Arndt K analyzed data; Bergemann C, Zaatreh S and Wegner K wrote the manuscript; Podbielski A, Bader R, Prinz C and Nebe JB designed the study.

Supported by the Federal Ministry of Education and Research of Germany (BMBF, project CEMOSTOBAS), No. 13N12105.

Institutional review board statement: This study does not involve any human or animal subjects.

Institutional animal care and use committee statement: This study does not involve any animal subjects.

Conflict-of-interest statement: To the best of authors' knowledge, no conflict of interest exists.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Dr. J Barbara Nebe, Professor, Department of Cell Biology, University Medical Center Rostock, Schillingallee 69, 18057 Rostock, Germany. barbara.nebe@med.uni-rostock.de
Telephone: +49-381-4947771
Fax: +49-381-4947764

Received: November 28, 2016
Peer-review started: December 3, 2016
First decision: March 28, 2017
Revised: April 25, 2017
Accepted: May 12, 2017
Article in press: May 15, 2017
Published online: June 24, 2017

Abstract

AIM

To investigate osteoconductive and antimicrobial properties of a titanium-copper-nitride (TiCuN) film and an additional BONIT® coating on titanium substrates.

METHODS

For micro-structuring, the surface of titanium test samples was modified by titanium plasma spray (TPS). On the TPS-coated samples, the TiCuN layer was deposited by physical vapor deposition. The BONIT® layer was coated electrochemically. The concentration of copper ions released from TiCuN films was measured by atomic absorption spectrometry. MG-63 osteoblasts on TiCuN and BONIT® were analyzed for cell adhesion, viability and spreading. In parallel, *Staphylococcus epidermidis* (*S. epidermidis*) were cultivated on the samples and planktonic and biofilm-bound bacteria were quantified by

counting of the colony-forming units.

RESULTS

Field emission scanning electron microscopy (FESEM) revealed rough surfaces for TPS and TiCuN and a special crystalline surface structure on TiCuN + BONIT®. TiCuN released high amounts of copper quickly within 24 h. These release dynamics were accompanied by complete growth inhibition of bacteria and after 2 d, no planktonic or adherent *S. epidermidis* were found on these samples. On the other hand viability of MG-63 cells was impaired during direct cultivation on the samples within 24 h. However, high cell colonization could be found after a 24 h pre-incubation step in cell culture medium simulating the *in vivo* dynamics closer. On pre-incubated TiCuN, the osteoblasts span the ridges and demonstrate a flattened, well-spread phenotype. The additional BONIT®-coating reduced the copper release of the TiCuN layer significantly and showed a positive effect on the initial cell adhesion.

CONCLUSION

The TiCuN-coating inhibits the formation of bacterial biofilms on orthopedic implants by influencing the "race for the surface" to the advantage of osteoblasts.

Key words: Implant-coating; Antimicrobial effect; Titanium plasma spray; Titanium-copper-nitride; BONIT®; Osteoconductivity

Core tip: Implant-associated infection is the most feared complication after joint replacement. We investigated the osteoconductive and antimicrobial properties of a titanium-copper-nitride (TiCuN) film and an additional BONIT® coating on titanium. TiCuN released high amounts of copper quickly within 24 h and after 2 d, no planktonic or adherent Staphylococcus epidermidis were found on these samples. A high colonization by osteoblast-like MG-63 cells was found after pre-incubation in medium for 24 h. TiCuN inhibits the formation of bacterial bio-films on orthopedic implants by influencing the "race for the surface" to the advantage of osteoblasts.

Bergemann C, Zaatreh S, Wegner K, Arndt K, Podbielski A, Bader R, Prinz C, Lembke U, Nebe JB. Copper as an alternative antimicrobial coating for implants - An *in vitro* study. *World J Transplant* 2017; 7(3): 193-202 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i3/193.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i3.193

INTRODUCTION

Materials commonly used for permanent implants such as knee and hip prostheses are for the most part inert. However, researchers have recently taken up the challenge of designing biomaterials which have been physically and/or chemically modified to promote the regenerative processes of the affected tissues[1-3]. Increased surface area (roughness) on implants improves bone-to-implant contact after the implant placement and enhances functional activity of bone cells in contact with the biomaterial[4-7]. Titanium is one of the most common materials used for orthopedic implants[8,9] and surface modifications are created by sandblasting, plasma spraying or etching to accelerate osseointegration[10].

Despite aseptic operation conditions and perioperative antibiotic prophylaxis, implant-associated infections remain one of the most severe complications after joint replacement[11-14], occurring even more frequently after revision arthroplasty[15]. *Staphylococcus epidermidis* (*S. epidermidis*) and *Staphylococcus aureus* are the most frequently found microorganisms causing such implant-associated infections. The pathogenesis of infections associated with biomaterials is as follows: After an initial, reversible adhesion of the bacteria, a biofilm is formed[16-18] which enables the bacteria to avoid immune responses and circumvent antibiotics[19]. Antimicrobial agents do not succeed as well against biofilm bacteria as against planktonic bacteria[19]. In addition, infected medical devices continue to pose problems in orthopedic surgery, thus warranting further development of effective prevention and treatment strategies, including the use of thin coatings based on metal-ions[20]. There are several metal ions (Cu^{2+}, Ag^{+}, Zn^{2+}) which are known to have antibacterial properties and which could be deposited on the surface of implants[21,22]. Silver, for example, has been in use as an antibacterial coating for medical devices[23-26]. However, the lower toxicity and higher cytocompatibility of copper commends this metal ion for deposition on implant surfaces[22]. Furthermore, copper can be metabolized[27], whereas silver tends to resist metabolization, increasing body's silver serum level[28]. Although the general antimicrobial effects of copper have been recognized, to date researchers have little experience with the use of copper as an antimicrobial agent on medical implant surfaces[29-31]. This lack of data on the effects of copper prompted us to study its qualities as an antibacterial agent in this context. We studied the effects of the deposition of a copper-based inter-metallic thin film on titanium plasma spray optimized (TPS) titanium substrates. Our particular interest was in finding a deposited film which exhibits an antimicrobial effect while allowing for sufficient growth and vitality of osteoblasts on the surface. Taking these two factors into account, we investigated the properties and effects of titanium-copper-nitride (TiCuN) films deposited by physical vapor deposition (PVD). For this purpose we studied the chemical composition of the coating and the release of copper from it, investigating its antibacterial properties and the influence on cell growth, as well as determining the influence of an additional osteoconductive coating with a BONIT® layer.

MATERIALS AND METHODS

Preparation of coatings and test samples

Commercially pure titanium (grade 5, DOT, Rostock, Germany) of technical purity was used in the form of cylindrical plates of 11 mm in diameter and 2 mm thick. For micro-structuring, the surface of the test samples was modified by TPS. For the TPS coating, argon is ionized in a high temperature plasma flame in a vacuum chamber. The argon gas heats up and expands rapidly being expelled at high speed through an anode. Simultaneously titanium powder is inserted into the plasma flame and the molten titanium particles adhere to the substrate surface, cool rapidly and fuse to the implant surface. On the TPS-coated titanium test samples, a TiCuN layer with an average copper load of 1-3 μg/mm² was deposited by PVD (DOT). Copper and titanium were released from a target by electricity, ionized and deposited on the sample surface. The procedure developed a face-centered cubic network of titanium atoms with nitrogen ions inserted in the gaps. The TiCuN coating is very thin and only modifies the implant surface, leaving the mechanical properties of the implant unchanged[32-35]. The second coating on the TiCuN-layered samples was a BONIT® layer (DOT) applied using an electrochemical process. Samples were packed into sterilization foils (Direct, Konstanz, Germany), sealed, and gamma-sterilized with a minimum dose of 25 kGy of Co-60 radiation (BBF Sterilisationsservice, Kernen-Rommelshausen, Germany).

We refer to these different samples as follows: TPS: Commercially pure titanium modified by TPS; TiCuN: TPS + TiCuN; TiCuN + BONIT®: TPS + TiCuN + BONIT®.

Characterization of the coatings

Roughness of the sample surfaces was analyzed by a Hommel tester (Hommel Etanic T 8000, Jenoptik, Jena, Germany). Coating thickness and porosity was determined according to the Standard Test Method for Stereological Evaluation of Porous Coatings on Medical Implants ASTM F 1854. Adhesive strength of the coatings was determined according to DIN EN 582 with the universal tensile testing machine Shimadzu AG-50KNG (Shimadzu, Kyoto, Japan). To investigate the surfaces of the different materials, samples were gold sputtered by a coater (SCD 004, BAL-TEC, Balzers, Liechtenstein) and the surfaces were examined by field emission scanning electron microscopy (FESEM, SUPRA 25, Carl Zeiss, Oberkochen, Germany).

Copper release measurement

The concentration of copper released from the samples was measured by atomic absorption spectrometry (AAS) (ZEEnit 650, Analytik Jena AG, Jena, Germany) with electro-thermal atomization as described earlier[36]. Briefly, the substrates were stored in 1 mL Dulbecco's modified Eagle medium (DMEM, Invitrogen, Darmstadt, Germany) with 10% fetal calf serum (FCS, Superior, Biochrome, Berlin, Germany) and 1% gentamicin (Ratiopharm, Ulm, Germany) at 37 ℃ in a humidified atmosphere with 5% CO_2. The copper concentration of this DMEM solution was measured after 24 h and after incubation for another 24 h on three samples each per coating method. Nitric acid was used to stabilize copper ions released in the DMEM after storage. The supernatant was diluted to 1:100000 and a volume of 20 μL of the diluted solution was used for analysis. The intensity measured was compared with the standard reference intensity to obtain the number of copper atoms released from the sample (n = 3). Copper release from samples seeded with MG-63 osteoblasts (see paragraph cell culture) was determined in the supernatant accordingly.

Investigations of antibacterial effects

Estimation of the antibacterial potential against *S. epidermidis* on test samples was completed according to the protocols described earlier[37,38]. The biofilm-forming strain of *S. epidermidis* (ATCC 35984, American Type Culture Collection, Manassas, VA, United States) was routinely cultured on Columbia blood agar plates (Thermo Fisher Scientific, Waltham, MA, United States). Previous to the test, an overnight culture (37 ℃, microaerobic conditions) of *S. epidermidis* was prepared in a tryptic soya broth medium (Sigma-Aldrich, St. Louis, MO, United States). Afterwards, the overnight culture was centrifuged at 4000 rpm for 10 min at 4 ℃, after a washing step the bacteria pellet was diluted in 1 × PBS and adjusted to its strain-specific OD at 600 nm to obtain 1×10^8 CFU/mL in tryptic soya broth medium. For the experiments, bacteria were diluted in DMEM containing 10% FCS until 1×10^3 CFU/mL was achieved. After 2 d of incubation at 37 ℃, 5% CO_2, *S. epidermidis* within the biofilm on the test samples were detached by ultrasonic treatment with a sonication bath for 4 min at 35 kHz (BactoSonic, Bandelin Electronic, Berlin, Germany) and deposited into glass test tubes (Greiner Bio-One, Kremsmünster, Austria) with 1 mL of PBS. Subsequently, the solution was serially diluted in PBS and afterwards plated on TSB-agar with the help of a spiral plater (Eddy Jet 2, IUL, S.A., Barcelona, Spain). After 24 h of incubation at 37 ℃, 5% CO_2, colony-forming units were determined. To analyze the planktonic, unbound *S. epidermidis,* supernatants of the test-samples were shifted into 15 mL centrifuge tubes (Greiner Bio-One) with 1 mL of PBS after 2 d of incubation. Supernatants were centrifuged at 4000 rpm for 10 min at 4 ℃ and diluted consecutively in PBS. To determine the quantity of colony-forming units, dilutions were plated on TSB-agar plates as described above (n = 6).

Cell culture

Human MG-63 osteoblast-like cells (ATCC, No. CRL-1427™, LGC Promochem, Wesel, Germany) were cultured in Dulbecco's modified Eagle medium (DMEM) with 10% FCS and 1% gentamicin at 37 ℃ in a humidified atmosphere containing 5% CO_2. At subconfluency, cells were detached with 0.05% trypsin/0.02% EDTA (PAA Laboratories, Cölbe, Germany) for 5 min at 37 ℃.

Table 1 Characterization of the coatings

Coating	TPS + TiCuN	TiCuN + BONIT®
Coating thickness (μm)	200-400	10-30
Roughness Ra (μm)	30-60	-
Porosity (%)	20-40	60
Adhesive strength (MPa)	74	15

TPS: Titanium plasma spray; TiCuN: Titanium-copper-nitride.

After stopping the trypsinization by the addition of complete cell culture medium, an aliquot of 100 μL was put into 10 mL of CASY® ton buffer solution (Roche Innovatis, Reutlingen, Germany) and the cell number was measured in the counter CASY® Model DT (Schärfe System, Reutlingen, Germany). An appropriate cell number was seeded onto the samples as described for the following applications. Two different experimental arrangements were used: (1) the MG-63 cells were directly cultivated on the samples; and (2) to simulate the *in vivo* dynamics closer, the samples were pre-incubated in cell culture medium DMEM with 10% FCS and 1% gentamicin at 37 ℃ in a humidified atmosphere with 5% CO_2 for 24 h, then the medium was changed and the cells were seeded onto the surfaces and cultivated for another 24 h.

Cell viability

To study the influence of TiCuN on cell metabolism and vitality the MTS assay (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega, Mannheim, Germany) was performed. Forty thousand cells were seeded onto the samples in 24-well plates either directly or on pre-incubated samples at a volume of 1 mL. After 24 h, the cell culture medium was replaced by 800 μL of fresh medium and 200 μL of the MTS solution and incubated for 3 h at 37 ℃ in a 5% CO_2 atmosphere. The spectrophotometric absorption of 5 × 100 μL of the culture medium of 3 samples was analyzed on a 96-well plate by an ELISA reader (Anthos 2010, Anthos Labtec Instruments, Wals-Siezenheim, Austria) at 490 nm (n = 3). The extinction is proportional to the number and the metabolic activity of the cells.

Flow cytometric measurement of cell adhesion

The cell adhesion of MG-63 osteoblasts on the different material surfaces was determined as already described[39]. Briefly, suspended MG-63 cells in DMEM with 10% FCS (5 × 10^4 cells/0.3 mL) were seeded directly onto sample discs. To avoid the seeding of cells beside samples, discs were laterally fixed in adhesive tapes (Carl Roth, Karlsruhe, Germany). After 10 min to allow cell sedimentation and adhesion to the surface, the supernatant containing the non-adherent cells was then drawn up with a pipette, transferred into 12 mm × 75 mm test tubes (BD Biosciences, Heidelberg, Germany) and analyzed by flow cytometry (FACSCalibur™; BD Biosciences). Cell adhesion of 3 independent experiments was then calculated in percent (n = 3).

Cell morphology and spreading

Material samples were pre-incubated in DMEM with 10% FCS and 1% gentamicin at 37 ℃ in a humidified atmosphere with 5% CO_2. After 24 h the medium was changed and 4.0 × 10^4 MG-63 cells were seeded onto the samples. After cultivation for 24 h, cells were washed with PBS, fixed with 4% glutaraldehyde (1 h, Merck, Darmstadt, Germany), dehydrated through a graded series of ethanol (30% 5 min, 50% 5 min, 75% 10 min, 90% 10 min, and 100% 2 × 10 min) and dried in a critical point dryer (K850, EMITECH, Cambridge, United Kingdom). Gold sputtering was performed with the coater (SCD 004, BAL-TEC). The morphology of the cells on the substrate surfaces was investigated by scanning electron microscopy (SEM, DSM 960A, Carl Zeiss). Spreading of the cells was quantified by ImageJ (Rasband, W.S., ImageJ, United States National Institutes of Health, Bethesda, Maryland, United States, http://imagej.nih. gov/ij/, 1997-2016). The cell area of 30 cells in 2 independent experiments was analyzed (n = 60).

Statistical analysis

The statistical significance was calculated using SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, United States). Data are expressed as mean values ± standard deviation (SD) and analyzed using Mann-Whitney U test or the t-test. Values were compared to TPS at the same time point and differences for all experiments were considered statistically significant at $P < 0.05$ ($^aP < 0.05$, $^bP < 0.01$, $^dP < 0.001$).

RESULTS

Sample characteristics

We tested TPS-coated titanium samples equipped with both a TiCuN layer and a BONIT® layer in order to determine their suitability as bone implants encompassing antimicrobial and osteoconductive characteristics. Samples were purchased from DOT Coating (Rostock, Germany). The characteristics of the different coatings are shown in Table 1.

Figure 1 shows FESEM images of the surfaces of the different samples. The visibly rough surface of the samples is caused by the titanium plasma spray technique for TPS and TiCuN. A special crystalline surface structure is visible on TiCuN + BONIT®. BONIT® is an absorbable composite layer of two thin crystalline calcium phosphate phases with different solubility, the more soluble outer calcium phosphate phase (brushite) and the inner crystalline hydroxyapatite phase (≥ 70% brushite and ≤ 30% hydroxyapatite). BONIT® was shown to promote a fast on growth of bone cells and bone formation on implant materials in earlier studies[40-42]. Therefore, we used this coating additionally on the TiCuN films to study the antimicrobial as well as osteoconductive properties combined in one sample.

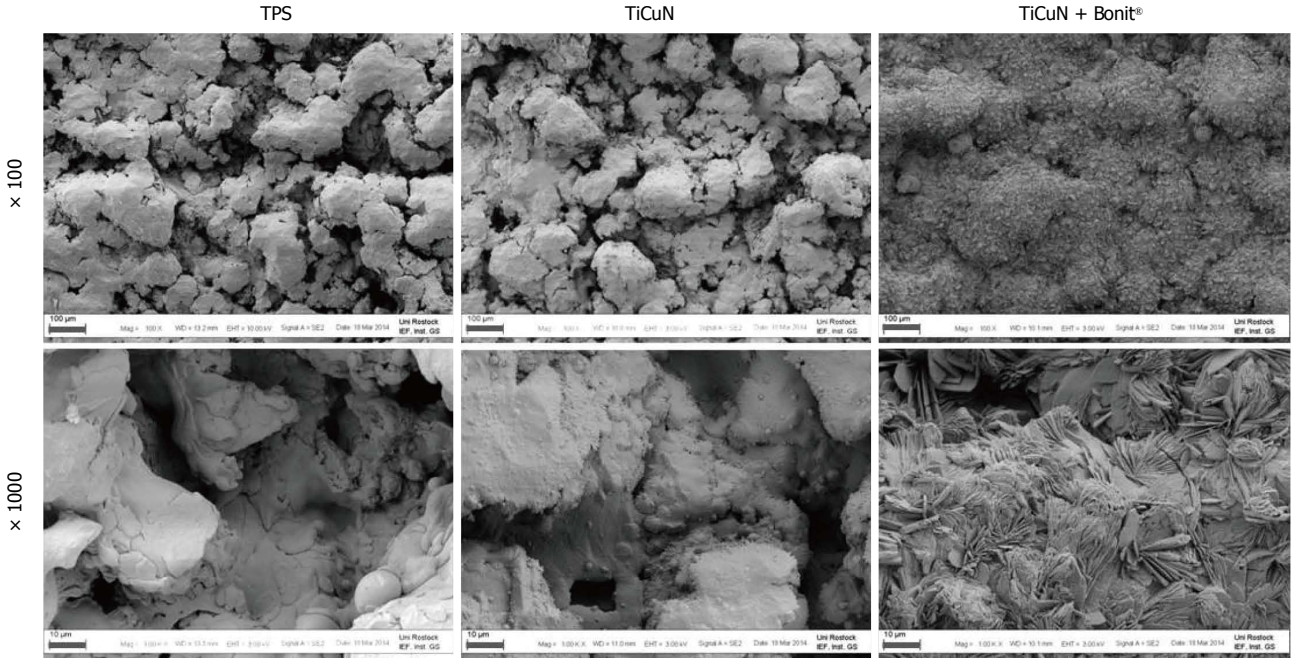


Figure 1 Surface topography of the coated materials *vs* titanium plasma spray control (field emission scanning electron microscopy, magnification × 100, × 1000, bars = 100 μm, 10 μm, respectively). TPS: Titanium plasma spray; TiCuN: Titanium-copper-nitride.

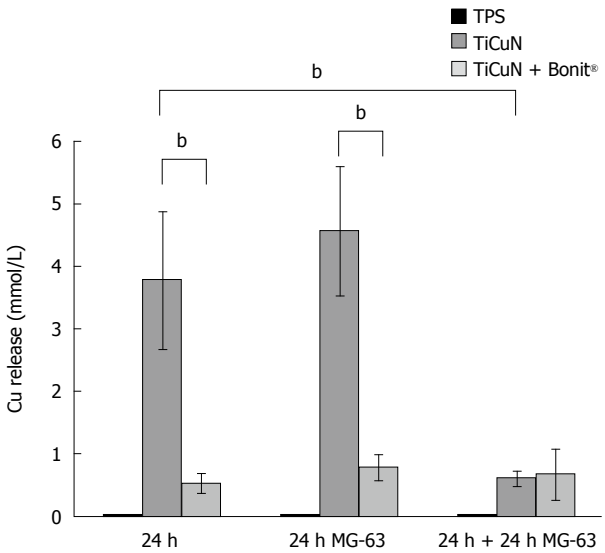


Figure 2 Copper release in Dulbecco's modified Eagle medium. A high amount of copper is released from the TiCuN layer after incubation in DMEM for 24 h. The copper release is reduced on TiCuN + BONIT® due to the BONIT® layer. A complete exchange of the medium and seeding with MG-63 cells for another 24 h reveals significantly reduced copper release from TiCuN. The amount is equalized to the level on TiCuN + BONIT® (n = 3, mean value ± SD, t-test, $^bP < 0.01$). TPS: Titanium plasma spray; TiCuN: Titanium-copper-nitride; DMEM: Dulbecco's modified Eagle medium.

Copper release

The results of copper release measurements from the samples in 1 mL of DMEM, indicated as mmol/L unit and dependent upon storage conditions, are shown in Figure 2. The highest copper release after 24 h was measured for TiCuN samples at about 3.8 mmol/L. Copper release was further elevated when samples were seeded with MG-63 osteoblastic cells and incubated for 24 h (around 4.6 mmol/L). For TiCuN samples which were pre-incubated in DMEM for 24 h and seeded with cells for another 24 h after exchanging the medium, copper release was significantly reduced to 0.6 mmol/L. TiCuN + BONIT® samples showed nearly constant low copper values between 0.5 and 0.8 mmol/L independently of the storage conditions. The BONIT® coating seems to slow down the release of copper from the TiCuN layer, resulting in a prolonged time of release.

Antibacterial effect

Heavy metal ions like copper ion can deactivate the central catabolic and biosynthetic pathways and become toxic[43]. We employed *S. epidermidis* strain RP 62A (ATCC35984) to study the influence of the TiCuN samples on the growth of bacteria. The antimicrobial effect of TiCuN films on *S. epidermidis* is presented in Figure 3. Only the TiCuN coating demonstrated growth inhibition; this indicates that the copper species was released into the medium at a high rate of diffusion. After 2 d, no planktonic or adherent *S. epidermidis* were found on the TiCuN samples. In contrast, the TPS discs proved to have 7.62×10^7 CFU/mL planktonic bacteria in the incubation fluids and 2.52×10^8 CFU/mL adherent bacteria in the rinsed fluids. The concentration of planktonic bacteria reached 1.08×10^8 CFU/mL in the incubation fluids from the TiCuN + BONIT® samples. An equal amount of biofilm-bound bacteria (1.33×10^8 CFU/mL) could be detected. Thus, no antibacterial potential was found after 24 h for TiCuN + BONIT®; it can be surmised that the low amount of copper released by this coating (between 0.5 and 0.8 mmol/L, see Figure 2) prevented any

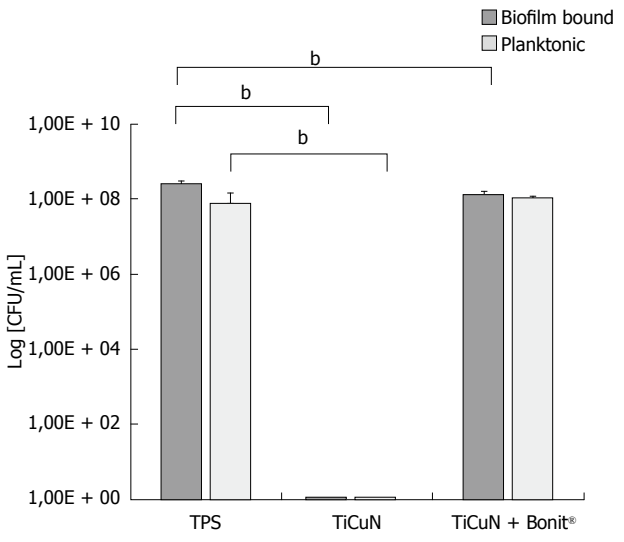


Figure 3 Antibacterial effect of the Titanium-copper-nitride coating on *Staphylococcus epidermidis* bacteria for planktonic and biofilm state after 2 d. On TiCuN, planktonic and biofilm bound bacteria were killed completely. On TiCuN + BONIT®, no antibacterial effect could be observed (n = 6, mean value ± SD, U-test, $^bP < 0.01$). TPS: Titanium plasma spray; TiCuN: Titanium-copper-nitride.

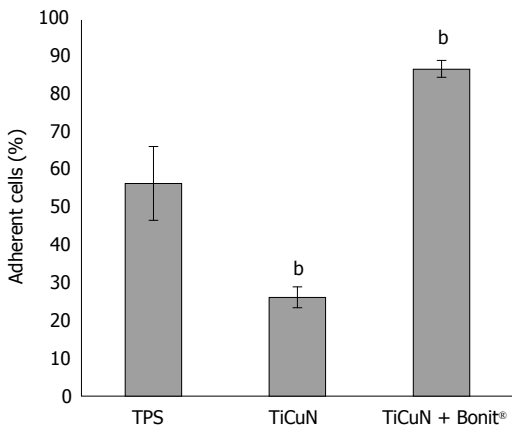


Figure 4 Initial cell adhesion of MG-63 osteoblasts on the titanium-copper-nitride. Surfaces compared to the titanium plasma spray control after 10 min. The MG-63 cells were directly seeded onto the samples and cultivated for 10 min. Cell adhesion was significantly reduced on TiCuN, but TiCuN + BONIT® enhanced cell adherence significantly (n = 3, mean value ± SD, t-test, $^bP < 0.01$). TPS: Titanium plasma spray; TiCuN: Titanium-copper-nitride.

significant antibacterial effect. The fast copper release from TiCuN samples can efficiently kill bacteria in the initial state of implantation and we assume that the risk of implant infection can thereby be significantly reduced.

Copper ions attack the bacteria at different sites[44-46]. They can interact with the outer membrane of bacteria and subsequently disintegrate the bacterial cell wall which is known as the bacteriolytic effect. If copper ions get into the bacteria, they can bind to the DNA and become involved in cross-linking within nucleic acid strands with the result that the bacteria cannot replicate. Furthermore copper ions generate reactive oxygen species and can cause lipid peroxidation and protein oxidation[47].

In addition, copper is an essential trace element present in many cell processes; a defect in the homeostasis of copper is a direct cause of certain human diseases[48]. Copper also plays a role in the control of cell proliferation[56]. Thus bioceramic scaffolds loaded with copper sulphate were shown to stimulate osteoblast activity and proliferation and the angiogenesis[49,50].

To determine the influence of the TiCuN and BONIT® coating on osteoblasts, we investigated the initial cell adhesion, the cell viability, the cell morphology and the cell spreading of MG-63 osteoblast-like cells after culturing on these surfaces.

Initial cell adhesion

Initial osteoblast cell adhesion was analyzed after 10 min of culturing (Figure 4). After direct seeding of MG-63 cells onto the samples, the non-adherent cells in the supernatant were measured by FACS. The adhesion of the cells was significantly reduced on TiCuN to about 26% compared to TPS where around 56% of the cells were adherent after 10 min. On the other hand, TiCuN + BONIT® enhanced initial cell adherence significantly (to about 87%).

Cell viability and spreading

The experiments to determine cell viability employed two different setups: (1) MG-63 cells were cultivated on the surfaces themselves; and (2) the samples underwent pre-incubation in cell culture medium DMEM for 24 h and cells were seeded onto the surfaces after a complete exchange of the medium. In this way the *in vivo* situation was simulated more closely, where dead cells and the persistent bacteria inside these cells are removed and new cells can adhere and proliferate on the surface. After incubation of the cells for 24 h, the cell viability was determined (Figure 5). Cultivation of the cells for 24 h directly on TiCuN reduced cell viability of the MG-63 cells to about 10% and on TiCuN + BONIT® for the same period to about 29% compared to TPS. Cells on TiCuN + BONIT® showed higher viability in comparison with TiCuN. This corresponds with the lower copper release values on these samples due to the BONIT® coating. Interestingly, the incubation of TiCuN samples for 24 h in DMEM prior to cultivating the cells led to an increase in cell viability by about 30%. During the pre-incubation period, a substantial amount of copper is released from the TiCuN film (Figure 2), after which the cells are able to grow onto the substrate surface. Although present, this effect is not as pronounced for TiCuN + BONIT®: Here, cell viability is increased by only 10%. So, on both samples cell viability reached around 40%. This corresponds to the low copper release measured on TiCuN and TiCuN + BONIT® after pre-incubation (between 0.6 and 0.7 mmol/L). The copper amounts released are slightly higher than the copper concentration limit identified for cell survival in earlier studies[51,57]. These studies showed that cell proliferation of hMSC is stimulated by copper concentrations below 0.3 mmol/L, whereas cell viability decreases significantly to around 30% at copper concentrations higher than

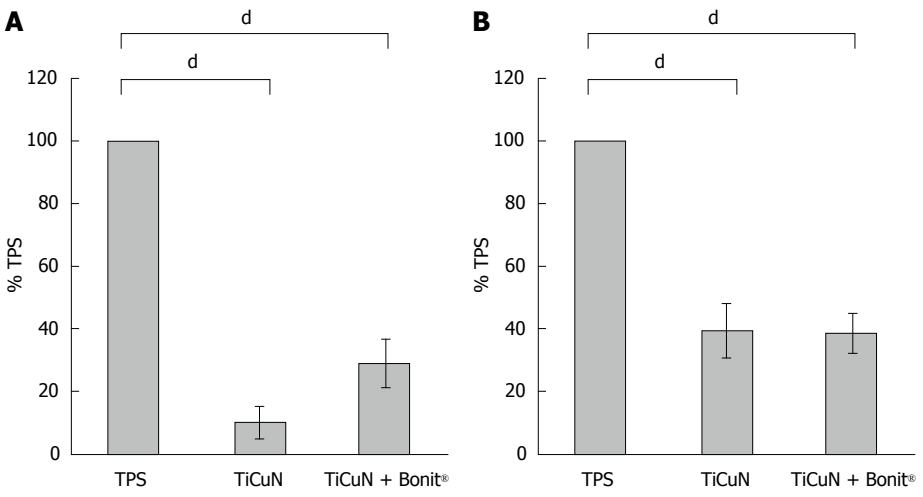


Figure 5 Viability of MG-63 osteoblasts on the titanium-copper-nitride surfaces. Two different experimental arrangements were used: (A) the MG-63 cells were directly cultivated on the TiCuN surfaces for 24 h and (B) the samples were pre-incubated in DMEM for 24 h and after this the cells were seeded onto the surfaces for another 24 h. Cell viability was significantly reduced after direct seeding on TiCuN. Cell viability was higher on TiCuN + BONIT® compared to TiCuN. Pre-incubation of the samples in DMEM for 24 h before seeding elevated cell viability on both samples (n = 3, mean value ± SD, t-test, $^{d}P < 0.001$). TPS: Titanium plasma spray; TiCuN: Titanium-copper-nitride; DMEM: Dulbecco's modified Eagle medium.

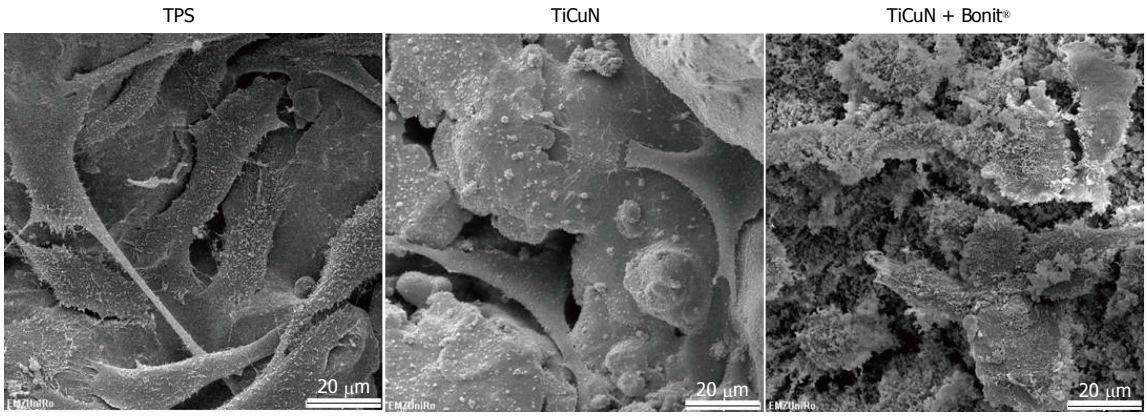


Figure 6 Scanning electron microscopy images of MG-63 osteoblasts on the pre-incubated surfaces. Samples were pre-incubated in DMEM for 24 h. After a complete exchange of medium, cells were seeded onto the surface and cultivated for another 24 h. Cells spread well on TPS and TiCuN surfaces but seem to be smaller on TiCuN + BONIT® (magnification × 1000). TPS: Titanium plasma spray; TiCuN: Titanium-copper-nitride; DMEM: Dulbecco's modified Eagle medium.

0.5 mmol/L.

Figure 6 shows SEM images of the osteoblasts growing on the sample surfaces**.** MG-63 osteoblasts were seeded onto the pre-incubated samples and cultivated for 24 h. It can be seen that the osteoblasts on the TPS reference and the TiCuN surfaces exhibit a flattened, well-spread phenotype and bridge the gaps between the ridges. The cells spread less readily on TiCuN + BONIT® and seem to be covered by small crystals evolved from the BONIT® layer. This is understandable, considering that BONIT® consists of a brushite and a hydroxyapatite phase. The more soluble brushite is metastable at a physiological pH and converts to a less soluble apatite phase[52,53]. During this phase transformation, loose crystal particles are released onto the settled cells and the surface cannot be considered solid. This explains the reduction in cell area on TiCuN + BONIT® compared to TiCuN and TPS, as revealed by the statistical analysis (Figure 7).

DISCUSSION

Our cell biological investigation revealed a cytotoxic effect on osteoblasts within 24 h by the TiCuN coating. On the other hand, the TiCuN surface showed a strong antibacterial influence on both planktonic and biofilm-bound *S. epidermidis*. The BONIT® coating reduced the copper release significantly within 24 h and as a consequence, no antibacterial effect could be demonstrated on TiCuN + BONIT® samples. The viability of osteoblasts on the TiCuN samples could be enhanced by a pre-incubation step. The copper-coated materials and controls were incubated in cell culture medium for 24 h and cell seeding was performed after a complete exchange of the medium. In this way the *in vivo* dynamics were simulated: Dead cells and the persistent bacteria inside these cells are removed and new cells can adhere and proliferate on the surface. Using this approach the osteoblasts were able to grow

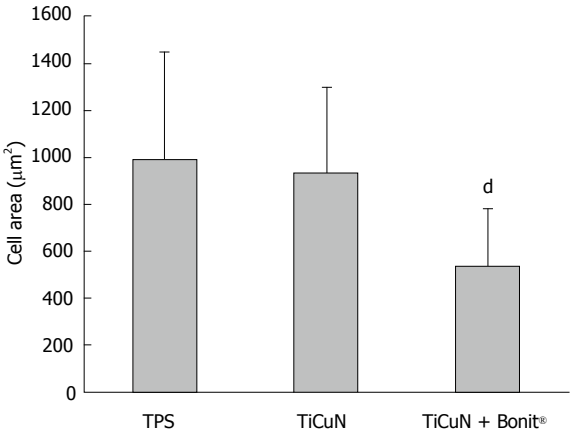


Figure 7 Spreading of MG-63 osteoblasts on pre-incubated samples after 24 h. Cell area is unchanged on TiCuN compared to TPS but significantly reduced on TiCuN + BONIT® due to the additional BONIT® layer (n = 60, mean value ± SD, t-test, $^{d}P < 0.001$). Titanium plasma spray; TiCuN: Titanium-copper-nitride.

properly. Stranak *et al*[36] found similar results for copper-doped titanium surfaces: Over a short period of time these released significant amounts of copper. Stranak *et al*[36] used dual high-power impulse magnetron sputtering which produced copper containing films on TiAlV alloys that released high amounts of copper (about 6 mmol/L) completely and quickly within 24 h. They were able to show an initial antibacterial effect within 24 h and high colonization by osteoblasts after replacement of the cell culture medium and cell seeding for another 24 h. A critical step in the development of implant-related infections is the surface adhesion of bacteria; this represents the first stage in the colonization process, the so-called "race for the surface" on the biomaterial[14,18]. Burghardt *et al*[57] demonstrated that complete killing of adherent bacteria within 24 h could be achieved by a final concentration of 1.75 mmol/L copper in the culture medium. The indicated bactericidal properties of copper can be used to hamper the settlement of an implant material by bacteria. It is, however, important to take into consideration the sensitivity to concentration displayed by copper's functional effects. It was found that copper acts as an antibacterial agent above concentrations of 0.5 mmol/L[51] and an osteoinductive one in the range of 0.05-0.3 mmol/L copper[57]. Therefore, it is suggested to use implants which initially introduce copper onto the surface at a high concentration to create an antibacterial effect in the vicinity of the implant. The stimulating effect on osteoblasts will prevail at a greater distance from the implant surface and later on. Some studies reported an additional advantage of depositing copper: It has lower toxicity and higher cytocompatibility compared to other antimicrobial metals. A relatively lower concentration of silver or zinc could have strong toxicity to the tissue cells; however, a relatively higher concentration of copper still had no toxic effect on the cells[22,27]. Further, copper represents an essential cofactor in collagen formation through its facilitation of the enzyme lysyl oxidase[54]. Recent studies which introduced copper combined with hyaluronan into elastin-vascular constructs were able to demonstrate increased synthesis of lysyl oxidase and collagen as well as stimulated elastin-crosslinking[55]. Various studies have shown the proliferation of human mesenchymal stem cells to be stimulated by copper ions; this makes the incorporation of copper into implant surfaces an interesting approach for tissue engineering in regenerative medicine[36,48,50,51,56,57]. In the study presented here we could show that TiCuN coating on TPS-optimized titanium combines a rough TPS surface with the antibacterial function of copper ions while maintaining the excellent properties required for good osteoblast cell growth. Our data were acquired by *in vitro* experiments, investigating processes within the first 48 h of material cell contact with osteoblast-like MG-63 cells. In future research, data will be verified by *in vitro* analyses after longer periods of time and with primary osteoblasts. In an animal study, we will examine the *in vivo* acceptance of the TiCuN and BONIT® coating on TPS-optimized titanium implants. Patients' first experiences provided in a clinical case report indicated that TiCuN-coated implants can be suitable as temporary spacers for two-stage septic joint revisions[31]. In conclusion, the TiCuN coating is indicated as a suitable method of reducing bacteria adhesion and promoting the growth of osteoblasts on implants. The additional BONIT® layer could be accomplished by another TiCuN coating or usage of an antibiotic to preserve the antibacterial effect and the osteoinductive influence.

In this study the antibacterial effect of TiCuN-coated, TPS-optimized titanium was examined. We showed that TiCuN has a strong ability to kill planktonic bacteria as well as bacteria adhering as a biofilm, and after pre-incubation we found low cytotoxicity. The antibacterial role should inhibit the formation of bacterial bio-films on orthopedic implants by influencing the "race for the surface" to the advantage of the osteoblasts.

ACKNOWLEDGMENTS

The authors thank H. G. Neumann (DOT GmbH Rostock) for industrial support. We would like to thank U. Schweers and S. Krüger (University Rostock) for their help. We thank M. Frank, W. Labs, G. Fulda (EMZ Rostock, Rostock University Medical Center) and R. Lange (Institute for Electronic Appliances and Circuits, University Rostock) for their assistance and support with the scanning electron microscopy and field emission scanning electron microscopy.

COMMENTS

Background

Titanium is one of the most common materials used for orthopedic implants. Increasing the roughness of the implant surface improves bone-to-implant contact after implant placement and enhances the functional activity of bone cells in contact with the biomaterial. Implant-associated infections remain one of the most severe complications after joint replacement. Bacteria interact with the surface of the material and after an initial reversible adhesion, a biofilm is formed. Such biofilms enable bacteria to evade antibiotics and immune responses.

Research frontiers

The problems associated with infected medical devices in orthopedic surgery necessitate further research and the development of alternative treatment and

prevention strategies, such as thin metal-ion based surfaces.

Innovations and breakthroughs

Some studies reported that copper represents a promising metal ion for deposition applications because of its lower toxicity and higher cytocompatibility compared to other antimicrobial metals. The authors investigated the properties and effects of titanium-copper-nitride (TiCuN) films deposited by physical vapor deposition. They studied the chemical composition and copper release with respect to antibacterial properties and cell growth and the influence of an additional osteoconductive coating with a BONIT® layer. The authors were able to show that a TiCuN coating on TPS-optimized titanium combines the rough TPS surface with the antibacterial function of copper ions, while maintaining the excellent properties required for good osteoblast cell growth.

Applications

In conclusion, the TiCuN coating is an interesting agent to inhibit the formation of bacterial bio-films on orthopedic implants by influencing the "race for the surface" to the advantage of the osteoblasts.

Peer-review

This is a very interesting topic and very well-presented scientific research. The study design is solid and meticulously and flawlessly conducted, the results of this study can be very important to professionals who perform these procedures.

REFERENCES

1 **de Jonge LT**, Leeuwenburgh SC, Wolke JG, Jansen JA. Organic-inorganic surface modifications for titanium implant surfaces. *Pharm Res* 2008; **25**: 2357-2369 [PMID: 18509601 DOI: 10.1007/s11095-008-9617-0]

2 **Hench LL**, Polak JM. Third-generation biomedical materials. *Science* 2002; **295**: 1014-1017 [PMID: 11834817 DOI: 10.1126/science.1067404]

3 **Goodman SB**, Yao Z, Keeney M, Yang F. The future of biologic coatings for orthopaedic implants. *Biomaterials* 2013; **34**: 3174-3183 [PMID: 23391496 DOI: 10.1016/j.biomaterials.2013.01.074]

4 **Passeri G**, Cacchioli A, Ravanetti F, Galli C, Elezi E, Macaluso GM. Adhesion pattern and growth of primary human osteoblastic cells on five commercially available titanium surfaces. *Clin Oral Implants Res* 2010; **21**: 756-765 [PMID: 20636730 DOI: 10.1111/j.1600-0501.2009.01906.x]

5 **Lüthen F**, Lange R, Becker P, Rychly J, Beck U, Nebe JG. The influence of surface roughness of titanium on beta1- and beta3-integrin adhesion and the organization of fibronectin in human osteoblastic cells. *Biomaterials* 2005; **26**: 2423-2440 [PMID: 15585246 DOI: 10.1016/j.biomaterials.2004.07.054]

6 **Anselme K**, Linez P, Bigerelle M, Le Maguer D, Le Maguer A, Hardouin P, Hildebrand HF, Iost A, Leroy JM. The relative influence of the topography and chemistry of TiAl6V4 surfaces on osteoblastic cell behaviour. *Biomaterials* 2000; **21**: 1567-1577 [PMID: 10885729 DOI: 10.1016/S0142-9612(00)00042-9]

7 **Bächle M**, Kohal RJ. A systematic review of the influence of different titanium surfaces on proliferation, differentiation and protein synthesis of osteoblast-like MG63 cells. *Clin Oral Implants Res* 2004; **15**: 683-692 [PMID: 15533129 DOI: 10.1111/j.1600-0501.2004.01054.x]

8 **Geetha M**, Singh AK, Asokamani R, Gogia AK. Ti based biomaterials, the ultimate choice for orthopaedic implants - A review. *Prog Mater Sci* 2009; **54**: 397-425 [DOI: 10.1016/j.pmatsci.2008.06.004]

9 **Mantripragada VP**, Lecka-Czernik B, Ebraheim NA, Jayasuriya AC. An overview of recent advances in designing orthopedic and craniofacial implants. *J Biomed Mater Res A* 2013; **101**: 3349-3364 [PMID: 23766134 DOI: 10.1002/jbm.a.34605]

10 **Wennerberg A**, Albrektsson T. On implant surfaces: a review of current knowledge and opinions. *Int J Oral Maxillofac Implants* 2010; **25**: 63-74 [PMID: 20209188]

11 **Moyad TF**, Thornhill T, Estok D. Evaluation and management of the infected total hip and knee. *Orthopedics* 2008; **31**: 581-598; quiz 581-588 [PMID: 18661881 DOI: 10.3928/01477447-20080601-22]

12 **Geipel U**, Herrmann M. [The infected implant. Part 1: bacteriology]. *Orthopade* 2004; **33**: 1411-1426 [PMID: 15551049 DOI: 10.1007/s00132-004-0741-1]

13 **Harris WH**, Sledge CB. Total hip and total knee replacement. *N Engl J Med* 1990; **323**: 801-807 [PMID: 2136367 DOI: 10.1056/Nejm199009203231206]

14 **Montanaro L**, Speziale P, Campoccia D, Ravaioli S, Cangini I, Pietrocola G, Giannini S, Arciola CR. Scenery of Staphylococcus implant infections in orthopedics. *Future Microbiol* 2011; **6**: 1329-1349 [PMID: 22082292 DOI: 10.2217/Fmb.11.117]

15 **Saleh KJ**, Celebrezze M, Kassim R, Dykes DC, Gioe TJ, Callaghan JJ, Salvati EA. Functional outcome after revision hip arthroplasty: a metaanalysis. *Clin Orthop Relat Res* 2003; **(416)**: 254-264 [PMID: 14646768 DOI: 10.1097/01.blo.0000093006.90435.43]

16 **Costerton JW**, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999; **284**: 1318-1322 [PMID: 10334980]

17 **Busscher HJ**, van der Mei HC, Subbiahdoss G, Jutte PC, van den Dungen JJ, Zaat SA, Schultz MJ, Grainger DW. Biomaterial-associated infection: locating the finish line in the race for the surface. *Sci Transl Med* 2012; **4**: 153rv10 [PMID: 23019658 DOI: 10.1126/scitranslmed.3004528]

18 **Gristina AG**. Biomaterial-centered infection: microbial adhesion versus tissue integration. *Science* 1987; **237**: 1588-1595 [PMID: 3629258 DOI: 10.1126/science.3629258]

19 **Davies D**. Understanding biofilm resistance to antibacterial agents. *Nat Rev Drug Discov* 2003; **2**: 114-122 [PMID: 12563302 DOI: 10.1038/nrd1008]

20 **Borkow G**, Gabbay J. Copper as a biocidal tool. *Curr Med Chem* 2005; **12**: 2163-2175 [PMID: 16101497 DOI: 10.2174/0929867054637617]

21 **Grass G**, Rensing L, Rensing C. Metal toxicity. *Metallomics* 2011; **3**: 1095-1097 [PMID: 22025262 DOI: 10.1039/c1mt90048j]

22 **Heidenau F**, Mittelmeier W, Detsch R, Haenle M, Stenzel F, Ziegler G, Gollwitzer H. A novel antibacterial titania coating: metal ion toxicity and in vitro surface colonization. *J Mater Sci Mater Med* 2005; **16**: 883-888 [PMID: 16167096 DOI: 10.1007/s10856-005-4422-3]

23 **Collinge CA**, Goll G, Seligson D, Easley KJ. Pin tract infections: silver vs uncoated pins. *Orthopedics* 1994; **17**: 445-448 [PMID: 8036188 DOI: 10.3928/0147-7447-19940501-11]

24 **Oloffs A**, Grosse-Siestrup C, Bisson S, Rinck M, Rudolph R, Gross U. Biocompatibility of silver-coated polyurethane catheters and silver-coated Dacron material. *Biomaterials* 1994; **15**: 753-758 [PMID: 7986938 DOI: 10.1016/0142-9612(94)90028-0]

25 **Wassall MA**, Santin M, Isalberti C, Cannas M, Denyer SP. Adhesion of bacteria to stainless steel and silver-coated orthopedic external fixation pins. *J Biomed Mater Res* 1997; **36**: 325-330 [PMID: 9260103 DOI: 10.1002/(Sici)1097-4636(19970905)36: 3<325: : Aid-Jbm7>3.0.Co; 2-G]

26 **Bosetti M**, Massè A, Tobin E, Cannas M. Silver coated materials for external fixation devices: in vitro biocompatibility and genotoxicity. *Biomaterials* 2002; **23**: 887-892 [PMID: 11771707 DOI: 10.1016/S0142-9612(01)00198-3]

27 **Shirai T**, Tsuchiya H, Shimizu T, Ohtani K, Zen Y, Tomita K. Prevention of pin tract infection with titanium-copper alloys. *J Biomed Mater Res B Appl Biomater* 2009; **91**: 373-380 [PMID: 19507137 DOI: 10.1002/jbm.b.31412]

28 **Massè A**, Bruno A, Bosetti M, Biasibetti A, Cannas M, Gallinaro P. Prevention of pin track infection in external fixation with silver coated pins: clinical and microbiological results. *J Biomed Mater Res* 2000; **53**: 600-604 [PMID: 10984710 DOI: 10.1002/1097-4636(200009)53: 5<600: : AID-JBM21>3.0.CO; 2-D]

29 **Jing H**, Yu Z, Li L. Antibacterial properties and corrosion resistance of Cu and Ag/Cu porous materials. *J Biomed Mater Res A* 2008; **87**: 33-37 [PMID: 18080302 DOI: 10.1002/jbm.a.31688]

30 **Wheeldon LJ**, Worthington T, Lambert PA, Hilton AC, Lowden CJ, Elliott TS. Antimicrobial efficacy of copper surfaces against spores and vegetative cells of Clostridium difficile: the germination theory.

J Antimicrob Chemother 2008; **62**: 522-525 [PMID: 18544601 DOI: 10.1093/jac/dkn219]

31 **Ellenrieder M**, Haenle M, Lenz R, Bader R, Mittelmeier W. Titanium-copper-nitride coated spacers for two-stage revision of infected total hip endoprostheses. *GMS Krankenhhyg Interdiszip* 2011; **6**: Doc16 [PMID: 22242097 DOI: 10.3205/dgkh000173]

32 **Prinz C**. Antibakterielle Optimierung von Implantatoberflächen [Dissertation]. Rostock: Universität Rostock, Agrar-und Umwelt wissenschaftliche Fakultät, 2010. Available from: URL: http://rosdok.uni-rostock.de/file/rosdok_derivate_000000004369/Dissertation_Prinz_000000002010.pdf

33 **Lembke U**, Neumann HG, Prinz C. inventor; DOT GmbH, Antibakterielle Beschichtung für ein Implantat. Germany patent DE 102010054046, [accessed 2012 June 14]. Available from: URL: http://www.freepatentsonline.com/de102010054046.html&ie=utf-8&sc_us=14696478605722856047

34 **Neumann HG**, Prinz C, Lembke U. inventor; DOT GmbH, Antibacterial coating for an implant and method for producing said coating. United States patent US 9107981. [accessed 2013 Sept 26]. Available from: URL: http://www.freepatentsonline.com/9107981.html&ie=utf-8&sc_us=6643309015689531805

35 **Neumann HG**, Prinz C, Lembke U. inventor; DOT GmbH, Antibacterial coating for an implant and method for producing said coating. European patent EP 2648773. [accessed 2015 Jan 14]. Available from: URL: http://www.freepatentsonline.com/9107981.html&ie=utf-8&sc_us=6643309015689531805

36 **Stranak V**, Wulff H, Rebl H, Zietz C, Arndt K, Bogdanowicz R, Nebe B, Bader R, Podbielski A, Hubicka Z, Hippler R. Deposition of thin titanium-copper films with antimicrobial effect by advanced magnetron sputtering methods. *Mat Sci Eng C-Mater* 2011; **31**: 1512-1519 [DOI: 10.1016/j.msec.2011.06.009]

37 **Zaatreh S**, Wegner K, Strauß M, Pasold J, Mittelmeier W, Podbielski A, Kreikemeyer B, Bader R. Co-Culture of S. epidermidis and Human Osteoblasts on Implant Surfaces: An Advanced In Vitro Model for Implant-Associated Infections. *PLoS One* 2016; **11**: e0151534 [PMID: 26982194 DOI: 10.1371/journal.pone.0151534]

38 **Patenge N**, Arndt K, Eggert T, Zietz C, Kreikemeyer B, Bader R, Nebe B, Stranak V, Hippler R, Podbielski A. Evaluation of antimicrobial effects of novel implant materials by testing the prevention of biofilm formation using a simple small scale medium-throughput growth inhibition assay. *Biofouling* 2012; **28**: 267-277 [PMID: 22435853 DOI: 10.1080/08927014.2012.671305]

39 **Kunz F**, Rebl H, Quade A, Matschegewski C, Finke B, Nebe JB. Osteoblasts with impaired spreading capacity benefit from the positive charges of plasma polymerised allylamine. *Eur Cell Mater* 2015; **29**: 177-188; discussion 188-189 [PMID: 25738585 DOI: 10.22203/eCM.v029a13]

40 **Reigstad O**, Franke-Stenport V, Johansson CB, Wennerberg A, Røkkum M, Reigstad A. Improved bone ingrowth and fixation with a thin calcium phosphate coating intended for complete resorption. *J Biomed Mater Res B Appl Biomater* 2007; **83**: 9-15 [PMID: 17318821 DOI: 10.1002/jbm.b.30762]

41 **ten Broeke RH**, Alves A, Baumann A, Arts JJ, Geesink RG. Bone reaction to a biomimetic third-generation hydroxyapatite coating and new surface treatment for the Symax hip stem. *J Bone Joint Surg Br* 2011; **93**: 760-768 [PMID: 21586774 DOI: 10.1302/0301-620X.93B6.24986]

42 **Reigstad O**, Johansson C, Stenport V, Wennerberg A, Reigstad A, Røkkum M. Different patterns of bone fixation with hydroxyapatite and resorbable CaP coatings in the rabbit tibia at 6, 12, and 52 weeks. *J Biomed Mater Res B Appl Biomater* 2011; **99**: 14-20 [PMID: 21648067 DOI: 10.1002/jbm.b.31866]

43 **Macomber L**, Imlay JA. The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. *Proc Natl Acad Sci USA* 2009; **106**: 8344-8349 [PMID: 19416816 DOI: 10.1073/pnas.0812808106]

44 **Kaur G**, Kumar S, Dilbaghi N, Bhanjana G, Guru SK, Bhushan S, Jaglan S, Hassan PA, Aswal VK. Hybrid surfactants decorated with copper ions: aggregation behavior, antimicrobial activity and anti-proliferative effect. *Phys Chem Chem Phys* 2016; **18**: 23961-23970 [PMID: 27523253 DOI: 10.1039/c6cp03070j]

45 **Wang X**, Liu S, Li M, Yu P, Chu X, Li L, Tan G, Wang Y, Chen X, Zhang Y, Ning C. The synergistic antibacterial activity and mechanism of multicomponent metal ions-containing aqueous solutions against Staphylococcus aureus. *J Inorg Biochem* 2016; **163**: 214-220 [PMID: 27569414 DOI: 10.1016/j.jinorgbio.2016.07.019]

46 **Dupont CL**, Grass G, Rensing C. Copper toxicity and the origin of bacterial resistance--new insights and applications. *Metallomics* 2011; **3**: 1109-1118 [PMID: 21984219 DOI: 10.1039/c1mt00107h]

47 **Tamayo L**, Azócar M, Kogan M, Riveros A, Páez M. Copper-polymer nanocomposites: An excellent and cost-effective biocide for use on antibacterial surfaces. *Mater Sci Eng C Mater Biol Appl* 2016; **69**: 1391-1409 [PMID: 27612841 DOI: 10.1016/j.msec.2016.08.041]

48 **Turski ML**, Thiele DJ. New roles for copper metabolism in cell proliferation, signaling, and disease. *J Biol Chem* 2009; **284**: 717-721 [PMID: 18757361 DOI: 10.1074/jbc.R800055200]

49 **Ewald A**, Käppel C, Vorndran E, Moseke C, Gelinsky M, Gbureck U. The effect of Cu(II)-loaded brushite scaffolds on growth and activity of osteoblastic cells. *J Biomed Mater Res A* 2012; **100**: 2392-2400 [PMID: 22528604 DOI: 10.1002/jbm.a.34184]

50 **Barralet J**, Gbureck U, Habibovic P, Vorndran E, Gerard C, Doillon CJ. Angiogenesis in calcium phosphate scaffolds by inorganic copper ion release. *Tissue Eng Part A* 2009; **15**: 1601-1609 [PMID: 19182977 DOI: 10.1089/ten.tea.2007.0370]

51 **Lüthen F**, Bergemann C, Bulnheim U, Prinz C, Neumann H, Podbielski A, Bader R, Rychly J. A dual role of copper on the surface of bone implants. *Mater Sci Forum* 2010; **638-642**: 600-605 [DOI: 10.4028/www.scientific.net/MSF.638-642.600]

52 **Bohner M**, Theiss F, Apelt D, Hirsiger W, Houriet R, Rizzoli G, Gnos E, Frei C, Auer JA, von Rechenberg B. Compositional changes of a dicalcium phosphate dihydrate cement after implantation in sheep. *Biomaterials* 2003; **24**: 3463-3474 [PMID: 12809775 DOI: 10.1016/S0142-9612(03)00234-5]

53 **Tamimi F**, Sheikh Z, Barralet J. Dicalcium phosphate cements: brushite and monetite. *Acta Biomater* 2012; **8**: 474-487 [PMID: 21856456 DOI: 10.1016/j.actbio.2011.08.005]

54 **Kim BE**, Nevitt T, Thiele DJ. Mechanisms for copper acquisition, distribution and regulation. *Nat Chem Biol* 2008; **4**: 176-185 [PMID: 18277979 DOI: 10.1038/Nchembio.72]

55 **Kothapalli CR**, Ramamurthi A. Biomimetic regeneration of elastin matrices using hyaluronan and copper ion cues. *Tissue Eng Part A* 2009; **15**: 103-113 [PMID: 18847363 DOI: 10.1089/ten.tea.2007.0390]

56 **Itoh S**, Kim HW, Nakagawa O, Ozumi K, Lessner SM, Aoki H, Akram K, McKinney RD, Ushio-Fukai M, Fukai T. Novel role of antioxidant-1 (Atox1) as a copper-dependent transcription factor involved in cell proliferation. *J Biol Chem* 2008; **283**: 9157-9167 [PMID: 18245776 DOI: 10.1074/jbc.M709463200]

57 **Burghardt I**, Lüthen F, Prinz C, Kreikemeyer B, Zietz C, Neumann HG, Rychly J. A dual function of copper in designing regenerative implants. *Biomaterials* 2015; **44**: 36-44 [PMID: 25617124 DOI: 10.1016/j.biomaterials.2014.12.022]

P- Reviewer: Gürel P, Hilmi I, Lopez-Jornet P **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 June 24; 7(3): 203-212

DOI: 10.5500/wjt.v7.i3.203

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Developing a donation after cardiac death risk index for adult and pediatric liver transplantation

Shirin Elizabeth Khorsandi, Emmanouil Giorgakis, Hector Vilca-Melendez, John O'Grady, Michael Heneghan, Varuna Aluvihare, Abid Suddle, Kosh Agarwal, Krishna Menon, Andreas Prachalias, Parthi Srinivasan, Mohamed Rela, Wayel Jassem, Nigel Heaton

Shirin Elizabeth Khorsandi, Emmanouil Giorgakis, Hector Vilca-Melendez, John O'Grady, Michael Heneghan, Varuna Aluvihare, Abid Suddle, Kosh Agarwal, Krishna Menon, Andreas Prachalias, Parthi Srinivasan, Mohamed Rela, Wayel Jassem, Nigel Heaton, Institute of Liver Studies, King's College Hospital, London SE5 9RS, United Kingdom

Author contributions: Khorsandi SE designed and wrote study; Giorgakis E performed the analysis and was involved in writing the manuscript; Vilca-Melendez H, O'Grady J, Heneghan M, Aluvihare V, Suddle A, Agarwal K, Menon K, Prachalias A, Srinivasan P, Rela M and Jassem W were all involved in the editing and review of the manuscript; Heaton N provided guidance in the manuscript composition and gave final institutional approval.

Institutional review board statement: There was institutional approval for the present work.

Conflict-of-interest statement: None of the authors have any conflict of interest declare that is applicable to the present manuscript.

Data sharing statement: No additional data is available.

Manuscript source: Unsolicited manuscript

Correspondence to: Nigel Heaton, Professor, Institute of Liver Studies, King's College Hospital, London SE5 9RS, United Kingdom. nigel.heaton@nhs.net
Telephone: +44-20-32994801
Fax: +44-20-32993575

Received: November 18, 2016
Peer-review started: November 23, 2016
First decision: December 29, 2016
Revised: January 21, 2017
Accepted: March 12, 2017
Article in press: March 13, 2017
Published online: June 24, 2017

Abstract

AIM

To identify objective predictive factors for donor after cardiac death (DCD) graft loss and using those factors, develop a donor recipient stratification risk predictive model that could be used to calculate a DCD risk index (DCD-RI) to help in prospective decision making on organ use.

METHODS

The model included objective data from a single institute DCD database (2005-2013, n = 261). Univariate survival analysis was followed by adjusted Cox-regressional hazard model. Covariates selected *via* univariate regression were added to the model *via* forward selection, significance level P = 0.3. The warm ischemic threshold was clinically set at 30 min. Points were given to each predictor in proportion to their hazard ratio. Using this model, the DCD-RI was calculated. The cohort was stratified to predict graft loss risk and respective graft survival calculated.

RESULTS

DCD graft survival predictors were primary indication for transplant (P = 0.066), retransplantation (P = 0.176), MELD > 25 (P = 0.05), cold ischemia > 10 h (P = 0.292) and donor hepatectomy time > 60 min (P = 0.028).

According to the calculated DCD-RI score three risk classes could be defined of low (DCD-RI < 1), standard (DCD-RI 2-4) and high risk (DCD-RI > 5) with a 5 years graft survival of 86%, 78% and 34%, respectively.

CONCLUSION

The DCD-RI score independently predicted graft loss (P < 0.001) and the DCD-RI class predicted graft survival (P < 0.001).

Key words: Liver transplant; Donor after cardiac death; Pediatric; Adult; Survival

Core tip: Calculating the donor after cardiac death (DCD) Risk Index score using objective variables from the donor (cold ischemic time, warm ischemic time, donor hepatectomy time) and from the selected recipient (primary indication for transplant, model for end-stage liver disease, retransplantation) can help rationalize the risk of using a DCD liver in a given recipient in order to produce good results.

Khorsandi SE, Giorgakis E, Vilca-Melendez H, O'Grady J, Heneghan M, Aluvihare V, Suddle A, Agarwal K, Menon K, Prachalias A, Srinivasan P, Rela M, Jassem W, Heaton N. Developing a donation after cardiac death risk index for adult and pediatric liver transplantation. *World J Transplant* 2017; 7(3): 203-212 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i3/203.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i3.203

INTRODUCTION

The continuing organ shortage combined with an expanding transplant waiting list is the main determinant of death on the waiting list. From UNOS and Eurotransplant data, death on the waiting list stands at 12% and 27% respectively[1,2]. This has driven the need and usage of the marginal liver or extended criteria organ, a term that encompasses the donor after cardiac death (DCD) liver. DCD liver transplantation has grown exponentially in countries that have utilized this form of donation[3]. Institutionally, DCD donation accounts for over 20% of transplants performed[4]. However, reports of poor patient and graft survival highlight that this is an organ with risks. A number of different factors have been identified as contributing to good outcome after DCD liver transplantation including donor factors of age, cold ischemic time (CIT), warm ischemic time (WIT), donor weight > 100 kg[5-8] and recipient factors of retransplantation (reTPL), on Intensive Therapy Unit (ITU) at time of transplant or renal dysfunction. Additionally, the use of a low risk DCD into a low risk recipient, can produce a good outcome that is equivalent to donor after brainstem death (DBD) liver transplantation[5].

Balancing the risk in DCD liver transplantation to achieve good results is still poorly understood and is both subjectively and experience driven. The aim of this study was to identify objective predictive factors for DCD graft loss and to use these factors to develop a donor recipient stratification risk predictive model that could be used to calculate a DCD risk index (DCD-RI) score to help in prospective decision making on DCD use.

MATERIALS AND METHODS

DCD practice and definitions

Institutionally the DCD programme started in 2001 and practice has been relatively consistent. In brief, recipient eligibility for DCD liver transplantation is decided at the liver transplant listing multidisciplinary meeting. Recipients offered a DCD liver are typically primary transplants for chronic liver disease (CLD) +/- hepatocellular cancer (HCC). The DCD liver was normally avoided in acute liver failure (ALF) or where a prolonged/difficult recipient hepatectomy was anticipated such as redo transplantation, young adult extrahepatic biliary atresia or the presence of an extensive portomesenteric venous thrombosis, as it would be anticipated to add to the CIT. Consent specifically for DCD transplantation would be obtained from the recipient.

For procurement a modified super rapid Casavilla technique was used[9]. Withdrawal of the DCD donor would either occur in the anaesthetic room or ITU depending on donor hospital preference. After declaration of cardiac death, there would be a 5 min stand off before the donor was brought into the operating room, where the donor team would be scrubbed and ready. After making a thoraco-abdominal incision, venting of blood would be in the chest, followed in sequence, by aortic cannulation, cross clamp in the chest and then portal/superior mesenteric vein cannulation. Adherence to WIT limits was consistent and a DCD liver would be discarded if the WIT exceeded 30 min[10]. The WIT was defined as the time from systolic of 50mmHg or oxygen saturations of 70%, depending on which agonal donor observation occurred first, to time of aortic cannulation.

Once perfusion had started, the gall bladder would be flushed until clear of bile followed by copious *in situ* flushing of the bile duct with chilled (4 ℃) normal saline. Topically, sterile crushed ice would then be placed around the organs to be retrieved. For perfusion *in situ* 4 L (aortic) and 2 L (portal) University of Wisconsin (UW) with 20000 IU heparin/L would be used. Pressure bags would only be used if flow by gravity was not sufficient. Attention to rapid donor hepatectomy was encouraged. On the back bench the portal vein (500 mL), hepatic artery (250 mL) and bile duct (250 mL) would be flushed further with chilled UW. Finally, the organ would be bagged for cold static storage.

Before proceeding with transplant the liver would be assessed on the backbench by the implanting surgeon. A severely steatotic liver on visual inspection would be discarded. If need be, a fresh frozen trucut liver biopsy

would be taken to assess degree of steatosis or to exclude donor pathology. DCD liver steatosis > 30% led to non-usage. Implantation technique was typically piggyback with a temporary portocaval shunt. The majority of livers were re-perfused *via* the portal vein. Standard immunosuppression was calcineurin inhibitor (tacrolimus) and steroid based. The cold ischemic time was the time from aortic cannulation in the donor to reperfusion in the recipient. Donor hepatectomy time (dHepT) was from the start of donor aortic perfusion to completion of hepatectomy. Model for end-stage liver disease (MELD) was defined as laboratory MELD and exception points have not been applied. The diagnosis of primary ischemic cholangiopathy (PIC) was based on review of biliary imaging by two consultant radiologists that demonstrated diffuse intrahepatic stricturing with no associated hepatic artery thrombosis (HAT).

Patient population and statistical analysis

The data analysed was extracted from a prospectively populated DCD database of a single institute, with a minimum follow up of 2 years (January 2005 - January 2013, n = 261). The pediatric age group was ⩽ 16 years. The study had full ethical approval in accordance with the declaration of Helsinki. Descriptive statistics were calculated for objective variables of the donor and the recipient, and for the calculated DCD-RI score. The developed DCD-RI model only included objective donor and recipient data. So subjectively assessed factors, such as liver steatosis were excluded from the analysis and the model. The primary end point was DCD graft loss. Survival analysis was performed using a Cox proportional hazard model and Kaplan-Meier estimator. Donor and recipient variables were tested independently to assess their uncontrolled effect on DCD graft survival. Significant predictive factors were then further analyzed separately with Kaplan-Meier and their respective ranges adjusted according to their level of significance. Similarly, primary indication for liver transplant was divided into 3 groups of high, standard and low risk according to their representation on the Kaplan-Meier survival curves.

For the development of the prediction model, etiology of liver disease was used as first indicator, which was then controlled for selected variables. Variables were added to the Cox regression model using forward selection with a significance level entry set at P = 0.3. Points were given to each variable in proportion to their calculated hazard ratio. WIT threshold was clinically set at 30 min and retained in the model. Using this model, the DCD-RI score was calculated for the study DCD cohort (n = 261). The DCD cohort was then stratified according to predicted graft loss risk as defined by the calculated DCD-RI score into three risk classes of low, standard and high. Respective predicted graft survivals were then calculated using Kaplan-Meier. Internal validation of the developed DCD-RI score was undertaken by performing a retrospective analysis on an earlier DCD cohort n = 37 (04/2001-12/2004), the experience of which has been previously published[11]. The receiver operator curve (ROC) and the area under the curve (AUROC) or c-statistic were then calculated to assess the performance of the DCD-RI score. The DCD-RI ROC curve was also compared to other scoring systems that have been used to predict graft survival after transplant. Statistical analysis was performed using SPSS® IBM® Statistics V22.0.

Table 1 Summary of the descriptive statistics for donor (d) and the recipient (r) that form the study donor after cardiac death cohort from which the donor after cardiac death risk index score was developed

DCD donor and recipient variables			All (n = 261)
Donor	dAge (yr)		46.1 ± 17.9
	dBMI		26 ± 4.9
	ITU Stay (d)		3.9 ± 5.8
	COD (CVA: Other: HBI: Trauma)		52.5:13.8:16.9:16.9
	dSodium (mmol/L)		144.51 ± 11.8
	dBilirubin (μmol/L)		9.81 ± 6.88
	Split/reduced (%)		2.30%
	WIT (min)		16.7 ± 9.8
	dHepT (min)		24.3 ± 10.6
	Liver Weight (g)		1518.28 ± 397.507
	CIT (min)		431 ± 118
Recipient	rAge (yr)		49.45 ± 15.36
	rGender		70.1%M/39.9%F
	rBMI		25.9 ± 4.7
	ALF (%)		1.50%
	rBilirubin (mmol/L)		89.36 ± 116.38
	rINR		1.89 ± 1.88
	MELD		14.8 ± 6.4
	Location (inpatient/home)		20.3%/79.6%
	Prior abdominal surgery (yr)		13.40%
	reTPL (yr)		5.70%
	Indication for TPL	Low	68 (26%)
		Standard	176 (67.5%)
		High	17 (6.5%)

Data presented as mean ± SD or % where appropriate. Primary indication for transplant has been divided into three risk groups of low, standard and high risk, as defined by their survival curves. BMI: Body mass index; COD: Cause of death; CVA: Cerebrovascular accident; HBI: Hypoxic brain injury; WIT: Warm ischemic time; CIT: Cold ischemic time; dHepT: Donor hepatectomy time; ALF: Acute liver failure; MELD: Model for end stage liver disease; reTPL: Retransplantation; TPL: Transplant.

RESULTS

Descriptive statistics for studied DCD transplant population

Table 1 summarizes the objective donor and recipient variables for the DCD study cohort (n = 261). The mean DCD recipient age was 49.45 ± 15.36 years, of which 15 (5.7%) were ⩽ 16 years. The mean DCD donor age was 46.1 ± 17.9 years, of which 18 (6.9%) were in the pediatric age group. Redo liver transplantation (reTPL) was a small component of the DCD programme accounting for 3.4% of activity in the period of study. The DCD liver was only used in a few cases of ALF (1.5%) and split/reduction (2.3%) was uncommon (Table 1). In the DCD study cohort the incidence of primary non

DCD risk group	Liver disease indication for transplant	Graft survival (%)					*P* value
		3 mo	6 mo	1 yr	3 yr	5 yr	
Low (*n* = 68)	AIH, PSC, NASH, HBV, cholestatic	96	94.5	91	89	86	0.066
Standard (*n* = 176)	Metabolic	91	89	85	79	76	
High (*n* = 17)	ALD, HCC, HCV, cryptogenic, other	87	84.5	76	69.5	64.5	

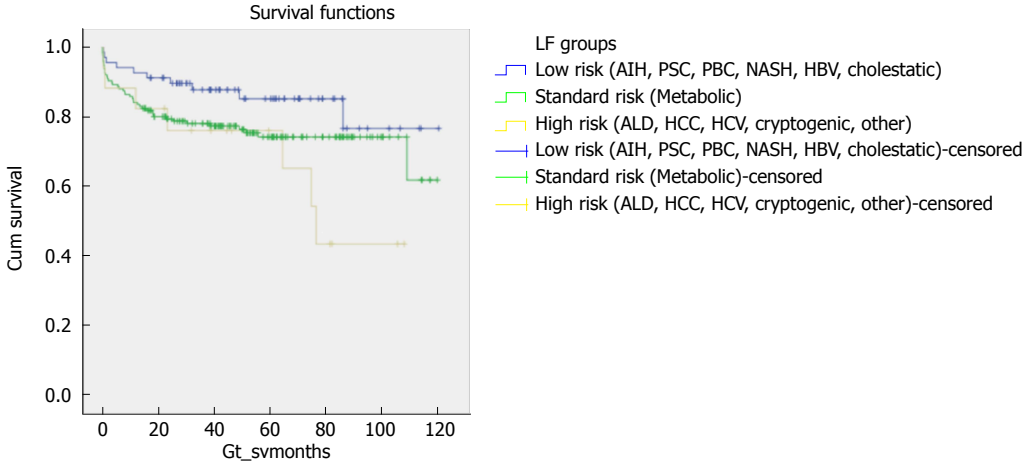


Figure 1 Stratified Kaplan-Meier curves for the cumulative donor after cardiac death graft survival in relation to primary indication for transplant and respective 3 mo, 6 mo, 1 year, 3 years and 5 years survival (χ^2 5.1 log-rank, *P* = 0.066). This stratification of indication for transplant defining the three risk groups of low, standard and high. Low DCD risk indications for transplant included autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), non-alcoholic steatohepatitis (NASH), hepatitis B virus (HBV) and cholestatic liver disease (primary familial intrahepatic cholestasis, extrahepatic biliary atresia and Crigler Najjar). Standard risk indications were metabolic diseases that included Wilson's, Hemochromatosis and Familial Amyloid Polyneuropathy. High risk indications for DCD transplant were alcohol related liver disease (ALD), hepatocellular carcinoma (HCC), hepatitis C virus (HCV), cryptogenic and Budd Chiari.

function (PNF) was 3.4% (*n* = 9), HAT 5% (*n* = 13), anastomotic biliary stricture 11.1% (*n* = 29) and PIC 3.5% (*n* = 9). Overall, there were 15% (*n* = 39) deaths and 3.5% (*n* = 9) retransplants.

Univariate analysis of donor and recipient risk factors for DCD graft loss

Univariate analysis of independent donor and recipient variables was initially performed to determine which variables were associated with DCD graft loss. Recipient variables analyzed were age (rAge), gender (rGender), weight (rWeight), BMI (rBMI), MELD, primary indication for transplant, patient location (home/hospital), reTPL, prior abdominal surgery and ALF/CLD. The donor variables analyzed were age (dAge), weight (dWeight), BMI (dBMI), cause of death (COD), sodium, CIT, WIT, liver weight, hepatectomy time (dHepT) and length of ITU stay (see Table 1).

On univariate analysis, the donor and recipient variables that were found to have a significant effect on DCD graft survival were MELD > 25 (χ^2 3.8 log-rank *P* = 0.05) and dHepT > 60 min (χ^2 4.8 log-rank *P* = 0.028). The variables that reached the significance level of entry into the forward selection regression model (*P* = 0.3) were primary indication for liver transplant (χ^2 5.1 log-rank *P* = 0.066), reTPL (χ^2 1.8 log-rank *P* = 0.176) and CIT > 10 h (χ^2 1.1 log-rank *P* = 0.292).

On grouping of survival curves based on primary liver disease indication for transplant, three DCD risk groups were defined of low, standard and high. Better survival was demonstrated when a DCD liver was used in a low risk indication for transplant (86% graft survival at 5 years) and poorer survival was found when the DCD liver was used in a high risk indication for transplant (64.5% graft survival at 5 years) (Figure 1). Low DCD risk indications for transplant included autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), non-alcoholic steatohepatitis (NASH), hepatitis B virus (HBV) and cholestatic liver disease. The cholestatic low risk indications for transplant encompassed primary familial intrahepatic cholestasis (PFIC), extrahepatic biliary atresia (EHBA) and Crigler Najjar. The standard risk indications for transplant were metabolic diseases that included Wilson's, Hemochromatosis and Familial Amyloid Polyneuropathy. The high risk indications for DCD transplant were alcohol related liver disease (ALD), HCC, hepatitis C virus (HCV), cryptogenic and Budd Chiari. Survival analysis for these three DCD risk groups as defined by primary indication for transplant is illustrated in Figure 1 (χ^2 5.1 log-rank *P* = 0.066).

In the period of study, the use of DCD for reTPL was rare, 5.7% (*n* = 9). In the cases that DCD was used for reTPL, there was significantly worse DCD graft survival. At 5 years DCD graft survival in reTPL was 65% compared to 78% when used in primary liver transplant (see Figure 2 for survival curves, χ^2 1.8 log-rank *P* = 0.176). Use of DCD in recipients with higher MELDs ⩾ 26 (*n* = 11) was also found to be associated with worse DCD graft survival (χ^2 3.8 log-rank *P* = 0.05), with a 5-year survival of 56% compared to 78%, when used in recipients with a MELD < 25 (Figure 3 for DCD survival curves according to

DCD-RI independent variable	Graft survival (%)					*P* value
	3 mo	6 mo	1 yr	3 yr	5 yr	
reTPL						
Yes (*n* = 9)	91	78	78	65	65	0.176
No (*n* = 252)	91	90	87	82	78	

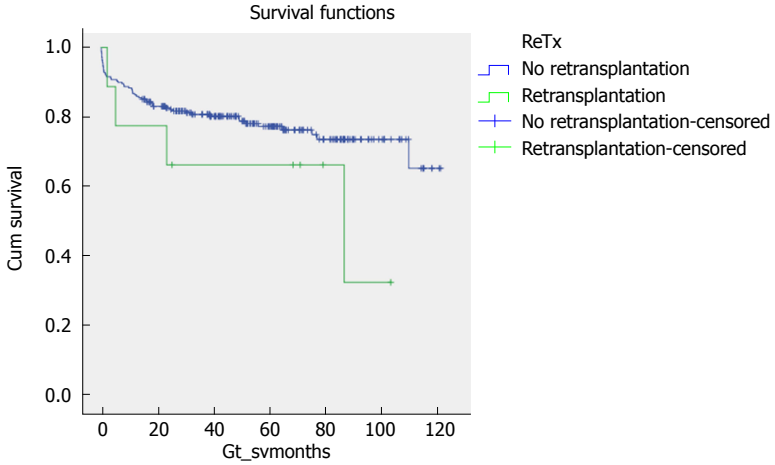


Figure 2 Stratified Kaplan-Meier curves for the cumulative DCD graft survival in relation to use in retransplantation or not and respective 3 mo, 6 mo, 1 year, 3 years and 5 years survival rates (χ^2 1.8 log-rank, *P* = 0.176).

MELD). Additionally, the donor hepatectomy time (dHepT) was found to be a determinant of DCD graft survival (χ^2 4.8 log-rank *P* = 0.028), with a dHepT ⩾ 60 min associated with early graft loss and a poor 5 year graft survival of 32% (Figure 4 for survival curves according to the dHepT groups).

Clinically, the warm ischemic threshold was set at 30 min and the WIT was retained in the DCD-RI model, despite not being found significant on univariate analysis, as it is institutionally regarded as a constant variable in determining outcome in DCD transplantation. After serial Kaplan-Meier analysis the CIT threshold was statistically set at ⩾ 10 h (*n* = 13) and < 10 h (*n* = 248) (χ^2 1.1 log-rank *P* = 0.292). However, many programmes are more stringent aiming for shorter CIT < 8 h (17). For the WIT, Kaplan-Meier analysis produced a cut off value of 25 min (*n* = 240) that had the lowest *P* value (χ^2 0.589 log-rank *P* = 0.443) and was the value incorporated into the developed DCD-RI model.

There was no difference in DCD graft survival between adult (*n* = 243) and pediatric (*n* = 18) donors (HR = 0.819, CI: 0.343-1.958, *P* = 0.653). Similarly, there was no difference in DCD graft survival between adult (*n* = 246) and pediatric (*n* = 15) recipients (HR = 1.268, CI: 0.389-4.132, *P* = 0.699). Therefore for the developed DCD-RI model adult and pediatric age groups have been combined.

Multivariate analysis and defining the DCD-RI score

Using primary indication for liver transplant as the primary indicator adjusted for the identified donor (WIT, CIT, dHepT) and recipient variables (MELD, reTPL) multivariate Cox regression analysis was undertaken. For the DCD-RI model points were given to each variable in proportion to the calculated hazard ratio (Table 2). According to the DCD-RI score three DCD-RI risk classes were defined, low risk (DCD-RI < 1), standard risk (DCD-RI 2-4) and high risk (DCD-RI > 5). Transplantation with a high risk DCD-RI score > 5 produced a 1 year graft survival of 40% and at 5 years 34% (Figure 5 for the survival curves according to DCD-RI score, Log-Rank and

Table 2 Point allocation system for the donor after cardiac death risk index score

Donor/recipient predictor variables	HR (CI)	Points
Primary indication for transplant		
Low (*P* = 0.07)		
Standard (*P* = 0.05)	2 (1-4.04)	2
High (*P* = 0.04)	2.83 (1.04-7.24)	3
reTPL (*P* = 0.26)	1.87 (0.63-5.58)	2
MELD > 25 (*P* = 0.04)	2.75 (1.04-7.24)	3
CIT > 10 h (*P* = 0.6)	1.37 (0.4-4.04)	1
WIT > 25 min (*P* = 0.4)	1.48 (0.6-3.63)	1
dHepT		
40-60 min (*P* = 0.5)	1.36 (0.53-3.53)	1
> 60 min (P = 0.05)	4.4 (1.02-19.04)	4

Points were given to each variable in proportion to their calculated hazard ratio (HR). Primary indication for liver transplant has been divided into three risk groups of low, standard and high, as defined by their survival curves. Low DCD risk indications for transplant include autoimmune hepatitis, primary sclerosing cholangitis, primary biliary cirrhosis, non-alcoholic steatohepatitis, Hepatitis B virus and cholestatic liver disease (primary familial intrahepatic cholestasis, extrahepatic biliary atresia and Crigler Najjar). Standard risk indications were metabolic diseases that included Wilson's, Hemochromatosis and Familial Amyloid Polyneuropathy. High risk indications for DCD transplant were alcohol related liver disease; HCV: Hepatitis C virus, cryptogenic and Budd Chiari. reTPL: Retransplantation; MELD: Model for end stage liver disease; CIT: Cold ischemic time; WIT: Warm ischemic time; dHepT: Donor hepatectomy time; HCC: Hepatocellular carcinoma.

DCD-RI independent variable	Graft survival (%)					*P* value
	3 mo	6 mo	1 yr	3 yr	5 yr	
MELD ⩾ 26 Yes (*n* = 11)	82	82	72	56	56	0.05
MELD ⩽ 25 No (*n* = 11)	92	90	83	81	78	

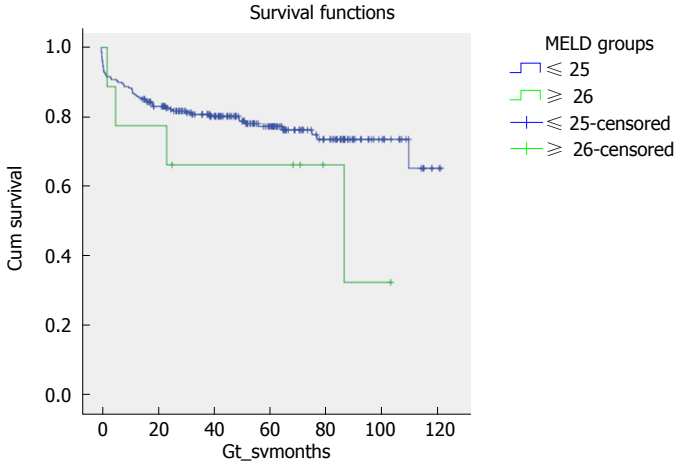


Figure 3 Stratified Kaplan-Meier curves for the cumulative DCD graft survival in relation to MELD and respective 3 mo, 6 mo, 1 year, 3 year and 5 year survival rates (χ^2 3.8 Log-Rank, *P* = 0.05).

DCD-RI independent variable	Graft survival (%)					*P* value
	3 mo	6 mo	1 yr	3 yr	5 yr	
dHepT						
⩽ 39 min (*n* = 237)	93	91	88	81	78	0.028
40-59 min (*n* = 21)	81	81	81	77	77	
⩾ 60 min (*n* = 3)	99	32	32	32	32	

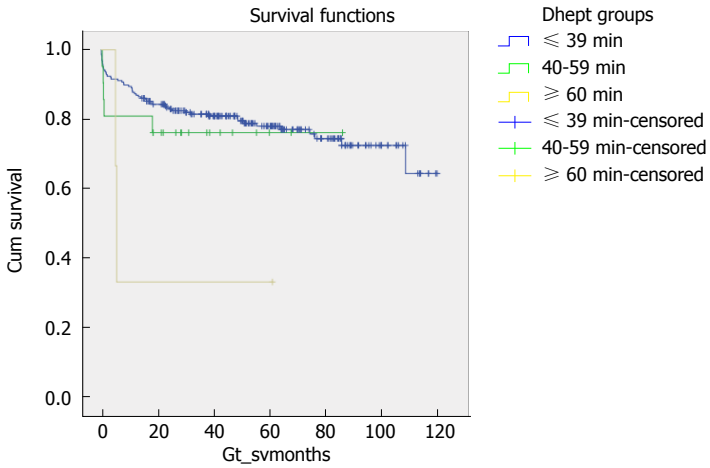


Figure 4 Stratified Kaplan-Meier curves for the cumulative DCD graft survival in relation to DCD donor hepatectomy time (dHepT) and respective 3 mo, 6 mo, 1 year, 3 years and 5 years survival rates (χ^2 4.8 Log-Rank, *P* = 0.028).

Breslow pooled over strata $P < 0.001$).

DCD-RI score internal validation

For internal validation of the developed DCD-RI score a retrospective analysis of an earlier DCD cohort 04/2001-12/2004 *n* = 37 were undertaken. Table 3 for summary of actual and predicted survival using the DCD-RI class subdivision. There was good concordance between actual graft survival and predicted DCD-RI survival, with actual graft survival falling within the confidence interval of the DCD-RI risk class predicted survival.

DCD-RI ROC and comparison to other predictive models

Based on the DCD-RI ROC, a DCD-RI score 1.5 cut off had a good positive predictive value (PPV = 0.993). A low risk DCD-RI score ⩽ 1.5, graft survival was predicted with 99.3% sensitivity and 98.3% specificity. Whereas, with a high risk DCD-RI score > 5, specificity was better than sensitivity (Figure 6 for DCD-RI ROC

DCD-RI risk class	DCD-RI score	Graft survival (%)					*P* value
		3 mo	6 mo	1 yr	3 yr	5 yr	
Low (*n* = 54, 20.6%)	0-1	96	95	93	89	86	0.000
Standard (*n* = 193, 74%)	2-4	93	90	87	82	78	
High (*n* = 14, 5.3%)	⩾ 5	80	75	40	34	34	

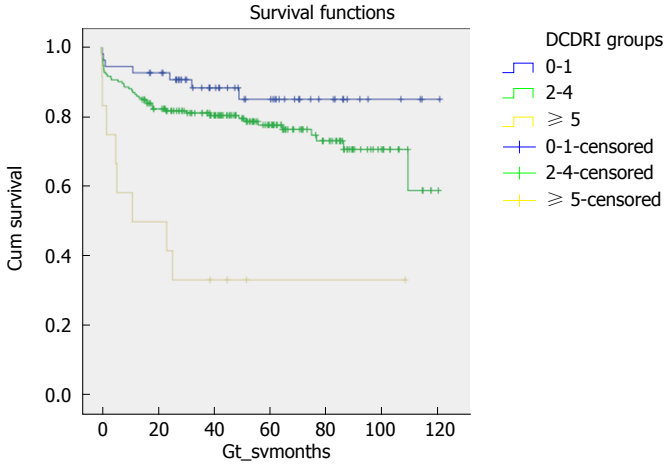


Figure 5 Stratified Kaplan-Meier curves for the cumulative DCD graft survival in relation to their DCD-RI score and respective 3 mo, 6 mo, 1 year, 3 years and 5 years survival (log-rank *P* = 0.000). The DCD-RI score divides the study cohort into three DCD-RI risk classes of Low (DCD-RI = 0-1), Standard (DCD-RI = 2-4) and High (DCD-RI ⩾ 5).

Table 3 Internal validation of the donor after cardiac death risk index in predicting donor after cardiac death graft survival

DCD Graft Survival (mo)	DCD-RI class		
	DCD-RI ⩽ 1, low (*n* = 10/27%)	DCD-RI 2-4, standard (*n* = 8/21.6%)	DCD-RI ⩾ 5, high (*n* = 19/51.4%)
3			
Actual	100	92.6	75
Predicted	96 (100-83.8)	90 (100-76.8)	80 (96.2-63.8)
6			
Actual	100	85.2	75
Predicted	95 (100-83.7)	90 (100-83.8)	75 (91.2-58.8)
12			
Actual	100	77.8	75
Predicted	93 (100-76.8)	87 (100-70.8)	40 (56.2-23.8)
60			
Actual	100	63	50
Predicted	86 (100-83.8)	78 (94.2-61.8)	34 (50.2-17.8)

The DCD-RI was calculated for an earlier DCD transplant cohort (2001 - 2004). The table summarizes actual and predicted graft survival as calculated with the DCD-RI. The DCD-RI predicted survival showed good correlation with actual graft survival, as actual graft survival fell within the confidence interval of DCD-RI predicted graft survival. DCD: Donor after cardiac death; DCD-RI: Donor after cardiac death risk index.

curve). To determine how the DCD-RI score compared to other transplant predictive scoring systems for graft outcome, the DCD-RI ROC curve was compared to other systems (see Figure 6). Based on the c-statistic (or AUROC) the DCD-RI (c-statistic = 0.657) was found to be better than MELD (c-statistic = 0.514) and better than the donor risk index (DRI) (c-statistic = 0.53) in predicting DCD graft loss when applied to the validation cohort.

DISCUSSION

The DCD liver is regarded as an extended criteria donor graft, in terms of the poorer outcomes that have been reported in the literature. Particular, concerns with this organ are the increased occurrence of PNF (0%-12%), early graft dysfunction (20%-30%), and PIC (15%) that result in the higher rates of graft loss and recipient death[5,6,12-18]. A large component in determining good results in DCD liver transplantation is the ability to balance risk through judicious matching of the donor and recipient. The aim of this work was to develop a formula, the DCD-RI that is valid and easy to apply with readily available objective variables relating to the donor and the recipient to help rationalize this risk balance.

The objective recipient variables that were found to have a significant effect on DCD graft survival were the primary indication for transplant, MELD and reTPL. While from the donor, CIT and hepatectomy time (dHepT) were important. These five variables combined with

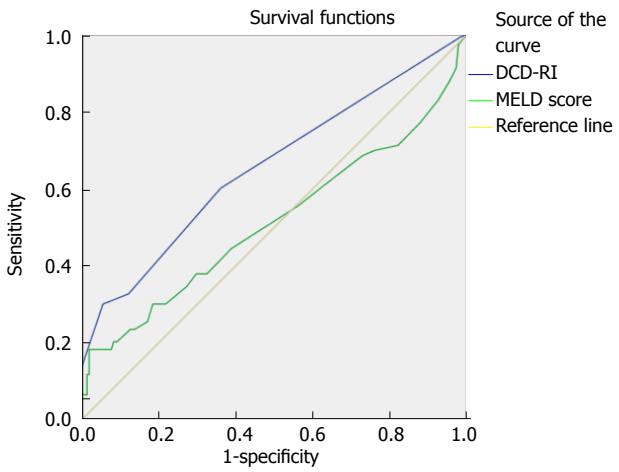


Figure 6 DCD-RI receiver operator curve and comparison to other predictive models illustrating that the DCD-RI performed better than model for end-stage liver disease in predicting graft survival. DCD-RI c-statistic = 0.657 and MELD c-statistic = 0.514). MELD: Model for end-stage liver disease; DCD-RI: Donor after cardiac death risk index.

the fundamental determinant of DCD graft outcome of WIT[7] formed the basis of the developed DCD-RI score. According to primary indication for transplant, three DCD risk groups of low, standard and high were defined (Figure 1). By applying this stratification, good graft survival of over 86% at 5 years was found when the DCD liver was used in recipients with low risk indications for transplant of AIH, PSC, PBC, NASH, HBV, and cholestatic diseases that included PFIC, EHBA and Crigler Najjar. While in the standard risk indication for transplant of metabolic diseases that encompassed Wilson's, Haemochromatosis and Amyloid, 5 year survival fell by 10%, to 76%. Similarly, 5 year DCD graft survival fell a further 10% to 64%, in the high risk recipient group of HCV, HCC, ALD, cryptogenic and Budd Chiari (Figure 1). When MELD was used to define DCD risk, recipients with a MELD ⩽ 25 were found to have a graft survival of 76% at 5 years, with survival falling a further 20% in higher MELD recipients. The use of DCD in reTPL was uncommon, accounting for 3.5% of DCD usage and graft survival was poorer (65% *vs* 78% at 5 years reTPL v primary transplant). Additionally, a donor hepatectomy time over one hour resulted in poor graft survival of 32% at 5 years. Allocating points, to the risk associated with each of these variables produced the DCD-RI score (Table 2).

A DCD-RI score over 5 was high risk for early graft loss, and predictive for poor long term survival of 34% at 5 years (Figure 5). In order to minimize DCD graft loss, the ideal is to aim for a DCD-RI score less than 5, which can be achieved either by minimizing the risk from the donor by selecting/aiming for a short WIT < 25 min, short CIT < 10 h, dHepT < 60 min, or by negating the DCD risk of the donor by selecting a low risk recipient, *i.e.,* MELD < 25, not a reTPL or belonging to the low risk primary indication group for DCD transplant. Internal validation of the DCD-RI on an earlier cohort supported the validity of the developed DCD-RI score by its ability to accurately predict graft survival for that cohort. Additionally, comparison, of the DCD-RI score showed it to out perform other predictive scoring systems such as the DRI and MELD.

Calculating the DCD-RI score helps to provide a framework to rationalize some of the risks involved in DCD liver transplantation. However, there are limitations to the data that were used to build the DCD-RI scoring system. The transplanted DCD livers whose data was used to design the DCD-RI score were already highly selected[10], automatically introducing bias into the study. By preselecting good quality DCD livers[21] as reflected by young donor age and short ischemic times (cold and warm), the majority (94.6%) of DCD transplants used to develop the DCD-RI belong to the low and standard DCD-RI score risk classes, while high risk DCD-RI transplants (DCD-RI score > 5) were rare in the programme. But by being stringent in DCD selection good outcomes can be achieved, comparable to DBD in both the short and long term[22].

Another factor that is well recognized to be a determinant of outcome in DCD transplantation but was not included in the DCD-RI was steatosis. The main reason for exclusion was assessment of liver steatosis by the surgeon is highly subjective[23] and histological assessment of the donor liver pre-perfusion is not routinely performed in transplant, and is in itself, a subjective assessment. Institutionally, the steatotic (> 30%) DCD liver is not used which may explain why donor BMI and donor liver weight, both surrogate markers of liver steatosis, were not found to have any bearing on graft survival. Neither, donor or recipient age, were included in the DCD-RI model as they were not found to be determinants of outcome in the data analyzed. This again reflects institutional practice, which is not to use donor/recipient age on its own, as a reason for DCD non consideration. Therefore, the developed DCD-RI has been able to combine adult and pediatric data. However, donor age has been identified in other series as a risk factor for graft failure[18] but older donors can be a valuable source of organs, and the risk from age can be balanced by reducing the risk from an alternative donor or recipient factor(s), *e.g.*, CIT[24,25].

Other predictive models for outcome after liver transplantation have been explored but they all, as does the DCD-RI, have various limitations. The donor recipient index (DRI) considers only donor factors[26]. While, the MELD score, that is the foundation of liver allocation on transplant waiting lists[27-29] is a poor predictor of outcome after transplant[30]. A number of other complex models detailing interactions between donor and recipient risk profiles have been developed to predict graft and patient survival after liver transplantation[30-39]. But none consider DCD in isolation and it is well recognized, that the DCD liver is a different type of graft in comparison to DBD, and DBD predictors of outcome have not been found to be applicable to DCD[40].

Only one other group has tried to design a DCD

prognostic scoring system, admittedly with smaller numbers (n = 81), in adults only and is yet to be validated[41]. However, they found similar variables to that of the present DCD-RI to be important predictors of DCD graft survival, such as primary indication for transplant, retransplantation, donor warm ischemic time and cold ischemic time (< 6 h). But with their DCD data, unlike the present data, they found recipient BMI (> 30) and donor HBV core antibody status influenced DCD graft outcomes. They did not consider donor hepatectomy time.

In conclusion, the developed DCD-RI score helps to rationalize and balance the risk between the donor and the recipient in DCD liver transplantation, in order to achieve good graft survival. To determine the true utility of the system it will need to be prospectively validated in other large volume DCD programmes.

ACKNOWLEDGMENTS

Sue Landymore, who maintains and updates the liver transplant database.

COMMENTS

Background

In Liver Transplant programmes in the West, there is a reliance on cadaveric donors over living related. The availability of cadaveric organs is insufficient to meet transplant need. Therefore, more marginal cadaveric organs have to be used, one such organ, is the donor after cardiac death (DCD) liver. However, this is a high risk organ and difficult to use with good results, most of which depends on subjective experience driven knowledge. The aim of this work was to create a scoring system using pre transplant objective data points from the donor and recipient to rationalize this risk.

Research frontiers

There is a lack of data on how to achieve the balance of risk between objective clinical variables from the DCD liver and selected recipient to produce good results. The present work aims to address this lack of information.

Innovations and breakthroughs

The DCD Risk Index (DCD-RI) score that was developed using objective variables from the donor and recipient was able to predict graft loss and DCD-RI class predicted graft survival.

Applications

The DCD-RI is a tool that can help in decision making on whether to use a given DCD liver in the selected recipient to produce good results.

Peer-review

Both internal and external was performed in order for this manuscript to be accepted for publication.

REFERENCES

1 **Hart A**, Smith JM, Skeans MA. OPTN/SRTR annual data report 2014. *Am J Transplant* 2016; **16**: 11-46

2 **Eurotransplant International Foundation**. Annual Report 2013. Available from: URL: http: //www.eurotransplant.org/cms/mediaobject.php?file=AR20135.pdf

3 **Monbaliu D**, Pirenne J, Talbot D. Liver transplantation using Donation after Cardiac Death donors. *J Hepatol* 2012; **56**: 474-485 [PMID: 21782762 DOI: 10.1016/j.jhep.2011.07.004]

4 **Khorsandi SE**, Yip VS, Cortes M, Jassem W, Quaglia A, O'Grady J, Heneghan M, Aluvihare V, Agarwal K, Menon K, Vilca-Melendez H, Prachalias A, Srinivasan P, Suddle A, Rela M, Heaton N. Does Donation After Cardiac Death Utilization Adversely Effect Hepatocellular Cancer Survival? *Transplantation* 2016; **100**: 1916-1924 [DOI: 10.1097/TP.0000000000001150]

5 **Mateo R**, Cho Y, Singh G, Stapfer M, Donovan J, Kahn J, Fong TL, Sher L, Jabbour N, Aswad S, Selby RR, Genyk Y. Risk factors for graft survival after liver transplantation from donation after cardiac death donors: an analysis of OPTN/UNOS data. *Am J Transplant* 2006; **6**: 791-796 [PMID: 16539637 DOI: 10.1111/j.1600-6143.2006.01243.x]

6 **Lee KW**, Simpkins CE, Montgomery RA, Locke JE, Segev DL, Maley WR. Factors affecting graft survival after liver transplantation from donation after cardiac death donors. *Transplantation* 2006; **82**: 1683-1688 [PMID: 17198260 DOI: 10.1097/01.tp.0000250936.73034.98]

7 **Taner CB**, Bulatao IG, Willingham DL, Perry DK, Sibulesky L, Pungpapong S, Aranda-Michel J, Keaveny AP, Kramer DJ, Nguyen JH. Events in procurement as risk factors for ischemic cholangiopathy in liver transplantation using donation after cardiac death donors. *Liver Transpl* 2012; **18**: 100-111 [PMID: 21837741 DOI: 10.1002/lt.22404]

8 **Mathur AK**, Heimbach J, Steffick DE, Sonnenday CJ, Goodrich NP, Merion RM. Donation after cardiac death liver transplantation: predictors of outcome. *Am J Transplant* 2010; **10**: 2512-2519 [PMID: 20977642 DOI: 10.1111/j.1600-6143.2010.03293.x]

9 **Casavilla A**, Ramirez C, Shapiro R, Nghiem D, Miracle K, Fung JJ, Starzl TE. Liver and kidney transplantation from non-heart beating donors: the Pittsburgh experience. *Transplant Proc* 1995; **27**: 710-712 [PMID: 7879154]

10 **Davila D**, Ciria R, Jassem W, Briceño J, Littlejohn W, Vilca-Meléndez H, Srinivasan P, Prachalias A, O'Grady J, Rela M, Heaton N. Prediction models of donor arrest and graft utilization in liver transplantation from maastricht-3 donors after circulatory death. *Am J Transplant* 2012; **12**: 3414-3424 [PMID: 23016623 DOI: 10.1111/j.1600-6143.2012.04242.x]

11 **Muiesan P**, Girlanda R, Jassem W, Melendez HV, O'Grady J, Bowles M, Rela M, Heaton N. Single-center experience with liver transplantation from controlled non-heartbeating donors: a viable source of grafts. *Ann Surg* 2005; **242**: 732-738 [PMID: 16244548 DOI: 10.1097/01.sla.0000186177.26112.d2]

12 **Abt PL**, Desai NM, Crawford MD, Forman LM, Markmann JW, Olthoff KM, Markmann JF. Survival following liver transplantation from non-heart-beating donors. *Ann Surg* 2004; **239**: 87-92 [PMID: 14685105 DOI: 10.1097/01.sla.0000103063.82181.2c]

13 **Jay CL**, Lyuksemburg V, Ladner DP, Wang E, Caicedo JC, Holl JL, Abecassis MM, Skaro AI. Ischemic cholangiopathy after controlled donation after cardiac death liver transplantation: a meta-analysis. *Ann Surg* 2011; **253**: 259-264 [PMID: 21245668 DOI: 10.1097/SLA.0b013e318204e658]

14 **Foley DP**, Fernandez LA, Leverson G, Anderson M, Mezrich J, Sollinger HW, D'Alessandro A. Biliary complications after liver transplantation from donation after cardiac death donors: an analysis of risk factors and long-term outcomes from a single center. *Ann Surg* 2011; **253**: 817-825 [PMID: 21475025 DOI: 10.1097/SLA.0b013e3182104784]

15 **Foley DP**, Fernandez LA, Leverson G, Chin LT, Krieger N, Cooper JT, Shames BD, Becker YT, Odorico JS, Knechtle SJ, Sollinger HW, Kalayoglu M, D'Alessandro AM. Donation after cardiac death: the University of Wisconsin experience with liver transplantation. *Ann Surg* 2005; **242**: 724-731 [PMID: 16244547 DOI: 10.1097/01.sla.0000186178.07110.92]

16 **Abt P**, Crawford M, Desai N, Markmann J, Olthoff K, Shaked A. Liver transplantation from controlled non-heart-beating donors: an increased incidence of biliary complications. *Transplantation* 2003; **75**: 1659-1663 [PMID: 12777852 DOI: 10.1097/01.TP.0000062574.18648.7C]

17 **Jay CL**, Lyuksemburg V, Kang R, Preczewski L, Stroupe K, Holl JL, Abecassis MM, Skaro AI. The increased costs of donation after cardiac death liver transplantation: caveat emptor. *Ann Surg* 2010; **251**: 743-748 [PMID: 20224367 DOI: 10.1097/SLA.0b013e3181d3d3da]

18 **de Vera ME**, Lopez-Solis R, Dvorchik I, Campos S, Morris W, Demetris AJ, Fontes P, Marsh JW. Liver transplantation using donation after cardiac death donors: long-term follow-up from a single center.

Am J Transplant 2009; **9**: 773-781 [PMID: 19344466 DOI: 10.1111/j.1600-6143.2009.02560.x]

19 **Chan EY**, Olson LC, Kisthard JA, Perkins JD, Bakthavatsalam R, Halldorson JB, Reyes JD, Larson AM, Levy AE. Ischemic cholangiopathy following liver transplantation from donation after cardiac death donors. *Liver Transpl* 2008; **14**: 604-610 [PMID: 18433032 DOI: 10.1002/lt.21361]

20 **Axelrod DA**, Dzebisashvilli N, Lentine KL, Xiao H, Schnitzler M, Tuttle-Newhall JE, Segev DL. National assessment of early biliary complications after liver transplantation: economic implications. *Transplantation* 2014; **98**: 1226-1235 [PMID: 25119126 DOI: 10.1097/TP.0000000000000197]

21 **Andrews PA**, Burnapp L, Manas D. Summary of the British Transplantation Society guidelines for transplantation from donors after deceased circulatory death. *Transplantation* 2014; **97**: 265-270 [PMID: 24448588 DOI: 10.1097/01.TP.0000438630.13967.c0]

22 **DeOliveira ML**, Jassem W, Valente R, Khorsandi SE, Santori G, Prachalias A, Srinivasan P, Rela M, Heaton N. Biliary complications after liver transplantation using grafts from donors after cardiac death: results from a matched control study in a single large volume center. *Ann Surg* 2011; **254**: 716-722; discussion 722-723 [PMID: 22042467 DOI: 10.1097/SLA.0b013e318235c572]

23 **Yersiz H**, Lee C, Kaldas FM, Hong JC, Rana A, Schnickel GT, Wertheim JA, Zarrinpar A, Agopian VG, Gornbein J, Naini BV, Lassman CR, Busuttil RW, Petrowsky H. Assessment of hepatic steatosis by transplant surgeon and expert pathologist: a prospective, double-blind evaluation of 201 donor livers. *Liver Transpl* 2013; **19**: 437-449 [PMID: 23408461 DOI: 10.1002/lt.23615]

24 **Cescon M**, Grazi GL, Cucchetti A, Ravaioli M, Ercolani G, Vivarelli M, D'Errico A, Del Gaudio M, Pinna AD. Improving the outcome of liver transplantation with very old donors with updated selection and management criteria. *Liver Transpl* 2008; **14**: 672-679 [PMID: 18433035 DOI: 10.1002/lt.21433]

25 **Firl DJ**, Hashimoto K, O'Rourke C, Diago-Uso T, Fujiki M, Aucejo FN, Quintini C, Kelly DM, Miller CM, Fung JJ, Eghtesad B. Impact of donor age in liver transplantation from donation after circulatory death donors: A decade of experience at Cleveland Clinic. *Liver Transpl* 2015; **21**: 1494-1503 [PMID: 26334196 DOI: 10.1002/lt.24316]

26 **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]

27 **Kamath PS**, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470 [PMID: 11172350 DOI: 10.1053/jhep.2001.22172]

28 **Malinchoc M**, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**: 864-871 [PMID: 10733541 DOI: 10.1053/he.2000.5852]

29 **Wiesner R**, Edwards E, Freeman R, Harper A, Kim R, Kamath P, Kremers W, Lake J, Howard T, Merion RM, Wolfe RA, Krom R. Model for end-stage liver disease (MELD) and allocation of donor livers. *Gastroenterology* 2003; **124**: 91-96 [PMID: 12512033 DOI: 10.1053/gast.2003.50016]

30 **Desai NM**, Mange KC, Crawford MD, Abt PL, Frank AM, Markmann JW, Velidedeoglu E, Chapman WC, Markmann JF. Predicting outcome after liver transplantation: utility of the model for end-stage liver disease and a newly derived discrimination function. *Transplantation* 2004; **77**: 99-106 [PMID: 14724442 DOI: 10.1097/01.TP.0000101009.91516.FC]

31 **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]

32 **Ghobrial RM**, Gornbein J, Steadman R, Danino N, Markmann JF, Holt C, Anselmo D, Amersi F, Chen P, Farmer DG, Han S, Derazo F, Saab S, Goldstein LI, McDiarmid SV, Busuttil RW. Pretransplant model to predict posttransplant survival in liver transplant patients. *Ann Surg* 2002; **236**: 315-322; discussion 322-323 [PMID: 12192318 DOI: 10.1097/01.SLA.0000026684.68004.1E]

33 **Avolio AW**, Agnes S, Gasbarrini A, Nure E, Siciliano M, Castagneto M. Prognostic value of MELD score and donor quality in liver transplantation: implications for the donor recipient match. *Transplant Proc* 2006; **38**: 1059-1062 [PMID: 16757263 DOI: 10.1016/j.transproceed.2006.03.008]

34 **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]

35 **Zamboni F**, Franchello A, David E, Rocca G, Ricchiuti A, Lavezzo B, Rizzetto M, Salizzoni M. Effect of macrovescicular steatosis and other donor and recipient characteristics on the outcome of liver transplantation. *Clin Transplant* 2001; **15**: 53-77 [DOI: 10.1034/j.1399-0012.2001.150109.x]

36 **Haydon GH**, Hiltunen Y, Lucey MR, Collett D, Gunson B, Murphy N, Nightingale PG, Neuberger J. Self-organizing maps can determine outcome and match recipients and donors at orthotopic liver transplantation. *Transplantation* 2005; **79**: 213-218 [PMID: 15665770]

37 **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]

38 **Schaubel DE**, Guidinger MK, Biggins SW, Kalbfleisch JD, Pomfret EA, Sharma P, Merion RM. Survival benefit-based deceased-donor liver allocation. *Am J Transplant* 2009; **9**: 970-981 [PMID: 19341419 DOI: 10.1111/j.1600-6143.2009.02571.x]

39 **Briceño J**, Cruz-Ramírez M, Prieto M, Navasa M, Ortiz de Urbina J, Orti R, Gómez-Bravo MÁ, Otero A, Varo E, Tomé S, Clemente G, Bañares R, Bárcena R, Cuervas-Mons V, Solórzano G, Vinaixa C, Rubín A, Colmenero J, Valdivieso A, Ciria R, Hervás-Martínez C, de la Mata M. Use of artificial intelligence as an innovative donor-recipient matching model for liver transplantation: results from a multicenter Spanish study. *J Hepatol* 2014; **61**: 1020-1028 [PMID: 24905493 DOI: 10.1016/j.jhep.2014.05.039]

40 **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]

41 **Hong JC**, Yersiz H, Kositamongkol P, Xia VW, Kaldas FM, Petrowsky H, Farmer DG, Lipshutz G, Markovic D, Hiatt JR, Busuttil RW. Liver transplantation using organ donation after cardiac death: a clinical predictive index for graft failure-free survival. *Arch Surg* 2011; **146**: 1017-1023 [PMID: 21930997 DOI: 10.1001/archsurg.2011.240]

P- Reviewer: Kang KJ, Quak SH, Sugawara Y **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 June 24; 7(3): 213-221

DOI: 10.5500/wjt.v7.i3.213

ISSN 2220-3230 (online)

CASE REPORT

Tuberculosis in kidney transplant recipients: A case series

Manish Anand, Ekta Nayyar, Beatrice Concepcion, Megha Salani, Heidi Schaefer

Manish Anand, Department of Medicine, Division of Nephrology, University of Cincinnati, Cincinnati, OH 45221, United States

Ekta Nayyar, Trihealth Infectious Disease, Good Samaritan Hospital, Cincinnati, OH 45220, United States

Beatrice Concepcion, Megha Salani, Heidi Schaefer, Department of Medicine, Division of Nephrology, Vanderbilt University, Nashville, TN 37232, United States

Author contributions: All authors contributed to the acquisition of data, drafting the article, and making critical revisions related to the content of the manuscript.

Institutional review board statement: This case report was exempt from Institutional Review Board Standards at Vanderbilt University at Nashville.

Conflict-of-interest statement: The authors of this manuscript have no conflicts of interest to disclose.

Manuscript source: Invited manuscript

Correspondence to: Heidi Schaefer, MD, Associate Professor of Medicine, Department of Medicine, Division of Nephrology, Vanderbilt University, 1161 21st Avenue South, Nashville, TN 37232, United States. heidi.schaefer@vanderbilt.edu
Telephone: +1-615-3226976
Fax: +1-615-9360695

Received: October 25, 2016
Peer-review started: October 28, 2016
First decision: December 1, 2016
Revised: January 14, 2017
Accepted: April 23, 2017
Article in press: April 24, 2017
Published online: June 24, 2017

Abstract

Solid organ transplant recipients have an elevated risk of tuberculosis (TB) with high mortality. Data about TB in this population in the United States is sparse. We present four cases of active tuberculosis in kidney transplant recipients at our center. All patients had possible TB exposure prior to transplant and all were diagnosed with active TB within the first year of transplant. Disseminated TB was seen in half of the patients with extra-pulmonary TB being more common affecting lymph nodes, pericardium, and the kidney allograft. Delay in diagnosis from onset of symptoms ranged from fifteen days to two months. Two patients died from TB. TB is a largely preventable and curable disease. However, challenges remain in the diagnosis due to most recipients presenting with atypical symptoms. Physicians should maintain a high degree of suspicion for TB to promptly diagnose and treat post-transplant thereby minimizing complications. A review of the literature including the epidemiology, pathogenesis, clinical presentation, diagnosis and treatment options are discussed.

Key words: Mycobacterium tuberculosis; Kidney transplant; Disseminated disease; Tuberculosis

Core tip: Tuberculosis is a largely preventable and curable disease that should be suspected in all solid organ transplant recipients who present with unexplained fevers, pulmonary or extra-pulmonary symptoms. This case report describes the varied presentations of tuberculosis in kidney transplant recipients and provides the most recent recommendations regarding diagnosis and treatment.

Anand M, Nayyar E, Concepcion B, Salani M, Schaefer H. Tuberculosis in kidney transplant recipients: A case series. *World J Transplant* 2017; 7(3): 213-221 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i3/213.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i3.213

INTRODUCTION

The overall incidence and prevalence of mycobacterium tuberculosis (TB) in solid organ transplant recipients is not well defined. The rates of TB in this population are mostly based on data available from individual study cohorts reported in the literature. In the western world, TB is a rare opportunistic infection with significant morbidity and mortality. Clinical presentation in immunocompromised individuals, including transplant recipients is often atypical and diverse. This leads to delay in the diagnosis and advanced disease at the time of diagnosis. In addition, inadequate host response in this setting poses a treatment challenge. The higher toxicity of treatment and concurrent use of immunosuppressive medications with drug interactions further generate complexity in management. We describe four cases of active TB in our kidney transplant recipients and explore the epidemiology, clinical presentation, management and outcomes of TB disease in this population.

CASE REPORT

Case 1

A 63-year-old Vietnamese male with end stage renal disease due to IgA nephropathy received an expanded criteria deceased donor kidney transplant (DDKT) in 2012 (5 antigen mismatch, 5% panel reactive antibody, PRA). He received induction with alemtuzumab and solumedrol and was maintained on tacrolimus and mycophenolate mofetil. There were no surgical complications or episodes of acute rejection in the post-transplant period. Allograft function stabilized with a serum creatinine (Cr) of 1.8 mg/dL. His past medical history was notable for incarceration in Vietnam, prior hepatitis B exposure with protective anti-Hepatitis B surface antibody, positive tuberculin skin test (TST) and a non-calcified nodule on chest X-ray (CXR). He had been in the United States for twenty years prior to his transplant. He did not receive isoniazid (INH) prophylaxis before undergoing kidney transplant. At one-year post-transplant, he was admitted with fever, palpitations and 3 cm non-tender submental lymph node. Labs were notable for acute kidney injury (AKI) with Cr of 3 mg/dL and urinary retention that resolved with urinary catheter placement and treatment for an enlarged prostate. CXR revealed bilateral pleural effusions and a large pericardial effusion. Fine needle aspiration of the lymph node and pericardial fluid grew Mycobacterium tuberculosis (MTB). He received anti-tubercular therapy (ATT) with 2 mo of Rifampin, INH, Pyrazinamide and Ethambutol (RIPE) and 4.5 mo of INH and Rifampin (IR). His treatment course was complicated by transaminitis with reactivation of hepatitis B leading to end stage liver disease. He was treated with tenofovir with resolution of transaminitis. Patient completed 6.5 mo of ATT and has been cured of TB. His kidney transplant failed three years later due to BK nephropathy, and he was initiated on hemodialysis.

Case 2

A 67-year-old Caucasian male, Vietnam War veteran with ESRD presumed secondary to hypertension received a DDKT in 2013 (0 antigen mismatch, PRA 36%). He received induction with alemtuzumab and solumedrol and was maintained on tacrolimus, mycophenolate mofetil, and prednisone. Pre-transplant CXR showed prior granulomatous disease. He was not tested for latent TB infection (LTBI). Two months after transplant, he was admitted with fever and progressive shortness of breath. CXR revealed a miliary pattern of infiltrates. He developed acute respiratory failure and septic shock requiring intubation and multiple vasopressors. The day after admission, sputum samples returned positive for acid-fast bacilli (AFB), and later grew MTB. Clinical course was complicated by development of presumed macrophage activation syndrome (MAS). He received neupogen for pancytopenia but bone marrow biopsy could not be obtained due to agitation. He did not receive intravenous steroids or chemotherapy for MAS. Patient died within three days of admission.

Case 3

A 38-year-old Indonesian woman living in United States for ten years with ESRD due to IgA nephropathy on hemodialysis for 10 years received a DDKT in 2015 (6 antigen mismatch, PRA 0%). She received induction with alemtuzumab and solumedrol and was maintained on tacrolimus, mycophenolate mofetil, and prednisone. There were no surgical complications or episodes of acute rejection in the post-transplant period. Allograft function was excellent with serum Cr of 1.0 mg/dL. Pre-transplant work up was notable for positive TST with normal CXR. She was started on INH immediately after transplant and received nine months of therapy for LTBI. One month after completing INH therapy, she was admitted with persistent fevers, night sweats and acute kidney injury, serum Cr of 2 mg/dL. Fever work up showed adenovirus in the blood and urine. There was increased flurodeoxyglucose uptake in the kidney allograft on positron emission tomography scan. Biopsy of the kidney transplant showed necrotizing granulomatous interstitial nephritis. Differential diagnosis of the granulomatous interstitial nephritis included renal transplant TB and adenovirus infection. Renal pathology changes were not consistent with adenovirus infection. AFB smear and cultures were negative in the urine and renal biopsy specimens. Due to persistent fevers, worsening renal function and clinical suspicion for TB, she was started on RIPE and Moxifloxacin. Moxifloxacin was added as a fifth agent due to concern for INH resistance given she was treated with INH monotherapy for LTBI. Fevers, night sweats and AKI resolved on treatment without addition of cidofovir, which supported the diagnosis of renal transplant TB. Her IS was modified with discontinuation of MMF. She is currently maintained on tacrolimus and prednisone. She completed 6 mo of ATT and is cured of TB. Renal allograft function is stable

with Cr of 1.3 mg/dL.

Case 4

A 67-year-old Caucasian male with ESRD, secondary to diabetes mellitus on hemodialysis for 2 years received a DDKT in 2015 (4 antigen mismatch, PRA 0%, A2 to B kidney). He received induction with alemtuzumab and solumedrol and was maintained on tacrolimus, mycophenolate mofetil, and prednisone. His pre-transplant CXR showed calcified lung nodules, and he had a negative interferon gamma release assay (quantiferon gold). He presented two and a half months' post-transplant with two weeks of intermittent fever, malaise, progressive dyspnea and lower extremity swelling. He was diagnosed with bilateral lower extremity deep vein thrombus and pulmonary embolism for which anticoagulation was initiated. Due to intermittent fevers, computed tomography (CT) of the chest was done that showed a few scattered sub centimeter non-calcified pulmonary nodules and a 2 cm right paratracheal lymph node concerning for granulomatous disease. Fungal testing including serum galactomannan, serum cryptococcal antigen, beta-D-glucan levels and urine histoplasma antigen, was negative. Bronchoscopy was performed with AFB stain positive in the bronchoalveolar lavage (BAL). AFB and non-necrotizing granulomas were seen on trans-bronchial lung biopsy. MTB complex polymerase chain reaction (PCR) was positive in both the BAL and blood, and cultures from both grew MTB. Sputum cultures later grew pan susceptible MTB. He was discharged on a four-drug regimen with RIPE. Two weeks later, he was readmitted with recurrence of fever, altered mental status and partial loss of vision. Repeat CT of the chest showed worsening bilateral pulmonary infiltrates.

Moxifloxacin was added to his regimen. ATT drug levels were obtained and found to be therapeutic. Sputum, urine and blood cultures returned negative for AFB. Neurology work up including magnetic resonance imaging of the brain and lumbar puncture was negative. Patient developed AKI with serum Cr of 3 mg/dL. Ethambutol dose was decreased from 1600 mg daily to 1600 mg every 36 h and pyrazinamide dose was lowered from 2000 mg daily to 2000 mg thrice weekly. Ethambutol was subsequently discontinued due to worsening visual changes and amikacin was added to the treatment regimen of isoniazid, rifampin, pyrazinamide and moxifloxacin. His IS was ultimately tapered to prednisone alone due to worsening of TB with persistent fever and progressive pulmonary infiltrates. Renal allograft function continued to decline likely due to tapering off IS and aminoglycoside toxicity ultimately leading to allograft failure. He was started on hemodialysis 4 mo after initiation of ATT and died three months later.

DISCUSSION

Epidemiology

Even before MTB was discovered, Laennec described the diseased lung cavities on autopsies. Historically this was referred to as "consumption" owing to significant weight loss and finally death that consumed patients. In 1839, Johann Schonle coined the term tuberculosis from the Latin word "tuberculum" which means small pimple or a bump. The bacillus was identified by Robert Koch as Mycobacterium tuberculosis on March 24, 1882 which is commemorated as World TB day.

The global TB incidence and prevalence has been declining per the most recent WHO Global TB report[1]. The incidence of TB globally is 18% lower in 2014 as compared to 2000 and TB prevalence is 42% lower as compared to 1990. TB mortality has also fallen 47% since 1990. The incidence rate is highest in South East Asia and the Western Pacific and lowest in Western Europe, Canada, United States, Australia and New Zealand. The CDC Morbidity and Mortality Report in early 2016 shows leveling of TB incidence in the United States at 3 cases/100000 persons in 2013-2015, after two decades of annual decline[2]. Approximately 70% of the cases are in foreign-born individuals, with Mexico, the Philippines, India, Vietnam and China accounting for the top five countries of origin. In our case series, two out of four were from Southeast Asia which is considered to be endemic for TB. Among those born in the United States, native Hawaiians/other Pacific Islanders have the highest incidence followed by American Indians and Alaskan Natives. Almost half of all reported TB cases in the United States are reported from California, Florida, New York and Texas. The TB incidence in foreign born individuals has been steadily declining compared to stabilization of TB incidence among those born in the United States, pointing to TB transmission in the United States. This has been confirmed by molecular genotyping. Risk factors for TB outbreaks include substance abuse, incarceration and homelessness.

Data regarding the prevalence and incidence of TB in solid organ transplant recipients is sparse. Prevalence of active TB is estimated to be 1.2%-6.4% in developed countries and up to 15% in highly endemic areas[3]. A study in 1998 estimated a 0.35%-1.2% incidence in renal transplant recipients in the United States[4]. Risk in solid organ transplant recipients is estimated to be 20-74 times higher than the general population with a high mortality rate of up to 30%. Mortality of TB is higher in patients with disseminated disease, prior rejection and those who received anti-T cell antibody therapy[4]. Another study found higher mortality with graft rejection, steroid treatment and concomitant other opportunistic infection[3]. Diabetes mellitus and chronic liver disease have also been associated with greater mortality[5]. Our case series show a mortality of 50%. Half of our TB cases had disseminated disease. All four patients received anti-T cell antibody therapy and three were on steroids. Half of our patients had diabetes mellitus. Baseline characteristics of our patients are listed in Table 1.

Over 50% of renal transplant recipients develop TB within the first year of transplant[4]. TB develops earlier

Table 1 Baseline characteristics of patients

	Patient 1	Patient 2	Patient 3	Patient 4
Age (yr)	63	67	38	67
Ethnicity	Vietnamese	Caucasian	Indonesia	Caucasian
Sex	Male	Male	Female	Male
BMI (kg/m^2)	32	33	21	32
Prior TB exposure	Incarceration in Vietnam	Vietnam war veteran	Lived in Indonesia till age 25	None
PPD/IGRA	Positive	Not done	Positive	Negative
Pre-transplant CXR	Non-calcified lymph nodes	Prior granulomatous disease	Normal	Calcified lung nodules
Smoking	Yes	No	No	No
Diabetes mellitus	Yes	No	No	Yes
Hepatitis C	No	No	No	No
Chronic liver disease	Prior hepatitis B exposure	No	No	No
Pre-transplant INH prophylaxis	No	No	No	No

BMI: Body mass index; TB: Tuberculosis; PPD: Purified protein derivative; IGRA: Interferon gamma release assay; CXR: Chest X-ray; INH: Isoniazid.

in those with prior TB exposure[3]. Markers for prior infection include cellular response to TB specific antigens (positive TST or interferon gamma release assay, IGRA) or sequelae of granulomatous infection on CXR. Older patients are more likely to have reactivation following transplantation than younger patients, particularly in the developed world. All of our cases had prior TB exposure and developed TB early after transplant, half developed disease within the first 3 mo following transplant.

Factors predisposing to TB both in the general population and transplant recipient include country of origin, history of untreated latent TB infection, cigarette smoking, body mass index < 18.5, diabetes mellitus, chronic kidney disease, chronic liver disease, lupus, human immunodeficiency virus, silicosis, gastrectomy, jejuno-ileal bypass, as well as social risk factors (homelessness, incarceration, alcoholism and known TB contact)[6,7]. The main predisposing factor in our center's experience was residence from or previous travel to an endemic region (Table 2).

Pathogenesis

TB is usually acquired *via* inhalation of bacilli into the lungs. Progression to clinical disease depends on the infecting dose and virulence of the Mycobacteria as well as the development of host cell mediated immunity. The most common reason for post-transplant TB is reactivation of previous infection. In patients with prior exposure, the risk is generally inversely related to the time from acquisition to transplantation. Rarely, TB can be donor-derived and transmitted through the transplanted organ. TB can be acquired post-transplant, more commonly in TB endemic countries, or nosocomial as part of outbreaks in renal transplant units[8].

Clinical presentation

The clinical presentation of TB in transplant recipients differs from the general population in that symptoms are more unusual and varied, often leading to a delay in diagnosis and poor outcomes. Fever is seen more commonly, and approximately 30%-50% of TB after transplant is extra-pulmonary or disseminated[4,7]. Disseminated disease is defined as involvement of two or more non-contiguous organs with positive TB cultures, with or without granulomas[4].

CXR's in post-transplant TB show diffuse pulmonary infiltrates rather than cavitary lesions which are more commonly seen in the general population[7]. In our case series, fever was present in all four patients. Cervical lymphadenopathy was seen in one patient. Disseminated TB was seen in two of the four patients with extra-pulmonary involvement of lymph nodes, pericardium and the renal allograft. Two patients had pulmonary TB and one of them had disseminated disease. Only one presented with cough. Patients with pulmonary involvement showed military pattern and bilateral diffuse pulmonary nodules on CXR.

Diagnosis and pre-transplant screening

Diagnosis of latent TB is an indirect measure of possible infection by detection of a cellular response to MTB specific antigens in the absence of symptoms. The two types of tests are *in-vivo*: Tuberculin skin test (TST) done by intradermal injection of purified protein derivative (PPD); and *ex-vivo*: IGRA (Quantiferon gold test or T-spot TB test). PPD is a glycerol extract of the tubercle bacillus and is not species specific. Induration of 5 mm or more is considered to be positive in transplant candidates. If the first test is negative, a follow-up second test is recommended two weeks later. This leads to a "booster effect" due to amnestic recall of immunity and can identify an additional 10% of cases[9]. Limitations of PPD testing include a higher rate of false negatives in the immunocompromised host, confounding by non-tubercular mycobacteria and prior BCG vaccination, and need for trained staff and a second visit for interpretation of the test by a qualified provider. IGRA utilizes sensitized T cells that release interferon-gamma. The advantages of IGRA over PPD include improved specificity due to MTB specific antigens that do not cross-react with BCG and the use of positive and negative controls that may differentiate true negatives from those that result from anergy or overt immunosuppression[10]. Performance of IGRA is better in low prevalence countries as compared to endemic

Table 2 Post-transplant patient characteristics and outcomes

	Patient 1	Patient 2	Patient 3	Patient 4
INH prophylaxis	No	No	Yes	No
T cell depleting antibody	Yes	Yes	Yes	Yes
Immunosuppressive	Tacrolimus, MMF	Tacrolimus, MMF	Tacrolimus, MMF	Tacrolimus, MMF
Corticosteroid	No	Yes	Yes	Yes
Acute rejection (6 mo prior to TB diagnosis)	No	No	No	No
Clinical features	Fever, palpitations, cervical LN	Fever, shortness of breath, cough	Fever, acute kidney injury	Fever, shortness of breath, leg swelling
TB site	Disseminated	Pulmonary	Extra-pulmonary	Disseminated
Time to symptom onset (mo)	11.5	2	9	2
Time to diagnosis, post-transplant (mo)	12	3	11	3
Treatment regimen	RIPE	None	RIPE-M	RIPE-M, Amikacin
Treatment duration (mo)	6.5	N/A	6	7
Adverse drug reaction	Hepatotoxicity	N/A	None	Neurologica,vision loss
Other complication	HBV reactivation, acute liver injury	Septic shock, MAS	None	VTE, IRIS, allograft failure
Outcome	Cured	Death	Cured	Death

INH: Isoniazid; MMF: Mycophenolate mofetil; TB: Tuberculosis; LN: Lymphadenopathy; RIPE: Rifampin, isoniazid, pyrazinamide, ethambutol; RIPE-M: Rifampin, isoniazid, pyrazinamide, ethambutol, moxifloxacin; HBV: Hepatitis B virus; MAS: Macrophage activation syndrome; VTE: Venous thromboembolism; IRIS: Immune reconstitution inflammatory syndrome.

areas[11]. Both these tests cannot differentiate between latent TB and active TB. ESRD and immunosuppressant use are responsible for a higher rate of false negative or equivocal results of immune based T-cell assays. Uremia is associated with impaired co-stimulatory function of the antigen-specific T-cells leading to a defect in T-cell function. One of our transplant recipients had a negative IGRA in the presence of calcified nodules on chest imaging.

Immunosuppressants such as T-cell depleting antibodies, corticosteroids and calcineurin inhibitors cause a reduction in the number of T-cells, affect their interaction with antigen-presenting cells and impair cytokine induction[12].

Diagnosis of TB in transplant recipients is often delayed. In our case series, delay in diagnosis from onset of symptoms ranged between fifteen days and two months. Diagnosis of active TB is made by demonstration of AFB on smear microscopy and isolation of mycobacteria in culture of the body fluid. AFB blood cultures should be done if there is a suspicion for disseminated TB. For pulmonary TB, three samples of sputum are sent 8-24 h apart with at least one being an early morning sample. Sputum induction with aerosolized hypertonic saline can be employed for patients who are unable to expectorate. Invasive diagnostic tests such as bronchoscopy with bronchoalveolar lavage may be necessary for diagnosis. Sensitivity and specificity of sputum AFB smear microscopy is 45%-80% and 50%-80%, respectively[13].

Sensitivity and specificity of sputum culture is 80% and 98%, respectively[14,15]. Cultures need to be incubated for 6-8 wk to isolate MTB. Drug susceptibility testing should be done on all positive MTB cultures. Nucleic acid amplification (NAA) assays are available for rapid diagnosis of TB. These tests can be done from cultures or direct tissue samples. The Centers for Disease Control (CDC) recommends sending the first sputum sample for NAA testing. These assays can detect target specific MTB complex RNA/DNA sequences with nucleic acid probes in 24-48 h. Xpert MTB/Rif test is an automated NAA test that detects rifampin resistance simultaneously in two hours. Rifampin resistance is a marker of multi-drug resistant (MDR) TB. Sensitivity and specificity of NAA tests in AFB smear positive respiratory secretions is over 95% and is not affected by non-tuberculous *Mycobacteria* (NTM) or immunosuppression. They have lower sensitivity, 75%-85% in smear negative sputum[16-18]. These tests should be performed within the first few days of ATT and a negative NAA test does not exclude TB. Cultures are still required for species identification and for drug susceptibility testing. NAA assays do not perform as well for other clinical specimens and the overall evidence regarding their use in transplant patients is lacking at this time. Tissue biopsy of the involved organ and/or fluid for histopathology evaluation, AFB smear and culture should be obtained in suspected extra-pulmonary TB.

In our case series, we diagnosed TB disease if any of the following criteria were met: (1) isolation of MTB in culture of sputum, blood or any body fluid, with or without detection of AFB on smear; (2) clinical response to ATT in a patient with fever of unknown origin or compatible clinical syndrome with radiographic and histopathological features suggestive of TB, including tissue sample with granulomas; and (3) presence of MTB DNA using PCR.

Pre-transplant screening of donor and recipient for TB infection should be rigorous given the high risk of TB in the transplant setting and significant associated mortality. In transplant candidates and living donors, thorough history taking and comprehensive physical examination should be performed with a special focus on the medical and social risk factors for TB mentioned earlier. History of TB exposure is most essential and one should inquire about residence and travel history to endemic areas, contact with a known active TB case, and prior TST testing results. In patients with a history of prior LTBI or TB, details regarding treatment regimen and duration are essential, and active TB in these individuals should

be excluded. These patients may need additional testing and consultation with a transplant infectious disease specialist. Donors with active TB within 2 years have higher risk of relapse and transmission *via* the allograft[6]. Patients without prior history of known LTBI or TB disease should undergo testing for LTBI with a PPD test or IGRA. If the first PPD test is negative, a second skin test is recommended for booster effect as discussed earlier. A CXR is part of routine preoperative screening and should be evaluated for evidence of prior granulomatous disease. Patients with positive PPD or IGRA should be treated for LTBI prior to transplantation, whenever possible, after exclusion of active TB. Individuals with low risk of TB based on history and negative testing are cleared for transplantation. In high risk patients with negative TST/IGRA, indeterminate IGRA or chest imaging suggestive of prior granulomatous disease, it is recommended to treat with INH for presumed LTBI, prior to transplantation. Active TB needs to be ruled out by appropriate smears, cultures and molecular testing before treatment for latent TB is initiated. In high-risk patients, urine for AFB and renal imaging should also be performed to rule out genitourinary TB[19]. In our case series, two patients had known LTBI by PPD/IGRA but did not receive INH prophylaxis prior to the transplant. One of the patients received INH prophylaxis immediately post-transplant. One patient was not tested for LTBI, but was high risk based on prior exposure history and a CXR with old granulomatous changes. Interestingly, one recipient tested negative by IGRA and was low clinical risk. He had calcified nodules on imaging and later developed TB disease.

Pre-transplant evaluation is challenging in deceased donors given the limited history available. Efforts should be made to obtain a history regarding prior TB exposure, TB disease and treatment from family and healthcare givers. The evaluation is similar to living donors as above, prior to accepting the organ. In donors with a history of TB and reliable information about completed ATT, appropriate smears, cultures and molecular testing should be done to rule out active disease. In deceased donors with a history of TB disease and insufficient information about treatment or positive testing, it is recommended to reject the donor except in urgent transplants. In this scenario, recipients should be treated for active TB after informed consent with close monitoring under the guidance of an infectious disease specialist[6,8].

Management

Direct evidence regarding management of transplant recipients for prevention and treatment of latent and active TB infection is lacking. Their care is largely based on expert opinion and extrapolation from studies in immune-competent and other immunocompromised populations. Indications for treatment of LTBI in recipient candidates include a positive TST/IGRA as well as those with a negative TST/IGRA or indeterminate IGRA with risk factors: Radiographic evidence of prior TB in the absence of treatment, donor with recent TB exposure, positive TST or radiographic signs, or close prolonged contact with an active TB case[8]. Before treatment of LTBI, active TB needs to be excluded. One recipient in our case series with a positive PPD received INH prophylaxis soon after transplant for 9 mo. However, a month after finishing INH, she developed renal allograft TB. This patient was asymptomatic, but cultures were not obtained prior to initiation of prophylaxis. The other explanations for the development of active TB include possible low levels of INH due to concomitant steroids and inadequate host response in the setting of immunosuppressant use post-transplant. Treatment regimens for LTBI include INH 5 mg/kg daily (maximum dose 300 mg/d) for 9 mo with pyridoxine 25-50 mg daily to prevent neurotoxicity. INH 15 mg/kg twice weekly (maximum dose 900 mg/d) with pyridoxine, given as directly observed therapy has also been proposed. Rifampin 10 mg/kg daily (maximum dose 600 mg/d) for four months may be used prior to transplant but should be avoided if possible after transplant due to drug interaction with the immunosuppressant medications. Combination of pyrazinamide and rifampin daily for 2 mo is not recommended due to the high risk of hepatotoxicity in the transplant population. A shorter regimen of weekly INH and rifapentine for 12 wk, as directly observed therapy, to treat immune competent individuals is not recommended in renal transplant candidates[8]. Compliance with LTBI treatment is poor as seen in a North American study where only half of the patients initiated on therapy finished the complete course of treatment[6]. If treatment is interrupted for more than two months, patients should be excluded again for active TB[12]. Adverse effects are more common in solid organ transplant recipients with hepatotoxicity seen in 37% of kidney recipients and up to 50% in liver transplant recipients[8,20]. Monitoring should involve monthly physician examination and bi-monthly blood levels of liver function tests.

Medications will need to be discontinued or dose adjusted if liver function tests are more than three times the upper limit of normal with symptoms/signs, or more than five times the upper limit of normal without symptoms[12].

Treatment of active drug susceptible TB usually involves two months of an initial phase therapy with INH, rifampin/rifabutin, pyrazinamide, +/- ethambutol, followed by a continuation phase therapy of four months of INH and rifampin, with a total duration for six months. Cavitary TB, with positive sputum culture after two months of intensive phase therapy, is treated for nine months' duration with prolongation of continuation phase therapy. Bone and joint disease as well as severe disseminated disease are treated for a total of six to nine months. Central nervous system disease warrants treatment duration of nine to twelve months[8]. Since the majority of transplant recipients present with severe disseminated TB, 9 mo or longer duration of treatment may be preferred in the presence of response to ATT. Risk of recurrence was found to be lower when treatment is extended to beyond 12 mo[12]. Longer course of therapy is required if second line drugs are used

due to adverse effects or in cases of drug resistant TB.

MDR and extensively drug resistant TB fortunately has been rarely reported in solid organ transplant recipients. This should be treated according to drug susceptibility testing with at least four active drugs. The World Health Organization (WHO) suggests a total treatment duration of 18 mo after culture conversion. Adjunctive surgery may be required in some patients[12].

In the United States, patients with pulmonary TB have sputum cultures obtained monthly until two consecutive cultures are negative, and at two months of intensive phase therapy to further guide treatment. If the sputum culture at two months of treatment is positive, WHO recommends sputum smear microscopy at the end of the third month and if positive, sputum culture and drug susceptibility testing. Drug susceptibility testing should also be done if a patient develops positive cultures after a period of negative cultures. European guidelines in transplant recipients recommend sputum smear and culture at a minimum of two months and four months of treatment, at the end of ATT, and on two further occasions until the end of 12 mo[12]. Extra-pulmonary TB in general is followed clinically. Patients should have baseline laboratory data including a comprehensive metabolic panel, complete blood counts, and uric acid levels. They should be monitored and managed for hepatotoxicity as described above. Baseline and monthly visual acuity and red-green discrimination testing should be done with ethambutol use.

If one suspects pulmonary TB, the patient should be isolated in a negative pressure room until active TB is excluded. Pulmonary TB patients should be isolated for at least two weeks with clinical improvement on therapy and until three consecutive negative sputum smears are obtained. In immunocompetent patients, rapid testing with Xpert MTB/Rif has been used in conjunction for decisions regarding discontinuation of TB isolation. However, this cannot be recommended in the transplant population at this time.

Drug interactions

Patients need to be monitored closely for drug interactions with immunosuppressive medications used in solid organ transplant given the increased risk of rejection. Rifampin is used in treatment of TB due to its potent MTB sterilizing action. Rifampin is a strong inducer of CYTP3A4 leading to increased metabolism of calcineurin inhibitors, mammalian target of rapamycin (mTOR) inhibitors, mycophenolate mofetil and corticosteroids. Rifabutin is a less potent cytochrome inducer. Drug levels need to be monitored closely at initiation of TB therapy, after discontinuation of rifampin or rifabutin, or with any adjustment of immunesuppressant dosing[8]. Spanish guidelines recommend rifamycin free regimens for treatment, except for disseminated TB and INH resistant TB[19]. We prefer rifamycin based regimens for treatment of TB in our renal transplant recipients. Other drug interactions to consider include the following: INH may increase corticosteroid levels and its adverse effects, streptomycin with cyclosporine and sirolimus may cause additive nephrotoxicity, fluoroquinolones can further increase risk of tendon rupture with concomitant corticosteroids, and corticosteroids may decrease INH levels[12].

Complications

Complications of TB besides primary organ involvement include septic shock, venous thromboembolism (VTE), immune reconstitution inflammatory syndrome (IRIS) and macrophage activation syndrome (MAS) or hemophagocytic syndrome[21,22]. Septic shock with TB is associated with high mortality[23]. Pulmonary and extra-pulmonary TB both predispose to VTE with the risk being much higher than other hospitalized patients, in general[24,25]. IRIS is recognized by the paradoxical symptom worsening of fever, cough, enlarging lymph nodes or worsening of findings on imaging after initiation of treatment. This is seen primarily in the first few months of initiation of therapy. MAS is rare and has high mortality. It manifests as fever, hepatosplenomegaly, pancytopenia and liver abnormalities. Diagnosis is usually made by bone marrow biopsy showing infiltration of non-malignant macrophage phagocytizing red blood cells[12,21]. In our case series, one patient presented with septic shock and presumed MAS succumbing to his illness. The other patient presented with VTE and developed IRIS two months after initiation of ATT.

In conclusion, TB remains a challenging opportunistic infection in the solid organ transplant population. Efforts should be made to prevent active TB *via* recognition and treatment of LTBI in potential donors and transplant candidates, ideally prior to transplantation. Current tests for LTBI (PPD and IGRA) can be falsely negative in patients with ESRD and those on immunosuppressive medications. IGRA has not been evaluated for use in deceased donors. There is a need for better diagnostics for LTBI. Exclusion of active TB is of paramount interest prior to LBTI therapy by culture, smear, imaging and molecular testing as needed. Given the changes in the allocation system, older and longer dialysis vintage recipients are being transplanted, increasing the risk of active TB. Due to the organ shortage with more high risk donors being utilized, the risk for donor derived TB might increase as well. More widespread use of rapid NAA assays and line probe assays is needed to screen high-risk TB donors, and for diagnosis of TB in recipients. As disseminated and extra-pulmonary disease are more common in transplant recipients, studies are needed to assess the performance of NAA assays in body fluids, other than sputum, in this population. Given diagnostic limitations, physicians need to maintain a high clinical suspicion for TB post transplantation in order to initiate early treatment and decrease morbidity and mortality. Studies are needed to investigate the efficacy of shorter treatment regimens given the interactions with immunosuppressive medications and significant adverse effects. Lastly, public health efforts are needed both at the

global and domestic level to minimize this disease.

COMMENTS

Case characteristics

Four kidney transplant recipients, aged 38-67 years, presenting with fever within one year of kidney transplantation.

Clinical diagnosis

Lymphadenopathy, pleural effusion, pericardial effusion, acute respiratory failure, septic shock, acute kidney injury, bilateral lower extremity deep venous thrombosis and pulmonary embolism.

Differential diagnosis

Bacterial infections, fungal infections such as histoplasmosis, cryptococcosis, interstitial nephritis due to adenovirus infection, post-transplant lymphoproliferative disorder.

Laboratory diagnosis

Demonstration of acid-fast bacilli in sputum and bronchoalveolar lavage. Mycobacterium tuberculosis grew in cultures from sputum, blood, lymph node aspirate and pericardial fluid. Positive Mycobacterium tuberculosis PCR in blood and bronchoalveolar lavage.

Imaging diagnosis

Radiological features included calcified/non-calcified lung nodules, diffuse lung infiltrates, pleural effusion, lymphadenopathy, pulmonary embolism and increased flurodeoxyglucose uptake in the kidney allograft on positron emission tomography scan.

Pathological diagnosis

Necrotizing and non-necrotizing granulomas seen on kidney allograft and trans-bronchial lung biopsies respectively. Demonstration of acid-fast bacilli on lung biopsy.

Treatment

Two months of Rifampin, Isoniazid, Ethambutol and Pyrazinamide followed by 4 mo of Rifampin and Isoniazid. Second-line drugs moxifloxacin and amikacin were used in selected cases.

Related reports

Tuberculosis in solid organ transplant recipients is rare in the developed countries. A study in 1998 estimated 0.35%-1.2% incidence in the United States.

Term explanation

Tuberculosis is a rare opportunistic infection caused by acid fast bacillus Mycobacterium tuberculosis that was identified by Robert Koch in 1884.

Experiences and lessons

Tuberculosis should be considered in solid organ transplant recipients presenting with unexplained fever to avoid delayed or missed diagnosis. TB carries high morbidity and mortality. Transplant recipients should have comprehensive screening for risk factors for TB along with testing for latent TB. Active TB needs to be ruled out prior to the treatment of latent TB. Ideally patients should be treated for latent TB prior to transplant due to drug interactions and suboptimal response to therapy in the setting of immunosuppression.

Peer-review

The data across the different trials is reviewed well. The benefits and adverse effects are clearly illustrated and summarized.

REFERENCES

1 Global Tuberculosis Report 2015. 20th ed. Available from: URL: http://www.who.int/tb/publications/global_report/en

2 CDC Morbidity and Mortality Weekly Report. 2016; **65**: 273-278

3 **Muñoz P**, Rodríguez C, Bouza E. Mycobacterium tuberculosis infection in recipients of solid organ transplants. *Clin Infect Dis* 2005; **40**: 581-587 [PMID: 15712081 DOI: 10.1086/427692]

4 **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]

5 **John GT**, Shankar V, Abraham AM, Mukundan U, Thomas PP, Jacob CK. Risk factors for post-transplant tuberculosis. *Kidney Int* 2001; **60**: 1148-1153 [PMID: 11532111 DOI: 10.1046/j.1523-1755.2001.0600031148.x]

6 **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]

7 **Benito N**, García-Vázquez E, Horcajada JP, González J, Oppenheimer F, Cofán F, Ricart MJ, Rimola A, Navasa M, Rovira M, Roig E, Pérez-Villa F, Cervera C, Moreno A. Clinical features and outcomes of tuberculosis in transplant recipients as compared with the general population: a retrospective matched cohort study. *Clin Microbiol Infect* 2015; **21**: 651-658 [PMID: 25882369 DOI: 10.1016/j.cmi.2015.03.010]

8 **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]

9 **Muñoz P**, Palomo J, Muñoz R, Rodríguez-Creixéms M, Pelaez T, Bouza E. Tuberculosis in heart transplant recipients. *Clin Infect Dis* 1995; **21**: 398-402 [PMID: 8562750]

10 **Sester M**, van Leth F, Bruchfeld J, Bumbacea D, Cirillo DM, Dilektasli AG, Domínguez J, Duarte R, Ernst M, Eyuboglu FO, Gerogianni I, Girardi E, Goletti D, Janssens JP, Julander I, Lange B, Latorre I, Losi M, Markova R, Matteelli A, Milburn H, Ravn P, Scholman T, Soccal PM, Straub M, Wagner D, Wolf T, Yalcin A, Lange C. Risk assessment of tuberculosis in immunocompromised patients. A TBNET study. *Am J Respir Crit Care Med* 2014; **190**: 1168-1176 [PMID: 25303140 DOI: 10.1164/rccm.201405-0967OC]

11 **Kim SH**, Lee SO, Park JB, Park IA, Park SJ, Yun SC, Jung JH, Kim YH, Kim SC, Choi SH, Jeong JY, Kim YS, Woo JH, Park SK, Park JS, Han DJ. A prospective longitudinal study evaluating the usefulness of a T-cell-based assay for latent tuberculosis infection in kidney transplant recipients. *Am J Transplant* 2011; **11**: 1927-1935 [PMID: 21749641 DOI: 10.1111/j.1600-6143.2011.03625.x]

12 **Bumbacea D**, Arend SM, Eyuboglu F, Fishman JA, Goletti D, Ison MG, Jones CE, Kampmann B, Kotton CN, Lange C, Ljungman P, Milburn H, Morris MI, Muller E, Muñoz P, Nellore A, Rieder HL, Sester U, Theodoropoulos N, Wagner D, Sester M. The risk of tuberculosis in transplant candidates and recipients: a TBNET consensus statement. *Eur Respir J* 2012; **40**: 990-1013 [PMID: 22496318 DOI: 10.1183/09031936.00000712]

13 Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. *Am J Respir Crit Care Med* 2000; **161**: 1376-1395 [PMID: 10764337 DOI: 10.1164/ajrccm.161.4.16141]

14 **Morgan MA**, Horstmeier CD, DeYoung DR, Roberts GD. Comparison of a radiometric method (BACTEC) and conventional culture media for recovery of mycobacteria from smear-negative specimens. *J Clin Microbiol* 1983; **18**: 384-388 [PMID: 6194175]

15 **Ichiyama S**, Shimokata K, Takeuchi J. Comparative study of a biphasic culture system (Roche MB Check system) with a conventional egg medium for recovery of mycobacteria. Aichi Mycobacteriosis Research Group. *Tuber Lung Dis* 1993; **74**: 338-341 [PMID: 8260667]

16 Rapid diagnostic tests for tuberculosis: what is the appropriate use? American Thoracic Society Workshop. *Am J Respir Crit Care Med* 1997; **155**: 1804-1814 [PMID: 9154896 DOI: 10.1164/ajrccm.155.5.9154896]

17 **Perry S**, Catanzaro A. Use of clinical risk assessments in evaluation

of nucleic acid amplification tests for HIV/tuberculosis. *Int J Tuberc Lung Dis* 2000; **4**: S34-S40 [PMID: 10688146]

18 **Laraque F**, Griggs A, Slopen M, Munsiff SS. Performance of nucleic acid amplification tests for diagnosis of tuberculosis in a large urban setting. *Clin Infect Dis* 2009; **49**: 46-54 [PMID: 19476429 DOI: 10.1086/599037]

19 **Aguado JM**, Torre-Cisneros J, Fortún J, Benito N, Meije Y, Doblas A, Muñoz P. Tuberculosis in solid-organ transplant recipients: consensus statement of the group for the study of infection in transplant recipients (GESITRA) of the Spanish Society of Infectious Diseases and Clinical Microbiology. *Clin Infect Dis* 2009; **48**: 1276-1284 [PMID: 19320593 DOI: 10.1086/597590]

20 **Aguado JM**, Herrero JA, Gavaldá J, Torre-Cisneros J, Blanes M, Rufí G, Moreno A, Gurguí M, Hayek M, Lumbreras C, Cantarell C. Clinical presentation and outcome of tuberculosis in kidney, liver, and heart transplant recipients in Spain. Spanish Transplantation Infection Study Group, GESITRA. *Transplantation* 1997; **63**: 1278-1286 [PMID: 9158022]

21 **Canet E**, Dantal J, Blancho G, Hourmant M, Coupel S. Tuberculosis following kidney transplantation: clinical features and outcome. A French multicentre experience in the last 20 years. *Nephrol Dial Transplant* 2011; **26**: 3773-3778 [PMID: 21467129 DOI: 10.1093/ndt/gfr156]

22 **Sun HY**, Munoz P, Torre-Cisneros J, Aguado JM, Lattes R, Montejo M, Garcia-Reyne A, Bouza E, Valerio M, Lara R, John GT, Bruno D, Singh N. Mycobacterium tuberculosis-associated immune reconstitution syndrome in solid-organ transplant recipients. *Transplantation* 2013; **95**: 1173-1181 [PMID: 23435454 DOI: 10.1097/TP.0b013e31828719c8]

23 **Kethireddy S**, Light RB, Mirzanejad Y, Maki D, Arabi Y, Lapinsky S, Simon D, Kumar A, Parrillo JE, Kumar A. Mycobacterium tuberculosis septic shock. *Chest* 2013; **144**: 474-482 [PMID: 23429859 DOI: 10.1378/chest.12-1286]

24 **Dentan C**, Epaulard O, Seynaeve D, Genty C, Bosson JL. Active tuberculosis and venous thromboembolism: association according to international classification of diseases, ninth revision hospital discharge diagnosis codes. *Clin Infect Dis* 2014; **58**: 495-501 [PMID: 24280089 DOI: 10.1093/cid/cit780]

25 **Heit JA**, Melton LJ, Lohse CM, Petterson TM, Silverstein MD, Mohr DN, O'Fallon WM. Incidence of venous thromboembolism in hospitalized patients vs community residents. *Mayo Clin Proc* 2001; **76**: 1102-1110 [PMID: 11702898]

P- Reviewer: Figueiredo AA, Fulop T, Gupta NP **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Lu YJ

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

ISSN 2220-3230 (online)

World Journal of *Transplantation*

World J Transplant 2017 August 24; 7(4): 222-249

Published by Baishideng Publishing Group Inc

WJT World Journal of Transplantation

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

Editorial Board

2016-2019

The *World Journal of Transplantation* Editorial Board consists of 361 members, representing a team of worldwide experts in transplantation. They are from 43 countries, including Argentina (1), Australia (7), Austria (3), Belgium (4), Brazil (7), Bulgaria (1), Canada (13), China (32), Cuba (1), Czech Republic (1), Denmark (1), Finland (1), France (4), Georgia (1), Germany (14), Greece (5), Hungary (2), India (7), Iran (7), Israel (4), Italy (33), Japan (18), Jordan (1), Macedonia (1), Mexico (2), Morocco (1), Netherlands (4), Nigeria (1), Norway (1), Pakistan (1), Poland (2), Qatar (1), Saudi Arabia (3), Singapore (1), South Korea (16), Spain (9), Sweden (1), Switzerland (3), Thailand (2), Tunisia (1), Turkey (6), United Kingdom (17), and United States (120).

WJT World Journal of Transplantation

Contents

Bimonthly Volume 7 Number 4 August 24, 2017

Contents

World Journal of Transplantation
Volume 7 Number 4 August 24, 2017

ABOUT COVER

Editorial Board Member of *World Journal of Transplantation*, Alessandro Busca, MD, Doctor, Stem Cell Transplant Unit, Department of Hematology, AOU San Giovanni Battista, Corso Bramante 88, Turin 10126, 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, PubMed Central.

FLYLEAF

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Ya-Jing Lu*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Fang-Fang Ji*
Proofing Editorial Office Director: *Ze-Mao Gong*

NAME OF JOURNAL
World Journal of Transplantation

ISSN
ISSN 2220-3230 (online)

LAUNCH DATE
December 24, 2011

FREQUENCY
Bimonthly

EDITORIAL OFFICE
Xiu-Xia Song, Director

World Journal of Transplantation
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
August 24, 2017

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 August 24; 7(4): 222-234

DOI: 10.5500/wjt.v7.i4.222

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Observational Study

Histopathological analysis of infiltrating T cell subsets in acute T cell-mediated rejection in the kidney transplant

Francisco Salcido-Ochoa, Susan Swee-Shan Hue, Siyu Peng, Zhaoxiang Fan, Reiko Lixiang Li, Jabed Iqbal, John Carson Allen Jr, Alwin Hwai Liang Loh

Francisco Salcido-Ochoa, Tregs and HLA Research Force and Renal Medicine Department, Singapore General Hospital, Singapore 169856, Singapore

Susan Swee-Shan Hue, Tregs and HLA Research Force and Department of Pathology, National University Hospital, Singapore 119074, Singapore

Siyu Peng, Zhaoxiang Fan, Tregs and HLA Research Force and Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119077, Singapore

Reiko Lixiang Li, Department of Pathology and Laboratory Medicine, KK Women's and Children's Hospital, Singapore 229899, Singapore

Jabed Iqbal, Alwin Hwai Liang Loh, Department of Pathology, Singapore General Hospital, Singapore 169856, Singapore,

John Carson Allen Jr, Centre for Quantitative Medicine, Duke-NUS Graduate Medical School, Singapore 169856, Singapore

Author contributions: Salcido-Ochoa F designed the study, revised all the collected data, analysed data, wrote and revised the paper; Hue SSS, Iqbal J and Loh AHL analysed histopathological data and revised the paper; Peng S and Fan Z collected clinical data and plotted the data; Li RL performed the immunohistochemistry experiments and collected clinical data; Allen Jr JC performed and supervised statistical analysis, and revised the paper.

Supported by National Kidney Foundation Singapore, No. NKFRC/2008/07/22; the Medicine Academic Clinical Program (a SingHealth-Duke/National University of Singapore Joint Partnership); and the Khoo Scholar Programme (Duke/National University of Singapore).

Institutional review board statement: The study protocol was approved by the Centralised Institutional Review Board of SingHealth, Singapore (approval No. 2009/615/E).

Informed consent statement: Signed informed consent was taken from all participants before being subjected to a kidney transplant biopsy, which was clinically indicated and not an experimental procedure in our protocol.

Conflict-of-interest statement: There is no conflict of interest among the authors or the participating institutions, and the authors do not have any financial relationships to disclose.

Data sharing statement: De-identified data was shared among few of the authors for the purpose of data analysis. No data was shared to third parties.

Manuscript source: Invited manuscript

Correspondence to: Dr. Francisco Salcido-Ochoa, MD (Mex), MRCP (UK), MSc (UK), PhD (UK), Consultant and Transplant Immunologist, Tregs and HLA Research Force and Renal Medicine Department, Singapore General Hospital, 20 College Road, Academia, Level 3, Singapore 169856, Singapore. francisco.salcido.ochoa@singhealth.com.sg
Telephone: +65-63266165
Fax: +65-62602308

Received: January 28, 2017
Peer-review started: February 8, 2017
First decision: May 8, 2017
Revised: June 6, 2017
Accepted: June 30, 2017
Article in press: July 3, 2017
Published online: August 24, 2017

Abstract

AIM

To compare the differential immune T cell subset com-

position in patients with acute T cell-mediated rejection in the kidney transplant with subset composition in the absence of rejection, and to explore the association of their respective immune profiles with kidney transplant outcomes.

METHODS

A pilot cross-sectional histopathological analysis of the immune infiltrate was performed using immunohistochemistry in a cohort of 14 patients with acute T cell-mediated rejection in the kidney transplant and 7 kidney transplant patients with no rejection subjected to biopsy to investigate acute kidney transplant dysfunction. All patients were recruited consecutively from 2012 to 2014 at the Singapore General Hospital. Association of the immune infiltrates with kidney transplant outcomes at up to 54 mo of follow up was also explored prospectively.

RESULTS

In a comparison to the absence of rejection, acute T cell-mediated rejection in the kidney transplant was characterised by numerical dominance of cytotoxic T lymphocytes over Foxp3$^+$ regulatory T cells, but did not reach statistical significance owing to the small sample size in our pilot study. There was no obvious difference in absolute numbers of infiltrating cytotoxic T lymphocytes, Foxp3$^+$ regulatory T cells and Th17 cells between the two patient groups when quantified separately. Our exploratory analysis on associations of T cell subset quantifications with kidney transplant outcomes revealed that the degree of Th17 cell infiltration was significantly associated with shorter time to doubling of creatinine and shorter time to transplant loss.

CONCLUSION

Although this was a small pilot study, results support our suspicion that in kidney transplant patients the immune balance in acute T cell-mediated rejection is tilted towards the pro-rejection forces and prompt larger and more sophisticated studies.

Key words: Acute T cell-mediated rejection in the kidney transplant; Banff classification; Cytotoxic T cell; Regulatory T cell; Th17 cell

Core tip: In the clinical setting, acute T cell-mediated rejection in the kidney transplant (ATCMR-KTx) is only confirmed through a kidney transplant biopsy, which is scored according to the Banff classification. The Banff classification is largely based on the estimation of mononuclear cell infiltration instead of the identification and quantification of the actual T cell subsets recruited to mediate rejection. Therefore, a more detailed analysis of the inflammatory infiltrate of ATCMR-KTx is likely to enhance the diagnostic accuracy of the Banff classification. In our analyses, ATCMR-KTx appeared to be characterised by a numerical dominance of cytotoxic T lymphocytes over regulatory T cells in comparison to the absence of acute rejection. We also found an association of the numbers of infiltrating Th17 cells with kidney transplant outcomes. Although this is a small pilot study, it further supports our suspicion that the immune balance in ATCMR-KTx is tilted towards the pro-rejection forces.

Salcido-Ochoa F, Hue SSS, Peng S, Fan Z, Li RL, Iqbal J, Allen Jr JC, Loh AHL. Histopathological analysis of infiltrating T cell subsets in acute T cell-mediated rejection in the kidney transplant. *World J Transplant* 2017; 7(4): 222-234 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i4/222.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i4.222

INTRODUCTION

Acute T cell-mediated rejection in the kidney transplant (ATCMR-KTx) is a common encounter in kidney transplantation. It can perpetuate itself as chronic T cell-mediated rejection or transform into antibody-mediated rejection, which progressively can destroy the renal parenchyma, leading to reduction of kidney transplant survival with potential transplant loss and the return to dialysis[1,2]. Therefore, adequate maintenance immunosuppression to prevent the occurrence of ATCMR-KTx, prompt and accurate identification, and early initiation of anti-rejection therapy are needed to minimise patient's complications and to improve long-term kidney transplant outcomes.

In the current state of the art, confirmation of ATCMR-KTx is based on scoring kidney transplant histopathological changes using the Banff classification[3]. Despite being the gold standard, there are a few limitations. The Banff classification relies on a semi-quantitative estimation of the infiltrating mononuclear cells. This approach, however, does not distinguish the actual cellular program that is operating within the transplant tissue. We believe that identification of the actual T cell subsets infiltrating the kidney transplant provides better insight into the immunologic events in ATMCR-KTx. In other words, a more detailed analysis of the inflammatory infiltrate of kidney transplant biopsies undergoing ATCMR-KTx is expected to reflect more accurately the status of alloactivation within the kidney transplant and to lead to a better understanding of the immunopathogenesis of ATCMR-KTx. Similarly, this information could be used in the future to improve the accuracy and the predictive value of the Banff classification in kidney transplantation.

The immunologically-mediated damage of ATCMR-KTx is mediated and executed by different subtypes of effector T cells, including cytotoxic T lymphocytes (CTL), T helper (Th) 17 cells and Th1 cells, as well as natural killer cells and monocytes. In addition, Foxp3$^+$ regulatory T cells (Treg cells) are known to migrate also to the transplant tissue to modulate the inflammatory response[4-9].

CTL are central effectors of alloimmune damage to the parenchymal cells of the kidney transplant[10,11]. Therefore, the detection of their cytotoxic products inside the kidney transplant is commonly used as a surrogate of their presence and their allotoxicity. To highlight a few examples, at the molecular level intra-graft detection of granzyme B mRNA has been shown to be able to differentiate ATCMR-KTx from the absence of rejection[12,13]. Concomitant detection of both granzyme B and perforin mRNA[14,15], or of both granzyme B and CD178 mRNA[13,16] have also been shown to identify ATCMR-KTx with higher accuracy. It has also been reported that the detection of granulysin mRNA, another CTL product, helped to differentiate patients with ATCMR-KTx from those with no rejection in their biopsies[17]. A similar result has also been observed at the protein level by immunohistochemical detection of granzyme B and perforin expression[10]. Although the outcome of kidney transplantation after an episode of ATCMR-KTx is difficult to predict, there are some indications that the detection of markers of CTL in the kidney transplant may offer some value. One study demonstrated that a higher degree of granzyme B^+ cell infiltration in the allograft was associated with poorer allograft survival[18], and another study showed that the intra-graft expression of granzyme B was associated with the severity of the rejection process[10]. Likewise, the expression of CD178[19] or the co-expression of both CD178 and granzyme B[13] conferred poorer prognosis to patients suffering from ATCMR-KTx. Despite the aforementioned findings, it has been suggested that expression of granzyme B by itself may have limited clinical predictive value[19].

Th17 cells are another type of effector T cells involved in alloimmunity and in biopsies are usually identified by the detection of IL-17. It has been reported that the magnitude of Th17 cell infiltration over Treg cell infiltration correlated with kidney transplant function, the degree of interstitial inflammation and tubular atrophy, the refractoriness to treatment and the recurrence of ATCMR-KTx[20-22].

Despite the belief that Th1 cells are believed to be crucial mediators of the rejection process, the detection of interferon-gamma, as a surrogate marker for their presence, was no better than the detection of cytotoxic molecules for the diagnosis of pure ATCMR-KTx[13]. In addition, intra-graft expression of T-bet, also a surrogate marker for Th1 cells, was not able to distinguish ATCMR-KTx from the absence of rejection. In this respect, the role of Th2 cells in the rejection process appears to be less dramatic and less understood; and the identification of Th2 cells through the detection of intra-graft IL-4 mRNA was also not useful for the diagnosis of ACTMR-KTx[13].

Although several reports have implicated $Foxp3^+$ Treg cells in alloregulation and transplantation tolerance in animal models[8,23] and in humans[24], the detection of $Foxp3^+$ Treg cells to aid in the diagnosis of ATCMR in the kidney transplant and their clinical significance has been beset with controversy[25]. Some authors have published that higher infiltration by $Foxp3^+$ Treg cells appeared to associate with more favourable transplant outcomes in patients with ATCMR-KTx[26] and in patients with subclinical rejection found in protocol biopsies[27,28], in comparison to those cases of much lower infiltration by $Foxp3^+$ Treg cells. Likewise, patients with ATCMR-KTx having higher expression of Foxp3 mRNA were more likely to respond to therapy that those with lower levels[20]. However, other studies reported were not very supportive of the detection of Treg cells in ATCMR-KTx. The detection of intra-graft Foxp3 mRNA, as a surrogate marker for $Foxp3^+$ Treg cells, was not associated with the diagnosis ATCMR-KTx in one study[12]. In addition, no association was found in another study of ATCMR-KTx between the detection of $Foxp3^+$ T cells by immunofluorescence and kidney transplant outcomes[29].

We have hypothesised that the balance between effector and $Foxp3^+$ Treg cells could play a role in determining the occurrence and severity of ATCMR-KTx, as well as predicting the potential outcome of the kidney transplant[25]. However, as discussed previously, the clinical significance of the immune infiltrate in ATCMR-KTx or its balance is controversial. Therefore, in this study performed in a cohort of Asian patients, we aimed to identify and quantify the main T cell subsets infiltrating the kidney transplant undergoing ATCMR and to compare with that in the absence of rejection. We use immunohistochemistry as our detection technology as it is inexpensive, easily reproducible and accessible to many laboratories. Based on the literature presented above, we focused our immunodetection on the most promising markers, *i.e.*, granzyme B and IL-17 (representing CTL and Th17 cells, respectively) and Foxp3 (representing $Foxp3^+$ Treg cells). To assess their immune balance, we arbitrarily measured their numerical ratios within the immune infiltrate found in both kidney transplant patients with ATCMR-KTx and with no rejection. Then, we explored the association of the numbers of these subsets and their ratios with kidney transplant outcomes up to fifty-four months of clinical follow up. We focused our outcome analysis on the risk of subsequent rejection episodes, deterioration of kidney transplant function and immunologically-mediated transplant loss.

MATERIALS AND METHODS

Study design

Cross-sectional immunohistochemical analysis performed in formalin-fixed paraffin-embedded tissue collected in a consecutive cohort of 21 kidney transplant patients that were subjected to kidney transplant biopsy for the investigation of acute kidney transplant dysfunction at any time post-transplantation. Patients satisfying our inclusion and exclusion criteria were subdivided post-hoc into two groups: (1) ATCMR-KTx; and (2) no rejection. All patients were recruited

between 1 January 2012 to 1 January 2014 at the Singapore General Hospital (SGH), the largest tertiary care and academic centre in Singapore; and followed for kidney transplant outcomes up to fifty-four months from the time of transplant biopsy.

Patient characteristics

Inclusion criteria: Adult kidney transplant patients (aged 21-80 years) who were of low immunological risk (ABO-compatible, lack of donor-specific antibodies, negative cross-match, no history of antibody-mediated rejection); who had acute kidney transplant dysfunction due to: (1) ATCMR-KTx (category 4 of the Banff 2009 classification); or (2) found with absence of rejection in the biopsy (category 1 of the Banff 2009 classification, or category 6 of the Banff 2009 classification of no inflammatory or infective nature, *i.e.*, with no BK virus nephropathy, other infections affecting the transplant, glomerulonephritis or interstitial nephritis).

Exclusion criteria: Human immunodeficiency virus infection, history of haematological malignancies, children, pregnant women, poor cognitive capacity, prisoners and the inability to understand the research protocol and give consent. Patients whose biopsies showed borderline rejection (category 3 of the Banff 2009 classification) or antibody-mediated rejection (category 2 of the Banff 2009 classification) were also excluded from the analysis. Biopsies in the non-rejection group were revised according to the Banff 2013 update before the final analysis, to ensure they still satisfy the non-rejection group criteria according to the Banff 2013 update.

Clinical data

Baseline demographic and clinical characteristics as well as clinical outcomes were retrieved from clinical hard-copy case notes and our electronic medical records. Use and type of immunosuppressants prescribed were also recorded.

Routine laboratory investigations

Serum creatinine and urine protein to creatinine ratio (or total urinary protein in a 24-h collection) were measured. Calculated estimated glomerular filtration rate (eGFR) was obtained through the "modification of diet in renal disease" equation. All laboratory parameters were retrieved prospectively from electronic medical records from the time of kidney transplant biopsy and at 3, 6, 12, 18, 24, 30, 36, 42, 48 and 54 mo of follow up post-biopsy. All laboratory investigations were conducted at the SGH's clinical laboratory, which is accredited by the College of American Pathologists.

T cell subset detection in kidney transplant biopsies by immunohistochemistry

Immunohistochemistry for detection of T cell subsets in kidney transplant tissue biopsies was performed in both the Renal Laboratory and the Pathology Laboratory at the SGH. In brief, slides prepared from formalin-fixed paraffin-embedded kidney tissue specimens were stained with monoclonal antibodies conjugated with either horseradish peroxidase or alkaline phosphatase and directed against different phenotypic markers, including CD4, CD8, CD19, IL-17, granzyme B and Foxp3. The binding of the different antibodies onto the kidney tissue samples was revealed using the respective chromogenic substrates for those enzymes. Isotype-matched antibodies were used as negative controls. Tonsil tissue served as positive control. Staining was visualized and quantified directly by light microscopy and adjusted to biopsy tubulo-interstitial area (vessels and glomeruli excluded) measured by Olympus CellSens software. Percentage of infiltration of $CD4^+$, $CD8^+$ and $CD19^+$ cells, as well as the number of Foxp3-, IL-17- or granzyme B-expressing cells per square millimetre of kidney tubulo-interstitial area in the biopsy (cell density) was reported. The ratios between the cell densities of granzyme B- and IL-17-expressing cells over Foxp3-expressing cells were calculated.

Statistical analysis

Sample size: As this was an exploratory study on consecutively recruited patients, sample size was not calculated a priori.

To determine whether tissue-infiltrating T cell profiles differ between kidney transplant patients with: (1) biopsy-proven ATCMR-KTx; and (2) no rejection, median cell densities of tissue-infiltrating: (1) granzyme B^+ CTL; (2) IL-17^+ Th17 cells; (3) $Foxp3^+$ Treg cells were compared between these two groups of patients. In addition, ratios of the cell densities of tissue-infiltrating; (4) granzyme B^+ CTL over $Foxp3^+$ Treg cells; and of (5) IL-17^+ Th17 cells over $Foxp3^+$ Treg cells were compared between kidney transplant patients with: (1) biopsy-proven ATCMR-KTx; and (2) no rejection. Medians were compared using the Wilcoxon rank-sum test. Spearman correlation was used to assess strength of association of densities and ratios of infiltrating immune cells with different kidney transplant outcomes, including: (1) changes in serum creatinine; (2) eGFR; and (3) proteinuria. Longitudinal analysis of variance was used to display and compare changes in these same outcome variables between the two groups of patients over the follow up period. The analysis was performed on log-transformed values in order to achieve normality of residuals. The log-rank test was used to compare time-to-event curves between the biopsy-proven ATCMR-KTx and the no-rejection groups for the following outcomes: (1) time to any rejection (a composite outcome including borderline rejection, ATCMR-KTx or antibody-mediated rejection occurring post-biopsy during the follow up period); (2) time to doubling of creatinine post-biopsy; and (3) time to confirmed or suspected immune-mediated transplant loss. The date for re-initiation of dialysis was taken as the date of transplant

Table 1 Baseline clinical and demographic characteristics of the kidney transplant patients

Characteristic	n^3	No rejection	n^4	ATCMR	*P* value
Age (yr)[1]	7	60.8	14	44.9	0.0101
Male sex (%)	7	57.14	14	71.43	0.6384
Race Chinese (%)	7	86.71	14	57.14	0.3371
Dialysis vintage (yr)[1]	7	2.08	14	5.015	0.6888
Transplant vintage (yr)[1]	7	13.75	14	3.935	0.0031
Deceased donor (%)	6	66.67	13	53.85	> 0.9999
Delayed graft function (%)	6	33.33	12	41.67	> 0.9999
Cold ischaemia time (h)	5	3	9	10	0.6973
Total HLA mismatch (#)[1]	6	3	11	3	0.9973
Very high immune risk (%)[2]	6	16.67	11	43.45	0.3334
% Panel of reactive antibodies[1]	3	8	9	0	0.2318
History of ATCMR (%)	7	14.29	14	50	0.1736
Re-transplant (%)	7	0	14	7.14	> 0.9999
GFR at biopsy (mL/min per 1.73 m^2)[1]	7	41.2	14	17.95	0.0767
Proteinuria at biopsy (g/d)[1]	7	3.5	14	1.23	0.2028
t score[1]	7	0	14	2	0.0116
I score[1]	7	1	14	2	0.0007
v score[1]	7	0	14	0	0.1196
Tacrolimus use at biopsy (%)	7	0	14	50	0.0468
Ciclosporin use at biopsy (%)	7	100	14	35.71	0.0071
MTORI use at biopsy (%)	7	0	14	14.29	0.5333
Steroids use at biopsy (%)	7	100	14	100	> 0.9999
Mycophenolate use at biopsy (%)	7	57.14	14	85.71	0.28
Azathioprine use at biopsy (%)	7	28.57	14	0	0.10
Anti-CD25 induction (%)	5	0	12	41.67	0.2445
Prior thymoglobulin use (%)	7	14.29	14	14.29	> 0.9999

[1]Results reported as median values; [2]According to United Kingdom Fuggle's classification based on HLA-DRB1 and HLA-B mismatches[30]; [3]Indicates the number of patients with available data in the non-rejection group; [4]Indicates the number of patients with available data in the ATCMR-KTx group. ATCMR: Acute T cell-mediated rejection; GFR: Glomerular filtration rate; HLA: Human leukocyte antigen; MTORI: Mammalian target of rapamycin inhibitor.

loss. Cox regression analysis was used to investigate the effect of the cell densities of the tissue infiltrating T cells and their ratios and other clinical parameters (potential confounders taken from Table 1) on different kidney transplant outcomes including: (1) time to any rejection post-biopsy; (2) time to doubling of creatinine post-biopsy; and (3) time to confirmed or suspected immune-mediated transplant loss. All analyses were performed using SAS V9.4 software (SAS Inc., Cary NC, United States).

RESULTS

Table 1 shows the main clinical and demographic characteristics of the 21 recruited kidney transplant patients that were subjected to transplant biopsy for the investigation of acute kidney transplant dysfunction (14 with ATCMR-KTx and 7 with no rejection, inflammation nor infection found in their biopsy). Overall, in the ATCMR-KTx group the need for transplant biopsy occurred earlier post-transplantation than for the non-rejection group and had worse kidney function at presentation. History of previous rejection episodes occurred preferentially in this group too. They also had slightly higher rate of delayed graft function and longer cold-ischaemia than the non-rejection group. The HLA mismatches and the immune risk according to Fuggle's classification[30] was similar in both groups. In our patient cohort, all the non-rejection patients were taking ciclosporin as maintenance immunosuppression at the time of the biopsy, while half of the patients in the ATCMR-KTx group were on tacrolimus. The acute rejection scores (*t*, *i* and *v*) of the Banff classification were higher in the ATCMR-Tx group, as expected. Tables 2 and 3 provide the detailed clinical and demographic characteristics of each recruited patient, as well as their particular immune variables and main kidney transplant outcomes.

Comparable infiltration of CTL, Treg cells and Th17 cells in ATCMR-KTx and the absence of rejection

Figure 1 shows a representative panel of the immunohostochemical analysis of T cell subsets in a patient with ATCMR-KTx. The percentage of $CD4^+$ and $CD8^+$ cell infiltration was higher in patients with ATCMR-KTx (Figure 2A and B), and there was no significant difference in B cell infiltration (Figure 2C) in comparison to patients with no rejection. The infiltration of granzyme B^+ cells (surrogates for CTL), $Foxp3^+$ cells (surrogates for Treg cells) and IL-17^+ cells (surrogates for Th17 cells), quantified as cell densities (number of cells per mm^2 of tubulo-interstitial biopsy area), were not statistically different between the two patient groups (Figure 2D-F). Nonetheless, a few ATCMR-KTx patients

Table 2 Baseline clinical and demographic characteristics of the kidney transplant patients

Patient	Group	Age	Sex	Race	Dialysis vintage (yr)	Tx vintage (yr)	Donor type	DGF	CIT (h)	HLA MM (#)	Immune risk	PRA (%)	ATCMR Hx	Re-Tx	Anti-CD25 induction	ATG use	Immuno-suppression at Bx
1	ATCMR	49.9	M	Ma	0.36	14.26	Living	No	0	0	Low	UNK	Yes	No	No	No	CsA + MPA
2	ATCMR	32.1	F	Ch	0.38	0.17	Living	No	UNK	1	Very high	20	Yes	No	Yes	No	MTORI + MPA
3	ATCMR	25.7	M	Ma	1.21	6.80	UNK	UNK	UNK	UNK	UNK	UNK	Yes	No	UNK	No	Tac + MPA
4	ATCMR	36.7	M	Ma	9.48	0.45	Deceased	Yes	10	3	High	7	Yes	No	Yes	No	Tac + MPA
5	ATCMR	59.4	M	Ch	8.68	3.90	Deceased	No	9	4	Very high	7	Yes	No	Yes	No	CsA + MPA
6	ATCMR	46.0	F	Ch	1.20	2.34	Living	No	0	1	Moderate	0	No	No	Yes	No	CsA + MPA
7	ATCMR	40.6	M	Ch	0.31	1.03	Living	No	UNK	UNK	UNK	UNK	No	No	No	Yes	Tac + MPA
8	ATCMR	44.1	M	Ch	9.52	8.09	Deceased	Yes	23	2	High	0	Yes	Yes	No	Yes	Tac
9	ATCMR	56.9	M	Ch	7.98	13.8	Deceased	Yes	UNK	3	High	UNK	No	No	No	No	CsA + MPA
10	ATCMR	45.6	M	Ch	1.08	1.26	Living	UNK	UNK	UNK	UNK	UNK	No	No	UNK	No	Tac + MPA
11	ATCMR	51.5	M	In	8.29	5.34	Deceased	No	19	4	Very high	0	Yes	No	No	No	Tac + MPA
12	ATCMR	57.4	F	Ma	9.31	2.38	Deceased	Yes	15	3	High	0	No	No	Yes	No	Tac + MPA
13	ATCMR	43.6	M	Ch	8.87	3.97	Deceased	Yes	14	5	Very high	3	No	No	No	No	CsA
14	ATCMR	30.6	F	Ma	2.05	11.86	Living	No	5	2	Very high	0	No	No	No	No	MTORI + MPA
15	NR	51.9	M	Ch	0.65	13.75	Living	No	0	4	High	UNK	No	No	No	Yes	CsA + MPA
16	NR	65.1	M	Ch	2.08	18.21	Living	No	UNK	0	Low	UNK	No	No	UNK	No	CsA + MPA
17	NR	61.9	M	Ch	5.88	10.31	Deceased	No	3	3	High	8	No	No	No	No	CsA
18	NR	64.4	F	Ch	2.03	18.36	Deceased	No	16	1	Moderate	33	Yes	No	No	No	CsA + AZA
19	NR	51.0	M	Ch	1.44	11.34	UNK	UNK	UNK	UNK	UNK	UNK	No	No	UNK	No	CsA + MPA
20	NR	43.6	F	Ch	3.24	19.81	Deceased	Yes	1.2	3	High	UNK	No	No	No	No	CsA + AZA
21	NR	60.8	F	Ma	4.42	8.86	Deceased	Yes	18	4	Very high	0	No	No	No	No	CsA + MPA

ATCMR: Acute T cell-mediated rejection; ATG: Anti-thymocyte globulin; AZA: Azathioprine; Bx: Biopsy; Ch: Chinese; CIT: Cold ischaemia time; CsA: Ciclosporin; DGF: Delayed graft function; F: Female; HLA: Human leukocyte antigen; Hx: History; In: Indian; M: Male; Ma: Malay; MM: Mismatch; MPA: Mycophenolic acid analogue; MTORI: Mammalian target of rapamycin inhibitor; NR: No rejection; PRA: Panel of reactive antibodies; Tac: Tacrolimus; Tx: Transplant; UNK: Data unknown.

Table 3 Immune infiltrate characteristics and outcomes of the kidney transplant patients

Patient	Group	t	i	v	CD4 (%)	CD8 (%)	CD19 (%)	Granzyme B (cells/mm^2)	IL-17 (cells/mm^2)	Foxp3 (cells/mm^2)	CTL/Treg ratio	Th17/Treg ratio	GFR at Bx	GFR last follow-up	Proteinuria at Bx	Proteinuria last follow-up	Time to any rejection (d)	Time to doubling of creatinine (d)	Time to Tx loss (d)	Total follow-up (d)
1	ATCMR	1	3	1	35	25	15	68	5	35	2	0.1	18.5	4.7	4.28	UNK	NA	38	116	116
2	ATCMR	3	2	0	60	60	10	346	2	149	2.3	0	33.6	67.2	0.51	0.16	28	NA	NA	1643
3	ATCMR	2	2	1	30	35	30	31	15	73	0.4	0.2	48.1	30.9	0	UNK	92	NA	NA	1623
4	ATCMR	2	2	0	30	25	30	55	17	56	1	0.3	15.2	9.5	0.41	1.71	NA	NA	513	513
5	ATCMR	3	2	1	85	80	25	544	19	311	1.8	0.1	11.2	15	1.08	1.61	NA	NA	645	645
6	ATCMR	2	1	0	30	15	10	26	52	3	8.8	17.9	30.1	6.7	0.39	UNK	1037	941	1176	1176
7	ATCMR	0	1	1	10	20	10	42	4	6	6.6	0.6	49.8	70.3	0.32	0.09	NA	NA	NA	1327
8	ATCMR	0	2	1	5	10	0	13	0	8	1.5	0	16.9	6.2	2.43	6.66	164	164	164	164
9	ATCMR	2	2	0	10	5	10	4	43	1	4.4	47.4	17.4	14.3	2.34	UNK	NA	NA	759	759
10	ATCMR	1	2	0	35	50	15	81	20	17	4.7	1.2	25.8	8.6	1.53	2.46	404	911	933	933
11	ATCMR	1	1	1	10	5	5	9	22	2	4.7	11.5	112.1	44.7	2.07	0.07	NA	520	NA	950
12	ATCMR	1	2	0	20	15	10	18	5	4	4.8	1.3	16.4	15.1	0.58	UNK	NA	NA	NA	917
13	ATCMR	3	2	0	80	70	20	322	32	35	9.3	0.9	9.2	9.2	1.39	1.39	NA	NA	NA	1
14	ATCMR	2	2	0	20	10	10	38	62	10	3.9	6.3	15.2	8.2	6.09	UNK	NA	NA	NA	913
15	NR	0	1	0	20	15	10	36	55	34	1	1.6	21.1	8.8	6.77	UNK	NA	598	862	862
16	NR	0	1	0	5	10	10	1	5	2	0.5	2.5	43.4	35.8	0.13	1.2	NA	NA	NA	1507
17	NR	0	1	0	5	15	10	5	15	4	1.3	3.8	41.2	63.5	0.39	0.57	NA	NA	NA	1306
18	NR	1	1	0	35	30	30	92	16	32	2.8	0.5	56	9.8	2.4	2.24	NA	974	1118	1118
19	NR	0	1	0	0	5	0	21	2	8	2.7	0.2	64.6	53.2	3.62	7.57	1168	NA	NA	1173
20	NR	1	0	0	20	15	10	25	73	15	1.7	5	28.4	7.1	3.5	UNK	NA	188	520	520
21	NR	1	1	0	25	15	10	81	81	10	8.1	8.1	20.9	12.4	10.59	7.46	NA	141	163	163

ATCMR: Acute T cell-mediated rejection; Bx: Biopsy; GFR: Glomerular filtration rate; HLA: Human leukocyte antigen; i: i score; NA: Not applicable; NR: No rejection; t: t score; Tx: Transplant; UNK: Data unknown; v: v score.

had higher infiltration by granzyme B$^+$ and Foxp3$^+$ cells and are referred subsequently in the text as 'high infiltration outliers'.

Infiltrating CTL appear to numerically overwhelm Treg cells in ATCMR-KTx

As an arbitrary measurement of immune balance within

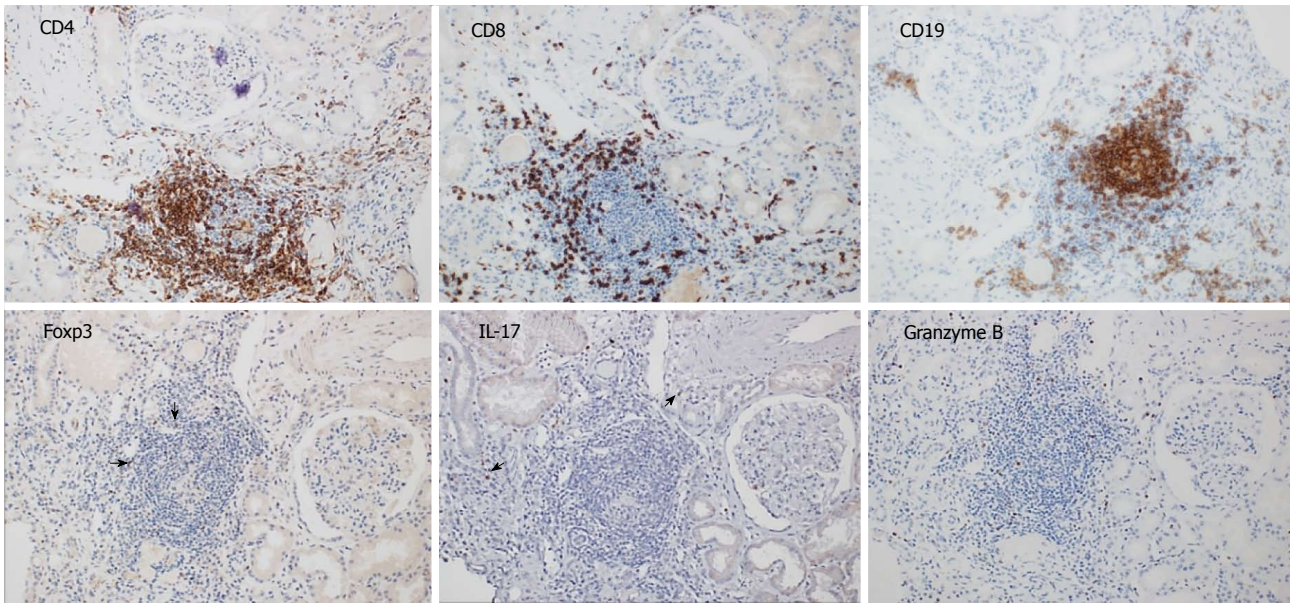


Figure 1 Representative T cell subsets infiltrating a kidney transplant undergoing acute T cell-mediated rejection using antibodies to CD4, CD8, CD19, Foxp3, IL-17 and granzyme B as labeled on the pictures (the arrows indicate positive cells). All pictures derived from the same region cut at consecutive levels (immunohistochemistry staining, magnification × 200).

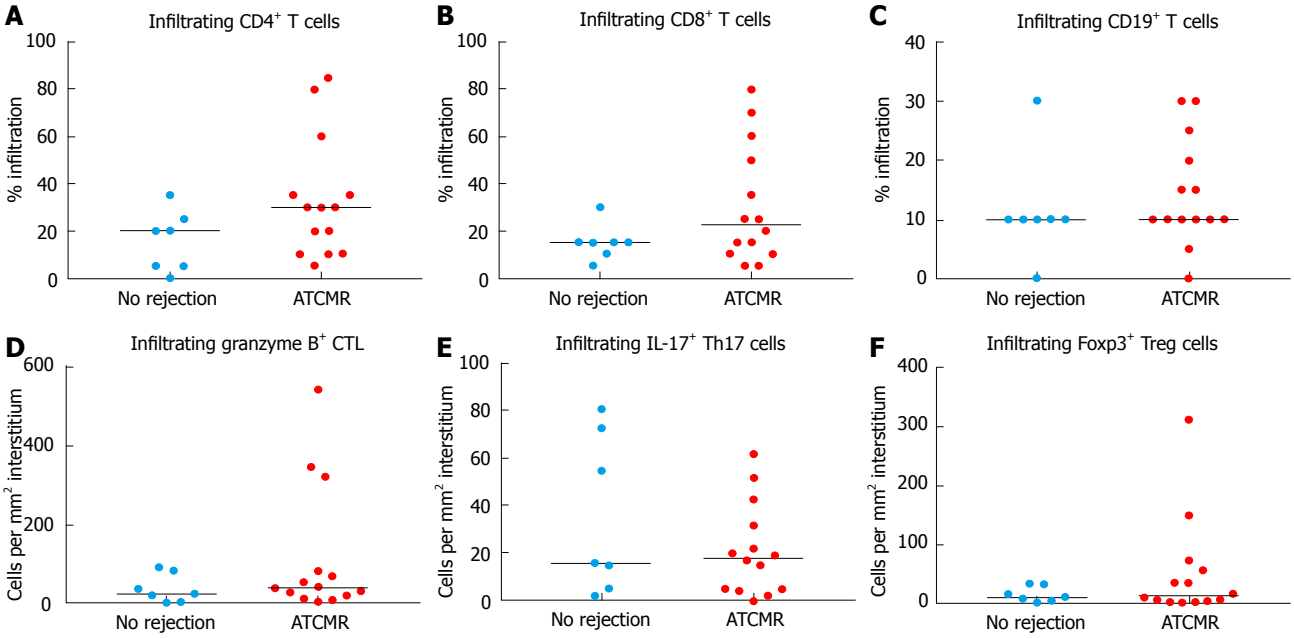


Figure 2 T cell subsets infiltrating kidney tissue, including %CD4$^+$ cells (A), %CD8$^+$ cells (B), %CD19$^+$ cells (C), granzyme B$^+$ cells/mm^2 (D), IL-17$^+$ cells/mm^2 (E) and Foxp3$^+$ cells/mm^2 (F) (all detected by immunohistochemistry) are compared between patients with acute T cell-mediated rejection in the kidney transplant (n = 14) and patients with no rejection (n = 7). The horizontal lines indicate the median values. Wilcoxon rank-sum test P values for all comparisons were statistically non-significant. ATCMR: Acute T cell-mediated rejection; CTL: Cytotoxic T lymphocyte.

the kidney transplant, the granzyme B$^+$ cell to Foxp3$^+$ cell density ratio was found to be higher in patients with ATCMR-KTx than for patients in which rejection was not observed (Figure 3A). However, the ratio of infiltrating IL-17-producing cells over Foxp3$^+$ cells was not much different in patients with ATCMR-KTx than in patients not experiencing rejection (Figure 3B). Given our small sample size, these comparisons did not achieve statistical significance. However, once more there were a few "high infiltration outliers" for the ratio of infiltrating Th17 cells over Foxp3$^+$ Treg cells.

Th17 cell infiltration in ATCMR-KTx associates with worse kidney transplant function

The numbers of infiltrating Th17 cells in the ATCMR-KTx patients were significantly positively correlated with serum creatinine levels and proteinuria, and negatively correlated with eGFR at different time points during follow up. The numbers of infiltrating Th17 cells and the ratio of Th17 cells over Foxp3$^+$ Treg cells in the non-rejection patients were

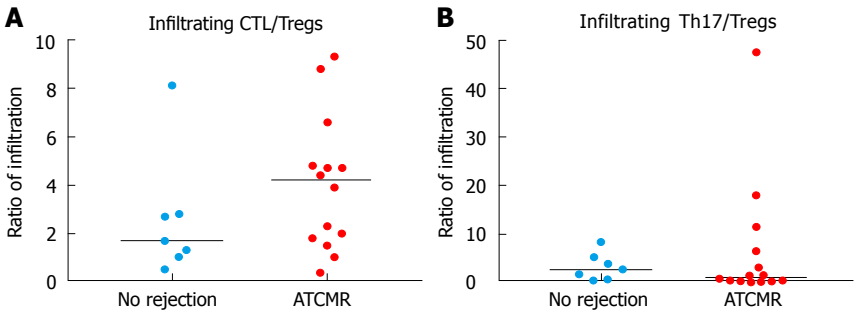


Figure 3 The ratios of (A) infiltrating granzyme B$^+$ cells (CTL) over Foxp3+ cells (Tregs) and of (B) of infiltrating IL-17$^+$ cells (Th17) over Foxp3$^+$ cells (Tregs) are compared between patients with acute T cell-mediated rejection in the kidney transplant (*n* = 14) and patients with no rejection (*n* = 7). All cell types were detected by immunohistochemistry. The horizontal lines indicate the median values. Wilcoxon rank-sum test p values for both comparisons were statistically non-significant. ATCMR: Acute T cell-mediated rejection; CTL: Cytotoxic T lymphocyte.

Table 4 Correlation (R) between numbers and ratios of infiltrating immune cells and kidney transplant outcomes

Group	Immune parameter	*vs*	Outcome	R	*P* value
No rejection	Infiltrating Th17 cells		Creatinine t3	0.9429	0.0167
No rejection	Infiltrating Th17 cells		GFR t0	-0.8571	0.0238
No rejection	Infiltrating Th17/Tregs		GFR t0	-0.7857	0.048
No rejection	Infiltrating Th17 cells		GFR t3	-0.9429	0.0167
No rejection	Infiltrating Th17/Tregs		GFR t3	-0.9429	0.0167
No rejection	Infiltrating Th17 cells		GFR t6	-0.8929	0.0123
ATCMR-KTx	Infiltrating CTL/Tregs		Creatinine t3	-0.6694	0.0145
ATCMR-KTx	Infiltrating Th17 cells		Creatinine t24	0.6485	0.049
ATCMR-KTx	Infiltrating Th17 cells		Creatinine t30	0.7619	0.0368
ATCMR-KTx	Infiltrating Th17 cells		GFR t30	-0.8333	0.0154
ATCMR-KTx	Infiltrating Th17 cells		Proteinuria t12	0.8095	0.0218

ATCMR-KTx: Acute T cell-mediated rejection in the kidney transplant; GFR: Glomerular filtration rate.

significantly positively correlated with serum creatinine levels and negatively correlated with eGFR at different time points during follow up. Correlation estimates and *P* values of the statistically significant associations are shown in Table 4. The numbers of infiltrating CTL and infiltrating Foxp3$^+$ Treg cells were not significantly associated with any of the clinical outcomes tested including changes in serum creatinine, eGFR or proteinuria. However, a significant negative correlation of the ratio of infiltrating CTL over Foxp3$^+$ Tregs with creatinine at 3 mo was observed in ATCMR-KTx patients. Figure 4 shows the dynamic changes in serum creatinine, eGFR and proteinuria throughout the follow up period. The ATCMR-KTx group had overall worse kidney transplant function during follow up than the non-rejection group, while the non-rejection group had overall higher levels of proteinuria. There was no more rapid deterioration in the ATCMR-KTx patients in comparison to the non-rejection patients, as indicated by the absence of statistically significant differences between respective mean values for changes in serum creatinine, eGFR and proteinuria. The time-to-event plots for any rejection post-biopsy (borderline, ATCMR-KTx or antibody-mediated rejection), time to doubling of creatinine post-biopsy, and time to confirmed or suspected immune-mediated transplant loss are found in Figure 5. Table 5 contains the respective median times to event. The comparisons of the time-to-event curves by log rank test were not statistically significant. The effect of the cell densities of the infiltrating immune cells and their ratios, as well as the effect of clinical parameters suspected to influence kidney transplant outcomes (*i.e.*, the potential confounders for kidney transplant outcomes taken from Table 1) were tested using cox regression model. Their respective hazard ratios and 95%CI are shown in Table 6. In the univariate analysis, younger age was associated significantly with shorter time to any rejection. In addition, the number of infiltrating Th17 cells and the degree of proteinuria at biopsy were significantly associated with shorter time to doubling of creatinine. The number of infiltrating Th17 cells, serum creatinine at biopsy and the occurrence of delayed graft function were significantly associated with shorter time to transplant loss. Multivariate analysis was not performed in consequence of the small sample size.

Finally, for ATCMR-KTx patients, Kaplan-Meier time-to-event curves for kidney transplant outcomes corresponding to "high infiltration outlier" patients were compared to outcomes for "non-outlier" patients relative to: (1) number of infiltrating CTL; (2) number of infiltrating Foxp3$^+$ Treg cells; and (3) ratio of Th17 cell to Foxp3$^+$ Treg cell. Owing to the small sample sizes, median time-to-event was not obtainable for any outcome, and differences between "outlier" and "non-outlier" survival curves were non-significant for all three outcome variables (data not shown).

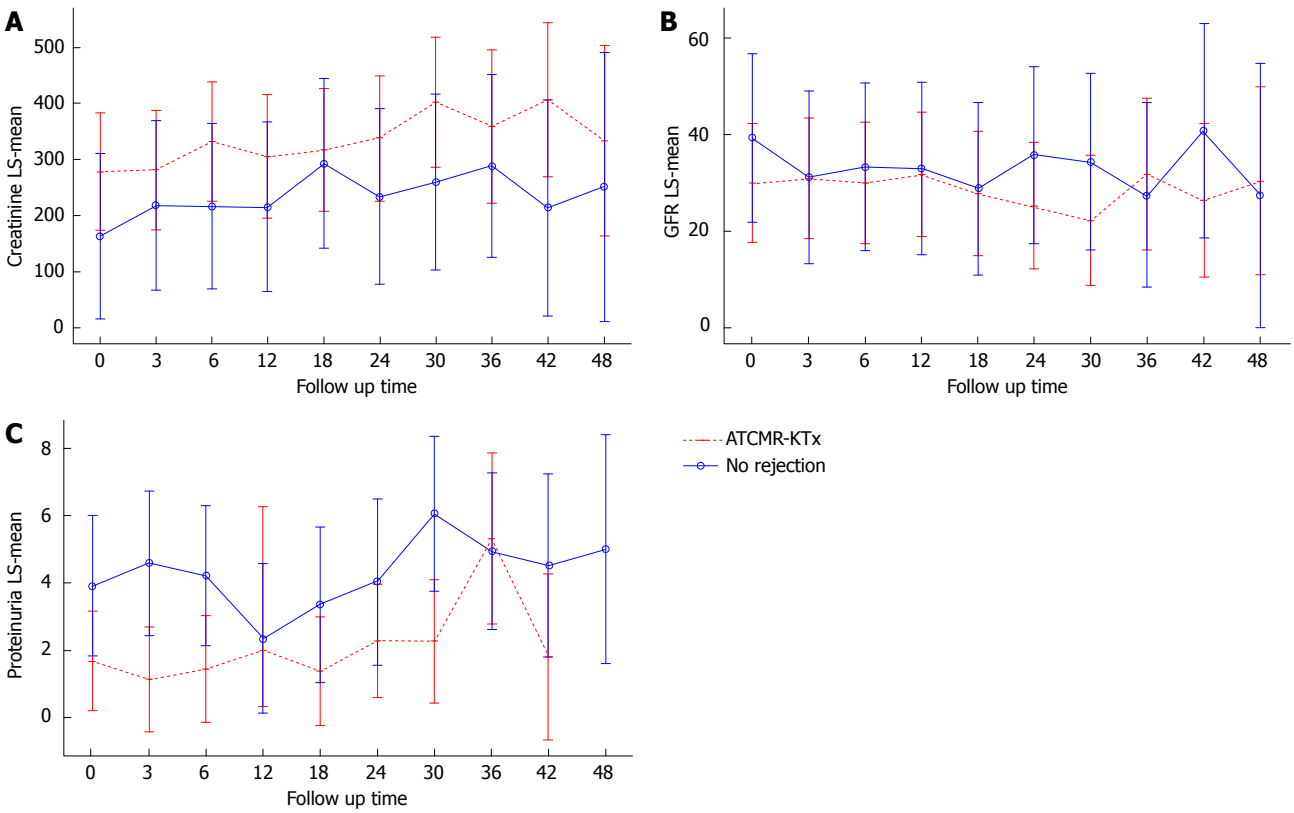


Figure 4 Longitudinal analysis comparing the dynamic changes in serum creatinine (A), glomerular filtration rate (B) and proteinuria (C) throughout the follow up period in the acute T cell-mediated rejection in the kidney transplant (red non-continuous line) and non-rejection (blue continuous line) groups. The comparisons between overall mean values and mean values at follow-up times were statistically non- significant. Upper and lower limits for 95%CIs at the different time points are indicated. ATCMR-KTx: Acute T cell-mediated rejection in the kidney transplant; GFR: Glomerular filtration rate.

Table 5 Comparison of time to transplant outcomes in the kidney transplant patients

Outcomes	Group	Median time-to-event	*P* values
Any rejection	ATCMR	1037	0.0941
	No rejection	Undefined[1]	
Doubling of creatinine	ATCMR	941	0.7452
	No rejection	974	
Transplant loss	ATCMR	1176	0.956
	No rejection	1118	

[1]Median time-to-event was not obtainable (see Figure 4A). ATCMR: Acute T cell-mediated rejection.

DISCUSSION

In this study, our main aim was to determine whether the T cell subset composition in ATCMR-KTx differed qualitatively or quantitatively from that in the absence of rejection. Our main focus was on the numbers and respective ratios of CTL, Th17 cells and Foxp3$^+$ Treg cells, thought to be the most relevant subsets implicated in ATCMR-KTx, according the previously presented literature. ATCMR-KTx appeared to be characterised by a numerical dominance of CTL over Foxp3$^+$ Treg cells in comparison to the absence of acute rejection, suggesting that the immune balance in ATCMR-KTx appears to be tilted to the pro-rejection forces; which might be overwhelming the regulatory forces. This finding is congruent with the literature reports, where the presence of CTL infiltrating the kidney transplant undergoing ATCMR is a characteristic to differentiate ATCMR-KTx from the absence of rejection[10,12,13]; and with the published observation that a lower infiltration by Foxp3$^+$ Treg cells in the kidney transplant undergoing ATCMR was associated with poorer transplant outcomes[26], or with poorer responsiveness to anti-rejection therapy[20].

Our analysis of kidney transplant outcomes revealed that the number of infiltrating Th17 cells was significantly associated with faster time to doubling of creatinine and transplant loss; and the ratio of infiltrating Th17 cells over Foxp3$^+$ Treg cells was significantly associated with a decline in eGFR. These findings parallel and further support the published observations where the magnitude of Th17 cell infiltration over Treg cell infiltration correlated with kidney transplant dysfunction, the degree of interstitial inflammation and tubular atrophy, the refractoriness to treatment and the recurrence of ATCMR in the kidney transplant[21,22]. However, the associations observed in our study were not very strong. The observation that the numbers of infiltrating Th17 cells and the ratio of Th17 cells over Foxp3$^+$ Treg cells associated negatively with kidney transplant outcomes in the non-rejection patients was unexpected, but interesting. Alloimmune responses in transplant patients

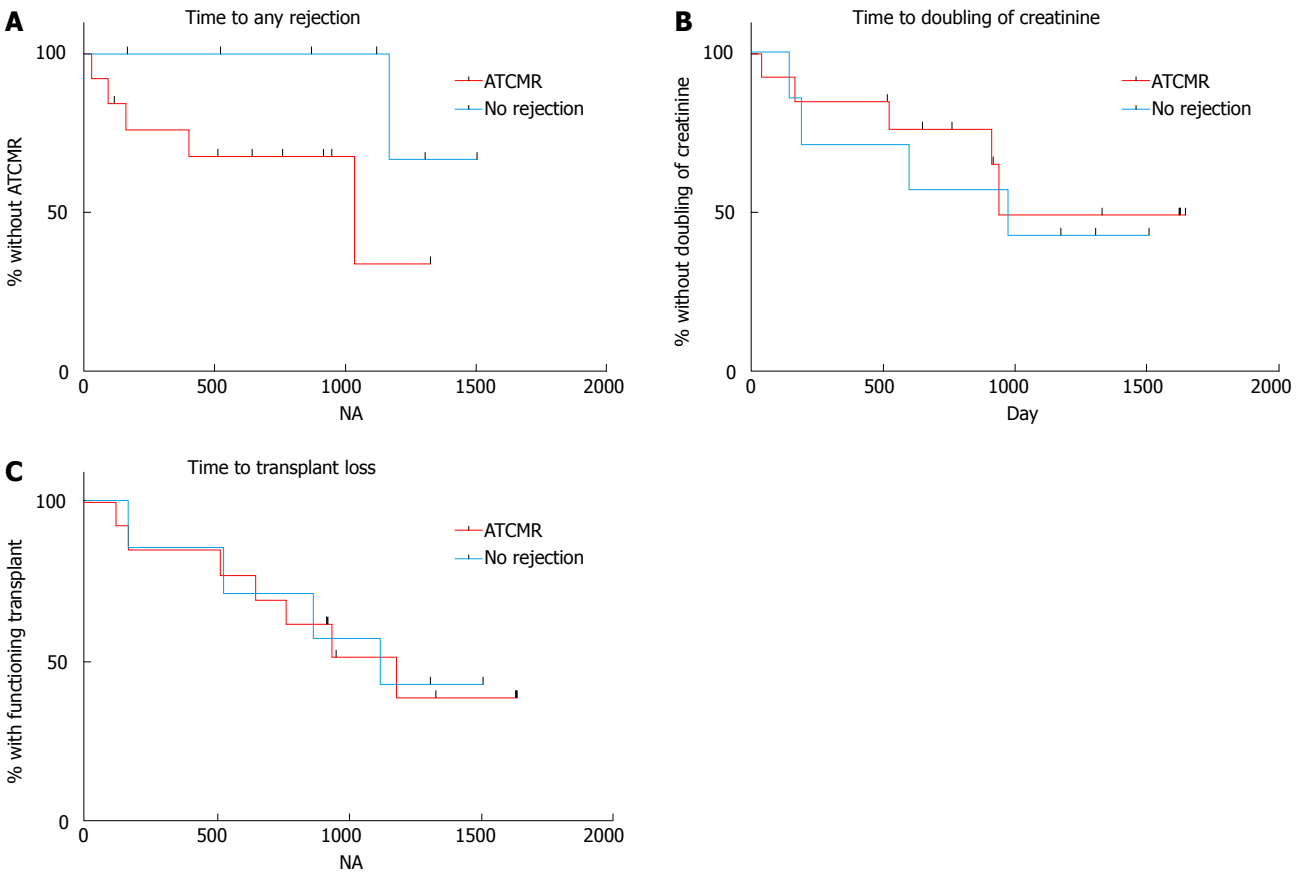


Figure 5 Time-to-event plots of (A) time to any rejection (borderline, acute T cell-mediated rejection in the kidney transplant or antibody-mediated rejection) post-biopsy, of (B) time to doubling of creatinine post-biopsy, and of (C) time to confirmed or suspected immune-mediated transplant loss in patients with acute T cell-mediated rejection in the kidney transplant (*n* = 14) and patients with no rejection (*n* = 7). Log-rank test *P* values for all the comparisons were statistically not significant. ATCMR: Acute T cell-mediated rejection.

Table 6 Effect of immune and clinical variables on kidney transplant outcomes

Outcomes	Risk factor	HR	95%CI	*P* value
Time to any rejection	Age	0.898	0.821, 0.983	0.0193
Time to doubling of creatinine	Infiltrating Th17 cells	1.031	1.002, 1.061	0.0359
Time to doubling of creatinine	Proteinuria	1.382	1.087, 1.757	0.0083
Time to transplant loss	Infiltrating Th17 cells	1.026	1.000, 1.052	0.0472
Time to transplant loss	Serum creatinine	1.009	1.003, 1.016	0.0036
Time to transplant loss	Delayed graft function	5.456	1.238, 24.036	0.0160

are detectable even in patients with apparent stable kidney function. Different sorts of immune cells are as a consequence "waiting for a chance" to flip over the silencing effects of maintenance immunosuppression and the deployed immunoregulatory mechanisms if "given the chance" (*i.e.*, reduction of immunosuppression, sensitizing events or the occurrence of concomitant infections or inflammatory disorders). Hence, it is possible that many transplants patients have certain degree of Th17 cell activation and infiltration. Thus, patients with higher degree of Th17 infiltration, irrespective of reaching the current thresholds for ATCMR-KTx or not, could be bound to worse outcomes due to the possibility that Th17 cells could be mediating smoldering inflammation or slow-motion chronic rejection or have the potential to mediate transformation into a rejection phenotype if the alloimmune milieu changes to a pro-inflammatory one. The use of more sophisticated technologies like the molecular microscope and a better classification of chronic T cell mediated rejection and i-IFTA (for inflamed areas of interstitial fibrosis and tubular atrophy) could help us in the future to assign a more accurate clinical significance to this interesting observation.

In contrast to published literature, in which a greater degree of infiltration by CTL in patients with ATCMR-KTx was associated with poorer allograft survival[18], and the magnitude of granzyme B expression was associated with the severity of the rejection process[10]; we found no association of CTL infiltration or the ratio of infiltrating CTL over Treg cells with kidney transplant outcomes.

However, we believe that statistical significance was not reached due to our small pilot sample size.

One of the merits of our study is the use of immunohistochemistry for our immunodetection as it is a highly available and inexpensive technology, easy to correlate to conventional light microscopy findings. Furthermore, in comparison to most available reports, our study provides a more comprehensive tissue staining, including the three markers that showed the best potential in the published literature: Granzyme B, IL-17 and Foxp3. Thus, our study hints that a more detailed immunohistochemical analysis of the cell infiltrate in kidney transplant biopsies can reflect more accurately the immune balance between the pro-rejection and anti-rejection forces and opens avenues for larger more powered and comprehensive confirmatory studies to address whether a detailed immunophenotyping of ATCMR-KTx can indeed improve the accuracy of the Banff classification; which is undergoing continuous improvement. It is important to comment that more sophisticated technologies like microarray technology have been used for the detection of CTL-associated transcripts and were reported to be more accurate than the detection of individual genes like perforin or granzyme B to cluster together patients with ATCMR-KTx[31]. However, this latter technology is not widely available and not as practical as immunohistochemistry; but indeed, microarray and high-throughput technologies such as the "omics" play a crucial role in biomarker discovery and identification of disease classifiers.

In addressing sample size, based upon our pilot study results, assuming a 1:2 sample size ratio of non-rejection:ATCMR-KTx patients, a common standard deviation (σ) and coefficient of variation (CV = $\sigma/\mu_{NoReject}$) 1.0 to 1.7, respective optimistic and pessimistic sample sizes to give 80% power to detect a two-fold ratio of CTL (CTL: Non-rejection/ATCMR-KTx $\geqslant$ 2) to Foxp3$^+$ Treg cells were calculated to be 18/36 (CV = 1.0) and 41/82 (CV = 1.7).

Participating patients were very heterogeneous in their clinical characteristics, which likely confounded our observations (Tables 1-3). For instance, we observed that the time to transplant loss from biopsy (not from transplant surgery) was similar in both patient groups. However, most kidney transplant biopsies in the non-rejection group were performed late post-transplantation, closer to their maximum transplant survival. In addition, the non-rejection group had higher proteinuria during the follow up period, which could be related also to their vintage in transplantation and likely higher degree of glomerulosclerosis, or perhaps proteinuria was an important factor in the decision to perform biopsy for those patients. Kidney transplant biopsies were indicated when transplant dysfunction ensued and recommended by treating nephrologists according to their own criteria and specific thresholds. The incorporation of selected immune parameters in a larger study including patients from the time of transplant surgery, subjected to more protocolised immunosuppressive regimens, or their incorporation in a clinical trial are anticipated to circumvent many of the biases in our study.

Finally, it would have been interesting to extend our protocol to assess the immune infiltrate inside the kidney transplant in protocol biopsies with subclinical ATCMR and without evidence of rejection. This could have helped us to address whether our observed immune changes mirror the events occurring in subclinical ATCMR-KTx, and to use negative protocol biopsies as better controls for a stable kidney transplant function. However, protocol biopsies are not performed in our institution.

The immune balance in ATCMR-KTx appears to be tilted numerically towards the pro-rejection forces, which seem to overwhelm counter-regulatory mechanisms. Similarly, the degree of infiltration of the kidney transplant by effector T cells could be associated with kidney transplant outcome prognosis. Although our findings are not conclusive, mainly due to our small sample size, they further elucidate the immunopathogenesis of ATCMR-KTx and open new avenues for a more detailed dissection of the complex immune mechanisms implicated in kidney transplant rejection. Upon further validation, ideally tested in randomised controlled trials, it is possible that these and other new signatures could be incorporated into the current diagnostic and therapeutic algorithms in order to deliver more personalised and precise management in kidney transplantation.

ACKNOWLEDGMENTS

We would like to acknowledge the Department of Clinical Research of the Singapore General Hospital for allowing to use their computers for data collection. One thousand thank-you's to Madam Rachel Liew, our library technician, for helping us obtain some of the least accessible articles; and to the anonymous reviewers of the journal for their useful comments. We are also much obliged to the National Kidney Foundation Singapore; the Medicine Academic Clinical Program (a SingHealth-Duke/National University of Singapore Joint Partnership); and the Khoo Scholar Programme (Duke/National University of Singapore) for generously funding different aspects of our research on T cell subset analysis in kidney transplantation.

COMMENTS

Background

In the clinical setting, acute T cell-mediated rejection in the kidney transplant (ATCMR-KTx) is only confirmed through a kidney transplant biopsy, which is scored according to the Banff classification. The Banff classification is largely based on the estimation of mononuclear cell infiltration instead of the identification and quantification of the actual T cell subsets recruited to mediate rejection.

Research frontiers

The identification of the actual T cell subsets involved in ATCMR-KTx likely

reflects more accurately the immune balance between effector and regulatory T cells, which has been implicated as an important factor determining the risk for ATCMR-KTx.

Innovations and breakthroughs

The detection of specific T cell subsets inside the kidney transplant suffering ATCMR adds new light to elucidate its immunopathogenesis, and opens new avenues for the development of novel biomarkers focusing on cytotoxic, Th17 cell-mediated and regulatory T cell responses.

Applications

A more detailed analysis of the inflammatory infiltrate of ATCMR-KTx, in particular of cytotoxic T lymphocytes and Th17 cells, is likely to enhance the diagnostic accuracy of the Banff classification.

Terminology

CD178: CD equivalent for Fas ligand, a membrane molecule able to trigger apoptosis upon ligation of CD95 in target allogeneic cells; Cytotoxic T lymphocytes: A subset of effector T cells able to cause direct cytotoxicity of transplanted parenchymal cells; Foxp3: Transcription factor crucial for the development and function of regulatory T cells; Granzyme B: Enzyme released by cytotoxic T lymphocytes able to trigger apoptosis in target transplanted cells; Regulatory T cells: A subset of T cells regarded as the master moderators of immune responses, thought to be able to regulate alloimmune responses and potentially to aid in the achievement of transplantation tolerance; Th17 cells: A subset of effector T cells implicated in the defence against exogenous microorganisms and implicated in the pathogenesis of several autoimmune disorders and effector alloresponses, whose characteristic cytokine product is IL-17.

Peer-review

This is a good article.

REFERENCES

1 **Opelz G**, Döhler B. Influence of time of rejection on long-term graft survival in renal transplantation. *Transplantation* 2008; **85**: 661-666 [PMID: 18337655 DOI: 10.1097/TP.0b013e3181661695]

2 **Schnuelle P**, Lorenz D, Trede M, Van Der Woude FJ. Impact of renal cadaveric transplantation on survival in end-stage renal failure: evidence for reduced mortality risk compared with hemodialysis during long-term follow-up. *J Am Soc Nephrol* 1998; **9**: 2135-2141 [PMID: 9808102]

3 **Loupy A**, Haas M, Solez K, Racusen L, Glotz D, Seron D, Nankivell BJ, Colvin RB, Afrouzian M, Akalin E, Alachkar N, Bagnasco S, Becker JU, Cornell L, Drachenberg C, Dragun D, de Kort H, Gibson IW, Kraus ES, Lefaucheur C, Legendre C, Liapis H, Muthukumar T, Nickeleit V, Orandi B, Park W, Rabant M, Randhawa P, Reed EF, Roufosse C, Seshan SV, Sis B, Singh HK, Schinstock C, Tambur A, Zeevi A, Mengel M. The Banff 2015 Kidney Meeting Report: Current Challenges in Rejection Classification and Prospects for Adopting Molecular Pathology. *Am J Transplant* 2017; **17**: 28-41 [PMID: 27862883 DOI: 10.1111/ajt.14107]

4 **Benghiat FS**, Graca L, Braun MY, Detienne S, Moore F, Buonocore S, Flamand V, Waldmann H, Goldman M, Le Moine A. Critical influence of natural regulatory CD25+ T cells on the fate of allografts in the absence of immunosuppression. *Transplantation* 2005; **79**: 648-654 [PMID: 15785370 DOI: 10.1097/01.TP.0000155179.61445.78]

5 **Chai JG**, Xue SA, Coe D, Addey C, Bartok I, Scott D, Simpson E, Stauss HJ, Hori S, Sakaguchi S, Dyson J. Regulatory T cells, derived from naïve CD4+CD25- T cells by in vitro Foxp3 gene transfer, can induce transplantation tolerance. *Transplantation* 2005; **79**: 1310-1316 [PMID: 15912097 DOI: 10.1097/01.TP.0000159147.56408.9C]

6 **Joffre O**, Santolaria T, Calise D, Al Saati T, Hudrisier D, Romagnoli P, van Meerwijk JP. Prevention of acute and chronic allograft rejection with CD4+CD25+Foxp3+ regulatory T lymphocytes. *Nat Med* 2008; **14**: 88-92 [PMID: 18066074 DOI: 10.1038/nm1688]

7 **Zheng XX**, Sanchez-Fueyo A, Domenig C, Strom TB. The balance of deletion and regulation in allograft tolerance. *Immunol Rev* 2003; **196**: 75-84 [PMID: 14617199 DOI: 10.1046/j.1600-065X.2003.00089.x]

8 **Graca L**, Cobbold SP, Waldmann H. Identification of regulatory T cells in tolerated allografts. *J Exp Med* 2002; **195**: 1641-1646 [PMID: 12070291 DOI: 10.1084/jem.20012097]

9 **Hu M**, Wang C, Zhang GY, Saito M, Wang YM, Fernandez MA, Wang Y, Wu H, Hawthorne WJ, Jones C, O'Connell PJ, Sparwasser T, Bishop GA, Sharland AF, Alexander SI. Infiltrating Foxp3(+) regulatory T cells from spontaneously tolerant kidney allografts demonstrate donor-specific tolerance. *Am J Transplant* 2013; **13**: 2819-2830 [PMID: 24102948 DOI: 10.1111/ajt.12445]

10 **Wagrowska-Danilewicz M**, Danilewicz M. Immunoexpression of perforin and granzyme B on infiltrating lymphocytes in human renal acute allograft rejection. *Nefrologia* 2003; **23**: 538-544 [PMID: 15002789]

11 **Rascio F**, Divella C, Grandaliano G. CTL and transplantation: tissue in vivo characterization. *Methods Mol Biol* 2014; **1186**: 283-294 [PMID: 25149314 DOI: 10.1007/978-1-4939-1158-5_16]

12 **Ashton-Chess J**, Dugast E, Colvin RB, Giral M, Foucher Y, Moreau A, Renaudin K, Braud C, Devys A, Brouard S, Soulillou JP. Regulatory, effector, and cytotoxic T cell profiles in long-term kidney transplant patients. *J Am Soc Nephrol* 2009; **20**: 1113-1122 [PMID: 19357258 DOI: 10.1681/ASN.2008050450]

13 **Desvaux D**, Schwarzinger M, Pastural M, Baron C, Abtahi M, Berrehar F, Lim A, Lang P, le Gouvello S. Molecular diagnosis of renal-allograft rejection: correlation with histopathologic evaluation and antirejection-therapy resistance. *Transplantation* 2004; **78**: 647-653 [PMID: 15371663 DOI: 10.1097/01.TP.0000133530.26680.DC]

14 **Heng B**, Li Y, Shi L, Du X, Lai C, Cheng L, Su Z. A Meta-analysis of the Significance of Granzyme B and Perforin in Noninvasive Diagnosis of Acute Rejection After Kidney Transplantation. *Transplantation* 2015; **99**: 1477-1486 [PMID: 25643139 DOI: 10.1097/TP.0000000000000567]

15 **Sabek O**, Dorak MT, Kotb M, Gaber AO, Gaber L. Quantitative detection of T-cell activation markers by real-time PCR in renal transplant rejection and correlation with histopathologic evaluation. *Transplantation* 2002; **74**: 701-707 [PMID: 12352889 DOI: 10.1097/00007890-200209150-00019]

16 **Graziotto R**, Del Prete D, Rigotti P, Anglani F, Baldan N, Furian L, Valente M, Antonello A, Marchini F, D'Angelo A, Gambaro G. Perforin, Granzyme B, and fas ligand for molecular diagnosis of acute renal-allograft rejection: analyses on serial biopsies suggest methodological issues. *Transplantation* 2006; **81**: 1125-1132 [PMID: 16641597 DOI: 10.1097/01.tp.0000208573.16839.67]

17 **Sarwal MM**, Jani A, Chang S, Huie P, Wang Z, Salvatierra O, Clayberger C, Sibley R, Krensky AM, Pavlakis M. Granulysin expression is a marker for acute rejection and steroid resistance in human renal transplantation. *Hum Immunol* 2001; **62**: 21-31 [PMID: 11165712 DOI: 10.1016/S0198-8859(00)00228-7]

18 **Mengel M**, Mueller I, Behrend M, von Wasielewski R, Radermacher J, Schwarz A, Haller H, Kreipe H. Prognostic value of cytotoxic T-lymphocytes and CD40 in biopsies with early renal allograft rejection. *Transpl Int* 2004; **17**: 293-300 [PMID: 15221125 DOI: 10.1007/s00147-004-0691-x]

19 **Nickel P**, Lacha J, Ode-Hakim S, Sawitzki B, Babel N, Frei U, Volk HD, Reinke P. Cytotoxic effector molecule gene expression in acute renal allograft rejection: correlation with clinical outcome; histopathology and function of the allograft. *Transplantation* 2001; **72**: 1158-1160 [PMID: 11579318 DOI: 10.1097/00007890-200109270-00031]

20 **Matignon M**, Aissat A, Canoui-Poitrine F, Grondin C, Pilon C, Desvaux D, Saadoun D, Barathon Q, Garrido M, Audard V, Rémy P, Lang P, Cohen J, Grimbert P. Th-17 Alloimmune Responses in Renal Allograft Biopsies From Recipients of Kidney Transplants Using Extended Criteria Donors During Acute T Cell-Mediated Rejection. *Am J Transplant* 2015; **15**: 2718-2725 [PMID: 25989263 DOI: 10.1111/ajt.13304]

21 **Chung BH**, Oh HJ, Piao SG, Sun IO, Kang SH, Choi SR, Park HS, Choi BS, Choi YJ, Park CW, Kim YS, Cho ML, Yang CW. Higher infiltration by Th17 cells compared with regulatory T cells is associated

with severe acute T-cell-mediated graft rejection. *Exp Mol Med* 2011; **43**: 630-637 [PMID: 21865860 DOI: 10.3858/emm.2011.43.11.071]

22 **Chung BH**, Oh HJ, Piao SG, Hwang HS, Sun IO, Choi SR, Park HS, Choi BS, Choi YJ, Park CW, Kim YS, Cho ML, Yang CW. Clinical significance of the ratio between FOXP3 positive regulatory T cell and interleukin-17 secreting cell in renal allograft biopsies with acute T-cell-mediated rejection. *Immunology* 2012; **136**: 344-351 [PMID: 22444300 DOI: 10.1111/j.1365-2567.2012.03588.x]

23 **Wood KJ**, Sakaguchi S. Regulatory T cells in transplantation tolerance. *Nat Rev Immunol* 2003; **3**: 199-210 [PMID: 12658268 DOI: 10.1038/nri1027]

24 **Louis S**, Braudeau C, Giral M, Dupont A, Moizant F, Robillard N, Moreau A, Soulillou JP, Brouard S. Contrasting CD25hiCD4+T cells/FOXP3 patterns in chronic rejection and operational drug-free tolerance. *Transplantation* 2006; **81**: 398-407 [PMID: 16477227 DOI: 10.1097/01.tp.0000203166.44968.86]

25 **Salcido-Ochoa F**, Yusof N, Hue SS, Haase D, Kee T, Rotzschke O. Are we ready for the use of foxp3(+) regulatory T cells for immunodiagnosis and immunotherapy in kidney transplantation? *J Transplant* 2012; **2012**: 397952 [PMID: 22690325 DOI: 10.1155/2012/397952]

26 **Martin L**, Funes de la Vega M, Bocrie O, Harzallah A, Justrabo E, Rifle G, Mousson C. Detection of Foxp3+ cells on biopsies of kidney transplants with early acute rejection. *Transplant Proc* 2007; **39**: 2586-2588 [PMID: 17954183 DOI: 10.1016/j.transproceed.2007.08.037]

27 **Bestard O**, Cruzado JM, Mestre M, Caldés A, Bas J, Carrera M, Torras J, Rama I, Moreso F, Serón D, Grinyó JM. Achieving donor-specific hyporesponsiveness is associated with FOXP3+ regulatory T cell recruitment in human renal allograft infiltrates. *J Immunol* 2007; **179**: 4901-4909 [PMID: 17878390 DOI: 10.4049/jimmunol.179.7.4901]

28 **Bestard O**, Cuñetti L, Cruzado JM, Lucia M, Valdez R, Olek S, Melilli E, Torras J, Mast R, Gomà M, Franquesa M, Grinyó JM. Intragraft regulatory T cells in protocol biopsies retain foxp3 demethylation and are protective biomarkers for kidney graft outcome. *Am J Transplant* 2011; **11**: 2162-2172 [PMID: 21749644 DOI: 10.1111/j.1600-6143.2011.03633.x]

29 **Kollins D**, Stoelcker B, Hoffmann U, Bergler T, Reinhold S, Banas MC, Rümmele P, Farkas S, Krämer BK, Banas B. FOXP3+ regulatory T-cells in renal allografts: correlation with long-term graft function and acute rejection. *Clin Nephrol* 2011; **75**: 91-100 [PMID: 21255537]

30 **Fuggle SV**, Belger MA, Johnson RJ, Ray TC, Morris PJ. A new national allocation scheme for adult kidneys in the United Kingdom. United Kingdom Transplant Support Service Authority (UKTSSA) Users' Kidney Advisory Group and its Task Forces. *Clin Transpl* 1998: 107-113 [PMID: 10503089]

31 **Hidalgo LG**, Einecke G, Allanach K, Mengel M, Sis B, Mueller TF, Halloran PF. The transcriptome of human cytotoxic T cells: measuring the burden of CTL-associated transcripts in human kidney transplants. *Am J Transplant* 2008; **8**: 637-646 [PMID: 18294160 DOI: 10.1111/j.1600-6143.2007.02129.x]

P- Reviewer: Taheri S **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 August 24; 7(4): 235-242

DOI: 10.5500/wjt.v7.i4.235

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Observational Study

Lymphocyte recovery is an independent predictor of relapse in allogeneic hematopoietic cell transplantation recipients for acute leukemia

Moussab Damlaj, Samer Ghazi, Walid Mashaqbeh, Gamal Gmati, Hend Salama, Khadega A Abuelgasim, Mushtaq Rather, Ali Hajeer, Mohsen Al-Zahrani, Abdul-Rahman Jazieh, Ayman Hejazi, Ahmad Al Askar

Moussab Damlaj, Samer Ghazi, Walid Mashaqbeh, Gamal Gmati, Hend Salama, Khadega A Abuelgasim, Mushtaq Rather, Mohsen Al-Zahrani, Abdul-Rahman Jazieh, Ayman Hejazi, Ahmad Al Askar, Division of Hematology and HSCT, Department of Oncology, King Abdulaziz Medical City, Riyadh 11426, Saudi Arabia

Moussab Damlaj, Samer Ghazi, Walid Mashaqbeh, Gamal Gmati, Hend Salama, Khadega A Abuelgasim, Mushtaq Rather, Mohsen Al-Zahrani, Abdul-Rahman Jazieh, Ayman Hejazi, Ahmad Al Askar, King Abdullah International Medical Research Center, Riyadh 11426, Saudi Arabia

Ali Hajeer, King Saud bin Abdulaziz University for Health Sciences, Riyadh 11426, Saudi Arabia

Author contributions: Damlaj M designed the study; Damlaj M, Ghazi S and Mashaqbeh W collected data; all authors analysed the data, provided patients, wrote and reviewed the manuscript, and approved final version of the manuscript.

Institutional review board statement: This study was approved by the institutional review board at King Abdulaziz Medical City (KAMC) - King Abdallah International Medical Research Center (KAIMRC).

Informed consent statement: The institutional review board waived informed consent due to the retrospective study design without patient contact or intervention; thus representing minimal risk study.

Conflict-of-interest statement: There are no conflicts of interest relevant to the conduct of this study.

Data sharing statement: There are no additional data available.

Manuscript source: Invited manuscript

Correspondence to: Moussab Damlaj, MD, FRCPC, FACP, Division of Hematology and HSCT, Department of Oncology, King Abdulaziz Medical City, P.O. Box 22490, Riyadh 11426, Saudi Arabia. damlajmo@ngha.med.sa
Fax: +966-11-8011111

Received: February 11, 2017
Peer-review started: February 15, 2017
First decision: March 27, 2017
Revised: July 6, 2017
Accepted: July 21, 2017
Article in press: July 24, 2017
Published online: August 24, 2017

Abstract

AIM

To examine the optimal absolute lymphocyte count (ALC) cut-off utilizing receiver operator characteristics (ROC) in addition to graft characteristics associated with early ALC recovery.

METHODS

Patients who received T-cell replete peripheral hematopoietic cell transplantation (HCT) for acute leukemia were identified. ALC cut-off was established using ROC analysis and subsequently the cohort was stratified. Time to endpoint analysis and cox regression modelling was computed to analyze outcomes.

RESULTS

A total of 72 patients met the inclusion criteria and

were analyzed. Optimal ALC cut-off was established to be on day 14 (D14) with ALC > 0.3 × 10^9/L. At 2 years, cumulative incidence of relapse was 16.9% *vs* 46.9% (P = 0.025) for early and delayed lymphocyte recovery cohorts, respectively. Chronic graft *vs* host disease was more prevalent in the early lymphocyte recovery (ELR) group at 70% *vs* 27%, respectively (P = 0.0006). On multivariable analysis for relapse, ELR retained its prognostic significance with HR = 0.27 (0.05-0.94, P = 0.038).

CONCLUSION

ELR is an independent predictor for relapse in patients receiving allogeneic HCT for acute leukemia. ELR was influenced by graft characteristics particularly CD34 count.

Key words: Acute leukemia; Allogeneic transplant; Absolute lymphocyte count

Core tip: Disease relapse remains the most common cause of treatment failure after allogeneic hematopoietic stem cell transplantation for acute leukemia. Previous studies have identified that early lymphocyte recovery can be a surrogate of graft *vs* leukemia effect hence identifying high risk patients for relapse. However, published reports are heterogeneous with regards to timeline and magnitude of lymphocyte recovery. Using receiver operator characteristics with area under the curve, we identified that absolute lymphocyte count > 0.3 × 10^9/L at day 14 is associated with half the relapse risk which was statistically significant at the multivariable analysis. There was a trend towards improved progression free survival and overall survival for patients with early lymphocyte recovery. In conclusion, we observed that lymphocyte recovery is an independent predictor of relapse in allogeneic transplant recipients for acute leukemia. This would help identify high risk patients who may benefit from maintenance strategies post-transplant.

Damlaj M, Ghazi S, Mashaqbeh W, Gmati G, Salama H, Abuelgasim KA, Rather M, Hajeer A, Al-Zahrani M, Jazieh AR, Hejazi A, Al Askar A. Lymphocyte recovery is an independent predictor of relapse in allogeneic hematopoietic cell transplantation recipients for acute leukemia. *World J Transplant* 2017; 7(4): 235-242 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i4/235.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i4.235

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HCT) is widely used to cure a number of hematologic malignancies including acute myeloid leukemia (AML) and lymphoblastic leukemia/lymphoma (ALL)[1-4]. Relapse of the primary disease remains the most frequent cause of treatment failure in contemporary HCT recipients[5]. Several factors are associated with relapse such as status at HCT, associated cytogenetic abnormalities, conditioning regimen and occurrence of chronic graft *vs* host disease[6]. Prognosis after overt relapse post-HCT is very poor and a minority of patients are able to achieve durable remissions[7]. Hence, identification of patients at risk of relapse may permit preemptive interventions for relapse prevention[8].

Immune reconstitution post HCT, particularly lymphocyte recovery, can be a surrogate for graft *vs* leukemia (GVL) effect hence improved long term disease control. Several groups reported that early absolute lymphocyte count (ALC) recovery is associated with decreased relapse rates in hematologic malignancies. However, there is heterogeneity regarding the predictive optimal threshold and timing of lymphocyte recovery. For example Michelis *et al*[9] reported that ALC ⩾ 0.5 × 10^9/L on day 28 in AML patients is associated with reduction of the relapse risk at multivariable analysis with hazard ratio (HR) = 0.49 (0.26-0.92, P = 0.03) without a survival advantage. On the other hand, Kumar *et al*[10,11] showed that ALC ⩾ 0.15 × 10^9/L on day +30 resulted in a 3 fold reduction in relapse risk in AML patients but an ALC of > 0.17 × 10^9/L on day +21 was protective from relapse in ALL patients. Thoma et al. showed that ALC > 0.3 × 10^9/L on day +100 is associated with improved overall survival (OS)[12].

In light of the above discrepancies, we examined the impact of ALC recovery on post HCT outcomes; where optimal ALC threshold and timeline was analyzed using receiver operator characteristics (ROC) and area under the curve (AUC). We also analyzed infused allograft cellular content for factors predicting early ALC recovery.

MATERIALS AND METHODS

Patient selection

After due institutional review board (IRB) approval, patients ⩾ 14 years of age with AML or ALL who underwent HCT at our institution between 2010 - 2015 were identified.

The selection criteria included patients receiving myeloablative (MAC) or reduced intensity conditioning (RIC) from related or unrelated donors. Classification of the conditioning intensity was based on the criteria suggested by the Centre of International Blood and Marrow Transplant Research (CIBMTR)[13]. Selection of regimen intensity was at the discretion of the treating physician and generally patients with a hematopoeitic stem cell co-morbidity index (HCT-CI) < 3 were considered for MAC regimen[14]. Patients with ALL who were candidates for MAC, preferentially received a total body irradiation (TBI) based regimen. Exclusion criteria were for patients who received a bone marrow graft or cord blood stem cell source, second transplant and those who underwent *in vivo* or *in vitro* T-cell depletion. Data were collected retrospectively from the patient's electronic medical records. Cytogenetic data

at the time of diagnosis was collected and stratified as previously described for AML patients[15]. ALL patients with hypodiploid karyotype, translocations at (4;11), (11q23), (9;22) and (1;19) were deemed high risk, and remaining patients were classified as standard risk[16-20].

Preparative regimens and GVHD prophylaxis

Patients candidates for MAC intensity received one of two regimens based on the underlying diagnosis; patients with ALL received cyclosphosphamide 60 mg/kg intravenously (IV) for two days followed by 1200 cGy of TBI fractioned twice daily for three days. Patients with AML received fludarabine 30 mg/m^2 daily for five days in addition to busulfan 3.2 mg/kg IV daily for four days in addition to cyclophosphamide 60 mg/kg IV daily for 2 d. Mesna was given for bladder protection. For RIC regimens, patients received either fludarabine 30 mg/m^2 IV daily for 5 d with busulfan 3.2 mg/kg IV daily for two days or fludarabine 30 mg/m^2 IV daily for 5 d with melphalan 70 mg/m^2 IV for two days. Phenytoin loading and maintenance was given for seizure prophylaxis if busulfan was used until 24 h post last dose. Graft *vs* host disease (GVHD) prophylaxis consisted of methotrexate and cyclosporine. Methotrexate was given at 15 mg/m^2 on day +1 followed by 10 mg/m^2 on days +3, +6 and +11 with leucovorin rescue 24 h post each methotrexate dose. Day +11 was omitted if there is evidence of significant liver toxicity or grade $\geqslant$ 2 mucositis.

Definitions and transplant related outcomes

OS was calculated from the date of transplant until the date of death of any cause or last documented follow-up date. Progression free survival (PFS) was calculated from the time of transplant until death of any cause or relapse. Cumulative incidence of relapse (CIR) was calculated from the date of transplant until relapse or date of last follow up. Cumulative incidence of non relapse mortality (NRM) was calculated from the date of transplant until death of any cause without evidence of disease relapse. Acute and chronic GVHD was diagnosed according to standard criteria. Neutrophil engraftment was defined as an absolute neutrophil count (ANC) of 0.5 × 10^9/L or higher for 3 consecutive days. Platelet engraftment was defined as platelet count higher than 20 × 10^9/L for 7 consecutive days without transfusion support.

End points

The primary end point was to examine the impact of early ALC recovery (ELR) on CIR. Secondary endpoints were to examine effect of ELR on other post HCT outcomes (OS, PFS and NRM) and to examine infused allo-graft cellular content for factors predicting ELR. ALC was abstracted on days +7, +14, +21 and +28 from the Complete Blood Count (CBC) post HCT using either the automated or manual differential method[21].

Statistical analysis

Baseline patient, disease and treatment related variables were reported using descriptive statistics (counts, medians and percentages). Categorical and continuous variables were compared using Pearson's χ^2 and Wilcoxon/Kruskal-Wallis, respectively. Probability of OS was computed using the Kaplan-Meier method. Group comparisons were made using the log-rank test. Time to event was calculated from the date of transplant until the event of interest or point of last clinical encounter, in which case the event will be censored. Cumulative incidence was computed as competing events using Grey's model, considering death as a competing event for relapse and relapse as a competing event for NRM. Univariable and multivariable analyses were performed using Cox proportional hazard regression modelling and expressed as HR with 95%CI and *P* value. Any variable with a $P \leqslant 0.1$ was incorporated into the multivariable model in a stepwise selection process. Thresholds of ALC recovery post HCT as well as infused allograft characteristics, if present, were assessed using the ROC and AUC for the end point of relapse. Statistical analysis were performed using JMP Pro Version 11 (SAS Institute, Cary, NC, United States) software and EZR on R commander version 1.28[22].

RESULTS

Patient and transplant characteristics

A total of 72 patients met the inclusion criteria and their data were analysed. Baseline characteristics of the cohort are shown in Table 1. Majority of transplants were from related donors (88%), while the remaining minority (12%) were from unrelated donors. Transplants were from peripheral blood stem cells, while cord blood and bone marrow grafts were excluded due to different immune reconstitution kinetics. All patients were from the Middle East and North Africa Region. The median follow up was 17 mo (range: 2-64.8) at which point the CIR was 35.2% and OS was 67.3%.

Optimal ALC threshold

ROC curves with AUC were used to determine the best cut-off value for ALC on days +7, +14, +21 and +28 based on their utility as a marker for the binary outcome of relapse *vs* no relapse. ALC on day +14 > 0.3 × 10^9/L was identified as the optimal cut-off point. Patients were subsequently stratified as ELR if ALC on day +14 > 0.3 × 10^9/L and delayed lymphocyte recovery (DLR) if day +14 ALC was $\leqslant$ 0.3 × 10^9/L. Patient's disease and HCT related variables are stratified per lymphocyte recovery as shown in Table 1. Cohorts were similar with regards to age, gender, diagnosis, performance status, cytogenetic risk, status at HCT, stem cell source, donor gender, ABO matching and conditioning intensity. Regimens containing TBI were more common in the DLR group at 63% *vs* 33% ($P = 0.019$).

Infused allo-graft characteristics influencing ELR

We examined infused allo-graft cellular contents for factors predicting ELR in our patients. Optimal thresholds

Table 1 Baseline characteristics of patients stratified by lymphocyte recovery *n* (%)

Variable	ALC > 0.3 (*n* = 24)	ALC ≤ 0.3 (*n* = 48)	*P* value
Patient age in years, median (range)	28 (16-57)	23 (14-63)	0.57
Recipient gender, male	13 (54)	28 (58)	0.74
Diagnosis			0.54
AML	13 (54)	22 (45)	
ALL	11 (46)	26 (54)	
ECOG	1 (0-2)	0 (0-3)	0.86
Cytogenetics (AML)			0.5
Favorable	3 (25)	2 (10)	
Intermediate	7 (58)	15 (71)	
High risk	2 (17)	4 (67)	
Cytogenetics (ALL)			0.78
Standard	5 (56)	11 (50)	
High risk	4 (44)	11 (50)	
Female donor/male recipient	4 (17)	11 (23)	0.53
Related donor	21 (88)	42 (88)	1
Status at HCT			0.33
CR1	13 (54)	31 (66)	
≥ CR2	11 (46)	16 (34)	
ABO Matching			0.89
Match	16 (67)	31 (64)	
Major/bidirectional	3 (12)	8 (17)	
Minor	5 (21)	9 (19)	
TBI containing regimen	8 (33)	30 (63)	0.019
Conditioning intensity			0.19
MAC	18 (75)	42 (88)	
RIC	6 (25)	6 (12)	

HCT: Hematopoietic stem cell transplant; AML: Acute myeloid leukemia; ALL: Acute lymphoblastic leukemia; ALC: Absolute lymphocyte count; ECOG: Eastern cooperative oncology group; TBI: Total body irradiation; CR: Complete remission; MAC: Myeloablative conditioning; RIC: Reduced intensity conditioning.

were again determined by ROC with AUC analysis. We observed that infusing grafts with the following characteristics was associated with higher incidence of ELR; CD 34 of < 6 × 10^6/kg (71% *vs* 42%, *P* = 0.018), CD3 > 24 × 10^7/kg (19% *vs* 2%, *P* = 0.017), infused ALC > 1.3 × 10^8/kg (96% *vs* 74%, *P* = 0.015), infused lymphocyte-monocyte ratio (LMR) > 4 (33% *vs* 11%, *P* = 0.022) and CD 34 < 6 × 10^6/kg with ALC > 1.3 × 10^8/kg (67% *vs* 27%, *P* = 0.0012). These results are show in Table 2.

Impact of ELR on post HCT outcomes

Stratified by lymphocyte recovery, after 2 years of follow up, the CIR was significantly higher for the DLR *vs* ELR groups at 46.9% *vs* 16.9%, respectively (*P* = 0.025). On the other hand, at 2 years, there was a non-significant difference of NRM between the two cohorts at 14.2% *vs* 23.3% for the DLR and ERL groups, respectively (*P* = 0.51). There was a trend towards improved 2 year PFS for the ELR at 61.9% *vs* 40.1% (*P* = 0.09), but no significant difference of OS was observed at 70.1% *vs* 53.9% for ELR *vs* DLR, respectively (*P* = 0.12) (Figure 1). Median time to ANC and platelet engraftment was similar for both groups at 17 (12-29) d and 24 (21-37) for ELR

Table 2 Graft characteristics as predictors of lymphocyte recovery *n* (%)

Graft characteristic	ALC > 0.3 (*n* = 24)	ALC ≤ 0.3 (*n* = 48)	*P* value
CD 34 × 10^6/kg < 6	17 (71)	20 (42)	0.018
TNC > 7 × 10^7/kg	5 (21)	10 (21)	1
CD 3 > 24 × 10^7/kg	4 (19)	1 (2)	0.017
CD 34 < 6 × 10^6/kg, CD 3 > 24 × 10^7/kg	3 (100)	0 (0)	0.0088
MNC > 2.7 × 10^8/kg	20 (83)	33 (69)	0.17
ALC > 1.3 × 10^8/kg	23 (96)	35 (74)	0.015
AMC > 1.75 × 10^8/kg	3 (13)	14 (30)	0.093
ALC > 1.3 × 10^8/kg, CD34 < 6 × 10^8/kg	16 (67)	13 (27)	0.0012
LMR > 4	8 (33)	5 (11)	0.022

ALC: Absolute lymphocyte count; TNC: Total nuclear count; MNC: Mononuclear count; AMC: Absolute monocyte count; LMR: Lymphocyte-monocyte ratio.

and 17 (12-25) and 24 (7-42), respectively (*P* = 0.76, 0.98). Incidence of aGVHD was similar but cGVHD was significantly higher in the ELR groups at 70% *vs* 27% (*P* = 0.0006). These results are shown in Table 3.

Six variables were found to influence relapse at univariable analysis; age at HCT HR = 0.97 (0.94-1.01, *P* = 0.1), single marital status HR = 2.59 (1.13-6.65, *P* = 0.023), female donor to male recipient HR = 2.15 (0.91-4.7, *P* = 0.079), CR1 remission HR = 0.52 (0.23-1.15, *P* = 0.1), cGVHD HR = 0.24 (0.079-0.59, *P* = 0.0013) and ELR 0.31 (0.09-0.8, *P* = 0.014). We also examined the impact of TBI on relapse given the higher incidence of TBI based conditioning in the DLR group, but did not see an apparent impact with HR = 1.003 (0.46-2.2, *P* = 0.99). Three factors remained prognostic at the multivariable analysis which were ELR HR = 0.27 (0.05-0.94, *P* = 0.038), CR1 remission HR = 0.36 (0.15-0.87, *P* = 0.024) and cGVHD 0.33 (0.1-0.92, *P* = 0.035). These results are shown in Table 4.

Causes of mortality in the ELR and DLR cohorts were related to relapse of primary disease in 3/8 (38%) and 18/24 (75%), infection 1/8 (12%) *vs* 0/24, organ failure 0/8 *vs* 1/24 (4.2%), aGVHD 1/8 (12) *vs* 2/24 (8.3%) and cGVHD 3/8 (38%) *vs* 3/24 (12%). These results are shown in Table 5.

DISCUSSION

The present analysis highlights again the value of ELR as a protective factor from disease relapse in acute leukemia. In particular, we report that ALC > 0.3 × 10^8/kg on day +14 post allogeneic HCT for acute leukemia is an independent factor predicting decreased CIR at multivariable analysis. We also observed a trend towards improved PFS and OS; however this did not meet statistical significance. NRM was not significant between both cohorts, however both the incidence of cGVHD and cGVHD related deaths were more frequent in the ELR group. Incidence of cGVHD related deaths

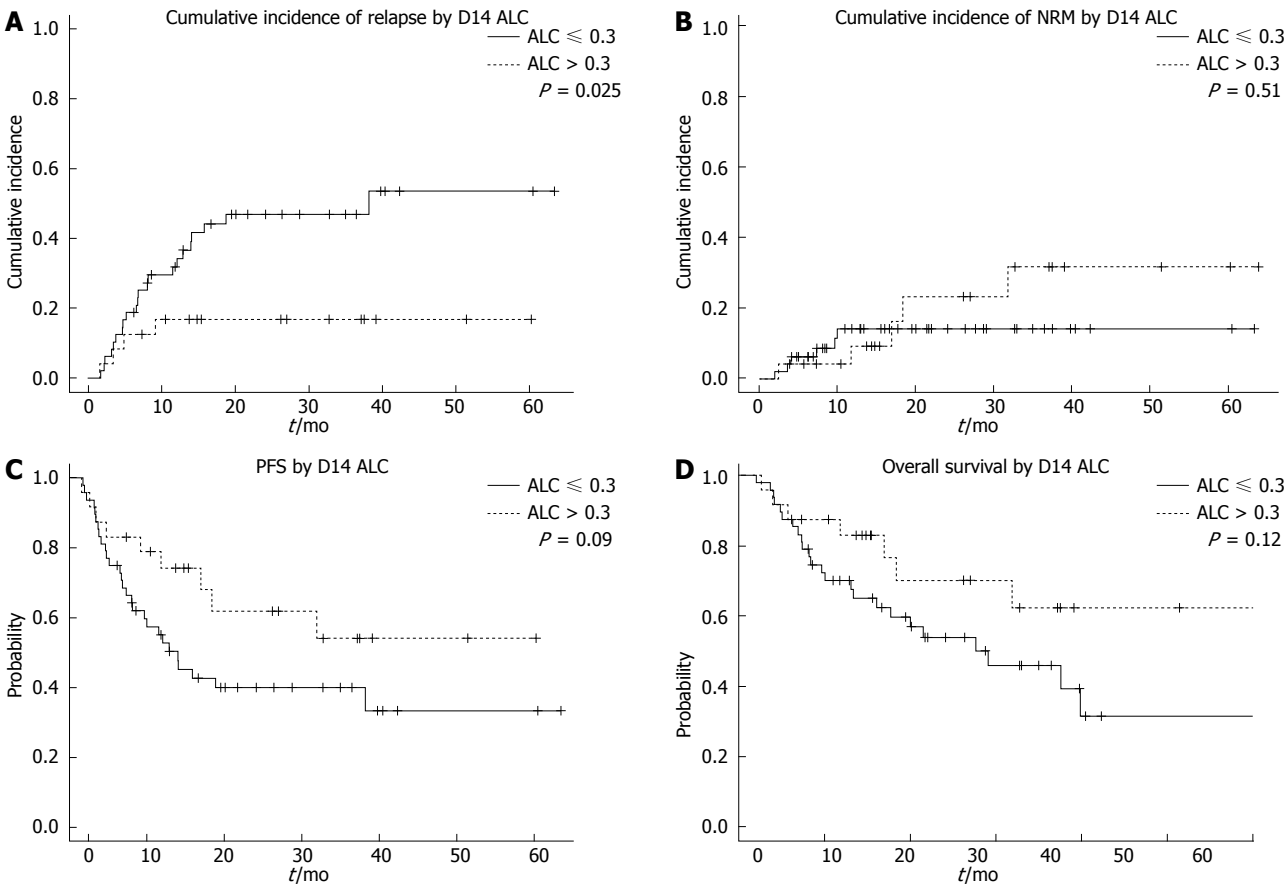


Figure 1 Post transplantation outcome of cumulative incidence of relapse (A), cumulative incidence of non-relapse mortality (B), progression free survival (C), and overall survival (D) stratified by lymphocyte recovery on day 14. ALC: Absolute lymphocyte count; PFS: Progression free survival.

Table 3 Transplant related outcomes

Variables	ALC > 0.3 (n = 24)	ALC ≤ 0.3 (n = 48)	P value
CIR (2-yr)	16.90%	46.90%	0.025
NRM (2-yr)	23.20%	14.20%	0.51
PFS (2-yr)	61.90%	40.10%	0.09
OS (2-yr)	70.10%	53.90%	0.12
Plt engraftment (median, d)	24 (21-37)	24 (7-42)	0.98
ANC engraftment (median, d)	17 (12-29)	17 (12-25)	0.76
aGVHD	5 (22)	15 (31)	0.4
cGVHD	16 (70)	13 (27)	0.0006

ALC: Absolute lymphocyte count; CIR: Cumulative incidence of relapse; NRM: Non-relapse mortality; PFS: Progression free survival; OS: Overall survival; Plt: Platelet; ANC: Absolute neutrophil count; aGVHD: Acute or chronic graft *vs* host disease; cGVHD: Chronic graft *vs* host disease.

was 37.5% (3/8) in the ELR group compared to 12.5% (3/24) in the DLR group. This perhaps explains the lack of statistical significance seen for PFS and OS.

Give that graft source and manipulation can affect cellular reconstitution post-transplant, we excluded patients who received bone marrow or cord blood grafts in addition to those receiving T-cell depleted manipulation of the graft[23,24]. TBI was administered more frequently in the DLR group, but we did not observe an impact on relapse using TBI at the univariable analysis level with HR: 1 (0.46-2.2, P = 0.99).

At multivariable analysis, three factors had an impact on relapse: CR1, cGVHD and ELR. cGVHD is well described to decrease incidence of relapse due to a parallel GVL effect[25]. The current analysis supports the hypothesis that ELR is a surrogate for GVL as cGVHD incidence was significantly higher in the ELR group. Incidence of cGVHD related deaths were also more frequent in the ELR group, which likely accounts for the observed NRM, PFS and OS rates.

Although lymphocyte subsets were not identified in this analysis, the most plausible subset implicated in our analysis would likely be the natural killer (NK) cells as they represent the bulk of recovered lymphocytes by two weeks post HCT[26]. Previously, NK cells were found to be an independent factor predicting post HCT outcomes in T-cell depleted grafts[27]. However, this finding was not reproduced when T-cell replete grafts were used[28]. That said, this observed protective effect from ELR is likely a complex interplay between various lymphocyte subsets, such as NK cells, cytotoxic T-lymphocytes (CD8+) and regulatory T-cells (CD4+ and CD25+)[29,30]. Furthermore, the infused graft cellular content likely impacts post HCT reconstitution, and this has been well demonstrated in the autologous HCT setting and to a lesser extent allogeneic HCT[12,31-33].

Infused allo-graft cellular content predicts post HCT reconstitution. We observed that higher T-cell

Table 4 Univariable and multivariable risk factors influencing incidence of relapse

IR	Univariable HR (95%CI, *P* value)	Multivariable HR (95%CI, *P* value)
Age at HCT	0.97 (0.94-1.01, *P* = 0.1)	0.13 (0.0096-1.38, *P* = 0.093)
Single Marital status	2.59 (1.13-6.65, *P* = 0.023)	0.82 (0.21-3.27, *P* = 0.77)
AML *vs* ALL	0.82 (0.36-1.8, *P* = 0.62)	
Female D *vs* Male R	2.15 (0.91-4.7, *P* = 0.079)	2.24 (0.88-5.31, *P* = 0.086)
Match *vs* Mismatch	1.9 (0.3-6.7, *P* = 0.42)	
MRD *vs* Other	1.6 (0.47-10, *P* = 0.49)	
D14 ALC > 0.3	0.31 (0.09-0.8, *P* = 0.014)	0.27 (0.05-0.94, *P* = 0.038)
MAC *vs* RIC	1.38 (0.46-3.4, *P* = 0.53)	
CR1 *vs* other	0.52 (0.23-1.15, *P* = 0.1)	0.36 (0.15-0.87, *P* = 0.024)
aGVHD	0.54 (0.16-1.43, *P* = 0.23)	
cGVHD	0.24 (0.079-0.59, *P* = 0.0013)	0.33 (0.1-0.92, *P* = 0.035)

ALC: Absolute lymphocyte count; HR: Hazard ratio; CR1: First complete remission; R: Recipient; D: Donor; AML: Acute myeloid leukemia; ALL: Acute lymphoblastic leukemia; cGVHD: Chronic graft *vs* host disease; MAC: Myeloablative conditioning; RIC: Reduced intensity conditioning; MRD: Matched related donor.

Table 5 Causes of mortality stratified by absolute lymphocyte count recovery

Variables	ALC > 0.3 (*n* = 8)	ALC ≤ 0.3 (*n* = 24)
Primary disease	3	18
Infection	1	N/A
Organ failure	N/A	1
aGVHD	1	2
cGVHD	3	3

ALC: Absolute lymphocyte count; aGVHD: Acute or chronic graft *vs* host disease; cGVHD: Chronic graft *vs* host disease; N/A: Not available.

and absolute lymphocyte content was significantly associated with ELR. Higher CD34 content is typically associated with faster engraftment and decreased rejection[34,35]. The National Marrow Donor Program (NMDP) reported on a cohort of over 900 unrelated HCTs using peripheral blood stem cells indicating that higher CD34 doses resulted in rapid engraftment, decreased transplant related mortality (TRM) and improved OS using various conditioning regimens[36]. However, the median stem cell dose administered was 6×10^6/kg and 5×10^6/kg in myeloablative (MAC) and reduced intensity RIC transplants, respectively. We found that infusing $< 6 \times 10^6$/kg stem cells was significantly associated with ELR. This is consistent with other reports indicating that administering higher doses of stem cells leads to detrimental outcomes both in MAC and RIC regimens[37-40]. Collectively, it appears that the optimal stem cell dose is 6-8 $\times 10^6$/kg, thus striking a balance between (GVL) and GVHD[41].

This analysis has inherent limitations, primarily due to the retrospective nature and sample size. We excluded patients who had T-cell manipulation or grafts other than peripheral blood stem cells as these factors can impact immune reconstitution. However, a number of important observations were made. First, similar to prior reports, we observed that ELR is protective of relapse but the timing post HCT and lymphocyte thresholds were determined using ROC-AUC and not empirically. Second, a higher incidence of cGVHD and cGVHD related deaths was seen with ELR, which confirms the likely mechanism of lower CIR seen in this cohort. Interestingly, marital status was significantly associated with decreased CIR although it did not retain significance at the multivariable analysis. Lastly, we reported that infusing less stem cells correlates better with ELR thus challenging the notion of "more is better".

In summary, the presented study demonstrates an independent protective effect of ALC at 14 d post allogeneic HCT. Given that patients with acute leukemia relapsing after allogeneic HCT have a dismal prognosis. Early identification of these cases may facilitate pre-emptive decisions such as early cessation of immune-suppression or use of lymphocyte infusion in order to better harness the GVL effect, or other maintenance strategies such as hypomethylating agents. These important observations warrant further study.

COMMENTS

Background

Disease relapse remains the most common cause of treatment failure after allogeneic hematopoietic stem cell transplantation for acute leukemia. Several factors are associated with relapse such as status at hematopoietic cell transplantation (HCT), associated cytogenetic abnormalities, conditioning regimen and occurrence of chronic graft *vs* host disease. Prognosis after overt relapse post-HCT is very poor and a minority of patients are able to achieve durable remissions. Hence, identification of patients at risk of relapse may permit preemptive interventions for relapse prevention. Immune reconstitution post HCT, particularly lymphocyte recovery, can be a surrogate for GVL effect hence improved long term disease control.

Research frontiers

Several groups reported that early absolute lymphocyte count (ALC) recovery is associated with decreased relapse rates in hematologic malignancies. However, there is heterogeneity regarding the predictive optimal threshold and timing of lymphocyte recovery.

Innovations and breakthroughs

The authors examined the impact of ALC recovery on post HCT outcomes. Using receiver operator characteristics with area under the curve, the authors identified that absolute lymphocyte count > 0.3×10^9/L at day 14 is associated with half the relapse risk which was statistically significant at the multivariable analysis. The authors also observed that infused graft content influences ALC

recovery.

Applications

Given that patients with acute leukemia relapsing after allogeneic HCT have a dismal prognosis. Early identification of these cases may facilitate pre-emptive decisions such as early cessation of immune-suppression or use of lymphocyte infusion in order to better harness the GVL effect, or other maintenance strategies such as hypomethylating agents.

Terminology

ALC recovery post allogeneic HCT is an easy to measure marker and can be used as a surrogate to identify high risk patients for relapse. Using receiver operator characteristics with area under the curve can help identify the optimal ALC threshold to exhibit this protective effect.

Peer-review

This is an interesting study, demonstrating lymphocyte recovery as independent predictor for relapse in allogenic hematopoietic stem cell transplantation for acute leukemia.

REFERENCES

1 **Farag SS**, Ruppert AS, Mrózek K, Mayer RJ, Stone RM, Carroll AJ, Powell BL, Moore JO, Pettenati MJ, Koduru PR, Stamberg J, Baer MR, Block AW, Vardiman JW, Kolitz JE, Schiffer CA, Larson RA, Bloomfield CD. Outcome of induction and postremission therapy in younger adults with acute myeloid leukemia with normal karyotype: a cancer and leukemia group B study. *J Clin Oncol* 2005; **23**: 482-493 [PMID: 15534356 DOI: 10.1200/JCO.2005.06.090]

2 **Yanada M**, Matsuo K, Emi N, Naoe T. Efficacy of allogeneic hematopoietic stem cell transplantation depends on cytogenetic risk for acute myeloid leukemia in first disease remission: a metaanalysis. *Cancer*2005; **103**: 1652-1658 [PMID: 15742336 DOI: 10.1002/cncr.20945]

3 **Koreth J**, Schlenk R, Kopecky KJ, Honda S, Sierra J, Djulbegovic BJ, Wadleigh M, DeAngelo DJ, Stone RM, Sakamaki H, Appelbaum FR, Döhner H, Antin JH, Soiffer RJ, Cutler C. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials. *JAMA* 2009; **301**: 2349-2361 [PMID: 19509382 DOI: 10.1001/jama.2009.813]

4 **Majhail NS**, Farnia SH, Carpenter PA, Champlin RE, Crawford S, Marks DI, Omel JL, Orchard PJ, Palmer J, Saber W, Savani BN, Veys PA, Bredeson CN, Giralt SA, LeMaistre CF. Indications for Autologous and Allogeneic Hematopoietic Cell Transplantation: Guidelines from the American Society for Blood and Marrow Transplantation. *Biol Blood Marrow Transplant* 2015; **21**: 1863-1869 [PMID: 26256941 DOI: 10.1016/j.bbmt.2015.07.032]

5 **Barrett AJ**, Battiwalla M. Relapse after allogeneic stem cell transplantation. *Expert Rev Hematol* 2010; **3**: 429-441 [PMID: 21083034 DOI: 10.1586/ehm.10.32]

6 **Ossenkoppele GJ**, Janssen JJ, van de Loosdrecht AA. Risk factors for relapse after allogeneic transplantation in acute myeloid leukemia. *Haematologica* 2016; **101**: 20-25 [PMID: 26721801 DOI: 10.3324/haematol.2015.139105]

7 **Savani BN**, Mielke S, Reddy N, Goodman S, Jagasia M, Rezvani K. Management of relapse after allo-SCT for AML and the role of second transplantation. *Bone Marrow Transplant* 2009; **44**: 769-777 [PMID: 19855439 DOI: 10.1038/bmt.2009.300]

8 **de Lima M**, Porter DL, Battiwalla M, Bishop MR, Giralt SA, Hardy NM, Kröger N, Wayne AS, Schmid C. Proceedings from the National Cancer Institute's Second International Workshop on the Biology, Prevention, and Treatment of Relapse After Hematopoietic Stem Cell Transplantation: part III. Prevention and treatment of relapse after allogeneic transplantation. *Biol Blood Marrow Transplant* 2014; **20**: 4-13 [PMID: 24018392 DOI: 10.1016/j.bbmt.2013.08.012]

9 **Michelis FV**, Messner HA, Loach D, Uhm J, Gupta V, Lipton JH, Seftel MD, Kuruvilla J, Kim DD. Early lymphocyte recovery at 28 d post-transplant is predictive of reduced risk of relapse in patients with acute myeloid leukemia transplanted with peripheral blood stem cell grafts. *Eur J Haematol* 2014; **93**: 273-280 [PMID: 24725056 DOI: 10.1111/ejh.12338]

10 **Kumar S**, Chen MG, Gastineau DA, Gertz MA, Inwards DJ, Lacy MQ, Tefferi A, Litzow MR. Effect of slow lymphocyte recovery and type of graft-versus-host disease prophylaxis on relapse after allogeneic bone marrow transplantation for acute myelogenous leukemia. *Bone Marrow Transplant* 2001; **28**: 951-956 [PMID: 11753550 DOI: 10.1038/sj.bmt.1703262]

11 **Kumar S**, Chen MG, Gastineau DA, Gertz MA, Inwards DJ, Lacy MQ, Tefferi A, Litzow MR. Lymphocyte recovery after allogeneic bone marrow transplantation predicts risk of relapse in acute lymphoblastic leukemia. *Leukemia* 2003; **17**: 1865-1870 [PMID: 12970788 DOI: 10.1038/sj.leu.2403055]

12 **Thoma MD**, Huneke TJ, DeCook LJ, Johnson ND, Wiegand RA, Litzow MR, Hogan WJ, Porrata LF, Holtan SG. Peripheral blood lymphocyte and monocyte recovery and survival in acute leukemia postmyeloablative allogeneic hematopoietic stem cell transplant. *Biol Blood Marrow Transplant* 2012; **18**: 600-607 [PMID: 21843495 DOI: 10.1016/j.bbmt.2011.08.007]

13 **Bacigalupo A**, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, Apperley J, Slavin S, Pasquini M, Sandmaier BM, Barrett J, Blaise D, Lowski R, Horowitz M. Defining the intensity of conditioning regimens: working definitions. *Biol Blood Marrow Transplant* 2009; **15**: 1628-1633 [PMID: 19896087 DOI: 10.1016/j.bbmt.2009.07.004]

14 **Sorror ML**, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, Storer B. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT.*Blood* 2005; **106**: 2912-2919 [PMID: 15994282 DOI: 10.1182/blood-2005-05-2004]

15 **Grimwade D**, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, Wheatley K, Harrison CJ, Burnett AK; National Cancer Research Institute Adult Leukaemia Working Group. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood* 2010; **116**: 354-365 [PMID: 20385793 DOI: 10.1182/blood-2009-11-254441]

16 **Moorman AV**, Ensor HM, Richards SM, Chilton L, Schwab C, Kinsey SE, Vora A, Mitchell CD, Harrison CJ. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. *Lancet Oncol* 2010; **11**: 429-438 [PMID: 20409752 DOI: 10.1016/S1470-2045(10)70066-8]

17 **Thirman MJ**, Gill HJ, Burnett RC, Mbangkollo D, McCabe NR, Kobayashi H, Ziemin-van der Poel S, Kaneko Y, Morgan R, Sandberg AA. Rearrangement of the MLL gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. *N Engl J Med* 1993; **329**: 909-914 [PMID: 8361504 DOI: 10.1056/NEJM199309233291302]

18 **Rambaldi A**, Attuati V, Bassan R, Neonato MG, Viero P, Battista R, Di Bona E, Rossi G, Pogliani E, Ruggeri M, Amaru R, Rivolta A, Giudici G, Biondi A, Barbui T. Molecular diagnosis and clinical relevance of t(9;22), t(4;11) and t(1 ;19) chromosome abnormalities in a consecutive group of 141 adult patients with acute lymphoblastic leukemia. *Leuk Lymphoma* 1996; **21**: 457-466 [PMID: 9172811 DOI: 10.3109/10428199609093444]

19 **Crist WM**, Carroll AJ, Shuster JJ, Behm FG, Whitehead M, Vietti TJ, Look AT, Mahoney D, Ragab A, Pullen DJ. Poor prognosis of children with pre-B acute lymphoblastic leukemia is associated with the t(1;19)(q23;p13): a Pediatric Oncology Group study. *Blood* 1990; **76**: 117-122 [PMID: 2364165]

20 **Harrison CJ**, Moorman AV, Broadfield ZJ, Cheung KL, Harris RL, Reza Jalali G, Robinson HM, Barber KE, Richards SM, Mitchell CD, Eden TO, Hann IM, Hill FG, Kinsey SE, Gibson BE, Lilleyman J, Vora A, Goldstone AH, Franklin IM, Durrant J, Martineau M; Childhood and Adult Leukaemia Working Parties. Three distinct subgroups of hypodiploidy in acute lymphoblastic leukaemia. *Br J Haematol* 2004; **125**: 552-559 [PMID: 15147369 DOI: 10.1111/j.1365-2141.2004.04948.x]

21 **Cox CJ**, Habermann TM, Payne BA, Klee GG, Pierre RV. Evaluation of the Coulter Counter model S-Plus IV. *Am J Clin Pathol* 1985; **84**: 297-306 [PMID: 4036859]

22 **Kanda Y**. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013; **48**: 452-458 [PMID: 23208313 DOI: 10.1038/bmt.2012.244]

23 **Anasetti C**, Logan BR, Lee SJ, Waller EK, Weisdorf DJ, Wingard JR, Cutler CS, Westervelt P, Woolfrey A, Couban S, Ehninger G, Johnston L, Maziarz RT, Pulsipher MA, Porter DL, Mineishi S, McCarty JM, Khan SP, Anderlini P, Bensinger WI, Leitman SF, Rowley SD, Bredeson C, Carter SL, Horowitz MM, Confer DL; Blood and Marrow Transplant Clinical Trials Network. Peripheral-blood stem cells versus bone marrow from unrelated donors. *N Engl J Med* 2012; **367**: 1487-1496 [PMID: 23075175 DOI: 10.1056/NEJMoa1203517]

24 **Jacobson CA**, Turki AT, McDonough SM, Stevenson KE, Kim HT, Kao G, Herrera MI, Reynolds CG, Alyea EP, Ho VT, Koreth J, Armand P, Chen YB, Ballen K, Soiffer RJ, Antin JH, Cutler CS, Ritz J. Immune reconstitution after double umbilical cord blood stem cell transplantation: comparison with unrelated peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant* 2012; **18**: 565-574 [PMID: 21875503 DOI: 10.1016/j.bbmt.2011.08.018]

25 **Signori A**, Crocchiolo R, Oneto R, Sacchi N, Sormani MP, Fagioli F, Rambaldi A, Ciceri F, Bacigalupo A. Chronic GVHD is associated with lower relapse risk irrespective of stem cell source among patients receiving transplantation from unrelated donors. *Bone Marrow Transplant* 2012; **47**: 1474-1478 [PMID: 22465976 DOI: 10.1038/bmt.2012.58]

26 **Storek J**, Geddes M, Khan F, Huard B, Helg C, Chalandon Y, Passweg J, Roosnek E. Reconstitution of the immune system after hematopoietic stem cell transplantation in humans. *Semin Immunopathol* 2008; **30**: 425-437 [PMID: 18949477 DOI: 10.1007/s00281-008-0132-5]

27 **Savani BN**, Mielke S, Adams S, Uribe M, Rezvani K, Yong AS, Zeilah J, Kurlander R, Srinivasan R, Childs R, Hensel N, Barrett AJ. Rapid natural killer cell recovery determines outcome after T-cell-depleted HLA-identical stem cell transplantation in patients with myeloid leukemias but not with acute lymphoblastic leukemia. *Leukemia* 2007; **21**: 2145-2152 [PMID: 17673900 DOI: 10.1038/sj.leu.2404892]

28 **Bühlmann L**, Buser AS, Cantoni N, Gerull S, Tichelli A, Gratwohl A, Stern M. Lymphocyte subset recovery and outcome after T-cell replete allogeneic hematopoietic SCT. *Bone Marrow Transplant* 2011; **46**: 1357-1362 [PMID: 21113185 DOI: 10.1038/bmt.2010.306]

29 **Edinger M**, Hoffmann P, Ermann J, Drago K, Fathman CG, Strober S, Negrin RS. CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. *Nat Med* 2003; **9**: 1144-1150 [PMID: 12925844 DOI: 10.1038/nm915]

30 **Di Ianni M**, Falzetti F, Carotti A, Terenzi A, Castellino F, Bonifacio E, Del Papa B, Zei T, Ostini RI, Cecchini D, Aloisi T, Perruccio K, Ruggeri L, Balucani C, Pierini A, Sportoletti P, Aristei C, Falini B, Reisner Y, Velardi A, Aversa F, Martelli MF. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood* 2011; **117**: 3921-3928 [PMID: 21292771 DOI: 10.1182/blood-2010-10-311894]

31 **Porrata LF**, Inwards DJ, Ansell SM, Micallef IN, Johnston PB, Hogan WJ, Markovic SN. Infused autograft lymphocyte-to-monocyte ratio and survival in T-cell lymphoma post-autologous peripheral blood hematopoietic stem cell transplantation. *J Hematol Oncol* 2015; **8**: 80 [PMID: 26138828 DOI: 10.1186/s13045-015-0178-5]

32 **Porrata LF**, Inwards DJ, Ansell SM, Micallef IN, Johnston PB, Hogan WJ, Markovic SN. Day 100 Peripheral Blood Absolute Lymphocyte/Monocyte Ratio and Survival in Classical Hodgkin's Lymphoma Postautologous Peripheral Blood Hematopoietic Stem Cell Transplantation. *Bone Marrow Res* 2013; **2013**: 658371 [PMID: 23710362 DOI: 10.1155/2013/658371]

33 **DeCook LJ**, Thoma M, Huneke T, Johnson ND, Wiegand RA, Patnaik MM, Litzow MR, Hogan WJ, Porrata LF, Holtan SG. Impact of lymphocyte and monocyte recovery on the outcomes of allogeneic hematopoietic SCT with fludarabine and melphalan conditioning. *Bone Marrow Transplant* 2013; **48**: 708-714 [PMID: 23103674 DOI: 10.1038/bmt.2012.211]

34 **Storb R**, Prentice RL, Thomas ED. Marrow transplantation for treatment of aplastic anemia. An analysis of factors associated with graft rejection. *N Engl J Med* 1977; **296**: 61-66 [PMID: 136605 DOI: 10.1056/NEJM197701132960201]

35 **Bittencourt H**, Rocha V, Chevret S, Socié G, Espérou H, Devergie A, Dal Cortivo L, Marolleau JP, Garnier F, Ribaud P, Gluckman E. Association of CD34 cell dose with hematopoietic recovery, infections, and other outcomes after HLA-identical sibling bone marrow transplantation. *Blood* 2002; **99**: 2726-2733 [PMID: 11929759]

36 **Pulsipher MA**, Chitphakdithai P, Logan BR, Leitman SF, Anderlini P, Klein JP, Horowitz MM, Miller JP, King RJ, Confer DL. Donor, recipient, and transplant characteristics as risk factors after unrelated donor PBSC transplantation: beneficial effects of higher CD34+ cell dose. *Blood* 2009; **114**: 2606-2616 [PMID: 19608747 DOI: 10.1182/blood-2009-03-208355]

37 **Remberger M**, Törlén J, Ringdén O, Engström M, Watz E, Uhlin M, Mattsson J. Effect of Total Nucleated and CD34(+) Cell Dose on Outcome after Allogeneic Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant* 2015; **21**: 889-893 [PMID: 25662230 DOI: 10.1016/j.bbmt.2015.01.025]

38 **Mehta J**, Mehta J, Frankfurt O, Altman J, Evens A, Tallman M, Gordon L, Williams S, Winter J, Krishnamurthy J, Duffey S, Singh V, Meagher R, Grinblatt D, Kaminer L, Singhal S. Optimizing the CD34 + cell dose for reduced-intensity allogeneic hematopoietic stem cell transplantation. *Leuk Lymphoma* 2009; **50**: 1434-1441 [PMID: 19603344 DOI: 10.1080/10428190903085944]

39 **Przepiorka D**, Smith TL, Folloder J, Khouri I, Ueno NT, Mehra R, Körbling M, Huh YO, Giralt S, Gajewski J, Donato M, Cleary K, Claxton D, Braunschweig I, van Besien K, Andersson BS, Anderlini P, Champlin R. Risk factors for acute graft-versus-host disease after allogeneic blood stem cell transplantation. *Blood* 1999; **94**: 1465-1470 [PMID: 10438735]

40 **Mohty M**, Bilger K, Jourdan E, Kuentz M, Michallet M, Bourhis JH, Milpied N, Sutton L, Jouet JP, Attal M, Bordigoni P, Cahn JY, Sadoun A, Ifrah N, Guyotat D, Faucher C, Fegueux N, Reiffers J, Maraninchi D, Blaise D. Higher doses of CD34+ peripheral blood stem cells are associated with increased mortality from chronic graft-versus-host disease after allogeneic HLA-identical sibling transplantation. *Leukemia* 2003; **17**: 869-875 [PMID: 12750699 DOI: 10.1038/sj.leu.2402909]

41 **Shimoni A**, Nagler A. Stem-cell dose for allogeneic hematopoietic stem cell transplantation in hematological malignancies: is more better? *Leuk Lymphoma* 2009; **50**: 1395-1396 [PMID: 19672777 DOI: 10.1080/10428190903174367]

P- Reviewer: Li Z, Ramírez M **S- Editor**: Kong JX **L- Editor**: A
E- Editor: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 August 24; 7(4): 243-249

DOI: 10.5500/wjt.v7.i4.243

ISSN 2220-3230 (online)

CASE REPORT

De novo intraocular amyloid deposition after hepatic transplantation in familial amyloidotic polyneuropathy

Ivo Filipe Gama, Leonor Duarte Almeida

Ivo Filipe Gama, Leonor Duarte Almeida, Ophthalmology Department, Santa Maria University Hospital (North Lisbon Hospital Center), 1649-035 Lisbon, Portugal

Author contributions: Gama IF and Almeida LD designed the report; Almeida LD evaluated and followed-up the patient; Gama IF performed the complimentary exams and collected all clinical data; Gama IF and Almeida LD wrote the paper.

Institutional review board statement: The retrospective review and publication of patient's clinical data were approved by the Santa Maria University Hospital Ethics Committee.

Informed consent statement: The patient gave written consent for publication of his clinical data.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest to disclose and they did not receive any funding for this paper.

Manuscript source: Invited manuscript

Correspondence to: Ivo Filipe Gama, MD, Ophthalmology Department, Santa Maria University Hospital (North Lisbon Hospital Center), Av. Prof. Egas Moniz, 1649-035 Lisbon, Portugal. ivo.gama@chln.min-saude.pt
Telephone: +351-93-9160876

Received: September 15, 2016
Peer-review started: September 17, 2016
First decision: October 21, 2016
Revised: May 29, 2017
Accepted: June 19, 2017
Article in press: June 20, 2017
Published online: August 24, 2017

Abstract

The familiar amyloid polyneuropathy (FAP) is a rare autosomal-dominant systemic amyloidosis. Amyloid deposition occurs more frequently and extensively in the vitq. The increase in intraocular pressure (IOP) is a result of deposition of transthyretin (TTR) in trabecular meshwork. Rarely, the amyloid deposition in anterior segment can be more exuberant than in posterior segment. A 42 years old man, with FAP (Val30Met mutation), liver transplantation in 1997. He was asymptomatic, without any significant ocular abnormality until 2011. In 2011 he had an episode of pain in right eye (RE). Scalloped pupils, pupillary amyloid deposits and subtle vitreous opacities were detected. The IOP was 40 mmHg in RE and 28 mmHg in left eye (LE) with open angle. Optical coherence tomography detected a temporal superior retinal nerve fiber layer defect in LE and perimetry was normal. Topical timolol was initiated, and brimonidine was subsequently added to improve IOP control, which was achieved with topical medication until last evaluation. No progression occurred since 2011. Actually, with longer life expectancies, there is an increased risk of ocular involvement in FAP, even after liver transplantation. Although rare, a more exuberant amyloid deposition in anterior segment *vs* posterior segment can occur, and supports an important role of amyloid production in ciliary pigment epithelium in these patients. Medical control of IOP and a stable course are unusual in this secondary glaucoma. Ophthalmologists have an important task in the follow-up of patients and early diagnosis of risk factors for secondary glaucoma, such as scalloped pupils with amyloid deposits.

Key words: Familial amyloid polyneuropathy; Glaucoma; Scalloped pupils; Pupillary amyloid deposits; Liver transplantation

Core tip: Ocular manifestations of familial amyloidotic

polyneuropathy (FAP) can appear after liver transplantation due to *de novo* ocular production of amyloid. Rarely, amyloid deposition in vitreous is relatively less exuberant than in anterior segment. Our case illustrates this asymmetry of amyloid deposition and emphasizes the association between scalloped pupils and glaucoma, a major ocular complication of FAP. Our case had a stable course, with excellent visual function and the intraocular pressure was controlled by medical therapy, which are unusual in this type of glaucoma. This case-report also highlights the importance of the long-term ophthalmological follow-up in FAP patients.

Gama IF, Almeida LD. *De novo* intraocular amyloid deposition after hepatic transplantation in familial amyloidotic polyneuropathy. *World J Transplant* 2017; 7(4): 243-249 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i4/243.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i4.243

INTRODUCTION

Transthyretin (TTR)-related familial amyloid polyneuropathy (FAP) is a group of autosomal-dominant diseases of variable penetrance caused by the deposition of polymerized mutated TTR in the peripheral nerves, gastrointestinal tract, heart, ocular tissues, and other organs. These protein aggregates have affinity for Congo red stain and apple-green birefringence when viewed under polarized light[1-3]. They are caused by mutations of TTR gene (18q11.2-12.1)[2]. Peripheral neuropathy is progressive and frequently the first manifestation of the disease[1-3]. Type 1 FAP, the Portuguese type (FAP1) was described for the first time in 1952 by Corino de Andrade[4]. FAP1 is the most frequent type of FAP and caused by a mutational substitution of the valine for methionine in position 30 of TTR gene (Val30Met)[2].

There are many ophthalmological manifestations of FAP caused by deposition of amyloid in various intra-ocular tissues: Vitreous, iris, pupillary border, anterior capsule and trabecular meshwork. The pupillary margin may have a scalloped/indented configuration (scalloped pupils) and pupils may be slow or nonreactive to both light and near stimulation, caused by disturbance of autonomic innervation[1-3,5-8]. Fleck deposits resembling pseudoexfoliation (PEX) may be found on the anterior lens capsule and pupillary margin[1-3,7]. *Pseudopodia lentis* is a hallmark of vitreous amyloidosis, where multiple small dots or footplates are formed on the posterior lens surface[2]. Trabecular meshwork deposition of amyloid causes obstruction of aqueous humor outflow and subsequent elevation of intra-ocular pressure (IOP)[9]. Secondary glaucoma can develop rapidly with high IOP, which if left untreated it can lead to severe damage[3,9]. Other manifestations include dry eye by decreased tear production, conjunctival microaneurysms and reduced corneal sensitivity with subsequent neurotrophic corneal ulcers[2].

TTR is a normal constituent of blood plasma, acts as a thyroxine transport protein and is important in vitamin A transport[2,3]. TTR is synthesized mainly in liver (90%), but there is also intra-ocular production[1,3,6,7,10-12]. Retinal pigment epithelium (RPE) is a source of TTR synthesis in rat eyes[10]. Recently, it was demonstrated that TTR production also occurs in the ciliary pigment epithelium (CPE)[12].

Liver transplantation (LT) improved the quality and survival of FAP patients, but does not prevent ocular manifestations of FAP, because of persistent intra-ocular production of amyloidotic TTR (ATTR). A case of vitreous amyloidosis appearing 2 years after LT was described and mutant protein ATTR was detected in aqueous humor of a Japanese patient after LT[13,14].

Secondary glaucoma is a major complication of FAP, which can be the first ocular manifestation and cause irreversible visual loss. Thus, early diagnosis is fundamental to avoid rapid progression of glaucoma[9].

The authors want to emphasize the importance of the recognition of ophthalmological signs that are associated with increased risk of ocular hypertension and glaucoma in FAP1 patients after LT as well as to report an unusual asymmetric pattern of intraocular amyloid deposition, with a case report and bibliographic revision.

CASE REPORT

A 42-year-old man had a diagnosis of FAP since 1995, with a positive genetic test for ATTR Val30Met mutation, and was subjected to LT in 1997. The peripheral neuropathy improved after LT. His brother and mother had type 1 FAP. The patient did not have any other previous ophthalmological diagnosis besides myopia. No FAP-related ophthalmological abnormalities were detected on routine ophthalmology evaluations for 14 years after LT.

In November 2011, he had an episode of ocular pain in right eye (RE) and attended the emergency room. Best-corrected visual acuity was 20/20 in RE and left eye (LE). Pupils were isochoric with slow pupillary responses to light and near stimulation. Ocular movements were normal. Biomicroscopy showed bilateral whitish fleck flocculent deposits of amyloid in the pupillary borders, scalloped pupils and few deposits in anterior vitreous (Figure 1). The detection of abnormalities led to the measurement of intraocular pressure (IOP) by Goldmann applanation tonometry (GAT), being 40 mmHg in RE and 28 mmHg in LE. Gonioscopy showed open angles - Shaffer grade of 4. Fundoscopy and retinography showed few vitreous opacities and clearly visible normal posterior poles, with normal appearing optic discs (Figures 2 and 3). Central corneal thickness was 559 μm in RE and 550 μm in LE. Ophthalmic ultrasound (US) showed few vitreous opacities bilaterally (Figure 4). Optical coherence tomography (OCT) only showed a superior-temporal

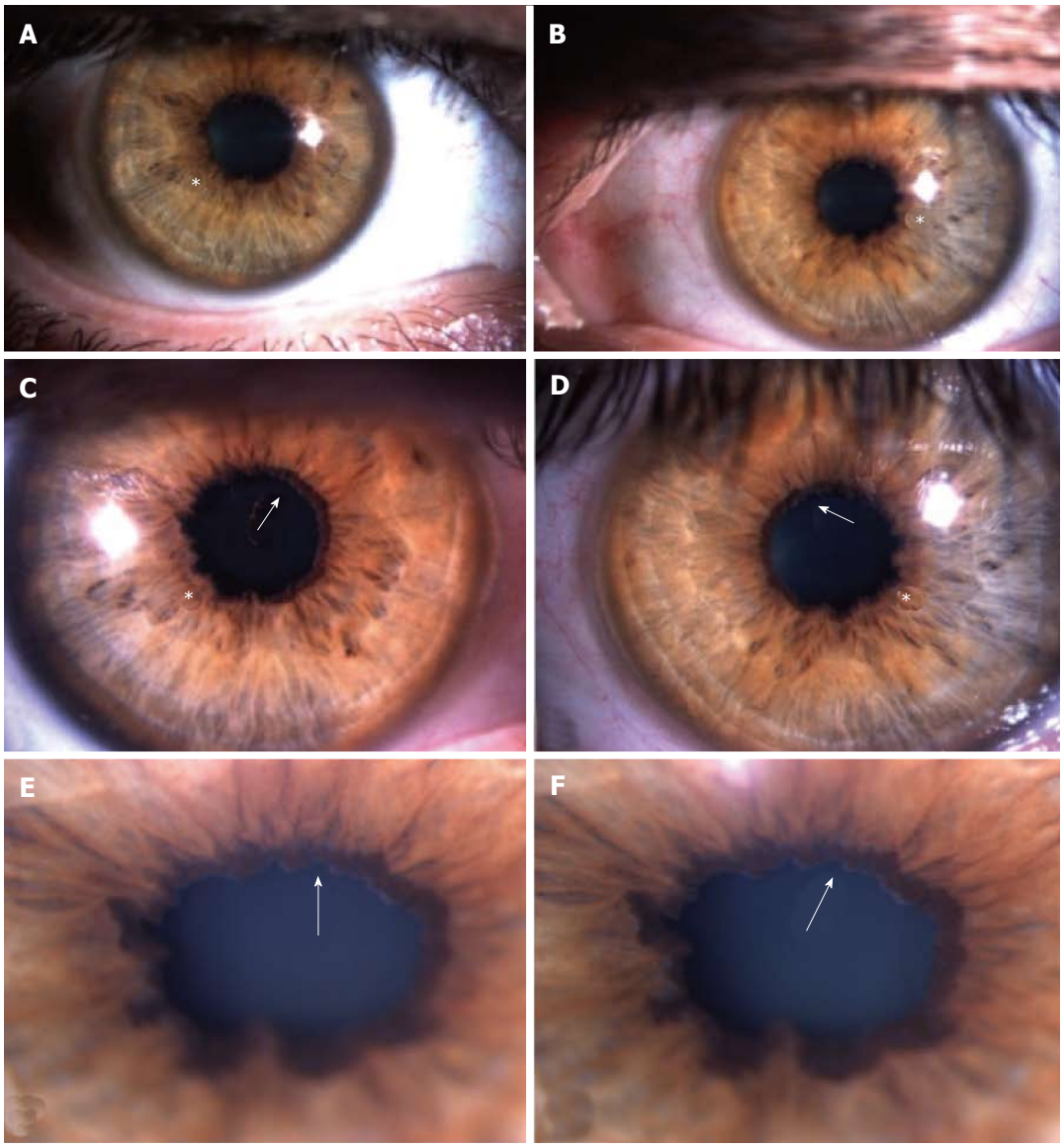


Figure 1 Slit-lamp photos showing scalloped pupils (astherisks) and amyloid deposition in the pupillary border (arrows) in both eyes. A and B: Slit-lamp photos of anterior segment of right (A) and left eyes (B) at low magnification; C and D: Slit-lamp photos at higher magnification to show pupillary margins of right (C) and left (D) eyes with more detail, in order to highlight the irregular pupillary margins, the scalloped pupils (astherisks) with amyloid deposits (arrows); E and F: Slit-lamp photos of the right eye (E and F) at the highest magnification to enhance visualization of the pupillary amyloid deposits (arrows), which resemble those seen in pseudoexfoliation syndrome.

peripapillary retinal fiber layer retinal nerve fiber layer (RNFL) defect in LE (Figure 5). Automated perimetry was unremarkable in both eyes (Figure 6). Topical monotherapy with timolol 0.5% was initiated at that time, and the IOP lowered to 26 mmHg in RE and to 21 mmHg in LE. To optimize IOP control, brimonidine was associated with timolol further lowering the IOP to 14 mmHg in both eyes. The patient was followed up closely in the glaucoma clinic until present, with controlled IOP. Last CSP and OCT exams excluded glaucoma progression.

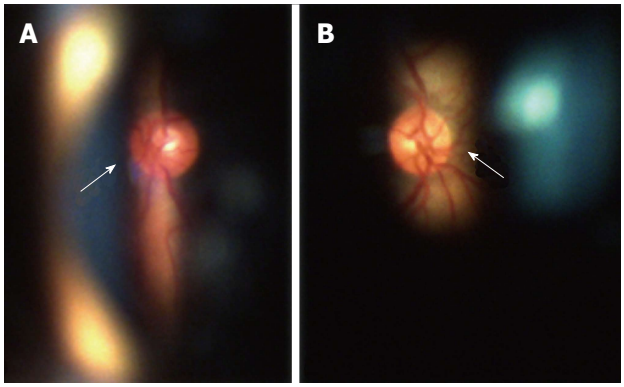


Figure 2 Fundoscopy of right (A) and left (B) eyes showed normal-appearing optic discs (arrows) and absence of abnormalities in posterior pole and peripheral retina. Ocular fundus was perfectly visible due to mild amyloid deposition in the vitreous.

DISCUSSION

Although ATTR levels after LT decline to < 1% of pre-transplant levels, FAP patients are still at risk of

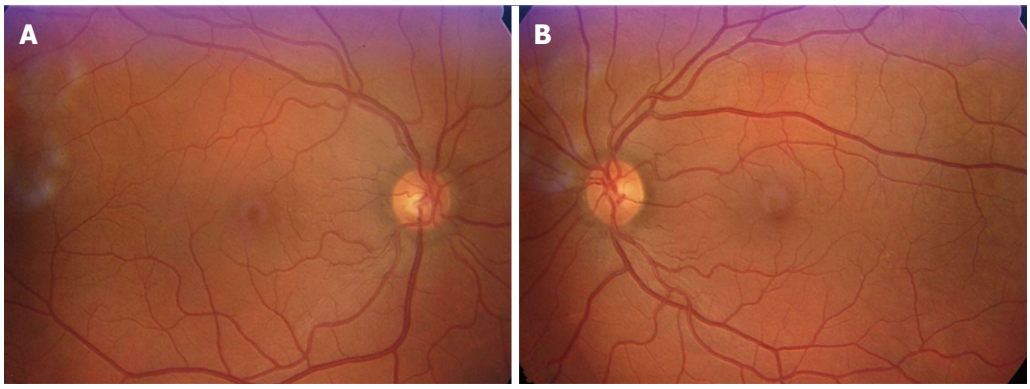


Figure 3 Retinography of right eye (A) and left eye (B) showed normal posterior poles, which were clearly visible due to the mild amyloid deposition in the vitreous, with only few opacities, which did not compromise visual acuity.

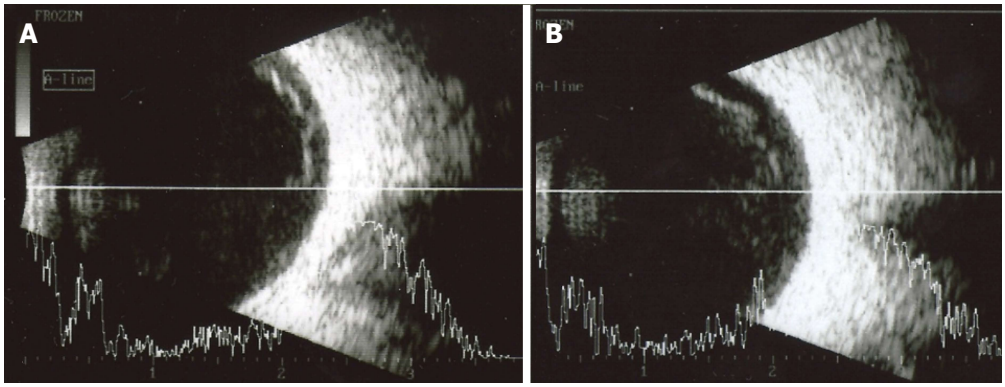


Figure 4 Ophthalmic ultrasound of right (A) and left (B) eyes showing some vitreous opacities corresponding to amyloid deposits in the vitreous. This amyloid deposition in the vitreous is relatively mild compared to the degree of amyloid deposition in anterior segment.

ophthalmological complications of the disease because of continued intra-ocular production in RPE and CPE and continued amyloid deposition in various ocular tissues, such as vitreous, pupil, anterior lens capsule and trabecular meshwork[1,3,6,7,10,12-18].

LT improved survival and consequently there is increased risk of ocular complications of FAP in transplantation era, because ocular manifestations are dependent of the duration of systemic disease. Glaucoma is a major ocular complication of FAP and a major cause of visual loss in these patients[1,3,19,20].

The study of Kimura *et al*[1] reported glaucoma in 24% of all FAP patients and in 17% of patients with Val30Met mutation, but the prevalence of glaucoma differs in various studies, from 5.4% to 27%[1,20,21]. Glaucoma is secondary to amyloid deposition in trabecular meshwork and if not recognized or treated adequately can have a rapid progression and devastating visual consequences in these patients, who have already a great morbidity from the systemic disease[1,3,19,20]. The pathophysiology of glaucoma in FAP1 after LT is related to the deposition of amyloid fibrillar aggregates in intertrabecular spaces of corneoscleral and uveoscleral meshworks and degeneration of endothelium cells of trabecular meshwork[6]. The trabecular outflow resistance increases, which raises IOP. Perivascular deposition of amyloid in conjunctival and scleral tissues can increase episcleral pressure and, consequently, the outflow resistance, but this mechanism is mainly dependent on systemic production of ATTR, playing an important role only before LT[6].

Most of the ophthalmological studies of FAP are focused in vitreous opacities (VO) and there are only few studies about secondary glaucoma. In the study of Kimura *et al*[1], VO were found in 35% of patients and amyloid deposition in pupil and anterior lens capsule in 31% of patients. Scalloped pupils are caused by autonomic abnormalities, which are associated with a higher degree of amyloid deposition in anterior segment and can also predict glaucoma[3]. They occur in 8% of FAP patients and glaucoma in 20% of patients. Glaucoma was diagnosed in all cases (100%) with scalloped pupils and in 57% of cases with amyloid deposition in anterior segment (pupil and anterior lens capsule). Only 49% of cases with VO had glaucoma[1]. Vitreous opacities are a classic ocular manifestation of FAP, but accordingly to the studies of Kimura *et al*[1] and Sandgren *et al*[3], the association between VO and glaucoma is weaker than between glaucoma and pupillary abnormalities (scalloped pupils, ATTR deposition in pupillary margin)[1,3]. This finding is supported by our clinical case.

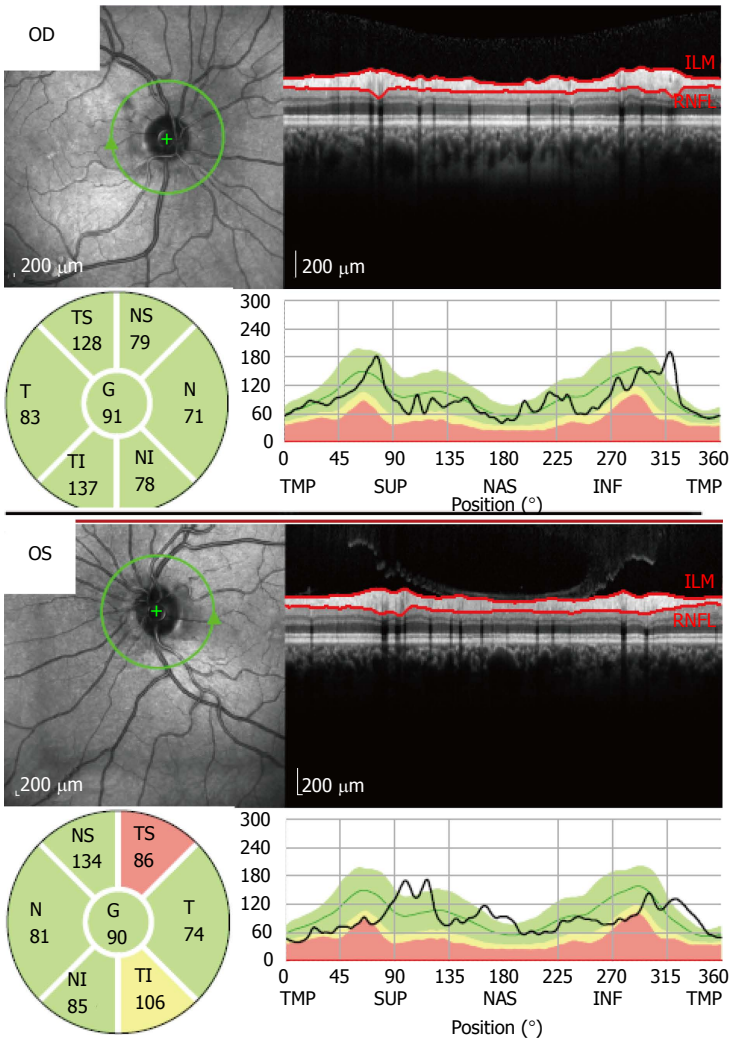


Figure 5 Optical coherence tomography showed a localized defect in the temporal-superior area of the peripapillary retinal nerve fiber layer of the left eye (OS). In the right eye (OD), the retinal nerve fiber layer thickness was normal in all peripapillary locations.

Sandgren *et al*[3,7] suggested that amyloid deposits in pupil and anterior lens capsule are more precocious than in the vitreous, which can explain the existence of rare cases, such as our case, which have much more ATTR deposition in anterior segment than in vitreous. These rare cases, such as our clinical case, corroborate the hypothesis raised by the study from Kawaji *et al*[12] postulating that the ATTR accumulated in anterior segment may have origin in CPE. This hypothesis can explain this asymmetry between ATTR deposition in anterior and posterior segments, as occurred our clinical case[12].

Amyloid is transported in the aqueous. Thus, pupillary amyloid deposits are an indirect sign of exuberant amyloid deposition in anterior segment, including the trabecular meshwork. This results in an increased resistance to aqueous humor outflow[19]. Kimura *et al*[1] have found that pupillary amyloid deposits have preceded the diagnosis of glaucoma by an average period of 2.55 ± 1.43 years (range 0.2-4.0 years). In the presented clinical case, the recognition of the pupillary abnormalities raised the clinical suspicion of glaucoma that was confirmed by appropriate investigation. Preperimetric glaucoma was confirmed by the finding of a localized defect of nerve fiber layer without perimetric functional repercussion.

Most cases of glaucoma secondary to FAP are usually refractory to medical treatment and have a fast progression and bad prognosis. This type of glaucoma usually requires surgical treatment[8]. Tube shunts, specially the Ahmed valve have been extensively used for surgical treatment of FAP1-related glaucoma in Portugal[8]. Recently minimal invasive options for glaucoma treatment are available for primary open-angle glaucoma and some types of secondary glaucoma, having the advantage of being less traumatic to the eye. However, prospective studies of efficacy in FAP-related glaucoma are lacking. Our clinical case had an unusual clinical course, with a good IOP control with medical treatment and stable visual fields and RNFL thicknesses.

Pars plana vitrectomy can be performed if vitreous opacities impair visual acuity, but this was not the case of our patient. Also, glaucoma can occur or be aggravated after pars plana vitrectomy in FAP patients, which is an important aspect to consider when managing ocular manifestations of FAP patients also affected by secondary glaucoma.

In an era that FAP patients have a greater life expectance with liver transplant, there is an increased probability of serious ocular disease caused by FAP, such as glaucoma that requires a regular ophthalmologic follow-up.

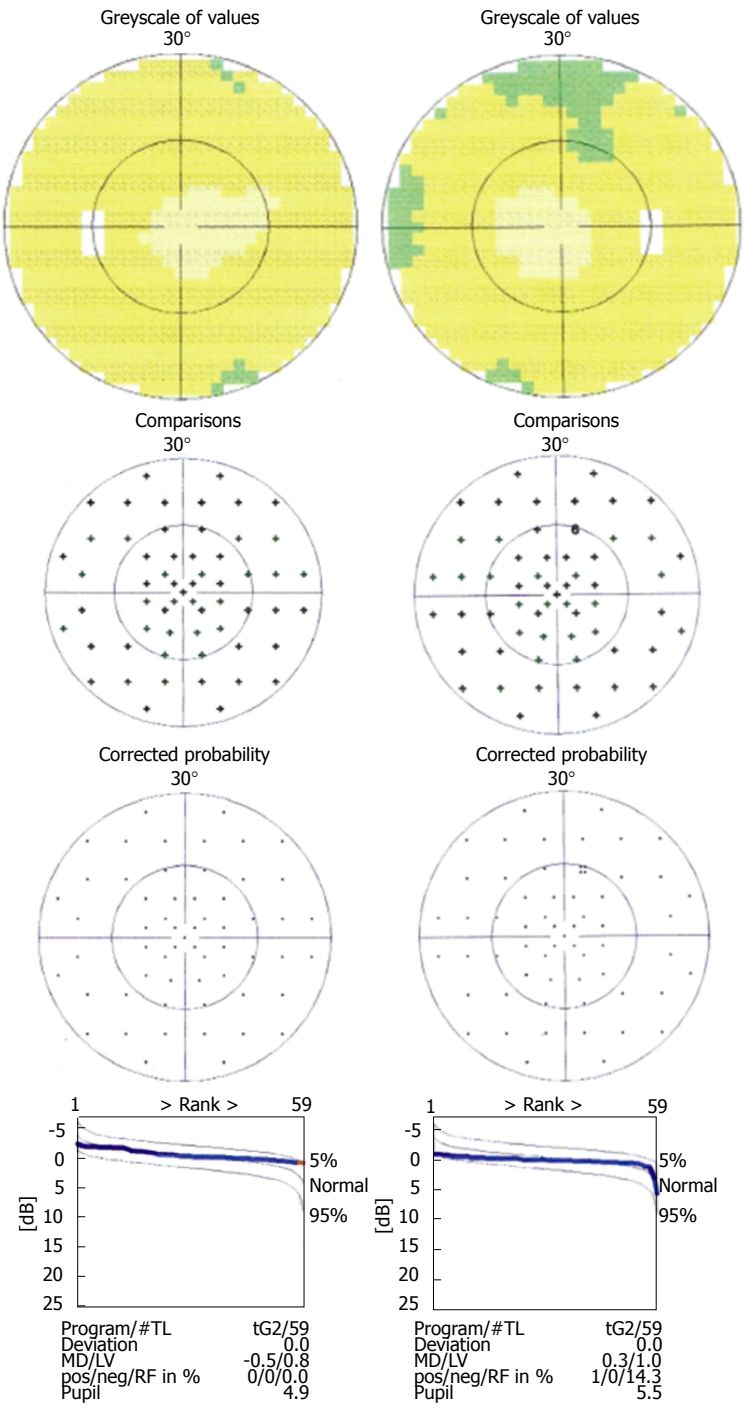


Figure 6 Computerized static perimetry of right eye (at the right) and left eye (at the left) (tendency-oriented perimetry, TOP - 30º program, Octopus 101 perimeter, Haag-Streit Diagnostics, Switzerland) in November 2011, showed the absence of clinically significant abnormalities in the visual fields - preperimetic glaucoma.

COMMENTS

Case characteristics

A 41-year-old man with type 1 familiar amyloid polyneuropathy (FAP) subjected to liver transplantation in 1997, presented with ocular pain.

Clinical diagnosis

Ophthalmological examination showed ocular hypertension, scalloped pupils associated to exuberant amyloid pupillary deposits, which contrasted with the mild vitreous opacities on ultrasound.

Differential diagnosis

FAP-related secondary open-angle glaucoma, FAP-related secondary ocular hypertension, pseudoexfoliation glaucoma, ocular hypertension associated to pseudoexfoliation syndrome.

Imaging diagnosis

Ocular ultrasound showed mild vitreous opacities due to amyloid deposition. Retinography showed normal posterior poles. Optical coherence tomography only showed a peripapillary temporal-superior retinal nerve fiber layer defect in OS. Perimetry did not show significant visual field abnormalities.

Treatment

Treatment with topical timolol and brimonidine achieved intraocular pressure (IOP) control. This treatment was continued, permitting disease stabilization with IOP control. This is a rare clinical course of this disease.

Related reports

De novo intraocular amyloid synthesis and deposition occurs after liver transplantation, having the potential to cause serious ocular complications. Most reported cases of FAP-related secondary glaucoma with scalloped

pupils have exuberant vitreous amyloid deposition. The asymmetry between exuberant amyloid deposition in anterior segment *vs* mild vitreous deposition that was reported in this clinical case is rare, and suggests a role of ciliary pigment epithelium in intraocular amyloid synthesis. This clinical case had a rare clinical course.

Term explanation

FAP-related glaucoma after liver transplantation is a secondary type of glaucoma, caused by an increase in trabecular outflow resistance associated to trabecular amyloid deposition, with amyloid fibrillar aggregates in intertrabecular spaces of corneoscleral and uveoscleral meshworks and degeneration of endothelium cells of trabecular meshwork.

Experiences and lessons

Rarely, amyloid deposition in anterior segment can be much more exuberant than vitreous deposition. This asymmetry supports a significant role of the ciliary pigmented epithelium in the intraocular amyloid synthesis in these cases. Pupillary amyloid deposition and scalloped pupils have a stronger correlation to glaucoma than other ocular manifestations. Rarely, FAP-related glaucoma can be stable and well controlled by medical treatment alone.

Peer-review

This case is very rare and an interesting case.

REFERENCES

1 **Kimura A**, Ando E, Fukushima M, Koga T, Hirata A, Arimura K, Ando Y, Negi A, Tanihara H. Secondary glaucoma in patients with familial amyloidotic polyneuropathy. *Arch Ophthalmol* 2003; **121**: 351-356 [PMID: 12617705 DOI: 10.1001/archopht.121.3.351]

2 **Rutar TRM**. Diseases of the Vitreous. In: Albert DM, Miller JW, Azar DT BB, ed. Albert & Jakobiec's Principles and Practice of Ophthalmology. 3rd ed. Philadelphia: W. B. Saunders Company, 2008: 2391-2398

3 **Sandgren O**, Kjellgren D, Suhr OB. Ocular manifestations in liver transplant recipients with familial amyloid polyneuropathy. *Acta Ophthalmol* 2008; **86**: 520-524 [PMID: 18435819 DOI: 10.1111/j.1600-0420.2007.01098.x]

4 **Andrade C**. A peculiar form of peripheral neuropathy; familiar atypical generalized amyloidosis with special involvement of the peripheral nerves. *Brain* 1952; **75**: 408-427 [PMID: 12978172 DOI: 10.1093/brain/75.3.408]

5 **Lessell S**, Wolf PA, Benson MD, Cohen AS. Scalloped pupils in familial amyloidosis. *N Engl J Med* 1975; **293**: 914-915 [PMID: 1177989 DOI: 10.1056/NEJM197510302931808]

6 **Rosa AM**, Quadrado MJ, Ferrão J, Manifesta ções Oculares de Polineuropatia Amiloidótica Familiar Tipo I em Doentes Submetidos a Transplante Hepático. *Oftalmol* (Port Ophthalmol Soc Journal) 2009; **33**: 177-183

7 **Sandgren O**. Ocular amyloidosis, with special reference to the hereditary forms with vitreous involvement. *Surv Ophthalmol* 1995; **40**: 173-196 [PMID: 8599154 DOI: 10.1016/S0039-6257(95)80025-5]

8 **Sampaio I**, Queirós J, Borges P, Reimão P, Beirão M. MMAC. Glaucoma em Doentes Portugueses com Polineuropatia Amiloidótica Familiar. *Oftalmol* (Port Ophthalmol Soc Journal) 2011; **35**: 311-318

9 **Doft BH**, Machemer R, Skinner M, Buettner H, Clarkson J, Crock J, McLeod D, Michels R, Scott J, Wilson D. Pars plana vitrectomy for vitreous amyloidosis. *Ophthalmology* 1987; **94**: 607-611 [PMID: 3627709 DOI: 10.1016/S0161-6420(87)33402-5]

10 **Cavallaro T**, Martone RL, Dwork AJ, Schon EA, Herbert J. The retinal pigment epithelium is the unique site of transthyretin synthesis in the rat eye. *Invest Ophthalmol Vis Sci* 1990; **31**: 497-501 [PMID: 1690688]

11 **Futa R**, Inada K, Nakashima H, Baba H, Kojima Y, Okamura R, Araki S. Familial amyloidotic polyneuropathy: ocular manifestations with clinicopathological observation. *Jpn J Ophthalmol* 1984; **28**: 289-298 [PMID: 6098757]

12 **Kawaji T**, Ando Y, Nakamura M, Yamamoto K, Ando E, Takano A, Inomata Y, Hirata A, Tanihara H. Transthyretin synthesis in rabbit ciliary pigment epithelium. *Exp Eye Res* 2005; **81**: 306-312 [PMID: 16129098 DOI: 10.1016/j.exer.2005.02.003]

13 **Munar-Qués M**, Salva-Ladaria L, Mulet-Perera P, Solé M, López-Andreu FR, Saraiva MJ. Vitreous amyloidosis after liver transplantation in patients with familial amyloid polyneuropathy: ocular synthesis of mutant transthyretin. *Amyloid* 2000; **7**: 266-269 [PMID: 11132095 DOI: 10.3109/13506120009146440]

14 **Haraoka K**, Ando Y, Ando E, Sun X, Nakamura M, Terazaki H, Misumi S, Tanoue Y, Tajiri T, Shoji S, Ishizaki T, Okabe H, Tanihara H. Presence of variant transthyretin in aqueous humor of a patient with familial amyloidotic polyneuropathy after liver transplantation. *Amyloid* 2002; **9**: 247-251 [PMID: 12557753 DOI: 10.3109/13506120209114101]

15 **Ando E**, Ando Y, Haraoka K. Ocular amyloid involvement after liver transplantation for polyneuropathy. *Ann Intern Med* 2001; **135**: 931-932 [PMID: 11712896 DOI: 10.7326/0003-4819-135-10-200111200-00025]

16 **Ando Y**, Ando E, Tanaka Y, Yamashita T, Tashima K, Suga M, Uchino M, Negi A, Ando M. De novo amyloid synthesis in ocular tissue in familial amyloidotic polyneuropathy after liver transplantation. *Transplantation* 1996; **62**: 1037-1038 [PMID: 8878404]

17 **Hara R**, Kawaji T, Ando E, Ohya Y, Ando Y, Tanihara H. Impact of liver transplantation on transthyretin-related ocular amyloidosis in Japanese patients. *Arch Ophthalmol* 2010; **128**: 206-210 [PMID: 20142544 DOI: 10.1001/archophthalmol.2009.390]

18 **Neto E**, Ferreira A, Almeida L, Pinto F GM. Paramiloidose ocular após transplante hepático. *Oftalmol* (Port Ophthalmol Soc Journal) 2009; **33**: 51-56

19 **Silva-Araújo AC**, Tavares MA, Cotta JS, Castro-Correia JF. Aqueous outflow system in familial amyloidotic polyneuropathy, Portuguese type. *Graefes Arch Clin Exp Ophthalmol* 1993; **231**: 131-135 [PMID: 8385054]

20 **Tsukahara S**, Matsuo T. Secondary glaucoma accompanied with primary familial amyloidosis. *Ophthalmologica* 1977; **175**: 250-262 [PMID: 896156]

21 **Ando E**, Ando Y, Okamura R, Uchino M, Ando M, Negi A. Ocular manifestations of familial amyloidotic polyneuropathy type I: long-term follow up. *Br J Ophthalmol* 1997; **81**: 295-298 [PMID: 9215058 DOI: 10.1136/bjo.81.4.295]

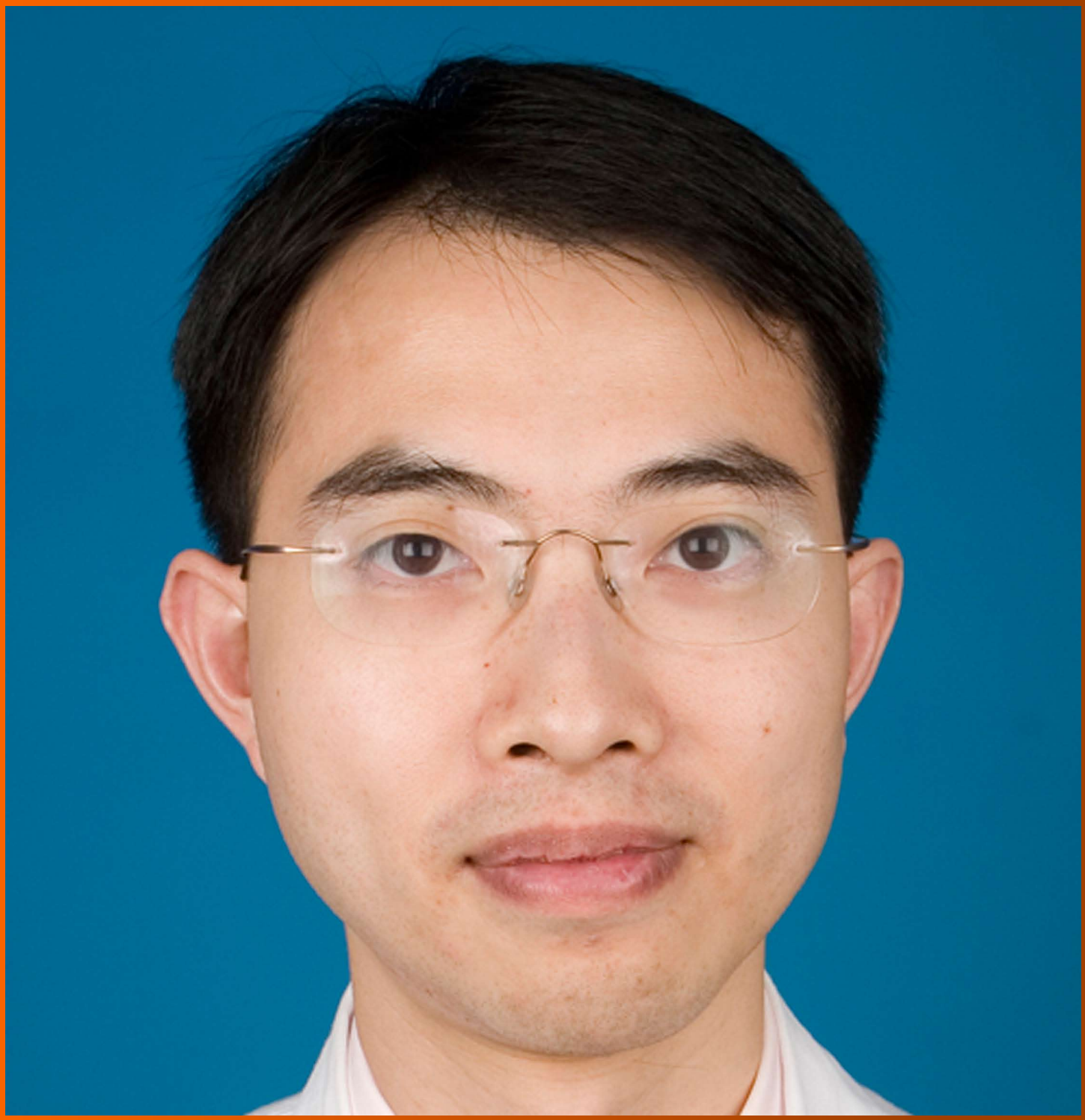
P- Reviewer: Hong YJ, Nowak MS **S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Lu YJ

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

ISSN 2220-3230 (online)

World Journal of *Transplantation*

World J Transplant 2017 October 24; 7(5): 250-275



Published by Baishideng Publishing Group Inc

WJT World Journal of Transplantation

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

Editorial Board

2016-2019

The *World Journal of Transplantation* Editorial Board consists of 361 members, representing a team of worldwide experts in transplantation. They are from 43 countries, including Argentina (1), Australia (7), Austria (3), Belgium (4), Brazil (7), Bulgaria (1), Canada (13), China (32), Cuba (1), Czech Republic (1), Denmark (1), Finland (1), France (4), Georgia (1), Germany (14), Greece (5), Hungary (2), India (7), Iran (7), Israel (4), Italy (33), Japan (18), Jordan (1), Macedonia (1), Mexico (2), Morocco (1), Netherlands (4), Nigeria (1), Norway (1), Pakistan (1), Poland (2), Qatar (1), Saudi Arabia (3), Singapore (1), South Korea (16), Spain (9), Sweden (1), Switzerland (3), Thailand (2), Tunisia (1), Turkey (6), United Kingdom (17), and United States (120).

WJT World Journal of Transplantation

Contents

Bimonthly Volume 7 Number 5 October 24, 2017

World Journal of Transplantation
Volume 7 Number 5 October 24, 2017

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Transplantation*, Daniel KL Cheuk, MD, Doctor, Department of Pediat and Adolescent Medical, University of Hong Kong, Hong Kong, China

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, PubMed Central.

FLYLEAF

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Ya-Jing Lu*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Li-Jun Cui*
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL
World Journal of Transplantation

ISSN
ISSN 2220-3230 (online)

LAUNCH DATE
December 24, 2011

FREQUENCY
Bimonthly

EDITORIAL OFFICE
Xiu-Xia Song, Director

World Journal of Transplantation
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
October 24, 2017

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 October 24; 7(5): 250-259

DOI: 10.5500/wjt.v7.i5.250

ISSN 2220-3230 (online)

REVIEW

Peripheral blood stem cell mobilization in multiple myeloma: Growth factors or chemotherapy?

Whitney D Wallis, Muzaffar H Qazilbash

Whitney D Wallis, Muzaffar H Qazilbash, the University of Texas MD Anderson Cancer Center, Houston, TX 77030, United States

ORCID number: Whitney D Wallis (0000-0002-6233-8811); Muzaffar H Qazilbash (0000-0003-2876-2886).

Author contributions: Authors contributed equally to this work.

Conflict-of-interest statement: Authors have no relevant conflicts of interest to disclose.

Manuscript source: Invited manuscript

Correspondence to: Muzaffar H Qazilbash, MD, the University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030,
United States. mqazilba@mdanderson.org
Telephone: +1-713-7922466
Fax: +1-713-7944902

Received: April 28, 2017
Peer-review started: April 28, 2017
First decision: June 16, 2017
Revised: August 25, 2017
Accepted: September 12, 2017
Article in press: September 13, 2017
Published online: October 24, 2017

Abstract

High-dose therapy followed by autologous hematopoietic stem cell (HSC) transplant is considered standard of care for eligible patients with multiple myeloma. The optimal collection strategy should be effective in procuring sufficient HSC while maintaining a low toxicity profile. Currently available mobilization strategies include growth factors alone, growth factors in combination with chemotherapy, or growth factors in combination with chemokine receptor antagonists; however, the optimal strategy has yet to be elucidated. Herein, we review the risks and benefits of each approach.

Key words: Multiple myeloma; Stem cell; Mobilization; Growth factors; Chemotherapy

Core tip: Obtaining an adequate peripheral blood stem cell yield is essential for the successful outcome of autologous hematopoietic stem cell transplant in multiple myeloma. While growth factor mobilization continues to be largely successful, suboptimal collection rates have been noted, particularly as use of novel therapies continue to increase. Chemomobilization remains toxic and has not been associated with better disease control. The newest mobilizing agent, plerixafor, is capable of overcoming suboptimal mobilization even in patients who are at a high risk of mobilization failure. Each mobilization strategy should be selected based on patient specific variables as well as risk factors for mobilization failure.

Wallis WD, Qazilbash MH. Peripheral blood stem cell mobilization in multiple myeloma: Growth factors or chemotherapy? *World J Transplant* 2017; 7(5): 250-259 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i5/250.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i5.250

INTRODUCTION

High-dose therapy followed by autologous hematopoietic

stem cell (HSC) transplant (auto-HCT) is considered standard of care for eligible patients with multiple myeloma (MM). MM remains the most common indication for auto-HCT, accounting for over 6000 transplants in the United States alone in 2013[1]. Auto-HCT has been shown to prolong progression-free survival and overall survival in patients with MM[2-4], a benefit that has been maintained even after the availability of immunomodulatory drugs such as thalidomide and lenalidomide[5,6], and proteasome inhibitors like bortezomib. Mobilization and collection of an optimal number of HSC is a fundamental requirement for auto-HCT. The optimal collection strategy should be effective in procuring sufficient HSC while maintaining a low toxicity profile. Currently available mobilization strategies include growth factors alone, growth factors in combination with chemotherapy, or growth factors in combination with chemokine receptor antagonists; however, the optimal strategy has yet to be elucidated. Herein, we review the data surrounding each approach.

SOURCE OF HSCs

Historically, bone marrow (BM) was used as the sole source of HSC for transplantation[7,8]. However, the ability to mobilize HSC to peripheral blood (PB) has eliminated the risk of general anesthesia, intubation, and painful aspirations associated with BM harvesting. Peripheral blood stem cell (PBSC) collection can be performed in the outpatient setting with a shorter recovery time. Additionally, use of PBSC reduces time to hematopoietic reconstitution, hospital stay, and need for transfusions[9-11]. Consequently, PB has largely replaced BM as the source of HSC for auto-HCT[12].

PBSC DOSE

The number of CD34 expressing mononuclear cells in PBSC collection correlates well with engraftment kinetics and thus is used as a surrogate marker of HSC[13-16] (Figure 1). A dose of > 2 million CD34$^+$ cells per kilogram (cells/kg) is considered the minimum acceptable dose for timely engraftment[17]. However, larger cell doses have been associated with a more rapid time to platelet and neutrophil recovery[18,19] and therefore ⩾ 3-5 million CD34 cells/kg is considered an optimal target[20,21].

PBSC MOBILIZATION APPROACHES

HSC primarily reside in the BM and account for 1%-4% of all mononuclear cells[13,15,22]. Retention of HSC in the BM is dependent on interactions between cell adhesion molecules on the surface of HSC, such as chemokine receptor 4 and very late antigen 4 (VLA4), and BM stromal factors, such as vascular cell adhesion molecule (VCAM-1) and stromal cell-derived factor-1 (SDF-1)[23]. Mobilization of HSC from BM to PB is the result of induced chemical disruption of these interactions between HSC and BM stroma. Cytokines,

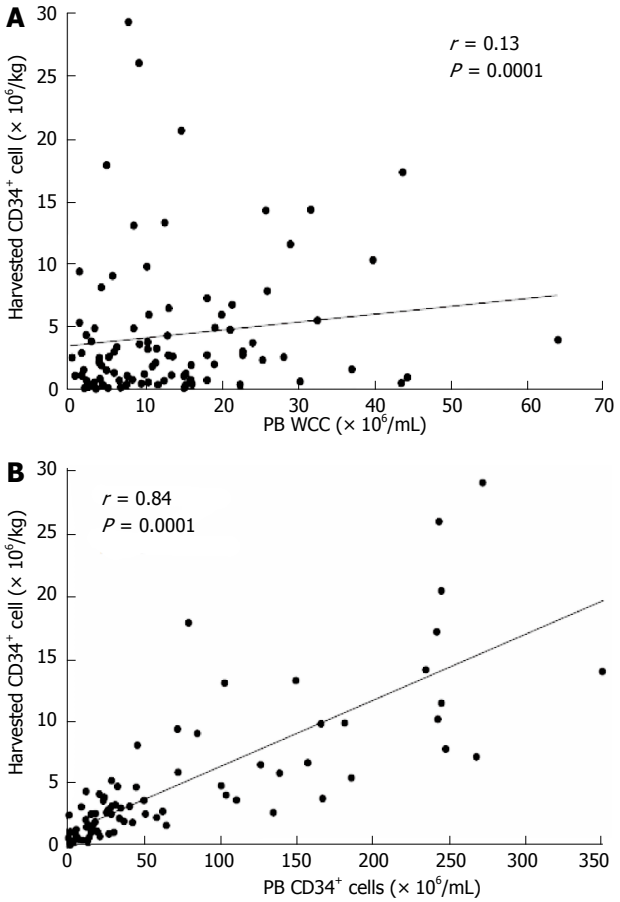


Figure 1 Correlation of harvested CD34$^+$ cells counts with white blood cell count and peripheral blood CD34$^+$ cell count. A: Correlation of harvested CD34$^+$ cells counts with white blood cell count; B: Correlation of harvested CD34$^+$ cells counts with peripheral blood CD34$^+$ cell count. Reprinted by permission from Macmillan Publishers Ltd: *Bone Marrow Transplant* 1997[16]. http://www.nature.com/bmt/index.html.

such as granulocyte-colony stimulating factor (G-CSF), and chemotherapy drugs like cyclophosphamide play an important role in releasing HSC from their niches in the BM[23-25] (Figure 2).

Growth factors alone

Historically, growth factors alone have been largely successful in mobilizing an adequate cell yield in MM patients undergoing auto-HCT[26,27] (Table 1). G-CSF has well established kinetics as well as favorable toxicity and cost profiles[28-30] but has been associated with suboptimal mobilization in over 20% of MM patients[31-33]. Data regarding a dose-response relationship between G-CSF and CD34$^+$ cell yield is discordant but doses up to 40 μg per kilogram per day (μg/kg per day) have been studied[34-36]. A widely accepted G-CSF dose for PBSC mobilization is 10 μg/kg per day as single or divided doses.

Other growth factors such as granulocyte-macrophage-colony stimulating factor (GM-CSF), pegylated G-CSF, and tbo G-CSF have also been studied for PBSC mobilization in MM patients[37-42]. When G-CSF was compared to GM-CSF in MM patients, CD34$^+$ cell yield was similar between

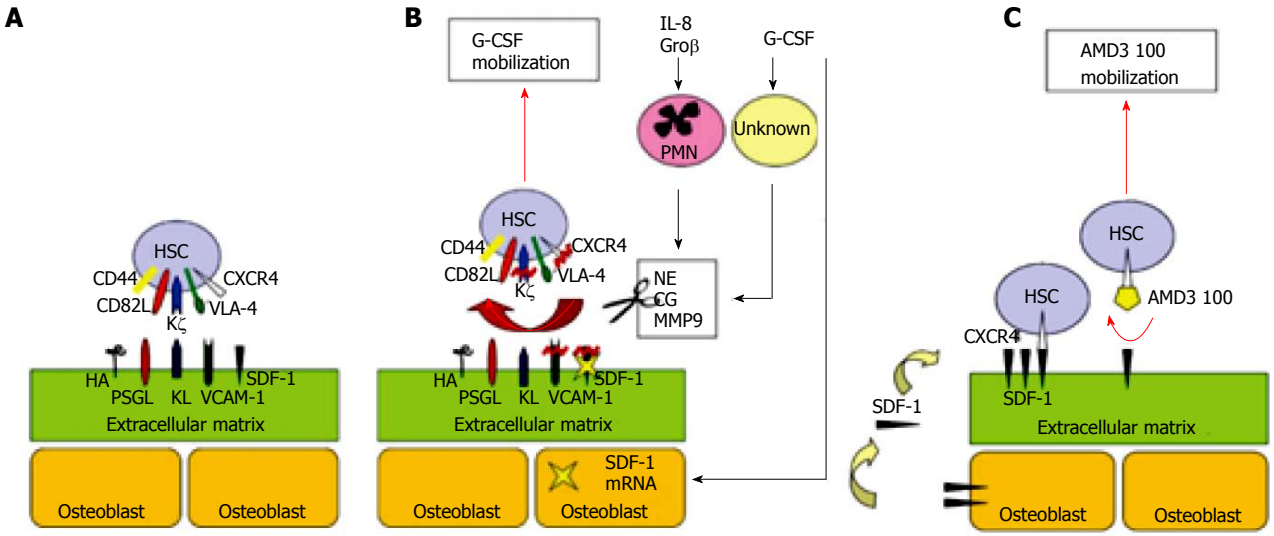


Figure 2 Bone marrow microenvironment (A) at physiologic state and effects of (B) granulocyte colony stimulating factor mobilization and (C) Plerixafor mobilization. Reprinted from *Journal of Cellular Biochemistry*, Vol 99/edition 3, Bruno Nervi, Dan C. Link, John F DiPersio, Cytokines and Hematopoietic Stem Cell Mobilization, 690-705, 2010, with permission from Wiley[26]. G-CSF: Granulocyte colony stimulating factor; HSC: Hematopoietic stem cell; SDF-1: Stromal cell-derived factor-1; VCAM-1: Vascular cell adhesion molecule.

Table 1 Growth factor mobilization

Ref.	Disease	Collection strategy	*n*	CD34$^+$ yield (× 10^{-6} cell/kg): Median (range)	Failure *n* (%)
Desikan *et al*[26]	MM	G-CSF 10-16 μg/kg per day	117	6.2 (0.6-34.1)	NR
Kröger *et al*[27]	MM	G-CSF 10-24 μg/kg per day	25	3.8 (0.3-17)	3 (12)
Popat *et al*[31]	MM	G-CSF	302	NR	9%
Pusic *et al*[90]	MM	G-CSF 10 μg/kg per day	384	4.6	24 (6.3)
	NHL HD	G + C	17	8.5	1 (5.9)
Weaver *et al*[34]	BC	G-CSF 10 μg/kg per day	14	0.6 (0.1-2.8)	NR
		G-CSF 20 μg/kg per day	13	1 (0.2-5.2)	
		G-CSF 30 μg/kg per day	14	2.4 (0.6-6.8)	
		G-CSF 40 μg/kg per day	14	1.4 (0.1-4.8)	
Weisdorf *et al*[42]	NHL	GM-CSF 250 μg/m^2 per day	16	4.78 (3.02-10.68)	0
	HD	G-CSF 250 μg/m^2 per day	15	8.01 (3.17-29)	0
Spitzer *et al*[41]	BC GCT	GCSF 10 mcg/kg per day	26	21.45 (1.63-182.91)	NR
	NHL HD	GCSF 10 mcg/kg per day +	24	13.33 (0.56-102.08)	
	MM	GM-CSF 5 mcg/kg per day			
Hosing *et al*[39]	MM	PEG 12 mg × 1	19	8.4 (4.1-15.8)	0
		G-CSF 10 μg/kg per day	8	8.1 (5.17-19.2)	0

MM: Multiple myeloma; G-CSF: Granulocyte colony stimulating factor; NR: Not reported; BC: Breast cancer; NHL: Non-hodgkin's lymphoma; GM-CSF: Granulocyte macrophage colony stimulating factor; HD: Hodgkin's disease; GCT: Germ cell tumor; PEG: Pegylated filgrastim.

the two groups, but GM-CSF-mobilized patients had a longer duration of neutropenia[43]. *In-vitro* data suggest that combination of G-CSF + GM-CSF may improve PBSC yield[44,45], but clinical trial data has not found a significant difference in CD34$^+$ cell yield or time to hematopoietic recovery with combination therapy[41].

Pegylated (PEG) filgrastim, a covalent conjugate of G-CSF and monomethoxy-polyethylene glycol, has a terminal half-life of 15-80 h, which enables less frequent administration compared to G-CSF. Given as a single 12 mg injection followed by PBSC collection, all MM patients who received PEG filgrastim successfully collected their target CD34$^+$ cells/kg dose[39]. Similarly, a multi-dose regimen of PEG filgrastim had a higher CD34$^+$ cells yield on first apheresis compared to G-CSF, but no differences in overall cell yield was observed[46]. Its convenient dosing schedule makes it an attractive option for PBSC mobilization.

Tbo-filgrastim is a non-glycosylated recombinant methionyl human G-CSF manufactured using the bacterium strain *E. coli* K802[47]. While not FDA approved for stem cell mobilization, retrospective data in MM patients found no difference in overall cell yield, number of apheresis sessions required for collection, nor need for rescue therapy with plerixafor[38,48].

Myelosuppressive chemotherapy

Transient circulation of PBSC occurs during the recovery phase of chemotherapy-induced pancytopenia[22,49,50] and is augmented by growth factor support[22] (Table 2). This

Table 2 Growth factors following chemotherapy

Ref.	Disease	Collection strategy	*n*	CD34$^+$ yield (× 10^{-6} cell/kg): Median (range)	Failure rates *n* (%)
Weaver *et al*[91]	MM ML	G-CSF 6 μg/kg per day	49	12 (0.1-54)	2 (4.1)
	BC	GM-CSF 250 μg/m^2 per day	49	5.4 (0.02-64)	4 (8.2)
		GM-CSF × 5 d then G-CSF 6 μg/kg per day	52	10.5 (0.4-96)	1 (1.9)
Arora *et al*[43]	MM	G-CSF 250 μg/m^2 per day	35	16.4 (1.1-71.7)	NR
		GM-CSF 250 μg/m^2 per day	37	12.8 (0.4-94.5)	
Tricot *et al*[46]	MM	PEG 6 mg q7d × 2	97	NR; no difference	NR
		G-CSF 10 μg/kg per day	140		
Fruehauf *et al*[92]	MM	PEG 12 mg × 1	26	9.7 (4.9-40.5)	3 (11.5)
Steidl *et al*[93]	MM	PEG 12 mg × 1	12	7.4 (4.9-38)	0
		G-CSF 8.5 μg/kg per day	12	10.8 (5-87)	0

MM: Multiple myeloma; ML: Malignant lymphoma; BC: Breast cancer; G-CSF: Granulocyte colony stimulating factor; GM-CSF: Granulocyte macrophage colony stimulating factor; NR: Not reported; NHL: Non-hodgkin's lymphoma; PEG: Pegylated filgrastim.

Table 3 Impact of chemotherapy on cell yield and morbidity

Ref.	Collection strategy	*n*	CD34$^+$ yield (× 10^{-6} cell/kg): median (range)	Hospital days: median (range)	Infection (%)	Transfusions (%) platelet/PRBC
Desikan *et al*[32]	CY 6 g/m^2 + G-CSF 6 μg/kg per day	22	33.4 (NR)	No difference	18	86/86
	G-CSF 16 μg/kg per day	22	5.8 (NR)		0	18/55
Alegre *et al*[51]	CY 4 g/m^2 + GM-CSF	18	6.8 (1.8-34.8)	21 (16-34)	11	33.3/27.7
	G-CSF 10 μg/kg per day	22	4.85 (2.1-10.05)	0	0	0/0
Fitoussi *et al*[60]	CY 7 g/m^2 + HGF	74	8.6 (0.4-166)	15 (9-34)	17.6	75.7/94.6
	CY 4 g/m^2 + HGF	42	13.4 (0.7-66.8)	22 (13-55)	16.7	26.2/52.4
Jantunen *et al*[61]	CY 4 g/m^2 + G-CSF 5-10 μg/kg per day	32	4.9 (0.8-47.4)[1]	9 (6-14)	NR	34/53
	CY 1.2-2 g/m^2 + G-CSF 5 μg/kg per day	42	5.6 (0.9-19)[1]	5 (3-12)	NR	0/28
Gojo *et al*[65]	CY 4.5 g/m^2 + G-CSF	28	21.38 (0-106.8)	8 (4-24)	25	57/NR
	CY 4.5 g/m^2 + VP-16 + G-CSF	49	22.39 (0-114.71)	7 (3-68)	53	67/NR
Hamadani *et al*[94]	CY 3-4 g/m^2 + G-CSF	55	16.6 (2-82)	4 (1-9)	NR	21.8/34.5
	CY 1.5 g/m^2 + G-CSF	68	7.5 (0-18)	3 (1-5)	NR	2.9/8.8
Hiwase *et al*[95]	CY 3-4 g/m^2 + G-CSF	26	7.71	7 (3-22)	19	No difference
	CY 1-2 2 g/m^2 + G-CSF	61	5.17	6 (3-18)	5	

[1]1st apheresis session. PRBC: Packed red blood cells; CY: Cyclophosphamide; G-CSF: Granulocyte colony stimulating factor; NR: Not reported; HGF: Hematopoietic growth factor; VP-16: Etoposide.

process, chemomobilization (CM), provides not only higher cell yields than G-CSF alone, but also affords anti-myeloma activity[32,51-54]. Cyclophosphamide (CY) 2-4 g/m^2, either alone or in combination with other chemotherapeutic agents, is commonly used in CM and has been a successful mobilization technique even in patients who underwent induction therapy with novel agents[31,55-59]. The impact of increased doses of CY on PBSC yields has shown conflicting results but was consistently associated with a longer duration of neutropenia as well as the use of antibiotics and blood products[54,60-64]. No additional impact on cell yield or objective response rate has been seen with the use of combination chemotherapy followed by growth factor[55,65] (Table 3). Furthermore, despite the potential benefit of cytoreduction, CM has not been associated with a better disease control or survival in MM[32,51,52,66-68].

Chemokine receptor antagonist

The newest mobilizing agent, plerixafor, rapidly and reversibly inhibits chemokine receptor CXCR4 on HSC, thereby preventing the binding of SDF-1a to CXCR4. Synergistic effect on PBSC mobilization is observed when plerixafor is given in combination with G-CSF[69,70]. A phase Ⅲ randomized, placebo controlled trial in MM patients compared mobilization with plerixafor + G-CSF to placebo + G-CSF. Use of plerixafor resulted in an increase in proportion of patients that were able to collect a cell yield of ⩾ 6 × 10^6/kg with fewer apheresis procedures compared to the G-CSF only group. Transplant outcomes were similar between groups[71]. Plerixafor can overcome suboptimal mobilization seen with prolonged prior lenalidomide therapy and other conventional chemotherapy agents[72,73]. Following failed attempts to mobilize, MM patients received a combination of G-CSF and plerixafor. In this population, at least 70% of patients were able to achieve a sufficient PBSC yield, without any evidence of tumor mobilization[73,74]. Plerixafor is successful when used as the initial mobilization strategy but at an increased drug acquisition cost and in patients that presumably could have attained an appropriate cell yield with G-CSF alone[75,76].

Risk adaptive strategies use initial mobilization with G-CSF alone and utilize plerixafor only in patients whose

Table 4 International Myeloma Working Group Consensus guidelines and recommendations on mobilization in malignant lymphoma[20]

Strategy	Recommendations
Mobilization	
G-CSF alone	Limit use to patients Treated with ≤ 1 line of therapy Never exposed to melphalan Received ≤ 4 cycles of lenalidomide Use doses from 10-16 μg/kg per day Monitor PB CD34$^+$ count
Chemomobilization + G-CSF	Limit to patients who have not adequately responded to salvage therapy
Plerixafor	Suitable for all patients particularly if goals include Highest cell yield obtainable Fewer apheresis sessions
Remobilization	
Plerixafor	P + G-CSF or P + CM + G-CSF
Chemomobilization	Acceptable in patients who failed cytokine mobilization
Bone marrow harvest	Use as third-line option in patients in whom ASCT is compelling

PBCD34$^+$: Peripheral blood CD34$^+$ cells; P + G-CSF: Plerixafor + granulocyte colony stimulating factor; P + CM + G-CSF: Plerixafor + chemomobilization + granulocyte colony stimulating factor.

Table 5 Advantages and disadvantages of mobilization strategies

Mobilization strategy	Advantages	Disadvantages
Growth factor	Cost effective Successful mobilization in most patients Predictable schedule	No anti-myeloma effect Multiple injections and collections Potential sub-optimal yield
CM	Anti-myeloma effect Increased cell yield Fewer apheresis sessions	Cytopenias Infection risk Hospital admission Potential transfusion requirement Unpredictable count recovery
Plerixafor	Rapid kinetics Increased cell yield Fewer apheresis sessions	Higher drug cost

CM: Chemomobilization.

PB CD34$^+$ count on day 4 is less than a predetermined threshold (10 × 10^6/L-10 × 10^9/L). Such strategies are associated with fewer mobilization failures when compared to traditional mobilization methods and appear to be cost effective[76-79]. Additional methods of cost reduction, namely the use of tbo-filgrastim, in combination with plerixafor has been studied. Prospective data in MM patients found similar overall cell yields without any mobilization failures[80].

PREDICTORS OF SUBOPTIMAL MOBILIZATION

Mobilization failure is generally defined as the inability to procure 2 × 10^6 CD34$^+$ cells/kg in 4 apheresis sessions. Despite recent advances in PBSC collection strategies, failure to obtain an adequate cell dose continues to delay and preclude auto-HCT in otherwise suitable transplant candidates. Factors associated with inadequate HSC mobilization in MM patients include: Thrombocytopenia[81], age > 60 years[36,58,82], extensive treatment course[17], prior radiotherapy, prior exposure to alkylating agents[17,83], and prolonged use of lenalidomide[20,21,31,84,85]. Such factors have been incorporated in consensus guidelines on stem cell mobilization (Table 4).

Lenalidomide's impact on cell yield is of particular concern due to its common use in frontline therapy[86]. While known to cause neutropenia and thrombocytopenia, the exact mechanism of lenalidomide induced myelosuppression is not fully known. In one study, lenalidomide was associated with a significant decrease in expression of transcription factor PU. 1, which is critical for myeloid maturation[87]. In another study, lenalidomide-treated patients were found to have decreased BM CD34$^+$ cells after six cycles of therapy[88]. This supports the literature that identifies lenalidomide as a risk factor for suboptimal stem cell collection and suggests that transplant eligible patients receiving lenalidomide should proceed to mobilization as early as feasible.

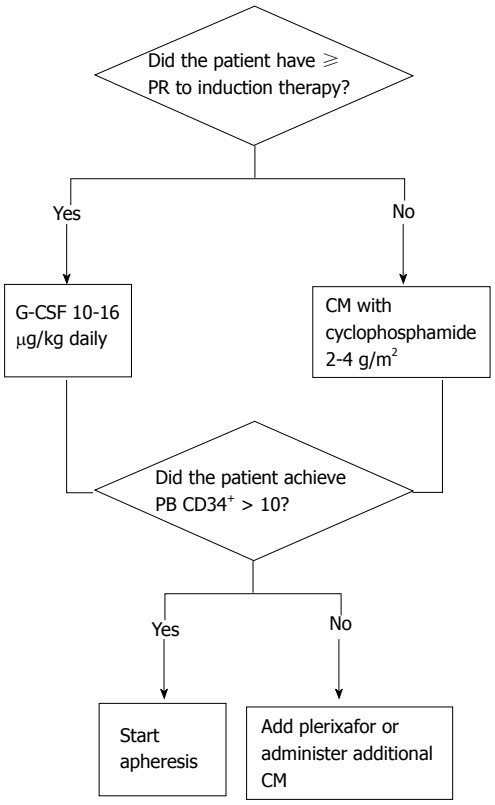


Figure 3 Mobilization strategies at authors' institution. CM: Chemomobilization; G-CSF: Granulocyte colony stimulating factor.

Despite identification of risk factors for poor mobilization, predictive algorithms have not correctly identified poor mobilizers[89]. The best predictor of adequate $CD34^+$ cell collection is the pre-collection PB $CD34^+$ cell count. A strong correlation exists with PB $CD34^+$ cell count and the final $CD34^+$ cell collection (Figure 1). PB $CD34^+$ count ⩾ 20×10^3 $CD34^+$ cells/mL was associated with an adequate HSC collection in 94% of patients[16,90].

CONCLUSION

In summary, obtaining an adequate PBSC yield is essential for the successful outcome of auto-HCT in MM. Each mobilization strategy reviewed here has its own advantages and disadvantages (Table 5) and should be selected based on patient specific variables. Current practice at the authors' institution is detailed in Figure 3; however, practitioners should be cognizant of risk factors for mobilization failure and utilize appropriate algorithms to optimize stem cell collection.

REFERENCES

1 **Pasquini MC**, Zhu X. Current uses and outcomes of hematopoietic stem cell transplantation. 2014 CIBMTR Summary Slides. [accessed 2016 June 1]. Available from: URL: http://www.cibmtr.org

2 **Attal M**, Harousseau JL, Stoppa AM, Sotto JJ, Fuzibet JG, Rossi JF, Casassus P, Maisonneuve H, Facon T, Ifrah N, Payen C, Bataille R. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Français du Myélome. *N Engl J Med* 1996; **335**: 91-97 [PMID: 8649495 DOI: 10.1056/NEJM199607113350204]

3 **Child JA**, Morgan GJ, Davies FE, Owen RG, Bell SE, Hawkins K, Brown J, Drayson MT, Selby PJ; Medical Research Council Adult Leukaemia Working Party. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. *N Engl J Med* 2003; **348**: 1875-1883 [PMID: 12736280 DOI: 10.1056/NEJMoa022340]

4 **Palumbo A**, Bringhen S, Petrucci MT, Musto P, Rossini F, Nunzi M, Lauta VM, Bergonzi C, Barbui A, Caravita T, Capaldi A, Pregno P, Guglielmelli T, Grasso M, Callea V, Bertola A, Cavallo F, Falco P, Rus C, Massaia M, Mandelli F, Carella AM, Pogliani E, Liberati AM, Dammacco F, Ciccone G, Boccadoro M. Intermediate-dose melphalan improves survival of myeloma patients aged 50 to 70: results of a randomized controlled trial. *Blood* 2004; **104**: 3052-3057 [PMID: 15265788 DOI: 10.1182/blood-2004-02-0408]

5 **Palumbo A**, Cavallo F, Gay F, Di Raimondo F, Ben Yehuda D, Petrucci MT, Pezzatti S, Caravita T, Cerrato C, Ribakovsky E, Genuardi M, Cafro A, Marcatti M, Catalano L, Offidani M, Carella AM, Zamagni E, Patriarca F, Musto P, Evangelista A, Ciccone G, Omedé P, Crippa C, Corradini P, Nagler A, Boccadoro M, Cavo M. Autologous transplantation and maintenance therapy in multiple myeloma. *N Engl J Med* 2014; **371**: 895-905 [PMID: 25184862 DOI: 10.1056/NEJMoa1402888]

6 **Gay F**, Oliva S, Petrucci MT, Conticello C, Catalano L, Corradini P, Siniscalchi A, Magarotto V, Pour L, Carella A, Malfitano A, Petrò D, Evangelista A, Spada S, Pescosta N, Omedè P, Campbell P, Liberati AM, Offidani M, Ria R, Pulini S, Patriarca F, Hajek R, Spencer A, Boccadoro M, Palumbo A. Chemotherapy plus lenalidomide versus autologous transplantation, followed by lenalidomide plus prednisone versus lenalidomide maintenance, in patients with multiple myeloma: a randomised, multicentre, phase 3 trial. *Lancet Oncol* 2015; **16**: 1617-1629 [PMID: 26596670 DOI: 10.1016/S1470-2045(15)00389-7]

7 **Körbling M**, Freireich EJ. Twenty-five years of peripheral blood stem cell transplantation. *Blood* 2011; **117**: 6411-6416 [PMID: 21460243 DOI: 10.1182/blood-2010-12-322214]

8 **Kessinger A**, Sharp JG. The whys and hows of hematopoietic progenitor and stem cell mobilization. *Bone Marrow Transplant* 2003; **31**: 319-329 [PMID: 12634722 DOI: 10.1038/sj.bmt.1703837]

9 **Beyer J**, Schwella N, Zingsem J, Strohscheer I, Schwaner I, Oettle H, Serke S, Huhn D, Stieger W. Hematopoietic rescue after high-dose chemotherapy using autologous peripheral-blood progenitor cells or bone marrow: a randomized comparison. *J Clin Oncol* 1995; **13**: 1328-1335 [PMID: 7538556 DOI: 10.1200/JCO.1995.13.6.1328]

10 **Henon PR**, Liang H, Beck-Wirth G, Eisenmann JC, Lepers M, Wunder E, Kandel G. Comparison of hematopoietic and immune recovery after autologous bone marrow or blood stem cell transplants. *Bone Marrow Transplant* 1992; **9**: 285-291 [PMID: 1376185]

11 **To LB**, Roberts MM, Haylock DN, Dyson PG, Branford AL, Thorp D, Ho JQ, Dart GW, Horvath N, Davy ML. Comparison of haematological recovery times and supportive care requirements of autologous recovery phase peripheral blood stem cell transplants, autologous bone marrow transplants and allogeneic bone marrow transplants. *Bone Marrow Transplant* 1992; **9**: 277-284 [PMID: 1350938]

12 **Pasquini MC**, Wang Z. Current use and outcome of hematopoietic stem cell transplantation: 2011 CIBMTR summary slides. [accessed 2016 June 1]. Available from: URL: http://www.cibmtr.org

13 **Andrews RG**, Singer JW, Bernstein ID. Monoclonal antibody 12-8 recognizes a 115-kd molecule present on both unipotent and multipotent hematopoietic colony-forming cells and their precursors. *Blood* 1986; **67**: 842-845 [PMID: 3947749]

14 **Berenson RJ**, Andrews RG, Bensinger WI, Kalamasz D, Knitter G, Buckner CD, Bernstein ID. Antigen CD34+ marrow cells engraft lethally irradiated baboons. *J Clin Invest* 1988; **81**: 951-955 [PMID: 2893812 DOI: 10.1172/JCI113409]

15 **Civin CI**, Strauss LC, Brovall C, Fackler MJ, Schwartz JF, Shaper JH. Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. *J Immunol* 1984; **133**: 157-165 [PMID: 6586833]

16 **Armitage S**, Hargreaves R, Samson D, Brennan M, Kanfer E, Navarrete C. CD34 counts to predict the adequate collection of peripheral blood progenitor cells. *Bone Marrow Transplant* 1997; **20**: 587-591 [PMID: 9337061 DOI: 10.1038/sj.bmt.1700938]

17 **Tricot G**, Jagannath S, Vesole D, Nelson J, Tindle S, Miller L, Cheson B, Crowley J, Barlogie B. Peripheral blood stem cell transplants for multiple myeloma: identification of favorable variables for rapid engraftment in 225 patients. *Blood* 1995; **85**: 588-596 [PMID: 7529066]

18 **Bensinger W**, Appelbaum F, Rowley S, Storb R, Sanders J, Lilleby K, Gooley T, Demirer T, Schiffman K, Weaver C. Factors that influence collection and engraftment of autologous peripheral-blood stem cells. *J Clin Oncol* 1995; **13**: 2547-2555 [PMID: 7595706 DOI: 10.1200/JCO.1995.13.10.2547]

19 **Weaver CH**, Hazelton B, Birch R, Palmer P, Allen C, Schwartzberg L, West W. An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. *Blood* 1995; **86**: 3961-3969 [PMID: 7579367]

20 **Giralt S**, Costa L, Schriber J, Dipersio J, Maziarz R, McCarty J, Shaughnessy P, Snyder E, Bensinger W, Copelan E, Hosing C, Negrin R, Petersen FB, Rondelli D, Soiffer R, Leather H, Pazzalia A, Devine S. Optimizing autologous stem cell mobilization strategies to improve patient outcomes: consensus guidelines and recommendations. *Biol Blood Marrow Transplant* 2014; **20**: 295-308 [PMID: 24141007 DOI: 10.1016/j.bbmt.2013.10.013]

21 **Giralt S**, Stadtmauer EA, Harousseau JL, Palumbo A, Bensinger W, Comenzo RL, Kumar S, Munshi NC, Dispenzieri A, Kyle R, Merlini G, San Miguel J, Ludwig H, Hajek R, Jagannath S, Blade J, Lonial S, Dimopoulos MA, Einsele H, Barlogie B, Anderson KC, Gertz M, Attal M, Tosi P, Sonneveld P, Boccadoro M, Morgan G, Sezer O, Mateos MV, Cavo M, Joshua D, Turesson I, Chen W, Shimizu K, Powles R, Richardson PG, Niesvizky R, Rajkumar SV, Durie BG; IMWG. International myeloma working group (IMWG) consensus statement and guidelines regarding the current status of stem cell collection and high-dose therapy for multiple myeloma and the role of plerixafor (AMD 3100). *Leukemia* 2009; **23**: 1904-1912 [PMID: 19554029 DOI: 10.1038/leu.2009.127]

22 **Siena S**, Bregni M, Brando B, Ravagnani F, Bonadonna G, Gianni AM. Circulation of CD34+ hematopoietic stem cells in the peripheral blood of high-dose cyclophosphamide-treated patients: enhancement by intravenous recombinant human granulocyte-macrophage colony-stimulating factor. *Blood* 1989; **74**: 1905-1914 [PMID: 2478216]

23 **Nervi B**, Link DC, DiPersio JF. Cytokines and hematopoietic stem cell mobilization. *J Cell Biochem* 2006; **99**: 690-705 [PMID: 16888804 DOI: 10.1002/jcb.21043]

24 **Liu F**, Poursine-Laurent J, Link DC. The granulocyte colony-stimulating factor receptor is required for the mobilization of murine hematopoietic progenitors into peripheral blood by cyclophosphamide or interleukin-8 but not flt-3 ligand. *Blood* 1997; **90**: 2522-2528 [PMID: 9326216]

25 **Semerad CL**, Christopher MJ, Liu F, Short B, Simmons PJ, Winkler I, Levesque JP, Chappel J, Ross FP, Link DC. G-CSF potently inhibits osteoblast activity and CXCL12 mRNA expression in the bone marrow. *Blood* 2005; **106**: 3020-3027 [PMID: 16037394 DOI: 10.1182/blood-2004-01-0272]

26 **Desikan KR**, Tricot G, Munshi NC, Anaissie E, Spoon D, Fassas A, Toor A, Zangari M, Badros A, Morris C, Vesole DH, Siegel D, Jagannath S, Barlogie B. Preceding chemotherapy, tumour load and age influence engraftment in multiple myeloma patients mobilized with granulocyte colony-stimulating factor alone. *Br J Haematol* 2001; **112**: 242-247 [PMID: 11167811]

27 **Kröger N**, Zeller W, Hassan HT, Krüger W, Renges H, Hummel K, Gutensohn K, Lölliger C, Zander AR. Successful mobilization of peripheral blood stem cells in heavily pretreated myeloma patients with G-CSF alone. *Ann Hematol* 1998; **76**: 257-262 [PMID: 9692813]

28 Neupogen® [package insert]. Thousand Oaks, CA. Amgen Pharmaceuticals Inc; 2016. [accessed 2016 June 1]. Available from: URL: http://pi.amgen.com/united_states/neupogen/neupogen_pi_hcp_english.pdf

29 **Grigg AP**, Roberts AW, Raunow H, Houghton S, Layton JE, Boyd AW, McGrath KM, Maher D. Optimizing dose and scheduling of filgrastim (granulocyte colony-stimulating factor) for mobilization and collection of peripheral blood progenitor cells in normal volunteers. *Blood* 1995; **86**: 4437-4445 [PMID: 8541532]

30 **Stroncek DF**, Clay ME, Petzoldt ML, Smith J, Jaszcz W, Oldham FB, McCullough J. Treatment of normal individuals with granulocyte-colony-stimulating factor: donor experiences and the effects on peripheral blood CD34+ cell counts and on the collection of peripheral blood stem cells. *Transfusion* 1996; **36**: 601-610 [PMID: 8701455]

31 **Popat U**, Saliba R, Thandi R, Hosing C, Qazilbash M, Anderlini P, Shpall E, McMannis J, Körbling M, Alousi A, Andersson B, Nieto Y, Kebriaei P, Khouri I, de Lima M, Weber D, Thomas S, Wang M, Jones R, Champlin R, Giralt S. Impairment of filgrastim-induced stem cell mobilization after prior lenalidomide in patients with multiple myeloma. *Biol Blood Marrow Transplant* 2009; **15**: 718-723 [PMID: 19450756 DOI: 10.1016/j.bbmt.2009.02.011]

32 **Desikan KR**, Barlogie B, Jagannath S, Vesole DH, Siegel D, Fassas A, Munshi N, Singhal S, Mehta J, Tindle S, Nelson J, Bracy D, Mattox S, Tricot G. Comparable engraftment kinetics following peripheral-blood stem-cell infusion mobilized with granulocyte colony-stimulating factor with or without cyclophosphamide in multiple myeloma. *J Clin Oncol* 1998; **16**: 1547-1553 [PMID: 9552064 DOI: 10.1200/JCO.1998.16.4.1547]

33 **Pozotrigo M**, Adel N, Landau H, Lesokhin A, Lendvai N, Chung DJ, Chimento D, Riedel E, Chen X, Reich L, Comenzo R, Giralt S, Hassoun H. Factors impacting stem cell mobilization failure rate and efficiency in multiple myeloma in the era of novel therapies: experience at Memorial Sloan Kettering Cancer Center. *Bone Marrow Transplant* 2013; **48**: 1033-1039 [PMID: 23334269 DOI: 10.1038/bmt.2012.281]

34 **Weaver CH**, Birch R, Greco FA, Schwartzberg L, McAneny B, Moore M, Oviatt D, Redmond J, George C, Alberico T, Johnson P, Buckner CD. Mobilization and harvesting of peripheral blood stem cells: randomized evaluations of different doses of filgrastim. *Br J Haematol* 1998; **100**: 338-347 [PMID: 9488624]

35 **Martínez C**, Urbano-Ispizua A, Marín P, Merino A, Rovira M, Carreras E, Montserrat E. Efficacy and toxicity of a high-dose G-CSF schedule for peripheral blood progenitor cell mobilization in healthy donors. *Bone Marrow Transplant* 1999; **24**: 1273-1278 [PMID: 10627634 DOI: 10.1038/sj.bmt.1702073]

36 **de la Rubia J**, Bladé J, Lahuerta JJ, Ribera JM, Martínez R, Alegre A, García-Laraña J, Fernández P, Sureda A, de Arriba F, Carrera D, Besalduch J, García Boyero R, Palomera Bernal L, Hernández MT, García PR, Pérez-Calvo J, Alcalá A, Casado LF, San Miguel J. Effect of chemotherapy with alkylating agents on the yield of CD34+ cells in patients with multiple myeloma. Results of the Spanish Myeloma Group (GEM) Study. *Haematologica* 2006; **91**: 621-627 [PMID: 16627253]

37 **Bruns I**, Steidl U, Kronenwett R, Fenk R, Graef T, Rohr UP, Neumann F, Fischer J, Scheid C, Hübel K, Haas R, Kobbe G. A single dose of 6 or 12 mg of pegfilgrastim for peripheral blood progenitor cell mobilization results in similar yields of CD34+ progenitors in patients with multiple myeloma. *Transfusion* 2006; **46**: 180-185 [PMID: 16441592 DOI: 10.1111/j.1537-2995.2006.00699.x]

38 **Elayan MM**, Horowitz JG, Magraner JM, Shaughnessy PJ, Bachier C. Tbo-Filgrastim versus Filgrastim during Mobilization and Neutrophil Engraftment for Autologous Stem Cell Transplantation. *Biol Blood Marrow Transplant* 2015; **21**: 1921-1925 [PMID: 26033279 DOI: 10.1016/j.bbmt.2015.05.024]

39 **Hosing C**, Qazilbash MH, Kebriaei P, Giralt S, Davis MS, Popat U, Anderlini P, Shpall EJ, McMannis J, Körbling M, Champlin RE. Fixed-dose single agent pegfilgrastim for peripheral blood progenitor cell mobilisation in patients with multiple myeloma. *Br J Haematol* 2006; **133**: 533-537 [PMID: 16681642 DOI: 10.1111/j.1365-2141.2006.06054.x]

40 **Publicover A**, Richardson DS, Davies A, Hill KS, Hurlock C, Hutchins D, Jenner MW, Johnson PW, Lamb J, Launders H, McKeag N, Newman J, Orchard KH. Use of a biosimilar granulocyte colony-stimulating factor for peripheral blood stem cell mobilization: an

analysis of mobilization and engraftment. *Br J Haematol* 2013; **162**: 107-111 [PMID: 23614650 DOI: 10.1111/bjh.12345]

41 **Spitzer G**, Adkins D, Mathews M, Velasquez W, Bowers C, Dunphy F, Kronmueller N, Niemeyer R, McIntyre W, Petruska P. Randomized comparison of G-CSF + GM-CSF vs G-CSF alone for mobilization of peripheral blood stem cells: effects on hematopoietic recovery after high-dose chemotherapy. *Bone Marrow Transplant* 1997; **20**: 921-930 [PMID: 9422470 DOI: 10.1038/sj.bmt.1700999]

42 **Weisdorf D**, Miller J, Verfaillie C, Burns L, Wagner J, Blazar B, Davies S, Miller W, Hannan P, Steinbuch M, Ramsay N, McGlave P. Cytokine-primed bone marrow stem cells vs. peripheral blood stem cells for autologous transplantation: a randomized comparison of GM-CSF vs. G-CSF. *Biol Blood Marrow Transplant* 1997; **3**: 217-223 [PMID: 9360784]

43 **Arora M**, Burns LJ, Barker JN, Miller JS, Defor TE, Olujohungbe AB, Weisdorf DJ. Randomized comparison of granulocyte colony-stimulating factor versus granulocyte-macrophage colony-stimulating factor plus intensive chemotherapy for peripheral blood stem cell mobilization and autologous transplantation in multiple myeloma. *Biol Blood Marrow Transplant* 2004; **10**: 395-404 [PMID: 15148493 DOI: 10.1016/j.bbmt.2004.02.001]

44 **Bot FJ**, van Eijk L, Schipper P, Backx B, Löwenberg B. Synergistic effects between GM-CSF and G-CSF or M-CSF on highly enriched human marrow progenitor cells. *Leukemia* 1990; **4**: 325-328 [PMID: 1697008]

45 **Hara H**, Namiki M. Mechanism of synergy between granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor in colony formation from human marrow cells in vitro. *Exp Hematol* 1989; **17**: 816-821 [PMID: 2473914]

46 **Tricot G**, Barlogie B, Zangari M, van Rhee F, Hoering A, Szymonifka J, Cottler-Fox M. Mobilization of peripheral blood stem cells in myeloma with either pegfilgrastim or filgrastim following chemotherapy. *Haematologica* 2008; **93**: 1739-1742 [PMID: 18728024 DOI: 10.3324/haematol.13204]

47 Granix® [package insert]. North Wales, PA: Teva Pharmaceuticals Inc; 2014. [accessed 2016 June 9]. Available from: URL: http://www.granixhcp.com/Pdf/prescribing-information.pdf

48 **Martino M**, Recchia AG, Moscato T, Fedele R, Neri S, Gentile M, Alati C, Vincelli ID, Piro E, Penna G, Musolino C, Ronco F, Molica S, Morabito F. Efficacy of biosimilar granulocyte colony-stimulating factor versus originator granulocyte colony-stimulating factor in peripheral blood stem cell mobilization in de novo multiple myeloma patients. *Cytotherapy* 2015; **17**: 1485-1493 [PMID: 26188967 DOI: 10.1016/j.jcyt.2015.05.010]

49 **Bell AJ**, Figes A, Oscier DG, Hamblin TJ. Peripheral blood stem cell autografting. *Lancet* 1986; **1**: 1027 [PMID: 2871297]

50 **Richman CM**, Weiner RS, Yankee RA. Increase in circulating stem cells following chemotherapy in man. *Blood* 1976; **47**: 1031-1039 [PMID: 1276467]

51 **Alegre A**, Tomás JF, Martínez-Chamorro C, Gil-Fernández JJ, Fernández-Villalta MJ, Arranz R, Díaz MA, Granda A, Bernardo MR, Escudero A, López-Lorenzo JL, Fernández-Rañada JM. Comparison of peripheral blood progenitor cell mobilization in patients with multiple myeloma: high-dose cyclophosphamide plus GM-CSF vs G-CSF alone. *Bone Marrow Transplant* 1997; **20**: 211-217 [PMID: 9257889 DOI: 10.1038/sj.bmt.1700867]

52 **Dingli D**, Nowakowski GS, Dispenzieri A, Lacy MQ, Hayman S, Litzow MR, Gastineau DA, Gertz MA. Cyclophosphamide mobilization does not improve outcome in patients receiving stem cell transplantation for multiple myeloma. *Clin Lymphoma Myeloma* 2006; **6**: 384-388 [PMID: 16640814 DOI: 10.3816/CLM.2006.n.014]

53 **Wood WA**, Whitley J, Moore D, Sharf A, Irons R, Rao K, Serody J, Coghill J, Gabriel D, Shea T. Chemomobilization with Etoposide is Highly Effective in Patients with Multiple Myeloma and Overcomes the Effects of Age and Prior Therapy. *Biol Blood Marrow Transplant* 2011; **17**: 141-146 [PMID: 20637882 DOI: 10.1016/j.bbmt.2010.06.021]

54 **Gertz MA**, Kumar SK, Lacy MQ, Dispenzieri A, Hayman SR, Buadi FK, Dingli D, Gastineau DA, Winters JL, Litzow MR. Comparison of high-dose CY and growth factor with growth factor alone for mobilization of stem cells for transplantation in patients with multiple myeloma. *Bone Marrow Transplant* 2009; **43**: 619-625 [PMID: 18997825 DOI: 10.1038/bmt.2008.369]

55 **Gettys SC**, Gulbis A, Wilhelm K, Sasaki K, Dinh Y, Rondon G, Qazilbash MH. Modified CVAD and modified CBAD compared to high-dose cyclophosphamide for peripheral blood stem cell mobilization in patients with multiple myeloma. *Eur J Haematol* 2017; **98**: 388-392 [PMID: 28009447 DOI: 10.1111/ejh.12843]

56 **Green DJ**, Bensinger WI, Holmberg LA, Gooley T, Till BG, Budde LE, Pagel JM, Frayo SL, Roden JE, Hedin L, Press OW, Gopal AK. Bendamustine, etoposide and dexamethasone to mobilize peripheral blood hematopoietic stem cells for autologous transplantation in patients with multiple myeloma. *Bone Marrow Transplant* 2016; **51**: 1330-1336 [PMID: 27214069 DOI: 10.1038/bmt.2016.123]

57 **Güner ŞI**, Yanmaz MT, Selvi A, Usul C. The High Effect of Chemomobilization with High-Dose Etopside + Granulocyte-Colony Stimulating Factor in Autologous Hematopoietic Peripheral Blood Stem Cell Transplantation: A Single Center Experience. *Hematol Rep* 2016; **8**: 6319 [PMID: 27103979 DOI: 10.4081/hr.2016.6319]

58 **Musto P**, Simeon V, Grossi A, Gay F, Bringhen S, Larocca A, Guariglia R, Pietrantuono G, Villani O, D'Arena G, Cuomo C, Musto C, Morabito F, Petrucci MT, Offidani M, Zamagni E, Tacchetti P, Conticello C, Milone G, Palumbo A, Cavo M, Boccadoro M. Predicting poor peripheral blood stem cell collection in patients with multiple myeloma receiving pre-transplant induction therapy with novel agents and mobilized with cyclophosphamide plus granulocyte-colony stimulating factor: results from a Gruppo Italiano Malattie EMatologiche dell'Adulto Multiple Myeloma Working Party study. *Stem Cell Res Ther* 2015; **6**: 64 [PMID: 25889496 DOI: 10.1186/s13287-015-0033-1]

59 **Mark T**, Stern J, Furst JR, Jayabalan D, Zafar F, LaRow A, Pearse RN, Harpel J, Shore T, Schuster MW, Leonard JP, Christos PJ, Coleman M, Niesvizky R. Stem cell mobilization with cyclophosphamide overcomes the suppressive effect of lenalidomide therapy on stem cell collection in multiple myeloma. *Biol Blood Marrow Transplant* 2008; **14**: 795-798 [PMID: 18541199 DOI: 10.1016/j.bbmt.2008.04.008]

60 **Fitoussi O**, Perreau V, Boiron JM, Bouzigon E, Cony-Makhoul P, Pigneux A, Agape P, Nicolini F, Dazey B, Reiffers J, Salmi R, Marit G. A comparison of toxicity following two different doses of cyclophosphamide for mobilization of peripheral blood progenitor cells in 116 multiple myeloma patients. *Bone Marrow Transplant* 2001; **27**: 837-842 [PMID: 11477441 DOI: 10.1038/sj.bmt.1702879]

61 **Jantunen E**, Putkonen M, Nousiainen T, Pelliniemi TT, Mahlamäki E, Remes K. Low-dose or intermediate-dose cyclophosphamide plus granulocyte colony-stimulating factor for progenitor cell mobilisation in patients with multiple myeloma. *Bone Marrow Transplant* 2003; **31**: 347-351 [PMID: 12634725 DOI: 10.1038/sj.bmt.1703840]

62 **Corso A**, Arcaini L, Caberlon S, Zappasodi P, Mangiacavalli S, Lorenzi A, Rusconi C, Troletti D, Maiocchi MA, Pascutto C, Morra E, Lazzarino M. A combination of dexamethasone, cyclophosphamide, etoposide, and cisplatin is less toxic and more effective than high-dose cyclophosphamide for peripheral stem cell mobilization in multiple myeloma. *Haematologica* 2002; **87**: 1041-1045 [PMID: 12368158]

63 **Goldschmidt H**, Hegenbart U, Haas R, Hunstein W. Mobilization of peripheral blood progenitor cells with high-dose cyclophosphamide (4 or 7 g/m2) and granulocyte colony-stimulating factor in patients with multiple myeloma. *Bone Marrow Transplant* 1996; **17**: 691-697 [PMID: 8733683]

64 **Sizemore CA**, LaPorte J, Sanacore M, Holland HK, Mccollum J, Westerman J, Morris LE, Bashey A, Solomon SR. A Comparison of Toxicity and Mobilization Efficacy Following Two Different Doses of Cyclophosphamide for Mobilization of Hematopoietic Stem Cells in Multiple Myeloma Patients [abstract]. *Biol Blood Marrow Tr* 2010; **16**: S206-S206 [DOI: 10.1016/j.bbmt.2009.12.125]

65 **Gojo I**, Guo C, Sarkodee-Adoo C, Meisenberg B, Fassas A, Rapoport AP, Cottler-Fox M, Heyman M, Takebe N, Tricot G. High-dose cyclophosphamide with or without etoposide for mobilization of peripheral blood progenitor cells in patients with multiple myeloma: efficacy and toxicity. *Bone Marrow Transplant* 2004; **34**: 69-76 [PMID: 15133484 DOI: 10.1038/sj.bmt.1704529]

66 **Stewart AK**, Vescio R, Schiller G, Ballester O, Noga S, Rugo H, Freytes C, Stadtmauer E, Tarantolo S, Sahebi F, Stiff P, Meharchard J, Schlossman R, Brown R, Tully H, Benyunes M, Jacobs C, Berenson R, White M, DiPersio J, Anderson KC, Berenson J. Purging of autologous peripheral-blood stem cells using CD34 selection does not improve overall or progression-free survival after high-dose chemotherapy for multiple myeloma: results of a multicenter randomized controlled trial. *J Clin Oncol* 2001; **19**: 3771-3779 [PMID: 11533101 DOI: 10.1200/JCO.2001.19.17.3771]

67 **Tuchman SA**, Bacon WA, Huang LW, Long G, Rizzieri D, Horwitz M, Chute JP, Sullivan K, Morris Engemann A, Yopp A, Li Z, Corbet K, Chao N, Gasparetto C. Cyclophosphamide-based hematopoietic stem cell mobilization before autologous stem cell transplantation in newly diagnosed multiple myeloma. *J Clin Apher* 2015; **30**: 176-182 [PMID: 25293363 DOI: 10.1002/jca.21360]

68 **Uy GL**, Costa LJ, Hari PN, Zhang MJ, Huang JX, Anderson KC, Bredeson CN, Callander NS, Cornell RF, Perez MA, Dispenzieri A, Freytes CO, Gale RP, Garfall A, Gertz MA, Gibson J, Hamadani M, Lazarus HM, Kalaycio ME, Kamble RT, Kharfan-Dabaja MA, Krishnan AY, Kumar SK, Kyle RA, Landau HJ, Lee CH, Maiolino A, Marks DI, Mark TM, Munker R, Nishihori T, Olsson RF, Ramanathan M, Rodriguez TE, Saad AA, Savani BN, Schiller GJ, Schouten HC, Schriber JR, Scott E, Seo S, Sharma M, Ganguly S, Stadtmauer EA, Tay J, To LB, Vesole DH, Vogl DT, Wagner JL, Wirk B, Wood WA, D'Souza A. Contribution of chemotherapy mobilization to disease control in multiple myeloma treated with autologous hematopoietic cell transplantation. *Bone Marrow Transplant* 2015; **50**: 1513-1518 [PMID: 26301967 DOI: 10.1038/bmt.2015.190]

69 **Broxmeyer HE**, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA, Liles WC, Li X, Graham-Evans B, Campbell TB, Calandra G, Bridger G, Dale DC, Srour EF. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med* 2005; **201**: 1307-1318 [PMID: 15837815 DOI: 10.1084/jem.20041385]

70 **Liles WC**, Broxmeyer HE, Rodger E, Wood B, Hübel K, Cooper S, Hangoc G, Bridger GJ, Henson GW, Calandra G, Dale DC. Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. *Blood* 2003; **102**: 2728-2730 [PMID: 12855591 DOI: 10.1182/blood-2003-02-0663]

71 **DiPersio JF**, Stadtmauer EA, Nademanee A, Micallef IN, Stiff PJ, Kaufman JL, Maziarz RT, Hosing C, Früehauf S, Horwitz M, Cooper D, Bridger G, Calandra G; 3102 Investigators. Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma. *Blood* 2009; **113**: 5720-5726 [PMID: 19363221 DOI: 10.1182/blood-2008-08-174946]

72 **Costa LJ**, Abbas J, Hogan KR, Kramer C, McDonald K, Butcher CD, Littleton A, Shoptaw K, Kang Y, Stuart RK. Growth factor plus preemptive ('just-in-time') plerixafor successfully mobilizes hematopoietic stem cells in multiple myeloma patients despite prior lenalidomide exposure. *Bone Marrow Transplant* 2012; **47**: 1403-1408 [PMID: 22484324 DOI: 10.1038/bmt.2012.60]

73 **Tricot G**, Cottler-Fox MH, Calandra G. Safety and efficacy assessment of plerixafor in patients with multiple myeloma proven or predicted to be poor mobilizers, including assessment of tumor cell mobilization. *Bone Marrow Transplant* 2010; **45**: 63-68 [PMID: 19543330 DOI: 10.1038/bmt.2009.130]

74 **Kim JS**, Yoon DH, Park S, Yoon SS, Cho SG, Min CK, Lee JJ, Yang DH, Kwak JY, Eom HS, Kim WS, Kim H, Do YR, Moon JH, Lee J, Suh C. Prognostic factors for re-mobilization using plerixafor and granulocyte colony-stimulating factor (G-CSF) in patients with malignant lymphoma or multiple myeloma previously failing mobilization with G-CSF with or without chemotherapy: the Korean multicenter retrospective study. *Ann Hematol* 2016; **95**: 603-611 [PMID: 26754633 DOI: 10.1007/s00277-016-2589-y]

75 **Kim SS**, Renteria AS, Steinberg A, Banoff K, Isola L. Pharmacoeconomic impact of up-front use of plerixafor for autologous stem cell mobilization in patients with multiple myeloma. *Cytotherapy* 2014; **16**: 1584-1589 [PMID: 24927717 DOI: 10.1016/j.jcyt.2014.05.003]

76 **Cooper DL**, Medoff E, Patel N, Baker J, Pratt K, Foss F, Seropian SE, Perreault S, Wu Y. Autologous Stem Cell Mobilization in the Age of Plerixafor. *Clin Lymphoma Myeloma Leuk* 2016; **16**: 411-416 [PMID: 27245311 DOI: 10.1016/j.clml.2016.04.007]

77 **Costa LJ**, Alexander ET, Hogan KR, Schaub C, Fouts TV, Stuart RK. Development and validation of a decision-making algorithm to guide the use of plerixafor for autologous hematopoietic stem cell mobilization. *Bone Marrow Transplant* 2011; **46**: 64-69 [PMID: 20383210 DOI: 10.1038/bmt.2010.78]

78 **Smith VR**, Popat U, Ciurea S, Nieto Y, Anderlini P, Rondon G, Alousi A, Qazilbash M, Kebriaei P, Khouri I, de Lima M, Champlin R, Hosing C. Just-in-time rescue plerixafor in combination with chemotherapy and granulocyte-colony stimulating factor for peripheral blood progenitor cell mobilization. *Am J Hematol* 2013; **88**: 754-757 [PMID: 23749720 DOI: 10.1002/ajh.23499]

79 **Vishnu P**, Roy V, Paulsen A, Zubair AC. Efficacy and cost-benefit analysis of risk-adaptive use of plerixafor for autologous hematopoietic progenitor cell mobilization. *Transfusion* 2012; **52**: 55-62 [PMID: 21658047 DOI: 10.1111/j.1537-2995.2011.03206.x]

80 **Fiala MA**, Schwab D, Vij R, Cashen AF, Stockerl-Goldstein K, Abboud CN. Randomized Trial of Tbo-Filgrastim versus Filgrastim for Autologous Stem Cell Mobilization in Patients with Multiple Myeloma or Non-Hodgkin Lymphoma [abstract]. *Blood* 2015; **23**: 516

81 **Lacativa CP**, Lacativa PG, Garnica M, Portugal RD, Schaffel R, Dutra Hdos S, Nogueira CM, Nucci M, Maiolino A. Risk factors for unsuccessful peripheral blood stem cell harvesting using granulocyte-colony stimulating factor mobilization in patients with multiple myeloma. *Transfus Apher Sci* 2012; **47**: 331-335 [PMID: 22874435 DOI: 10.1016/j.transci.2012.06.013]

82 **Sinha S**, Gertz MA, Lacy MQ, Dispenzieri A, Hayman SR, Buadi FK, Dingli D, Micallef IN, Hogan WJ, Gastineau DA, Rajkumar SV, Kumar SK. Majority of patients receiving initial therapy with lenalidomide-based regimens can be successfully mobilized with appropriate mobilization strategies. *Leukemia* 2012; **26**: 1119-1122 [PMID: 22033494 DOI: 10.1038/leu.2011.308]

83 **Prince HM**, Imrie K, Sutherland DR, Keating A, Meharchand J, Crump RM, Girouard C, Trip K, Stewart AK. Peripheral blood progenitor cell collections in multiple myeloma: predictors and management of inadequate collections. *Br J Haematol* 1996; **93**: 142-145 [PMID: 8611448]

84 **Kumar S**, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, Gastineau DA, Litzow MR, Fonseca R, Roy V, Rajkumar SV, Gertz MA. Impact of lenalidomide therapy on stem cell mobilization and engraftment post-peripheral blood stem cell transplantation in patients with newly diagnosed myeloma. *Leukemia* 2007; **21**: 2035-2042 [PMID: 17581613 DOI: 10.1038/sj.leu.2404801]

85 **Waterman J**, Rybicki L, Bolwell B, Copelan E, Pohlman B, Sweetenham J, Dean R, Sobecks R, Andresen S, Kalaycio M. Fludarabine as a risk factor for poor stem cell harvest, treatment-related MDS and AML in follicular lymphoma patients after autologous hematopoietic cell transplantation. *Bone Marrow Transplant* 2012; **47**: 488-493 [PMID: 21572461 DOI: 10.1038/bmt.2011.109]

86 **NCCN Clinical Practice Guidelines in Oncology**. Multiple Myeloma (Version 3.2016). [accessed 2016 Feb 2]. Available from: URL: https://www.nccn.org/professionals/physician_gls/pdf/myeloma.pdf

87 **Pal R**, Monaghan SA, Hassett AC, Mapara MY, Schafer P, Roodman GD, Ragni MV, Moscinski L, List A, Lentzsch S. Immunomodulatory derivatives induce PU.1 down-regulation, myeloid maturation arrest, and neutropenia. *Blood* 2010; **115**: 605-614 [PMID: 19965623 DOI: 10.1182/blood-2009-05-221077]

88 **Wilk CM**, Heinzler N, Boquoi A, Cadeddu RP, Strapatsas T, Dienst A, Majidi F, Deenen R, Bruns I, Schroeder T, Köhrer K, Haas R, Kobbe G, Fenk R. Lenalidomide consolidation treatment in patients with multiple myeloma suppresses myelopoieses but spares erythropoiesis. *Int J Cancer* 2016; **139**: 2343-2352 [PMID: 27389073 DOI: 10.1002/ijc.30257]

89 **Costa LJ**, Nista EJ, Buadi FK, Lacy MQ, Dispenzieri A, Kramer CP, Edwards KH, Kang Y, Gertz MA, Stuart RK, Kumar S. Prediction of poor mobilization of autologous CD34+ cells with growth factor in

multiple myeloma patients: implications for risk-stratification. *Biol Blood Marrow Transplant* 2014; **20**: 222-228 [PMID: 24211319 DOI: 10.1016/j.bbmt.2013.11.003]

90 **Pusic I**, Jiang SY, Landua S, Uy GL, Rettig MP, Cashen AF, Westervelt P, Vij R, Abboud CN, Stockerl-Goldstein KE, Sempek DS, Smith AL, DiPersio JF. Impact of mobilization and remobilization strategies on achieving sufficient stem cell yields for autologous transplantation. *Biol Blood Marrow Transplant* 2008; **14**: 1045-1056 [PMID: 18721768 DOI: 10.1016/j.bbmt.2008.07.004]

91 **Weaver CH**, Schulman KA, Wilson-Relyea B, Birch R, West W, Buckner CD. Randomized trial of filgrastim, sargramostim, or sequential sargramostim and filgrastim after myelosuppressive chemotherapy for the harvesting of peripheral-blood stem cells. *J Clin Oncol* 2000; **18**: 43-53 [PMID: 10623692 DOI: 10.1200/JCO.2000.18.1.43]

92 **Fruehauf S**, Ehninger G, Hübel K, Topaly J, Goldschmidt H, Ho AD, Müller S, Moos M, Badel K, Calandra G. Mobilization of peripheral blood stem cells for autologous transplant in non-Hodgkin's lymphoma and multiple myeloma patients by plerixafor and G-CSF and detection of tumor cell mobilization by PCR in multiple myeloma patients. *Bone Marrow Transplant* 2010; **45**: 269-275 [PMID: 19597422 DOI: 10.1038/bmt.2009.142]

93 **Steidl U**, Fenk R, Bruns I, Neumann F, Kondakci M, Hoyer B, Gräf T, Rohr UP, Bork S, Kronenwett R, Haas R, Kobbe G. Successful transplantation of peripheral blood stem cells mobilized by chemotherapy and a single dose of pegylated G-CSF in patients with multiple myeloma. *Bone Marrow Transplant* 2005; **35**: 33-36 [PMID: 15531906 DOI: 10.1038/sj.bmt.1704702]

94 **Hamadani M**, Kochuparambil ST, Osman S, Cumpston A, Leadmon S, Bunner P, Watkins K, Morrison D, Speir E, Deremer D, Kota V, Jillella A, Craig M, Awan F. Intermediate-dose versus low-dose cyclophosphamide and granulocyte colony-stimulating factor for peripheral blood stem cell mobilization in patients with multiple myeloma treated with novel induction therapies. *Biol Blood Marrow Transplant* 2012; **18**: 1128-1135 [PMID: 22248715 DOI: 10.1016/j.bbmt.2012.01.005]

95 **Hiwase DK**, Bollard G, Hiwase S, Bailey M, Muirhead J, Schwarer AP. Intermediate-dose CY and G-CSF more efficiently mobilize adequate numbers of PBSC for tandem autologous PBSC transplantation compared with low-dose CY in patients with multiple myeloma. *Cytotherapy* 2007; **9**: 539-547 [PMID: 17882718 DOI: 10.1080/14653240701452800]

P- Reviewer: Spyridonidis A, Zhang JJ **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 October 24; 7(5): 260-268

DOI: 10.5500/wjt.v7.i5.260

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Prediction of delayed graft function using different scoring algorithms: A single-center experience

Magda Michalak, Kristien Wouters, Erik Fransen, Rachel Hellemans, Amaryllis H Van Craenenbroeck, Marie M Couttenye, Bart Bracke, Dirk K Ysebaert, Vera Hartman, Kathleen De Greef, Thiery Chapelle, Geert Roeyen, Gerda Van Beeumen, Marie-Paule Emonds, Daniel Abramowicz, Jean-Louis Bosmans

Magda Michalak, Rachel Hellemans, Amaryllis H Van Craenenbroeck, Marie M Couttenye, Gerda Van Beeumen, Daniel Abramowicz, Jean-Louis Bosmans, Department of Nephrology-Hypertension, Antwerp University Hospital, B-2650 Edegem, Belgium

Kristien Wouters, Department of Medical Statistics, Antwerp University Hospital, B-2650 Edegem, Belgium

Erik Fransen, StatUa Center for Statistics, University of Antwerp, B-2610 Wilrijk, Belgium

Bart Bracke, Dirk K Ysebaert, Vera Hartman, Kathleen De Greef, Thiery Chapelle, Geert Roeyen, Department of Hepatobiliary, Endocrine and Transplantation Surgery, Antwerp University Hospital, B-2650 Edegem, Belgium

Marie-Paule Emonds, Histocompatibility and Immunogenetic Laboratory, Belgian Red Cross-Flanders, 2800 Mechelen, Belgium

Author contributions: Michalak M, Abramowicz D and Bosmans JL designed the study; Michalak M collected and analyzed the data and wrote the first draft of the manuscript; Abramowicz D and Bosmans JL contributed to the analysis of the data and the finalization of the manuscript; Wouters K and Fransen E provided the statistical analysis; Van Beeumen G and Emonds MP contributed to the collection of the patient-related data; Hellemans R, Van Craenenbroeck AH, Couttenye MM, Bracke B, Ysebaert DK, Hartman V, De Greef K, Chapelle T and Roeyen G were responsible for the medical and surgical management of the patients.

Institutional review board statement: This study was approved by the Ethic Committee of the Antwerp University Hospital (Ref. 16/34/339).

Informed consent statement: Since this was a retrospective study, a written informed consent could not be obtained from the patients. However, all patients agreed verbally for the data collection.

Conflict-of-interest statement: This project was an investigator driven study without any support from external organizations. The authors have no conflict of interest.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Jean-Louis Bosmans, MD, PhD, Professor of Medicine, Department of Nephrology-Hypertension, Antwerp University Hospital, Wilrijkstraat 10, B-2650 Edegem, Belgium. jeanlouis.bosmans@uantwerpen.be
Telephone: +32-3-8213421
Fax: +32-3-8290100

Received: December 19, 2016
Peer-review started: December 25, 2016
First decision: February 17, 2017
Revised: March 31, 2017
Accepted: May 3, 2017
Article in press: May 5, 2017
Published online: October 24, 2017

Abstract

AIM

To compare the performance of 3 published delayed graft

function (DGF) calculators that compute the theoretical risk of DGF for each patient.

METHODS

This single-center, retrospective study included 247 consecutive kidney transplants from a deceased donor. These kidney transplantations were performed at our institution between January 2003 and December 2012. We compared the occurrence of observed DGF in our cohort with the predicted DGF according to three different published calculators. The accuracy of the calculators was evaluated by means of the c-index (receiver operating characteristic curve).

RESULTS

DGF occurred in 15.3% of the transplants under study. The c index of the Irish calculator provided an area under the curve (AUC) of 0.69 indicating an acceptable level of prediction, in contrast to the poor performance of the Jeldres nomogram (AUC = 0.54) and the Chapal nomogram (AUC = 0.51). With the Irish algorithm the predicted DGF risk and the observed DGF probabilities were close. The mean calculated DGF risk was significantly different between DGF-positive and DGF-negative subjects (P < 0.0001). However, at the level of the individual patient the calculated risk of DGF overlapped very widely with ranges from 10% to 51% for recipients with DGF and from 4% to 56% for those without DGF. The sensitivity, specificity and positive predictive value of a calculated DGF risk ⩾ 30% with the Irish nomogram were 32%, 91% and 38%.

CONCLUSION

Predictive models for DGF after kidney transplantation are performant in the population in which they were derived, but less so in external validations.

Key words: Delayed graft function; Kidney transplantation; Nomogram; Receiver operating characteristic curve; Risk calculation

Core tip: In this single centre, retrospective study we compared the incidence of observed delayed graft function (DGF) in 247 consecutive kidney transplant recipients with the predicted risk of DGF according to 3 different nomograms. Although the Irish nomogram provided an acceptable predictive value for the global study population, this calculator did not allow to make an accurate prediction of DGF at the individual level. Our study suggests that currently available predictive models for the risk of DGF after kidney transplantation are predictive in the population in which they were derived, but they lose their predictive value in external validations.

Michalak M, Wouters K, Fransen E, Hellemans R, Van Craenenbroeck AH, Couttenye MM, Bracke B, Ysebaert DK, Hartman V, De Greef K, Chapelle T, Roeyen G, Van Beeumen G, Emonds MP, Abramowicz D, Bosmans JL. Prediction of delayed graft function using different scoring algorithms: A single-center experience. *World J Transplant* 2017; 7(5): 260-268 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i5/260.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i5.260

INTRODUCTION

Delayed graft function (DGF) is classically defined as the need for at least one postoperative dialysis session during the first week after transplantation[1,2]. This definition has some limitations since the postoperative requirement of dialysis is not standardized and the decision to dialyze is subjective. For this and other reasons, the frequency of DGF varies worldwide between 10% and 40% for deceased donor kidney transplants[1,3]. DGF leads to prolonged hospitalization, higher cost of transplantation, and increased complexity of management of immunosuppressive drugs[4-6]. DGF is associated with an increased risk of acute rejection and may negatively impact long-term allograft function and outcome[7,8].

There are currently neither clinical practice guidelines nor an approved therapy to prevent DGF. In addition, the use of "extended criteria donors" (ECD) and kidneys from donors after cardiac death (DCD), which are associated with a higher incidence of DGF, is rising. The ability to predict DGF at the time of the transplant offer might help in clinical decisions making, such as declining the offer, selecting a recipient who would have a lower DGF risk, or modifying the transplantation strategy. This may include efforts to shorten the cold ischemia time (CIT), or delay the initiation of calcineurin inhibitors (CNIs) under the cover of induction therapy with anti-lymphocyte antibodies, or even to machine-perfuse the kidney.

Recently several DGF-scoring systems have been developed. In 2003, Irish *et al*[6], using a combination of 16 donor- and recipient-related risk factors known at the time of transplantation, developed a nomogram to predict/quantify the risk of DGF after renal transplantation. In 2010 they refined their previously published model using a more recent data set and adding two risk factors (in total 18) to the analysis (Table 1)[9]. This predictive model has an area under the receiver operating characteristic curve (ROC AUC) of 0.70, which indicates a good degree of discrimination[9]. In 2009, Jeldres *et al*[10] developed a simpler but equally accurate scoring system on 532 patients (6 variables, AUC = 0.74) (Table 1). More recently Chapal *et al*[11] proposed a predictive score that could be calculated by computing only 5 variables with a ROC AUC of 0.73 (Table 1).

The main aim of our study was to conduct a single-center retrospective analysis of a cohort of 247 adult patients to evaluate the performance of available nomograms to predict DGF in our patients, *i.e.*, in a different population than the one they have been tested in. We also studied separately recipients of standard criteria,

Table 1 Comparison of variables used in different scoring systems

	DGF risk calculator (Irish *et al*[9])	DGFS scoring system (Chapal *et al*[11])	Jeldres scoring system (Jeldres *et al*[10])
Recipient variables			
Recipient BMI	+	+	-
Recipient age	+	-	+
No. of HLA mismatches	+	-	+
Peak PRA (%)	+	-	+
Recipient race	+	-	-
Recipient gender	+	-	-
Duration of dialysis	+	-	-
History of diabetes mellitus	+	-	-
Previous transplantation or blood transfusion	+	-	-
Single or multiple organ transplant	+	-	-
Recipient weight	-	-	+
Donor variables			
Donor age	+	+	+
Duration of CIT	+	+	+
Terminal serum creatinine	+	+	-
Donor weight	+	-	-
Primary cause of death	+	-	-
History of hypertension	+	-	-
Duration of WIT	+	-	-
Type of the donor (living, DCD)	+	-	-
Type of induction therapy	-	+	-

PRA: Panel-reactive antibody; WIT: Warm ischemia time; CIT: Cold ischemia time; DCD: Donation after cardiac death; DGF: Delayed graft function.

extended criteria and donation after cardiac death donors.

MATERIALS AND METHODS

Patient characteristics

From January 1st 2003 to December 31st 2012, 349 renal transplantations were performed at the Antwerp University Hospital. Data were collected from our prospective institutional database and the database of Eurotransplant International Foundation. We excluded 27 pediatric transplants (aged < 18), 16 combined solid organ transplantations in adults (13 pancreases and 3 hearts), 31 transplantations performed with living donors (10.1%), 2 pre-emptive transplantations and 15 machine perfused kidneys. Moreover, we excluded 5 patients because of missing data for CIT. Thus, a total of 253 kidney transplantations from a deceased donor (87% first and 13% re-grafts), performed on 243 patients were considered for study. Six out of those 253 grafts (2%) were lost due to primary non function (PNF). These patients were excluded from further analysis and the final data set comprised 247 transplantations. Recipient and donor characteristics at the time of transplantation are summarized in Tables 2 and 3.

Definition of DGF, PNF and ECD

DGF was defined as the requirement of at least one dialysis within the first 7 d post-transplantation. The duration of DGF was defined as the number of days between the transplantation and the day of the last dialysis. PNF was defined as the absence of allograft function starting immediately after transplantation, and requiring maintenance dialysis. An ECD was defined as: A donor aged ⩾ 60 years, or a donor aged 50-59 years with at least 2 of the following conditions: History of hypertension, terminal serum creatinine level greater than 1.5 mg/dL, or death resulting from a cerebrovascular accident/stroke (CVA).

Post-transplant immunosuppressive therapy

One hundred and sixty-one patients (63.6%) were given an induction with an inhibitor of the IL2-receptor (basiliximab of daclizumab). Ninety-two patients (36.4%) were induced with antithymocyte globulin (ATG). According to our induction immunosuppression protocols ATG was given to immunized patients (peak PRA > 50%), patients of North-African origin, patients with a history of acute rejection during the first year after previous transplantation or in the case of kidney transplantation with ECD or DCD donor kidneys. Most patients (n = 244, 96.4%) received a CNI as initial therapy in addition to the treatment with corticosteroids and mycophenolate mofetil. Cyclosporin A was initiated at a starting dose of 2 × 4 mg/kg at post-transplant day 1. Only 7 patients (2.8%) were given mTOR-inhibitors. Two remaining patients (0.8%) [Eurotransplant Senior Program (ESP)] did not receive either medication but only ATG, MMF and prednisolone.

Data collection and DGF risk assessment

Risk factors for DGF included donor[12-15] and recipient factors known before and at the time of the transplantation

Table 2 Recipient characteristics at the time of transplantation

Age (yr)	50.2 ± 11.9[2]
Origin (%)	
Blacks	4.5
Caucasians	95.5
Gender (%)	
Male	61.9
History of diabetes mellitus (%)	
Yes	16.6
Body mass index (kg/m²)	25.1 ± 3.8[2]
Pretransplant transfusions (%)	
Yes	38.1
No	56.7
Unknown	5.3
Duration of the pre-transplant renal replacement therapy (mo)	26.7 (16.4-43.5)[1]
Peak panel-reactive antibodies (%)	88.5
⩽ 5%	9.5
5%-80%	2
⩾ 80%	
Proportion of kidney re-graft (%)	12.6
Total HLA mismatches	3 (2-3)[1]

[1]Median with P25-75; [2]Mean ± standard deviation (SD).

Table 3 Donor characteristics at the time of transplantation

Age (yr)	45.1 ± 14.1[2]
Weight (kg)	76.2 ± 16.4[2]
History of hypertension (%)	
Yes	23.1
No	74.5
Unknown	2.4
Terminal serum creatinine (mg/dL)	0.78 (0.61-1.00)[1]
Donor type (%)	
Standard criteria donor	68.8
Extended criteria donor	17
Donation after cardiac death donor	14.2
Primary cause of death (%)	
Cerebrovascular accident/stroke	27.1
Anoxia	8.1
Other	64.8
Cold ischemia time (h)	14 ± 4.7[2]
Second warm ischemia time (min)	32.8 ± 7.9[2]

[1]Median with P25-75; [2]Mean ± standard deviation (SD).

and were required to calculate the risk of DGF with the DGF risk calculator[9] (www.transplantcalculator.com/DGF), the Jeldres scoring system (Jeldres *et al*[10]) and the DGFS scoring system[11]. Recipient variables included: Age, gender, race, body mass index (BMI), history of diabetes mellitus, previous transplantation, pretransplant blood transfusion, duration of renal replacement therapy (RRT), the percentage of serum panel-reactive antibodies (peak PRA), and the number of HLA mismatches. Donor variables included: Age, gender, weight, donor type [standard criteria donor (SCD), ECD, DCD], primary cause of death, history of hypertension, duration of cold (CIT) and second warm ischemia time (WIT), and the terminal serum creatinin (mg/dL).

Statistical analysis

The statistical methods were performed and reviewed by Kristien Wouters (Department of Medical Statistics, Antwerp University Hospital, B-2650 Edegem, Belgium) and by Erik Fransen (StatUa Center for Statistics, University of Antwerp, B-2610 Wilrijk, Belgium).

Normality was tested with the Shapiro-Wilk and the Q-Q plot test. Normally distributed data are represented as mean and standard deviation; non-normally distributed data as median with P25 and P75. Categorical data are presented as numbers and percentages. Comparison of predicted DGF probability between DGF positive and negative patients was done by means of the Mann-Whitney *U* test. Receiver operating characteristic (ROC) curves were generated to evaluate the performance of explanatory scoring systems in predicting outcomes. The c-statistic (or AUC = area under ROC curve) was used as a measure of the predictive performance of the studied scoring systems. Additionally, the performance of the 3 nomograms was evaluated using a Hosmer-Lemeshow goodness-of-fit test. All data were analyzed using IBM SPSS statistics (version 21). Statistical significance was predefined as a *P*-value < 0.05. Goodness-of-fit was set at *P* > 0.05 for the Hosmer-Lemeshow test.

RESULTS

DGF occurred in 38 of the 247 transplants under study (15.3%). The mean duration of DGF was 11.3 ± 15.1 d (range 1-71 d). Graft survival at one year was comparable in patients with or without DGF (94.6% *vs* 93.3% respectively, *P* = ns). However, graft function was significantly inferior in patients with DGF both at 30 d (creatinine clearance according to MDRD formula 31 ± 16 mL/min *vs* 46 ± 17 mL/min, *P* = 0.001) and at 1 year (42 ± 14 mL/min *vs* 52 ± 17 mL/min, *P* < 0.001).

Analysis according to the algorithm of Irish et al[9]

At the population level, the average DGF risk calculated with the DGF risk calculator was 18.5%, which was close to the observed data (DGF rate: 15.3%). The AUC was 0.69 (Figure 1). Figure 2A illustrates the relatively good calibration of the Irish model. The predicted DGF risk and the observed DGF probabilities were close (*P* = 0.74, Hosmer-Lemeshow statistic). The mean calculated DGF risk was significantly different (*P* < 0.0001) between DGF-positive and DGF-negative subjects (Figure 3). However, at the level of the individual patient the calculated risk of DGF overlapped very widely (Figure 3). Indeed, it ranged from 10% to 51% for recipients with DGF and from 4% to 56% for those without DGF. The sensitivity, specificity and positive predictive value of a calculated DGF risk ⩾ 30% were 32%, 91% and 38% respectively.

Analysis according to the algorithm of Jeldres et al[10]

At the population level, the average DGF risk calculated with Jeldres nomogram was 27.9%, which is almost the double of the observed DGF rate (15.3%). The AUC of the ROC curve was poor at 0.54 (Figure 1). The

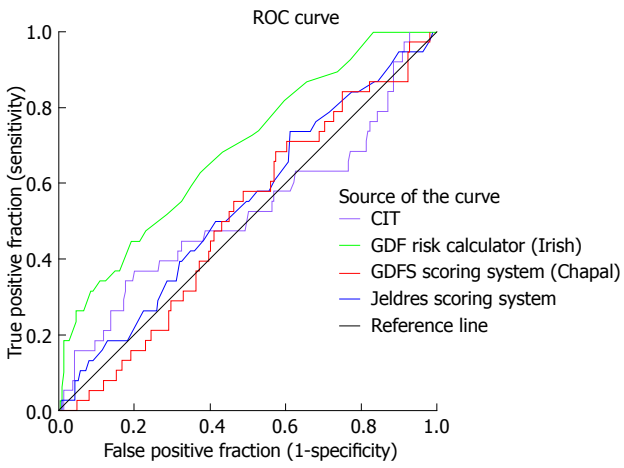


Figure 1 Receiver operating characteristic curves to evaluate the prognostic capacity of cold ischemia time, the delayed graft function risk calculator, the Jeldres scoring system[10] and the DGFS scoring system[11] to predict delayed graft function. The cold ischemia time (purple-line): Area under ROC curve (AUC) = 0.52. The DGF risk calculator (green-line) proposed by Irish *et al*[9]: AUC = 0.69. The scoring system (blue-line) proposed by Jeldres *et al*[10]: AUC = 0.54. The DGFS scoring system (red-line) proposed by Chapal *et al*[11]: AUC = 0.51. ROC: Receiver operating characteristic; CIT: Cold ischemia time; DGF: Delayed graft function.

Hosmer-Lemeshow "goodness-of-fit" test demonstrated a significant difference ($P < 0.05$) between the predicted DGF risk and the observed DGF, which indicates that the DGF risk was not well estimated by the Jeldres scoring system (Figure 2B). The calculated risk of DGF showed a wide range of values from 5%-82% in the DGF-group and 3%-83% in the non- DGF-group with a very large overlap between both groups (Figure 4). The sensitivity, specificity and positive predictive value of a calculated DGF risk ⩾ 30% was 44.7%, 61.7% and 17.5% respectively.

Analysis according to the algorithm of Chapal et al[11]

The average DGFS value was -0.48 [(-0.46) ± 0.76; 95%CI: (-0.43) - (-0.71)] in the DGF positive group and (-0.48) ± 0.89; 95%CI: (-0.46) - (-0.60) in the DGF negative group] (Figure 5A), indicating the inability of the Chapal score to predict DGF in our population. The sensitivity, specificity and negative predictive value of a DGFS value ⩽ (-0.5) were 45.6%, 70.3% and 85.8% respectively. Only 3 patients (1.2%) had a DGFs score ⩾ 1.2, which should in theory point to a high risk of DGF. None of these 3 patients developed DGF (sensitivity and positive predictive value for DGFs score ⩾ 1.2 was 0).

The average DGF risk calculated with the DGFS nomogram was 20%. The ROC curve analysis showed a c-index of 0.51 (Figure 1), indicating the absence of any predictive value. There was no difference between the median calculated DGF risk in the DGF-positive and the DGF-negative subjects (Figure 5B). The calibration plot of this model (Figure 2C) showed a significant difference ($P = 0.02$) between the predicted DGF risk and the observed DGF, which indicates that the DGF risk was not well calibrated by the Chapal nomogram. The

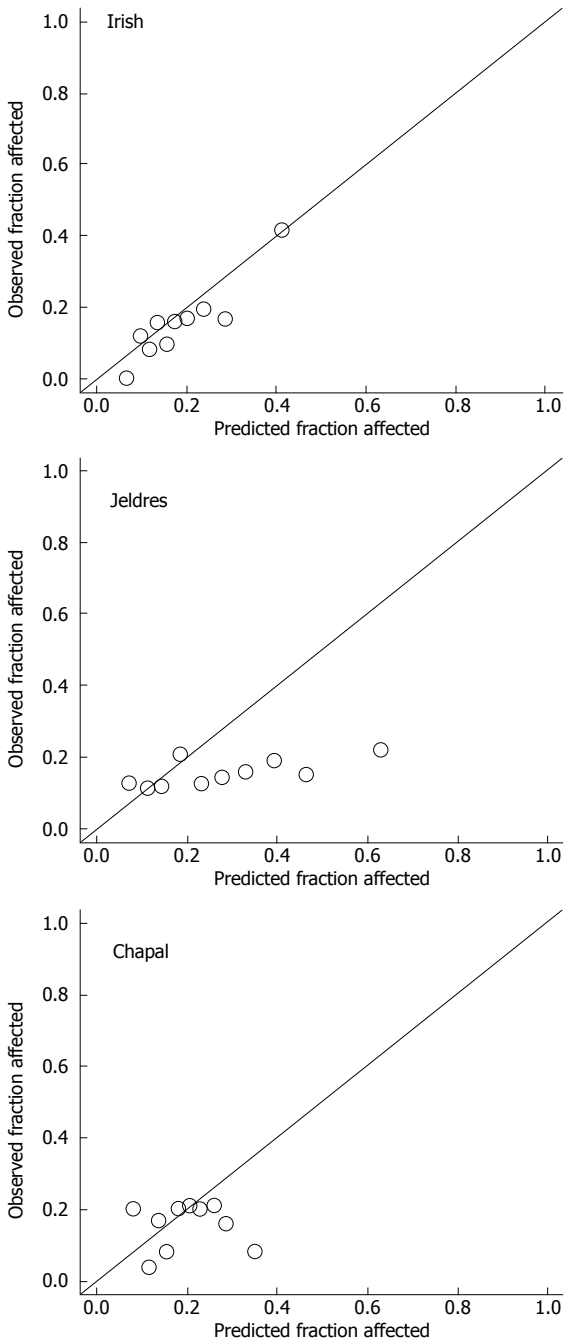


Figure 2 Calibration plot of: The delayed graft function risk calculator (Irish *et al*[9]), the Jeldres scoring system[10] and the DGFS scoring system (Chapal *et al*[11]) to predict delayed graft function. Patients were divided into 10 subgroups (deciles of increased DGF risk), based upon the risk prediction. Each figure plots the mean predicted probability (X-axis) of DGF against the observed prevalence of DGF (Y-axis) (Hosmer-Lemeshow). The *P*-values were 0.74 for the Irish score, < 0.05 for the Jeldres score and 0.02 for the Chapal score. DGF: Delayed graft function.

sensitivity, specificity and positive predictive value of a calculated DGF risk ⩾ 30% were 5.2%, 88% and 8% respectively.

Analysis in the subgroups with a higher risk of DGF

Next, we studied how well the three nomograms can predict DGF in subgroups of patients considered to be at increased risk of DGF such as ECD and DCD donors

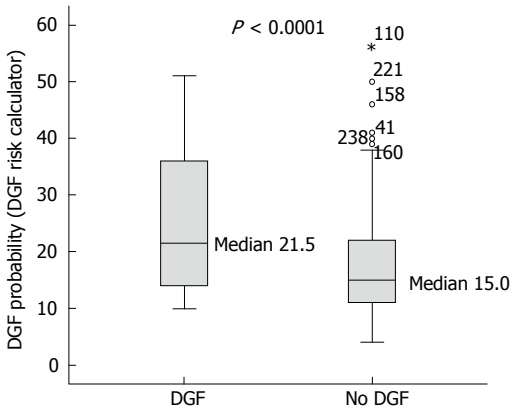


Figure 3 Correlation between the predicted delayed graft function probability according to the delayed graft function risk calculator (Irish *et al*[9]) and the presence or absence of delayed graft function. DGF: Delayed graft function.

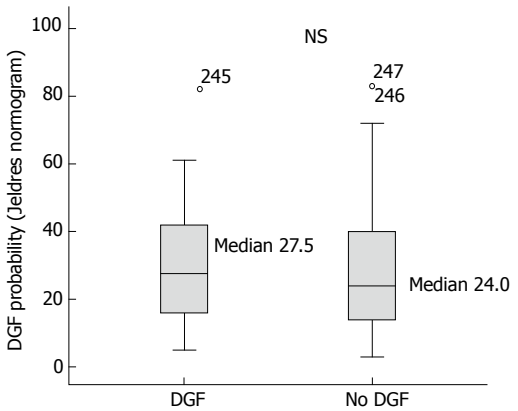


Figure 4 Correlation between the predicted delayed graft function probability according to the Jeldres scoring system (Jeldres *et al*[10]) and the presence or absence of delayed graft function. DGF: Delayed graft function.

(Table 4). The results presented in Table 4 suggest an acceptable agreement between the observed prevalence of DGF and the Irish DGF score for DCD donors, but not for ECD donors. The DGFS scoring system and the Jeldres scoring system[10] could not predict DGF in these high-risk groups (Table 4).

DISCUSSION

The first finding from our study is that our mean DGF rate was in the low range (15%), with a stepwise increase according to the risk categories (SCD, ECD, DCD donors). Next, we found that, at a population level, the observed DGF rate and the median calculated DGF risk according to the Irish calculator (16%) were similar. In our study the AUC calculated according to the Irish calculator was 0.69 which is similar to the results obtained in the 2010 Irish model (AUC of 0.70) and indicates an acceptable degree of discrimination. Along this line, the Hosmer-Lemeshow "goodness-of-fit" test demonstrated that the DGF risk was well calibrated by the DGF risk calculator. With regards to the ECD and DCD high-risk groups, there was a good agreement for

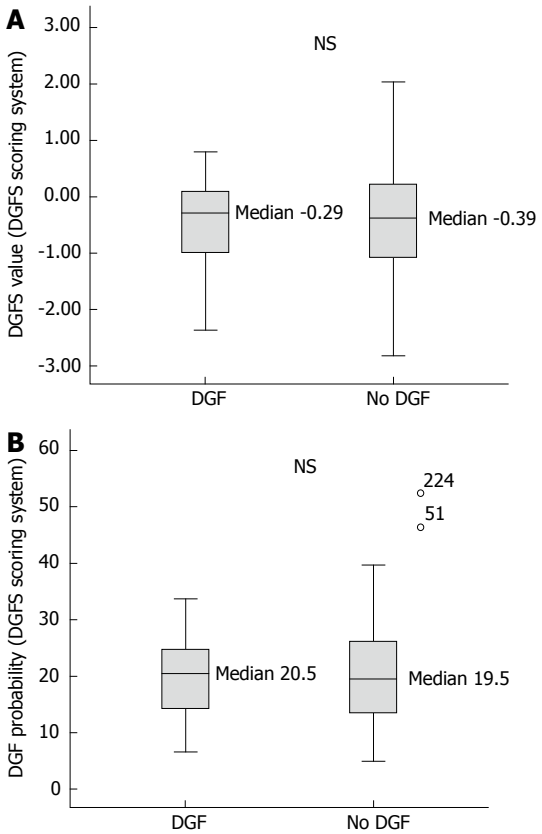


Figure 5 Correlation between the DGFS value (A: Y-axis) and the predicted delayed graft function probability according to the DGFS scoring system (Chapal *et al*[11]) (B: Y-axis) and the presence or absence of delayed graft function (A and B: X-axis). DGF: Delayed graft function.

DCD but not for ECD. This could be due to the smaller number of patients tested with these conditions in our center. While it appears that the DGF risk calculator can relatively well predict the percentage of DGF in our global study group, it is obvious that we cannot use this tool to take clinical decisions for individual patients. Indeed, as seen in Figure 3, because of the large overlap in DGF risk prediction between patients who developed DGF and those who did not, a high- or low-risk score did not correspond with the presence or absence of DGF. The specificity, sensitivity and positive predictive value of the DGF-risk calculator are too low to help with clinical-decision making regarding the immunosuppressive strategy. This nomogram has been previously tested in Australian[8], North American[16] and European[17] populations, but yielded conflicting results. In the Australian cohort from Kaisar *et al*[8] the nomogram was applied to 598 deceased donor renal transplantations, and showed a slightly better AUC value of 0.76 with a sensitivity of 74% and a specificity of 71%. Of note, however, no data are given about the overlap between the DGF and no DGF patients in this series, and it is thus difficult to evaluate its predictive value at individual patient level. Moore *et al*[17] evaluated the nomogram of Irish on 210 United Kingdom patients and showed a similar predictive value with an AUC of 0.71 with a high specificity (95%) but a very poor

Table 4 Observed prevalence *vs* predicted probability of delayed graft function in the overall population and by risk group

Kidney graft according to donor type	Observed prevalence of DGF (%)	Probability of DGF predicted by the DGF risk calculator (%) (Irish *et al*[9])	Probability of DGF predicted by the DGF scoring system (%) (Chapal *et al*[11])	Probability of DGF predicted by the Jeldres scoring system (%) (Jeldres *et al*[10])
Overall population (n = 247)	15.3	16[1] 12-24[2] 0.69[3]	19.7[1] 13.6-26[2] 0.51[3]	25[1] 14-40[2] 0.54[3]
Standard criteria donor (n = 170)	11.8	14[1] 10-20[2] 0.73[3]	20.1[1] 14.5-26.4[2] 0.60[3]	21[1] 13.7-34.2[2] 0.54[3]
Extended criteria donor (n = 42)	19	19.5[1] 14-25[2] 0.39[3]	21.2[1] 14.4-27.6[2] 0.34[3]	41.5[1] 25.7-60[2] 0.38[3]
Donation after cardiac death (n = 35)	28.6	30[1] 18-38[2] 0.65[3]	11.8[1] 9.1-20.4[2] 0.58[3]	21[1] 8-39[2] 0.64[3]

[1]Median; [2]P25-P75; [3]AUC of the ROC curve. DGF: Delayed graft function.

sensitivity (25%) at a score > 150. They concluded that the utility of the nomogram score in predicting DGF was moderate at best. Grossberg *et al*[16] showed a poor association between the Irish nomogram and DGF (the average DGF risk in DGF-positive patients was 0.45 ± 0.14 *vs* 0.40 ± 0.14 in DGF-negatives, P = 0.07) in a US population of 169 patients, but they did not report a c-index.

In 2012, Rodrigo *et al*[18], used the web-based calculator to predict DGF on 342 European renal transplants. Similar to the Irish group[9] they found an AUC of 0.71. The reported specificity and sensitivity of a calculated DGF risk ⩾ 30% were 75.8% and 51.8% respectively. They concluded, like us, that there was overlap in DGF risk prediction, which limited the utility of the score for individual patients. Finally, a large number of variables are needed to calculate the Irish DGF risk score, which limits its usefulness in daily clinical practice.

For this particular reason, two other independent and easier scoring systems were developed[10,11]. Jeldres *et al*[10] developed a more user-friendly nomogram based on the analysis of 6 risk factors. The c-statistic for assessing the predictive ability of Jeldres score for DGF (internal validation) was very similar to the Irish scoring system (AUC of 0.74). However, Chapal *et al*[11] tried to validate Jeldres score on their patients and showed an inferior predictive capacity of this scoring system to predict DGF (AUC = 0.61). The ROC curve analysis based on our population showed that the predictive utility of the Jeldres scoring system was poor, with a c-index of 0.54. This poor predictive value was confirmed by the Hosmer-Lemeshow "goodness-of-fit" test that showed a bad calibration of this model. The median calculated DGF risk in the DGF-positive group did not differ significantly from the DGF-negative group and there was a large overlap between both groups. Jeldres *et al*[10] proposed no cut-off to classify patients according to their DGF risk in their original study.

In our study the predictive capacity of the DGFS scoring system from Chapal was poor with an AUC of 0.51. In our population the negative predictive value of the DGFS score was 0.86 which implies that with the DGFS scoring system we can fairly well recognize the patients at a low risk of DGF. In contrast, the threshold for high risk of DGF was clinically useless in our study (none of the patients with DGFS score ⩾ 1.2 actually developed DGF). The failure of the DGFS scoring system in the prediction of DGF in our study may be explained by a lower incidence of DGF in our population (15.3% in our study *vs* 25.5% in the study of Chapal *et al*[11]). This difference is the consequence of shorter CIT [14 h (range 2.8 to 29.9 h) *vs* 19.2 h (range 6.0 to 58.6 h)], use of kidneys from younger donors (45.1 years *vs* 51.9 years) and lower terminal donor serum creatinine (69 μmol/L *vs* 91 μmol/L) in respectively our study population and in the study by Chapal[11]. According to these data our center seems to be more stringent in the selection of donors. This could also explain why the algorithm proposed by Chapal *et al*[11] fails to predict adequately DGF in our population.

There are some limitations to our study. First, the need to dialyse within the first week after the transplantation is an endpoint that could be influenced by several clinical factors (such as for instance heart failure, hyperkalemia…). This can lead to obvious mistakes in the validation of different scoring systems. Second, the sample size in our study is relatively small, particularly when compared to large-population-based transplant registers. Finally, the composition of our study population differs from the initial studies [*e.g.*, 4.5% blacks in our population *vs* 30.1% blacks in the study of Irish; relatively short CIT in our study (14 ± 4.7 h *vs* 19.2 ± 7 h in the study of Chapal or 17.8 ± 7.8 h in the study of Irish)]. And finally, according to our induction immunosuppression protocols ATG was de facto given to the patients at increased risk for DGF. The delayed introduction of CNIs could have attenuated the incidence of DGF in our population at risk. Another issue not captured by any scoring system is the policy of peri-operative volemia control, which has been shown to play an important role in the incidence of DGF (Mikhalski

et al[4]).

In summary, our study suggests that currently available predictive models for the risk of DGF after kidney transplantation are predictive in the population in which they were derived, but they lose their predictive value in external validations. This is not surprising, as none of these scores has been previously rigorously validated in external population of patients. Along this line, there were large variations between centers regarding demographic values (donor age, CIT, proportion of ECD/DCD, *etc*...) explaining why external validation like the one we tried, failed. This means that we still need better predictive tools for the kidney allocation to individual patients, especially those patients who are at high risk of DGF. Currently we are unable to further improve the outcome of a single patient by altering our management on the basis of available scores for the risk of DGF.

COMMENTS

Background

Delayed graft function (DGF) occurs in 10% to 40% of deceased donor kidney transplantations, and leads to prolonged hospitalization, higher costs of transplantation, and increased complexity of management of immunosuppressive drugs. The ability to predict DGF at the time of the transplant offer might help in clinical decision making, such as declining the offer, selecting a recipient who would have a lower DGF risk, or modifying the transplantation strategy. Three predictive scoring systems for DGF were previously developed and published (Irish *et al*, Jeldres *et al* and Chapal *et al*). However, since these scores were not validated in an external study population, we decided to analyse the performance of these three scoring systems in a single centre cohort of 247 consecutive kidney transplant recipients at our institution between 2003 and 2013.

Research frontiers

Three different scoring systems for the prediction of DGF have been developed and validated in the past in respectively well-defined study populations, specific for each study. However, these scoring systems were never validated in an external study population (*i.e.*, different from the initial study population). To explore the validity of these three predictive models, we retrospectively analysed their performance in a cohort of 247 consecutive kidney transplant recipients at our institution.

Innovations and breakthroughs

DGF occurred in 15% of this study population. Only the Irish calculator provided an acceptable level of prediction for DGF with an AUC of the ROC curve of 0.69. However, at the level of the individual patient the calculated risk of DGF overlapped very widely, and therefore this predictive score was not useful in clinical decision making in our study population.

Applications

Based on the reported literature and on our data, we conclude that predictive models for DGF are performant in the population in which they were derived, but these models require additional validation in an external study population.

Terminology

DGF: Delayed graft function; AUC: Area under the curve; ROC: Receiver operator curve; C index: The index of concordance is a "global" index for validating the predictive ability of an algorithm (*e.g.*, for the occurrence of DGF); Nomogram: Is a prediction tool based on information from large numbers of patients. Predictive data are put in a mathematical model that enables to calculate a hypothetical outcome measure.

Peer-review

It is very well-conducted study with some interesting findings, mostly pointed out that we still cannot predict with accuracy the development of DGF. The study design and method, and statistical analysis were all well-thought and accurately followed throughout the paper.

REFERENCES

1 **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]

2 **Siedlecki A**, Irish W, Brennan DC. Delayed graft function in the kidney transplant. *Am J Transplant* 2011; **11**: 2279-2296 [PMID: 21929642 DOI: 10.1111/j.1600-6143.2011.03754.x]

3 **Yarlagadda SG**, Coca SG, Garg AX, Doshi M, Poggio E, Marcus RJ, Parikh CR. Marked variation in the definition and diagnosis of delayed graft function: a systematic review. *Nephrol Dial Transplant* 2008; **23**: 2995-3003 [PMID: 18408075 DOI: 10.1093/ndt/gfn158]

4 **Mikhalski D**, Wissing KM, Ghisdal L, Broeders N, Touly M, Hoang AD, Loi P, Mboti F, Donckier V, Vereerstraeten P, Abramowicz D. Cold ischemia is a major determinant of acute rejection and renal graft survival in the modern era of immunosuppression. *Transplantation* 2008; **85**: S3-S9 [PMID: 18401260 DOI: 10.1097/TP.0b013e318169c29e]

5 **Kayler LK**, Srinivas TR, Schold JD. Influence of CIT-induced DGF on kidney transplant outcomes. *Am J Transplant* 2011; **11**: 2657-2664 [PMID: 22051325 DOI: 10.1111/j.1600-6143.2011.03817.x]

6 **Irish WD**, McCollum DA, Tesi RJ, Owen AB, Brennan DC, Bailly JE, Schnitzler MA. Nomogram for predicting the likelihood of delayed graft function in adult cadaveric renal transplant recipients. *J Am Soc Nephrol* 2003; **14**: 2967-2974 [PMID: 14569108]

7 **Pieringer H**, Biesenbach G. Risk factors for delayed kidney function and impact of delayed function on patient and graft survival in adult graft recipients. *Clin Transplant* 2005; **19**: 391-398 [PMID: 15877804 DOI: 10.1111/j.1399-0012.2005.00360.x]

8 **Kaisar MO**, Johnson DW. Validation of a nomogram for predicting the likelihood of delayed graft function in Australian adult deceased donor renal transplant recipients. *Nephrology* (Carlton) 2006; **11**: 78 [PMID: 16509938 DOI: 10.1111/j.1440-1797.2006.00532.x]

9 **Irish WD**, Ilsley JN, Schnitzler MA, Feng S, Brennan DC. A risk prediction model for delayed graft function in the current era of deceased donor renal transplantation. *Am J Transplant* 2010; **10**: 2279-2286 [PMID: 20883559 DOI: 10.1111/j.1600-6143.2010.03179.x]

10 **Jeldres C**, Cardinal H, Duclos A, Shariat SF, Suardi N, Capitanio U, Hébert MJ, Karakiewicz PI. Prediction of delayed graft function after renal transplantation. *Can Urol Assoc J* 2009; **3**: 377-382 [PMID: 19829730]

11 **Chapal M**, Le Borgne F, Legendre C, Kreis H, Mourad G, Garrigue V, Morelon E, Buron F, Rostaing L, Kamar N, Kessler M, Ladrière M, Soulillou JP, Launay K, Daguin P, Offredo L, Giral M, Foucher Y. A useful scoring system for the prediction and management of delayed graft function following kidney transplantation from cadaveric donors. *Kidney Int* 2014; **86**: 1130-1139 [PMID: 24897036 DOI: 10.1038/ki.2014.188]

12 **Port FK**, Bragg-Gresham JL, Metzger RA, Dykstra DM, Gillespie BW, Young EW, Delmonico FL, Wynn JJ, Merion RM, Wolfe RA, Held PJ. Donor characteristics associated with reduced graft survival: an approach to expanding the pool of kidney donors. *Transplantation* 2002; **74**: 1281-1286 [PMID: 12451266 DOI: 10.1097/01.TP.0000034060.18738.0B]

13 **Nyberg SL**, Matas AJ, Kremers WK, Thostenson JD, Larson TS, Prieto M, Ishitani MB, Sterioff S, Stegall MD. Improved scoring system to assess adult donors for cadaver renal transplantation. *Am J Transplant* 2003; **3**: 715-721 [PMID: 12780563]

14 **Schold JD**, Kaplan B, Baliga RS, Meier-Kriesche HU. The broad spectrum of quality in deceased donor kidneys. *Am J Transplant* 2005; **5**: 757-765 [PMID: 15760399 DOI: 10.1111/j.1600-6143.2005.00770.x]

15 **Rao PS**, Schaubel DE, Guidinger MK, Andreoni KA, Wolfe RA, Merion RM, Port FK, Sung RS. A comprehensive risk quantification score for deceased donor kidneys: the kidney donor risk index. *Transplantation* 2009; **88**: 231-236 [PMID: 19623019 DOI: 10.1097/TP.0b013e3181ac620b]

16 **Grossberg JA**, Reinert SE, Monaco AP, Gohh R, Morrissey PE. Utility of a mathematical nomogram to predict delayed graft function: a single-center experience. *Transplantation* 2006; **81**: 155-159 [PMID: 16436956 DOI: 10.1097/01.tp.0000188621.54448.c8]

17 **Moore J**, Tan K, Cockwell P, Krishnan H, McPake D, Ready A, Mellor S, Hamsho A, Ball S, Lipkin G, Borrows R. Predicting early renal allograft function using clinical variables. *Nephrol Dial Transplant* 2007; **22**: 2669-2677 [PMID: 17556406 DOI: 10.1093/ndt/gfm249]

18 **Rodrigo E**, Miñambres E, Ruiz JC, Ballesteros A, Piñera C, Quintanar J, Fernández-Fresnedo G, Palomar R, Gómez-Alamillo C, Arias M. Prediction of delayed graft function by means of a novel web-based calculator: a single-center experience. *Am J Transplant* 2012; **12**: 240-244 [PMID: 22026730 DOI: 10.1111/j.1600-6143.2011.03810.x]

P- Reviewer: Hilmi I, Sheashaa HA, Taheri S **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 October 24; 7(5): 269-275

DOI: 10.5500/wjt.v7.i5.269

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Observational Study

Graft loss among renal-transplant recipients with early reduction of immunosuppression for BK viremia

Marwan M Azar, Roland Assi, Aziz K Valika, David B Banach, Isaac E Hall, Marie-Louise Landry, Maricar F Malinis

Marwan M Azar, Department of Pathology, Section of Microbiology, Massachusetts General Hospital, Boston, MA 02145, United States

Marwan M Azar, Maricar F Malinis, Section of Infectious Diseases, Yale School of Medicine, New Haven, CT 06510, United States

Roland Assi, Maricar F Malinis, Department of Surgery, Yale University School of Medicine, New Haven, CT 06510, United States

Aziz K Valika, Adventist Health Partners, Chicago, IL 60521, United States

David B Banach, Division of Infectious Diseases, University of Connecticut School of Medicine, Farmington, CT 06032, United States

Isaac E Hall, Division of Hypertension and Nephrology, University of Utah School of Medicine, Dalt Lake City, UT 84132, United States

Marie-Louise Landry, Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06510, United States

Author contributions: Azar MM and Malinis MF analyzed data; Azar MM drafted manuscript; Assi R, Valika AK, Banach DB, Hall IE, Landry ML and Malinis MF revised and edited manuscript; all authors contributed significantly to this work.

Institutional review board statement: The Yale University Institutional Review Board approved this study and all procedures conducted were in accordance with the Helsinki Declaration of 1975.

Conflict-of-interest statement: The authors have no conflicts of interest.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Marwan M Azar, MD, Microbiology Fellow, Department of Pathology, Section of Microbiology, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02145, United States. mmazar@mgh.harvard.edu
Telephone: +1-617-6434393
Fax: +1-888-3244639

Received: February 12, 2017
Peer-review started: February 15, 2017
First decision: April 17, 2017
Revised: June 28, 2017
Accepted: August 2, 2017
Article in press: August 2, 2017
Published online: October 24, 2017

Abstract

AIM

To review the incidence of graft loss and acute rejection among renal transplant recipients with early reduction of immunosuppression for BK viremia.

METHODS

We performed a retrospective analysis of consecutive *de-novo* kidney-only transplants from January 2009 to December 2012 to evaluate the incidence of Polyomavirus associated nephropathy (PyVAN). Recipient plasma was screened for BKV DNA *via* quantitative polymerase chain reaction (PCR) at months 1, 3, 6, 9 and 12 post-transplant and on worsening graft function.

Immunosuppression was reduced at ⩾ 3-log copies/mL. Those with viremia of ⩾ 4-log copies/mL (presumptive PyVAN) underwent renal transplant biopsy. Presumptive PyVAN (PP) and definitive PyVAN (DP; biopsy-proven) were treated by immunosuppression reduction (IR) only.

RESULTS

Among 319 kidney transplant recipients, the median age was 53 years (range 19-83), 65.8% were male, and 58.9% were white. Biopsy-proven acute rejection was found in 18.5% within 0-168 wk. Death-censored graft loss occurred in 5.3% (n = 17) and graft loss attributable to PyVAN was 0.6% (n = 2). Forty-seven patients were diagnosed with PP (14.7%) and 18 (5.6%) with DP. Graft loss among participants with PyVAN (8.5%) and those without (4.8%) was not significantly different. Deceased donor kidney transplantation (OR = 2.3, 95%CI = 1.1-4.6) and AR (OR = 2.3, 95%CI = 1.2-4.7) were associated with PyVAN in the multivariate analysis. BK viremia between 3 and 4-log copies/mL occurred in 27 patients, all of whom underwent IR. Of these, 16 (59%) never developed PyVAN while 11 (41%) developed PyVAN (4 DP, 7 PP) within a range of 11-39 wk.

CONCLUSION

Instituting an early reduction of immunosuppression, in the absence of adjunctive antivirals, is effective at preventing PyVAN and may be associated with a lower incidence of graft-loss without a reciprocal increase in the incidence of acute rejection.

Key words: BK virus; Renal transplant; Screening; PyVAN; Prevention; Graft loss

Core tip: The authors describe results of a retrospective study of 319 renal transplant recipients who underwent reduction of immunosuppression for BK viremia at a BK viral of ⩾ 3-log copies/mL. Instituting early reduction of immunosuppression in the absence of adjunctive antivirals was effective in reducing the incidence of graft loss due to Polyoma-virus associated nephropathy (PyVAN) without a reciprocal increase in acute rejection. Our study also emphasizes that efforts to implement universal BK virus polymerase chain reaction assay standards recently developed by the World Health Organization are key in establishing a preventative strategy for PyVAN that is widely applicable and highly effective.

Azar MM, Assi R, Valika AK, Banach DB, Hall IE, Landry ML, Malinis MF. Graft loss among renal-transplant recipients with early reduction of immunosuppression for BK viremia. *World J Transplant* 2017; 7(5): 269-275 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i5/269.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i5.269

INTRODUCTION

BK virus (BKV) is a polyomavirus that causes widespread sub-clinical infection at a young age and subsequently establishes long-term latency in cells of the renal and urinary systems. In recipients of renal allograft transplants and allogeneic hematopoietic stem cell transplants, high-level BKV replication may lead to overt clinical disease. BK-polyoma virus associated nephropathy (PyVAN) is a major complication of kidney transplantation, occurring in 1%-10% of renal transplant recipients[1,2]. PyVAN is directly associated with graft failure[3,4] due to progressive interstitial nephritis and indirectly linked to allograft rejection due to immunosuppression reduction (IR), which is the cornerstone of PyVAN treatment[5]. Guidelines currently recommend prospective screening for BKV reactivation post-transplantation, by using urine cytology to detect decoy cells or testing for high-level BK viruria and/or viremia. In the event of a sustained BK viremia of ≥ 4-log copies/mL for more than 3 wk, a renal biopsy is recommended to confirm the diagnosis of PyVAN by demonstrating polyomavirus cytopathic changes with confirmation by immunohistochemical staining[6]. In addition, a prompt reduction of immunosuppression is critical in an attempt to abrogate the full-fledged manifestations of the disease. Although agents with anti-BK activity such as leflunomide[7], cidofovir[8] and quinolones[9] have been used[10], randomized controlled trials proving their efficacy are lacking.

The positive predictive value of BK viremia at a cut-off of 7×10^3 copies/mL (approximately 4 log copies/mL) has been estimated at 50% to 60% for detecting proven PyVAN within 2 to 6 wk but rises to 90% when a threshold of 6-log copies/mL is implemented[1,11,12]. A lower threshold of 3-log copies/mL may increase the sensitivity, leading to the identification of more cases, and earlier in the natural course when intervention may be more effective and graft loss more likely to be averted. In this paper, we aim to assess the incidence of PyVAN and graft loss in a single transplant center while implementing a reduction of immunosuppression at BKV loads of ⩾ 3-log BKV copies/mL.

MATERIALS AND METHODS

We performed a retrospective analysis of consecutive de-novo kidney transplants at Yale-New Haven Hospital (YNHH), who underwent screening for PyVAN screening and prevention, from January 2009 to December 2012. The Yale University Institutional Review Board approved this study and all procedures conducted were in accordance with the Helsinki Declaration of 1975. Individuals included in the study were above the age of 18 years and underwent primary kidney-only transplant. Medical records were reviewed for data on demographics,

comorbidities, and transplant parameters including type of transplant (deceased or living donor), CMV donor and recipient serostatus, induction and maintenance immunosuppression, presence of delayed graft function (DGF), biopsy-proven acute rejection (AR), graft loss and its etiology, last follow-up visit, deaths, BK viral load (copies/mL) and biopsy-proven PyVAN. Graft loss was censored for episodes of death with a functioning graft.

Presumptive PyVAN (PP) was defined as sustained BK viremia ⩾ 4-log copies/mL while definitive PyVAN (DP) required cytopathic changes on renal biopsy that were confirmed by positive BKV immunohistochemistry[6]. Renal allograft rejection was graded in accordance with the BANFF working classification of renal allograft pathology[13]. DGF was defined as acute renal failure requiring dialysis within 7 d of transplantation. Graft failure was defined as chronic allograft nephropathy leading to the resumption of chronic renal replacement therapy. Primary outcomes included both presumptive and definitive PyVAN while the secondary outcomes were graft survival and acute rejection.

The YNHH kidney transplant program has been active since 1967 and performs approximately 100 kidney transplantations annually. The standard maintenance immunosuppressive regimen consists of tacrolimus, mycophenolate mofetil and low dose prednisone (5-10 mg daily). The target tacrolimus trough level for the first 30 d post-transplant was 8-10 ng/mL and 5-7 ng/mL thereafter. As part of the institutional protocol for PyVAN screening and prevention, transplant recipient plasma is screened for BKV DNA *via* quantitative PCR. For the first two years of the study, an NIH-developed, real-time BKV PCR assay targeting the viral T antigen gene was used[14]. Due to concerns about potential under-quantitation of some BKV subtypes, a multiplex real-time PCR assay developed at the University of Washington (UW) that targets both VP1 and T genes using two primer sets and three probes was implemented in December 2010[15].

Per protocol, a serum BKV DNA viral load (VL) is obtained at months 1, 3, 6, 9 and 12 post-transplant and in case of worsening graft function. A BKV DNA VL between 3 and 3.99 log copies/mL prompted a 50% dose reduction of mycophenolate mofetil, a reduced target tacrolimus trough level of 5 ng/mL and monthly plasma BKVL until negative. Additionally, mycophenolate mofetil was discontinued and a renal biopsy was with immunohistochemical staining was performed if the serum BKV VL was above ⩾ 4-log copies/mL PP and DP were treated by reduction of immunosuppression, without adjunctive anti-viral treatment.

Statistical analysis

Statistical analysis was performed using SPSS software, version 16.0.0.0. In univariate analyses, χ^2 and Fisher's exact test (when appropriate) were used to evaluate categorical variables and Mann-Whitney *U* test was used for continuous variables. Predictors of PyVAN were identified using a multivariate logistic regression model. Only variables with a *P*-value < 0.10 on univariate analysis were entered into a stepwise multivariate logistic regression model to identify factors independently associated with Presumptive PyVAN. All tests were double-tailed, with an assumed type 1 error risk a equal to 5%. Kaplan-Meier survival curves were plotted using GraphPad Prism version 6.03 (GraphPad Software, San Diego, CA, United States).

RESULTS

A total of 330 primary kidney transplant recipients were identified and were followed for a median time of 42 ± 14.7 mo. BK screening data on 11 patients was unavailable and thus they were excluded, leaving 319 patients available for analysis. The median age was 53 years (range 19-83), 65.8% were male, 58.9% were white, and 27.0% had diabetes mellitus. A CMV D+/R- serostatus was present in 18.2% of transplants and 54.5% of recipients underwent a deceased-donor kidney transplantation (DDKT). Induction immunosuppressive therapy consisted of basiliximab (44.8%), anti-thymocyte globulin (37.0%) or daclizumab (17.6%). Maintenance immunosuppressive therapy included both a calcineurin inhibitor and mycophenolate mofetil in 95% of cases and 95% received steroids. Biopsy-proven rejection was found in 18.5% (*n* = 59) of transplant recipients within 0-168 wk. Graft loss occurred in 5.3% (*n* = 17) and PyVAN-associated graft los occurred in 0.6% (*n* = 2). Causes of graft loss included: AR (*n* = 7), antibody-mediated chronic rejection (*n* = 2), PyVAN (*n* = 2), CMV nephropathy (*n* = 1), hypoplastic kidney disease (*n* = 1), ureteral obstruction (*n* = 1), renal graft vein thrombosis (*n* = 1) and unknown cause (*n* = 2). Death ensued in 6.6% (*n* = 21) of the sample. A detailed list of demographics is found in Table 1.

BK viremia of ⩾ 3-log copies/mL was detected in 63 (19.7%) recipients. Of these, 47 (14.7%) were subsequently diagnosed with PP at a median time of 25 wk from initial screening. A renal biopsy was performed in 34 of these recipients and 18 (5.6% of the original sample) were confirmed to have DP. Two patients with DP progressed to graft failure and 4 developed AR within 90 d after reduction of immunosuppression.

The majority of the 319 patients included in the study (85.3%) never developed PyVAN. Time to first BK viremia was 190 d in patients with PyVAN and 235 d in those without. Graft loss occurred in 8.5% of patients with PyVAN vs. 4.8% of those without. Graft survival for 1-year, 3-year and 5-years were 99%, 95% and 92% respectively. A Kaplan-Meier curve of graft survival over time for recipients with and without PP (Figure 1) showed that survival was not significantly different between groups (logrank *P* = 0.93).

In a univariate analysis of recipients diagnosed with PP (*n* = 47) compared to recipients without PyVAN (*n* = 272), black race, DDKT and AR were significantly associated with PyVAN (*P* < 0.10). In a subsequent multivariate analysis, only DDKT (OR = 2.24; 95%CI

Table 1 Demographics and outcomes *n* (%)

Variable	All sample (*n* = 319)	PyVAN negative (*n* = 272)	PyVAN positive (*n* = 47)
Age (mean, yr)	53	51	53.1
Male	210 (65.8)	177 (65.1)	33 (70.2)
Black	86 (27.0)	67 (24.6)	19 (40.4)
Diabetes mellitus	86 (27.0)	71 (26.1)	15 (32.0)
CMV D+/R-	58 (18.2)	49 (18.0)	9 (19.1)
DDKT	174 (54.5)	140 (51.5)	34 (72.3)
Induction immunosuppression			
Thymoglobulin	118 (37.0)	97 (35.7)	21 (44.7)
Basiliximab	143 (44.8)	124 (45.6)	19 (40.4)
Daclizumab	56 (17.6)	50 (18.4)	6 (12.8)
Delayed graft function	58 (18.2)	47 (17.3)	11 (23.4)
Acute rejection	59 (18.5)	44 (16.2)	15 (32.0)
Graft loss	17 (5.3)	13 (4.8)	4 (8.5)
Death	21 (6.6)	15 (5.5)	6 (12.8)

DDKT: Deceased-donor kidney transplantation; PyVAN: Polyoma-virus associated nephropathy.

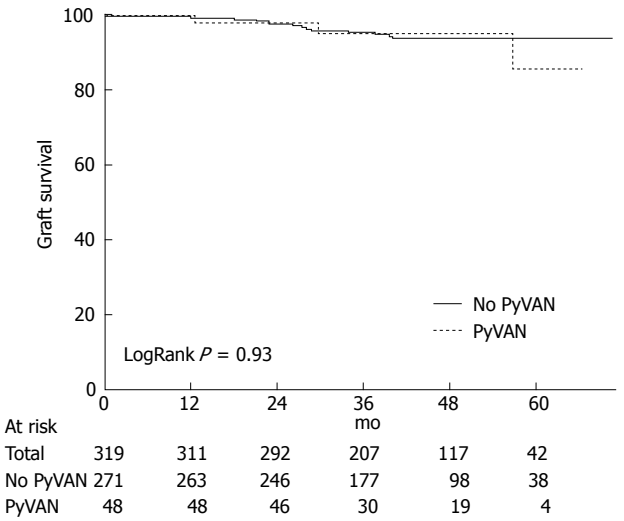


Figure 1 Kaplan-Meier Survival curve showing graft survival over time for recipients with and without presumptive polyoma-virus associated nephropathy. PyVAN: Polyoma-virus associated nephropathy.

= 1.1-4.54) and AR (OR = 2.42; 95%CI = 1.19-4.29) were significantly associated with PyVAN ($P < 0.05$). In this model, PyVAN was not associated with delayed graft function, graft loss or increased mortality. A full description covariates included in the fit-model is found in Table 2.

While the majority of patients with high-level viremia were found to have an initial BKVL above 4 log copies/mL, an initial BK viral load between 3 and 4-log copies/mL was reported in 27 transplant recipients, all of whom underwent reduction of immunosuppression, without administration of adjunctive anti-viral therapy. Of these, 16 (59%) never developed PyVAN while 11 (41%) developed PyVAN within a range of 11-39 wk. Among the 11 recipients with PyVAN, 4 were proven by renal biopsy and 7 were presumptive. Two of 27 recipients developed AR and none developed graft loss.

Since the BK PCR assay changed midway through the study, we compared the incidence of PyVAN when using the NIH assay (January 2009 to December 2010) to the incidence when using the UW assay (January 2011 to December 2012). In a univariate chi-square analysis of all recipients with first BK viremia, 17/33 (52%) had PyVAN before the assay change versus 30/59 (51%) after the change ($P = 0.95$).

In addition, we reviewed BKVL data of patients with PP to evaluate for adherence or deviation from the set protocol for post-transplant viral load screening. Among 47 patients with PP, 16 patients underwent screening beyond the recommended interval during the study period and were found to have high-level viremia on belated screening. For 3 patients, screening was done within 10 d of recommended time point. A summary of patients in whom protocol deviation occurred is found in Table 3.

DISCUSSION

Though established guidelines recommend a reduction of immunosuppression at a sustained BK viremia of ⩾ 4-log copies/mL, studies vary significantly with regards to the implemented threshold. Cutoffs of any viremia[16,17], of ⩾ 3-log copies/mL[18] and of ⩾ 4-log copies/mL[19] have been used with varying rates of PyVAN, graft loss and acute rejection. The incidence of PP in this study, using a lower threshold (⩾ 3-log copies/mL) of BK viremia for reduction of immunosuppression, was consistent with previously reported rates. In contrast to several investigations conducted in the last decade, which reported rates of graft loss of 15%-60% within 5 years of transplant, our incidence of graft loss, was 5.3%[5,20-23]. The incidence of PyVAN-associated graft loss in this study (0.6%) was commensurate with more recently published data from 2009-2013, in which BK associated graft loss ranged from 0%-0.85%[1,19,24-26]. Early reduction of immunosuppression in the setting of BK viremia, though potentially associated with decreased rates of graft loss due to BK nephropathy, carries the potential for increased rates of acute rejection. However, in this study, the incidence of acute rejection (18.5%) was also in keeping with previously reported rates[19,25], suggesting that early reduction of

Table 2 Predictors of polyoma-virus associated nephropathy

Predictor	Univariate analysis			Multivariate analysis		
	PyVAN negative (n = 272)	PyVAN positive (n = 47)	P value	OR	CI	P value
Age (mean, yr)	51	53.1	0.343			
Male	177	33	0.493			
Black race	67	19	0.024[a]	1.68	0.86-3.31	0.13
Diabetes mellitus	71	15	0.407			
CMV D+/R-	49	9	0.839			
DDKT	140	34	0.008[a]	2.24	1.1-4.54	0.03[a]
Thymoglobulin	97	21	0.237			
Basiliximab	124	19	0.511			
Daclizumab	50	6	0.412			
Delayed graft function	47	11	0.315			
Acute rejection	44	15	0.010[a]	2.42	1.19-4.29	0.02[a]
Graft loss	13	4				
Death	16	6	0.112			

[a]P < 0.05. DDKT: Deceased-donor kidney transplantation; PyVAN: Polyoma-virus associated nephropathy.

Table 3 Protocol deviation among patients with presumptive polyoma-virus associated nephropathy[1]

Patient	Time point of protocol deviation post-transplant	Target days between serial screening	Actual days between serial screening	BKVL change (copies/mL)
1	Month 1 to month 3	60 d	100 d	ND to 1065190
2	Month 1 to month 3	60 d	138 d	ND to 17478
3	Month 9 to month 12	90 d	214 d	< 1000 to 1076120
4	Month 6 to month 9	90 d	393 d	< 1000 to 1269650
5	Month 6 to month 9	90 d	113 d	ND to 57982
6	Month 1 to month 3	60 d	134 d	ND to 392527
7	Month 3 to month 6	90 d	133 d	ND to 627218
8	Month 3 to month 6	90 d	108 d	ND to 82354
9	DOT to month 1	30 d	121 d	157939 at month 1
10	Month 3 to month 6	90 d	137 d	ND to 74389
11	Month 1 to month 3	60 d	179 d	ND to 28592
12	Month 1 to month 3	60 d	94 d	ND to 39000
13	DOT to month 1	30 d	58 d	17558 at month 1

[1]Three patients with < 10 d of deviation from protocol were not included. ND: Not detectable.

immunosuppression may not necessarily increase the risk of acute rejection.

In the past decade, there has been a steady trend towards decreased rates of graft loss. This is thought to be the result of improved diagnostic tools including immunostaining and PCR, which better differentiate virus-induced nephropathy from acute rejection, as well as targeted interventions to promptly identify BK viremia and reduce immunosuppression earlier[27]. A multitude of factors including the potency of induction and maintenance immunosuppressive regimens, demographic differences such as in age and race, frequency of BK viral load monitoring and use of adjunctive anti-virals may account for observed differences. The heterogeneity of these studies is further compounded by variation in the sensitivity, lower limit of detection of the BK virus PCR assay and most importantly a lack of equivalence of quantitation between different assays. Complicating matters is the presence of multiple viral subtypes, some of which (serotypes 3 and 4) are particularly prone to under-quantitation. In this study, we found no statistical difference in the number of patients with any viremia when sequentially comparing two different molecular assays but results from laboratories using different assays have been shown to vary significantly, even when performed on the same sample[15]. Since specific BK VL cutoffs are used to trigger interventions, assay variability is a critical issue and may indeed explain the variability in thresholds used across different transplant centers. A BK PCR standard that can be applied across laboratories is paramount in implementing a uniform BK viremia threshold for reduction of immunosuppression. In 2015, the World Health Organization (WHO) took steps to establish an international standard for BKV DNA nucleic acid amplification technique-based assays, using purified virions from BKV infected cell cultures[28]. This standard, however, has not yet been widely adopted and additional *in-vitro* verification data and in-vivo clinical data are needed to ascertain its performance characteristics. Until then, performing serial testing on individual patients using the same assay within the same laboratory, eschewing over-interpretation of small viral load changes as biologically important and establishing center-specific viral load cutoffs to guide clinical decision making in local

patient populations will facilitate the interpretation of current BK viral load testing.

Certainly, adequate implementation of screening protocols is another critical factor in optimizing preventative strategies. In our study, a substantial number of patients with presumptive PyVAN did not adhere to the scheduled BKVL screening time-points. Strict adherence to screening protocol is likely to reduce the incidence of PyVAN by identifying viremia earlier and allowing for early interventions.

Instituting an early reduction of immunosuppression at ⩾ 3-log copies/mL, in the absence of adjunctive antivirals, was effective at preventing PyVAN in our center and may be associated with a lower incidence of graft-loss without an increased rate of acute rejection compared to published data. However, efforts to implement the WHO BK standard will be key in establishing a universal preventive strategy for PyVAN that is both highly effective and widely applicable.

COMMENTS

Background

Polyoma virus associated nephropathy (PyVAN) caused by BK virus (BKV) is a major complication occurring in 1%-10% of renal transplant recipients that is directly associated with graft loss and indirectly associated with graft rejection.

Research frontiers

Guidelines currently recommend prospective screening for BKV reactivation post-transplantation, with reduction of immunosuppression at > 4-log copies/mL of BK virus. Additional research is needed to determine the best screening strategy.

Innovations and breakthroughs

The present study describes results of early reduction of immunosuppression (at ⩾ 3-log copies/mL) in the absence of antivirals. This strategy effective at preventing PyVAN and was associated with a lower incidence of graft-loss without a reciprocal increase in the incidence of acute rejection.

Applications

Early reduction of immunosuppression should be considered as a strategy for prospective screening for BKV reactivation post-transplantation.

Terminology

PyVAN is a disease of the kidney that results from reactivation of BK virus in the setting of immune suppression, leading to cytopathic effect on renal tubular cells and secondary inflammation.

Peer-review

The information provided by the authors adds to the existing knowledge on the subject and will prove useful to the transplant community.

REFERENCES

1 **Hirsch HH**, Knowles W, Dickenmann M, Passweg J, Klimkait T, Mihatsch MJ, Steiger J. Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. *N Engl J Med* 2002; **347**: 488-496 [PMID: 12181403 DOI: 10.1056/NEJMoa020439]

2 **Ramos E**, Drachenberg CB, Portocarrero M, Wali R, Klassen DK, Fink JC, Farney A, Hirsch H, Papadimitriou JC, Cangro CB, Weir MR, Bartlett ST. BK virus nephropathy diagnosis and treatment: experience at the University of Maryland Renal Transplant Program. *Clin Transpl* 2002: 143-153 [PMID: 12971444]

3 **Hirsch HH**. Polyomavirus BK nephropathy: a (re-)emerging complication in renal transplantation. *Am J Transplant* 2002; **2**: 25-30 [PMID: 12095052 DOI: 10.1034/j.1600-6143.2002.020106.x]

4 **Randhawa PS**, Demetris AJ. Nephropathy due to polyomavirus type BK. *N Engl J Med* 2000; **342**: 1361-1363 [PMID: 10793170 DOI: 10.1056/NEJM200005043421809]

5 **Ramos E**, Drachenberg CB, Papadimitriou JC, Hamze O, Fink JC, Klassen DK, Drachenberg RC, Wiland A, Wali R, Cangro CB, Schweitzer E, Bartlett ST, Weir MR. Clinical course of polyoma virus nephropathy in 67 renal transplant patients. *J Am Soc Nephrol* 2002; **13**: 2145-2151 [PMID: 12138148 DOI: 10.1097/01.ASN.0000023435.07320.81]

6 **Hirsch HH**, Randhawa P; AST Infectious Diseases Community of Practice. BK polyomavirus in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 179-188 [PMID: 23465010 DOI: 10.1111/ajt.12110]

7 **Josephson MA**, Gillen D, Javaid B, Kadambi P, Meehan S, Foster P, Harland R, Thistlethwaite RJ, Garfinkel M, Atwood W, Jordan J, Sadhu M, Millis MJ, Williams J. Treatment of renal allograft polyoma BK virus infection with leflunomide. *Transplantation* 2006; **81**: 704-710 [PMID: 16534472 DOI: 10.1097/01.tp.0000181149.76113.50]

8 **Wu SW**, Chang HR, Lian JD. The effect of low-dose cidofovir on the long-term outcome of polyomavirus-associated nephropathy in renal transplant recipients. *Nephrol Dial Transplant* 2009; **24**: 1034-1038 [PMID: 19059933 DOI: 10.1093/ndt/gfn675]

9 **Sharma BN**, Li R, Bernhoff E, Gutteberg TJ, Rinaldo CH. Fluoroquinolones inhibit human polyomavirus BK (BKV) replication in primary human kidney cells. *Antiviral Res* 2011; **92**: 115-123 [PMID: 21798289 DOI: 10.1016/j.antiviral.2011.07.012]

10 **Wright AJ**, Gill JS. Strategies to prevent BK virus infection in kidney transplant recipients. *Curr Opin Infect Dis* 2016; **29**: 353-358 [PMID: 27257795 DOI: 10.1097/QCO.0000000000000278]

11 **Bressollette-Bodin C**, Coste-Burel M, Hourmant M, Sebille V, Andre-Garnier E, Imbert-Marcille BM. A prospective longitudinal study of BK virus infection in 104 renal transplant recipients. *Am J Transplant* 2005; **5**: 1926-1933 [PMID: 15996241 DOI: 10.1111/j.1600-6143.2005.00934.x]

12 **Dall A**, Hariharan S. BK virus nephritis after renal transplantation. *Clin J Am Soc Nephrol* 2008; **3** Suppl 2: S68-S75 [PMID: 18309005 DOI: 10.2215/CJN.02770707]

13 **Solez K**, Axelsen RA, Benediktsson H, Burdick JF, Cohen AH, Colvin RB, Croker BP, Droz D, Dunnill MS, Halloran PF. International standardization of criteria for the histologic diagnosis of renal allograft rejection: the Banff working classification of kidney transplant pathology. *Kidney Int* 1993; **44**: 411-422 [PMID: 8377384 DOI: 10.1038/ki.1993.259]

14 **Ryschkewitsch C**, Jensen P, Hou J, Fahle G, Fischer S, Major EO. Comparison of PCR-southern hybridization and quantitative real-time PCR for the detection of JC and BK viral nucleotide sequences in urine and cerebrospinal fluid. *J Virol Methods* 2004; **121**: 217-221 [PMID: 15381359 DOI: 10.1016/j.jviromet.2004.06.021]

15 **Hoffman NG**, Cook L, Atienza EE, Limaye AP, Jerome KR. Marked variability of BK virus load measurement using quantitative real-time PCR among commonly used assays. *J Clin Microbiol* 2008; **46**: 2671-2680 [PMID: 18562587 DOI: 10.1128/JCM.00258-08]

16 **Knight RJ**, Gaber LW, Patel SJ, DeVos JM, Moore LW, Gaber AO. Screening for BK viremia reduces but does not eliminate the risk of BK nephropathy: a single-center retrospective analysis. *Transplantation* 2013; **95**: 949-954 [PMID: 23545506 DOI: 10.1097/TP.0b013e31828423cd]

17 **Hardinger KL**, Koch MJ, Bohl DJ, Storch GA, Brennan DC. BK-virus and the impact of pre-emptive immunosuppression reduction: 5-year results. *Am J Transplant* 2010; **10**: 407-415 [PMID: 20055811 DOI: 10.1111/j.1600-6143.2009.02952.x]

18 **Schaub S**, Hirsch HH, Dickenmann M, Steiger J, Mihatsch MJ, Hopfer H, Mayr M. Reducing immunosuppression preserves allograft function in presumptive and definitive polyomavirus-associated nephropathy. *Am J Transplant* 2010; **10**: 2615-2623 [PMID: 21114642 DOI: 10.1111/j.1600-6143.2010.03310.x]

19 **Sood P**, Senanayake S, Sujeet K, Medipalli R, Zhu YR, Johnson CP, Hariharan S. Management and outcome of BK viremia in renal transplant recipients: a prospective single-center study. *Transplantation* 2012; **94**: 814-821 [PMID: 23018881 DOI: 10.1097/TP.0b013e31826690c6]

20 **Brennan DC**, Agha I, Bohl DL, Schnitzler MA, Hardinger KL, Lockwood M, Torrence S, Schuessler R, Roby T, Gaudreault-Keener M, Storch GA. Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. *Am J Transplant* 2005; **5**: 582-594 [PMID: 15707414 DOI: 10.1111/j.1600-6143.2005.00742.x]

21 **Vasudev B**, Hariharan S, Hussain SA, Zhu YR, Bresnahan BA, Cohen EP. BK virus nephritis: risk factors, timing, and outcome in renal transplant recipients. *Kidney Int* 2005; **68**: 1834-1839 [PMID: 16164661 DOI: 10.1111/j.1523-1755.2005.00602.x]

22 **Wadei HM**, Rule AD, Lewin M, Mahale AS, Khamash HA, Schwab TR, Gloor JM, Textor SC, Fidler ME, Lager DJ, Larson TS, Stegall MD, Cosio FG, Griffin MD. Kidney transplant function and histological clearance of virus following diagnosis of polyomavirus-associated nephropathy (PVAN). *Am J Transplant* 2006; **6**: 1025-1032 [PMID: 16611340 DOI: 10.1111/j.1600-6143.2006.01296.x]

23 **Drachenberg CB**, Papadimitriou JC, Wali R, Nogueira J, Mendley S, Hirsch HH, Cangro CB, Klassen DK, Weir MR, Bartlett ST, Ramos E. Improved outcome of polyoma virus allograft nephropathy with early biopsy. *Transplant Proc* 2004; **36**: 758-759 [PMID: 15110653 DOI: 10.1016/j.transproceed.2004.03.040]

24 **Koukoulaki M**, Grispou E, Pistolas D, Balaska K, Apostolou T, Anagnostopoulou M, Tseleni-Kotsovili A, Hadjiconstantinou V, Paniara O, Saroglou G, Legakis N, Drakopoulos S. Prospective monitoring of BK virus replication in renal transplant recipients. *Transpl Infect Dis* 2009; **11**: 1-10 [PMID: 18811631 DOI: 10.1111/j.1399-3062.2008.00342.x]

25 **Alméras C**, Vetromile F, Garrigue V, Szwarc I, Foulongne V, Mourad G. Monthly screening for BK viremia is an effective strategy to prevent BK virus nephropathy in renal transplant recipients. *Transpl Infect Dis* 2011; **13**: 101-108 [PMID: 21371220 DOI: 10.1111/j.1399-3062.2011.00619.x]

26 **Thakur R**, Arora S, Nada R, Minz M, Joshi K. Prospective monitoring of BK virus reactivation in renal transplant recipients in North India. *Transpl Infect Dis* 2011; **13**: 575-583 [PMID: 21507160 DOI: 10.1111/j.1399-3062.2011.00634.x]

27 **Hariharan S**. BK virus nephritis after renal transplantation. *Kidney Int* 2006; **69**: 655-662 [PMID: 16395271 DOI: 10.1038/sj.ki.5000040]

28 **Sheila Govind JH**, Clare Morris, Collaborative Study Group. Collaborative Study to establish the 1st WHO International Standard for BKV DNA for nucleic acid amplification technique (NAT)-based assays. *Biologicals* 2016; **44**: 423-433

P- Reviewer: Shrestha BM **S- Editor**: Kong JX **L- Editor**: A
E- Editor: Lu YJ

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

ISSN 2220-3230 (online)

World Journal of *Transplantation*

World J Transplant 2017 December 24; 7(6): 276-363

Published by Baishideng Publishing Group Inc

WJT World Journal of Transplantation

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

Editorial Board

2016-2019

The *World Journal of Transplantation* Editorial Board consists of 361 members, representing a team of worldwide experts in transplantation. They are from 43 countries, including Argentina (1), Australia (7), Austria (3), Belgium (4), Brazil (7), Bulgaria (1), Canada (13), China (32), Cuba (1), Czech Republic (1), Denmark (1), Finland (1), France (4), Georgia (1), Germany (14), Greece (5), Hungary (2), India (7), Iran (7), Israel (4), Italy (33), Japan (18), Jordan (1), Macedonia (1), Mexico (2), Morocco (1), Netherlands (4), Nigeria (1), Norway (1), Pakistan (1), Poland (2), Qatar (1), Saudi Arabia (3), Singapore (1), South Korea (16), Spain (9), Sweden (1), Switzerland (3), Thailand (2), Tunisia (1), Turkey (6), United Kingdom (17), and United States (120).

WJT World Journal of Transplantation

Contents

Bimonthly Volume 7 Number 6 December 24, 2017

World Journal of Transplantation
Volume 7 Number 6 December 24, 2017

Contents

NAME OF JOURNAL
World Journal of Transplantation

ISSN
ISSN 2220-3230 (online)

LAUNCH DATE
December 24, 2011

FREQUENCY
Bimonthly

World Journal of Transplantation
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
December 24, 2017

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 December 24; 7(6): 276-284

DOI: 10.5500/wjt.v7.i6.276

ISSN 2220-3230 (online)

EDITORIAL

Biomarkers and a tailored approach for immune monitoring in kidney transplantation

Francisco Salcido-Ochoa, John Carson Allen Jr

Francisco Salcido-Ochoa, Tregs and HLA Research Force, Francisco Kidney and Medical Centre, Mount Elizabeth Novena Hospital, Singapore 329563, Singapore

John Carson Allen Jr, Centre for Quantitative Medicine, Duke-NUS Graduate Medical School, Singapore 169856, Singapore

ORCID number: Francisco Salcido-Ochoa (0000-0001-7518-0462); John Carson Allen Jr (0000-0003-4570-109X).

Author contributions: Both Salcido-Ochoa F and Allen JC Jr wrote and revised the paper.

Conflict-of-interest statement: There is no conflict of interest between the authors or the participating institutions, and the authors do not have any financial relationships to disclose. The opinions expressed in this editorial are those of the authors and attach to the ideology of the Tregs and HLA Research Force, but might or not reflect their other scientific affiliations. The recommendations and suggestions presented by the authors are mainly aimed to inspire scientific reflections and serve as prototype for future clinical guidelines of biomarker research in kidney transplantation. The authors can be contacted for scholarly discussions, suggestions or corrections.

Manuscript source: Invited manuscript

Correspondence to: Francisco Salcido-Ochoa, MD, MRCP, MSc, PhD, Consultant Nephrologist and Transplant Immunologist, Tregs and HLA Research Force, Francisco Kidney and Medical Centre, Mount Elizabeth Novena Hospital, 38 Irrawaddy Road, Singapore 329563, Singapore. fsalcidoochoa@gmail.com
Telephone: +65-66906814
Fax: +65-66906828

Received: May 17, 2017
Peer-review started: May 23, 2017
First decision: July 20, 2017
Revised: October 16, 2017
Accepted: November 22, 2017
Article in press: November 22, 2017
Published online: December 24, 2017

Abstract

A literature review on immune monitoring in kidney transplantation produced dozens of research articles and a multitude of promising biomarkers, all in the quest for the much sought after - but perennially elusive - "holy grail" of kidney biomarkers able to unequivocally predict acute transplant rejection *vs* non-rejection. Detection methodologies and study designs were many and varied. Hence the motivation for this editorial, which espouses the notion that in today's kidney transplantation milieu, the judicious use of disease classifiers tailored to specific patient immune risks may be more achievable and productive in the long run and confer a greater advantage for patient treatment than the pursuit of a single "omniscient" biomarker. In addition, we desire to direct attention toward greater scrutiny of biomarker publications and decisions to implement biomarkers in practice, standardization of methods in the development of biomarkers and consideration for adoption of "biomarker-driven" biopsies. We propose "biomarker-driven" biopsies as an adjunctive to and/or alternative to random surveillance (protocol) biopsies or belated indication biopsies. The discovery of a single kidney transplantation biomarker would represent a major breakthrough in kidney transplantation practice, but until that occurs - if ever it does occur, other approaches offer substantial potential for unlocking prognostic, diagnostic and therapeutic options. We conclude our editorial with suggestions and recommendations for productively incorporating current biomarkers into diagnostic algorithms and for testing future biomarkers of acute

rejection in kidney transplantation.

Key words: Acute rejection; Banff classification; Biomarker; Human leukocyte antigen matching; Immune monitoring; Immunological risk; Kidney transplantation; Protocol biopsy

Core tip: In kidney transplantation, a multitude of biomarkers have been proposed to predict transplant rejection *vs* non-rejection, but few - if any - have gained acceptance as reliable tools for predicting rejection. However, an approach more likely to be successful would include improved timing of kidney transplant biopsies and judicious use of multiple diagnostic methodologies based on different immune risks and events throughout transplantation. This approach could also aid in improving diagnostic and prognostic kidney transplantation algorithms and in developing more impactful therapeutic options.

Salcido-Ochoa F, Allen JC Jr. Biomarkers and a tailored approach for immune monitoring in kidney transplantation. *World J Transplant* 2017; 7(6): 276-284 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i6/276.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i6.276

INTRODUCTION

Kidney transplantation provides kidney failure patients the best opportunity to live longer and fuller lives. Indeed, kidney transplantation is recommended as the first option for suitable patients. However, immunosuppressive drugs currently in use for kidney transplantation are not one-hundred per cent effective in preventing acute or subclinical rejection episodes, or premature transplant failure. In addition, immunosuppressive drugs bring a constellation of side effects linked to significant morbidity and mortality. Thus, until the advent of more targeted and effective, less toxic and tolerogenic immunotherapies, the best strategy appears to be tailoring current immunosuppressive ammunition to the specific immune systems affected by kidney transplant patients. However, the tailored immunosuppressive approach stands in contrast to the current protocolised indiscriminate minimisation of immunosuppression, which has proven to be counterproductive in many instances[1]. Tailoring immune monitoring strategies to a patient's particular risks of rejection, of transplant loss and of transplant-related complications would certainly be more impactful and cost-effective than the non-judicious use of "in vogue" biomarkers.

Conventional monitoring of kidney transplant patients consists of assessing dynamic changes in serum creatinine levels as well as other laboratory parameters such as proteinuria and immunosuppressive drug levels. Additionally, some transplant programmes perform surveillance biopsies, and many measure donor-specific alloantibodies (DSA). DSA are clearly markers of an ongoing anti-allograft response and traditionally viewed as late and ominous markers of rejection that are difficult to counteract. DSA are currently under thorough evaluation in the United Kingdom[2]. Importantly, most immunosuppressive dose changes in kidney transplantation are guided by drug blood levels and their associated toxicities or as a consequence of infections or rejection episodes. But conventional immunomonitoring strategies are unable to reveal the actual state of the immune system and the body's innate defense system. It would be expected then that accurate information on the detector, effector and regulatory arms of the immune system would aid researchers in their quest for more clinically useful biomarkers with improvements in diagnostic accuracy and outcome prediction. It would be anticipated that biomarkers derived from actual immune processes occurring *in vivo* in kidney transplantation would be more likely to guide physicians in choosing the most suitable immunosuppressive strategy.

A substantial impediment to biomarker discovery and application is that the activated targetable immune pathways vary with the immune risk profile of each donor-recipient pair, as well as with the immunosuppressive regimens selected for the recipient. Making the situation even more complex, activated pathways change dynamically throughout the various transplantation stages in response to immunological and infective events occurring throughout the duration of the kidney transplant, and due to modifications in immunosuppressive drugs. Therefore, it is unlikely that one or even a few universal biomarkers can guide transplant physicians in the best use of immunosuppressive regimens throughout all stages of transplantation. However, a combination of clinical parameters and biomarkers revealing distinct immunological, inflammatory and tolerogenic processes occurring at different stages post-transplantation could provide a more useful guide to clinicians. In striving to provide a more accurate picture of the state of the immune system, and hence of the requirements for specific kidney transplant patients, the ideal strategy would complement the immune biomarker analysis with biomarkers revealing parenchymal tissue injury, repair, fibrosis and senescence. Finally, knowledge of the kinetics and interplay of these processes is essential for a proper interrogation and utilization of the biomarker universe.

PERFECT BIOMARKER *VS* TODAY'S REALITY

Biomarker preferred definitions and conceptual framework have been formulated by the Biomarkers Definitions Working Group[3]. Our definition of the perfect tailored immunosuppressive biomarker combines the following properties and characteristics: (1) is easily obtained non-invasively from patients to allow

multiple and sequential analyses; (2) is easily detected and detectable prior to clinically observable events; (3) reflects physiopathogenic mechanisms; (4) demonstrates strong immunodiagnostic and theragnostic value to guide selection and changes in immunosuppressive therapies and possess immunopredictive value; (5) correlates with treatment response; (6) anticipates potential clinical outcomes before and after interventions; (7) indicates over-immunosuppression and risk of infection and cancers; (8) inexpensive with rapid turnaround time; and (9) spares the patient from a kidney transplant biopsy. However, given the complexity of the immune system and alloresponses, the perfect biomarker may be just a pipe-dream.

In kidney transplantation, urine is the most attractive sample source for non-invasive biomarker testing and discovery. Urine is also very accessible, and several urine biomarkers have shown great promise. For instance, chemokines CXCL10 and CXCL9, measured by ELISA, were found to be elevated in urine up to 30 d prior to the episode of acute rejection, and importantly, levels decreased with anti-rejection treatment and displayed prognostic value[4]. Similarly, higher levels of urinary transcripts for cytotoxic cell products like perforin and granzyme B are found in patients with rejection as opposed to non-rejection states[5,6]. Despite the anatomical relationship with the transplant, the kidney does not leak all molecules released by the immune system or injured parenchymal cells into the urine. Many of the leaked molecules are not reliable surrogate markers of rejection, and are even less reliable as markers of tolerance.

On the other hand, whole blood and serum are very accessible, and transcripts for cytotoxic cell products like granzyme B, perforin and granulolysin top the list of promising biomarkers to differentiate rejection from non-rejection[7]. However, many of the molecules participating in transplant rejection or inflammation are not leaked into the blood compartment or they are diluted. Many cells involved in alloimmune processes and detectable in tissue[8] remain or die inside the kidney, or migrate preferentially to draining lymph nodes, which make them inaccessible to the physician's tools. In spite of these limitations, alloreactive memory/effector T cell responses in peripheral blood using an IFN-gamma ELISPOT[9], and the detection of a 17-gene set in peripheral blood using the so-called kidney solid organ response test (kSORT)[10] have shown promise to identify kidney transplant rejection at both the subclinical and clinical stages. Thus, as physicians we must learn to take full advantage of available biomarkers by using them in the correct combinations and at optimal sampling times post-transplantation.

It is important to remember that in many cases serum creatinine levels and glomerular filtration rate are of uninformative for detecting kidney transplant dysfunction as a consequence of rejection. Elevation of serum creatinine levels occurs late in the rejection process and indicates overt kidney transplant injury and nephron loss. At this point, significant alloaggressive mechanisms have commenced, portending the possibility of permanent and irreparable tissue damage and increased risk of refractory rejection. In addition, serum creatinine monitoring precludes the possibility of detecting acute rejection pre-emptively at the state of subclinical rejection. Moreover, small elevations of serum creatinine indicating initiation or progression of the rejection process, may be ignored by patients and physicians with opportunity for early intervention delayed. Serum creatinine is recognized as an imperfect marker for acute kidney dysfunction and a very poor marker for acute rejection; however, its utility might be augmented if taken in combination with other promising non-invasive biomarkers, including certain cytotoxic cell products described above[4-7] or others. Taken in combination, immunodiagnostic and immunopredictive properties might be enhanced.

It would be absurd to suggest that urine and blood biomarkers - given the current state of the art - are able to replace kidney transplant biopsy, which is the gold standard for diagnosis of allograft rejection[11]. However, the realistic and practical utility of these biomarkers would be to aid physicians in decisions that ultimately expedite a confirmatory transplant biopsy and initiation of anti-rejection therapy thereby minimizing damage to the kidney and enhancing chances of therapeutic success.

CURSE OF THE SPECIFIC "MAGICAL'' BIOMARKER

The kidney transplant literature is rife with research in pursuit of a "magical" biomarker capable of identifying onset of kidney transplant rejection with perfect accuracy - with an aim to supplanting the kidney transplant biopsy. But inevitably, pre-study optimism is confuted by post-study outcomes demonstrating that tested markers are not specific for kidney rejection - they cannot distinguish indicators of rejection from those of other disease processes such as BK virus infection, non-rejection sources of inflammation or nonspecific tissue injury. Markers are then labelled as "not-very useful" and dismissed - a possibly premature verdict considering a marker might be still useful for signalling at least that some pathologic events are in progress in the transplant kidney and thereby alerting to the need of a confirmatory biopsy.

For researchers engaged in the perennial search for a biomarker to replace the kidney transplant biopsy, a concomitant enterprise could be mining the depth and breadth of information that remains untapped in a transplant biopsy. It is highly unlikely that urine or blood markers can surpass those extracted from the transplant kidney and the draining lymph nodes as more informative of the condition of the kidney transplant - the invasive nature of biopsy and inaccessibility of lymph nodes notwithstanding. In today's world of kidney transplants, current non-invasive biomarkers will not be perfect predictors as they reveal only partially the complex

interplay of immune and non-immune factors and events occurring inside the kidney transplant. However, we can use them more effectively by understanding their precise biological meaning and clinical value.

On the other hand, tissue biomarkers, classifiers and archetypes obtained from the molecular microscope on kidney transplant biopsies, when combined with the constellation of non-invasive biomarkers, could give physicians the most comprehensive and accurate information upon which to base therapeutic decisions. In this respect, citing the INTERCOMEX Study, the analysis of transcripts in kidney transplant biopsies was able to classify patients with acute kidney transplant dysfunction with high accuracy in those having pure T cell-mediated rejection (TCMR), antibody-mediated rejection (ABMR), mixed rejection and no rejection[12].

The most effective solution - although not the simplest - will involve a finer dissection of the immunopathogenesis of rejection. The purpose would be to achieve greater understanding of the biological meaning and derivation of the presently available biomarkers and potential new biomarkers, to rank them physiopathologically and address their clinical contributions individually and in combination with other biomarkers.

SURVEILLANCE KIDNEY TRANSPLANT BIOPSIES RELOADED

Surveillance kidney transplant biopsies play an important role in kidney transplant immune monitoring, especially in patients at high immunological risk for antibody-mediated rejection whose biopsies were performed in the early stages post-transplantation when risk of rejection is higher. The purpose of surveillance biopsies is straightforward: To find remediable problems as early as possible. However, many centres do not perform surveillance biopsies for various reasons, including the following: Biopsies are not part of their academic culture, feasibility issues, historic poor yields and/or poor outcomes - making crucial judicious patient selection - or use of more effective combinations of immunosuppressive drugs. However, surveillance biopsy schedules tend to be somewhat arbitrary and unit specific. They reflect varying physician experience and thresholds among transplant units and are imperfect in consequence of the limited and equivocal signs and symptoms manifested by the alloresponses. Thresholds adjudged warranting a kidney transplant biopsy vary among transplant units and physicians - even thresholds attributable to indication biopsies (also referred to as for-cause or episode biopsies) - when something is obviously going wrong. In addressing arbitrariness in selecting surveillance biopsy time points, current and future biomarkers could be designed not only for diagnosis of rejection - as they might not replace biopsy - but to identify the onset of specific problems or simply to confirm with a transplant biopsy when something wrong (yet to be defined) is occurring at the subclinical stage. These types of biopsies would not be called surveillance biopsies or indication biopsies, but might be referred to as "biomarker-driven or biomarker-triggered biopsies". Biomarker-driven biopsies would enhance the diagnostic yield of the biopsy procedure as accuracy would likely be higher than a conventional and arbitrarily mandated protocol surveillance biopsy, and they would be more opportune than an indication biopsy. An exciting prospect is the potential to enhance the diagnostic yield and outcome prediction potential of any surveillance, indication or "biomarker-triggered" transplant biopsy by coupling gene expression analysis (the molecular microscope) with the conventional histopathologic grading of the Banff classification like in the INTERCOMEX Study[12].

Inherent in the concept of a "biomarker-triggered" transplant biopsy, is the notion of a more impactful search for biomarkers of subclinical rejection rather than markers of acute rejection. Subclinical rejection biomarkers could trigger an opportune diagnostic kidney transplant biopsy enabling initiation of anti-rejection strategies much earlier. Performance of a marker of acute rejection might not be as good if tested for utility in identifying subclinical rejection. Nevertheless, biomarkers of acute rejection could still have a role in confirming suspicious cases of rejection, as prognosticators of transplant outcomes, or for hypothesis generation in the search for novel biomarkers of subclinical rejection.

Although kidney transplant biopsy is considered the gold standard for diagnosing acute rejection, it is far from ideal. The vision provided of what is occurring reveals patchy, non-uniform rejection throughout the kidney tissue. Consequently, acute rejection can be missed by performing biopsies in randomly selected areas of the kidney transplant. In addition, a biopsy cannot quantify the degree to which the renal parenchyma is inflamed. One possible solution - not yet developed - is an imaging technique that could give a quantifiable assessment of inflammation in the kidney parenchyma. Imaging findings in combination with biopsy results would allow quantification of the extent of rejection and guide better "tailoring" of corrective immunosuppression after rejection episodes.

STATISTICAL ADVANTAGE IN IMMUNE MONITORING

From the discussion above and the examples presented, we can also expect that the development of predictors for immune monitoring strategies that incorporate multiple biomarkers, as opposed to just a single biomarker, would have the greatest potential for considerably enhancing prognostic accuracy, especially if incorporated into comprehensive monitoring algorithms that include clinical parameters. One approach to accomplishing this more effectively would be to incorporate tests assessing different biomarkers in prospective studies and clinical trials under more controllable and less heterogeneous

circumstances to investigate potential utility as predictors in kidney transplantation. In clinical trials, biomarkers could be investigated as theragnostic markers to guide the use of interventions or assess response to interventions thereby providing data enabling better kidney transplant outcomes.

LAYING THE FOUNDATIONAL STONES IN IMMUNE MONITORING

A better understanding of the immunopathogenesis of kidney transplant rejection and the mechanisms of immune adaptation that could potentially lead to transplant tolerance is crucial for the development of more accurate and precise biomarkers in kidney transplantation.

Technological advances now allow us to interrogate the immune system in peripheral blood and other fluids and tissues of kidney transplant patients that give a multidimensional and multifaceted perspective. We are currently able to obtain a very detailed picture of the state of many genes involved in the body's response to kidney transplantation, specifically of their transcriptional and translational products. Nevertheless, a multitude of genetic interactions, their hierarchy and precise clinical translation remain to be deciphered. Sophisticated biomolecular technologies and mass spectrometry-based technologies are robust to identify and discover novel biomarkers, which once validated, will open the way for implementation of other less expensive and more accessible technologies to serve in the clinical detection of those biomarkers. Thus, a multidimensional and multisystem interrogation of different biological systems in kidney transplantation would provide a combinatorial (phenotypic and functional picture) of the actual state of the immune system and its inter-relationships with other bodily systems. Well-equipped and experienced labs will be able to eventually reveal the secret world underlying alloresponses, especially if they commit their full resources and capabilities to achieving the goal.

Until the advent of more robust non-invasive biomarkers able to detect subclinical rejection with greater accuracy, *i.e.*, "biomarker-triggered transplant biopsies", protocoled surveillance biopsies and indication biopsies will continue to play a central role in the discovery of molecular signatures and the evaluation and correlation of novel biomarker candidates.

A comprehensive review article on different types of biomarkers tested and those showing promise in kidney transplantation immunodiagnosis was published recently in this journal[13]. However, more critical reviews of the available literature are needed to identify the most promising biomarkers. Admittedly, this is a difficult task given the multitude of biomarker candidates obtained from diverse sources using a range of technologies in typically heterogeneous patient populations. Thus, laboratories aiming to discover and validate biomarkers should consider protocol standardization and judicious selection of testing time points as essential elements of adequate and well-controlled biomarker-led clinical trials. The creation of advisory and work groups, and opportunities for collaboration and grant applications, should also be promoted with the ultimate aim of advancing the science of biomarker use and immunomonitoring in kidney transplantation.

RECOMMENDATIONS AND SUGGESTIONS FOR USING BIOMARKERS AND SURVEILLANCE BIOPSIES IN KIDNEY TRANSPLANTATION

It is quite apparent that we are still far from finding biomarkers that can supplant kidney transplant biopsy. Nevertheless, we can proceed methodically and persistently, perhaps not expecting to find the "magical" biomarker but towards a more in-depth and informative interrogation of the patient immune system. With this view in mind, our recommendations and suggestions for utilizing and testing biomarkers in kidney transplantation are summarized in Table 1. These are presented in the context of eight scenarios representing somewhat typically encountered cases. Given the complexity of clinical kidney transplantation, they are by no means all-inclusive or exhaustive. For each scenario, the necessity for customization in addressing different immunological risks should be recognized. Challenges confronting researchers engaged in biomarker development and utilization in kidney transplantation are encountered as well in other branches of nephrology (*e.g.,* biomarkers of acute kidney injury) and other disciplines of Medicine. We believe that the recommendations and suggestions offered have general applicability in other areas of biomarker research. Standard measures for assessing kidney transplant status are omitted from Table 1 as they are standard practice. Therapeutic recommendations or choice of immunosuppressants are not given as they are not within the scope of our biomarker-centred recommendations and suggestions. The interested reader is referred to the references cited[1,14].

IMMUNOLOGICAL RISK AND HOW IT AFFECTS BIOMARKER RESEARCH

Approaches for objective quantification of immunological risk have been attempted but as yet no reliable risk score has been developed. Immunological risk depends largely on the distinct genetic and antigenic differences between recipients and donors (along with other factors), type and amount of immunosuppression used, the degree of activation of the innate defense system and the set of dynamic alloresponses occurring throughout transplantation. The current or proposed attempts to quantify immunological risk would require an editorial or review article of its own - which will likely come with imperfect approximations - but we would like to bring attention one an important point, which is the

Table 1 Recommendations and suggestions on the incorporation of biomarkers and surveillance biopsies in kidney transplantation

Scenario A: Patients with acute kidney transplant dysfunction on whom a kidney transplant biopsy has been performed to exclude rejection	
Recommendations	
A1	Diagnose rejection if present in kidney transplant biopsies according to the Banff classification (using the most current update; now the 2015 update), and report it in a systematic way
A2	Quantify BK viremia[a] and BK virus (BKV) nephropathy by specific staining
A3	Detect anti-HLA antibodies/DSA[d] and define their immunoglobulin class, complement fixing capacities and titres through dilutions
Suggestions	
A4	Bank serum, plasma, urine, peripheral blood mononuclear cells (PBMC) and kidney transplant tissue for future biomarker research[c]
A5	Exclude active infection by cytomegalovirus (CMV) and Epstein-Barr virus (EBV)[a]
A6	Generate a data base with detailed clinical and immunological variables, ideally, using a standardized data base from a consortium or a large multicentre/multinational collaboration
A7	Test any experimental biomarker(s) of your choice and correlate it/them with standard clinical variables and a detailed immune profile. The use of validated disease classifiers and archetypes appears to have more diagnostic accuracy than the use of single biomarkers
A8	Perform a surveillance biopsy if kidney function and other clinical or laboratory parameters do not improve as expected after treatment to exclude persisting rejection or transformation to another type of rejection[b]
Scenario B: Patients with acute kidney transplant dysfunction on whom a kidney transplant biopsy is being considered to exclude rejection	
Recommendations	
B1	Quantify BK viremia[a]
B2	Detect anti-HLA antibodies/DSA[d] and define their immunoglobulin class, complement fixing capacities and titres through dilutions; and perform a kidney transplant biopsy if DSA are detected
B3	Use validated disease classifiers and archetypes (if available) to enhance to pre-test probability for rejection, and perform a kidney transplant biopsy if positive
B4	If a kidney transplant biopsy is performed, consider the recommendations and suggestions for Scenario A
Suggestions	
B4	Bank serum, plasma, urine and PBMC for future biomarker research[c]
B5	Exclude CMV and EBV infection[a]
B6	Generate a data base with detailed clinical and immunological variables, ideally, using a standardized data base from a consortium or a large multicentre/multinational collaboration
B7	Test any experimental biomarker(s) of your choice and correlate it/them with standard clinical variables and a detailed immune profile. The use of validated disease classifiers and archetypes appears to have more diagnostic accuracy than the use of single biomarkers
Scenario C: Patients with: (1) stable kidney function; (2) low immunological risk for ABMR with lack of preformed DSA; and (3) low immunological risk for TCMR or for the synthesis of *de novo* DSA due to no or low degree of HLA mismatch[16-18]	
Recommendations	
C1	Detect anti-HLA antibodies/DSA[d] after a sensitization event (transfusions, pregnancies or other transplants *e.g.*, pancreas after kidney transplantation) and define their immunoglobulin class, complement fixing capacities and titres through dilutions
C2	Perform a kidney transplant biopsy if DSA are detected, diagnose it according to the Banff classification 2015 update and exclude intra-graft BKV infection by specific staining
C3	In case of kidney dysfunction, consider the recommendations and suggestions for Scenarios A or B
Suggestions	
C4	Test any experimental biomarker(s) of your choice at pre-selected time points and correlate it/them with standard clinical variables and a detailed immune profile. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry
C5	Consider surveillance biopsies that exclude subclinical rejection and banking of kidney transplant tissue for biomarker research[c]. Recommendation to select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry
C6	Detect anti-HLA antibodies/DSA[d] at your pre-selected time points, to define their immunoglobulin class, complement fixing capacities and titres through dilutions, and correlate them with standard clinical variables and a detailed immune profile. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry. There are published consensus guidelines[19], but their recommendations are relatively arbitrary as well
C7	Bank serum, plasma, urine and PBMC at your pre-selected sampling time points and when kidney biopsies are performed[c]
C8	Exclude CMV and EBV infection[a]
C9	Perform a biomarker-driven biopsy if your chosen validated biomarker for rejection (or any other anomaly) turns positive, and bank tissue for further biomarker research
Scenario D: Patients with: (1) stable kidney function; and (2) high immunological risk for ABMR due to preformed DSA (desensitized or not)	
Recommendations	
D1	Ensure adequate levels of immunosuppression and prevent non-compliance with treatment[e]
D2	Perform surveillance biopsies to exclude subclinical rejection and banking of kidney transplant tissue for biomarker research[c]. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry, but available guidelines[19] recommend them within the first 3 (or 6) mo post-transplantation
D3	Monitor anti-HLA antibodies/DSA[d] and define their immunoglobulin class, complement fixing capacities and titres through dilutions at your pre-selected time points and correlate them with standard clinical variables and a detailed immune profile. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry; although there are published consensus guidelines[19]
D4	Detect anti-HLA antibodies/DSA[d] after a sensitization event (transfusions, pregnancies or other transplants, *e.g.*, pancreas after kidney transplantation) and define their immunoglobulin class, complement fixing capacities and titres through dilutions
D5	Perform a kidney transplant biopsy if DSA are detected, to diagnose it according to the Banff classification 2015 update and exclude intra-graft BKV infection by specific staining

D6	Perform a biomarker-driven biopsy if your chosen validated biomarker for rejection (or any other anomaly) turns positive, and bank tissue for further biomarker research
D7	In case of kidney dysfunction, we recommend to perform a kidney transplant biopsy and to consider the recommendations and suggestions for Scenario A
Suggestions	
D8	Test any experimental biomarker(s) of your choice at pre-selected time points and correlate it/them with standard clinical variables and a detailed immune profile. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry
D9	Bank serum, plasma, urine and PBMC at your pre-selected sampling time points and when kidney biopsies are performed[c]
D10	Exclude CMV and EBV infection[a]
Scenario E: Patients with: (1) stable kidney function; (2) high immunological risk for TCMR and for the synthesis of *de novo* DSA due to high degree HLA mismatch[16-18]; and (3) without preformed DSA	
Recommendations	
E1	Ensure adequate levels of immunosuppression and prevent non-compliance with treatment[e]
E2	Detect anti-HLA antibodies/DSA[d], especially in those with HLA-B and HLA-DRB1 mismatches, thought to be more immunogenic[16], at your pre-selected time points and correlate them with standard clinical variables and a detailed immune profile. Define immunoglobulin class, complement fixing capacities and titres through dilutions. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry, although there are published consensus guidelines[19]
E3	Detect anti-HLA antibodies/DSA[d] after a sensitization event (transfusions, pregnancies or other transplants, *e.g.*, pancreas after kidney transplantation) and define their immunoglobulin class, complement fixing capacities and titres through dilutions
E4	Perform a kidney transplant biopsy if DSA are detected, diagnose according to the Banff classification 2015 update and exclude intra-graft BKV infection by specific staining
E5	In case of kidney dysfunction, perform a kidney transplant biopsy, especially in those with HLA-B and HLA-DRB1 mismatches, thought to be more immunogenic, and consider the recommendations and suggestions for Scenario A
Suggestions	
E6	Test any experimental biomarker(s) of your choice at pre-selected time points and correlate it/them with standard clinical variables and a detailed immune profile. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry
E7	Suggest surveillance biopsies exclude subclinical rejection and banking of kidney transplant tissue for biomarker research[c]. Select time points based on the modal distribution of rejection in a specific population of patients with similar immunological risk, ideally derived from your own registry
E8	Bank serum, plasma, urine and PBMC at your pre-selected sampling time points and when kidney biopsies are performed[c]
E9	Exclude CMV and EBV infection[a]
E10	Perform a biomarker-driven biopsy if your chosen validated biomarker for rejection (or any other anomaly) turns positive, and bank tissue for further biomarker research
Scenario F: Patients with: (1) stable kidney function; (2) high immunological risk for ABMR due to preformed DSA; and (3) high immunological risk for TCMR and for the synthesis of *de novo* DSA due to high degree HLA mismatch[16-18]	
Recommendation	
F1	Follow our recommendations and suggestions for Scenarios D and E
Scenario G: Patients with delayed graft function (DGF)	
Recommendations	
G1	Perform a kidney transplant biopsy if DGF extends beyond the first week post-transplantation without an obvious explanation, and subsequently every 7-10 d if DGF persists[14]
G2	Detect anti-HLA antibodies/DSA[d] if DGF extends beyond the first week post-transplantation without an obvious explanation, and subsequently every 7-10 d if DGF persists, and define their immunoglobulin class, complement fixing capacities and titres through dilutions
G3	Perform a kidney transplant biopsy if DSA are detected, to diagnose it according to the Banff classification 2015 update and exclude intra-graft BKV infection by specific staining
Suggestions	
G4	Define lower threshold for performing a kidney transplant biopsy in patients with DGF and pre-formed DSA or with HLA-B and HLA-DRB1 mismatches thought to be more immunogenic[16]
G5	Bank serum, plasma, urine and PBMC at the protocolised sampling time points and when kidney biopsies are performed[c]
G6	Bank kidney transplant tissue for biomarker research whenever a biopsy is performed[c]
G7	Test any experimental biomarker(s) of your choice at protocolised time points and correlate it/them with standard clinical variables and a detailed immune profile[c]
G8	Perform a biomarker-driven biopsy if your chosen validated biomarker for rejection (or any other anomaly) turns positive, and bank tissue for further biomarker research
G9	Exclude active CMV and EBV infection[a]
Scenario H: Every kidney transplant patient included in a clinical trial	
Recommendations	
H1	Bank serum, plasma, urine and PBMC at the protocolised sampling time points and when kidney biopsies are performed[cf]
H2	Bank kidney transplant tissue for biomarker research whenever a biopsy is performed[cf]
H3	Test any experimental biomarker(s) of your choice at the sampling points established by the trial designers and correlate it/them with
H4	Consider performing surveillance biopsies at important assessment points as per trial protocol (which can help to exclude subclinical rejection and to assess histopathological response to interventions) and banking of kidney transplant tissue for biomarker research[c]

[a]These infections can present with kidney dysfunction, trigger or appear around a rejection episode, but importantly viraemia, especially at high levels, will elicit cytotoxic-type and other immune responses that can interfere with the interpretation of biomarkers. [b]This is another opportunity for biomarker testing, especially if its kinetics post-treatment are known or being tested. [c]When banking samples, we suggest to process them and store them with the vision that they could be analysed using different technologies (*e.g.*, RNA- or proteomics-friendly sample processing), even if those technologies are not available in your lab, as the research world is developing towards more constructive collaborations and cross-validation approaches. In such way,

laboratories will end up with legacy sample banks from highly characterized patients with several follow up times points, in which future technologies (pending improvements or not developed yet) could be easily applied, saving huge time to researchers (no further recruitment and sample acquisition), minimizing the risk of including patients to similar protocols (just because the technology has changed) and maximizing previous patients effort and kindness; at least for pilot, exploratory and cross-validation studies. Seek advice on how to maximise your sample banking from an experienced laboratory. Strict protocols should be devised and followed up and biobanking details of the samples should be recorded (time and date of collection, type of tube, type of anti-coagulant, additives for preservation, if centrifuged the speed of centrifugation in "g", sample processor – if a person – or a machine, *etc.*). It is important to consider the easiness of the retrieval process of the data as it is inputted (any free text or absence of drop-down lists from choice answers will result in manual-dependent retrieval, which will be time consuming and expensive. [d]We recommend high resolution tissue typing of HLA-A; -B; -C; -DP; -DQ; and -DRB1,3,4,5 alleles for both donor and recipient. This will ensure more accurate detection of anti-HLA DSA, and the use of algorithms to assess degree HLA mismatching like the HLAMatchmaker[17,18]. [e]This recommendation is important for every kidney transplant patient, but seems crucial for patients with augmented immunological risk. [f]For clinical trials, we prefer to recommend rather than just suggest the inclusion of biomarker testing as the incorporation of biomarkers in diagnostic well-designed clinical trials is the best channel to validate biomarkers in a standardized controlled setting and maximize all the benefits from the trial.

differentiation of risk conferred by pre-formed anti-human leukocyte antigen (HLA) alloantibodies from risk conferred by HLA mismatches.

Many centres stratify patients according to the degree of immunological risk based primarily on presence or absence (or titres) of preformed anti-HLA alloantibodies (greater risk if DSA) and cross-match characteristics, and whether or not they have been desensitised. Some centres pay appropriate attention to the degree of HLA mismatches but others do not. Thus, a patient with a negative crossmatch and no anti-HLA antibodies could be deemed in some programmes to have a low immunological risk even with a high degree of HLA mismatch. This type of stratification, based on the presence of preformed alloantibodies, represents risk primarily for immediate or early ABMR due to preformed DSA, particularly in the absence of desensitization or subsequent ABMR episodes (either acute or chronic) and depicts previous sensitization events in the recipient (*e.g.*, pregnancies, transfusions, previous transplants). However, the immunological risk derived from the degree of HLA mismatch between recipients and donors must be considered more explicitly. The antigenic differences provided by the donor genes not present in the recipient are the main drivers of strong *de novo* alloresponses. These can trigger either the development of TCMR or the formation of *de novo* DSA with consequent progression to ABMR[15] or both, especially when current immunosuppression is not 100% effective to prevent rejection. In fact, pre-formed alloantibodies are derived from the same principle, *i.e.,* from mismatches in HLA molecules (or other polymorphic antigens) between the fetus and the mother, and the blood or tissue donor and the "pre-transplant" recipient. The degree of HLA mismatch has been traditionally quantified by counting, enumerating or stratifying the number and type of HLA mismatches[16], but more robust algorithms like the HLAMatchmaker[17,18] that more specifically assess HLA epitope mismatches can be applied to assess the risk for TCMR (acute or chronic variants) and synthesis of *de novo* DSA. To make things even more complex, kidney transplant patients usually have a combination of immunological factors that put them at risk for both types of rejection. So our recommendations and suggestions have to be tailored to the specific clinical and immunological characteristics of specific patient populations, and they would need to be implemented in the context of other available useful guidelines[14,19].

ACKNOWLEDGMENTS

We would like to thank Dr Maria Hernandez-Fuentes, an expert in the field of immune monitoring in solid organ transplantation, for reviewing our manuscript and her useful comments. Similar degree of gratitude goes to the four anonymous reviewers, whose constructive comments increased the quality of this paper. Once more, our deep gratitude to Ms Rachel Liew, our library technician, for helping us finding the least accessible references.

REFERENCES

1 **Zsom L**, Wagner L, Fülöp T. Minimization vs tailoring: Where do we stand with personalized immunosuppression during renal transplantation in 2015? *World J Transplant* 2015; **5**: 73-80 [PMID: 26421259 DOI: 10.5500/wjt.v5.i3.73]

2 **Dorling A**, Rebollo-Mesa I, Hilton R, Peacock JL, Vaughan R, Gardner L, Danzi G, Baker R, Clark B, Thuraisingham RC, Buckland M, Picton M, Martin S, Borrows R, Briggs D, Horne R, McCrone P, Kelly J, Murphy C. Can a combined screening/treatment programme prevent premature failure of renal transplants due to chronic rejection in patients with HLA antibodies: study protocol for the multicentre randomised controlled OuTSMART trial. *Trials* 2014; **15**: 30 [PMID: 24447519 DOI: 10.1186/1745-6215-15-30]

3 **Biomarkers Definitions Working Group**. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001; **69**: 89-95 [PMID: 11240971 DOI: 10.1067/mcp.2001.113989]

4 **Hirt-Minkowski P**, De Serres SA, Ho J. Developing renal allograft surveillance strategies - urinary biomarkers of cellular rejection. *Can J Kidney Health Dis* 2015; **2**: 28 [PMID: 26285614 DOI: 10.1186/s40697-015-0061-x]

5 **Li B**, Hartono C, Ding R, Sharma VK, Ramaswamy R, Qian B, Serur D, Mouradian J, Schwartz JE, Suthanthiran M. Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. *N Engl J Med* 2001; **344**: 947-954 [PMID: 11274620 DOI: 10.1056/NEJM200103293441301]

6 **van Ham SM**, Heutinck KM, Jorritsma T, Bemelman FJ, Strik MC, Vos W, Muris JJ, Florquin S, Ten Berge IJ, Rowshani AT. Urinary granzyme A mRNA is a biomarker to diagnose subclinical and acute cellular rejection in kidney transplant recipients. *Kidney Int* 2010; **78**: 1033-1040 [PMID: 20720522 DOI: 10.1038/ki.2010.274]

7 **Simon T**, Opelz G, Wiesel M, Ott RC, Süsal C. Serial peripheral blood perforin and granzyme B gene expression measurements for prediction of acute rejection in kidney graft recipients. *Am J*

Transplant 2003; **3**: 1121-1127 [PMID: 12919092 DOI: 10.1034/j.1600-6143.2003.00187.x]

8 **Salcido-Ochoa F**, Hue SS, Peng S, Fan Z, Li RL, Iqbal J, Allen JC Jr, Loh AHL. Histopathological analysis of infiltrating T cell subsets in acute T cell-mediated rejection in the kidney transplant. *World J Transplant* 2017; **7**: 222-234 [PMID: 28900605 DOI: 10.5500/wjt.v7.i4.222]

9 **Bestard O**, Cruzado JM, Lucia M, Crespo E, Casis L, Sawitzki B, Vogt K, Cantarell C, Torras J, Melilli E, Mast R, Martinez-Castelao A, Gomà M, Reinke P, Volk HD, Grinyó JM. Prospective assessment of antidonor cellular alloreactivity is a tool for guidance of immunosuppression in kidney transplantation. *Kidney Int* 2013; **84**: 1226-1236 [PMID: 23783240 DOI: 10.1038/ki.2013.236]

10 **Roedder S**, Sigdel T, Salomonis N, Hsieh S, Dai H, Bestard O, Metes D, Zeevi A, Gritsch A, Cheeseman J, Macedo C, Peddy R, Medeiros M, Vincenti F, Asher N, Salvatierra O, Shapiro R, Kirk A, Reed EF, Sarwal MM. The kSORT assay to detect renal transplant patients at high risk for acute rejection: results of the multicenter AART study. *PLoS Med* 2014; **11**: e1001759 [PMID: 25386950 DOI: 10.1371/journal.pmed.1001759]

11 **Loupy A**, Haas M, Solez K, Racusen L, Glotz D, Seron D, Nankivell BJ, Colvin RB, Afrouzian M, Akalin E, Alachkar N, Bagnasco S, Becker JU, Cornell L, Drachenberg C, Dragun D, de Kort H, Gibson IW, Kraus ES, Lefaucheur C, Legendre C, Liapis H, Muthukumar T, Nickeleit V, Orandi B, Park W, Rabant M, Randhawa P, Reed EF, Roufosse C, Seshan SV, Sis B, Singh HK, Schinstock C, Tambur A, Zeevi A, Mengel M. The Banff 2015 Kidney Meeting Report: Current Challenges in Rejection Classification and Prospects for Adopting Molecular Pathology. *Am J Transplant* 2017; **17**: 28-41 [PMID: 27862883 DOI: 10.1111/ajt.14107]

12 **Halloran PF**, Reeve J, Akalin E, Aubert O, Bohmig GA, Brennan D, Bromberg J, Einecke G, Eskandary F, Gosset C, Duong Van Huyen JP, Gupta G, Lefaucheur C, Malone A, Mannon RB, Seron D, Sellares J, Weir M, Loupy A. Real Time Central Assessment of Kidney Transplant Indication Biopsies by Microarrays: The INTERCOMEX Study. *Am J Transplant* 2017; **17**: 2851-2862 [PMID: 28449409 DOI: 10.1111/ajt.14329]

13 **Salvadori M**, Tsalouchos A. Biomarkers in renal transplantation: An updated review. *World J Transplant* 2017; **7**: 161-178 [PMID: 28698834 DOI: 10.5500/wjt.v7.i3.161]

14 **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]

15 **Salcido-Ochoa F.** Human Leukocyte Antigen (HLA) System in Solid Organ Transplantation and Few Novel Concepts in HLA Matching. In: Shabir Ahmad Mir, ed. Recent Trends in Immunology. Texas, USA: SM Online Publishers, 2015

16 **Fuggle SV**, Johnson RJ, Rudge CJ, Forsythe JL. Human leukocyte antigen and the allocation of kidneys from cadaver donors in the United Kingdom. *Transplantation* 2004; **77**: 618-620 [PMID: 15084948 DOI: 10.1097/01.TP.0000103726.37649.EF]

17 **Duquesnoy RJ**, Takemoto S, de Lange P, Doxiadis II, Schreuder GM, Persijn GG, Claas FH. HLAmatchmaker: a molecularly based algorithm for histocompatibility determination. III. Effect of matching at the HLA-A,B amino acid triplet level on kidney transplant survival. *Transplantation* 2003; **75**: 884-889 [PMID: 12660519 DOI: 10.1097/01.TP.0000055101.20821.AC]

18 **Duquesnoy RJ**, Witvliet M, Doxiadis II, de Fijter H, Claas FH. HLAMatchmaker-based strategy to identify acceptable HLA class I mismatches for highly sensitized kidney transplant candidates. *Transpl Int* 2004; **17**: 22-30 [PMID: 12955350 DOI: 10.1007/s00147-003-0641-z]

19 **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]

P- Reviewer: Fulop T, Lu K, Sheashaa HA, Taheri S **S- Editor**: Kong JX
L- Editor: A **E- Editor**: Yan JL

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 December 24; 7(6): 285-300

DOI: 10.5500/wjt.v7.i6.285

ISSN 2220-3230 (online)

REVIEW

De novo glomerular diseases after renal transplantation: How is it different from recurrent glomerular diseases?

Fedaey Abbas, Mohsen El Kossi, Jon Kim Jin, Ajay Sharma, Ahmed Halawa

Fedaey Abbas, Department of Nephrology, Jaber El Ahmed Military Hospital, Safat 13005, Kuwait

Fedaey Abbas, Mohsen El Kossi, Jon Kim Jin, Ajay Sharma, Ahmed Halawa, Faculty of Health and Science, University of Liverpool, Institute of Learning and Teaching, School of Medicine, Liverpool L69 3GB, United Kingdom

Mohsen El Kossi, Doncaster Royal Infirmary, Doncaster DN2 5LT, United Kingdom

Jon Kim Jin, Nottingham Children Hospital, Nottingham NG7 2UH, United Kingdom

Ajay Sharma, Royal Liverpool University Hospitals, Liverpool L7 8XP, United Kingdom

Ahmed Halawa, Department of Transplantation Surgery, Sheffield Teaching Hospitals, Sheffield S5 7AU, United Kingdom

ORCID number: Fedaey Abbas (0000-0001-8673-4344); Mohsen El Kossi (0000-0002-2490-2784); Jon Kim Jin (0000-0003-4307-8513); Ajay Sharma (0000-0003-4050-6586); Ahmed Halawa (0000-0002-7305-446X).

Author contributions: Abbas F designed the study, data collection, writing the manuscript; El Kossi M, Jin JK and Sharma A reviewed and edited the manuscript; Halawa A contributed to conceptualization, designing the study, supervising the data collection and reviewing and editing the manuscript.

Conflict-of-interest statement: No conflict of interest.

Manuscript source: Unsolicited manuscript

Correspondence to: Ahmed Halawa, FRCS (Gen Surg), MD, MSc, Surgeon, Consultant Transplant Surgeon, Department of Transplantation Surgery, Sheffield Teaching Hospitals, Herries Road, Sheffield S5 7AU, United Kingdom. ahmed.halawa@sth.nhs.uk
Telephone: +44-77-87542128
Fax: +44-11-42714604

Received: September 12, 2017
Peer-review started: September 15, 2017
First decision: October 24, 2017
Revised: November 2, 2017
Accepted: November 10, 2017
Article in press: November 10, 2017
Published online: December 24, 2017

Abstract

The glomerular diseases after renal transplantation can occur *de novo*, *i.e.*, with no relation to the native kidney disease, or more frequently occur as a recurrence of the original disease in the native kidney. There may not be any difference in clinical features and histological pattern between *de novo* glomerular disease and recurrence of original glomerular disease. However, structural alterations in transplanted kidney add to dilemma in diagnosis. These changes in architecture of histopathology can happen due to: (1) exposure to the immunosuppression specifically the calcineurin inhibitors (CNI); (2) in vascular and tubulointerstitial alterations as a result of antibody mediated or cell-mediated immunological onslaught; (3) post-transplant viral infections; (4) ischemia-reperfusion injury; and (5) hyperfiltration injury. The pathogenesis of the *de novo* glomerular diseases differs with each type. Stimulation of B-cell clones with subsequent production of the monoclonal IgG, particularly IgG3 subtype that has higher affinity to the negatively charged glomerular tissue, is suggested to be included in PGNMID pathogenesis. *De novo* membranous nephropathy can

be seen after exposure to the cryptogenic podocyte antigens. The role of the toxic effects of CNI including tissue fibrosis and the hemodynamic alterations may be involved in the *de novo* FSGS pathophysiology. The well-known deleterious effects of HCV infection and its relation to MPGN disease are frequently reported. The new concepts have emerged that demonstrate the role of dysregulation of alternative complement pathway in evolution of MPGN that led to classifying into two subgroups, immune complex mediated MPGN and complement-mediated MPGN. The latter comprises of the dense deposit disease and the C3 GN disease. *De novo* C3 disease is rather rare. Prognosis of *de novo* diseases varies with each type and their management continues to be empirical to a large extent.

Key words: *De novo* glomerulonephritis; Renal transplantation; New concepts of therapy

Core tip: The role of post-transplant glomerulonephritis in affecting both patient and allograft survival is well documented. For decades recurrent glomerular diseases after renal transplantation have been thoroughly investigated. On the other hand a group of a newly classified *de novo* glomerular diseases attained an increasing interest. However, the paucity of data concerned with *de novo* glomerular diseases after renal transplantation have been shown to be a great obstacle necessitating more active cooperation between transplant centers. A thorough work up is clearly warranted to declare not only their pathogenesis, but also to draw the proper therapeutic plan.

Abbas F, El Kossi M, Jin JK, Sharma A, Halawa A. *De novo* glomerular diseases after renal transplantation: How is it different from recurrent glomerular diseases? *World J Transplant* 2017; 7(6): 285-300 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i6/285.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i6.285

INTRODUCTION

De novo glomerular disease is a glomerular disease that damages the renal allograft and it is totally different from the native renal disease. The most common types of *de novo* glomerulonephritis (GN) are: Membranous nephropathy (MN), focal segmental glomerulosclerosis (FSGS), membranoproliferative glomerulonephritis (MPGN) and TMA secondary to drug intake[1,2]. Since immunofluorescence technique (IF) and electron microscopy (EM) are not used that often when assessing histopathology of a biopsy specimen in early post-transplant period, and the possibility of a range of renal diseases of unknown etiology, make it difficult to evaluate the real prevalence of *de novo* GN diseases[3]. *De novo* GN disease is reportedly uncommon[4-9]. In this review we shall discuss the most common *de novo* GN after renal transplantation in addition to the recently presented *de novo* proliferative GN with monoclonal IgG deposits (PGNMID). The *de novo* GN disease presents late, usually one year after renal transplantation, on the other hand recurrent GN might present earlier, sometimes within the first few weeks of renal transplantation. Unfortunately, both types of patterns of GN, whether *de novo* or recurrent, do have a lower graft survival as compared to patients without glomerular involvement[3].

DE NOVO GLOMERULAR DISEASES AFTER RENAL TRANSPLANTATION

De novo MN

Definition: *De novo* MN, is rather uncommon etiology among causes of allograft failure, can be defined as a MN lesion that is developed in the renal allograft of a patient originally suffered from another renal disease in native kidney[10].

***De novo* or recurrent MN:** The type of IgG subclass deposition is different in recurrent MN when compared to *de novo* MN, where IF is of immense use. Kearney *et al*[11] (2011) reported that IgG4 was dominant in glomerular deposits of recurrent MN, IgG1 was the dominant subtype in *de novo* MN. Honda *et al*[12] (2011) and others reported a clear predominance of IgG4 in idiopathic MN in comparison with the *de novo* type[13]. Another vital difference is the lack of phospholipase A2 receptor (PLA2R) staining in *de novo* MN, in contrast to the *recurrent* MN that is characterized by positive glomerular PLA2R staining[14,15].

Incidence: Of 1000 allograft biopsy, 19 cases of *de novo* MN were reported in a large French series[16], while the incidence was 1.8% in another French study[17], which means that 2% of renal transplant recipients can develop *de novo* MN[14]. In United Kingdom, *de novo* MN is considered to be the second most common cause of nephrotic syndrome after kidney transplantation[18]. The disease was reported to be 9% in a pediatric series[19]. *De novo* MN can be associated with: Alport's syndrome, ureteral obstruction, newly diagnosed HCV and recurrent IgA[10].

Pathogenesis: The new autoimmune disease IgG-related lesions have been recently shown to affect the renal allograft in several ways including *de novo* MN[20]. A novel regulatory protein (named: Pdlim2) has been recognized, with an observed decline of this protein in the podocytes of MN patients. A possible role of this protein in *de novo* MN pathogenesis has been suggested[21]. Various types of injury, *e.g.*, viral, ischemic, immunological and mechanical can induce podocyte damage, exposing the hidden or cryptogenic antigens, which could be different from that of the idiopathic

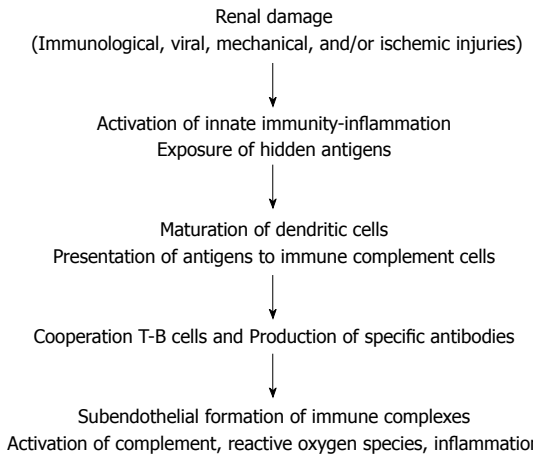


Figure 1 Any type of kidney injury can cause tissue damage. The danger signals released by the damaged tissue alert the recognition receptors, which activate the inflammatory cells and mediators of the innate immunity system. In this inflammatory environment, hidden podocyte antigens may be exposed, whereas dendritic cells become mature, migrate to lymphatic system, and present the antigen to immune competent cells. T cells cooperate with B cells favoring the production of antibodies directed against the exposed antigens planted in the subepithelium, with *in situ* formation of immune complexes, activation of complement, formation of free oxygen radicals, and inflammation. Adapted from: Ponticelli *et al*[10], 2012. *De novo* membranous nephropathy (MN) in kidney allografts. A peculiar form of all immune disease? With permission.

MN. This is quite evident, for example, in allogenic hematopoietic stem cell transplantation[22]. Consequently, these damaged cells will generate danger signals that intercepted by toll-like receptors and other receptors, which in turn initiate a cascade of signals activating transcription factors encoding the inflammatory gene[23]. Finally, the inflammatory cells of the innate system (PNLC, monocytes, macrophages and natural killers' cells) eventually release cytokines, inflammatory mediators and other mediators. Dendritic cells present the antigen to immunocompetent CD4 T cells that trigger B-cell induced antibody production. The end result is subepithelial immune complex deposition, complement activation and glomerular effector cell induced injury[24] (Figure 1).

No one single antigen can be "blamed" to be responsible of evolution of *de novo* MN, but rather a wide array of various antigens. An alloimmune response, viral infection and may be mechanical injury can create an environment that lead to release of various cryptogenic (hidden) autologous podocyte-antigen with subsequent production of auto- and alloantibodies (namely IgG1 subtype) that ultimately results in "*in situ*" immune complex formation, subepithelial deposits and eventually histological form of MN[10]. A thorough search for underlying malignancy and a hidden viral infection should be performed in view of the clear general association between MN and both cancer and infection[14]. Honda *et al*[12] (2011) reported frequent association of the AMR with *de novo* MN. El Kossi *et al*[25] (2008) suggested a possible evolutional role of DSA in development of *de novo* MN.

Role of HCV: HCV is a small RNA virus (30-38 nm) with lipid envelope and related to the *Flaviviridae* family. A robust relation to many glomerular (FSGS, immunotactoid, IgA, post-infectious and fibrillary GN)[26-31] and non-glomerular (tubulointerstitial and TMA) diseases has been reported[30,32]. Prevalence of HCV exceeds 8%-10% in many dialysis centers. HCV is known to be related to a range of renal diseases, such as MPGN Type Ⅰ associated type Ⅱ mixed cryoglobulinemia being the most common, other less common pathologies include MNGN and non-cryoglobulinemic MPGN[3]. Thereby, chronic HCV infection is a serious risk factor for development of *de novo* MN[27]. Another series reported an incidence of 3.6% in patients with positive HCV infection as compared to those with lack of HCV infection (0.36%)[13]. Genesis of *de novo* GN can be influenced by several factors on long-run including impact of immunosuppressive agents, HCV-induced modulation of lymphocyte response and the production of antibodies, so that an imbalance between antigens and antibodies will be created and the subjective allograft susceptibility of the allograft itself[33]. For patients with chronic HCV and post-transplant AMR, a particular focus of attention should be directed to *de novo* MN, with the IgG subtype staining being much helpful to differentiate recurrent from *de novo* MN[13].

The LM features of allograft biopsy of *de novo* lesions are similar to that in idiopathic MN, but with more foam cells in arterial intima and possibly with signs of AMR[34]. IF shows diffuse granular deposits of IgG in the subepithelial side of the glomerular basement membrane, with the IgG1 subclass being dominant in *de novo* MN, while the IgG4 is usually seen in *recurrent* type[11].

Clinical presentation: Clinical features vary much from no symptoms up to nephrotic range proteinuria[7,35,36], with some 25% of them would present with allograft dysfunction[19]. *De novo* MN usually presents a few years after renal transplantation[11,12,37,38].

Prognosis: There are no established risk factors for poor prognosis. In pediatric patients, 60% of Antignac *et al*[19] (1988) patients, for example, lost their grafts in 6 years after diagnosis of *de novo* MN, despite 20% have no proteinuria. In another series, 4 of 7 patients who received a second transplant, developed *de novo* MN for the second time[11]. Prognosis of *de novo* MN in adults is different. *De novo* MN was reported to have no impact on allograft function in a large French series as well as in Schwarz *et al*[39] (1991) study that reported similar 5 year survival in 21 patients with *de novo* MN to other RTR. On the other hand, Monga *et al*[34] (1993) reported a progression of the pathological stage and deposit extension to more glomeruli in serial biopsies. Accelerated allograft loss was also reported by Dische *et al*[40] (1981). Of note, most cases with deleterious outcome showed signs of chronic rejection in allograft biopsy[10]. The observed poor prognosis of *de novo* MN may be attributed by some authors to the associated AMR[41]. The latter is responsible of 20%-30% of allograft losses

in the literature[37]. The impact of *de novo* MN on allograft survival continues to be debatable. While a higher rate of allograft loss associated with signs of chronic rejection was reported in some series[37], no impact on allograft survival has been shown by others[14,37].

Anti-vascular endothelial growth factor therapy related de novo PLA2R-negative membranous nephropathy

The role of vascular endothelial growth factor (VEGF) in angiogenesis is well documented[42,43]. Local (intravitreal) and systemic (IV) anti-VEGF therapy have been recently introduced in many diseases. Systemic (IV) therapy has been used in the management of advanced cancer therapy. Unfortunately, this type of therapy has been associated with several untoward effects, *e.g.*, hypertension, hemorrhage, proteinuria and thromboembolic events[44]. On the other hands, local (intravitreal) route is usually well tolerated[45], due to its low administrated dose and the localized nature of injection. However, clearance of these agents has individual variations that may be reflected as systemic insults[46].

Recently, Wisit Cheungpasitporn *et al*[46] (2015) reported two cases of allograft dysfunction that are related to the administration of intravitreal anti-VEGF therapy. First case developed MN with spherular deposits after one year of initiation of therapy[47]. Moreover, PLA2R antibodies were reported to be negative in biopsy and no anti-PLA2R antibodies were detected in serum[48,49], which favours the *de novo* nature of MN, as only one third of idiopathic MN can express the lack of anti-PLA2R antibodies[48,49]. The increased level of proteinuria was not due to MN, as no evidence of immune complex GN in subsequent biopsy (4 mo), which is in agreement with other reports[49,50]. The second case has long standing decline but stable renal function, it showed progressing proteinuria observed few months after initiation of therapy with a clear evidence of both acute and chronic AMR in allograft biopsy[51].

Relation to proteinuria: Appearance of proteinuria in anti-VEGF treated patients is reported to be related to the start of anti-VEGF therapy, a finding that is supported by allograft biopsy findings (microspherule substructure variant of MN) properly due to a new antibody formation or unmasking of already present anti-HLA antibodies. The appearance of proteinuria is clearly related to the systemic use of anti-VEGF in cancer patients[44]. An observation that can be explained by the well documented effect of VEGF on preserving the glomerular filtration barrier integrity[42,52]. Moreover, an altered VEGF activity has been proposed to be a potential aetiology of mTOR inhibitors-induced proteinuria[53]. On the other hand local (intravitreal) administration of anti-VEGF may lack this effect[45]. A given explanation may be due to its different formulation and the local route of administration. However, clearance of these agents is ultimately systemic[45]. Furthermore, a recent report recorded a precipitous decline of allograft function (GFR < 25 mL/min per 1.73 m^2) in a group of anti-VEGF treated patients[54].

Mechanism of renal injury: The following mechanisms have been postulated as a given explanations for allograft injury: (1) Disruption of the normal survival signals mediated by VEGF leading to creation of alloreactive antibodies or exaggerated renal allograft injury induced by the already present antibodies; (2) Loss of the mitigating effect exerted by VEGF on CyA toxicity[55]; (3) Unmasking action on the already present anti-HLA antibodies; (4) Renal allograft susceptibility to anti-VEGF-induced injury leading to an increased tissue marker expression, including HLA and non-HLA antibodies; and (5) Evolution of antibody-mediated rejection through anti-HLA antibodies production[46]. The exact role of anti-VEGF agents' interference in allograft biology is complex, necessitating more extensive investigations[56-58].

Recommendations: Two recommendations have been proposed in the context of anti-VEGF therapy: (1) RTR should be strictly monitored through at least monthly determination of urinary proteins; and (2) The threshold index for allograft biopsy should be lowered, with application of both IF and EM studies[46].

De novo MPGN

The recent classification of MPGN relies primarily on the immunofluorescence (IF) findings. While cases with only capillary and mesangial complement deposition with lack of the Ig deposits are categorized as C3 glomerulopathy (C3 GN or DDD) or complement-mediated GN (CGN)[14,59], other cases with Ig mesangial and capillary deposits can be classified as immune complex-mediated GN (ICGN) (Table 1) (monoclonal, oligoclonal and polyclonal). Recurrence of MPGN post renal transplantation is frequent (mostly ICGN)[59]. On the other hand, *de novo* C3 glomerulopathy has not been reported[14]. In regards to *de novo* IC-mediated MPGN, it can be seen, but not frequently after renal transplantation, usually in association with HCV infection in about 50% of patients[60].

Incidence: In a large French study, only 13 of 399 (3.25%) patients develop *de novo* MPGN[60]. According to Ponticelli *et al*[14] (2014), *de novo* C3 glomerulopathy (CGN) subtype has not been reported, however, some case reports appear thereafter (see below section "VI").

Allograft biopsy: Typical pattern of hypercellularity with broad capillary loops due to reduplication of the glomerular basement membrane. In IF study, mesangial and subendothelial deposition of Ig as well as complement glomerular subendothelial electronic dense deposits (EDD), while fibrillary pattern is usually seen with cryoglobulinemia, most probably as a result of the associated HCV infection[61]. The impact of the associated systemic diseases is usually responsible of

Table 1 Prevalence of the *de novo vs* recurrent membranoproliferative glomerulonephritis according to the new membranoproliferative glomerulonephritis pathological classification depending on the mechanism of glomerular injury instead of deposits distribution[14,59]

No.	MPGN subtype	Pathological criteria	Recurrent MPGN	*De novo* MPGN
1	ICGN (immune complex-mediated GN)	Contains immune complexes + complement compounds	More common (most of the recurrent cases are ICGN)	Reported (3.25%)
2	CGN (complement-mediated GN)	Contains complement compounds only	Less prevalent (change from one type to another)	Not reported (Ponticelli *et al*[14], 2014)

MPGN: Membranoproliferative glomerulonephritis; ICGN: Immune complex mediated glomerulonephritis; CGN: Complement-mediated glomerulonephritis.

Table 2 Case reports in the literatures on *de novo* proliferative glomerulonephritis with monoclonal IgG deposits in renal allografts

Case	Age at diagnosis	Gender	Onset time (mo)	Type of IgG deposits	C1q deposition	Native kidney disease	Pattern of glomerular injury	Monoclonal gammopathy	Ref.
1	24	M	43	IgG3κ	N/A	T1DM	MPGN	None	Albawardi *et al*[64] (2011)
2	68	F	156	IgG1κ	N/A	PKD	MPGN	None	Albawardi *et al*[64] (2011)
3	38	F	72	IgG3κ	1+	T1DM	MesGN or EC	N/A	Hussain *et al*[72] (2017)
4	61	F	98	IgG3κ	C1q	MPGN	EC	None	Al-Rabadi *et al*[73] (2015)
5	40	F	132	IgG3κ	N/A	MPGN	MPGN	None	Al-Rabadi *et al*[73] (2015)
6	46	M	49	IgG1κ	1+	FSGS	MesGN	N/A	Li *et al*[77] (2017)
7	69	M	6	IgG3κ	1+	Obesity (FSGS?)	MPGN	N/A	Merhi *et al*[75] (2017)

EPGN: Endocapillary proliferative glomerulonephritis; FSGS: Focal segmental glomerulosclerosis; MesGN: Mesangioproliferaitve glomerulonephritis; MPGN: Membranoproliferative glomerulonephritis; N/A: Not available; PGNMID: Proliferative glomerulonephritis with monoclonal IgG deposits; PKD: Polycystic kidney disease; T1DM: Type 1 diabetes mellitus; EC: Endocapillary proliferative; M: Male; F: Female.

the *de novo* pattern of allograft biopsy[14].

Pathogenesis: The pathogenesis is not completely understood. However, the glomerular deposits of the hepatitis C virus as well as the anti-HCV antibodies may be responsible of the histological patterns in HCV positive patients[60]. Presence of cryoglobulin is seen in some patients[62]. Evolution of the clinical and histological pattern associated with *de novo* MPGN may be also triggered by the stress of rejection, calcineurin inhibitors (CNI) toxicity as well as viral infection[14].

Clinical features: Nearly, about 50% of cases presents with nephrotic syndrome, but the majority usually show non-nephrotic range proteinuria (*i.e.*, < one gram). Presence of signs of thrombotic microangiopathy in allograft biopsy is usually associated with the clinical and laboratory manifestations of hemolytic uremic syndrome. Some patients with normal kidney function and non-nephrotic range proteinuria usually experience a slow and silent course, while in others the evolution of *de novo* MPGN can trigger rapid graft loss[33].

De novo proliferative GN with monoclonal IgG deposits

De novo proliferative GN with monoclonal IgG deposits (PGNMID) is an extremely rare disease[63-68]. PGNMID is a unique type of GN that was first presented in the literature for the first time in 2004[69], 5 years later the largest series (37 case) was presented in 2009[70]. PGNMID is a proteinuria/hematuria syndrome with a reported incidence of only 0.17%, usually with a normal workup for paraproteinemia[70]. While the recurrent PGNMID presents early (within the initial two years after renal transplantation), *de novo* PGNMID appears several years later[63,64]. A handful of cases of *de novo* PGNMID have been reported in the literature (Table 2), since Nasr *et al*[70] (2009) presented his largest series of the native PGNMID. After a 30 mo follow up of these patients, 38% had complete or partial recovery, 22% developed ESRF, and (38%) of these patients experienced persistent allograft dysfunction. Only 10% of patients expressed low complement level. No M protein bands were detected, which indicates that PGNMID disease should not be considered a precursor for multiple myeloma development[9]. However, Batal *et al*[68] (2014) reported that 18% of their patient with native PGNMID disease showed an evidence of low grade lymphoma. Moreover, Barbour *et al*[71] (2011) and others also reported two patients with native PGNMID kidney disease with evidence of chronic lymphocytic lymphoma.

Case reports of *de novo* PGNMID in the literature: A detailed summary of the case reports of the *de novo* PGNMID in the literature, as regard age, gender, time elapsed since kidney transplantation, type of the deposited IgG, presence of C1q, native kidney disease, pattern of glomerular injury as well as presence of monoclonal gammopathy have been shown in Table 2[64,72-75].

Clinical presentation: Like recurrent PGNMID, *de novo* PGNMID usually manifests with allograft dysfunction associated with a variable degrees of proteinuria with or without hematuria in a white female patient, the disease generally can be seen in adults above 50 years of age[72,73].

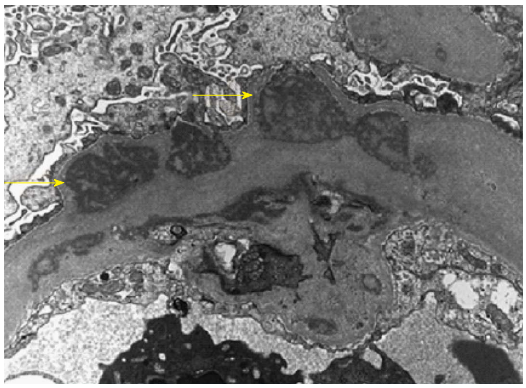


Figure 2 Glomerular capillaries are greatly distorted and thickened by the presence of numerous, sometimes large and/or confluent subendothelial electron-dense deposits (arrows). The electron-dense deposits have a variegated ("two-toned") appearance and are finely granular, but they do not show organized substructures. Adapted from: Al-Rabadi *et al*[73] (2015) (open access).

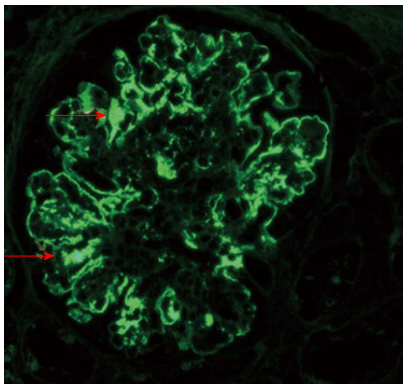


Figure 3 Diffuse irregular granular and pseudo linear deposition of IgG (3+/4+) (arrows). No staining is found in Bowman's capsule or the tubular BM. Adapted from: Al-Rabadi *et al*[73] (2015) (open access).

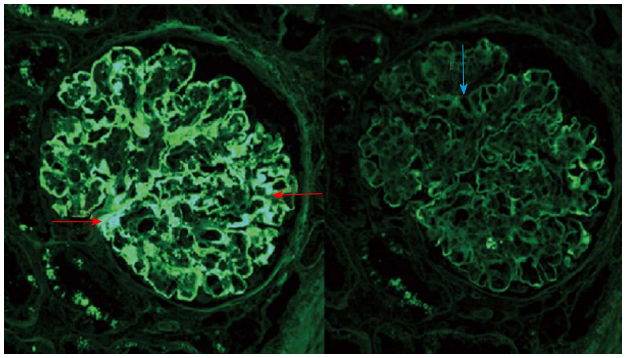


Figure 4 Kappa light chains stain strongly positive (3+/4+) (Rt side, red arrows) along the peripheral capillary walls and mesangial areas. Lambda light chains are negative in the deposits (Lt side, blue arrow). Adapted from: Al-Rabadi *et al*[73] (2015) (open access).

Pathogenesis: Pathogenesis of PGNMID is not clear. However, the reported recurrence of this disease suggests the presence of a circulating factor in RTR[72]. Other reports suggest deposition of a circulating non-deleted monoclonal IgG in the glomeruli, followed by complement fixation with outburst of inflammatory mediators[69]. A variety of intrinsic and extrinsic antigens would cause glomerular injury through stimulation of B-cell clones with subsequent production of the monoclonal IgG, particularly IgG3 subtype (8% of the total IgG). The latter is rapidly absorbed by the glomeruli so that it cannot be detected by immunofixation. Three criteria have been postulated to increase the avidity of IgG3 to glomerular deposition: (1) Positively charged nature; (2) The heaviest molecular weight; and (3) The greatest complement fixing capacity. So, these criteria would augment the affinity of IgG3 to the negatively charged glomerular elements, making it highly nephritogenic[63].

Histopathology: LM usually shows mesangioproliferative or endocapillary GN. EM shows prominent granular mesangial and subendothelial electron dense deposits (EDD) (Figure 2)[73]. Finally, IF study could ultimately establish the PGNMID diagnosis. A positive staining of one of the monoclonal IgG subtypes, with IgG3 being the most common and either *kappa* (most common) or the less common *lambda* subtype, strictly and exclusively in the glomerular constituents. C1q and complement 3 may be positive denoting complement activation (Figures 3 and 4)[73].

Differential diagnosis: PGNMID should be differentiated from other entities, *e.g.*, Type Ⅰ cryoglobulinemic GN, transplant glomerulopathy, primary MPGN, post infectious GN, immunotactoid and fibrillary GN. In comparison to Type Ⅰ cryoglobulinemic GN, PGNMID lacks the serologic evidence of cryoglobulinemia, and also the annular-tubular as well as the fibrillary substructure by EM are absent. The microtubules of 30-40 nm in EM that characterizing immunotactoid are missed, so did the negative Congo red randomly organized fibrils of 16-24 nm diameter of the fibrillary GN. Lack of IF and EM studies can lead to a suspicion of transplant glomerulopathy, but the absence of monoclonality and the faint staining of the IgG can differentiate it from PGNMID[64]. PGNMID may simulate LHCDD in many aspects, but the pathogenesis is not alike[73]. While the heavy and the light chains deposition involve the glomerular as well as the tubular basement membrane in LHCDD, deposition of the intact monoclonal Ig is usually confined to the glomerular constituents in PGNMID. Also, the EDD are of granular nature in PGNMID as opposed to the powdery nature of LCDD deposits[73].

In the last few years, PGNMID disease attained a particular entity. Given the lack of monoclonal bands either in urine or serum, with normal appearance of bone marrow biopsy, this entity should be differentiated from other diseases with similar presentation, a challenging insight during RTR preparation[73]. Once the features of MPGN have been observed in the LM of allograft kidney biopsy, PGNMID disease should be considered

among differential diagnoses. Long term monitoring of PGNMID is recommended to look for occult hematological malignancy[71].

De novo non-collapsing FSGS

De novo FSGS was reported to be the commonest form of *de novo* GN in some Canadian series[76]. While the recurrent FSGS can develop early post renal transplantation, usually in the form of nephrotic syndrome, *de novo* FSGS usually presents more than 12 mo after renal transplantation.

Clinically: *De novo* FSGS presents with a variable amounts of proteinuria up to nephrotic syndrome. Hypertension and progressive decline in allograft function can be seen[14].

Pathogenesis: The size discrepancy between the recipient's body mass and the nephron mass of his allograft as a single kidney will induce compensatory hyperfiltration of the residual nephrons. DM, hypertension, BK polyomavirus[77], or parvovirus B19[78] can be also included in the pathogenesis of FSGS in addition to any pathological event that results in nephron loss. CNI toxicity can result in development of *de novo* FSGS months after transplant procedures in the form of proteinuria, hypertension and progressive decline in allograft function. The robust vasoconstrictor effect in addition to the typical microvascular lesions induced by CNI could ultimately induce arteriolar ischemic changes with subsequent characteristic histopathological lesions. A pivotal role of transforming growth factor-β (TGF-β), the multipotent protein responsible of cell growth regulation, differentiation and matrix formation can be observed. CNI can augment the expression of the podocyte TGF-β[79], leading to podocyte apoptosis and detachment from the glomerular basement membrane with synechia and glomerulosclerosis[14].

Allograft biopsy: Focal and/or global glomerular sclerosis in addition to arteriolar occlusion, tubular atrophy, interstitial nephritis and striped interstitial fibrosis could be seen. On the other hand, RTR who converted their immunosuppression protocol from CNI to a high dose of sirolimus (SRL) have developed *de novo* FSGS with proteinuria and similar lesion to that seen in the classic FSGS. The immunohistochemical studies show decreased or lack of expression of the podocyte-specific epitopes synaptopodin p57 with acquired expression of cytokeratin and PAX2, which reflects an immature fetal phenotype[80]. This pattern reflects a state of podocyte dysregulation, which was confirmed in human by exposure of human podocytes to sirolimus. Decreased VEGF and protein kinase B phosphorylation have been also observed. Dose-dependent decline in Wilms' tumor 1, a transcription factor responsible of podocyte integrity was also observed[81]. *De novo* non-collapsing FSGS usually presents months or year after renal transplantation, with expected poor prognosis. The 5 years graft survival is only 40% after disease diagnosis in cases associated with CAN findings[82,83].

De novo collapsing FSGS

Collapsing FSGS (CG) is a distinct clinical and pathological variant from FSGS[14]. The reported incidence of *de novo* collapsing FSGS is about 0.6%[84]. *De novo* CG usually present 4-5 years after renal transplantation with heavy proteinuria and rapid decline of allograft function. Allograft biopsy usually shows segmental and/or global collapse of the glomerular capillary tuft. Prominent podocytes occupying the Bowman's capsule with marked tubulointerstitial damage as well as obliterative vascular disease are usually seen[85]. Pathogenesis is not clear, but these histological changes could be seen with acute rejection, diabetic nephropathy and immune complex GN disease. Altered hemodynamic stability may be included in the *de novo* CG behavior. Certain infections, *e.g.*, CMV and parvovirus B19 may be also associated with *de novo* CG disease[86,87]. Post-transplant antibody to angiotensin Ⅱ Type Ⅰ receptors, have been implicated in development of AMR[88].

A deleterious impact to the glomerular visceral and parietal epithelial cells integrity leading to cellular dedifferentiation with loss of glomerular filtration barrier function has been suggested[89-91]. Mitochondrial function disturbance has also been postulated as a deleterious mechanism in CG pathogenesis[92]. Ten cases of CG have been reported in serial biopsies in Mayo Clinic performed between 1994 and 2003[93]. CG is found to be prevalent in deceased donor kidney, presented usually with heavy proteinuria, higher serum creatinine level and poor response to plasmapheresis and ultimately allograft loss[94].

Prognosis: Outcomes of *de novo* CG is ultimately poor. All cases reported by Swaminathan *et al*[93] (2006), for example, lost their allograft within three years.

De novo C3 glomerulopathy

C3GN is a recently presented rare GN disease, characterized by predominant C3 glomerular deposits with similar morphology to that seen in DDD. However, in C3 GN there is lack of the ribbon-like intramembranous EDD. Recurrence of C3GN is reported, however, *de novo* C3GN disease is very rare[95].

In 2012, Sethi *et al*[96] (2012) presented the first two cases of recurrent C3GN, with subsequently reported 14 cases more in the next two years[97]. On the other hand, in 2008, Boyer *et al*[98] (2008) present two cases of *de novo* C3GN, however, these cases were presented as an aHUS or complement H deficiency. Furthermore, Nahm *et al*[95] (2016), reported a case of *de novo* C3 GN in a patient with no past history of alternative complement pathway abnormality, family history of renal disease or any symptoms related to glomerular disease. Tests related to complement factor H, complement factor H-related protein 5 genes and C3 nephritic factors were all negative[95]. They postulated an acquired complement

Table 3 Main characteristics of the more frequent *de novo* glomerulonephritis after transplantation (minimal change disease, nephrotic syndrome, membranous nephropathy, membranoproliferative glomerulonephritis, hepatitis C virus, IgAN)

Disease	Presentation	Time of onset	Difference with native GN	Treatment	Prognosis
MN	Proteinuria sometimes in nephrotic range	Late after transplant	Associated with trans-plant complications; IgG1 deposits instead of IgG4	No specific treatment	Slowly progressive
MPGN	Proteinuria, hematuria, NS, nephritic sediment	Months or years after transplant	Often associated with HCV, or with other diseases	Steroids + cytotoxic drugs if crescentic GN (?)	Slowly progressive; poor with many crescents.
FSGS	Proteinuria, rarely in nephrotic range	Months or years after transplant	NS is rare; signs of rejection or CNI toxicity at biopsy	Removal of associated events	Usually poor, particularly in collapsing GN
MCD	NS	Early after transplant	Mild mesangial sclerosis, hypercellularity	Steroids	Good

Adapted from: Ponticelli *et al*[14] (2014). *De novo* Glomerular Diseases after Renal Transplantation. *Clin J Am Soc Nephrol* 2014; 9: 1479-1487, with permission. MCD: Minimal change disease; NS: Nephrotic syndrome; MN: Membranous nephropathy; MPGN: Membranoproliferative GN; HCV: Hepatitis C virus.

abnormality after renal transplantation.

Histopathology: The C3GN early pathological changes usually show minimal mesangial expansion which may progress later to mesangial proliferation. EDD initially located in the mesangium, extend later to the subepithelial and subendothelial areas[97]. The EDD that present early in tubular basement membrane and in Bowman's capsule may change to band-like simulating that present in dense deposition disease (DDD) that is characterizing and specified to its diagnosis[99]. However, C3 GN showed segmental tubular basement membrane deposits[100]. The DDD disease may experience phenotypical transformation to C3GN in the native kidney[101]. However, DDD usually shows more profound MP features as well as more intense complement abnormalities as compared to C3 GN[102]. The presence of an overlap may justify using the term "C3 glomerulopathy" instead of exerting to separate the two pathological identities, DDD and C3 GN[95]. *De novo* C3GN is a rare subtype of post renal transplantation GN diseases. The fundamental role observed through both IF and E/M studies in diagnosis and serial follow up is quite mandatory[95]. Of note that despite the observed decline in C3 deposition, renal function as well as histopathological changes continue to progress.

De novo minimal change disease

De novo minimal change disease (MCD) is a rarely reported disease in RTR. Fulfilled criteria of MCD diagnosis is not always present in some cases, which suggests a misdiagnosis of FSGS disease. While Markowitz *et al*[103] (1998) succeeded to report eight cases with full criteria of MCD, Truong and his associates (2002) added five more cases[104]. Furthermore, *de novo* MCD have been reported in incompatible ABO transplants[105]. With evolution of *de novo* MCD, a nephrotic range proteinuria developed rapidly after renal transplantation, however, some cases reported eight years after transplantation[106].

Histopathology: LM show typically normal appearance of the glomeruli. Some cases show hypercellularity and IgM/C3 deposition[103,105].

Pathogenesis: The pathogenesis of *de novo* MCD still uncertain. An activation of the innate and/or the adaptive immunity with T cell dysfunction and cytokines release, *e.g.*, cardiotrophin-like cytokine-1[107] or the soluble urokinase-plasminogen receptor[108], leading to alteration of the glomerular capillary wall permeability has been suggested. The initial culprit agent is unknown, but certain viral-induced activity has been postulated. Another suggested factor, the costimulatory molecule B7-1 (CD80) in podocytes, has an additional impact on glomerular permselectivity. This agent [B7-1 (CD80)] has been proved to have a role in inducing an experimental nephrotic syndrome[109]. The role of this factor in inducing foot process fusion and proteinuria in the renal allograft is to be determined. The reported development of *de novo* MCD in a patient was on SRL therapy with clearance of the disease with drug withdrawal, has suggested a possible role of certain drugs in *de novo* MCD pathogenesis[110].

Prognosis: *De novo* MCD has a favorable prognosis in most cases[14]. Owing to its potential reversibility, *de novo* MCD has no deleterious impact on allograft survival on the long run. However, this disease is possibly still underestimated as a pivotal cause of nephrotic syndrome in the renal allograft (Table 3).

De novo IgAN

IgAN has been one of the most common GN worldwide. Graft loss has been frequently reported with *recurrent* IgAN[8]. On the other hand, this fate is rarely reported with *de novo* IgAN[111].

Incidence: *De novo* IgAN has been reported to be less common than recurrent IgAN[112]. Considering the high frequency of asymptomatic IgAN, some authors argue that *de novo* IgAN might be considered as "transmitted disease", which means that recipient received an allograft that already had a "latent" form of IgAN[14], this argument is supported by the finding that a considerable percentage of mesangial IgA deposition (16.1%) has been reported in 0-hour protocol biopsy performed by a

Table 4 The risk of recurrence of *de novo* glomerulonephritis after retransplantation is unknown

Disease	Indications to retransplant
MN	In view of the slow progression, there is no contraindication to retransplant
MPGN	The risk of recurrence is high in carriers of HCV, active autoimmune disease, or monoclonal gammopathy. These risk factors should be removed or inactivated before retransplant
FSGS	If FSGS was caused by calcineurin inhibitor or mTOR inhibitor toxicity, there is no contraindication to retransplant, but the dosage of the offending drug should be minimized. If FSGS was associated with AMR, the risk of recurrence is increased. Circulating antibodies should be removed before retransplant
Collapsing nephropathy	Risk of recurrence is probably high. Antiviral and/or removal of circulating AB before retransplant are recommended according to the possible role played by virus infection or AMR in the 1st transplant
MCD	In view of the favorable prognosis, there is no contraindication to retransplant
IgAN	No contraindication to retransplant

Adapted from: Ponticelli *et al*[14] (2014), De Novo Glomerular Diseases after Renal Transplantation. *Clin J Am Soc Nephrol* 2014; 9: 1479-1487. Published online 2014, with permission. MCD: Minimal change disease; NS: Nephrotic syndrome; MN: Membranous nephropathy; MPGN: Membranoproliferative GN; HCV: Hepatitis C virus; FSGS: Focal segmental glomerulosclerosis.

Japanese study[113].

Histopathology: Intracapillary proliferation with a possibility of crescent formation can be observed in many biopsies. IgA and C3 granular deposits in the glomerular capillary wall and mesangium are frequently seen in IF studies.

Clinical features: despite the presence of frequent IgA deposition, *de novo* IgA is frequently asymptomatic especially in Asian population that may be discovered only in protocol biopsy.

Course and prognosis: In case of presence of crescent formation in allograft biopsy, prognosis of *de novo* IgA is ultimately poor, otherwise course and prognosis is quiescent with mild mesangial hypercellularity[8]. For example, Robles *et al*[4] (1991) reported a case of *de novo* IgAN with progressive proteinuria, microscopic hematuria and rapid deterioration of allograft function after renal transplantation in a patient with ESRD due to MPGN. On the other hand, *de novo* Henoch-Schönlein purpura has been described post renal transplantation with a rapid graft loss[114,115].

THERAPY OF *DE NOVO* GN DISEASES

Treatment of de novo MN

Options of *de novo* MN therapy are variable, including rituximab, bortezomib, PE, and intravenous Ig[116-118]. Unfortunately, absence of randomized control prospective studies and the high cost would be an obvious obstacles[13]. Therapy of *de novo* MN is still unclear. There is no enough data to support the use of rituximab in *de novo* MN therapy and there no clear base supporting the introduction of cytotoxic therapy or the intensified immunosuppressive agents would be efficacious[37,119].

Indications for retransplantation: MN is a slowly progressive disease, there is no contraindication to retransplant (Table 4).

Treatment of de novo MPGN

Therapy of *de novo* MPGN is still elusive. Trial of intensification of immunosuppression and the use of steroids generally showed poor and unstable results. Re-transplantation, however, is not contraindicated as long as the HCV infection as well as other risk factors have been eliminated. In this instance, the newly introduced oral anti-HCV agents, *e.g.*, protease inhibitors and/or RNA polymerase inhibitors, should be considered before attempting renal transplantation[14].

Indications for retransplantation: The risk of recurrence is high in HCV carriers, active autoimmune disease, or in monoclonal gammopathy. Risk factors should be eliminated before retransplantation (Table 4).

Treatment of de novo PGNMID GN

There is no established therapy for *de novo* PGNMID[68]. However, a trial of rituximab, cyclophosphamide, plasmapheresis and high dose steroids have been introduced[63,65-67]. An observed reasonable response to rituximab and cyclophosphamide was reported with the recurrent disease, which was attributed by the authors to an early application of the protocol biopsy[63]. Multiple protocols have been tried by others including: High-dose steroids, RAS blocking agents, bortezomib, rituximab with and without steroids and plasmapheresis[78] (Table 3).

Rationale of rituximab use: B cells in PGNMID hypersecrete an abnormal IgG. The latter have the ability of self-aggregation and glomerular deposition. Rituximab, a monoclonal antibody has been widely used post renal transplantation for PTLPD, resistant antibody-mediated rejection and recurrent glomerular disease and as a prophylactic therapy for chronic antibody mediated rejection through inhibiting antibody production and hampering the B-cell immunity[120-127].

The recent advents of rituximab in PGNMID therapy have been shown to improve allograft function with better outcome[67,76,128]. Merhi *et al*[75] (2017), reported a unique results with the use of rituximab in two male

patients one *de novo* (with IgG3κ restriction) and the other is recurrent (with IgG1κ restriction). They reported better allograft function with continuous stability and return to basal creatinine level that have been continued for almost two years with persistent stable clinical and pathological response (Table 3). To declare the magnitude of benefits of rituximab, a clear insight on the pathogenesis of PGNMID depending in a wide scale of prospective controlled randomized trials should be accomplished. The role of allograft protocol biopsy in PGNMID in immunosuppressed patients is to be also declared[75].

Treatment of de novo non-collapsing FSGS

The early interference in the course of *de novo* FSGS by CNI withdrawal and introduction of MMF or mTOR inhibitors (mammalian target of rapamycin) may induce stabilization or even improvement of allograft function. One major drawback should be expected, *i.e.*, the increased risk of rejection, particularly so, if there is associated proteinuria or the CrCl was below 40 mL/min[129]. Allograft loss due *de novo* FSGS, however, does not preclude the attempt of retransplantation as long as the factors incriminated in the pathogenesis of FSGS would be eliminated. It will be also worthy to modulate the therapeutic strategies to decrease the risk of recurrence, *e.g.*, by CNI minimization and/or considering antiviral prophylaxis[14,129] (Table 3).

Retransplantation: In patients with *de novo* FSGS due to either CNI- or mTOR inhibitors-induced toxicity, there is no contraindication to retransplantation, however, the dose of the drug should be modified. If there was an associated AMR, the risk of recurrence would be high. Donors organs that are likely to trigger a repeat challenge by corresponding antigens leading to a rise in DSA should be excluded before retransplantation and, if feasible, desensitization be considered (Table 4).

Treatment of de novo CG

There is no particular therapy for *de novo* CG. With the presence of evidence of viral infection, antiviral agents may be suggested. Despite the unpredicted results, an attempt to use abatacept may be tried if there is B7-1 (CD80) expression in the podocytes[130]. In view of scarce data as regard re-transplantation in patients who lost their grafts due to *de novo* CG, there is no specific recommendation. However, an attempt to do re-transplantation in such a situation should be preceded by meticulous screening of antibodies to angiotensin II Type Ⅰ receptors, in addition to an intensive course of antiviral therapy[14].

Retransplantation: The risk of recurrence of FSGS is potentially high. Antiviral therapy and/or clearance of the circulating antibodies are recommended in view of the potential role of viral infection and/or AMR in the first transplant (Table 4).

Treatment of de novo C3 glomerulopathy

Impact of therapy on glomerular morphology: Eculizumab has been reported to induce partial reduction in glomerular inflammatory activity as well as decline in deposits distribution[100]. On the other hands, other reports showed that eculizumab may be associated with EDD[131]. However, Nahm *et al*[95] (2016) used pulse steroids, ATG, rituximab, PE and IVIG to treat the associated AMR, with good response as regard normalization of serum creatinine and reduction of glomerular C3 deposition, but unfortunately the EDD persist. They speculate that C3 deposits may be masked at the locations that they were hard to wash out.

Follow up: Serial biopsies show more intensified tubular basement membrane deposits as compared to glomerular deposits. So, the E/M examination can declare these deposits more precisely as compared to the IF studies as shown by Hou *et al*[132] (2014), with IF pattern changes in about 43% of cases in repeated biopsies.

Rationale of eculizumab use: Eculizumab has been used in 11 cases of C3GN, with mostly but not always favorable results[101,102,133-141]. Eculizumab is a humanized monoclonal antibodies with a potent affinity to complement 5 and prevents the generation of serum membrane attack complex (sMAC) and release of a very potent inflammatory mediator C5a, giving an effective target of therapy[142]. So, it has been suggested that eculizumab administration could be effective in C3GN therapy if given early in cases with minimal fibrosis, short disease course and in patients with increased sMAC with accepted results[138]. These benefits were confirmed by Kersnik Levart *et al*[143] (2016). They reported clinical as well as laboratory improvement, in addition to normalization of the sMAC levels. Moreover, a quite evident decline in glomerular inflammatory activity was observed in the latest biopsies in the form of absent neutrophilic infiltration and necrotic lesions as well as reduced glomerular proliferation activity. Active cellular crescents get transformed into inactive fibrous crescents.

Decision to commence eculizumab therapy should not be attempted until all other differential diagnoses have been excluded and failures of other immunosuppressive measures have been proved[144]. This will work only if properly guided by serial allograft biopsies as well as the clinical features before commencing to use such an expensive drug with a prolonged-term therapeutic approach[143]. Renal function recovery and decline of proteinuria could be expected even in a patient with crescentic GN with a rapidly progressive course[140]. Furthermore, patient already commenced dialysis can quit RRT after only five months of eculizumab therapy[141]. Six months, however, should be elapsed prior to reporting the failure of eculizumab therapy[141,144]. Long-term sequalae of this drug is uncertain, however, it has been tried successfully in paroxysmal nocturnal hemoglobinuria

without evidence of appearance of proteinuria or decline in renal function[145]. Serial long-term biopsies follow up declared also the new observation of eculizumab binding to the renal tissues, an evidence with no harmful impact, despite the fact that eculizumab deposits are similar to that of the monoclonal Ig deposits[143].

Treatment of de novo MCD

A sustained remission of the nephrotic syndrome is usually expected with intensification of steroid therapy and other immunosuppressive agents[14]. A good renal function can be maintained after remission with or without minimal proteinuria (Table 3).

Retransplantation: Prognosis is quite favorable, there is no contraindication to retransplantation (Table 4).

Treatment of de novo IgA

For mild and moderate *de novo* IgA, no specific therapy is advised. However, Shabaka *et al*[111] (2017) reported that potentiation of immunosuppressive therapy with CNI and augmentation of RAS blockade can lead to a complete remission and better renal function. On the other hand, Carneiro-Roza *et al*[146] (2006) reported a better initial response in decreasing urinary protein level with no improvement in renal function. In patients presented with crescentic IgAN and a rapidly progressive course, pulse steroid, cyclophosphamide and PE may be tried with expected poor results[14].

Retransplantation: No contraindication to retransplant (Table 4).

CONCLUSION

The management of *de novo* GN diseases poses unique set of challenges. For a transplanting team, it is paramount to be armed with as much information as possible about the original disease of the native kidney when proceeding with renal transplantation. A lacunae in information would raise the risk of graft loss due to recurrent GN disease. Moreover, awareness of the pathogenesis of these diseases, their clinical features as well as their potential prognosis would help in improving both allograft and patient survival. One of the greatest obstacles hampering the achievement of these targets is the scarce number of the reported *de novo* GN diseases after renal transplantation. A world-wide cooperation between transplantation centers through multicenter randomized controlled trials would address many questions in regards to making a clear diagnosis and defining a robust management plan.

ACKNOWLEDGMENTS

Authors of this article do appreciate the permission offered by Professor, Dr. Ponticelli C, allowing the use of the graphs included in his valuable articles.

REFERENCES

1 **Ivanyi B**. A primer on recurrent and de novo glomerulonephritis in renal allografts. *Nat Clin Pract Nephrol* 2008; **4**: 446-457 [PMID: 18560395 DOI: 10.1038/ncpneph0854]

2 **Denton MD**, Singh AK. Recurrent and de novo glomerulonephritis in the renal allograft. *Semin Nephrol* 2000; **20**: 164-175 [PMID: 10746858]

3 **Molina M**, Sánchez J, Sevillano ÁM, Bengoa I, Gutiérrez E, Martínez MA, Hernández E, Praga M. Two cases of glomerulonephritis in one patient infected with the hepatitis C virus. *Nefrologia* 2013; **33**: 593-600 [PMID: 23897193 DOI: 10.3265/Nefrologia.pre2013.May.12067]

4 **Robles NR**, Gomez Campdera FJ, Anaya F, Niembro De Rasche E, Galan A, Rengel MA, Valderrabano F. IgA nephropathy with rapidly progressive course after kidney transplantation. *Nephron* 1991; **58**: 487-488 [PMID: 1922620]

5 **Francisco S**, Wall BM, Cooke CR. Immunoglobulin A nephropathy in a renal allograft of a black transplant recipient. *Am J Nephrol* 1992; **12**: 121-125 [PMID: 1415357]

6 **Jeong HJ**, Kim YS, Kwon KH, Kim SI, Kim MS, Choi KH, Lee HY, Han DS, Park K. Glomerular crescents are responsible for chronic graft dysfunction in post-transplant IgA nephropathy. *Pathol Int* 2004; **54**: 837-842 [PMID: 15533226 DOI: 10.1111/j.1440-1827.2004.01751.x]

7 **Gough J**, Yilmaz A, Yilmaz S, Benediktsson H. Recurrent and de novo glomerular immune-complex deposits in renal transplant biopsies. *Arch Pathol Lab Med* 2005; **129**: 231-233 [PMID: 15679428 DOI: 10.1043/1543-2165(2005)129]

8 **Kowalewska J**, Yuan S, Sustento-Reodica N, Nicosia RF, Smith KD, Davis CL, Alpers CE. IgA nephropathy with crescents in kidney transplant recipients. *Am J Kidney Dis* 2005; **45**: 167-175 [PMID: 15696457]

9 **Tang Z**, Ji SM, Chen DR, Wen JQ, Chen JS, Liu ZH, Li LS. Recurrent or de novo IgA nephropathy with crescent formation after renal transplantation. *Ren Fail* 2008; **30**: 611-616 [PMID: 18661411 DOI: 10.1080/08860220802134516]

10 **Ponticelli C**, Glassock RJ. De novo membranous nephropathy (MN) in kidney allografts. A peculiar form of alloimmune disease? *Transpl Int* 2012; **25**: 1205-1210 [PMID: 22909324 DOI: 10.1111/j.1432-2277.2012.01548.x]

11 **Kearney N**, Podolak J, Matsumura L, Houghton D, Troxell M. Patterns of IgG subclass deposits in membranous glomerulonephritis in renal allografts. *Transplant Proc* 2011; **43**: 3743-3746 [PMID: 22172838 DOI: 10.1016/j.transproceed.2011.10.042]

12 **Honda K**, Horita S, Toki D, Taneda S, Nitta K, Hattori M, Tanabe K, Teraoka S, Oda H, Yamaguchi Y. De novo membranous nephropathy and antibody-mediated rejection in transplanted kidney. *Clin Transplant* 2011; **25**: 191-200 [PMID: 20236137 DOI: 10.1111/j.1399-0012.2010.01213.x]

13 **Doke T**, Sato W, Takahashi K, Hayashi H, Koide S, Sasaki H, Kusaka M, Shiroki R, Hoshinaga K, Takeda A, Yuzawa Y, Hasegawa M. Post-Transplant Membranous Nephropathy Associated with Chronic Active Antibody-Mediated Rejection and Hepatitis C Infection after Deceased Donor Renal Transplantation. *Intern Med* 2016; **55**: 375-380 [PMID: 26875963 DOI: 10.2169/internalmedicine.55.5468]

14 **Ponticelli C**, Moroni G, Glassock RJ. De novo glomerular diseases after renal transplantation. *Clin J Am Soc Nephrol* 2014; **9**: 1479-1487 [PMID: 24700797 DOI: 10.2215/CJN.12571213]

15 **Larsen CP**, Walker PD. Phospholipase A2 receptor (PLA2R) staining is useful in the determination of de novo versus recurrent membranous glomerulopathy. *Transplantation* 2013; **95**: 1259-1262 [PMID: 23514961 DOI: 10.1097/TP.0b013e31828a947b]

16 **Charpentier B**, Lévy M. [Cooperative study of de novo extramembranous glomerulonephritis in renal allografts in humans: report of 19 new cases in 1550 renal transplant patients of the transplantation group of the Ile de France]. *Nephrologie* 1982; **3**: 158-166 [PMID: 6759963]

17 **Aline-Fardin A**, Rifle G, Martin L, Justrabo E, Bour JB, D' Athis P, Tanter Y, Mousson C. Recurent and de novo membranous glomerulopathy after kidney transplantation. *Transplant Proc* 2009; **41**: 669-671 [PMID: 19328952 DOI: 10.1016/j.transproceed.2009.01.042]

18 **Davison AM**, Johnston PA. Allograft membranous nephropathy. *Nephrol Dial Transplant* 1992; **7** Suppl 1: 114-118 [PMID: 1343822]

19 **Antignac C**, Hinglais N, Gubler MC, Gagnadoux MF, Broyer M, Habib R. De novo membranous glomerulonephritis in renal allografts in children. *Clin Nephrol* 1988; **30**: 1-7 [PMID: 3061691]

20 **Cornell LD**. IgG4-related kidney disease. *Curr Opin Nephrol Hypertens* 2012; **21**: 279-288 [PMID: 22487688 DOI: 10.1097/MNH.0b013e32835265ac]

21 **Sistani L**, Dunér F, Udumala S, Hultenby K, Uhlen M, Betsholtz C, Tryggvason K, Wernerson A, Patrakka J. Pdlim2 is a novel actin-regulating protein of podocyte foot processes. *Kidney Int* 2011; **80**: 1045-1054 [PMID: 21814175 DOI: 10.1038/ki.2011.231]

22 **Stevenson WS**, Nankivell BJ, Hertzberg MS. Nephrotic syndrome after stem cell transplantation. *Clin Transplant* 2005; **19**: 141-144 [PMID: 15659148 DOI: 10.1111/j.1399-0012.2004.00294.x]

23 **Medzhitov R**. Toll-like receptors and innate immunity. *Nat Rev Immunol* 2001; **1**: 135-145 [PMID: 11905821 DOI: 10.1038/35100529]

24 **Couser WG**. Basic and translational concepts of immune-mediated glomerular diseases. *J Am Soc Nephrol* 2012; **23**: 381-399 [PMID: 22282593 DOI: 10.1681/ASN.2011030304]

25 **El Kossi M**, Harmer A, Goodwin J, Wagner B, Shortland J, Angel C, McKane W. De novo membranous nephropathy associated with donor-specific alloantibody. *Clin Transplant* 2008; **22**: 124-127 [PMID: 18217914 DOI: 10.1111/j.1399-0012.2007.00741.x]

26 **Ji F**, Li Z, Ge H, Deng H. Successful interferon-α treatment in a patient with IgA nephropathy associated with hepatitis C virus infection. *Intern Med* 2010; **49**: 2531-2532 [PMID: 21088367]

27 **Cruzado JM**, Torras J, Gil-Vernet S, Grinyó JM. Glomerulonephritis associated with hepatitis C virus infection after renal transplantation. *Nephrol Dial Transplant* 2000; **15** Suppl 8: 65-67 [PMID: 11261709]

28 **Gonzalo A**, Navarro J, Bárcena R, Quereda C, Ortuño J. IgA nephropathy associated with hepatitis C virus infection. *Nephron* 1995; **69**: 354 [PMID: 7753282]

29 **Cabezuelo JB**, Enríquez R, Andrada E, Amorós F, Sirvent AE, Reyes A. [Extracapillary IgA nephropathy associated with infection with hepatitis C virus and hepatic cirrhosis]. *Nefrologia* 2000; **20**: 379-382 [PMID: 11039265]

30 **Sumida K**, Ubara Y, Hoshino J, Suwabe T, Nakanishi S, Hiramatsu R, Hasegawa E, Hayami N, Yamanouchi M, Sawa N, Takemoto F, Takaichi K, Oohashi K. Hepatitis C virus-related kidney disease: various histological patterns. *Clin Nephrol* 2010; **74**: 446-456 [PMID: 21084048]

31 **Coccoli P**, Esposito P, Cianciaruso B, Pota A, Visciano B, Annecchini R, Parrilli G. Hepatitis C and kidney disease. *Dig Liver Dis* 2007; **39** Suppl 1: S83-S85 [PMID: 17936231]

32 **Meyers CM**, Seeff LB, Stehman-Breen CO, Hoofnagle JH. Hepatitis C and renal disease: an update. *Am J Kidney Dis* 2003; **42**: 631-657 [PMID: 14520615]

33 **Cruzado JM**, Carrera M, Torras J, Grinyó JM. Hepatitis C virus infection and de novo glomerular lesions in renal allografts. *Am J Transplant* 2001; **1**: 171-178 [PMID: 12099366]

34 **Monga G**, Mazzucco G, Basolo B, Quaranta S, Motta M, Segoloni G, Amoroso A. Membranous glomerulonephritis (MGN) in transplanted kidneys: morphologic investigation on 256 renal allografts. *Mod Pathol* 1993; **6**: 249-258 [PMID: 8346172]

35 **Heidet L**, Gagnadoux ME, Beziau A, Niaudet P, Broyer M, Habib R. Recurrence of de novo membranous glomerulonephritis on renal grafts. *Clin Nephrol* 1994; **41**: 314-318 [PMID: 8050213]

36 **Teixeira e Costa F**, Pinto JR, Carvalho F, Galvão MJ. An early case of de novo membranous nephropathy in a renal transplant patient. *Transplant Proc* 2002; **34**: 364 [PMID: 11959328 DOI: 10.1016/S0041-1345(01)]

37 **Truong L**, Gelfand J, D'Agati V, Tomaszewski J, Appel G, Hardy M, Pirani CL. De novo membranous glomerulonephropathy in renal allografts: a report of ten cases and review of the literature. *Am J Kidney Dis* 1989; **14**: 131-144 [PMID: 2667346 DOI: 10.1016/S0272-6386(89)80189-1]

38 **Cosyns JP**, Pirson Y, Squifflet JP, Alexandre GP, van Ypersele de Strihou C, Pinn VW, Sweet SJ, Shapiro KS, Cho S, Harrington JT. De novo membranous nephropathy in human renal allografts: report of nine patients. *Kidney Int* 1982; **22**: 177-183 [PMID: 6752531]

39 **Schwarz A**, Krause PH, Offermann G, Keller F. Recurrent and de novo renal disease after kidney transplantation with or without cyclosporine A. *Am J Kidney Dis* 1991; **17**: 524-531 [PMID: 2024653]

40 **Dische FE**, Herbertson BM, Melcher DH, Morley AR. Membranous glomerulonephritis in transplant kidneys: recurrent or de novo disease in four patients. *Clin Nephrol* 1981; **15**: 154-163 [PMID: 7023767]

41 **Kim M**, Martin ST, Townsend KR, Gabardi S. Antibody-mediated rejection in kidney transplantation: a review of pathophysiology, diagnosis, and treatment options. *Pharmacotherapy* 2014; **34**: 733-744 [PMID: 24753207 DOI: 10.1002/phar.1426]

42 **Schrijvers BF**, Flyvbjerg A, De Vriese AS. The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. *Kidney Int* 2004; **65**: 2003-2017 [PMID: 15149314 DOI: 10.1111/j.1523-1755.2004.00621.x]

43 **Carmeliet P**. Angiogenesis in life, disease and medicine. *Nature* 2005; **438**: 932-936 [PMID: 16355210 DOI: 10.1038/nature04478]

44 **Gordon MS**, Cunningham D. Managing patients treated with bevacizumab combination therapy. *Oncology* 2005; **69** Suppl 3: 25-33 [PMID: 16301833 DOI: 10.1159/000088481]

45 **Macugen AMD Study Group**, Apte RS, Modi M, Masonson H, Patel M, Whitfield L, Adamis AP. Pegaptanib 1-year systemic safety results from a safety-pharmacokinetic trial in patients with neovascular age-related macular degeneration. *Ophthalmology* 2007; **114**: 1702-1712 [PMID: 17509689 DOI: 10.1016/j.ophtha.2007.02.021]

46 **Cheungpasitporn W**, Chebib FT, Cornell LD, Brodin ML, Nasr SH, Schinstock CA, Stegall MD, Amer H. Intravitreal Antivascular Endothelial Growth Factor Therapy May Induce Proteinuria and Antibody Mediated Injury in Renal Allografts. *Transplantation* 2015; **99**: 2382-2386 [PMID: 25905984 DOI: 10.1097/TP.0000000000000750]

47 **Kowalewska J**, Smith KD, Hudkins KL, Chang A, Fogo AB, Houghton D, Leslie D, Aitchison J, Nicosia RF, Alpers CE. Membranous glomerulopathy with spherules: an uncommon variant with obscure pathogenesis. *Am J Kidney Dis* 2006; **47**: 983-992 [PMID: 16731293 DOI: 10.1053/j.ajkd.2006.03.004]

48 **El-Zoghby ZM**, Grande JP, Fraile MG, Norby SM, Fervenza FC, Cosio FG. Recurrent idiopathic membranous nephropathy: early diagnosis by protocol biopsies and treatment with anti-CD20 monoclonal antibodies. *Am J Transplant* 2009; **9**: 2800-2807 [PMID: 19845581 DOI: 10.1111/j.1600-6143.2009.02851.x]

49 **Herrmann SM**, Sethi S, Fervenza FC. Membranous nephropathy: the start of a paradigm shift. *Curr Opin Nephrol Hypertens* 2012; **21**: 203-210 [PMID: 22240444 DOI: 10.1097/MNH.0b013e32835026ed]

50 **Myslak M**, Amer H, Morales P, Fidler ME, Gloor JM, Larson TS, Stegall MD, Cosio FG. Interpreting post-transplant proteinuria in patients with proteinuria pre-transplant. *Am J Transplant* 2006; **6**: 1660-1665 [PMID: 16827868 DOI: 10.1111/j.1600-6143.2006.01361.x]

51 **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 meeting report writing committee. 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]

52 **Jiménez-Sousa MA**, Fernández-Rodríguez A, Heredia M, Tamayo E, Guzmán-Fulgencio M, Lajo C, López E, Gómez-Herreras JI, Bustamante J, Bermejo-Martín JF, Resino S. Genetic polymorphisms located in TGFB1, AGTR1, and VEGFA genes are

associated to chronic renal allograft dysfunction. *Cytokine* 2012; **58**: 321-326 [PMID: 22433249 DOI: 10.1016/j.cyto.2012.02.017]

53 **Ko HT**, Yin JL, Wyburn K, Wu H, Eris JM, Hambly BD, Chadban SJ. Sirolimus reduces vasculopathy but exacerbates proteinuria in association with inhibition of VEGF and VEGFR in a rat kidney model of chronic allograft dysfunction. *Nephrol Dial Transplant* 2013; **28**: 327-336 [PMID: 23229928 DOI: 10.1093/ndt/gfs453]

54 **Georgalas I**, Papaconstantinou D, Papadopoulos K, Pagoulatos D, Karagiannis D, Koutsandrea C. Renal injury following intravitreal anti-VEGF administration in diabetic patients with proliferative diabetic retinopathy and chronic kidney disease--a possible side effect? *Curr Drug Saf* 2014; **9**: 156-158 [PMID: 24517109]

55 **Alvarez Arroyo MV**, Suzuki Y, Yagüe S, Lorz C, Jiménez S, Soto C, Barat A, Belda E, González-Pacheco FR, Deudero JJ, Castilla MA, Egido J, Ortiz A, Caramelo C. Role of endogenous vascular endothelial growth factor in tubular cell protection against acute cyclosporine toxicity. *Transplantation* 2002; **74**: 1618-1624 [PMID: 12490797 DOI: 10.1097/01.TP.0000041449.31360.16]

56 **Peng W**, Chen J, Jiang Y, Shou Z, Chen Y, Wang H. Acute renal allograft rejection is associated with increased levels of vascular endothelial growth factor in the urine. *Nephrology* (Carlton) 2008; **13**: 73-79 [PMID: 18199108 DOI: 10.1111/j.1440-1797.2007.00856.x]

57 **Bieri M**, Oroszlan M, Farkas A, Ligeti N, Bieri J, Mohacsi P. Anti-HLA I antibodies induce VEGF production by endothelial cells, which increases proliferation and paracellular permeability. *Int J Biochem Cell Biol* 2009; **41**: 2422-2430 [PMID: 19577661 DOI: 10.1016/j.biocel.2009.06.009]

58 **Mittal RD**, Srivastava P, Singh V, Jaiswal P, Kapoor R. Association of common variants of vascular endothelial growth factor and interleukin-18 genes with allograft survival in renal transplant recipients of North India. *DNA Cell Biol* 2011; **30**: 309-315 [PMID: 21323573 DOI: 10.1089/dna.2010.1138]

59 **Alasfar S**, Carter-Monroe N, Rosenberg AZ, Montgomery RA, Alachkar N. Membranoproliferative glomerulonephritis recurrence after kidney transplantation: using the new classification. *BMC Nephrol* 2016; **17**: 7 [PMID: 26754737 DOI: 10.1186/s12882-015-0219-x]

60 **Roth D**, Cirocco R, Zucker K, Ruiz P, Viciana A, Burke G, Carreno M, Esquenazi V, Miller J. De novo membranoproliferative glomerulonephritis in hepatitis C virus-infected renal allograft recipients. *Transplantation* 1995; **59**: 1676-1682 [PMID: 7541575]

61 **Hammoud H**, Haem J, Laurent B, Alamartine E, Diab N, Defilippis JP, Berthoux P, Berthoux F. Glomerular disease during HCV infection in renal transplantation. *Nephrol Dial Transplant* 1996; **11** Suppl 4: 54-55 [PMID: 8918756]

62 **Faguer S**, Kamar N, Boulestin A, Esposito L, Durand D, Blancher A, Rostaing L. Prevalence of cryoglobulinemia and autoimmunity markers in renal-transplant patients. *Clin Nephrol* 2008; **69**: 239-243 [PMID: 18397697]

63 **Nasr SH**, Sethi S, Cornell LD, Fidler ME, Boelkins M, Fervenza FC, Cosio FG, D'Agati VD. Proliferative glomerulonephritis with monoclonal IgG deposits recurs in the allograft. *Clin J Am Soc Nephrol* 2011; **6**: 122-132 [PMID: 20876681 DOI: 10.2215/CJN.05750710]

64 **Albawardi A**, Satoskar A, Von Visger J, Brodsky S, Nadasdy G, Nadasdy T. Proliferative glomerulonephritis with monoclonal IgG deposits recurs or may develop de novo in kidney allografts. *Am J Kidney Dis* 2011; **58**: 276-281 [PMID: 21705124 DOI: 10.1053/j.ajkd.2011.05.003]

65 **Sumida K**, Ubara Y, Marui Y, Nakamura M, Takaichi K, Tomikawa S, Fujii T, Ohashi K. Recurrent proliferative glomerulonephritis with monoclonal IgG deposits of IgG2λ subtype in a transplanted kidney: a case report. *Am J Kidney Dis* 2013; **62**: 587-590 [PMID: 23474011 DOI: 10.1053/j.ajkd.2013.01.013]

66 **Ranghino A**, Tamagnone M, Messina M, Barreca A, Biancone L, Basolo B, Segoloni GP, Mazzucco G. A case of recurrent proliferative glomerulonephritis with monoclonal IgG deposits after kidney transplant treated with plasmapheresis. *Case Rep Nephrol Urol* 2012; **2**: 46-52 [PMID: 23197955 DOI: 10.1159/000339405]

67 **Wu D**, Chen JS, Cheng DR, Chen H, Li X, Ji SM, Xie KN, Ni XF, Liu ZH, Wen JQ. Recurrent Proliferative Glomerulonephritis With Monoclonal IgG Deposits After a Renal Transplant Which Was Insensitive to Pulse Therapy Remitted by Double Filtration Plasmapheresis. *Exp Clin Transplant* 2015; **13**: 467-470 [PMID: 25275829 DOI: 10.6002/ect.2014.0107]

68 **Batal I**, Bijol V, Schlossman RL, Rennke HG. Proliferative glomerulonephritis with monoclonal immunoglobulin deposits in a kidney allograft. *Am J Kidney Dis* 2014; **63**: 318-323 [PMID: 24051081 DOI: 10.1053/j.ajkd.2013.07.015]

69 **Nasr SH**, Markowitz GS, Stokes MB, Seshan SV, Valderrama E, Appel GB, Aucouturier P, D'Agati VD. Proliferative glomerulonephritis with monoclonal IgG deposits: a distinct entity mimicking immune-complex glomerulonephritis. *Kidney Int* 2004; **65**: 85-96 [PMID: 14675039 DOI: 10.1111/j.1523-1755.2004.00365.x]

70 **Nasr SH**, Satoskar A, Markowitz GS, Valeri AM, Appel GB, Stokes MB, Nadasdy T, D'Agati VD. Proliferative glomerulonephritis with monoclonal IgG deposits. *J Am Soc Nephrol* 2009; **20**: 2055-2064 [PMID: 19470674 DOI: 10.1681/ASN.2009010110]

71 **Barbour SJ**, Beaulieu MC, Zalunardo NY, Magil AB. Proliferative glomerulonephritis with monoclonal IgG deposits secondary to chronic lymphocytic leukemia. Report of two cases. *Nephrol Dial Transplant* 2011; **26**: 2712-2714 [PMID: 21633102 DOI: 10.1093/ndt/gfr251]

72 **Hussain SM**, Sureshkumar KK. Proliferative glomerulonephritis with monoclonal IgG deposits; an unusual cause of de novo disease in kidney allograft. *J Nephropathol* 2017; **6**: 220-224 [PMID: 28975104 DOI: 10.15171/jnp.2017.36]

73 **Al-Rabadi L**, Francis JM, Henderson J, Ghai S. Proliferative glomerulonephritis with monoclonal immunoglobulin in renal allografts. *Clin Kidney J* 2015; **8**: 722-728 [PMID: 26613031 DOI: 10.1093/ckj/sfv105]

74 **Tsuji T**, Miura M, Yanai M, Itami H, Ishii Y, Akimoto M, Fukasawa Y. De novo proliferative glomerulonephritis with monoclonal IgG deposits of the IgG1κ subtype in a kidney allograft. *Nephrology* (Carlton) 2016; **21** Suppl 1: 44-47 [PMID: 26976360 DOI: 10.1111/nep.12773]

75 **Merhi B**, Patel N, Bayliss G, Henriksen KJ, Gohh R. Proliferative glomerulonephritis with monoclonal IgG deposits in two kidney allografts successfully treated with rituximab. *Clin Kidney J* 2017; **10**: 405-410 [PMID: 28616219 DOI: 10.1093/ckj/sfx001]

76 **Chailimpamontree W**, Dmitrienko S, Li G, Balshaw R, Magil A, Shapiro RJ, Landsberg D, Gill J, Keown PA; Genome Canada Biomarkers in Transplantation Group. Probability, predictors, and prognosis of posttransplantation glomerulonephritis. *J Am Soc Nephrol* 2009; **20**: 843-851 [PMID: 19193778 DOI: 10.1681/ASN.2008050454]

77 **Li RM**, Branton MH, Tanawattanacharoen S, Falk RA, Jennette JC, Kopp JB. Molecular identification of SV40 infection in human subjects and possible association with kidney disease. *J Am Soc Nephrol* 2002; **13**: 2320-2330 [PMID: 12191976]

78 **Waldman M**, Kopp JB. Parvovirus B19 and the kidney. *Clin J Am Soc Nephrol* 2007; **2** Suppl 1: S47-S56 [PMID: 17699510 DOI: 10.2215/CJN.01060307]

79 **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]

80 **Letavernier E**, Bruneval P, Mandet C, Duong Van Huyen JP, Péraldi MN, Helal I, Noël LH, Legendre C. High sirolimus levels may induce focal segmental glomerulosclerosis de novo. *Clin J Am Soc Nephrol* 2007; **2**: 326-333 [PMID: 17699432 DOI: 10.2215/CJN.03751106]

81 **Letavernier E**, Bruneval P, Vandermeersch S, Perez J, Mandet C, Belair MF, Haymann JP, Legendre C, Baud L. Sirolimus interacts with pathways essential for podocyte integrity. *Nephrol Dial Transplant* 2009; **24**: 630-638 [PMID: 18927120 DOI: 10.1093/ndt/gfn574]

82 **Cosio FG**, Frankel WL, Pelletier RP, Pesavento TE, Henry ML,

Ferguson RM. Focal segmental glomerulosclerosis in renal allografts with chronic nephropathy: implications for graft survival. *Am J Kidney Dis* 1999; **34**: 731-738 [PMID: 10516356 DOI: 10.1016/S0272-6386(99)70400-2]

83 **Gupta R**, Sharma A, Mahanta PJ, Agarwal SK, Dinda AK. Focal and segmental glomerulosclerosis in renal allograft recipients: a clinico-pathologic study of 37 cases. *Saudi J Kidney Dis Transpl* 2013; **24**: 8-14 [PMID: 23354185]

84 **Meehan SM**, Pascual M, Williams WW, Tolkoff-Rubin N, Delmonico FL, Cosimi AB, Colvin RB. De novo collapsing glomerulopathy in renal allografts. *Transplantation* 1998; **65**: 1192-1197 [PMID: 9603167]

85 **Gupta R**, Sharma A, Agarwal SK, Dinda AK. Collapsing glomerulopathy in renal allograft biopsies: A study of nine cases. *Indian J Nephrol* 2011; **21**: 10-13 [PMID: 21655163 DOI: 10.4103/0971-4065.75220]

86 **Tomlinson L**, Boriskin Y, McPhee I, Holwill S, Rice P. Acute cytomegalovirus infection complicated by collapsing glomerulopathy. *Nephrol Dial Transplant* 2003; **18**: 187-189 [PMID: 12480980]

87 **Moudgil A**, Shidban H, Nast CC, Bagga A, Aswad S, Graham SL, Mendez R, Jordan SC. Parvovirus B19 infection-related complications in renal transplant recipients: treatment with intravenous immunoglobulin. *Transplantation* 1997; **64**: 1847-1850 [PMID: 9422430]

88 **Alachkar N**, Gupta G, Montgomery RA. Angiotensin antibodies and focal segmental glomerulosclerosis. *N Engl J Med* 2013; **368**: 971-973 [PMID: 23465123 DOI: 10.1056/NEJMc1207233]

89 **Schwimmer JA**, Markowitz GS, Valeri A, Appel GB. Collapsing glomerulopathy. *Semin Nephrol* 2003; **23**: 209-218 [PMID: 12704581 DOI: 10.1053/snep.2003.50019]

90 **Albaqumi M**, Soos TJ, Barisoni L, Nelson PJ. Collapsing glomerulopathy. *J Am Soc Nephrol* 2006; **17**: 2854-2863 [PMID: 16914539 DOI: 10.1681/ASN.2006030225]

91 **Albaqumi M**, Barisoni L. Current views on collapsing glomerulopathy. *J Am Soc Nephrol* 2008; **19**: 1276-1281 [PMID: 18287560 DOI: 10.1681/ASN.2007080926]

92 **Sauter M**, Jülg B, Porubsky S, Cohen C, Fischereder M, Sitter T, Schlondorff D, Gröne HJ. Nephrotic-range proteinuria following pamidronate therapy in a patient with metastatic breast cancer: mitochondrial toxicity as a pathogenetic concept? *Am J Kidney Dis* 2006; **47**: 1075-1080 [PMID: 16731304 DOI: 10.1053/j.ajkd.2006.02.189]

93 **Swaminathan S**, Lager DJ, Qian X, Stegall MD, Larson TS, Griffin MD. Collapsing and non-collapsing focal segmental glomerulosclerosis in kidney transplants. *Nephrol Dial Transplant* 2006; **21**: 2607-2614 [PMID: 16705026 DOI: 10.1093/ndt/gfl225]

94 **Sureshkumar KK**, Dosani I, Jasnosz KM, Arora S. De Novo Collapsing Glomerulopathy: An Unusual Cause of Early Graft Failure following Kidney Transplantation. *Case Rep Transplant* 2011; **2011**: 263970 [PMID: 23198255 DOI: 10.1155/2011/263970]

95 **Nahm JH**, Song SH, Kim YS, Cheong HI, Lim BJ, Kim BS, Jeong HJ. De novo C3 glomerulonephritis in a renal allograft. *Ultrastruct Pathol* 2016; **40**: 112-115 [PMID: 26986539 DOI: 10.3109/01913123.2016.1154634]

96 **Sethi S**, Fervenza FC, Zhang Y, Zand L, Vrana JA, Nasr SH, Theis JD, Dogan A, Smith RJ. C3 glomerulonephritis: clinicopathological findings, complement abnormalities, glomerular proteomic profile, treatment, and follow-up. *Kidney Int* 2012; **82**: 465-473 [PMID: 22673887 DOI: 10.1038/ki.2012.212]

97 **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 DOI: 10.1681/ASN.2013070715]

98 **Boyer O**, Noël LH, Balzamo E, Guest G, Biebuyck N, Charbit M, Salomon R, Frémeaux-Bacchi V, Niaudet P. Complement factor H deficiency and posttransplantation glomerulonephritis with isolated C3 deposits. *Am J Kidney Dis* 2008; **51**: 671-677 [PMID: 18371543 DOI: 10.1053/j.ajkd.2007.11.032]

99 **Medjeral-Thomas NR**, O'Shaughnessy MM, O'Regan JA, Traynor C, Flanagan M, Wong L, Teoh CW, Awan A, Waldron M, Cairns T, O'Kelly P, Dorman AM, Pickering MC, Conlon PJ, Cook HT. C3 glomerulopathy: clinicopathologic features and predictors of outcome. *Clin J Am Soc Nephrol* 2014; **9**: 46-53 [PMID: 24178974 DOI: 10.2215/CJN.04700513]

100 **Herlitz LC**, Bomback AS, Markowitz GS, Stokes MB, Smith RN, Colvin RB, Appel GB, D'Agati VD. Pathology after eculizumab in dense deposit disease and C3 GN. *J Am Soc Nephrol* 2012; **23**: 1229-1237 [PMID: 22677550 DOI: 10.1681/ASN.2011121186]

101 **Gurkan S**, Fyfe B, Weiss L, Xiao X, Zhang Y, Smith RJ. Eculizumab and recurrent C3 glomerulonephritis. *Pediatr Nephrol* 2013; **28**: 1975-1981 [PMID: 23689905 DOI: 10.1007/s00467-013-2503-y]

102 **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]

103 **Markowitz GS**, Stemmer CL, Croker BP, D'Agati VD. De novo minimal change disease. *Am J Kidney Dis* 1998; **32**: 508-513 [PMID: 9740171]

104 **Zafarmand AA**, Baranowska-Daca E, Ly PD, Tsao CC, Choi YJ, Suki WN, Truong LD. De novo minimal change disease associated with reversible post-transplant nephrotic syndrome. A report of five cases and review of literature. *Clin Transplant* 2002; **16**: 350-361 [PMID: 12225432]

105 **Mochizuki Y**, Iwata T, Nishikido M, Uramatsu T, Sakai H, Taguchi T. De novo minimal change disease after ABO-incompatible kidney transplantation. *Clin Transplant* 2012; **26** Suppl 24: 81-85 [PMID: 22747482 DOI: 10.1111/j.1399-0012.2012.01645.x]

106 **Madhan KK**, Temple-Camp CR. Late de novo minimal change disease in a renal allograft. *Saudi J Kidney Dis Transpl* 2009; **20**: 266-269 [PMID: 19237816]

107 **McCarthy ET**, Sharma M, Savin VJ. Circulating permeability factors in idiopathic nephrotic syndrome and focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol* 2010; **5**: 2115-2121 [PMID: 20966123 DOI: 10.2215/CJN.03800609]

108 **Wei C**, El Hindi S, Li J, Fornoni A, Goes N, Sageshima J, Maiguel D, Karumanchi SA, Yap HK, Saleem M, Zhang Q, Nikolic B, Chaudhuri A, Daftarian P, Salido E, Torres A, Salifu M, Sarwal MM, Schaefer F, Morath C, Schwenger V, Zeier M, Gupta V, Roth D, Rastaldi MP, Burke G, Ruiz P, Reiser J. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat Med* 2011; **17**: 952-960 [PMID: 21804539 DOI: 10.1038/nm.2411]

109 **Reiser J**, von Gersdorff G, Loos M, Oh J, Asanuma K, Giardino L, Rastaldi MP, Calvaresi N, Watanabe H, Schwarz K, Faul C, Kretzler M, Davidson A, Sugimoto H, Kalluri R, Sharpe AH, Kreidberg JA, Mundel P. Induction of B7-1 in podocytes is associated with nephrotic syndrome. *J Clin Invest* 2004; **113**: 1390-1397 [PMID: 15146236 DOI: 10.1172/JCI20402]

110 **Mainra R**, Mulay A, Bell R, Karpinski J, Hoar S, Knoll G, Robertson S, Wang D. Sirolimus use and de novo minimal change nephropathy following renal transplantation. *Transplantation* 2005; **80**: 1816 [PMID: 16378080]

111 **Shabaka A**, Pérez-Flores I, Cortés JA, Sánchez-Fructuoso AI. De Novo IgA Nephropathy in a Renal Allograft. *Exp Clin Transplant* 2017; Epub ahead of print [PMID: 28664821 DOI: 10.6002/ect.2016.0266]

112 **Habib R**, Antignac C, Hinglais N, Gagnadoux MF, Broyer M. Glomerular lesions in the transplanted kidney in children. *Am J Kidney Dis* 1987; **10**: 198-207 [PMID: 3307396 DOI: 10.1016/S0272-6386]

113 **Suzuki K**, Honda K, Tanabe K, Toma H, Nihei H, Yamaguchi Y. Incidence of latent mesangial IgA deposition in renal allograft donors in Japan. *Kidney Int* 2003; **63**: 2286-2294 [PMID: 12753320 DOI: 10.1046/j.1523-1755.63.6s.2.x]

114 **Shimizu T**, Tanabe K, Tokumoto T, Shimmura H, Koga S,

Ishikawa N, Oshima T, Toma H, Yamaguchi Y. A case of rapid progressive glomerulonephritis with IgA deposits after renal transplantation. *Clin Transplant* 2001; **15** Suppl 5: 11-15 [PMID: 11791788 DOI: 10.1034/j.1399-0012.2001.0150s5011.x]

115 **Nin M**, Cordero R, Doufrechou L, Larre-Borges A, Coria V, Acosta N, Gadola L, Orihuela S, González F, Noboa O. Post-transplant Henoch-Schonlein purpura de novo: clinical-histological discordance. *Nefrologia* 2012; **32**: 850-852 [PMID: 23169378 DOI: 10.3265/Nefrologia.pre2012.Jun.11468]

116 **Everly MJ**. An update on antibody reduction and rejection reversal following bortezomib use: a report of 52 cases across 10 centers. *Clin Transpl* 2010: 353-362 [PMID: 21696053]

117 **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 2045-2048 [PMID: 20077121 DOI: 10.1007/s00467-009-1386-4]

118 **Kim MG**, Kim YJ, Kwon HY, Park HC, Koo TY, Jeong JC, Jeon HJ, Han M, Ahn C, Yang J. Outcomes of combination therapy for chronic antibody-mediated rejection in renal transplantation. *Nephrology* (Carlton) 2013; **18**: 820-826 [PMID: 24033843]

119 **Poduval RD**, Josephson MA, Javaid B. Treatment of de novo and recurrent membranous nephropathy in renal transplant patients. *Semin Nephrol* 2003; **23**: 392-399 [PMID: 12923728]

120 **Faguer S**, Kamar N, Guilbeaud-Frugier C, Fort M, Modesto A, Mari A, Ribes D, Cointault O, Lavayssière L, Guitard J, Durand D, Rostaing L. Rituximab therapy for acute humoral rejection after kidney transplantation. *Transplantation* 2007; **83**: 1277-1280 [PMID: 17496547 DOI: 10.1097/01.tp.0000261113.30757.d1]

121 **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]

122 **Ponticelli C**, Glassock RJ. Posttransplant recurrence of primary glomerulonephritis. *Clin J Am Soc Nephrol* 2010; **5**: 2363-2372 [PMID: 21030574 DOI: 10.2215/CJN.06720810]

123 **Zimmermann H**, Trappe RU. Therapeutic options in post-transplant lymphoproliferative disorders. *Ther Adv Hematol* 2011; **2**: 393-407 [PMID: 23556105 DOI: 10.1177/2040620711412417]

124 **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]

125 **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]

126 **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]

127 **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]

128 **Ranghino A**, Segoloni GP, Lasaponara F, Biancone L. Lymphatic disorders after renal transplantation: new insights for an old complication. *Clin Kidney J* 2015; **8**: 615-622 [PMID: 26413290 DOI: 10.1093/ckj/sfv064]

129 **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; Sirolimus CONVERT Trial Study Group. 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]

130 **Yu CC**, Fornoni A, Weins A, Hakroush S, Maiguel D, Sageshima J, Chen L, Ciancio G, Faridi MH, Behr D, Campbell KN, Chang JM, Chen HC, Oh J, Faul C, Arnaout MA, Fiorina P, Gupta V, Greka A, Burke GW 3rd, Mundel P. Abatacept in B7-1-positive proteinuric kidney disease. *N Engl J Med* 2013; **369**: 2416-2423 [PMID: 24206430 DOI: 10.1056/NEJMoa1304572]

131 **Brealey JK**, Cassidy J. Electron-dense deposit in renal transplant patients on eculizumab may be drug-derived. *Ultrastruct Pathol* 2016; **40**: 2-6 [PMID: 26512451 DOI: 10.3109/01913123.2015.1090515]

132 **Hou J**, Markowitz GS, Bomback AS, Appel GB, Herlitz LC, Barry Stokes M, D'Agati VD. Toward a working definition of C3 glomerulopathy by immunofluorescence. *Kidney Int* 2014; **85**: 450-456 [PMID: 24067430 DOI: 10.1038/ki.2013.340]

133 **Radhakrishnan S**, Lunn A, Kirschfink M, Thorner P, Hebert D, Langlois V, Pluthero F, Licht C. Eculizumab and refractory membranoproliferative glomerulonephritis. *N Engl J Med* 2012; **366**: 1165-1166 [PMID: 22435384 DOI: 10.1056/NEJMc1106619]

134 **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]

135 **Zuber J**, Fakhouri F, Roumenina LT, Loirat C, Frémeaux-Bacchi V; French Study Group for aHUS/C3G. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. *Nat Rev Nephrol* 2012; **8**: 643-657 [PMID: 23026949 DOI: 10.1038/nrneph.2012.214]

136 **Kerns E**, Rozansky D, Troxell ML. Evolution of immunoglobulin deposition in C3-dominant membranoproliferative glomerulopathy. *Pediatr Nephrol* 2013; **28**: 2227-2231 [PMID: 23892798 DOI: 10.1007/s00467-013-2565-x]

137 **Vivarelli M**, Emma F. Treatment of C3 glomerulopathy with complement blockers. *Semin Thromb Hemost* 2014; **40**: 472-477 [PMID: 24799307 DOI: 10.1055/s-0034-1375299]

138 **Bomback AS**. Eculizumab in the treatment of membranoproliferative glomerulonephritis. *Nephron Clin Pract* 2014; **128**: 270-276 [PMID: 25402185 DOI: 10.1159/000368592]

139 **Payette A**, Patey N, Dragon-Durey MA, Frémeaux-Bacchi V, Le Deist F, Lapeyraque AL. A case of C3 glomerulonephritis successfully treated with eculizumab. *Pediatr Nephrol* 2015; **30**: 1033-1037 [PMID: 25796589 DOI: 10.1007/s00467-015-3061-2]

140 **Le Quintrec M**, Lionet A, Kandel C, Bourdon F, Gnemmi V, Colombat M, Goujon JM, Frémeaux-Bacchi V, Fakhouri F. Eculizumab for treatment of rapidly progressive C3 glomerulopathy. *Am J Kidney Dis* 2015; **65**: 484-489 [PMID: 25530108 DOI: 10.1053/j.ajkd.2014.09.025]

141 **Inman M**, Prater G, Fatima H, Wallace E. Eculizumab-induced reversal of dialysis-dependent kidney failure from C3 glomerulonephritis. *Clin Kidney J* 2015; **8**: 445-448 [PMID: 26251714 DOI: 10.1093/ckj/sfv044]

142 **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]

143 **Kersnik Levart T**, Ferluga D, Vizjak A, Mraz J, Kojc N. Severe active C3 glomerulonephritis triggered by immune complexes and inactivated after eculizumab therapy. *Diagn Pathol* 2016; **11**: 94 [PMID: 27717365 DOI: 10.1186/s13000-016-0547-6]

144 **Rodriguez-Osorio L**, Ortiz A. Timing of eculizumab therapy for C3 glomerulonephritis. *Clin Kidney J* 2015; **8**: 449-452 [PMID: 26251715 DOI: 10.1093/ckj/sfv065]

145 **Hillmen P**, Elebute M, Kelly R, Urbano-Ispizua A, Hill A, Rother RP, Khursigara G, Fu CL, Omine M, Browne P, Rosse W. Long-term effect of the complement inhibitor eculizumab on kidney function in patients with paroxysmal nocturnal hemoglobinuria. *Am J Hematol* 2010; **85**: 553-559 [PMID: 20658586 DOI: 10.1002/ajh.21757]

146 **Carneiro-Roza F**, Medina-Pestana JO, Moscoso-Solorzano G,

Franco M, Ozaki K, Mastroianni-Kirsztajn G. Initial response to immunosuppressive and renoprotective treatment in posttransplant glomerulonephritis. *Transplant Proc* 2006; **38**: 3491-3497 [PMID: 17175313 DOI: 10.1016/j.transproceed.2006.10.109]

P- Reviewer: Cheungpasitporn W, Fourtounas C, Salvadori M
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Yan JL

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 December 24; 7(6): 301-316

DOI: 10.5500/wjt.v7.i6.301

ISSN 2220-3230 (online)

REVIEW

Recurrence of primary glomerulonephritis: Review of the current evidence

Fedaey Abbas, Mohsen El Kossi, Jon Kim Jin, Ajay Sharma, Ahmed Halawa

Fedaey Abbas, Department of Nephrology, Jaber El Ahmed Military Hospital, Safat 13005, Kuwait

Fedaey Abbas, Mohsen El Kossi, Jon Kim Jin, Ajay Sharma, Ahmed Halawa, Faculty of Health and Science, University of Liverpool, Institute of Learning and Teaching, School of Medicine, Liverpool L69 3GB, United Kingdom

Mohsen El Kossi, Doncaster Royal Infirmary, Doncaster DN2 5LT, United Kingdom

Jon Kim Jin, Nottingham Children Hospital, Nottingham NG7 2UH, United Kingdom

Ajay Sharma, Royal Liverpool University Hospitals, Liverpool L7 8XP, United Kingdom

Ahmed Halawa, Department of Transplantation Surgery, Sheffield Teaching Hospitals, Sheffield S5 7AU, United Kingdom

ORCID number: Fedaey Abbas (0000-0001-8673-4344); Mohsen El Kossi (0000-0002-2490-2784); Jon Kim Jin (0000-0003-4307-8513); Ajay Sharma (0000-0003-4050-6586); Ahmed Halawa (0000-0002-7305-446X).

Author contributions: Abbas F designed the study, executed data collection, and writing the manuscript; El Kossi M, Jin JK and Sharma A reviewed and edited the manuscript; Halawa A contributed to conceptualization, designing of the study, supervising of the data collection and reviewing and editing of the manuscript.

Conflict-of-interest statement: None.

Manuscript source: Invited manuscript

Correspondence to: Dr. Ahmed Halawa, Consultant Transplant Surgeon, DNB, FRCS (Ed), FRCS (Gen Surg), MD, MSc, Department of Transplantation Surgery, Sheffield Teaching Hospital, Herries Road, Sheffield S5 7AU, United Kingdom. ahmed.halawa@sth.nhs.uk
Telephone: +44-778-7542128
Fax: +44-114-2714604

Received: May 6, 2017
Peer-review started: May 10, 2017
First decision: July 20, 2017
Revised: November 8, 2017
Accepted: November 22, 2017
Article in press: November 22, 2017
Published online: December 24, 2017

Abstract

In view of the availability of new immunosuppression strategies, the recurrence of allograft glomerulonephritis (GN) are reported to be increasing with time post transplantation. Recent advances in understanding the pathogenesis of the GN recurrent disease provided a better chance to develop new strategies to deal with the GN recurrence. Recurrent GN diseases manifest with a variable course, stubborn behavior, and poor response to therapy. Some types of GN lead to rapid decline of kidney function resulting in a frustrating return to maintenance dialysis. This subgroup of aggressive diseases actually requires intensive efforts to ascertain their pathogenesis so that strategy could be implemented for better allograft survival. Epidemiology of native glomerulonephritis as the cause of end-stage renal failure and subsequent recurrence of individual glomerulonephritis after renal transplantation was evaluated using data from various registries, and pathogenesis of individual glomerulonephritis is discussed. The following review is aimed to define current protocols of the recurrent primary glomerulonephritis therapy.

Key words: Recurrent glomerulonephritis; Renal transplantation; Primary glomerulonephritis

Core tip: Renal transplantation is the best-known therapy for end stage renal disease, with the glomerulonephritis represents a major aetiology for its prevalence. Unfortunately, recurrence of the glomerulonephritis (GN) disease after renal transplantation represents a real devastating impact on allograft survival. A clear understanding of their pathogenesis, will help not only in ameliorating GN recurrence, but also improves allograft survival.

Abbas F, El Kossi M, Jin JK, Sharma A, Halawa A. Recurrence of primary glomerulonephritis: Review of the current evidence. *World J Transplant* 2017; 7(6): 301-316 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i6/301.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i6.301

INTRODUCTION

The impact of glomerulonephritis (GN) recurrence varies widely from mild or negligible effect, *e.g.*, IgA nephropathy (IgAN), to a real detrimental impact on graft survival, *e.g.*, Focal Sclerosing Glomerulosclerosis (FSGS) and membranoproliferative GN (MPGN)[1]. Since it has been early recognized, the deleterious impact of the recurrent GN on allograft longevity, continuous efforts have been exerted to determine its real prevalence, clear pathogenesis and to tailor the best strategies for treatment and prevention[2]. Recently, several mechanisms have been postulated to address a clear pathogenesis of GN recurrence[1]. The prevalence of GN as an etiology of end-stage renal disease (ESRD) was reported to be exceeding 48% in China[3,4], 50% in Australian-New Zealand[2] and 30% according to USRDS 2015 report[5]. The frequent lack of kidney biopsy resulted in underestimation of the real prevalence of the GN recurrence[2]. Moreover, the distinction between recurrent GN and the *de novo* disease is not widely applied. Compared to an early (within the first year) post transplantation assessment of prevalence of about 4%, a value of 13% after 7.5 years[6], and 18% in other studies[7,8] have been recorded[2]. The reported wide variations in prevalence may be attributed to the variability in follow up periods of various studies[9].

The advent of the new immunosuppressive strategies in kidney transplantation have been reflected on the rates of acute and chronic rejection, but unfortunately has little (impact on the prevalence rates of GN recurrence as well as the *de novo* GN disease[10]). The expected improved allograft survival rate will be ultimately reflected in the future on the prevalence of the recurrent GN after kidney transplantation. It is noteworthy to mention that GN disease with a seemingly benign course, *e.g.,* IgAN is known to recur in 40% of patients but leads to graft loss only in 10%[11,12]. The magnitude of challenge, at times, seems insurmountable despite the progress in understanding the pathogenesis of certain recurrent GN, *e.g.,* permeability factors (suPAR in FSGS and ant-PLA2R AB in MN).

In this review, the authors have identified the most recent progress in understanding the pathogenesis of GN recurrence and its impact on the renal allograft survival. Further insights on the available strategies for treatment and prevention of GN recurrence, particularly so in the main primary GN is will be addressed.

GRAFT SURVIVAL IN RECURRENT GN DISEASE AFTER RENAL TRANSPLANTATION, GENERAL CONCEPTS

An assumed underestimation of the real prevalence of the GN recurrence has been proved By application of the "Protocol Biopsy" that defined as: biopsy at fixed time, with no relation to a clinical guide. Protocol biopsy delineates a higher incidence of GN recurrence (5%, 18%, 21%, 35%, 42% at 1, 3, 5, 8 and 10 years respectively)[5]. Many explanations have been postulated in this concept to shed the light on the reported discrepancy in prevalence of the GN recurrence: (1) absence of clear native kidney disease diagnosis; (2) absence of valid biomarker for GN recurrence; (3) difficulty in differential diagnosis from other pathological entities*, e.g.,* CAN and drug intoxication; (4) absence of clear stratification and characterization of GN recurrence nature in view of the advent of the new therapeutic approaches[13-15]; (5) the decision of biopsy is not always performed routinely whenever indicated (*e.g.,* proteinuria/hematuria, renal impairment); (6) IF/EM techniques are not routinely performed after each biopsy; (7) a wide discrepancy is found in certain diseases*, e.g.,* IgAN, between histopathologic characteristic changes and the appearance of clinical manifestations; (8) a trend to differentiate and isolate *de novo* disease from a true recurrent disease is usually not eventually attempted; (9) absence of basal data as regard etiology of ESRF and the native renal biopsy in many cases; and (10) data inconvenience may result in misdiagnosis of a recurrent disease as a *de novo* disease, which is in fact a true recurrence[2].

The detrimental impact of GN recurrence on allograft survival is irrefutable. The consideration of this impact relies on three points: (1) impact of recurrence of particular types of GN before transplantation on graft survival, *e.g.,* FSGS and MPGN Type Ⅰ *vs* other types of GN. A significantly higher risk of graft failure in these types[9,16]. The proper evaluation should involve a fairly large number of patients studied and followed for an enough period of time[2]; (2) evaluation of the risk of graft failure in case of GN recurrence: The etiology of graft failure should be considered, membranous nephropathy (MN), for example, has high recurrence rate leading to hazardous effect on graft survival[17]; and (3) global allograft GN particularly recurrent disease and its relation

to the death censored allograft survival: As the time of recurrence is not constant, it should be considered a time-dependent variable for a better and proper evaluation[2].

As reported by Cosio *et al*[2] in the American Transplant Congress, 2015, Type Ⅰ MPGN and FSGS showed the highest rate of GN recurrence with subsequent increased risk of allograft loss, followed by IgAN. These data are supported by some studies[12], but not agreed by others[6,9]. It was assumed that 18%-22% of the death-censored kidney allograft losses was attributed to allograft GN (*de novo* and recurrent)[7], the second most common cause of death-censored graft losses[18] and third most prevalent cause of uncensored graft losses[9,16]. However, Mashaly *et al*[19] observed that the best allograft survival of kidney transplantation was noted in recipients whose end stage renal failure was due to polycystic kidney disease followed by those who had urologic disease and then those who had GN as the cause of renal failure. The recurrent GN disease has a wide variety of drawbacks deranging allograft function, which made it occupy the third most common etiology of allograft loss after death with a functioning graft and chronic allograft glomerulopathy, an assumption that was agreed by Fairhead and Knoll[20] (2010) who declared that the recurrent GN disease is a major determinant of the long term graft survival (Figure 1). On the other hand, Toledo *et al*[21] (2011) denied the presence of any difference between GN recurrence and other causes of allograft dysfunction as regard their influence on long term allograft survival. This discrepancy could be a statistical artefact attributed to the small number of patients in their study, racial impacts and the different immunosuppression strategies.

SIGNIFICANCE OF "PROTOCOL BIOPSY" FOR EARLY DIAGNOSIS OF RECURRENT GN

A full detailed map of allograft deterioration due to GN recurrence, can be obtained through a standard protocol biopsy, a widely applied strategy in many centers, so that the earliest changes in allograft histology can be discovered and the native GN disease recurrence can be early anticipated. An intraoperative basal kidney biopsy, at discharge, then after 3 wk, 3-6 mo, 12 mo and after 3 years biopsy is performed serially[22]. The importance of the protocol biopsy could be observed in identification of the early course changes in some transmitted GN diseases, *e.g.,* IgAN, which accounts for more than 90% of transmitted GN[23]. Early recurrence can be detected within 1-2 mo after transplantation. At that time and after the confirmation of recurrence in the third month, no hematuria/proteinuria could be observed; only histological recurrence can be titrated with the frequent specimens[22].

Japanese pathologists pioneered protocol biopsy to understand primary and secondary GN recurrence, *e.g.,*

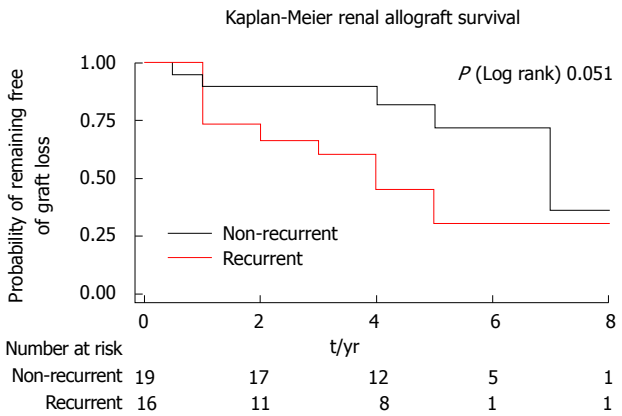


Figure 1 Kaplan Meier of allografts' survival in patients with membranoproliferative glomerulonephritis of immune complex mediated type as original disease (adapted from Alasfar *et al*[30] with permission).

FSGS[24,25], IgAN[26,27], atypical HUS[28] and light chain deposition disease[29].

Graft survival in MPGN type I recurrence

Green *et al*[23,24] (2015) reported that the risk of recurrence is higher in MPGN Type Ⅰ, with the following factors: (1) the HLA B49, HLA DR4; (2) previous transplantations; (3) acute tubular necrosis after transplantation; (4) shorter duration of dialysis before transplantation; and (5) Arab origin was all associated with decreased graft and patient survival[24].

A better allograft survival is expected in MPGN Type Ⅰ, with the following[24]: (1) unrelated living donors; and (2) absence of recurrence in the first year post transplantation.

The advent of the new concepts declaring the role of the alternative complement pathway in the pathogenesis of MPGN was addressed in appearance of the new classification of MPGN. It depends on the mechanism of glomerular injury instead of deposits distribution, which will be ultimately reflected on development of the new therapeutic policies (see therapy of GN recurrence) and its clinical interpretations[30]. So, MPGN will be immune complex mediated (ICGN), encompassing immune complexes and complement compounds, or complement-mediated (CGN) containing only complement, without immune complex (Table 1). Old studies were based on the old classification and data in this subject were very limited owing to the limited number of patients and short follow up durations. The highest prevalence rate has been observed with the previously named MPGN II[31,32].

Risk factors of MPGN recurrence

According to Alasfar *et al*[30] (2016) (Figure 2), the following risk factors have been proposed to be associated with more liability for MPGN recurrence: (1) preemptive renal transplantation[30]; (2) the living related donation[30]; (3) presence of monoclonal immunoglobulins[33]; (4) diminished complement levels[33]; (5) a higher level of proteinuria[32]; (6) human leukocyte antigen type: HLA B8, DR 3[34]; and (7) evidence of crescents in the original biopsy[34].

Table 1 The membranoproliferative glomerulonephritis new classification depends on the mechanism of glomerular injury instead of deposits distribution[30]

No	Type	Criteria	Prevalence
1	ICGN	Contains immune complexes + complement compounds	More common (most of the recurrent cases are ICGN)
2	CGN	Contains complement compounds only	Less prevalent (change from one type to another is possible)

ICGN: Immune complex-mediated glomerulonephritis; CGN: Complement-mediated glomerulonephritis.

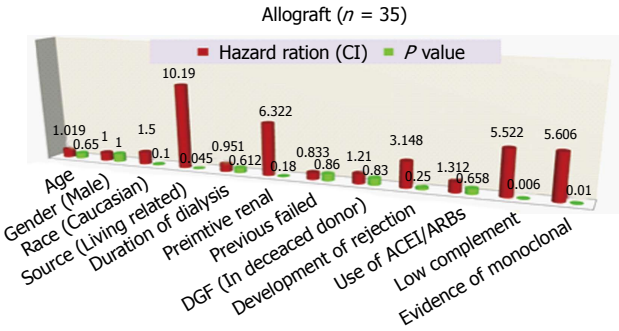


Figure 2 Variables associated with membranoproliferative glomerulonephritis immune complex mediated glomerulonephritis-type recurrence after kidney transplantation by univariate Cox analysis (adapted from Alasfar *et al*[30] with permission).

Impact of HLA typing on prevalence of MPGN recurrence: Green *et al*[24] (2013) concluded that the risk of recurrence is higher in MPGN Type Ⅰ, with certain human leukocyte antigen, *i.e.*, HLA B49, HLA DR4. Andresdottir et *al*[34] (1997) reported an increased risk of recurrence of MBGN Type Ⅰ was observed in patients with the HLA haplotype B8 DR3.

Graft survival in "recurrent MN"

The recurrence of primary MN after renal transplantation is obviously has deleterious impact on graft survival. For better evaluation of the death censored survival, timing of GN recurrence should be considered[17].

Anti-PLA2R autoantibodies in recurrent MN and graft survival: The pivotal role of anti-phospholipase A2 receptor (PLA2R) auto antibodies in the pathogenesis of primary MN before as well as after renal transplantation has an impressing popularity. The prevalence of anti-PLA2R antibodies in primary MN is approaching 70% and nearly the same percentage in RTR (70%-80%)[17,35,36], with about half of the patients are liable for recurrence after renal transplantation[17,37]. Patients with anti-PLA2R antibodies before transplantation have a 60%-76% chance of histologic recurrence, while absence of these autoantibodies decreases their risk of recurrence to less than 30%[17,36,38,39]. After transplantation the anti-PLA2R antibodies absorbed rapidly into the allograft and as a result of decreased antibodies production due to the immunosuppression medications leading to decline of their level in up to 50% of patients[36]. This decline is definitely associated not only by a lower risk of recurrence, but also by a slower rate of progression if MN does recur[36]. On the other hand, the significance of the anti-PLA2R post transplantation is greatly observed in their predictive value of recurrence and disease progression which is exceeding 80%[36], a high anti-PLA2R is usually accompanied by an increased risk of recurrence, rapid disease progression and probably more resistance to drug therapy[36,38].

Impact of anti-PLA2R on graft survival: Serial survey of the anti-PLA2R antibodies titer is of utmost importance for the following indications[2]: (1) evaluating the magnitude of recurrence risk; (2) determining the rate of disease progression; (3) prediction of the response to treatment[2]; and (4) differential diagnosis of proteinuria in recipients with native primary MN.

Non-anti-PLA2R MN recurrence: Not all the patients with primary MN express anti-PLA2R antibodies, 30% of these patients are negative to these antibodies. Instead, few patients have been reported to have antibodies against other types like cationic bovine serum albumin and thrombospondin type Ⅰ[40] but data, however, concerned with the real significance of these mediators are still deficient[40-42].

Of note that if the anti-PLA2R antibody titer is negative, we should search for the "glomerular PLA2R" staining, in such a case there is associated anti-PLA2R MN with negative anti-PLA2R serum level, which is present in 30% of cases[36,43].

Graft survival in recurrent "primary focal segmental"

Primary focal segmental (FSGS) is proved to be one of the highest glomerulonephritis (GN) in recurrence rate after kidney transplantation (KTx), with a percentage of prevalence exceeding 30% in the most recent series[2], with an expected very poor graft survival rate[43]. It can recur immediately post-transplantation, or recur lately, where its diagnosis is usually masked by the secondary FSGS resulting from the reduced total nephron mass, or due to other causes, *e.g.*, iatrogenic[20,44]. Of all causes of the FSGN, "genetic" subtype showed the least incidence of recurrence[19,45,46]. On the other hand, podocin mutations did not show a decreased risk of recurrence[45]. However, revising the recent series, there is consensus about certain clinical parameters that is considered the paramount risk factors for FSGS recurrence: (1) White race[43]; (2) higher level of proteinuria[46,47]; (3) rapid progression to ESRD (< 3 years); (4) younger age (< 15 years old) at time of diagnosis[46]; and (5) the most

reliable risk factor for recurrence is recurrence in a previous graft[2].

By far, the most reliable risk factor for recurrence is recurrence in a failed allograft, which will be ultimately reflected on allograft survival. Losing of allograft due to recurrent FSGS is associated of an 80% liability of recurrence of the original disease[2].

Graft survival in "recurrent IgAN"

The reported incidence if recurrent IgAN is quite variable according to the considered diagnosis and period of follow up. IgAN can remain silent for 5 years before it became clinically evident. So, an average incidence of 30% has been reported[48]. The histologic recurrence is by far more prevalent and discovered earlier before the disease became clinically evident. Rarely, crescentic disease with a rapidly progressing course can occur, which ultimately is associated with poor prognosis[48-50].

A growing body of evidence that three markers of an active disease indicates a great liability for recurrence: (1) galactose-deficient IgA1; (2) IgA-IgG immune complexes; and (3) lower levels of IgA-soluble CD89 circulating complexes, the myeloid cell receptor for IgA[51]. The only defect in considering these components is that they were considered on a clinically evident base of IgAN recurrence, therefore, silent disease - a quite common IgAN behavior - will be definitely missed, which means an easily missed diagnosis of IgAN recurrence[2].

Risk factors of IgAN recurrence include: (1) young RTR; (2) aggressive course of the disease before transplantation; (3) living *vs* deceased donation[52,53]; (4) polymorphisms in IL-10[54,55]; (5) HLA-B8-DR3 haplotype[56]; (6) steroid-free regimen[57,58]; and (7) impact of histological classification: could have prognostic implications[18,59,60].

Despite the reported excellent outcome after renal transplantation and the better graft survival in comparison with other GN diseases[61-63], recurrent IgA disease - on the other hand - have been proved to be detrimental to the allograft. So, definitely, patient with recurrent IgAN have a higher risk of losing their grafts in comparison with patients without recurrence[18,48,64,65].

ROLE OF IMMUNOSUPPRESSION

A definite role of immunosuppression on recurrent GN prevalence was previously denied by the early reports[13]. However, recently, some explanations were given to argue that immunosuppressive therapy could cure or at least modulate the recurrent GN course[2]: (1) certain GN recurrences show a diminished rate of recurrence[20,66-68]; (2) an increased rate of recurrence has been observed with steroid free regimen in pediatrics as well as in IgAN[57,58,64], but not in FSGS patients[69,70]; and (3) an observed decline in antibody level (anti-PLA2R), one of the essential effects that observed once the immunosuppressive agents have been commenced[36].

GENERAL RECOMMENDATIONS (EDTA DATABASE) FOR RECURRENT GN THERAPY

On behalf of the EDTA database, Floege *et al*[10] tried to shed the light on the most vital recommendations in dealing with a RTR with an underlying glomerular disease as follows: (1) defining the original native glomerular disease in RTR will help prevent its recurrence; (2) with "living-related" kidney donation, and expected familial GN such as IgAN, renal biopsy should be considered. Floege *et al*[10] accept living related donation for RTR with MN, MPGN Type Ⅰ, IgA and anti-GBM disease; (3) sharp limiting roles should judge the living related donation pool. A deep discussion with a patient with dense deposit disease (DDD) and a child with FSGS should be instituted; (4) the list of recipients with high risk of recurrence includes advanced mesangiocapillary alterations in renal biopsy, age of less than 15 years and short duration between established diagnosis and ESRD; (5) a benefit/risk ratio should be balanced properly between proceeding to kidney transplant and surviving on dialysis accordingly; (6) etiology of graft loss in a previously failed transplant is better to be elucidated; (7) avoid living donation in case of a previously failed transplant due to GN recurrence, the risk of recurrence and subsequent allograft loss will be enhanced in presence of the recurrence risk factors[71]; (8) the impact of modification of immunosuppression protocols still questionable by some authors; and (9) robust battery of investigations is required including renal biopsy with its related studies, *e.g.,* LM, IF, E/M and immune-studies should be accomplished with every renal biopsy, so that a perfect differential diagnosis from other possible lesions, *e.g.,* chronic allograft glomerulopathy could be established.

TREATMENT OF RECURRENT MPGN

The advent of a new classification of MPGN including the classic morphology as well as the other features enables not only a better understanding of the course of this disease, but also delineates the best tools of prevention and therapy of recurrence, which will be ultimately reflected on the allograft survival[72,73]. This fact is evolved from the observed wide discrepancy in the behavior of each subtype (see below) as regard the incidence and the intensity of recurrence as well as its impact on allograft survival[2].

One of the largest series about post-transplant MPGN recurrence in the literature was admitted by Alasfar *et al*[30], it was the first study that applied the new MPGN classification in evaluating post-transplant MPGN recurrence (Table 1, Figure 3). Despite the absence of worse survival in the recurrent cohort of Alasfar *et al*[30], the rate of allograft loss was higher (Figure 1).

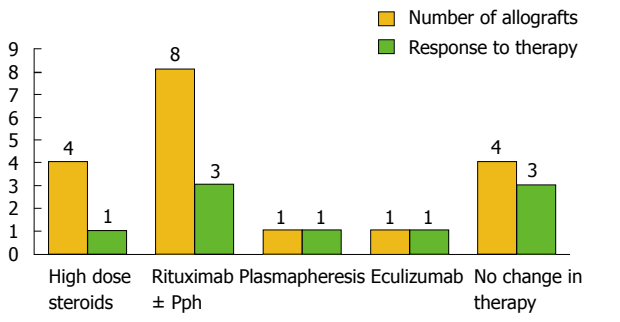


Figure 3 Response of post-transplant membranoproliferative glomerulonephritis recurrence to different treatments (response to therapy defined by improvement in GFR and no subsequent graft loss). Adapted from Alasfar *et al*[30] with permission.

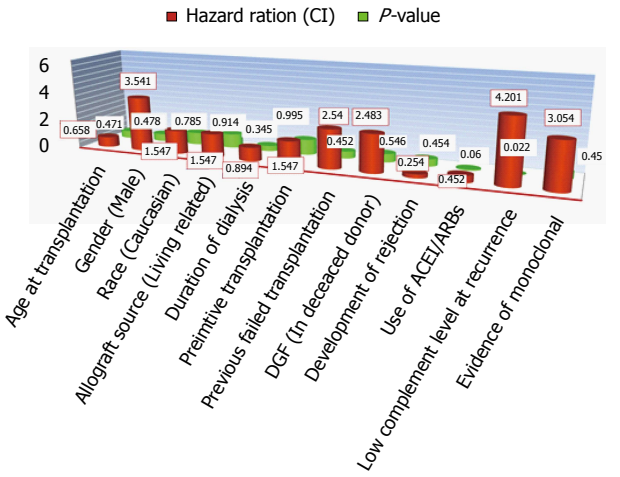


Figure 4 Variables associated with allograft loss among patients with membranoproliferative glomerulonephritis immune complex mediated glomerulonephritis-type recurrence after KTx by univariate Cox analysis (*n* = 16). Adapted from Alasfar *et al*[30] with permission.

They explained this discrepancy by the small sample size. Unfortunately, the response to immunosuppressive therapy in this study was poor, as less than 50% of their patients treated by high dose steroid therapy, rituximab and/or plasmapheresis, or eculizumab could attain allograft function stability and prevent graft loss (Figure 4). An assumed benefit of ACEi/ARBs therapy in prevention of graft loss was suggested by this study, which should be considered cautiously regarding the small number of cases[30]. Alasfar *et al*[30], however, showed that 43% of their patients who developed MPGN recurrence were of the immune complex-mediated GN (ICGN) type and were complicated by graft loss. On the other hand, one of the two patients with GN recurrence and subtyped as complement-mediated GN (CGN) developed graft loss. The average time of graft loss was 6.5 mo (2-18 mo). Interpreting these results showed non-significant results between recurrent and non-recurrent groups, despite the presence of tendency to worse survival[30]. Also, no significance could be detected with other factors, *e.g.,* (age, race, gender, mismatching degree, graft source, pre-emptive transplantation and degree of proteinuria). In contrary to other factors and despite of non-significance, ACEi/ARB therapy could ameliorate the tendency of graft loss (Figure 4). For more specified specific therapy, all the old biopsies before the advent of the new classification, should be reclassified. The CGN is generally less prevalent, on the other hand, ICGN is more common (Table 1) and most of the native as well as the recurrent MPGN appear to be classified as ICGN. It is noteworthy to remind that some of the reclassified cases may change their microscopy by time. Unfortunately, the latter change could be difficult to differentiate from a *de novo* GN disease, which will be ultimately resulted in a difficulty on choosing the mode of therapy[30].

Impact of the new classification on therapeutic options

MPGN with Ig deposits: We should focus in suppression of the antibody production, but there are no controlled trials.

MPGN with monoclonal Ig deposits: The anti-CD20 antibodies are proved to be effective in uncontrolled trials in native as well as in allograft recurrence[74]. Monoclonal deposits are proved to be associated with a higher rate of recurrence[75]. This association may suggest they have their role in the pathogenesis of MPGN, consequently, two important steps have been suggested: (1) meticulous screening for "monoclonal gammopathy" during preparation of a patient with MPGN for renal transplantation; and (2) a "hematologist consultation" may be advised with strict follow up in such situation for long periods[30].

MPGN C3GN: The use anti C5 monoclonal antibodies, eculizumab, is shown to be effective with mixed results[76-80], depending on the success of this drug in preventing the recurrence of atypical HUS, which own a similar pathogenesis[80-83].

Impact of subtype's behavior on therapeutic options

MPGN with polyclonal Ig deposits: Usually presented late, within the first 5 years, with a relatively benign course as regard low risk of recurrence and slow progression. Interestingly, the morphology of the lately recurred MPGN with polyclonal Ig deposits is difficult to be differentiated from the *de novo* GN which can behave similarly as regard the late presentation post transplantation as well as the presence of polyclonal Ig deposits[18]. The former group has C4d deposits in their glomeruli, fortunately help in differential diagnosis. Also, a higher risk of recurrence could be expected with the presence of reduced complement level (C3 and C4) level[74] (Figure 5).

C3GN: C3 glomerular deposits are abundant with absence or minimal Ig deposits[84,85]. The risk of recurrence in C3GN is very high, exceeding 70%, can be presented early with a very aggressive course that ultimately ends by graft failure in nearly half of the

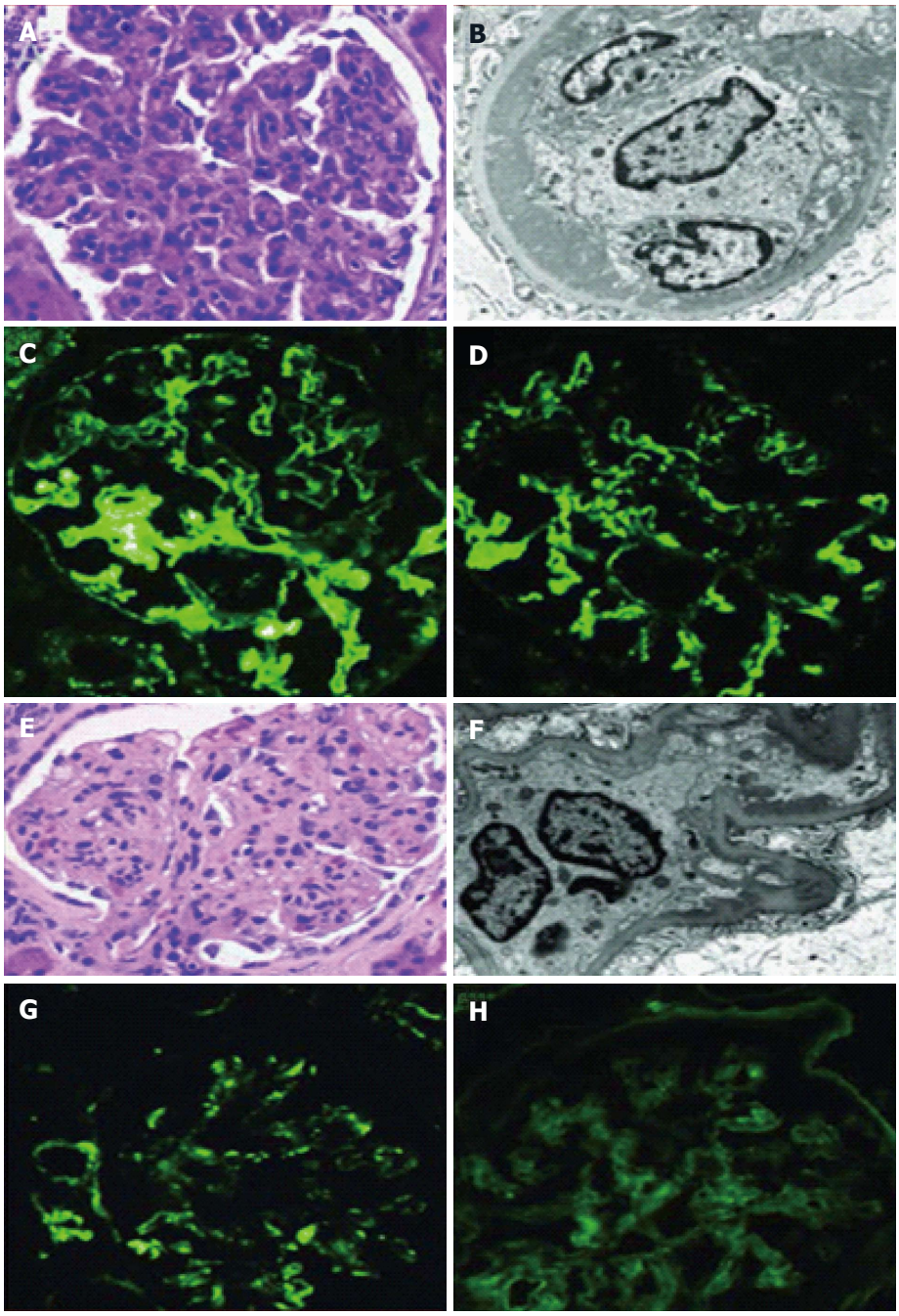


Figure 5 Histological changes of membranoproliferative glomerulonephritis in kidney transplant biopsies. Typical LM, EM and IF finding in cases previously classified as MPGN. First panel shows a case reclassified as ICGN with C3 abnormalities, including (A) the classic MPGN pattern GN on LM (B) large sub-endothelial electron dense deposits on EM and granular mesangial and capillary wall staining for both (C) IgG and (D) C3 on IF. Second panel shows a case reclassified as a C3 glomerulopathy, with (E) a similar MPGN pattern on LM, (F) smaller sub-endothelial deposits on EM and granular mesangial and capillary wall staining for (G) C3, but no significant staining for (H) IgG. Adapted from Alasfar *et al*[30] with permission. LM: Light microscopic; EM: Electron microscopy; IF: Immunofluorescence.

patients[86]. There is no established treatment for C3GN. For complement dysregulation in the pathogenesis of this disease, a supply of "normal plasma" has been suggested[87]. Recently, a new therapy targeting an alternative complement pathway using the anti-C5 AB[88-90] and soluble CR1 (a potent regulator of complement activity) has been reported[91]. However, controlled trials regarding the efficacy of these therapies have not yet been conducted.

Dense deposit disease subtype: The rate of recurrence of this subtype is extremely high (80%-90%), leading to reduced graft survival[32,92]. Two criteria characterize this subtype: It is usually slowly progressive with minimal or absent clinical manifestation, and the timing of recurrence is mostly delayed[92,93]. Both DDD and C3GN usually express an alteration in the alternative pathway with resultant overproduction of the activated C3[94,95]. Recently, polymorphism of the complement

regulating proteins, especially in alternative pathway are found to be propagated mostly in all subtypes of MPGN, with a possible alterations related to renal outcome were assumed[96]. In DDD and other C3 glomerulopathies: Eculizumab or anti-auto antibodies activating complement cascade therapy have been suggested[97].

"Monoclonal gammopathy with renal significance": Both C3 GN and DDD lack C4d, indicating alternative pathway activation[98]. Any MPGN subtype associated with monoclonal Ig deposits usually complicated by GN recurrence in 66% of cases and expressing a very aggressive course often complicated by allograft failure[99]. Interestingly, 70% of these cases do not express monoclonal IG either in serum or in urine, without any evidence of plasma cell dyscrasia in bone marrow and with low risk of progress into multiple myeloma[100,101].

Monoclonal proteins: Monoclonal proteins are present in 30% of cases with MPGN with monoclonal Ig deposits have serum monoclonal proteins[100] despite absence of any evidence of multiple myeloma. A subtype name of this group of patients called "monoclonal gammopathy with renal significance"[102,103], which obviously will express a very high risk of recurrence[104].

Stem cell transplantation: It is noteworthy to declare that in monoclonal gammopathy, stem cell transplantation can reverse the renal dysfunction through elimination of the light chain and immunoglobulins, with an expected general improvement. The observed link between C3GN and monoclonal and the complement (alternative pathway) activation by λ-light chain has been recorded in previous reports[105-107].

Recommendations for a better management

Extrapolating the aggressive behavior of these recurrent diseases, especially in the presence of monoclonal deposits and C3GN, rigorous precautions should be considered to strive against its activity. A prophylactic protocol to guard against MPGN with monoclonal deposits recurrence utilizing an anti-CD20 AB before transplantation is currently under evaluation by Cosio *et al*[2], with promising preliminary results. It is assumed that the C3GN remains silent until they exposed to a certain event, *e.g.,* ischemia/reperfusion injury of transplantation that results in dysregulation of complement activation with evolution of the pathological events associated to its aggressive course[108-110]. So, it is essential to reclassify the MPGN based on the recent MPGN classification, which will help not only in designing a therapeutic protocol, but also in instituting a prophylactic policy. It is noteworthy mentioning that the clinical course of MPGN pre- and post-transplantation are not the same, *i.e.,* slow preoperative course is not necessarily applied to the post-transplantation behavior[2].

TREATMENT OF RECURRENT MN

RTR with recurrent MN are better to be under RAAS-blockade as well as symptomatic therapy in the form of diuretics, statins and anticoagulants. Other lines include were listed below.

CNI

Referring to its efficacy in MN in the native kidney disease, many RTR with recurrent MN are utilizing CNI therapy relevant to the recent advances in understanding the pathogenesis of MN recurrence[111].

Corticosteroid/alkylating agents (cyclophosphamide or chlorambucil) combination

Again effective in both native and recurrent MN disease[112]. Unfortunately, leukopenia could be quite troublesome, so, holding MMF while commencing the alkylating agents' therapy is advised[112].

Anti-CD20 antibody

Rituximab is also successful in treating the native as well as the recurrent MN disease[113-117]. More than 80% of cases could achieve partial or complete remission, while 40% of cases could express subendothelial deposits resolution[17,117]. Despite the increased risk of infection with anti-CD20 therapy[17,113], rituximab is generally safe, effective, simpler to utilize and more tolerated as compared to alkylating agents. So, the anti-CD20, rituximab, is recommended as a primary line in treating MN recurrence, without alterations in the immunosuppression protocol and regardless the anti-PLA2R antibody level[2].

Resistant cases

Alternative therapy between rituximab and alkylating agents is suggested, once one of them failed, then shift to the other line[17,115]. As the level change of anti-PLA2R antibodies titre precedes the decline of proteinuria after rituximab therapy, serial follow up of the antibody titre can be used to anticipate the magnitude of response to therapy as well as the possibility of relapse[113].

Timing of therapy

Early intervention-in contrary to native MN[116] - with anti-CD20 therapy is recommended, exactly when the proteinuria approaching one gram per 24 h. A very high rate of success would be expected[17], which will be ultimately reflected on reduction of the rate of death censored allograft failure related to MN recurrence (45%).

Prophylaxis history

The anti-CD20 was used effectively by Cosio *et al*[2], to prevent MN recurrence in two patients with a previous allograft loss due to MN recurrence, with serial follow up through a protocol biopsy.

Prevention

The use of ant-CD20 few months pre-transplantation may be applied in an attempt to prevent recurrence through the reduction of the ant-PLA2R antibody titer. However, two reasons may prevent the application of this maneuver in a wider scale: (1) the ant-PLA2R antibody titer already declines soon after transplantation, which will decrease the chance of recurrence[36]; and (2) the expected high rate of success achieved by the anti-CD20 in case of early recurrence has been documented[17,117,118].

TREATMENT OF RECURRENT FSGS

The recent progress in understanding the pathogenesis of FSGS recurrence was unfortunately not supported by evidence-based controlled trials.

Plasmapheresis

In 1985 treating FSGS with plasmapheresis (PE) sessions has been commenced with variable success[119]. Plasmapheresis has the ability to induce remission in 70% of children and 63% of adults as reported by Ponticelli *et al*[120]. An overestimation of these reports is postulated due to retrospective nature of the study, short follow up period and lack of controlled design. Once the disease recurrence become clinically evident, we can extrapolate a satisfactory response with commencing the PE sessions early after transplantation. PE is usually prescribed as one to two times plasma volume exchanges, three times per week, with total 8-12 treatments until remission has been established. An intensified course for longer period was suggested by other researchers[121].

Prophylactic PE

Gohh *et al*[122] has admitted preoperative PE for eight sessions in ten patients. In case of living donation, the recipient received PE one week before and one week after the operation. In case of deceased donation, PE was only given 24 h preoperatively. No one case of FSGS recurrence has been diagnosed in the high risk group and only half of his patients has had their allograft failed. They concluded these results were less than previous reports[122], while others denied any benefits for the prophylactic PE[121,123]. A combination of PE and immunosuppressive agents has been proposed with limited data[124,125].

Higher dose of CyA

Only the intensified dose of CyA can reduce the proteinuria level, in contrary to the standard dose that can do nothing for FSGS recurrence[126]. Relevant to its lipophilic criteria, CyA has the ability to bind the LDL receptors on the cell surface of the peripheral lymphocytes. As a result of the rich lipid content (LDL cholesterol) in the nervous system, blood level of the drug is reduced, which could only be overcome through a higher dose augmentation. At this base, *i.v.* CyA 3 mg/kg/d for 3-4 wk, followed by oral route aiming at preserving the blood level at 250-350 ng/mL, have been successful in induction of remission[127]. However, this policy has been hampered by the multiple untoward effects of the high dosage.

Rituximab

An anti-CD20 chimeric monoclonal antibody depleting the B cells with a direct protective effect on the podocytes. It has the ability to abort the downregulation of sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b) protein and the acid sphingomyelinase (ASMase), both of them were documented to be present in the podocyte exposed to the sera of recipients with recurrent FSGS[128]. In 2006, beneficial benefits of rituximab in treating the recurrent FSGS post transplantation was suggested[126]. A remission rate of 64%, either partial or complete, has been reported with rituximab therapy[129]. A better response is expected with a normal albumin serum level, and fewer administered infusions as well as in young age recipients[130]. It is not well-proved if titrating rituximab dosage will be the best policy to deplete the B-cell or not. The typical published dosage of rituximab is 375 mg/m^2/dose/2-6 doses, with 1-2 wk apart.

PE and rituximab combination

An augmented benefit was assumed to be expected with the combined therapy including PE in addition to rituximab[131,132]. Tsagalis *et al*[131] utilized one gram rituximab per dose, in two doses with two wk apart with PE not performed before 72 h. Two of his patients commenced complete remission and the other two have a partial remission with a stable renal profile and absence of severe complications for 18-60 mo of follow up.

While the resolution of recurrent FSGS was assumed to be possible[2] through the use of the anti-CD 20 AB, rituximab[133], this efficacy, unfortunately, is not consistent but rather limited to certain subtypes. The use PE proved to be effective in removing the circulating permeability factors[134]. For instance, we cannot rely only on this effect in case of recurrent FSGS disease. On the other hand, rituximab was proved in a small pediatric group with recurrent FSGS to be effective in achieving PE independence successfully. The variability in response of recurrent FSGS to both PE as well as anti-CD 20 AB (rituximab) therapy is widely spread[135-137], which indicates a variable response that varied according to different subtypes. Despite the absence of well-designed randomized prospective studies, some trials attempted to prove an effective response of removing a putative permeability factor through PE sessions to guard against FSGS recurrence, which was not confirmed by others. A new strategy has been tailored by Cosio *et al*[2] to evaluate the ability of the anti-CD25, rituximab, before transplantation to prevent/decrease FSGS recurrence rate has been commenced with encouraging early results.

Renin-angiotensin system blockade

Few case reports have proved the efficacy of renin-

angiotensin system blockade on reducing proteinuria in recurrent FSGS[138,139], which shed the light on the fact that the recurrent FSGS is not completely pure immunological in origin, but additional factors including the primary as well as the adaptive form of FSGS have been incorporated.

Ability of "galactose infusion" therapy

In ameliorating the toxicity of the circulating permeability factor has been shown in one case series. Galactose therapy has been proposed by Savin *et al*[139] as a non-toxic agent for treatment of the FSGS-associated nephrotic syndrome. The focal segmental permeability factor (FSPF) has a high affinity to galactose. The latter has the ability to inactivate and clear FSPF from the circulation. In addition, the FSPF-galactose complex has a high liability to uptake and catabolism[139].

Cyclophosphamide

In addition to its untoward toxic manifestations with prolonged use, conflicting results have been determined with cyclophosphamide therapy. Kershaw *et al*[140] used a high dose of cyclophosphamide in three pediatric patients with recurrent FSGS, two achieved complete remission and the third one have had partial response. Cochat *et al*[141] reported sustained remission through a regimen composed of pulse steroid, cyclophosphamide and plasmapheresis. Cheong *et al*[142] reported sustained remission only in two of six patients with recurrent FSGS through a similar protocol. Dall'Amico *et al*[143] achieved sustained remission in seven of eleven pediatric patients through utilization of steroid pulse-free protocol composed of cyclophosphamide and PE only. Three major toxicities hampered the widespread use of cyclophosphamide, the immunosuppression burden, gonadal toxicities and the risk of malignancy[144].

Resistant recurrent FSGS to PE and rituximab therapy

A case report recorded a complete remission using the T-cell costimulatory protein B7-1 blocker abatacept, which was not confirmed by others[145-147].

TREATMENT OF IGAN

There is no recommended specific therapy in treating the recurrent IgAN. Treatment of recurrent IgAN is similar to that in native disease in non-transplant patient[1]. However, the following maneuvers have been reported.

ATG induction

The use of ATG as induction therapy is shown to be associated with less risk of IgA recurrence[148].

Low-dose steroids after transplantation

A protective impact against IgAN recurrence was reported[57,58].

"Tonsillectomy"

As advocated by the Japanese, a better prognosis post-tonsillectomy could be expected[149-151].

ACE inhibitors

The use of ACEi is proved to be of no benefit in improving the allograft survival[152]. Only the anti-proteinuric effect could be beneficial to the allograft[153,154]. All patients of the study of Floege *et al*[152] received ACEi with graft failure occurred in more the half of them.

Methylprednisolone pulse

In of the study of Floege *et al*[152], only 20% of patients received steroid pulses; again more than half have had their graft lost.

Maintenance immunosuppression

No benefit could be expected with any alterations on the immunosuppressive policy in regard to improvement of graft survival[155]. However, Moroni *et al*[12] assumed that immunosuppressive protocols including less than three agents is an independent risk factor of recurrence, however, this theory is still debatable. The choice of immunosuppressive strategy members has nothing to do with IgAN recurrence after renal transplantation[152].

CONCLUSION

One of the most challenges for renal allograft survival is the GN recurrence after renal transplantation. With improving long-term renal allograft survival, recurrent disease has increased prominence as a significant contributor to late graft loss. Knowledge on the risk factors for recurrence, onset time and impact on graft function is prerequisite to informed decisions. There are minimal data on the risk of recurrent disease with new immunosuppressive agents. The early recognition would slow down deterioration of renal function even if it may not slow down the course of progression of GN. Each of the GN types has a very unique natural history in renal allograft. With more advancement in understanding its pathogenesis in the future, prophylactic treatment for prevention of GN recurrence might be effective.

ACKNOWLEDGMENTS

Authors do acknowledging Dr. Sami Alasfar *et al* for permitting us adapting their graphs.

REFERENCES

1 **Sprangers B**, Kuypers DR. Recurrence of glomerulonephritis after renal transplantation. *Transplant Rev* (Orlando) 2013; **27**: 126-134 [PMID: 23954034 DOI: 10.1016/j.trre.2013.07.004]

2 **Cosio FG**, Cattran DC. Recent advances in our understanding of recurrent primary glomerulonephritis after kidney transplantation. *Kidney Int* 2017; **91**: 304-314 [PMID: 27837947 DOI: 10.1016/j.kint.2016.08.030]

3 **Liu ZH**. Nephrology in china. *Nat Rev Nephrol* 2013; **9**: 523-528 [PMID: 23877587 DOI: 10.1038/nrneph.2013.146]

4 **Li L**. End-stage renal disease in China. *Kidney Int* 1996; **49**: 287-301 [DOI: 10.1038/ki.1996.41]

5 **Cosio FG**, El Ters M, Cornell LD, Schinstock CA, Stegall MD. Changing Kidney Allograft Histology Early Posttransplant: Prognostic Implications of 1-Year Protocol Biopsies. *Am J Transplant* 2016; **16**: 194-203 [PMID: 26274817 DOI: 10.1111/ajt.13423]

6 **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]

7 **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]

8 **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]

9 **Briganti EM**, Russ GR, McNeil JJ, Atkins RC, Chadban SJ. Risk of renal allograft loss from recurrent glomerulonephritis. *N Engl J Med* 2002; **347**: 103-109 [PMID: 12110738 DOI: 10.1056/NEJMoa013036]

10 **Floege J**, Regele H, Gesualdo L; ERA-EDTA Immunonephrology Working Group. The ERA-EDTA Database on Recurrent Glomerulonephritis following renal transplantation. *Nephrol Dial Transplant* 2014; **29**: 15-21 [PMID: 23986077 DOI: 10.1093/ndt/gft299]

11 **Ponticelli C**, Glassock RJ. Posttransplant recurrence of primary glomerulonephritis. *Clin J Am Soc Nephrol* 2010; **5**: 2363-2372 [PMID: 21030574 DOI: 10.2215/CJN.06720810]

12 **Moroni G**, Longhi S, Quaglini S, Gallelli B, Banfi G, Montagnino G, Messa P. The long-term outcome of renal transplantation of IgA nephropathy and the impact of recurrence on graft survival. *Nephrol Dial Transplant* 2013; **28**: 1305-1314 [PMID: 23229925 DOI: 10.1093/ndt/gfs472]

13 **Mulay AV**, van Walraven C, Knoll GA. Impact of immunosuppressive medication on the risk of renal allograft failure due to recurrent glomerulonephritis. *Am J Transplant* 2009; **9**: 804-811 [PMID: 19353768 DOI: 10.1111/j.1600-6143.2009.02554.x]

14 **ameron JS**. Glomerulonephritis in renal transplants. *Transplantation* 1982; **34**: 237-245 [PMID: 6760479 DOI: 10.1097/00007890-198211000-00001]

15 **Hariharan S**, Adams MB, Brennan DC, Davis CL, First MR, Johnson CP, Ouseph R, Peddi VR, Pelz C, Roza AM, Vincenti F, George V. Recurrent and de novo glomerular disease after renal transplantation: a report from renal allograft disease registry. *Transplant Proc* 1999; **31**: 223-224 [PMID: 10083084 DOI: 10.1016/S0041-1345(98)01511-5]

16 **Hariharan S**, Peddi VR, Savin VJ, Johnson CP, First MR, Roza AM, Adams MB. Recurrent and de novo renal diseases after renal transplantation: a report from the renal allograft disease registry. *Am J Kidney Dis* 1998; **31**: 928-931 [PMID: 9631835 DOI: 10.1053/ajkd.1998.v31.pm9631835]

17 **Grupper A**, Cornell LD, Fervenza FC, Beck LH Jr, Lorenz E, Cosio FG. Recurrent Membranous Nephropathy After Kidney Transplantation: Treatment and Long-Term Implications. *Transplantation* 2015; Epub ahead of print [PMID: 26720301 DOI: 10.1097/TP.0000000000001056]

18 **El-Zoghby ZM**, Stegall MD, Lager DJ, Kremers WK, Amer H, Gloor JM, Cosio FG. Identifying specific causes of kidney allograft loss. *Am J Transplant* 2009; **9**: 527-535 [PMID: 19191769 DOI: 10.1111/j.1600-6143.2008.02519.x]

19 **Mashaly ME**, Ismail MI, Lotfy EE, Donia AF, Wafa IW, Foda MA, Denewar AA, Abbas MH, Shokeir AA. Frequency of the Original Kidney Disease and Its Effect on the Outcome of Kidney Transplant in the Urology-Nephrology Center Mansoura University. *Exp Clin Transplant* 2016; **14**: 157-165 [PMID: 26788876 DOI: 10.6002/ect.2015.0200]

20 **Fairhead T**, Knoll G. Recurrent glomerular disease after kidney transplantation. *Curr Opin Nephrol Hypertens* 2010; **19**: 578-585 [PMID: 20639758 DOI: 10.1097/MNH.0b013e32833d6904]

21 **Toledo K**, Pérez-Sáez MJ, Navarro MD, Ortega R, Redondo MD, Agüera ML, Rodríguez-Benot A, Aljama P. Impact of recurrent glomerulonephritis on renal graft survival. *Transplant Proc* 2011; **43**: 2182-2186 [PMID: 21839228 DOI: 10.1016/j.transproceed.2011.05.010]

22 **Morozumi K**, Takeda A, Otsuka Y, Horike K, Gotoh N, Watarai Y. Recurrent glomerular disease after kidney transplantation: an update of selected areas and the impact of protocol biopsy. *Nephrology* (Carlton) 2014; **19** Suppl 3: 6-10 [PMID: 24842814 DOI: 10.1111/nep.12255]

23 **Suzuki K**, Honda K, Tanabe K, Toma H, Nihei H, Yamaguchi Y. Incidence of latent mesangial IgA deposition in renal allograft donors in Japan. *Kidney Int* 2003; **63**: 2286-2294 [PMID: 12753320 DOI: 10.1046/j.1523-1755.63.6s.2.x]

24 **Green H**, Rahamimov R, Rozen-Zvi B, Pertzov B, Tobar A, Lichtenberg S, Gafter U, Mor E. Recurrent membranoproliferative glomerulonephritis type I after kidney transplantation: a 17-year single-center experience. *Transplantation* 2015; **99**: 1172-1177 [PMID: 25340602 DOI: 10.1097/TP.0000000000000459]

25 **Shimizu A**, Higo S, Fujita E, Mii A, Kaneko T. Focal segmental glomerulosclerosis after renal transplantation. *Clin Transplant* 2011; **25** Suppl 23: 6-14 [PMID: 21623907 DOI: 10.1111/j.1399-0012.2011.01452.x]

26 **Takeuchi O**, Oikawa T, Usami T, Koyama K, Takeda A, Uchida K, Morozumi K. A case of IgA nephropathy after ABO-incompatible living kidney transplantation. *Clin Transplant* 1999; **13** Suppl 1: 38-42 [PMID: 10751055]

27 **Moriyama T**, Nitta K, Suzuki K, Honda K, Horita S, Uchida K, Yumura W, Tanabe K, Toma H, Nihei H, Yamaguchi Y. Latent IgA deposition from donor kidney is the major risk factor for recurrent IgA nephropathy in renal transplantation. *Clin Transplant* 2005; **19** Suppl 14: 41-48 [PMID: 15955168 DOI: 10.1111/j.1399-0012.2005.00403.x]

28 **Watanabe S**, Yamaguchi Y, Suzuki T, Ikezoe M, Matsumoto N, Chikamoto H, Nagafuchi H, Horita S, Hattori M, Shiraga H, Tokumoto T, Tanabe K, Toma H, Ito K. Inherited factor H dysfunction and complement-associated glomerulonephritis in renal grafts of first and second transplantations. *Clin Transplant* 2001; **15** Suppl 5: 45-50 [PMID: 11791795 DOI: 10.1034/j.1399-0012.2001.0150s5045.x]

29 **Horike K**, Takeda A, Otsuka Y, Inaguma D, Goto N, Watarai Y, Uchida K, Morozumi K. A case of recurrent light chain deposition disease after living-related renal transplantation - detailed process of the recurrence. *Clin Transplant* 2012; **26** Suppl 24: 64-69 [PMID: 22747479 DOI: 10.1111/j.1399-0012.2012.01674.x]

30 **Alasfar S**, Carter-Monroe N, Rosenberg AZ, Montgomery RA, Alachkar N. Membranoproliferative glomerulonephritis recurrence after kidney transplantation: using the new classification. *BMC Nephrol* 2016; **17**: 7 [PMID: 26754737 DOI: 10.1186/s12882-015-0219-x]

31 **Appel GB**, Cook HT, Hageman G, Jennette JC, Kashgarian M, Kirschfink M, Lambris JD, Lanning L, Lutz HU, Meri S, Rose NR, Salant DJ, Sethi S, Smith RJ, Smoyer W, Tully HF, Tully SP, Walker P, Welsh M, Würzner R, Zipfel PF. Membranoproliferative glomerulonephritis type II (dense deposit disease): an update. *J Am Soc Nephrol* 2005; **16**: 1392-1403 [PMID: 15800116 DOI: 10.1681/ASN.2005010078]

32 **Braun MC**, Stablein DM, Hamiwka LA, Bell L, Bartosh SM, Strife CF. Recurrence of membranoproliferative glomerulonephritis type II in renal allografts: The North American Pediatric Renal Transplant Cooperative Study experience. *J Am Soc Nephrol* 2005; **16**: 2225-2233 [PMID: 15888559 DOI: 10.1681/ASN.2005020175]

33 **Lorenz EC**, Sethi S, Leung N, Dispenzieri A, Fervenza FC, Cosio FG. Recurrent membranoproliferative glomerulonephritis after kidney transplantation. *Kidney Int* 2010; **77**: 721-728 [PMID:

20130531 DOI: 10.1038/ki.2010.1]

34 **Andresdottir MB**, Assmann KJ, Hoitsma AJ, Koene RA, Wetzels JF. Recurrence of type I membranoproliferative glomerulonephritis after renal transplantation: analysis of the incidence, risk factors, and impact on graft survival. *Transplantation* 1997; **63**: 1628-1633 [PMID: 9197358]

35 **Beck LH Jr**, Fervenza FC, Beck DM, Bonegio RG, Malik FA, Erickson SB, Cosio FG, Cattran DC, Salant DJ. Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy. *J Am Soc Nephrol* 2011; **22**: 1543-1550 [PMID: 21784898 DOI: 10.1681/ASN.2010111125]

36 **Kattah A**, Ayalon R, Beck LH Jr, Sethi S, Sandor DG, Cosio FG, Gandhi MJ, Lorenz EC, Salant DJ, Fervenza FC. Anti-phospholipase A2 receptor antibodies in recurrent membranous nephropathy. *Am J Transplant* 2015; **15**: 1349-1359 [PMID: 25766759 DOI: 10.1111/ajt.13133]

37 **Cosyns JP**, Couchoud C, Pouteil-Noble C, Squifflet JP, Pirson Y. Recurrence of membranous nephropathy after renal transplantation: probability, outcome and risk factors. *Clin Nephrol* 1998; **50**: 144-153 [PMID: 9776417]

38 **Quintana LF**, Blasco M, Seras M, Pérez NS, López-Hoyos M, Villarroel P, Rodrigo E, Viñas O, Ercilla G, Diekmann F, Gómez-Roman JJ, Fernandez-Fresnedo G, Oppenheimer F, Arias M, Campistol JM. Antiphospholipase A2 Receptor Antibody Levels Predict the Risk of Posttransplantation Recurrence of Membranous Nephropathy. *Transplantation* 2015; **99**: 1709-1714 [PMID: 25675198 DOI: 10.1097/TP.0000000000000630]

39 **Seitz-Polski B**, Payré C, Ambrosetti D, Albano L, Cassuto-Viguier E, Berguignat M, Jeribi A, Thouret MC, Bernard G, Benzaken S, Lambeau G, Esnault VL. Prediction of membranous nephropathy recurrence after transplantation by monitoring of anti-PLA2R1 (M-type phospholipase A2 receptor) autoantibodies: a case series of 15 patients. *Nephrol Dial Transplant* 2014; **29**: 2334-2342 [PMID: 25063424 DOI: 10.1093/ndt/gfu252]

40 **Tomas NM**, Beck LH Jr, Meyer-Schwesinger C, Seitz-Polski B, Ma H, Zahner G, Dolla G, Hoxha E, Helmchen U, Dabert-Gay AS, Debayle D, Merchant M, Klein J, Salant DJ, Stahl RAK, Lambeau G. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N Engl J Med* 2014; **371**: 2277-2287 [PMID: 25394321 DOI: 10.1056/NEJMoa1409354]

41 **Ronco P**, Debiec H. Pathogenesis of membranous nephropathy: recent advances and future challenges. *Nat Rev Nephrol* 2012; **8**: 203-213 [PMID: 22371247 DOI: 10.1038/nrneph.2012.35]

42 **Debiec H**, Lefeu F, Kemper MJ, Niaudet P, Deschênes G, Remuzzi G, Ulinski T, Ronco P. Early-childhood membranous nephropathy due to cationic bovine serum albumin. *N Engl J Med* 2011; **364**: 2101-2110 [PMID: 21631322 DOI: 10.1056/NEJMoa1013792]

43 **Rudnicki M**. Focal Segmental Glomerulosclerosis Recurrence in Adults after Renal Transplantation. *BioMed Research International* 2016; **2016**: 3295618 [DOI: 10.1155/2016/3295618]

44 **Cosio FG**, Frankel WL, Pelletier RP, Pesavento TE, Henry ML, Ferguson RM. Focal segmental glomerulosclerosis in renal allografts with chronic nephropathy: implications for graft survival. *Am J Kidney Dis* 1999; **34**: 731-738 [PMID: 10516356 DOI: 10.1016/S0272-6386(99)70400-2]

45 **Bertelli R**, Ginevri F, Caridi G, Dagnino M, Sandrini S, Di Duca M, Emma F, Sanna-Cherchi S, Scolari F, Neri TM, Murer L, Massella L, Basile G, Rizzoni G, Perfumo F, Ghiggeri GM. Recurrence of focal segmental glomerulosclerosis after renal transplantation in patients with mutations of podocin. *Am J Kidney Dis* 2003; **41**: 1314-1321 [PMID: 12776285 DOI: 10.1016/S0272-6386(03)00364-0]

46 **Pinto J**, Lacerda G, Cameron JS, Turner DR, Bewick M, Ogg CS. Recurrence of focal segmental glomerulosclerosis in renal allografts. *Transplantation* 1981; **32**: 83-89 [PMID: 7027555]

47 **Cameron JS**. Recurrent primary disease and de novo nephritis following renal transplantation. *Pediatr Nephrol* 1991; **5**: 412-421 [PMID: 1911114 DOI: 10.1007/BF01453669]

48 **Ponticelli C**, Traversi L, Banfi G. Renal transplantation in patients with IgA mesangial glomerulonephritis. *Pediatr Transplant* 2004; **8**: 334-338 [PMID: 15265157 DOI: 10.1111/j.1399-3046.2004.00177.x]

49 **Kowalewska J**, Yuan S, Sustento-Reodica N, Nicosia RF, Smith KD, Davis CL, Alpers CE. IgA nephropathy with crescents in kidney transplant recipients. *Am J Kidney Dis* 2005; **45**: 167-175 [PMID: 15696457 DOI: 10.1053/j.ajkd.2004.09.030]

50 **Tang Z**, Ji SM, Chen DR, Wen JQ, Chen JS, Liu ZH, Li LS. Recurrent or de novo IgA nephropathy with crescent formation after renal transplantation. *Ren Fail* 2008; **30**: 611-616 [PMID: 18661411 DOI: 10.1080/08860220802134516]

51 **Berthelot L**, Robert T, Vuiblet V, Tabary T, Braconnier A, Dramé M, Toupance O, Rieu P, Monteiro RC, Touré F. Recurrent IgA nephropathy is predicted by altered glycosylated IgA, autoantibodies and soluble CD89 complexes. *Kidney Int* 2015; **88**: 815-822 [PMID: 26061544 DOI: 10.1038/ki.2015.158]

52 **Han SS**, Huh W, Park SK, Ahn C, Han JS, Kim S, Kim YS. Impact of recurrent disease and chronic allograft nephropathy on the long-term allograft outcome in patients with IgA nephropathy. *Transpl Int* 2010; **23**: 169-175 [PMID: 19761553 DOI: 10.1111/j.1432-2277.2009.00966.x]

53 **McDonald SP**, Russ GR. Recurrence of IgA nephropathy among renal allograft recipients from living donors is greater among those with zero HLA mismatches. *Transplantation* 2006; **82**: 759-762 [PMID: 17006322 DOI: 10.1097/01.tp.0000230131.66971.45]

54 **Bantis C**, Heering PJ, Aker S, Schwandt C, Grabensee B, Ivens K. Influence of interleukin-10 gene G-1082A polymorphism on recurrent IgA nephropathy. *J Nephrol* 2008; **21**: 941-946 [PMID: 19034880]

55 **Coppo R**, Amore A, Chiesa M, Lombardo F, Cirina P, Andrulli S, Passerini P, Conti G, Peruzzi L, Giraudi R, Messina M, Segoloni G, Ponticelli C. Serological and genetic factors in early recurrence of IgA nephropathy after renal transplantation. *Clin Transplant* 2007; **21**: 728-737 [PMID: 17988266 DOI: 10.1111/j.1399-0012.2007.00730.x]

56 **Andresdottir MB**, Haasnoot GW, Persijn GG, Claas FH. HLA-B8, DR3: a new risk factor for graft failure after renal transplantation in patients with underlying immunoglobulin A nephropathy. *Clin Transplant* 2009; **23**: 660-665 [PMID: 19674013 DOI: 10.1111/j.1399-0012.2009.01059.x]

57 **Sutherland S**, Li L, Concepcion W, Salvatierra O, Sarwal MM. Steroid-free immunosuppression in pediatric renal transplantation: rationale for and [corrected] outcomes following conversion to steroid based therapy. *Transplantation* 2009; **87**: 1744-1748 [PMID: 19502970 DOI: 10.1097/TP.0b013e3181a5df60]

58 **Von Visger JR**, Gunay Y, Andreoni KA, Bhatt UY, Nori US, Pesavento TE, Elkhammas EA, Winters HA, Nadasdy T, Singh N. The risk of recurrent IgA nephropathy in a steroid-free protocol and other modifying immunosuppression. *Clin Transplant* 2014; **28**: 845-854 [PMID: 24869763 DOI: 10.1111/ctr.12389]

59 **Working Group of the International IgA Nephropathy Network and the Renal Pathology Society**, Cattran DC, Coppo R, Cook HT, Feehally J, Roberts IS, Troyanov S, Alpers CE, Amore A, Barratt J, Berthoux F, Bonsib S, Bruijn JA, D'Agati V, D'Amico G, Emancipator S, Emma F, Ferrario F, Fervenza FC, Florquin S, Fogo A, Geddes CC, Groene HJ, Haas M, Herzenberg AM, Hill PA, Hogg RJ, Hsu SI, Jennette JC, Joh K, Julian BA, Kawamura T, Lai FM, Leung CB, Li LS, Li PK, Liu ZH, Mackinnon B, Mezzano S, Schena FP, Tomino Y, Walker PD, Wang H, Weening JJ, Yoshikawa N, Zhang H. The Oxford classification of IgA nephropathy: rationale, clinicopathological correlations, and classification. *Kidney Int* 2009; **76**: 534-545 [PMID: 19571791 DOI: 10.1038/ki.2009.243]

60 **Working Group of the International IgA Nephropathy Network and the Renal Pathology Society**, Roberts IS, Cook HT, Troyanov S, Alpers CE, Amore A, Barratt J, Berthoux F, Bonsib S, Bruijn JA, Cattran DC, Coppo R, D'Agati V, D'Amico G, Emancipator S, Emma F, Feehally J, Ferrario F, Fervenza FC, Florquin S, Fogo A, Geddes CC, Groene HJ, Haas M, Herzenberg AM, Hill PA, Hogg RJ, Hsu SI, Jennette JC, Joh K, Julian BA, Kawamura T, Lai FM, Li LS, Li PK, Liu ZH, Mackinnon B, Mezzano S, Schena FP, Tomino Y, Walker PD, Wang H, Weening JJ, Yoshikawa N, Zhang H. The Oxford classification of IgA nephropathy: pathology

definitions, correlations, and reproducibility. *Kidney Int* 2009; **76**: 546-556 [PMID: 19571790 DOI: 10.1038/ki.2009.168]

61 **Soler MJ**, Mir M, Rodriguez E, Orfila A, Munne A, Vázquez S, Lloveras J, Puig JM. Recurrence of IgA nephropathy and Henoch-Schönlein purpura after kidney transplantation: risk factors and graft survival. *Transplant Proc* 2005; **37**: 3705-3709 [PMID: 16386512 DOI: 10.1016/j.transproceed.2005.09.172]

62 **Kanaan N**, Mourad G, Thervet E, Peeters P, Hourmant M, Vanrenterghem Y, De Meyer M, Mourad M, Maréchal C, Goffin E, Pirson Y. Recurrence and graft loss after kidney transplantation for henoch-schonlein purpura nephritis: a multicenter analysis. *Clin J Am Soc Nephrol* 2011; **6**: 1768-1772 [PMID: 21734091 DOI: 10.2215/CJN.00520111]

63 **Samuel JP**, Bell CS, Molony DA, Braun MC. Long-term outcome of renal transplantation patients with Henoch-Schonlein purpura. *Clin J Am Soc Nephrol* 2011; **6**: 2034-2040 [PMID: 21700827 DOI: 10.2215/CJN.01410211]

64 **Clayton P**, McDonald S, Chadban S. Steroids and recurrent IgA nephropathy after kidney transplantation. *Am J Transplant* 2011; **11**: 1645-1649 [PMID: 21797974 DOI: 10.1111/j.1600-6143.2011.03667.x]

65 **Moroni G**, Gallelli B, Quaglini S, Leoni A, Banfi G, Passerini P, Montagnino G, Messa P. Long-term outcome of renal transplantation in patients with idiopathic membranous glomerulonephritis (MN). *Nephrol Dial Transplant* 2010; **25**: 3408-3415 [PMID: 20466669 DOI: 10.1093/ndt/gfq223]

66 **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]

67 **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]

68 **Geetha D**, Eirin A, True K, Valentina Irazabal M, Specks U, Seo P, Nachman P, Fervenza FC. Renal transplantation in antineutrophil cytoplasmic antibody-associated vasculitis: a multicenter experience. *Transplantation* 2011; **91**: 1370-1375 [PMID: 21508899 DOI: 10.1097/TP.0b013e31821ab9aa]

69 **Boardman R**, Trofe J, Alloway R, Rogers C, Roy-Chaudhury P, Cardi M, Safdar S, Groene B, Buell J, Hanaway M, Thomas M, Alexander W, Munda R, Woodle ES. Early steroid withdrawal does not increase risk for recurrent focal segmental glomerulosclerosis. *Transplant Proc* 2005; **37**: 817-818 [PMID: 15848542 DOI: 10.1016/j.transproceed.2004.12.065]

70 **Ibrahim H**, Rogers T, Casingal V, Sturdevant M, Tan M, Humar A, Gillingham K, Matas A. Graft loss from recurrent glomerulonephritis is not increased with a rapid steroid discontinuation protocol. *Transplantation* 2006; **81**: 214-219 [PMID: 16436965 DOI: 10.1097/01.tp.0000188656.44326.53]

71 **Briggs JD**, Jones E. Recurrence of glomerulonephritis following renal transplantation. Scientific Advisory Board of the ERA-EDTA Registry. European Renal Association-European Dialysis and Transplant Association. *Nephrol Dial Transplant* 1999; **14**: 564-565 [PMID: 10193799 DOI: 10.1093/ndt/14.3.564]

72 **Sethi S**, Fervenza FC. Membranoproliferative glomerulonephritis--a new look at an old entity. *N Engl J Med* 2012; **366**: 1119-1131 [PMID: 22435371 DOI: 10.1056/NEJMra1108178]

73 **Sethi S**, Haas M, Markowitz GS, D'Agati VD, Rennke HG, Jennette JC, Bajema IM, Alpers CE, Chang A, Cornell LD, Cosio FG, Fogo AB, Glassock RJ, Hariharan S, Kambham N, Lager DJ, Leung N, Mengel M, Nath KA, Roberts IS, Rovin BH, Seshan SV, Smith RJ, Walker PD, Winearls CG, Appel GB, Alexander MP, Cattran DC, Casado CA, Cook HT, De Vriese AS, Radhakrishnan J, Racusen LC, Ronco P, Fervenza FC. Mayo Clinic/Renal Pathology Society Consensus Report on Pathologic Classification, Diagnosis, and Reporting of GN. *J Am Soc Nephrol* 2016; **27**: 1278-1287 [PMID: 26567243 DOI: 10.1681/ASN.2015101160612]

74 **Guiard E**, Karras A, Plaisier E, Duong Van Huyen JP, Fakhouri F, Rougier JP, Noel LH, Callard P, Delahousse M, Ronco P. Patterns of noncryoglobulinemic glomerulonephritis with monoclonal Ig deposits: correlation with IgG subclass and response to rituximab. *Clin J Am Soc Nephrol* 2011; **6**: 1609-1616 [PMID: 21700823 DOI: 10.2215/CJN.10611110]

75 **Moroni G**, Casati C, Quaglini S, Gallelli B, Banfi G, Montagnino G, Messa P. Membranoproliferative glomerulonephritis type I in renal transplantation patients: a single-center study of a cohort of 68 renal transplants followed up for 11 years. *Transplantation* 2011; **91**: 1233-1239 [PMID: 21502910 DOI: 10.1097/TP.0b013e318218e94e]

76 **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]

77 **Herlitz LC**, Bomback AS, Markowitz GS, Stokes MB, Smith RN, Colvin RB, Appel GB, D'Agati VD. Pathology after eculizumab in dense deposit disease and C3 GN. *J Am Soc Nephrol* 2012; **23**: 1229-1237 [PMID: 22677550 DOI: 10.1681/ASN.2011121186]

78 **Le Quintrec M**, Lionet A, Kandel C, Bourdon F, Gnemmi V, Colombat M, Goujon JM, Frémeaux-Bacchi V, Fakhouri F. Eculizumab for treatment of rapidly progressive C3 glomerulopathy. *Am J Kidney Dis* 2015; **65**: 484-489 [PMID: 25530108 DOI: 10.1053/j.ajkd.2014.09.025]

79 **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]

80 **Zuber J**, Fakhouri F, Roumenina LT, Loirat C, Frémeaux-Bacchi V; French Study Group for aHUS/C3G. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. *Nat Rev Nephrol* 2012; **8**: 643-657 [PMID: 23026949 DOI: 10.1038/nrneph.2012.214]

81 **Zuber J**, Le Quintrec M, Sberro-Soussan R, Loirat C, Frémeaux-Bacchi V, Legendre C. New insights into postrenal transplant hemolytic uremic syndrome. *Nat Rev Nephrol* 2011; **7**: 23-35 [PMID: 21102542 DOI: 10.1038/nrneph.2010.155]

82 **Krid S**, Roumenina LT, Beury D, Charbit M, Boyer O, Frémeaux-Bacchi V, Niaudet P. Renal transplantation under prophylactic eculizumab in atypical hemolytic uremic syndrome with CFH/CFHR1 hybrid protein. *Am J Transplant* 2012; **12**: 1938-1944 [PMID: 22494769 DOI: 10.1111/j.1600-6143.2012.04051.x]

83 **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; French Study Group for Atypical HUS. 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]

84 **Servais A**, Frémeaux-Bacchi V, Lequintrec M, Salomon R, Blouin J, Knebelmann B, Grünfeld JP, Lesavre P, Noël LH, Fakhouri F. Primary glomerulonephritis with isolated C3 deposits: a new entity which shares common genetic risk factors with haemolytic uraemic syndrome. *J Med Genet* 2007; **44**: 193-199 [PMID: 17018561 DOI: 10.1136/jmg.2006.045328]

85 **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]

86 **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 DOI: 10.1681/ASN.2013070715]

87 **Kakita H**, Miyake T, Komiya T, Tsukamoto T, Muso E. A case report of recurrent C3 glomerulonephritis 18 mo after renal transplantation. *Renal Replacement Therapy* 2016; **2**: 38 [DOI: 10.1186/s41100-016-0049-8]

88 **Daina E**, Noris M, Remuzzi G. Eculizumab in a patient with

dense-deposit disease. *N Engl J Med* 2012; **366**: 1161-1163 [PMID: 22435382 DOI: 10.1056/NEJMc1112273]

89 **Vivarelli M**, Pasini A, Emma F. Eculizumab for the treatment of dense-deposit disease. *N Engl J Med* 2012; **366**: 1163-1165 [PMID: 22435383 DOI: 10.1056/NEJMc1111953]

90 **Radhakrishnan S**, Lunn A, Kirschfink M, Thorner P, Hebert D, Langlois V, Pluthero F, Licht C. Eculizumab and refractory membranoproliferative glomerulonephritis. *N Engl J Med* 2012; **366**: 1165-1166 [PMID: 22435384 DOI: 10.1056/NEJMc1106619]

91 **Athanasiou Y**, Voskarides K, Gale DP, Damianou L, Patsias C, Zavros M, Maxwell PH, Cook HT, Demosthenous P, Hadjisavvas A, Kyriacou K, Zouvani I, Pierides A, Deltas C. Familial C3 glomerulopathy associated with CFHR5 mutations: clinical characteristics of 91 patients in 16 pedigrees. *Clin J Am Soc Nephrol* 2011; **6**: 1436-1446 [PMID: 21566112 DOI: 10.2215/CJN.09541010]

92 **Eddy A**, Sibley R, Mauer SM, Kim Y. Renal allograft failure due to recurrent dense intramembranous deposit disease. *Clin Nephrol* 1984; **21**: 305-313 [PMID: 6380848]

93 **Curtis JJ**, Wyatt RJ, Bhathena D, Lucas BA, Holland NH, Luke RG. Renal transplantation for patients with type I and type II membranoproliferative glomerulonephritis: serial complement and nephritic factor measurements and the problem of recurrence of disease. *Am J Med* 1979; **66**: 216-225 [PMID: 371395 DOI: 10.1016/0002-9343(79)90530-8]

94 **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]

95 **Sethi S**, Fervenza FC, Zhang Y, Zand L, Vrana JA, Nasr SH, Theis JD, Dogan A, Smith RJ. C3 glomerulonephritis: clinicopathological findings, complement abnormalities, glomerular proteomic profile, treatment, and follow-up. *Kidney Int* 2012; **82**: 465-473 [PMID: 22673887 DOI: 10.1038/ki.2012.212]

96 **Iatropoulos P**, Noris M, Mele C, Piras R, Valoti E, Bresin E, Curreri M, Mondo E, Zito A, Gamba S, Bettoni S, Murer L, Fremeaux-Bacchi V, Vivarelli M, Emma F, Daina E, Remuzzi G. Complement gene variants determine the risk of immunoglobulin-associated MPGN and C3 glomerulopathy and predict long-term renal outcome. *Mol Immunol* 2016; **71**: 131-142 [PMID: 26895476 DOI: 10.1016/j.molimm.2016.01.010]

97 **Chen Q**, Müller D, Rudolph B, Hartmann A, Kuwertz-Bröking E, Wu K, Kirschfink M, Skerka C, Zipfel PF. Combined C3b and factor B autoantibodies and MPGN type II. *N Engl J Med* 2011; **365**: 2340-2342 [PMID: 22168663 DOI: 10.1056/NEJMc1107484]

98 **Sethi S**, Nasr SH, De Vriese AS, Fervenza FC. C4d as a Diagnostic Tool in Proliferative GN. *J Am Soc Nephrol* 2015; **26**: 2852-2859 [PMID: 25991041 DOI: 10.1681/ASN.2014040406]

99 **Nasr SH**, Sethi S, Cornell LD, Fidler ME, Boelkins M, Fervenza FC, Cosio FG, D'Agati VD. Proliferative glomerulonephritis with monoclonal IgG deposits recurs in the allograft. *Clin J Am Soc Nephrol* 2011; **6**: 122-132 [PMID: 20876681 DOI: 10.2215/CJN.05750710]

100 **Nasr SH**, Markowitz GS, Stokes MB, Seshan SV, Valderrama E, Appel GB, Aucouturier P, D'Agati VD. Proliferative glomerulonephritis with monoclonal IgG deposits: a distinct entity mimicking immune-complex glomerulonephritis. *Kidney Int* 2004; **65**: 85-96 [PMID: 14675039 DOI: 10.1111/j.1523-1755.2004.00365.x]

101 **Nasr SH**, Valeri AM, Cornell LD, Fidler ME, Sethi S, D'Agati VD, Leung N. Renal monoclonal immunoglobulin deposition disease: a report of 64 patients from a single institution. *Clin J Am Soc Nephrol* 2012; **7**: 231-239 [PMID: 22156754 DOI: 10.2215/CJN.08640811]

102 **Leung N**, Bridoux F, Hutchison CA, Nasr SH, Cockwell P, Fermand JP, Dispenzieri A, Song KW, Kyle RA; International Kidney and Monoclonal Gammopathy Research Group. Monoclonal gammopathy of renal significance: when MGUS is no longer undetermined or insignificant. *Blood* 2012; **120**: 4292-4295 [PMID: 23047823 DOI: 10.1182/blood-2012-07-445304]

103 **Bridoux F**, Leung N, Hutchison CA, Touchard G, Sethi S, Fermand JP, Picken MM, Herrera GA, Kastritis E, Merlini G, Roussel M, Fervenza FC, Dispenzieri A, Kyle RA, Nasr SH; International Kidney and Monoclonal Gammopathy Research Group. Diagnosis of monoclonal gammopathy of renal significance. *Kidney Int* 2015; **87**: 698-711 [PMID: 25607108 DOI: 10.1038/ki.2014.408]

104 **Czarnecki PG**, Lager DJ, Leung N, Dispenzieri A, Cosio FG, Fervenza FC. Long-term outcome of kidney transplantation in patients with fibrillary glomerulonephritis or monoclonal gammopathy with fibrillary deposits. *Kidney Int* 2009; **75**: 420-427 [PMID: 19037251 DOI: 10.1038/ki.2008.577]

105 **Bridoux F**, Desport E, Frémeaux-Bacchi V, Chong CF, Gombert JM, Lacombe C, Quellard N, Touchard G. Glomerulonephritis with isolated C3 deposits and monoclonal gammopathy: a fortuitous association? *Clin J Am Soc Nephrol* 2011; **6**: 2165-2174 [PMID: 21784830 DOI: 10.2215/CJN.06180710]

106 **Zand L**, Kattah A, Fervenza FC, Smith RJ, Nasr SH, Zhang Y, Vrana JA, Leung N, Cornell LD, Sethi S. C3 glomerulonephritis associated with monoclonal gammopathy: a case series. *Am J Kidney Dis* 2013; **62**: 506-514 [PMID: 23623956 DOI: 10.1053/j.ajkd.2013.02.370]

107 **Meri S**, Koistinen V, Miettinen A, Törnroth T, Seppälä IJ. Activation of the alternative pathway of complement by monoclonal lambda light chains in membranoproliferative glomerulonephritis. *J Exp Med* 1992; **175**: 939-950 [PMID: 1532415]

108 **Zhou W**, Farrar CA, Abe K, Pratt JR, Marsh JE, Wang Y, Stahl GL, Sacks SH. Predominant role for C5b-9 in renal ischemia/reperfusion injury. *J Clin Invest* 2000; **105**: 1363-1371 [PMID: 10811844]

109 **Collard CD**, Väkevä A, Morrissey MA, Agah A, Rollins SA, Reenstra WR, Buras JA, Meri S, Stahl GL. Complement activation after oxidative stress: role of the lectin complement pathway. *Am J Pathol* 2000; **156**: 1549-1556 [PMID: 10793066]

110 **Thurman JM**, Royer PA, Ljubanovic D, Dursun B, Lenderink AM, Edelstein CL, Holers VM. Treatment with an inhibitory monoclonal antibody to mouse factor B protects mice from induction of apoptosis and renal ischemia/reperfusion injury. *J Am Soc Nephrol* 2006; **17**: 707-715 [PMID: 16467447 DOI: 10.1681/ASN.2005070698]

111 **Cattran DC**, Appel GB, Hebert LA, Hunsicker LG, Pohl MA, Hoy WE, Maxwell DR, Kunis CL; North America Nephrotic Syndrome Study Group. Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. *Kidney Int* 2001; **59**: 1484-1490 [PMID: 11260412 DOI: 10.1046/j.1523-1755.2001.0590041484.x]

112 **Ponticelli C**, Zucchelli P, Passerini P, Cesana B, Locatelli F, Pasquali S, Sasdelli M, Redaelli B, Grassi C, Pozzi C. A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int* 1995; **48**: 1600-1604 [PMID: 8544420]

113 **El-Zoghby ZM**, Grande JP, Fraile MG, Norby SM, Fervenza FC, Cosio FG. Recurrent idiopathic membranous nephropathy: early diagnosis by protocol biopsies and treatment with anti-CD20 monoclonal antibodies. *Am J Transplant* 2009; **9**: 2800-2807 [PMID: 19845581 DOI: 10.1111/j.1600-6143.2009.02851.x]

114 **Ruggenenti P**, Chiurchiu C, Brusegan V, Abbate M, Perna A, Filippi C, Remuzzi G. Rituximab in idiopathic membranous nephropathy: a one-year prospective study. *J Am Soc Nephrol* 2003; **14**: 1851-1857 [PMID: 12819245 DOI: 10.1097/01.ASN.0000071511.35221.B3]

115 **Fervenza FC**, Abraham RS, Erickson SB, Irazabal MV, Eirin A, Specks U, Nachman PH, Bergstralh EJ, Leung N, Cosio FG, Hogan MC, Dillon JJ, Hickson LJ, Li X, Cattran DC; Mayo Nephrology Collaborative Group. Rituximab therapy in idiopathic membranous nephropathy: a 2-year study. *Clin J Am Soc Nephrol* 2010; **5**: 2188-2198 [PMID: 20705965 DOI: 10.2215/CJN.05080610]

116 **Fervenza FC**, Cosio FG, Erickson SB, Specks U, Herzenberg AM, Dillon JJ, Leung N, Cohen IM, Wochos DN, Bergstralh E, Hladunewich M, Cattran DC. Rituximab treatment of idiopathic membranous nephropathy. *Kidney Int* 2008; **73**: 117-125 [PMID:

17943078 DOI: 10.1038/sj.ki.5002628]
117 **Sprangers B**, Lefkowitz GI, Cohen SD, Stokes MB, Valeri A, Appel GB, Kunis CL. Beneficial effect of rituximab in the treatment of recurrent idiopathic membranous nephropathy after kidney transplantation. *Clin J Am Soc Nephrol* 2010; **5**: 790-797 [PMID: 20185599 DOI: 10.2215/CJN.04120609]
118 **Gallon L**, Chhabra D. Anti-CD20 monoclonal antibody (rituximab) for the treatment of recurrent idiopathic membranous nephropathy in a renal transplant patient. *Am J Transplant* 2006; **6**: 3017-3021 [PMID: 17294527 DOI: 10.1111/j.1600-6143.2006.01544.x]
119 **Zimmerman SW**. Plasmapheresis and dipyridamole for recurrent focal glomerular sclerosis. *Nephron* 1985; **40**: 241-245 [PMID: 3889681]
120 **Ponticelli C**. Recurrence of focal segmental glomerular sclerosis (FSGS) after renal transplantation. *Nephrol Dial Transplant* 2010; **25**: 25-31 [PMID: 19875378 DOI: 10.1093/ndt/gfp538]
121 **Hickson LJ**, Gera M, Amer H, Iqbal CW, Moore TB, Milliner DS, Cosio FG, Larson TS, Stegall MD, Ishitani MB, Gloor JM, Griffin MD. Kidney transplantation for primary focal segmental glomerulosclerosis: outcomes and response to therapy for recurrence. *Transplantation* 2009; **87**: 1232-1239 [PMID: 19384172 DOI: 10.1097/TP.0b013e31819f12be]
122 **Gohh RY**, Yango AF, Morrissey PE, Monaco AP, Gautam A, Sharma M, McCarthy ET, Savin VJ. Preemptive plasmapheresis and recurrence of FSGS in high-risk renal transplant recipients. *Am J Transplant* 2005; **5**: 2907-2912 [PMID: 16303004 DOI: 10.1111/j.1600-6143.2005.01112.x]
123 **Hubsch H**, Montané B, Abitbol C, Chandar J, Shariatmadar S, Ciancio G, Burke G, Miller J, Strauss J, Zilleruelo G. Recurrent focal glomerulosclerosis in pediatric renal allografts: the Miami experience. *Pediatr Nephrol* 2005; **20**: 210-216 [PMID: 15605284 DOI: 10.1007/s00467-004-1706-7]
124 **Fencl F**, Simková E, Vondrák K, Janda J, Chadimová M, Stejskal J, Seeman T. Recurrence of nephrotic proteinuria in children with focal segmental glomerulosclerosis after renal transplantation treated with plasmapheresis and immunoadsorption: case reports. *Transplant Proc* 2007; **39**: 3488-3490 [PMID: 18089416 DOI: 10.1016/j.transproceed.2007.09.045]
125 **Belson A**, Yorgin PD, Al-Uzri AY, Salvatierra O, Higgins J, Alexander SR. Long-term plasmapheresis and protein A column treatment of recurrent FSGS. *Pediatr Nephrol* 2001; **16**: 985-989 [PMID: 11793085 DOI: 10.1007/s004670100008]
126 **Cravedi P**, Kopp JB, Remuzzi G. Recent progress in the pathophysiology and treatment of FSGS recurrence. *Am J Transplant* 2013; **13**: 266-274 [PMID: 23312002 DOI: 10.1111/ajt.12045]
127 **Raafat RH**, Kalia A, Travis LB, Diven SC. High-dose oral cyclosporin therapy for recurrent focal segmental glomerulosclerosis in children. *Am J Kidney Dis* 2004; **44**: 50-56 [PMID: 15211437]
128 **Fornoni A**, Sageshima J, Wei C, Merscher-Gomez S, Aguillon-Prada R, Jauregui AN, Li J, Mattiazzi A, Ciancio G, Chen L, Zilleruelo G, Abitbol C, Chandar J, Seeherunvong W, Ricordi C, Ikehata M, Rastaldi MP, Reiser J, Burke GW 3rd. Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. *Sci Transl Med* 2011; **3**: 85ra46 [PMID: 21632984 DOI: 10.1126/scitranslmed.3002231]
129 **Araya CE**, Dharnidharka VR. The factors that may predict response to rituximab therapy in recurrent focal segmental glomerulosclerosis: a systematic review. *J Transplant* 2011; **2011**: 374213 [PMID: 22174985 DOI: 10.1155/2011/374213]
130 **Cravedi P**, Ruggenenti P, Remuzzi G. Low-dose rituximab for posttransplant recurrent membranous nephropathy. *Am J Transplant* 2010; **10**: 1336 [PMID: 20199509 DOI: 10.1111/j.1600-6143.2010.03029.x]
131 **Tsagalis G**, Psimenou E, Nakopoulou L, Laggouranis A. Combination treatment with plasmapheresis and rituximab for recurrent focal segmental glomerulosclerosis after renal transplantation. *Artif Organs* 2011; **35**: 420-425 [PMID: 20637013 DOI: 10.1111/j.1525-1594.2010.01068.x]
132 **Hristea D**, Hadaya K, Marangon N, Buhler L, Villard J, Morel P, Martin PY. Successful treatment of recurrent focal segmental glomerulosclerosis after kidney transplantation by plasmapheresis and rituximab. *Transpl Int* 2007; **20**: 102-105 [PMID: 17181660 DOI: 10.1111/j.1432-2277.2006.00395.x]
133 **Pescovitz MD**, Book BK, Sidner RA. Resolution of recurrent focal segmental glomerulosclerosis proteinuria after rituximab treatment. *N Engl J Med* 2006; **354**: 1961-1963 [PMID: 16672715 DOI: 10.1056/NEJMc055495]
134 **Savin VJ**, Sharma R, Sharma M, McCarthy ET, Swan SK, Ellis E, Lovell H, Warady B, Gunwar S, Chonko AM, Artero M, Vincenti F. Circulating factor associated with increased glomerular permeability to albumin in recurrent focal segmental glomerulosclerosis. *N Engl J Med* 1996; **334**: 878-883 [PMID: 8596570 DOI: 10.1056/NEJM199604043341402]
135 **Yu CC**, Fornoni A, Weins A, Hakroush S, Maiguel D, Sageshima J, Chen L, Ciancio G, Faridi MH, Behr D, Campbell KN, Chang JM, Chen HC, Oh J, Faul C, Arnaout MA, Fiorina P, Gupta V, Greka A, Burke GW 3rd, Mundel P. Abatacept in B7-1-positive proteinuric kidney disease. *N Engl J Med* 2013; **369**: 2416-2423 [PMID: 24206430 DOI: 10.1056/NEJMoa1304572]
136 **Yabu JM**, Ho B, Scandling JD, Vincenti F. Rituximab failed to improve nephrotic syndrome in renal transplant patients with recurrent focal segmental glomerulosclerosis. *Am J Transplant* 2008; **8**: 222-227 [PMID: 17979998 DOI: 10.1111/j.1600-6143.2007.02021.x]
137 **Canaud G**, Zuber J, Sberro R, Royale V, Anglicheau D, Snanoudj R, Gaha K, Thervet E, Lefrère F, Cavazzana-Calvo M, Noël LH, Méjean A, Legendre Ch, Martinez F. Intensive and prolonged treatment of focal and segmental glomerulosclerosis recurrence in adult kidney transplant recipients: a pilot study. *Am J Transplant* 2009; **9**: 1081-1086 [PMID: 19344432 DOI: 10.1111/j.1600-6143.2009.02580.x]
138 **Montagnino G**, Tarantino A, Banfi G, Maccario M, Costamagna L, Ponticelli C. Double recurrence of FSGS after two renal transplants with complete regression after plasmapheresis and ACE inhibitors. *Transpl Int* 2000; **13**: 166-168 [PMID: 10836656]
139 **Savin VJ**, McCarthy ET, Sharma R, Charba D, Sharma M. Galactose binds to focal segmental glomerulosclerosis permeability factor and inhibits its activity. *Transl Res* 2008; **151**: 288-292 [PMID: 18514139 DOI: 10.1016/j.trsl.2008.04.001]
140 **Kershaw DB**, Sedman AB, Kelsch RC, Bunchman TE. Recurrent focal segmental glomerulosclerosis in pediatric renal transplant recipients: successful treatment with oral cyclophosphamide. *Clin Transplant* 1994; **8**: 546-549 [PMID: 7865917]
141 **Cochat P**, Kassir A, Colon S, Glastre C, Tourniaire B, Parchoux B, Martin X, David L. Recurrent nephrotic syndrome after transplantation: early treatment with plasmaphaeresis and cyclophosphamide. *Pediatr Nephrol* 1993; **7**: 50-54 [PMID: 8439479]
142 **Cheong HI**, Han HW, Park HW, Ha IS, Han KS, Lee HS, Kim SJ, Choi Y. Early recurrent nephrotic syndrome after renal transplantation in children with focal segmental glomerulosclerosis. *Nephrol Dial Transplant* 2000; **15**: 78-81 [PMID: 10607771]
143 **Dall'Amico R**, Ghiggeri G, Carraro M, Artero M, Ghio L, Zamorani E, Zennaro C, Basile G, Montini G, Rivabella L, Cardillo M, Scalamogna M, Ginevri F. Prediction and treatment of recurrent focal segmental glomerulosclerosis after renal transplantation in children. *Am J Kidney Dis* 1999; **34**: 1048-1055 [PMID: 10585314 DOI: 10.1016/S0272-6386(99)70010-7]
144 **Vinai M**, Waber P, Seikaly MG. Recurrence of focal segmental glomerulosclerosis in renal allograft: an in-depth review. *Pediatr Transplant* 2010; **14**: 314-325 [PMID: 20519016 DOI: 10.1111/j.1399-3046.2009.01261.x]
145 **Garin EH**, Reiser J, Cara-Fuentes G, Wei C, Matar D, Wang H, Alachkar N, Johnson RJ. Case series: CTLA4-IgG1 therapy in minimal change disease and focal segmental glomerulosclerosis. *Pediatr Nephrol* 2015; **30**: 469-477 [PMID: 25239302 DOI: 10.1007/s00467-014-2957-6]
146 **Alachkar N**, Carter-Monroe N, Reiser J. Abatacept in B7-1-positive proteinuric kidney disease. *N Engl J Med* 2014; **370**: 1263-1264

[PMID: 24670180 DOI: 10.1056/NEJMc1400502#SA2]
147 **Alkandari O**, Nampoory N, Nair P, Atta A, Zakaria Z, Mossad A, Yagan J, Al-Otaibi T. Recurrent Focal Segmental Glomerulosclerosis and Abatacept: Case Report. *Exp Clin Transplant* 2016; **14**: 456-459 [PMID: 25432003 DOI: 10.6002/ect.2014.0154]
148 **Berthoux F**, El Deeb S, Mariat C, Diconne E, Laurent B, Thibaudin L. Antithymocyte globulin (ATG) induction therapy and disease recurrence in renal transplant recipients with primary IgA nephropathy. *Transplantation* 2008; **85**: 1505-1507 [PMID: 18497694 DOI: 10.1097/TP.0b013e3181705ad4]
149 **Hotta K**, Fukasawa Y, Akimoto M, Tanabe T, Sasaki H, Fukuzawa N, Seki T, Togashi M, Harada H. Tonsillectomy ameliorates histological damage of recurrent immunoglobulin A nephropathy after kidney transplantation. *Nephrology* (Carlton) 2013; **18**: 808-812 [PMID: 23941658 DOI: 10.1111/nep.12151]
150 **Kennoki T**, Ishida H, Yamaguchi Y, Tanabe K. Proteinuria-reducing effects of tonsillectomy alone in IgA nephropathy recurring after kidney transplantation. *Transplantation* 2009; **88**: 935-941 [PMID: 19935466 DOI: 10.1097/TP.0b013e3181b75374]
151 **Ushigome H**, Suzuki T, Fujiki M, Nobori S, Sakamoto S, Okamoto M, Urasaki K, Yoshimura N. Efficacy of tonsillectomy for patients with recurrence of IgA nephropathy after kidney transplantation. *Clin Transplant* 2009; **23** Suppl 20: 17-22 [PMID: 19594590 DOI: 10.1111/j.1399-0012.2009.01003.x]
152 **Floege J**, Gröne HJ. Recurrent IgA nephropathy in the renal allograft: not a benign condition. *Nephrol Dial Transplant* 2013; **28**: 1070-1073 [PMID: 23674835 DOI: 10.1093/ndt/gft077]
153 **Oka K**, Imai E, Moriyama T, Akagi Y, Ando A, Hori M, Okuyama A, Toki K, Kyo M, Kokado Y, Takahara S. A clinicopathological study of IgA nephropathy in renal transplant recipients: beneficial effect of angiotensin-converting enzyme inhibitor. *Nephrol Dial Transplant* 2000; **15**: 689-695 [PMID: 10809812 DOI: 10.1093/ndt/15.5.689]
154 **Courtney AE**, McNamee PT, Nelson WE, Maxwell AP. Does angiotensin blockade influence graft outcome in renal transplant recipients with IgA nephropathy? *Nephrol Dial Transplant* 2006; **21**: 3550-3554 [PMID: 16968729 DOI: 10.1093/ndt/gfl506]
155 **Rauen T**, Eitner F, Fitzner C, Sommerer C, Zeier M, Otte B, Panzer U, Peters H, Benck U, Mertens PR, Kuhlmann U, Witzke O, Gross O, Vielhauer V, Mann JF, Hilgers RD, Floege J; STOP-IgAN Investigators. Intensive Supportive Care plus Immunosuppression in IgA Nephropathy. *N Engl J Med* 2015; **373**: 2225-2236 [PMID: 26630142 DOI: 10.1056/NEJMoa1415463]

P- Reviewer: Kute VBB, Gheith O **S- Editor**: Kong JX
L- Editor: A **E- Editor**: Yan JL

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 December 24; 7(6): 317-323

DOI: 10.5500/wjt.v7.i6.317

ISSN 2220-3230 (online)

MINIREVIEWS

Hepatocyte transplantation: Consider infusion before incision

Ryan D Heath, Furkan Ertem, Bhupinder S Romana, Jamal A Ibdah, Veysel Tahan

Ryan D Heath, Bhupinder S Romana, Jamal A Ibdah, Veysel Tahan, Division of Gastroenterology and Hepatology, University of Missouri, Columbia, MO 65212, United States

Furkan Ertem, Department of Internal Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA 15260, United States

ORCID number: Ryan D Heath (0000-0002-2072-4028); Furkan Ertem (0000-0001-6151-7925); Bhupinder S Romana (0000-0002-2244-9701); Jamal A Ibdah (0000-0002-5646-9014); Veysel Tahan (0000-0001-6796-9359).

Author contributions: All authors contributed to the acquisition of data, writing, and revision of this manuscript.

Conflict-of-interest statement: All the authors have no conflicts of interests to declare. All authors of this article declare that there is no conflicting interests (including but not limited to commercial, personal, political, intellectual or religious interests) that are related to the work submitted for consideration of publication. The statement of "No conflict of interest" file is signed by each author and attached.

Manuscript source: Invited manuscript

Correspondence to: Veysel Tahan, MD, FACP, FACG, FESBGH, Associate Professor, Division of Gastroenterology and Hepatology, University of Missouri, 1 Hospital Dr, Columbia, MO 65212, United States. tahanv@health.missouri.edu
Telephone: +1-573-8846044
Fax: +1-573-8844595

Received: October 28, 2017
Peer-review started: October 29, 2017
First decision: November 20, 2017
Revised: November 27, 2017
Accepted: December 5, 2017
Article in press: December 6, 2017
Published online: December 24, 2017

Abstract

Human hepatocyte transplantation is undergoing study as a bridge, or even alternative, to orthotopic liver transplantation (OLT). This technique has undergone multiple developments over the past thirty years in terms of mode of delivery, source and preparation of cell cultures, monitoring of graft function, and use of immunosuppression. Further refinements and improvements in these techniques will likely allow improved graft survival and function, granting patients higher yield from this technique and potentially significantly delaying need for OLT.

Key words: Hepatocye; Transplantation; Cell therapy; Liver; Graft; Orthotopic

Core tip: Further human studies involving humans are needed, however, the current collectively suggest progress in terms of improved effectiveness of human hepatocyte transplantation (HTx). With improvements in optimizing delivery technique and assessing proper recipients of livers, monitoring graft function, as well as recognizing and treating graft rejection, HTx may be able to be used more widely in metabolic liver disease and potentially delay necessity of orthotopic liver transplantation.

Heath RD, Ertem F, Romana BS, Ibdah JA, Tahan V. Hepatocyte transplantation: Consider infusion before incision. *World J Transplant* 2017; 7(6): 317-323 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i6/317.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i6.317

INTRODUCTION

Human hepatocyte transplantation (HTx) is being studied as a potential future alternative and currently use as a bridge to orthotropic liver transplantation (OLT). Over the last 10 years it has been noted that the number of patients requiring transplant as well as total transplants being performed has been stable (NIHMS). Given the inadequate supply of donor organs in relation to patients who would benefit from transplantation, continued research into alternate therapies for treatment or to prolong time before transplantation becomes necessary is timely. HTx is a technique which has been refined over the past three decades which seeks to improve liver function *via* transplantation of donor hepatocytes directly, rather than transplanting an entire organ. While a number of disorders have been evaluated for efficacy of therapy with this technique, individuals with inborn errors of metabolism appear the greatest benefit[1,2]. Sustained benefits have not been observed, however, refinements in the practice may lead to greater temporal benefits. While this review aims to summarize use of HTx in studies, it also seeks to highlight potential shortcomings of previously utilized technique and focus on areas of future study which may lead to improved yield of HTx.

PREPARATION OF HEPATOCYTE CULTURES

While many consider avoidance and delay of surgery appealing when considering HTx compared to OLT, it should be noted that the source of hepatocytes utilized for HTx generally come from livers deemed unsuitable for OLT[1-4]. The most common reason for rejection of a liver for OLT being steatosis, which is associated with both lower cell viability and yield[5-7]. Ischemic damage to livers is also a common reason for rejection, similarly affecting the yield and viability of extracted hepatocytes[8]. That stated, there is evidence that high quality hepatocytes may be obtained from cardiac death donors with prolonged warm ischemia, though prolonged episodes of ischemia predictably decreases viability[9,10]. While a current argument in favor of expanding research and use of HTx is that one is able to utilize cells from a larger pool of donor organs, one suspects that use of hepatocytes for HTx cultured from livers deemed suitable for OLT would likely result in greater success of this therapy. Beyond simple increased efficacy, multiple recipients could benefit from a single donor liver. Admittedly, there are concerns regarding evaluating the fitness of a recipient to receive donor hepatocytes. For instance, the cytochrome P450 enzyme is involved metabolism of drugs and steroids, bile synthesis, cholesterol synthesis, and vitamin D production. This enzyme system has been noted to have different levels of expression and function within humans, however, and this variability may be partially responsible for variant viability of HTx[11-13]. Not every person may be fit to receive any donor hepatocyte culture due to pre-existing chronic condition or associated medication they take, however, increasing the donor pool would still likely increase overall access to HTx. Furthermore, cell cultures can be cryopreserved and stored until needed, whereas there is a finite amount of time a whole liver can be stored before it is no longer viable for OLT[14].

CLINICAL INDICATIONS FOR HEPATOCYTE TRANSPLANTATION

Further discussion of refinement in technique warrants first discussing potential clinical indications for its use. As previously noted, congenital metabolic disorders appear to hold the greatest promise for use of HTx as metabolism of substrates in questions occur almost exclusively in hepatocytes. The cases reviewed below demonstrate HTx as a successful bridge to OLT.

Crigler-Najjar syndrome (CN) Type Ⅰ is an autosomal recessive condition with complete absence of a uridine diphosphate glucuronosyltransferase (UDPGT) enzymes, resulting in life threatening unconjugated hyperbilirubinemia with long term risk of kernicterus. While phototherapy can be an effective treatment, its effectiveness has been observed to decrease with increased age[15]. The first hepatocyte transplantation was performed in a rat model deficient in UDPGT enzymes[16-18]. A minimal percentage of liver mass comprised of engrafted cells (0.2%), resulted in a 40% decrease in unconjugated bilirubin levels[18]. Humans with CN Type Ⅰ have subsequently undergone HTx with marked improvement in unconjugated bilirubinemia, however, all patients subsequently required OLT anywhere from 4 to 20 mo after HTx due to either loss of graft or insufficient improvement in symptoms[19-23].

Urea cycle disorders, comprising a group of disorder due to deficiencies in one of six different enzymes in the urea cycle, are another group seemingly optimally situated to benefit from HTx. These deficiencies collectively result in hyperammonemia with significant neurologic sequelae. Most patients present as neonates, with current therapy involving protein restriction, hemodialysis, or hemofiltration. Hyperammonemia is still noted despite these treatments, however, with OLT being the only current definitive treatment. Humans have successfully undergone HTx as a bridge to whole organ transplantation, with stabilization of ammonia metabolism noted between 4-13 mo before OLT became necessary[24,25].

Familial hypercholesterolemia (FH) is caused by absence of the low density lipoprotein receptor (LDLR) resulting in early onset severe coronary artery disease. Low density lipoprotein (LDL) apheresis or OLT are the only current treatments, however, a rabbit model of FH undergoing HTx was noted to have decreased levels of serum cholesterol by 30%-60% for 100 d[26-28]. In 1995, 5 patients between the ages of 7 and 41 underwent HTx, demonstrating up to a 20% reduction in LDL in three of the patients, the other 2 not responding to therapy[29].

Glycogen storage disease Type Ⅰ (GSD-I) is an

Table 1 Summary of hepatocyte transplantation reports in human patients

Ref.	Indication	No. of patients	Infusion site	Outcome
Ambrosino *et al*[21]	Criggler-Najjar Type I	A 9-year-old boy	Portal vein	Decreased bilirubin approximately 4 mo, underwent OLT
Lysy *et al*[22]	Criggler-Najjar Type I	A 9-year-old girl	Jejunal vein	Decreased bilirubin approximately 6 mo, underwent OLT
Lysy *et al*[22]	Criggler-Najjar Type I	A 1-year-old girl	Splenic vein	Decreased bilirubin approximately 4 mo, underwent OLT
Zhou *et al*[53]	Criggler-Najjar Type I	2 (4-mo-old boy and newborn boy)	Portal vein	Decreased bilirubin approximately 3-4 mo with subsequent OLT
Meyburg *et al*[24]	Urea cycle disorders	4 (1-d to 3-year-old)	Portal vein	Stable 4-13 mo before OLT, 1 death at 4-mo
Grossman *et al*[29]	Familial hyper-cholesterolemia	5 (7-year-old to 41-year-old)	Portal vein	Three patients with approximately 40% reduction in LDL lasting 4 mo
Lee *et al*[31]	Glycogen storage disorders	A 8-year-old kid	Portal vein	Followed for 7 mo, on tacrolimus and able to fast for 7 h without hypoglycemia
Muraca *et al*[1]	Glycogen storage disorders	47-year-old, female	Portal vein	Followed for 9 mo, on tacrolimus and able to fast for 7 h without hypoglycemia
Sokal *et al*[33]	Refsum disease	4-year-old girl	Portal vein	16 mo improvement
Dhawan *et al*[36]	Hemophilia A	2 (3-mo-old and 35-mo-old)	Portal vein	6 mo with 70% reduction in Factor VII requirements
Hansel *et al*[42]	A1AT deficiency	A 52-year-old	Portal vein	A1AT levels did increase before OLT available 2 d later
Soltys *et al*[52]	Phenyl-ketonuria	A 27-year-old female	Portal vein	7 mo of unrestricted diet

A1AT: Alpha 1 antitrypsin; OLT: Orthotopic liver transplantation.

autosomal recessive metabolic disorder resulting from deficiency of the hepatic enzymes glucose-6-phosphatase (Ia) or glucose-6-phosphate transporter (Ib), resulting in deficiency in glucose production with noted severe hypoglycemia, lactic acidosis, hyperlipidemia, growth retardation, hyperuricemia, and renal dysfunction. While many patients can be treated with consumption of starch, some are unresponsive to dietary therapy and require OLT to correct the underlying defect[30]. Two patients, 18 and 47 years old, underwent HTx with subsequent ability to maintain unaltered diet for up to 7-9 mo[1,31].

Infantile Refsum disease is an autosomal recessive disorder characterized by impaired peroxisome function, resulting in accumulation of very long chain fatty acids and branched chain fatty acids which are normally degraded in peroxisomes. Patients present with severe neurologic defects and rarely survive beyond age 10, with treatment generally centering around supportive care[32]. One 4 years old female patient underwent HTx, demonstrating significant biochemical improvement for more than 16 mo[33].

HTx has been suggested as a treatment for Hemophilia A and B; with murine models demonstrating in 5%-10% increase in factor Ⅷ and 1%-2% increase in factor Ⅸ[34,35]. These increases do result in decreased bleeding time and do provide a therapeutic benefit. In one 2004 study, a 3 mo and 35 mo old patients underwent HTx with 70% reduction in factor Ⅶ requirements noted after 6 mo, however, both patients eventually underwent OLT[36].

Progressive familial intrahepatic cholestasis (PFIC) encompasses a group of autosomal recessive liver diseases presenting in infancy and childhood with progressive cholestasis of hepatocellular origin, with three subtypes noted involving different components of bile metabolism[37]. Murine models of this disease process demonstrated improved bile metabolism using intrasplenic HTx[38]. Two children have been treated with HTx, however, both required OLT after 5 and 14 mo. Biopsies of the livers demonstrated extensive fibrosis and no donor cells on pathology before transplantation, the conclusion made that existing fibrosis likely impaired engraftment[39].

Phenylketonuria (PKU) is one of the most common inborn errors of metabolism, a deficiency of the enzyme phenylalanine hydroxyls (PAH) resulting in toxic concentrations of phenylalanine, the only current treatment involving phenylalanine restricted diet[40]. Murine models demonstrate significant improvement in PAH levels[41].

Alpha 1 antitrypsin (A1AT) deficiency - in 1997, a 52 years old patient underwent HTx as a bridge to transplant, with wild type A1AT levels were noted to increase in the interval between OLT, which occurred 2 d later[42].

This list is not exhaustive, however, it serves to illustrate the potential for this route of therapy in a large number of disorders mediated by hepatocyte dysfunction and subsequent metabolic derangements. While temporary improvements have been noted, others suspect the temporal benefits of HTx could be extended with transplantation of adequate cell mass, improved stock and implantation of transplanted hepatocytes, evaluating the ideal route of delivery, and improved and more accurate monitoring of graft function with emphasis on timely detection of rejection (Table 1).

METHODS OF DELIVERY

Regarding adequate transplantation, two issues warrant discussion. One, culturing of hepatocytes from livers deemed unsuitable for OLT, has been previously discussed. Another issue regarding viability deals with transplantation of "fresh"

vs cryopreserved hepatocytes. Fresh hepatocytes do demonstrate higher viability, with cryopreserved hepatocytes observed to have mitochondrial respiratory chain alterations and decreased ATP production[43]. Furthermore, protein synthesis has been noted to be impaired in cryopreserved cells relative to fresh hepatocytes[44,45]. A 2013 cohort study compared viability of freshly isolated hepatocytes against cryopreserved hepatocytes at 24, 48, and 72 h[46]. Freshly isolated hepatocytes demonstrated mean viability of approximately 81%, while means viability was approximately 61% at 24 h, 52% at 48 h, and 48% at 72 h. There was no noted increased caspase activity, an enzyme involved in apoptosis, though there did appear to some mild derangement in Cytochrome activities, previously noted above to be involved in hepatic metabolism of many different substrates.

Hepatocytes have been transplanted into the liver, spleen, and peritoneal cavity, with intraportal injection being the preferred and most physiological site for clinical transplantation[14,42]. This site may be accessed *via* percutaneous trans-hepatic puncture, cannulation of the umbilical vein, or open cannulation of a mesenteric vein[14]. Shear stress from catheterization can have an effect on viability, however, it has been demonstrated that catheters as small as 4.2 F are associated with acceptable viability[47]. Portal hypertension and any thrombosis are associated with lower engraftment levels, however, use of heparin infusion has been proposed as a potential mechanism to improve engraftment[48]. In cases of known portal hypertension, the spleen may be used as an alternate engraftment site, however, there are cases of splenic necrosis after injection into the splenic artery[8,49]. The peritoneal cavity is another alternate site, however, engraftment levels and long term viability of the graft have been observed to be significantly lower than portal vein infusion[50,51]. There are studies comparing efficacy of any method of delivery against another, and proper determination of the relative efficacy of each would be invaluable toward design of future studies evaluating HTx.

MONITORING GRAFT FUNCTION

Beyond just the method of delivery, appropriate pre-treatment of the recipient has been evaluated to improve efficacy of HTx. A 2017 case series details use of pre-operative liver-directed radiation[52]. Preoperative liver-directed irradiation has been noted to demonstrate complete correction of the bilirubin conjugation defect noted in rat models of Criger-Najjar syndrome Type Ⅰ following HTx[53]. This case series demonstrated improved function of HTx from porcine hepatocytes comparing primates receiving hepatic pre-irradiation *vs* those who did not. Function was assessed by measuring levels of porcine albumin after HTx; pre-irradiated subjects demonstrated significantly higher levels of this protein than control subjects. Using immunohistochemical staining, spatial analysis of stained recipient liver tissue post HTx demonstrated level of engraftment to be approximately 11.8% in experimental subjects *vs* approximately 5% in control subjects. Survival of the graft appears improved in the pre-treated group appears improved as well, with no evidence of infiltrating T cells or macrophages noted in cells of the experimental group. Given the promising nature of the above results, two children with urea cycle defects were subsequently infused after undergoing the irradiation preconditioning protocol. One child was 4 mo of the age, the other underwent HTx shortly after birth. Regarding the patient receiving HTx shortly after birth, at 26 h, cell viability was noted to be approximately 63% with ammonia metabolism noted at normal levels[52]. One patient was noted to have intermittent episodes of hyperammonemia, however, it was noted that goal tacrolimus levels post-transplant were not sustained. This patient did eventually undergo OLT at 3.5 mo of age. The other patient maintained normal levels of ammonia for approximately 40 d, however, was not to have intermittent episodes of hyperammonemia after this point. On day 84 acutely increased levels of ammonia, glutamine, and urinary orotic acid suggested graft failure.

This same case series included a 27 years old female patient with PKU also undergoing HTx after irradiation pretreatment, doing well for 7 mo on an unrestricted diet before demonstrating evidence of rejection. Tacrolimus levels were again noted to be below goal level, and the patient was treated with corticosteroids and augmented immunosuppression protocol with phenylalanine tolerance returning. Phenylalanine levels remained normal for over one year, however, the patient's follow became inconsistent and adequate monitoring of immunosuppression was not performed. Her brother, also afflicted with PKU, was used a control agent. At this point in the study, the graft was assumed to be rejected and immunosuppression discontinued without any adverse effects noted. All three cases demonstrated improved length of graft function after HTx has taken place, and suggests pre-operative irradiation may serve as standard pretreatment to improve HTx efficacy.

Also evident in the above case series, however, is the issue of recognizing and treating graft rejection. The case series did detail how rejection was diagnosed, however, there remains no consensus on pretreatment to reduce risk of rejection, graft monitoring, and treatment once rejection is recognized or suspected. This case series chose to utilize monitoring for CD154+ T-cytotoxic memory cells, previously demonstrated to be sensitive for acute rejection in pediatric liver or intestinal implants[53-56]. Increasing concentration of this T cell was noted to generally correlate with suspected decreased function of the hepatocytes, however, the authors do note that significant daily variance of measured total bilirubin in the CN Type Ⅰ patients and phenylalanine levels in the PKU patient[52]. Reviewing the aforementioned cases and use of immunosuppression, it

appears tacrolimus is an acceptable immunosuppressive agent, however, that closer monitoring of drug levels may be necessary to ensure continued appropriate function of the transplanted hepatocytes[1,31,52]. Further prospective cohort studies utilizing different monitoring intervals or alternate immunosuppressive therapy is likely necessary to ensure sustained and optimized graft function.

CONCLUSION

Further human studies involving humans are needed, however, the above collectively suggest progress in terms of improved effectiveness of HTx. With improvements in optimizing delivery technique and assessing proper recipients of livers, monitoring graft function, as well as recognizing and treating graft rejection, HTx may be able to be used more widely in metabolic liver disease and potentially delay necessity of OLT.

REFERENCES

1 **Muraca M**, Gerunda G, Neri D, Vilei MT, Granato A, Feltracco P, Meroni M, Giron G, Burlina AB. Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. *Lancet* 2002; **359**: 317-318 [PMID: 11830200 DOI: 10.1016/S0140-6736(02)07529-3]

2 **Hines RN**, McCarver DG. The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. *J Pharmacol Exp Ther* 2002; **300**: 355-360 [PMID: 11805191 DOI: 10.1124/jpet.300.2.355]

3 **Popescu I**, Dima SO. Domino liver transplantation: how far can we push the paradigm? *Liver Transpl* 2012; **18**: 22-28 [PMID: 21987415 DOI: 10.1002/lt.22443]

4 **Roels L**, Rahmel A. The European experience. *Transpl Int* 2011; **24**: 350-367 [PMID: 21265886 DOI: 10.1111/j.1432-2277.2011.01225.x]

5 **Fox IJ**, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI, Dorko K, Sauter BV, Strom SC. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. *N Engl J Med* 1998; **338**: 1422-1426 [PMID: 9580649 DOI: 10.1056/NEJM199805143382004]

6 **Donato MT**, Lahoz A, Jiménez N, Pérez G, Serralta A, Mir J, Castell JV, Gómez-Lechón MJ. Potential impact of steatosis on cytochrome P450 enzymes of human hepatocytes isolated from fatty liver grafts. *Drug Metab Dispos* 2006; **34**: 1556-1562 [PMID: 16763015 DOI: 10.1124/dmd.106.009670]

7 **Sagias FG**, Mitry RR, Hughes RD, Lehec SC, Patel AG, Rela M, Mieli-Vergani G, Heaton ND, Dhawan A. N-acetylcysteine improves the viability of human hepatocytes isolated from severely steatotic donor liver tissue. *Cell Transplant* 2010; **19**: 1487-1492 [PMID: 20587150 DOI: 10.3727/096368910X514620]

8 **Strom SC**, Fisher RA, Thompson MT, Sanyal AJ, Cole PE, Ham JM, Posner MP. Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. *Transplantation* 1997; **63**: 559-569 [PMID: 9047152 DOI: 10.1097/00007890-199702270-00014]

9 **Mitry RR**, Dhawan A, Hughes RD, Bansal S, Lehec S, Terry C, Heaton ND, Karani JB, Mieli-Vergani G, Rela M. One liver, three recipients: segment IV from split-liver procedures as a source of hepatocytes for cell transplantation. *Transplantation* 2004; **77**: 1614-1616 [PMID: 15239631 DOI: 10.1097/01.TP.0000122224.98318.19]

10 **Kawahara T**, Toso C, Douglas DN, Nourbakhsh M, Lewis JT, Tyrrell DL, Lund GA, Churchill TA, Kneteman NM. Factors affecting hepatocyte isolation, engraftment, and replication in an in vivo model. *Liver Transpl* 2010; **16**: 974-982 [PMID: 20677288 DOI: 10.1002/lt.22099]

11 **Gramignoli R**, Marongiu F, Tahan V, Dorko K, Geller D, Venkataramanan R, D'Antiga L, Ellis ECS, Strom SC. Isolation and evaluation of human hepatocytes for cell-based therapy. *Dig Liver Dis* 2011; **43**: S96 [DOI: 10.1016/S1590-8658(11)60097-2]

12 **Donato MT**, Lahoz A, Montero S, Bonora A, Pareja E, Mir J, Castell JV, Gómez-Lechón MJ. Functional assessment of the quality of human hepatocyte preparations for cell transplantation. *Cell Transplant* 2008; **17**: 1211-1219 [PMID: 19181215 DOI: 10.3727/093688908787236620]

13 **LeCluyse EL**. Human hepatocyte culture systems for the in vitro evaluation of cytochrome P450 expression and regulation. *Eur J Pharm Sci* 2001; **13**: 343-368 [PMID: 11408150 DOI: 10.1016/S0928-0987(01)00135-X]

14 **Jorns C**, Ellis EC, Nowak G, Fischler B, Nemeth A, Strom SC, Ericzon BG. Hepatocyte transplantation for inherited metabolic diseases of the liver. *J Intern Med* 2012; **272**: 201-223 [PMID: 22789058 DOI: 10.1111/j.1365-2796.2012.02574.x]

15 **Strauss KA**, Robinson DL, Vreman HJ, Puffenberger EG, Hart G, Morton DH. Management of hyperbilirubinemia and prevention of kernicterus in 20 patients with Crigler-Najjar disease. *Eur J Pediatr* 2006; **165**: 306-319 [PMID: 16435131 DOI: 10.1007/s00431-005-0055-2]

16 **Groth CG**, Arborgh B, Björkén C, Eriksson G, Lundren G. Hepatocellular transplantation in glucuronyltransferase-deficient rat. *Sven Kir* 1974; **31**: 42

17 **Groth CG**, Arborgh B, Björkén C, Sundberg B, Lundgren G. Correction of hyperbilirubinemia in the glucuronyltransferase-deficient rat by intraportal hepatocyte transplantation. *Transplant Proc* 1977; **9**: 313-316 [PMID: 405772]

18 **Matas AJ**, Sutherland DE, Steffes MW, Mauer SM, Sowe A, Simmons RL, Najarian JS. Hepatocellular transplantation for metabolic deficiencies: decrease of plasms bilirubin in Gunn rats. *Science* 1976; **192**: 892-894 [PMID: 818706 DOI: 10.1126/science.818706]

19 **Darwish AA**, Sokal E, Stephenne X, Najimi M, de Goyet Jde V, Reding R. Permanent access to the portal system for cellular transplantation using an implantable port device. *Liver Transpl* 2004; **10**: 1213-1215 [PMID: 15350017 DOI: 10.1002/lt.20228]

20 **Dhawan A**, Puppi J, Hughes RD, Mitry RR. Human hepatocyte transplantation: current experience and future challenges. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 288-298 [PMID: 20368738 DOI: 10.1038/nrgastro.2010.44]

21 **Ambrosino G**, Varotto S, Strom SC, Guariso G, Franchin E, Miotto D, Caenazzo L, Basso S, Carraro P, Valente ML, D'Amico D, Zancan L, D'Antiga L. Isolated hepatocyte transplantation for Crigler-Najjar syndrome type 1. *Cell Transplant* 2005; **14**: 151-157 [PMID: 15881424 DOI: 10.3727/000000005783983250]

22 **Lysy PA**, Najimi M, Stephenne X, Bourgois A, Smets F, Sokal EM. Liver cell transplantation for Crigler-Najjar syndrome type I: update and perspectives. *World J Gastroenterol* 2008; **14**: 3464-3470 [PMID: 18567072 DOI: 10.3748/wjg.14.3464]

23 **Allen KJ**, Mifsud NA, Williamson R, Bertolino P, Hardikar W. Cell-mediated rejection results in allograft loss after liver cell transplantation. *Liver Transpl* 2008; **14**: 688-694 [PMID: 18433045 DOI: 10.1002/lt.21443]

24 **Meyburg J**, Das AM, Hoerster F, Lindner M, Kriegbaum H, Engelmann G, Schmidt J, Ott M, Pettenazzo A, Luecke T, Bertram H, Hoffmann GF, Burlina A. One liver for four children: first clinical series of liver cell transplantation for severe neonatal urea cycle defects. *Transplantation* 2009; **87**: 636-641 [PMID: 19295306 DOI: 10.1097/TP.0b013e318199936a]

25 **Meyburg J**, Hoffmann GF. Liver, liver cell and stem cell transplantation for the treatment of urea cycle defects. *Mol Genet Metab* 2010; **100** Suppl 1: S77-S83 [PMID: 20156696 DOI: 10.1016/j.ymgme.2010.01.011]

26 **Eguchi S**, Rozga J, Lebow LT, Chen SC, Wang CC, Rosenthal R, Fogli L, Hewitt WR, Middleton Y, Demetriou AA. Treatment of hypercholesterolemia in the Watanabe rabbit using allogeneic hepatocellular transplantation under a regeneration stimulus.

Transplantation 1996; **62**: 588-593 [PMID: 8830820 DOI: 10.1097/00007890-199609150-00008]

27 **Gunsalus JR**, Brady DA, Coulter SM, Gray BM, Edge AS. Reduction of serum cholesterol in Watanabe rabbits by xenogeneic hepatocellular transplantation. *Nat Med* 1997; **3**: 48-53 [PMID: 8986740 DOI: 10.1038/nm0197-48]

28 **Chowdhury JR**, Grossman M, Gupta S, Chowdhury NR, Baker JR Jr, Wilson JM. Long-term improvement of hypercholesterolemia after ex vivo gene therapy in LDLR-deficient rabbits. *Science* 1991; **254**: 1802-1805 [PMID: 1722351 DOI: 10.1126/science.1722351]

29 **Grossman M**, Rader DJ, Muller DW, Kolansky DM, Kozarsky K, Clark BJ 3rd, Stein EA, Lupien PJ, Brewer HB Jr, Raper SE. A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia. *Nat Med* 1995; **1**: 1148-1154 [PMID: 7584986 DOI: 10.1038/nm1195-1148]

30 **Chou JY**, Jun HS, Mansfield BC. Glycogen storage disease type I and G6Pase-β deficiency: etiology and therapy. *Nat Rev Endocrinol* 2010; **6**: 676-688 [PMID: 20975743 DOI: 10.1038/nrendo.2010.189]

31 **Lee KW**, Lee JH, Shin SW, Kim SJ, Joh JW, Lee DH, Kim JW, Park HY, Lee SY, Lee HH, Park JW, Kim SY, Yoon HH, Jung DH, Choe YH, Lee SK. Hepatocyte transplantation for glycogen storage disease type Ib. *Cell Transplant* 2007; **16**: 629-637 [PMID: 17912954 DOI: 10.3727/000000007783465019]

32 **Steinberg SJ**, Dodt G, Raymond GV, Braverman NE, Moser AB, Moser HW. Peroxisome biogenesis disorders. *Biochim Biophys Acta* 2006; **1763**: 1733-1748 [PMID: 17055079 DOI: 10.1016/j.bbamcr.2006.09.010]

33 **Sokal EM**, Smets F, Bourgois A, Van Maldergem L, Buts JP, Reding R, Bernard Otte J, Evrard V, Latinne D, Vincent MF, Moser A, Soriano HE. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. *Transplantation* 2003; **76**: 735-738 [PMID: 12973120 DOI: 10.1097/01.TP.0000077420.81365.53]

34 **Ohashi K**, Waugh JM, Dake MD, Yokoyama T, Kuge H, Nakajima Y, Yamanouchi M, Naka H, Yoshioka A, Kay MA. Liver tissue engineering at extrahepatic sites in mice as a potential new therapy for genetic liver diseases. *Hepatology* 2005; **41**: 132-140 [PMID: 15619229 DOI: 10.1002/hep.20484]

35 **Tatsumi K**, Ohashi K, Shima M, Nakajima Y, Okano T, Yoshioka A. Therapeutic effects of hepatocyte transplantation on hemophilia B. *Transplantation* 2008; **86**: 167-170 [PMID: 18622295 DOI: 10.1097/TP.0b013e31817b9160]

36 **Dhawan A**, Mitry RR, Hughes RD, Lehec S, Terry C, Bansal S, Arya R, Wade JJ, Verma A, Heaton ND, Rela M, Mieli-Vergani G. Hepatocyte transplantation for inherited factor VII deficiency. *Transplantation* 2004; **78**: 1812-1814 [PMID: 15614156 DOI: 10.1097/01.TP.0000146386.77076.47]

37 **Arnell H**, Fischler B, Bergdahl S, Schnell PO, Jacobsson H, Nemeth A. Hepatobiliary scintigraphy during cholestatic and noncholestatic periods in patients with progressive familial intrahepatic cholestasis after partial external biliary diversion. *J Pediatr Surg* 2011; **46**: 467-472 [PMID: 21376194 DOI: 10.1016/j.jpedsurg.2010.09.042]

38 **De Vree JM**, Ottenhoff R, Bosma PJ, Smith AJ, Aten J, Oude Elferink RP. Correction of liver disease by hepatocyte transplantation in a mouse model of progressive familial intrahepatic cholestasis. *Gastroenterology* 2000; **119**: 1720-1730 [PMID: 11113093 DOI: 10.1053/gast.2000.20222]

39 **Loeber JG**. Neonatal screening in Europe; the situation in 2004. *J Inherit Metab Dis* 2007; **30**: 430-438 [PMID: 17616847 DOI: 10.1007/s10545-007-0644-5]

40 **Blau N**, van Spronsen FJ, Levy HL. Phenylketonuria. *Lancet* 2010; **376**: 1417-1427 [PMID: 20971365 DOI: 10.1016/S0140-6736(10)60961-0]

41 **Hamman KJ**, Winn SR, Harding CO. Hepatocytes from wild-type or heterozygous donors are equally effective in achieving successful therapeutic liver repopulation in murine phenylketonuria (PKU). *Mol Genet Metab* 2011; **104**: 235-240 [PMID: 21917493 DOI: 10.1016/j.ymgme.2011.07.027]

42 **Hansel MC**, Davila JC, Vosough M, Gramignoli R, Skvorak KJ, Dorko K, Marongiu F, Blake W, Strom SC. The Use of Induced Pluripotent Stem Cells for the Study and Treatment of Liver Diseases. *Curr Protoc Toxicol* 2016; **67**: 14.13.1-14.13.27 [PMID: 26828329 DOI: 10.1002/0471140856.tx1412s62]

43 **Stéphenne X**, Najimi M, Ngoc DK, Smets F, Hue L, Guigas B, Sokal EM. Cryopreservation of human hepatocytes alters the mitochondrial respiratory chain complex 1. *Cell Transplant* 2007; **16**: 409-419 [PMID: 17658131 DOI: 10.3727/000000007783464821]

44 **Gómez-Lechón MJ**, Lahoz A, Jiménez N, Vicente Castell J, Donato MT. Cryopreservation of rat, dog and human hepatocytes: influence of preculture and cryoprotectants on recovery, cytochrome P450 activities and induction upon thawing. *Xenobiotica* 2006; **36**: 457-472 [PMID: 16769645 DOI: 10.1080/00498250600674352]

45 **Terry C**, Hughes RD, Mitry RR, Lehec SC, Dhawan A. Cryopreservation-induced nonattachment of human hepatocytes: role of adhesion molecules. *Cell Transplant* 2007; **16**: 639-647 [PMID: 17912955 DOI: 10.3727/000000007783465000]

46 **Sharma S**, Ellis EC, Gramignoli R, Dorko K, Tahan V, Hansel M, Mattison DR, Caritis SN, Hines RN, Venkataramanan R, Strom SC. Hepatobiliary disposition of 17-OHPC and taurocholate in fetal human hepatocytes: a comparison with adult human hepatocytes. *Drug Metab Dispos* 2013; **41**: 296-304 [PMID: 23129211]

47 **Meyburg J**, Alexandrova K, Barthold M, Kafert-Kasting S, Schneider AS, Attaran M, Hoerster F, Schmidt J, Hoffmann GF, Ott M. Liver cell transplantation: basic investigations for safe application in infants and small children. *Cell Transplant* 2009; **18**: 777-786 [PMID: 19796496 DOI: 10.3727/096368909X470775]

48 **Baccarani U**, Adani GL, Sanna A, Avellini C, Sainz-Barriga M, Lorenzin D, Montanaro D, Gasparini D, Risaliti A, Donini A, Bresadola F. Portal vein thrombosis after intraportal hepatocytes transplantation in a liver transplant recipient. *Transpl Int* 2005; **18**: 750-754 [PMID: 15910305 DOI: 10.1111/j.1432-2277.2005.00127.x]

49 **Nagata H**, Ito M, Shirota C, Edge A, McCowan TC, Fox IJ. Route of hepatocyte delivery affects hepatocyte engraftment in the spleen. *Transplantation* 2003; **76**: 732-734 [PMID: 12973119 DOI: 10.1097/01.TP.0000081560.16039.67]

50 **Habibullah CM**, Syed IH, Qamar A, Taher-Uz Z. Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. *Transplantation* 1994; **58**: 951-952 [PMID: 7940741 DOI: 10.1097/00007890-199410270-00016]

51 **Pilichos C**, Perrea D, Demonakou M, Preza A, Donta I. Management of carbon tetrachloride-induced acute liver injury in rats by syngeneic hepatocyte transplantation in spleen and peritoneal cavity. *World J Gastroenterol* 2004; **10**: 2099-2102 [PMID: 15237443 DOI: 10.3748/wjg.v10.i14.2099]

52 **Soltys KA**, Setoyama K, Tafaleng EN, Soto Gutiérrez A, Fong J, Fukumitsu K, Nishikawa T, Nagaya M, Sada R, Haberman K, Gramignoli R, Dorko K, Tahan V, Dreyzin A, Baskin K, Crowley JJ, Quader MA, Deutsch M, Ashokkumar C, Shneider BL, Squires RH, Ranganathan S, Reyes-Mugica M, Dobrowolski SF, Mazariegos G, Elango R, Stolz DB, Strom SC, Vockley G, Roy-Chowdhury J, Cascalho M, Guha C, Sindhi R, Platt JL, Fox IJ. Host conditioning and rejection monitoring in hepatocyte transplantation in humans. *J Hepatol* 2017; **66**: 987-1000 [PMID: 28027971 DOI: 10.1016/j.jhep.2016.12.017]

53 **Zhou H**, Dong X, Kabarriti R, Chen Y, Avsar Y, Wang X, Ding J, Liu L, Fox IJ, Roy-Chowdhury J, Roy-Chowdhury N, Guha C. Single liver lobe repopulation with wildtype hepatocytes using regional hepatic irradiation cures jaundice in Gunn rats. *PLoS One* 2012; **7**: e46775 [PMID: 23091601 DOI: 10.1371/journal.pone.0046775]

54 **Ashokkumar C**, Bentlejewski C, Sun Q, Higgs BW, Snyder S, Mazariegos GV, Abu-Elmagd K, Zeevi A, Sindhi R. Allospecific CD154+ B cells associate with intestine allograft rejection in children. *Transplantation* 2010; **90**: 1226-1231 [PMID: 20881665 DOI: 10.1097/TP.0b013e3181f995ce]

55 **Ashokkumar C**, Shapiro R, Tan H, Ningappa M, Elinoff B, Fedorek S, Sun Q, Higgs BW, Randhawa P, Humar A, Sindhi R.

Allospecific CD154+ T-cytotoxic memory cells identify recipients experiencing acute cellular rejection after renal transplantation. *Transplantation* 2011; **92**: 433-438 [PMID: 21747326 DOI: 10.1097/TP.0b013e318225276d]

56 **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]

P- Reviewer: Kin T, Ramsay MA, Sureshkumar KK **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Yan JL

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 December 24; 7(6): 324-328

DOI: 10.5500/wjt.v7.i6.324

ISSN 2220-3230 (online)

MINIREVIEWS

Elderly donor graft for liver transplantation: Never too late

Harleen Chela, Mohamad H Yousef, Abdelmajeed A Albarrak, Bhupinder S Romana, Dania N Hudhud, Veysel Tahan

Harleen Chela, Dania N Hudhud, Department of Internal Medicine, University of Missouri, Columbia, MO 65212, United States

Mohamad H Yousef, Abdelmajeed A Albarrak, Bhupinder S Romana, Veysel Tahan, Division of Gastroenterology and Hepatology, Missouri University, Columbia, MO 65212, United States

ORCID number: Veysel Tahan (0000-0001-6796-9359).

Author contributions: All authors contributed to the acquisition of data, writing and revision of this manuscript.

Conflict-of-interest statement: All the authors have no conflicts of interests to declare. All authors of this article declare that was no conflicting interests (including but not limited to commercial, personal, political, intellectual or religious interests) that are related to the work submitted for consideration of publication.

Manuscript source: Invited manuscript

Correspondence to: Veysel Tahan, MD, FACP, FACG, FESBGH, Division of Gastroenterology and Hepatology, University of Missouri, 1 Hospital Dr., Columbia, MO 65212, United States. tahanv@health.missouri.edu
Telephone: +1-573-8846044
Fax: +1-573-8844595

Received: November 4, 2017
Peer-review started: November 5, 2017
First decision: November 20, 2017
Revised: November 27, 2017
Accepted: December 5, 2017
Article in press: December 6, 2017

Published online: December 24, 2017

Abstract

The definitive treatment for end stage liver disease remains a liver transplant and hence livers are needed for these patients along with cases of acute fulminant liver failure. Hence livers are a scarce and highly valuable commodity in the current time. By extending the pool of donors to include the elderly livers, it allows for increased availability of donors and reduces the mortality that is associated with the waiting list itself. There is an increasing prevalence of end stage liver disease due to conditions like chronic hepatitis B and C, non-alcoholic steatohepatitis, alcoholic liver disease. Many studies show non-inferior outcomes when elderly livers are used as a vigorous selection process is implemented. The process takes into account the characteristics of the donor, graft and recipient allowing for appropriate donor-recipient coupling. To meet the increasing demands of livers, elderly donors should be utilized for liver transplantation. The aim of this review article is to describe the aging process of the liver and the outcomes associated with use of elderly livers for transplantation.

Key words: Liver transplantation; Donor age; Elderly; Age; Outcome; Success

Core tip: There is an increasing demand of livers for transplantation. Several studies showed successful results with elderly donors. We reviewed the aging process of the liver and the transplant outcomes of elderly donors. We highlight that elderly donors can be utilized given the extensive screening process allowing for risk factor analysis and appropriate allocation. Hence they should be used to allow for treatment of

liver disease globally and help mitigate the shortage of hepatic grafts.

Chela H, Yousef MH, Albarrak AA, Romana BS, Hudhud DN, Tahan V. Elderly donor graft for liver transplantation: Never too late. *World J Transplant* 2017; 7(6): 324-328 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i6/324.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i6.324

INTRODUCTION

Orthotropic liver transplantation is an area of hepatology that is under continuous evolution. It is the definitive treatment for end stage liver disease as well as cases of acute fulminant liver failure. The fact that livers are a paucity and in high demand all over the world has forced the medical community to evaluate livers from marginal or extended criteria donors thus allowing the pool of donors to enlarge. The term marginal donors or extended criteria donors encompasses a group of criteria that allows for the enlargement of the donor pool. These comprise of elderly donors (aged > 60 years), steatosis > 30%, grafts with cold ischemia time > 12 h, hypernatremia, hepatitis B and C viral infections, split-liver grafts, donors who are living relatives, donors from cardiac arrest patients[1]. The need for livers greatly exceeds the supply and hence leads to prolonged waiting time and the associated subsequent mortality while awaiting transplant. The availability of younger donors is decreasing given the advances in medicine overall less motor vehicle accidents. The purpose of this review is to evaluate the impact of age on the liver and the implications that it carries on the outcome of the transplantation. Genetic and environmental factors also influence the aging process of the liver itself[2]. Multiple series of studies have revealed that the stigma that aging livers carry is not completely viable. The fear associated with elderly donors is that the increased risk of complications due to concern for impaired function and lack of a robust response to external and internal stressors as compared to younger livers[2]. There is a potential of transmission of occult malignancies as well as concern of overall decreased survival of the recipient and the graft itself[1,2]. Numerous variables are taken into account with regards to the donor, the recipient and the graft itself. Balancing these and carefully selecting the correct donor-recipient pair yields good outcomes which are comparable to those obtained from transplanting donor livers.

Impact of age on the liver

Similar to all organs in the human body, the liver undergoes many age related changes. Though as compared to other organs, the liver possesses the capacity to regenerate, abundant vascularity, as well as superior functional reserve[1]. Two of the main changes in the liver include a decrease in the overall hepatic mass and the blood flow[1]. Understanding the changes in the structural, morphological and functional changes that occur as the liver ages can help in making appropriate decisions regarding the use of older livers for transplantation.

Macroscopic and microscopic changes

As the liver ages, it tends to shrink in size and undergoes a process brown atrophy. Grossly, it acquires a brownish colored appearance and is due to the deposition of lipofuscin which are insoluble proteins[1-5]. The Glisson's capsule also acquires a fibrous thickening[1-5]. Microscopically, there is reduction of the number of hepatocytes though the cell volume increases[1-2]. There is increased variation in the cell size and the nuclear size increases as well as the amount of nuclear DNA along with aneuploidy[1]. Similar to the cells themselves, the mitochondria undergo a process of acquiring increased volume but reduction in the overall number of mitochondria[1,2,6]. These alterations reflect that the cells and their organelles are attempting to overcome the reduction in the overall number[1]. The cells are vulnerable to reperfusion injury due to the reduced mitochondrial adenosine-triphosphate content[2]. There is reduction of smooth endoplasmic reticulum and buildup of lysosomes[2]. Hepatic sinusoids exhibit increased thickness of the endothelial lining and reduction in the fenestration in the endothelial cells[2]. There is increased thickness of the hepatic arteriolar walls as well[2]. Reduction in the secretion of bile acids as well as reduced bile flow is also reported[1,2].

Vascular changes

Along with reduction in liver mass there is a substantial reduction in the hepatic blood flow with age especially after the age of 30 years[1,2,4]. This can significantly impact the clearance of medications hence leading to the potential for complications to arise[1]. Age related atherosclerotic changes affect the vascular tree and its branches. The branches of the abdominal aorta is predominantly impacted in the proximal and mid proximal regions, however in cases where there is occlusive pathology of the distal portions there can be involvement of the hepatic artery[1]. This predisposes to vascular complications and can hence impact the graft survival and overall outcome post-transplant.

Functional change

The overall synthetic function of the liver declines with age especially with regards to protein synthesis as well as synthesis of clotting factors[1]. The levels of serum bilirubin, alkaline phosphatase and transaminases are not impacted by age and instead are a measure of liver damage and not to the functional capacity of the liver[1]. It appears that overall age does not have a major effect on function of the liver itself but alters the response to stressors (especially external) including states of increased metabolic need or disease processes[1]. There is also report of diminished phase Ⅰ metabolism of

drugs and increased production of pro-inflammatory cytokines[2]. There is increased predisposition to the development of diseases due to decreased rates of DNA repair, decreased expression of growth regulatory genes and the impact of oxidative stress[2]. Regenerative capacity of the liver is not impaired but the rate of regeneration is decreased as a consequence of aging[1].

Evaluating the aging liver

Diligent and through assessment of the graft as well as the donor is required for selection for transplant. Marginal or extended criteria donors are associated with increased risk of complications including initial poor function and primary non-function[1]. Initial poor function is an aspartate transaminase (AST) value more than 2000 IU/L, ammonia level > 50 μmol/L, prothrombin time > 16 s on post-transplant days 2-6[1]. Primary non-function is defined as graft failure in the first week post-transplant or require a re-transplant for survival[1]. There are many variables associated with the donor that can predict failure of the graft and increased mortality of the recipient[1,2]. By use of Cox regression analysis there are seven major factors that are independently associated with graft failure[1,2,7]. These include donors aged > 40 years (especially > 60 years), prolonged warm ischemia, split/partial grafts, prolonged cold storage > 10 h, length of ICU stay > 5 d, decreased donor height, cerebrovascular accident, black race[1,2]. Scores like the marginal liver score have also been formulated to aid in identifying the higher risk factors associated with poor graft survival and overall outcomes[1]. The factors include donor age > 60 years, cold ischemia time > 13 h, length of intensive care unit (ICU) stay > 4 d, hypotensive episodes < 60 mmHg for > 1 h, alanine transaminase (ALT) > 170 U/L. AST > 140 U/L, dopamine dose > 10 mg/kg, serum sodium > 155 mEq/L, bilirubin > 2.0 mg/dL[1]. Each factor has a score of 2 and an overall score of 3 or above predicts poor survival of the graft[1]. When livers are being prepared for harvesting, caution has to be exercised to ensure adequate circulation to avoid ischemia, hypovolemia, hypoxemia as well as avoiding infection[1]. Dopamine is commonly used in cases of hypotension to augment renal and mesenteric circulation, however doses exceeding 10 mcg/kg per minute can result in acute tubular necrosis and doses beyond 15 mcg/kg per minute have been associated with graft preservation injury[1]. Hence a delicate balance exists and must be maintained to ensure adequate perfusion and oxygenation of the liver.

Increased length of stay in the ICU especially > 4 d can affect the post-transplant function of the liver due to the use of vasopressors and the resultant effect on hormonal status, hemodynamics and nutritional status[1,2,8]. Another factor that is recognized to have a negative impact is hypernatremia which leads to cell swelling and worsens ischemia reperfusion injury and graft dysfunction[1,2]. However increased transaminases in elderly donors were considered as marginal criteria in the past, however these are not commonly elevated in elderly donors that are used in successful transplants and this is indicative of the rigorous selection technique[2,9].

An ultrasound of abdomen is recommended to evaluate the donor liver for steatosis, tumors of the liver and other intra-abdominal malignancy or abscess[1,2]. Many experts also recommend obtaining a liver biopsy as well to assess for fibrosis, steatosis, hepatitis, cholestasis[1,2,9]. Microsteatosis may be linked to early allograft dysfunction, however macrosteatosis > 30% increases the risk of reperfusion injury and is a strong predictor of poor outcome especially when combined with prolonged cold or warm ischemia[1,2]. Some studies have revealed that microsteatosis may not impose any challenges regardless of the severity as opposed to macrosteatosis in which the outcome is negatively influenced with increasing severity of fat infiltration[10]. Prevalence of steatosis does increase with age and is linked to malnutrition, obesity, type Ⅱ diabetes, chronic alcohol intake[2].

Prolonged cold ischemia time leads to ischemia reperfusion injury which is a type of microvascular injury and leads to increased risk of rejection of the graft and morbidity[1,2,11]. There are 4 stages of injury: Pre-preservation, cold preservation, rewarming and reperfusion[1]. The chances of this injury and the severity are affected by various factors which can potentially be controlled hence minimizing the risk of injury and improving the outcome of transplant. Older livers are more vulnerable to this form of injury hence extra caution must be exerted to keep the cold ischemia time to a minimum in them[1,2]. Increased warm ischemia time also has deleterious effects and should also be kept minimized[1,2].

Overall outcome of using elderly donors

Underlying condition of the recipient does influence survival of the graft, however recurrent diseases are a major cause of graft failure[2]. Cirrhosis secondary to hepatitis C is a major cause of liver failure requiring transplantation and has exceedingly high recurrence rates[2]. Graft fibrosis after transplantation was linked to the organ age and hence elderly livers are avoided in such cases, however this may change with the advancements in antiviral therapies or hepatitis C reducing the risk of recurrence[2]. With time post transplantation there is occurrence of chronic hepatitis and eventual fibrosis and hence elderly livers are avoided for transplant in the pediatric population[1,2].

Due to the decreased number of hepatocytes and the alteration in the regenerative capacity of older livers there has been concern to use them for transplant due to fear of early allograft dysfunction and primary non function[2]. Due to the increased prevalence of advanced atherosclerotic disease in the elderly there is increased concern for vascular complications developing post-transplant when elderly donors are used[2]. Though the elderly have increased arteriosclerosis in the celiac axis, it appears that the hepatic arteries are not significantly

impacted by this and more distal portions of the hepatic arterial system is used for transplant[2]. Arteriosclerosis affects the graft by two methods: Decreased blood supply at time of organ harvesting due to stenosis of celiac axis ostium causing poor graft preservation and increasing chances of primary non-function. The second method is effect on the vascular reconstruction process if the donor arteries are diseased by arteriosclerosis leading to early as well as delayed difficulties[2]. Hepatic artery thrombosis is one of the major causes of graft failure and has increased prevalence with increasing age of the donor[1,2,11]. The major causes of mortality in recipients of elderly donors are medical complications, cirrhosis due to hepatitis C recurrence and *de novo* tumors[1]. In a study performed by Zhao *et al*[11], that involved the use of elderly brain-dead donors, it was found that there was no primary non functions or need for re-transplant in the patients receiving the elderly (> 60 years) livers. They also found that early graft function was similar between the elderly and the younger donor group[11]. If careful selection and risk stratification is performed then acceptable and even at times comparable outcomes can be achieved with elderly donors. For example, using high risk donors for low risk recipients so that the risk of the donor is offset by the lower risk of the recipient to achieve more favorable outcomes and to avoid the waiting list mortality[1]. Using marginal or extended criteria donors requires that multiple factors be taken into account to match the appropriate donor with the corresponding recipient. By detailed assessment of the graft characteristics and taking into account factors that will augment each other negatively, appropriate donors can be selected[10]. Evaluating the recipient's ability to accommodate high risk donor grafts allows appropriate matching to occur without yielding a negative outcome[10].

In a retrospective study performed by Zhao *et al*[11] in which they evaluated 106 donor liver transplants which were harvested from cadavers. They were used in total of 98 patients and 7 of these patients were recipients of elderly donor livers (age > 60 years). The patients were divided into two groups. Group Ⅰ received livers from elderly donors > 60 years, and Group Ⅱ received livers from donors < 60 years. They accounted for risk factors like age of the donor, body mass index, the etiology of death, duration of stay in the ICU, gender, blood pressure and the amount of vasopressor used[11]. There were no significant differences overall with regards to parameters like bilirubin levels, transaminases at one week post-operatively[11]. Outcome with regards to recipient and graft survival as well as complications like primary non-function, biliary complications, hepatic artery thrombosis, need for re-transplantation. Zhao *et al*[11] supported the use of elderly donors for liver transplantation.

A study conducted in Birmingham (United Kingdom) revealed that mortality due to primary non function was comparable between the donors less than 70 years (1.3%) and more than 70 years (2.0%)[2]. They noted that recipients of aged livers experienced fewer arterial complications such as hepatic artery thrombosis and though it was not significant at the statistical level but did reflect that elderly livers should not be associated with worse outcomes[2]. Other centers around the world have experienced similar outcomes hence promoting the use of elderly liver donors.

Another study conducted by Rodríguez González *et al*[12] using 100 liver allografts from elderly donors aged > 60 years also supported the use of aged donors > 60 years. They emphasized that elderly donors can be used as long as a comprehensive risk assessment and evaluation is performed. Assessing the pre-transplant conditions like the liver function tests, length of ICU stay, cold ischemia time, hemodynamic status, whether vasopressors were used or not[12]. Factors like cold ischemia time of less than 6 h ideally as well as macrovesicular steatosis < 30% were very important contributors to a favorable outcome when using the elderly donors in their study[12]. Hence their study showed favorable outcomes when elderly donors were used as long as pre-operative risks were assessed and minimized as much as possible[12].

A study conducted by Thorsen *et al*[13] focused on liver from decreased donors aged > 75 years and though they noted an increased rate of biliary complications, they did not see overall worse outcomes with regards to mortality rates in recipients or graft survival.

CONCLUSION

The use of elderly donors is becoming more favorable and helping to reduce the mortality associated with the waiting list itself. The liver is a remarkable organ that possesses several unique qualities though like other organs in the human body it is also subjected to the process of aging. Though advancing age is not an advantage for the process of transplantation, it should not preclude the use of elderly livers for transplant. A careful and meticulous selection process can be carried out allowing to risk stratify the donor, the graft and the recipient. Factors in the graft like the gross as well as microscopic appearance are evaluated to exclude donors with obvious abnormalities like tumors or significant macroscopic steatosis. An ultrasound is recommended along with a liver biopsy to evaluate for occult tumors along with pathologies like fibrosis, steatosis. A thorough evaluation of the donor should be performed including detailed medical history, intra-operative exploration of the abdominal as well as thoracic cavities to exclude malignancies. Recipients should be evaluated as well and accordingly matched to appropriate donors hence achieving optimal outcomes. With this rigorous selection process it has been shown in several studies that elderly donors are comparable to younger donors and have successful outcomes. We would like to emphasize that elderly donors can be utilized given the extensive screening process allowing for risk factor analysis and appropriate allocation. Hence they should be used to allow for treatment of liver disease globally and help

mitigate the shortage of hepatic grafts.

REFERENCES

1 **Jiménez-Romero C**, Caso Maestro O, Cambra Molero F, Justo Alonso I, Alegre Torrado C, Manrique Municio A, Calvo Pulido J, Loinaz Segurola C, Moreno González E. Using old liver grafts for liver transplantation: where are the limits? *World J Gastroenterol* 2014; **20**: 10691-10702 [PMID: 25152573 DOI: 10.3748/wjg.v20.i31.10691]

2 **Dasari BVM**, Schlegel A, Mergental H, Perera MTPR. The use of old donors in liver transplantation. *Best Pract Res Clin Gastroenterol* 2017; **31**: 211-217 [PMID: 28624109 DOI: 10.1016/j.bpg.2017.03.002]

3 **Jiménez Romero C**, Moreno González E, Colina Ruíz F, Palma Carazo F, Loinaz Segurola C, Rodríguez González F, González Pinto I, García García I, Rodríguez Romano D, Moreno Sanz C. Use of octogenarian livers safely expands the donor pool. *Transplantation* 1999; **68**: 572-575 [PMID: 10480418 DOI: 10.1097/00007890-199908270-00021]

4 **Wynne HA**, Cope LH, Mutch E, Rawlins MD, Woodhouse KW, James OF. The effect of age upon liver volume and apparent liver blood flow in healthy man. *Hepatology* 1989; **9**: 297-301 [PMID: 2643548 DOI: 10.1002/hep.1840090222]

5 **Jung T**, Bader N, Grune T. Lipofuscin: formation, distribution, and metabolic consequences. *Ann N Y Acad Sci* 2007; **1119**: 97-111 [PMID: 18056959 DOI: 10.1196/annals.1404.008]

6 **Watanabe T**, Tanaka Y. Age-related alterations in the size of human hepatocytes. A study of mononuclear and binucleate cells. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1982; **39**: 9-20 [PMID: 6123185 DOI: 10.1007/BF02892832]

7 **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]

8 **Novitzky D**, Cooper DK, Reichart B. Hemodynamic and metabolic responses to hormonal therapy in brain-dead potential organ donors. *Transplantation* 1987; **43**: 852-854 [PMID: 3296351]

9 **Nardo B**, Masetti M, Urbani L, Caraceni P, Montalti R, Filipponi F, Mosca F, Martinelli G, Bernardi M, Daniele Pinna A, Cavallari A. Liver transplantation from donors aged 80 years and over: pushing the limit. *Am J Transplant* 2004; **4**: 1139-1147 [PMID: 15196073 DOI: 10.1111/j.1600-6143.2004.00472.x]

10 **Nemes B**, Gámán G, Polak WG, Gelley F, Hara T, Ono S, Baimakhanov Z, Piros L, Eguchi S. Extended criteria donors in liver transplantation Part I: reviewing the impact of determining factors. *Expert Rev Gastroenterol Hepatol* 2016; **10**: 827-839 [PMID: 26838962 DOI: 10.1586/17474124.2016.1149061]

11 **Zhao Y**, Lo CM, Liu CL, Fan ST. Use of elderly donors (> 60 years) for liver transplantation. *Asian J Surg* 2004; **27**: 114-119 [PMID: 15140662 DOI: 10.1016/S1015-9584(09)60323-7]

12 **Rodríguez González F**, Jiménez Romero C, Rodríguez Romano D, Loinaz Segurola C, Marqués Medina E, Pérez Saborido B, García García I, Rodríguez Cañete A, Moreno González E. Orthotopic liver transplantation with 100 hepatic allografts from donors over 60 years old. *Transplant Proc* 2002; **34**: 233-234 [PMID: 11959260]

13 **Thorsen T**, Aandahl EM, Bennet W, Olausson M, Ericzon BG, Nowak G, Duraj F, Isoniemi H, Rasmussen A, Karlsen TH, Foss A. Transplantation With Livers From Deceased Donors Older Than 75 Years. *Transplantation* 2015; **99**: 2534-2542 [PMID: 25909464 DOI: 10.1097/TP.0000000000000728]

P- Reviewer: Salvadori M, Sugawara Y **S- Editor**: Cui LJ **L- Editor**: A
E- Editor: Yan JL

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 December 24; 7(6): 329-338

DOI: 10.5500/wjt.v7.i6.329

ISSN 2220-3230 (online)

MINIREVIEWS

Polyoma virus nephropathy in kidney transplantation

Jacob RW Scadden, Adnan Sharif, Kassi Skordilis, Richard Borrows

Jacob RW Scadden, University of Birmingham, Edgbaston, Birmingham B15 2TH, United Kingdom

Adnan Sharif, Richard Borrows, Department of Kidney Transplantation, Queen Elizabeth Hospital Birmingham, Birmingham B15 2TH, United Kingdom

Kassi Skordilis, Department of Renal Histopathology, Queen Elizabeth Hospital Birmingham, Birmingham B15 2TH, United Kingdom

Author contributions: Scadden JRW, Sharif A and Borrows R devised, wrote and edited manuscript; Skordilis K edited manuscript and provided histology.

Conflict-of-interest statement: Dr. Borrows has nothing to disclose.

Manuscript source: Invited manuscript

Correspondence to: Richard Borrows, Consultant Nephrologist, Department of Kidney Transplantation, Queen Elizabeth Hospital Birmingham, Edgbaston, Birmingham B15 2TH, United Kingdom. richard.borrows@uhb.nhs.uk
Telephone: +44-121-3716099
Fax: +44-121-3716098

Received: January 18, 2017
Peer-review started: January 19, 2017
First decision: February 15, 2017
Revised: November 17, 2017
Accepted: December 1, 2017
Article in press: December 4, 2017
Published online: December 24, 2017

Abstract

BK virus (BKV) is a polyomavirus that is able to cause renal dysfunction in transplanted grafts *via* BK virus-associated nephritis (BKVAN). This condition was mis-diagnosed in the past due to clinical and histopthological similarities with acute rejection. Due to the prevalence of the virus in the population, it is an important pathogen in this context, and so it is important to understand how this virus functions and its' relationship with the pathogenesis of BKVN. Screening for BKV often reveals viruria and/or viremia, which then manifests as BKVN, which can be asymptomatic or result in clinical features namely renal dysfunction. The pathogenesis of BKV infection is still unclear and needs to be further investigated; nevertheless there are a variety of hypotheses that indicate that there are a host of factors that play important roles. Treatments for BKVAN include a reduction in immunosuppression, the use of antiviral therapy or the combination of both treatment options.

Key words: Polyoma; Kidney; Transplant; Infection; Virus

Core tip: Prior to its recognition as a separate entity, kidney transplant infection with the polyoma virus, BK virus (BKV), and the ensuing viral nephropathy (BKVN) portended a poor prognosis. But with the advent of heightened clinical suspicion and improved diagnostics the prognosis has improved considerably. Blood and urine polymerase chain reaction testing allows invasive investigation (*i.e.*, transplant biopsy) to be selective and appropriate. Peripheral blood assays of anti-BKV cell mediated immunity offers potential for refining risk stratification. While conventional antiviral agents have failed to show utility to date, reduction of immunosuppression currently represents the most effective and proven treatment for BKVN.

Scadden JRW, Sharif A, Skordilis K, Borrows R. Polyoma virus nephropathy in kidney transplantation. *World J Transplant* 2017; 7(6): 329-338 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i6/329.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i6.329

INTRODUCTION

BK virus (BKV) was first isolated from the urine of a patient with transplant ureteric stenosis[1] in 1971. BKV is a member of the *Polyomaviridae* family, falling into the *Betapolyomavirus* subcategory with JC virus and Simian virus 40 (SV40)[2]. BKV and SV40 have approximately 70% similarity within their respective genomes[3,4]. This similarity between the genomes of these viruses enables SV40 to be a marker for immunohistochemical staining, which is vital in diagnosis of BKV-associated nephropathy (BKVAN)[4]. BKV primary infection occurs in early childhood and is asymptomatic in the majority of cases[5,6]. Transmission of BKV is thought to involve respiratory and oral routes[7], and results in a seroprevalence of 82% in adulthood[8]. BKV is a latent infection, which can lie dormant in tissues, the kidney being the most notable. Heritage *et al*[9] showed that BKV was present in 50% of kidneys based on DNA sequencing of BKV in renal samples. The virus is able to reside in renal tubular epithelial cells and in the uroepithelium. Therefore, another "artificial" route of BKV transmission is through kidney transplantation, particularly on the background of the immunosuppression required in this context to prevent organ rejection. Moreover, although primary infection in non-transplanted patients is generally asymptomatic, viral infection in the context of transplantation and immunosuppression may result in viral replication within epithelial tissues (in this case renal tubules), and the development of inflammation, BKVAN, which resembles other forms of tubule-interstitial nephritis and transplant rejection. If left untreated, these processes progress to result in allograft dysfunction and failure[10]. Indeed, North American and European series suggest that although not the leading overall cause of graft failure, it represents an important and potentially treatable (even preventable) cause in many[11,12]. For many years, BKVAN was mis-diagnosed as rejection, and therefore inappropriately treated, resulting in graft failure in many patients. Nevertheless, greater understanding of BKV has resulted in clinical advances in the field. In this literature review the virology of BKV, the mechanism of BKVAN pathogenesis, and advances in therapeutic strategies will be addressed.

VIROLOGY OF BKV

BKV is a member of the *Polyomavirus* genus of the *Polyomaviridae* family. These viruses are 40-45 nm in diameter[1,13] comprising of an icosahedral capsid surrounding double-stranded DNA, which is able to replicate in the host cell nucleus[14]. The BKV genome contains 5153 base pairs that can be translated bi-directionally[6,15,16]. However, recent analysis of BKV from a kidney transplant patient observed a genome size of 5141 base pairs[17]. The BKV genome is divided into three regions: Early, late and regulatory (non-coding control region or NCCR) regions. The early stage encompasses regulatory proteins such as small tumour antigens (tAg) and large tumour antigens (TAg) as well as late structural capsid proteins - viral protein (VP) 1, VP2, VP3 and the agnoprotein[18]. However, these late proteins are produced after the genome of the virus has be replicated[19]. VP1 is the most common protein found on the outer layer of the capsid and contains a small groove used for host cell receptor binding[20]. The NCCR encodes transcriptional control elements, such as the origin of replication and promoters for genes encoded within the early and late regions[11,21]. BKV enters cells *via* VP1 binding to sialic acid residues of glycoprotein receptors[22,23]. After receptor binding BKV is internalised *via* a caveolae-mediated endocytosis pathway, the virus then travelling to the cell nucleus to launch either a latent or acute infection[13,24,25]. According to Jin *et al*[26], four serotypes (Ⅰ-Ⅳ) of BKV are recognised based on the differences between amino acids 61-83 in the region coding for VP1, with the similarity of this region between different serotypes at 61%-70%[27]. Serotype Ⅰ is the most common within the worldwide population (80%), followed by type Ⅳ (15%)[28]. However, Sharma *et al*[29], using a phylogenetic whole-genome approach, suggested a classification system of BKV which contain serotypes V and Ⅵ.

PATHOGENESIS OF BKVAN

There are many proposed factors relating to the pathogenesis of BKVAN as shown in Figure 1: Source of the viral infection; host cellular immunity to BKV; the influence of immunosuppression; HLA matching; recipient and donor blood group matching.

Source: Donor vs recipient

BKVAN within transplanted kidneys arises from either primary infection from the transplanted kidney itself, or following reactivation from latency in the patient's native urinary tract. Epidemiological work has striven to understand the predominant mechanism in this context. Andrews *et al*[30] were the first to show that recipients of transplants from seropositive donors (either deceased or living), was associated with increased rate of BKV infection within the transplant kidney, thereby suggesting the importance of donor-derived infection in the pathogenesis of BKVAN. This was supported by data from Bohl *et al*[31] who observed BKV infection in 25 of 54 (46%) recipients of kidneys from seropositive donors compared with 4 of 27 (15%) recipients of kidneys from seronegative donors (P = 0.007). These authors also noted that the rate at which BK viruria occurred was faster in patients receiving kidneys from seropositive *vs* seronegative donors (median onset 45 d *vs* 370 d

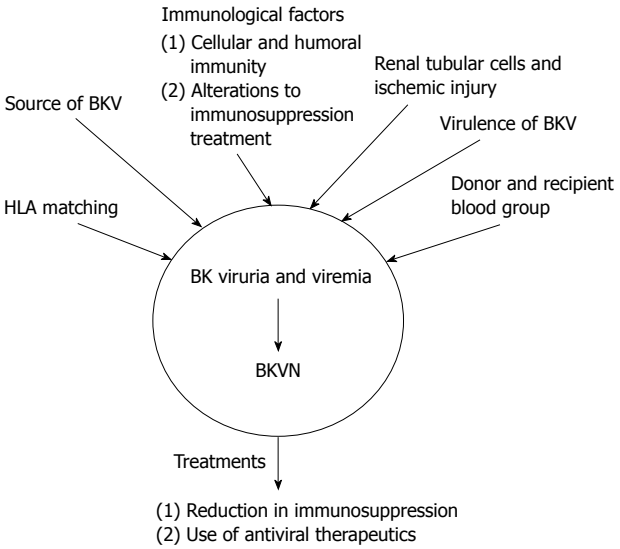


Figure 1 Proposed mechanisms for the pathogenesis of BK virus-associated nephritis after BK virus infection has occurred resulting in BK viruria or BK viremia. These mechanisms include immunological factors, such as alterations to immunosuppressive therapy and cellular and humoral immunity, the source of BKV, either from the recipient or the donor, HLA matching, donor and recipient blood group. The two main treatment options for BKVN are a reduction in immunosuppression and the use of antiviral therapies. These treatments can also be used for BK viruria and viremia in order to prevent progression to BKVAN. BKV: BK virus; BKVAN: BK virus-associated nephritis.

respectively; $P < 0.001$). The duration of BK viruria was also longer in the context of seropositive donors (median duration 157 d *vs* 7 d, $P = 0.009$). The authors also recorded that none of the 27 recipients from seronegative donors developed viremia or sustained viremia, whereas these numbers were 7 and 4 respectively in recipients from seropositive donors. In addition, this study demonstrated donor BKV antibody titre inversely correlated with the time to onset of post-transplant viruria ($P = 0.001$), and was positively correlated with duration of viruria ($P = 0.014$) and peak urine viral titres ($P = 0.005$). These studies did not evaluate the importance of recipient BKV serostatus, but this was done in a study of paediatric recipients[32], which suggested the importance of recipient BK seronegativity. In this study, all patients developed BKV viruria, with recipient seronegativity strongly associated with the development of nephropathy ($P = 0.01$). Finally, Saundh *et al*[33] studied 112 renal transplant patients before and after transplantation, and conducted a phylogenetic analysis of VP1 sequences and serotypes. Twelve patients developed BKV viremia, and 8 had a sufficiently high viral load to allow amplification of VP1. Based on this analysis the authors concluded that donor-derived infection was responsible for the majority of cases of BKV infection. A single patient had two differing VP1 subgroups present (Ib-1 after 6 mo followed by Ia after 12 mo post transplantation), perhaps suggesting a potential switch between donor and recipient strains, and means that cases of BKV infection due to reactivation from the recipient may be a real phenomenon. However, the burden of evidence from this, and the other aforementioned studies, is that donor-derived infection represents the major risk confronting the kidney transplant recipient.

Cellular and humoral immunity

Humoral and cellular immunity is thought to be implicated with the pathogenesis of BKVAN, and both $CD8^+$ and $CD4^+$ T cells are involved in the recognition and clearance of viruses such as BKV. The lack of BKV specific IgG may be important in the development of BKVAN[12]. As mentioned above, there is a greater risk for patients that are BKV seronegative at the time of their transplant, as they will have no BKV specific antibody; patients with previous exposure and who have developed immunity to BKV, may not develop the infection[34]. Yet the presence of a BKV-specific antibody response is clearly not protective, and it is likely that cellular immunity plays the central role in viral control. Certainly, patients with BKV specific antibodies remain at risk of developing BKVAN[35]. Comoli *et al*[36] showed that patients that had BKVAN had fewer BKV-specific lymphocytes that secreted interferon-γ (IFN-γ), with the mean frequency of BKV specific, IFN-γ being 151×10^6 cells. This was approximately 10 times less than other viruses related to transplantation, such as EBV. The researchers concluded that, based on their data that there is reduced BKV immunity, which in turn would increase the rate of active BKV infection.

Immunosuppression burden

Immunosuppression is required to ameliorate rejection of the transplanted kidney by the host immune system. However, with developments in immunosuppressive drugs such as mycophenolate mofetil (MMF) and tacrolimus (Tac), the reduction in rejection rate has been inversely paralleled by an increased incidence of BKV infection. Calcineurin inhibitor (CNI) based therapies have been shown to increase the risk of BKVAN and subsequent nephrotoxicity following renal transplant[37]. However, a recent study by Jacobi *et al*[38] observed no significant change in the number of patients with BKV infection ($n = 352$) when using CNI of either tacrolimus or cyclosporine A (CyA). This led to the conclusion that CNI immunosuppressants were not associated in BK viremia.

Mengel *et al*[39] suggested particularly increased risk of BKV nephropathy with a combination of Tac and MMF. Similarly, Brennan *et al*[40] showed that viruria was most common with a drug combination of Tac-MMF (46%) and lowest with cyclosporine-MMF (13%). These authors also confirmed the association between viruria and subsequent BKV viremia. In addition, when surveillance for BK viremia was undertaken for the purposes of this study, a reduction in immunosuppression in response to detectable viremia resulted in reductions in viral load in 95% of patients, and without increased risk of rejection. A more recent randomized study also supports the role of immunosuppression in this context. In a study of 682 patients, the combination of Tac and MMF was associated with greater rates of viremia at 6

and 12 mo post-transplantation than the combination of cyclosporine and MMF (16.3% *vs* 10.6%, P = 0.048 and 12.1% *vs* 4.8%, P = 0.004 respectively). Cumulative steroid dose up to month 3 was also a risk factor for viremia in this study, highlighting the role of overall immunosuppression burden in this disease[41]. Clinical observations of campth therapy were made by Kayler *et al*[42], they administered campth to two patients with BKV viruria and one with nephropathy. In all cases, there was increased viral replication and one of the patients with viruria developed nephropathy. The authors concluded that campth treatment does not permanently remove immune cells that are able to respond against BKV and that the therapy does not prevent stop the clearance of BKV from the blood.

Recent attention has focused on the role of lytic antibody induction, and particularly the role of alemtuzumab (anti-CD52 monoclonal antibody; "Campath") which is undergoing more recent widespread usage. A large study ("3C study") demonstrated double the incidence of BKV infection with alemtuzumab compared with basiliximab induction. However, absolute incidence was low (8% *vs* 4%), and this difference was driven by BK viremia (7% *vs* 3%) rather than BKV nephropathy, the rates of which were very low in both alemtuzumab and basiliximab arms of the study (1% and 2% respectively)[43]. Conversely, it has been shown that alemtuzumab did not remain significant risk factor after the adjusted hazard ratio for each variable had been calculated[44].

A United States OPTN database review showed that there was an increased risk of BKV infection with an induction therapy using thymoglobulin (P < 0.0001)[44]. In a study by Ott *et al*[45] renal transplants patients, under either basiliximab (n = 22) or thymoglobulin (n = 27) treatment regimens, were assessed for complication in a mean follow up period of three years. Of the 27 patients treated with thymoglobulin, two developed BKVAN whereas no patients had a BKV infection when treated with basiliximab. However, CMV infections were observed in both patient cohorts, with four and three patients infected for basiliximab and thymoglobulin therapies, respectively. This indicated that treatments using thymoglobulin carry a greater risk of BKV infection to renal transplant patients post transplant.

Effect of HLA matching

The adaptive immune response to viral infection is dependent on T-cell recognition of viral antigen presented in the context of self-MHC. In other transplant settings, it has been shown that immune responses to viral antigen presented in the context of donor-derived MHC (in this case cytomegalovirus) do not develop[46]. The donor-derived nature of BKV may therefore impair the magnitude or timing of effective immune clearance. In keeping with this concept, a study by Lee *et al*[47] used a mouse model to show ineffective clearance of BKV in the context of MHC mismatching. Clinical data also supports this concept of increased HLA mismatch as a risk factor for BKVAN[48-50]. However, in contrast, a clinical study from Drachenberg *et al*[51] found lesser degrees of HLA matching was actually associated with maintained graft function in patients with established BKVAN. This led to the proposal that even though reduction in HLA matching would decrease the recipient's ability to mount an effective immune response to BKV, there would be less tissue damage, thus reduced risk of graft loss. This in turn raises questions in regard to the mechanism of viral clearance in this context, and whether this is dependent on the T-cell response or other viral elimination mechanisms such as NK and NKT cell activation. Clinical data from other cohorts may also serve to clarify the current understanding.

Aside from HLA matching, the question arises as to whether BKV infection and nephropathy is associated with specific (donor or recipient) HLA alleles. Awadalla *et al*[50] found no such association(s), but another study from Bohl *et al*[31] found a possible link between HLA C7 and the severity of BKV infection. Although there was no association between BK viremia and either donor or recipient HLA-A, -B or -DR type, all 11 transplant recipients with persistent BK viremia received kidneys from HLA C7 negative donors, and 10 of these 11 recipients also lacked HLA C7. The possible mechanism underlying this observation is unclear. However, if confirmed, this raises implications for the relevance of HLA-C typing in transplantation, which is not currently recommended or undertaken, but which may identify individuals at greater risk of refractory infection.

Donor and recipient blood group

A fundamental feature of transplantation is the matching of blood groups of donor and recipient in order to avoid the risk of hyper-acute rejection. Nevertheless, with intensified preconditioning and antibody removal, blood group-incompatible transplantation is now commonplace[52]. However, Sharif *et al*[53] suggest a high rate of BKVAN in such patients. In a study of 62 blood group incompatible transplantations between 1998 and 2010, the risk of BKVAN was 17.7% (compared to 3% risk among blood group compatible patients). This data has been replicated in a different cohort by Bentall *et al*[54]. While we may infer this is due to desensitization and/or heightened immunosuppression for incompatible patients, the authors actually observed a lower risk among a cotemporaneous group of 221 HLA antibody incompatible transplants (5.9%, P = 0.008) who also underwent intensified preconditioning and received stronger induction therapy (ATG) compared to either blood group incompatible or compatible patients (Basiliximab). Therefore, pre-conditioning or heightened immunosuppression cannot be the sole explanation for this observation. Interestingly, the authors identified the lack of a typical accommodation-like phenotype (defined as C4d deposition in the absence of any micro-circulation inflammation) among blood-group incompatible transplant recipients with BKVAN

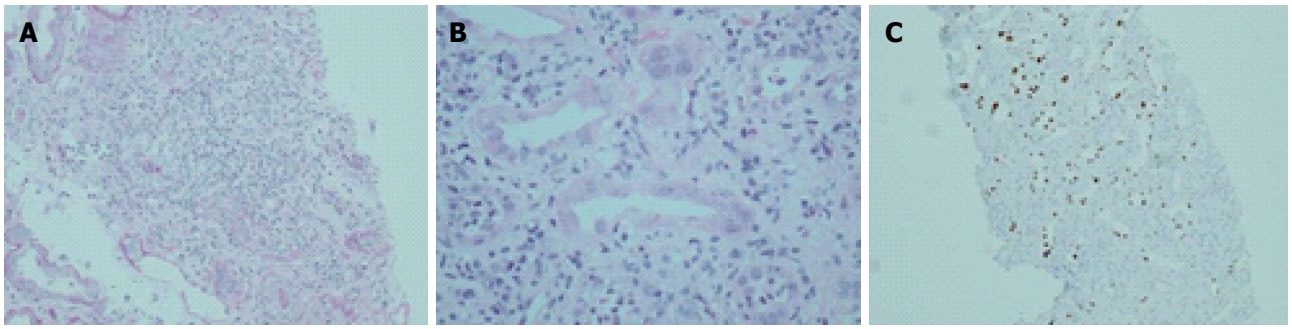


Figure 2 Histological features of BK virus nephropathy by light microscopy. A: Tubule-interstitial infiltrate and tubulitis classical for BKVN, but also compatible with any other form of interstitial nephritis such as acute cellular rejection; B: Higher power view of same biopsy sample, with characteristic viral inclusions seen within epithelial cells (circled); C: Positive SV40 immunoperoxidase staining on same specimen, confirming diagnosis of BKVN. BKVN: BK virus nephropathy.

compared to those without BKVAN (40.0% *vs* 75.8% respectively, $P = 0.04$). However, it is unclear from this data whether lack of accommodation-like phenotype development increases the risk for BKVAN or whether blood-group incompatible patients with BKVAN lose their accommodation-like phenotype but further studies are warranted to research this further.

OTHER RISK FACTORS

There are also a number of risk factors between the donor and recipient that can increase the risk of a BKV infection, including gender, race, age, diabetes mellitus or where the organ was sourced from a deceased donor[39,44,55,56].

CLINICAL FEATURES AND DIAGNOSIS OF BKVAN

The median time to clinically apparent BKVAN is within the first year after transplantation[57,58]. The recipient is characteristically asymptomatic, with the infection presenting as progressively worsening renal function, usually in the absence of significant or new-onset proteinuria[59]. This presentation generally results in a "for cause" biopsy, which shows the characteristic features of BKVAN. A number of histological grading systems have attempted to classify BKVAN[60-63], and whilst differences exist between these alternative systems, recurring themes are:

The separation into stages of BKVAN depending on the presence of viral infection in the absence of inflammation or significant chronic damage (Grade A), with inflammation dominating over chronic damage (Grade B), and with chronic damage (fibrosis and tubular atrophy) as a notable component, with or without inflammation (Grade C).

That prognosis is correlated with these stages of BKVAN, and especially with the presence of significant chronic damage (Grade C nephropathy).

In simultaneous biopsy cores, discordant findings (*i.e.*, the lack of evidence of BKVAN in one of the cores) was found in around a third of cases. Of note, in the core without evidence of virus, interstitial inflammation and/or acute tubular injury were frequent findings

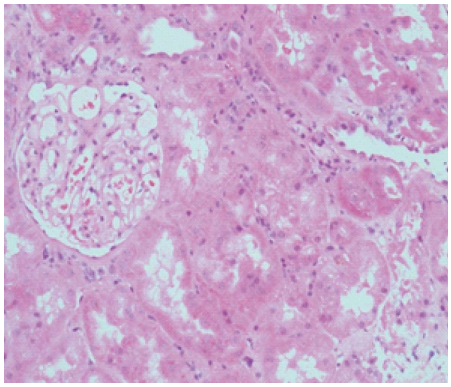


Figure 3 Kidney with preserved tubular architecture, without significant chronic damage or interstitial inflammation, but with BK virus nephropathy confirmed by virtue of positive SV40 staining (as shown in insert taken from immunoperoxidase sample from same biopsy specimen).

(approximately 80%), raising implications for the clinical interpretation of kidney biopsy specimens where only one core is retrieved, where the sampling is inadequate, and where there is collateral evidence that BKVAN may be a diagnosis[64].

Whilst concurrent viremia is almost universal with the finding of BKVAN on microscopy, the magnitude of circulating viral load seems to have little or no relationship with the extent of nephropathy[64]. Representative examples of the histological appearances of BKVAN are shown in Figures 2-4.

For many years, BKVAN was confused with acute rejection, as both have the appearance of an "interstitial nephritis". With more widespread recognition of BKVAN, the availability of blood and urine testing for viral load, and the utility of SV40 staining on biopsy samples, the pathological diagnosis of BKVAN has become more straightforward. However, acute rejection and BKVN are not mutually exclusive, and particularly in the period following BKVAN treatment (see below), the two may coincide, and it may be unclear which represents the dominant process. Despite efforts, there remains no consensus in regard to the most accurate way to separate these entities, although the presence of macro- or micro-vascular inflammation points to

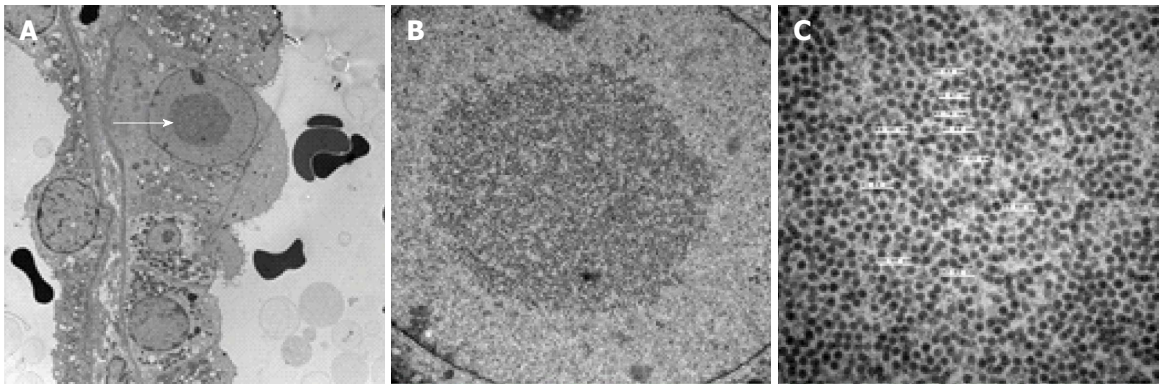


Figure 4 Histological features of BK virus nephropathy by electron microscopy. A: Electron microscopy evidence of viral inclusions (arrow) within epithelial cells, equivalent to those seen and circled in the light microscopy sample shown in Figure 2B; B: Higher power magnification of epithelial viral inclusions; C: Highest magnification demonstrating characteristic appearance and size (labelled) of BK virions.

rejection as a component at least.

Aside from the classical presentation described above, BKVAN may present in a "subclinical" manner, as is seen with other forms of transplant-related renal injury including "subclinical rejection". Recent clinical studies have used protocol renal biopsies to test for the presence of BKV in this setting. Buehrig *et al*[65] concluded that allograft biopsy allowed earlier detection of BKVAN, and the potential for this to enable earlier treatment, although this proposed approach has not yet been evaluated. Whether such a strategy translates into improved overall clinical outcome (and justifies the risk, inconvenience and cost of the biopsy) remains to be seen.

SCREENING FOR BKV INFECTION

Established BKV screening methods include testing urine for decoy cells, viral particles by electron microscopy, and viral DNA by PCR. However, plasma PCR for detection of viremia remains a more common approach to screening[66]. It has also been suggested that circulating viral loads above certain thresholds (approximately > 4 log copies/mL) can be considered presumptive of nephropathy even in the absence of histological evidence (see above). Whilst inter-laboratory standardization of such PCR assays is awaited, such discrete values remain subject to interpretation by individual centres.

A more recent study carried out by Singh *et al*[67] investigated whether the qualitative detection of three-dimensional aggregates of polyomavirus (Haufen crystals) within a patient's urine could be used as a diagnostic test for patients BKVAN. Of 21 patients known to have BKVAN 77 of the 143 samples taken contained Haufen. During follow up, the presence or absence of Haufen matched the course of renal disease. All control samples (194) were negative. The predictive values of Haufen for BKVAN were 97% for positive and 100% for negative. This leads to the conclusion that Haufen testing in urine is a more accurate approach than detection of viral DNA in urine or plasma, although the reproducibility and generalizability of these findings requires further clarification.

Finally, the role of urine profiling for VP1 (BK capsid protein) mRNA is under investigation, with exploratory[68] and validation studies[69] suggesting potential utility in predicting nephropathy, albeit in small patient numbers Further generalisation may yet provide additional important information in this regard.

CURRENT AND FUTURE TREATMENTS FOR BKV INFECTION

The first step in the treatment of BKV infection is reduction in immunosuppression. Certainly, this approach is not disputed for cases of nephropathy, although evidence guiding the order with which the component immunosuppressant are withdrawn is lacking. Less clear is whether immunosuppression should be altered in the face of viremia and in the absence of overt nephropathy. This question was addressed in a large and important study of 200 patients by Brennan *et al*[40], where reduction in immunosuppression in response to detectable viremia on protocolised plasma samples resolved 95% cases of viremia with no signal towards graft rejection, dysfunction or loss. Other smaller studies also support this approach of "pre-emptive" therapy[70]. The efficacy of this strategy is also supported by Saad *et al*[71]. In this study, MMF and/or Tac doses were reduced for the patients, who in this study were not restricted to those with viremia (24 patients: 66% BKVN, 34% Viremia). Overall, a decline in BKV viral load was seen. However, three patients developed acute cellular rejection, albeit with successful treatment with intravenous bolus steroids. One patient experienced BKVN relapse during pregnancy and lost the graft. Seventeen patients maintained or improved their graft function following this reduction in immunosuppression. In summary, the evidence from these studies support decreasing immunosuppression as the first line of treatment for patients that present with BKVAN, and possibly with detectable viremia, although clearly the risk of rejection needs careful consideration. Controlled studies are required to solidify these findings, although

in the meantime the recommendation from Kidney Disease: Improving Global Outcomes (KDIGO) expert panel is to reduce immunosuppression when plasma viral loads exceed a certain threshold (10000 copies per mL, whilst accounting for inter-laboratory variation)[72].

BKV interacts with the AKT/mammalian target of rapamycin (mTOR) pathway[73]. Everolimus and sirolimus are examples of mTOR inhibitors (mTOR-i)[73-76]. Everolimus was observed by Polanco *et al*[75] to increase renal function in BKVAN positive patients that had their treatments converted from tacrolimus to everolimus, with a suspension of mycophenolate. This study involved 15 patients, all presenting with BKVAN of which 9 underwent the immunosuppressant conversation. The serum creatinine of these patients decreased from 2 (± 0.21) mg/dL at the time of conversion to 1.6 (± 0.39) mg/dL at the final follow up. BK viremia became negative in 5 of the 9 patients and the remain 4 had a > 95% decrease in BKV. This decrease in BKVAN is also seen in conversion to sirolimus. In a recent single centre retrospective study by Tohme *et al*[77], patients were either placed on a tacrolimus or sirolimus based immunosuppression therapy. If the patients were < 62 years old they were converted from tacrolimus to sirolimus. Clinically significant BK viremia fell when converting from tacrolimus (P = 0.04) to sirolimus (P = 0.02), 17.9% to 4.3%, respectively. However, the hazard ratio for the male gender was also associated with the incidence of BK viremia (P = 0.03). Discontinuation of the sirolimus treatment occurred in 34% of patients due to various side effects. Thus, the use of mTOR-i as a treatment option of not only provides immunosuppression, reducing the risk of acute rejection, but also due to its behaviour as a metabolic pathway inhibitor for BKV it can also aid in the reduction in viral load, hence a lower risk of developing BKVAN.

The next question is whether detection of virus in urine (rather than waiting for it to appear in plasma) might represent a more efficient screening and intervention biomarker. In this regard, the clinical data is less optimistic. Specifically a series of retrospective studies have suggested increased rates of (or episodes of) acute rejection in the presence of viruria, even in the absence of viremia[78-80]. Whilst a proportion of these episodes were likely a response to immunosuppression weaning, there were clearly others which were unrelated, and which may potentially be a manifestation of low grade viral reactivation and inflammation inciting a secondary alloimmune response. However, irrespective of the mechanism, these observations (although limited by study design and interpretation) suggest that immunosuppression weaning in the context of viruria should not be recommended until and unless further information comes to light.

Even with successful treatment with immunosuppression reduction, the timeline of viral clearance is variable, although the reported median time to complete plasma clearance is 9 mo[81]. Serial renal histopathology following treatment is interesting, although reports are limited due to the nature of such studies. Of relevance though, the report from Menter *et al*[81] suggests that a self-limited "interstitial nephritis" is common during the phase of viral clearance and that this may represent an appropriate antiviral response rather than alloimmunity.

Specific antiviral therapy is generally used as a secondary line of treatment for BKVAN, and although an attractive approach, the role(s) of multiple agents remain unproven and unclear. Although better recognized as antibacterial agents, the quinolone antibiotics do display *in vitro* activity against polyoma viruses. Arroyo *et al*[82] retrospectively investigated the effects of ciprofloxacin on patients with BK viruria and viremia, after clinical failure with prior reduction in immunosuppression. The study showed that there were no adverse effects of ciprofloxacin and that out of the nine patients that received the treatment, three showed complete clearance of the virus and another three had the viral load in the plasma reduced by ⩾ 50%. Unfortunately, a subsequent randomized controlled trial of 3 mo levofloxacin (from post-operative day 5) in 154 kidney transplant recipients showed no effect on the development of BKV viruria compared with the control group (29% *vs* 33%)[83]. In addition, an increased incidence of antibiotic resistance to bacterial isolates, and also a signal towards increased tendonitis was seen in the levofloxacin treated arm. Observational data also comes from Jung *et al*[84], this time studying the effect of leflunomide on biopsy-proven BKVN in paediatric patients. Tac dosage was reduced and leflunomide and intravenous immunoglobulin treatment was instituted. Viral load then decreased and remained below 100 copies/mL over an 18 mo period with no loss of renal function, from a value of 474140 copies/mL of BKV viral load in patient serum. Intravenous immunoglobulin in the absence of adjunctive antiviral agents has also been reported as a treatment for BKV infection[85], and observational data supports a potential role for another antiviral agent, cidofovir[86]. Yet in the absence of more robust data, few conclusions can be drawn; it is also relevant to highlight the conclusion of a 2010 systematic review, which found no evidence of an effect for either leflunomide or cidofovir in treating this infection[87]. Adoptive cell therapy in the context of transplant-associated infections is perhaps best known in the context of EBV and post-transplant lymphoproliferative disease. Whilst no data exists for this strategy in the setting of BKV infection, it is possible this approach might hold promise. In the context of infection with the related polyoma virus, JC virus, a report describes a positive clinical response to this form of therapy in a patient following hematopoietic cell transplantation[88]. Intuitively, the same approach may be worthwhile for BKV infection.

CONCLUSION

This review focuses on the pathogenesis, risk factors, presentation and treatment of BKV infection in the setting of kidney transplantation, which remains clearly the most common scenario in which this polyoma virus

infection is encountered. Whilst important understanding has accumulated over recent years, and has certainly led to improved recognition of this infection and clinical management of patients, there is much more to be discovered and studied. We believe the most important tasks at hand are now to: (1) more accurately risk-stratify patients prior to (and also following) transplantation, with aim of individualizing immunosuppression and reducing the risk of (or duration/consequences of) BKV infection. This may include developing understanding of, and then monitoring strategies for, cell-mediated immune responses to this virus, which can then be interpreted in combination with peripheral blood and renal biopsy measures of viral load and (admittedly currently unavailable) standardised assays of alloreactivity to garner a more "holistic" understanding of the overall and antigen-specific immunosuppressive burden; and (2) to enhance the sizeable observational experience of treatment strategies with controlled studies of immunosuppression weaning and/or adjunctive antiviral agents. It is not unconceivable that with such refined approaches BKV infection (whilst not eradicated) may present a far less sinister complication for kidney transplant patients in the future.

REFERENCES

1 **Gardner SD**, Field AM, Coleman DV, Hulme B. New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet* 1971; **1**: 1253-1257 [PMID: 4104714 DOI: 10.1016/S0140-6736(71)91776-4]

2 **Shah KV**. Polyomaviruses. In: Fields BN, Knipe DM, eds. Fields Virology. 2nd ed. Philadelphia: Lippincott-Raven, 1996: 2027-2043

3 **Butel JS**, Lednicky JA. Cell and molecular biology of simian virus 40: implications for human infections and disease. *J Natl Cancer Inst* 1999; **91**: 119-134 [PMID: 9923853 DOI: 10.1093/jnci/91.2.119]

4 **Li RM**, Mannon RB, Kleiner D, Tsokos M, Bynum M, Kirk AD, Kopp JB. BK virus and SV40 co-infection in polyomavirus nephropathy. *Transplantation* 2002; **74**: 1497-1504 [PMID: 12490781 DOI: 10.1097/01.TP.0000042485.92082.5B]

5 **Shah KV**. Human polyomavirus BKV and renal disease. *Nephrol Dial Transplant* 2000; **15**: 754-755 [PMID: 10831621 DOI: 10.1093/ndt/15.6.754]

6 **Bohl DL**, Brennan DC. BK virus nephropathy and kidney transplantation. *Clin J Am Soc Nephrol* 2007; **2** Suppl 1: S36-S46 [PMID: 17699509 DOI: 10.2215/CJN.00920207]

7 **Imperiale MJ,** Major EO. Polyomaviruses. In: Fields BN, Knipe DM, Howley PM. Fields Virology. 5th ed. Philadelphia: Lippincott-Raven, 2007: 2175-2195

8 **Egli A**, Infanti L, Dumoulin A, Buser A, Samaridis J, Stebler C, Gosert R, Hirsch HH. Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. *J Infect Dis* 2009; **199**: 837-846 [PMID: 19434930 DOI: 10.1086/597126]

9 **Heritage J**, Chesters PM, McCance DJ. The persistence of papovavirus BK DNA sequences in normal human renal tissue. *J Med Virol* 1981; **8**: 143-150 [PMID: 6271922 DOI: 10.1002/jmv.1890080208]

10 **Sawinski D**, Goral S. BK virus infection: an update on diagnosis and treatment. *Nephrol Dial Transplant* 2015; **30**: 209-217 [PMID: 24574543 DOI: 10.1093/ndt/gfu023]

11 **El-Zoghby ZM**, Stegall MD, Lager DJ, Kremers WK, Amer H, Gloor JM, Cosio FG. Identifying specific causes of kidney allograft loss. *Am J Transplant* 2009; **9**: 527-535 [PMID: 19191769 DOI: 10.1111/j.1600-6143.2008.02519.x]

12 **Chand S**, Atkinson D, Collins C, Briggs D, Ball S, Sharif A, Skordilis K, Vydianath B, Neil D, Borrows R. The Spectrum of Renal Allograft Failure. *PLoS One* 2016; **11**: e0162278 [PMID: 27649571 DOI: 10.1371/journal.pone.0162278]

13 **Hirsch HH**, Steiger J. Polyomavirus BK. *Lancet Infect Dis* 2003; **3**: 611-623 [PMID: 14522260 DOI: 10.1016/S1473-3099(03)00770-9]

14 **Hariharan S**. BK virus nephritis after renal transplantation. *Kidney Int* 2006; **69**: 655-662 [PMID: 16395271 DOI: 10.1038/sj.ki.5000040]

15 **Garces JC**. BK Virus-Associated Nephropathy in Kidney Transplant Recipients. *Ochsner J* 2010; **10**: 245-249 [PMID: 21603392]

16 **Henriksen S**, Mittelholzer C, Gosert R, Hirsch HH, Rinaldo CH. Human BK Polyomavirus Plasmid pBKV (34-2) (Dunlop) Contains Mutations Not Found in the Originally Published Sequences. *Genome Announc* 2015; **3**: pii e00046-15 [PMID: 25814590 DOI: 10.1128/genomeA.00046-15]

17 **Ranjan R**, Rani A, Brennan DC, Finn PW, Perkins DL. Complete Genome Sequence of BK Polyomavirus Subtype Ib-1 Detected in a Kidney Transplant Patient with BK Viremia Using Shotgun Sequencing. *Genome Announc* 2017; **5**: pii e01474-16 [PMID: 28183780 DOI: 10.1128/genomeA.01474-16]

18 **Leuenberger D**, Andresen PA, Gosert R, Binggeli S, Ström EH, Bodaghi S, Rinaldo CH, Hirsch HH. Human polyomavirus type 1 (BK virus) agnoprotein is abundantly expressed but immunologically ignored. *Clin Vaccine Immunol* 2007; **14**: 959-968 [PMID: 17538118 DOI: 10.1128/CVI.00123-07]

19 **Barbanti-Brodano G**, Sabbioni S, Martini F, Negrini M, Corallini A, Tognon M. BK virus, JC virus and Simian Virus 40 infection in humans, and association with human tumors. *Adv Exp Med Biol* 2006; **577**: 319-341 [PMID: 16626046 DOI: 10.1007/0-387-32957-9_23]

20 **Anzivino E**, Bellizzi A, Mitterhofer AP, Tinti F, Barile M, Colosimo MT, Fioriti D, Mischitelli M, Chiarini F, Ferretti G, Taliani G, Pietropaolo V. Early monitoring of the human polyomavirus BK replication and sequencing analysis in a cohort of adult kidney transplant patients treated with basiliximab. *Virol J* 2011; **8**: 407 [PMID: 21849069 DOI: 10.1186/1743-422X-8-407]

21 **Mischitelli M**, Bellizzi A, Anzivino E, Fioriti D, Boldorini R, Miglio U, Chiarini F, Di Monaco F, Pietropaolo V. Complications post renal transplantation: literature focus on BK virus nephropathy and diagnostic tools actually available. *Virol J* 2008; **5**: 38 [PMID: 18315864 DOI: 10.1186/1743-422X-5-38]

22 **ugan AS**, Eash S, Atwood WJ. An N-linked glycoprotein with alpha(2,3)-linked sialic acid is a receptor for BK virus. *J Virol* 2005; **79**: 14442-14445 [PMID: 16254379 DOI: 10.1128/JVI.79.22.14442-14445.2005]

23 **Low JA**, Magnuson B, Tsai B, Imperiale MJ. Identification of gangliosides GD1b and GT1b as receptors for BK virus. *J Virol* 2006; **80**: 1361-1366 [PMID: 16415013 DOI: 10.1128/JVI.80.3.1361-1366.2006]

24 **Moriyama T**, Marquez JP, Wakatsuki T, Sorokin A. Caveolar endocytosis is critical for BK virus infection of human renal proximal tubular epithelial cells. *J Virol* 2007; **81**: 8552-8562 [PMID: 17553887 DOI: 10.1128/JVI.00924-07]

25 **Eash S**, Querbes W, Atwood WJ. Infection of vero cells by BK virus is dependent on caveolae. *J Virol* 2004; **78**: 11583-11590 [PMID: 15479799 DOI: 10.1128/JVI.78.21.11583-11590.2004]

26 **Jin L**, Gibson PE, Knowles WA, Clewley JP. BK virus antigenic variants: sequence analysis within the capsid VP1 epitope. *J Med Virol* 1993; **39**: 50-56 [PMID: 7678637 DOI: 10.1002/jmv.1890390110]

27 **Knowles WA**, Gibson PE, Gardner SD. Serological typing scheme for BK-like isolates of human polyomavirus. *J Med Virol* 1989; **28**: 118-123 [PMID: 2544676 DOI: 10.1002/jmv.1890280212]

28 **Takasaka T**, Goya N, Tokumoto T, Tanabe K, Toma H, Ogawa Y, Hokama S, Momose A, Funyu T, Fujioka T, Omori S, Akiyama H, Chen Q, Zheng HY, Ohta N, Kitamura T, Yogo Y. Subtypes of BK virus prevalent in Japan and variation in their transcriptional control region. *J Gen Virol* 2004; **85**: 2821-2827 [PMID: 15448343 DOI: 10.1099/vir.0.80363-0]

29 **Sharma PM**, Gupta G, Vats A, Shapiro R, Randhawa P. Phylogenetic analysis of polyomavirus BK sequences. *J Virol* 2006; **80**: 8869-8879 [PMID: 16940499 DOI: 10.1128/JVI.00510-06]

30 **Andrews CA**, Shah KV, Daniel RW, Hirsch MS, Rubin RH. A serological investigation of BK virus and JC virus infections in recipients of renal allografts. *J Infect Dis* 1988; **158**: 176-181 [PMID: 2839580 DOI: 10.1093/infdis/158.1.176]

31 **Bohl DL**, Storch GA, Ryschkewitsch C, Gaudreault-Keener M, Schnitzler MA, Major EO, Brennan DC. Donor origin of BK virus in renal transplantation and role of HLA C7 in susceptibility to sustained BK viremia. *Am J Transplant* 2005; **5**: 2213-2221 [PMID: 16095500 DOI: 10.1111/j.1600-6143.2005.01000.x]

32 **Smith JM**, McDonald RA, Finn LS, Healey PJ, Davis CL, Limaye AP. Polyomavirus nephropathy in pediatric kidney transplant recipients. *Am J Transplant* 2004; **4**: 2109-2117 [PMID: 15575916 DOI: 10.1111/j.1600-6143.2004.00629.x]

33 **Saundh BK**, Baker R, Harris M, Welberry Smith MP, Cherukuri A, Hale A. Early BK polyomavirus (BKV) reactivation in donor kidney is a risk factor for development of BKV-associated nephropathy. *J Infect Dis* 2013; **207**: 137-141 [PMID: 23100561 DOI: 10.1093/infdis/ji642]

34 **Beimler J**, Sommerer C, Zeier M. The influence of immunosuppression on the development of BK virus nephropathy-- does it matter? *Nephrol Dial Transplant* 2007; **22** Suppl 8: viii66-viii71 [PMID: 17890267 DOI: 10.1093/ndt/gfm646]

35 **Comoli P**, Cioni M, Basso S, Gagliardone C, Potenza L, Verrina E, Luppi M, Zecca M, Ghiggeri GM, Ginevri F. Immunity to Polyomavirus BK Infection: Immune Monitoring to Regulate the Balance between Risk of BKV Nephropathy and Induction of Alloimmunity. *Clin Dev Immunol* 2013; **2013**: 256923 [PMID: 24000288 DOI: 10.1155/2013/256923]

36 **Comoli P**, Azzi A, Maccario R, Basso S, Botti G, Basile G, Fontana I, Labirio M, Cometa A, Poli F, Perfumo F, Locatelli F, Ginevri F. Polyomavirus BK-specific immunity after kidney transplantation. *Transplantation* 2004; **78**: 1229-1232 [PMID: 15502726 DOI: 10.1097/01.TP.0000137932.44791.D3]

37 **Kuppachi S**, Kaur D, Holanda DG, Thomas CP. BK polyoma virus infection and renal disease in non-renal solid organ transplantation. *Clin Kidney J* 2016; **9**: 310-318 [PMID: 26985385 DOI: 10.1093/ckj/sfv143]

38 **Jacobi J**, Prignitz A, Büttner M, Korn K, Weidemann A, Hilgers KF, Heller K, Velden J, Knöll A, Wullich B, May C, Eckardt KU, Amann KU. BK viremia and polyomavirus nephropathy in 352 kidney transplants; risk factors and potential role of mTOR inhibition. *BMC Nephrol* 2013; **14**: 207 [PMID: 24088187 DOI: 10.1186/1471-2369-14-207]

39 **Mengel M**, Marwedel M, Radermacher J, Eden G, Schwarz A, Haller H, Kreipe H. Incidence of polyomavirus-nephropathy in renal allografts: influence of modern immunosuppressive drugs. *Nephrol Dial Transplant* 2003; **18**: 1190-1196 [PMID: 12748354 DOI: 10.1093/ndt/gfg072]

40 **Brennan DC**, Agha I, Bohl DL, Schnitzler MA, Hardinger KL, Lockwood M, Torrence S, Schuessler R, Roby T, Gaudreault-Keener M, Storch GA. Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. *Am J Transplant* 2005; **5**: 582-594 [PMID: 15707414 DOI: 10.1111/j.1600-6143.2005.00742.x]

41 **Hirsch HH**, Vincenti F, Friman S, Tuncer M, Citterio F, Wiecek A, Scheuermann EH, Klinger M, Russ G, Pescovitz MD, Prestele H. Polyomavirus BK replication in de novo kidney transplant patients receiving tacrolimus or cyclosporine: a prospective, randomized, multicenter study. *Am J Transplant* 2013; **13**: 136-145 [PMID: 23137180 DOI: 10.1111/j.1600-6143.2012.04320.x]

42 **Kayler LK**, Mohanka R, Morgan C, Basu A, Shapiro R, Randhawa PS. Clinical course of kidney transplant patients with acute rejection and BK virus replication following Campath therapy. *Clin Transplant* 2008; **22**: 348-353 [PMID: 18279421 DOI: 10.1111/j.1399-0012.2008.00791.x]

43 **3C Study Collaborative Group**, Haynes R, Harden P, Judge P, Blackwell L, Emberson J, Landray MJ, Baigent C, Friend PJ. Alemtuzumab-based induction treatment versus basiliximab-based induction treatment in kidney transplantation (the 3C Study): a randomised trial. *Lancet* 2014; **384**: 1684-1690 [PMID: 25078310 DOI: 10.1016/S0140-6736(14)61095-3]

44 **Dharnidharka VR**, Cherikh WS, Abbott KC. An OPTN analysis of national registry data on treatment of BK virus allograft nephropathy in the United States. *Transplantation* 2009; **87**: 1019-1026 [PMID: 19352121 DOI: 10.1097/TP.0b013e31819cc383]

45 **Ott U**, Busch M, Steiner T, Schubert J, Wolf G. Renal retransplantation: a retrospective monocentric study. *Transplant Proc* 2008; **40**: 1345-1348 [PMID: 18589102 DOI: 10.1016/j.transproceed.2008.01.068]

46 **Egli A**, Binggeli S, Bodaghi S, Dumoulin A, Funk GA, Khanna N, Leuenberger D, Gosert R, Hirsch HH. Cytomegalovirus and polyomavirus BK posttransplant. *Nephrol Dial Transplant* 2007; **22** Suppl 8: viii72-viii82 [PMID: 17890268 DOI: 10.1093/ndt/gfm648]

47 **Lee EDH**, Wang J, Dong Y, Lukacher AE, Newell KA, Pearson TC, Larsen CP. Inability to clear polyoma virus in MHC disparate murine kidney transplant model results in accelerated graft rejection [Abstract]. *Am J Kidney Dis* 2004; **4**: 599

48 **Hirsch HH**, Knowles W, Dickenmann M, Passweg J, Klimkait T, Mihatsch MJ, Steiger J. Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. *N Engl J Med* 2002; **347**: 488-496 [PMID: 12181403 DOI: 10.1056/NEJMoa020439]

49 **Masutani K**, Ninomiya T, Randhawa P. HLA-A2, HLA-B44 and HLA-DR15 are associated with lower risk of BK viremia. *Nephrol Dial Transplant* 2013; **28**: 3119-3126 [PMID: 24084328 DOI: 10.1093/ndt/gft298]

50 **Awadalla Y**, Randhawa P, Ruppert K, Zeevi A, Duquesnoy RJ. HLA mismatching increases the risk of BK virus nephropathy in renal transplant recipients. *Am J Transplant* 2004; **4**: 1691-1696 [PMID: 15367226 DOI: 10.1111/j.1600-6143.2004.00563.x]

51 **Drachenberg CB**, Papadimitriou JC, Mann D, Hirsch HH, Wali R, Ramos E. Negative impact of human leukocyte antigen matching in the outcome of polyomavirus nephropathy. *Transplantation* 2005; **80**: 276-278 [PMID: 16041275 DOI: 10.1097/01.TP.0000165096.01034.15]

52 **Muramatsu M**, Gonzalez HD, Cacciola R, Aikawa A, Yaqoob MM, Puliatti C. ABO incompatible renal transplants: Good or bad? *World J Transplant* 2014; **4**: 18-29 [PMID: 24669364 DOI: 10.5500/wjt.v4.i1.18]

53 **Sharif A**, Alachkar N, Bagnasco S, Geetha D, Gupta G, Womer K, Arend L, Racusen L, Montgomery R, Kraus E. Incidence and outcomes of BK virus allograft nephropathy among ABO- and HLA-incompatible kidney transplant recipients. *Clin J Am Soc Nephrol* 2012; **7**: 1320-1327 [PMID: 22626962 DOI: 10.2215/CJN.00770112]

54 **Bentall A**, Neil D, Sharif A, Ball S. ABO-incompatible kidney transplantation is a novel risk factor for BK nephropathy. *Transplantation* 2015; **99**: e8-e9 [PMID: 25651124 DOI: 10.1097/TP.0000000000000483]

55 **Merlino C**, Bergallo M, Gribaudo G, Gregori G, Paolo Segoloni G, Giacchino F, Ponzi AN, Cavallo R. Polyomavirus BK DNA quantification assay to evaluate viral load in renal transplant recipients. *J Clin Virol* 2003; **28**: 265-274 [PMID: 14522065 DOI: 10.1016/S1386-6532(03)00012-X]

56 **Vasudev B**, Hariharan S, Hussain SA, Zhu YR, Bresnahan BA, Cohen EP. BK virus nephritis: risk factors, timing, and outcome in renal transplant recipients. *Kidney Int* 2005; **68**: 1834-1839 [PMID: 16164661 DOI: 10.1111/j.1523-1755.2005.00602.x]

57 **Nickeleit V**, Klimkait T, Binet IF, Dalquen P, Del Zenero V, Thiel G, Mihatsch MJ, Hirsch HH. Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. *N Engl J Med* 2000; **342**: 1309-1315 [PMID: 10793163 DOI: 10.1056/NEJM200005043421802]

58 **Drachenberg CB**, Beskow CO, Cangro CB, Bourquin PM, Simsir A, Fink J, Weir MR, Klassen DK, Bartlett ST, Papadimitriou JC. Human polyoma virus in renal allograft biopsies: morphological findings and correlation with urine cytology. *Hum Pathol* 1999; **30**: 970-977 [PMID: 10452511 DOI: 10.1016/S0046-8177(99)90252-6]

59 **Krishna A**, Prasad N. BK virus nephropathy in renal transplantation. *Ind J Transplant* 2011; **5**: 182-190 [DOI: 10.1016/S2212-0017(11)60038-5]

60 **Drachenberg RC**, Drachenberg CB, Papadimitriou JC, Ramos E, Fink JC, Wali R, Weir MR, Cangro CB, Klassen DK, Khaled A, Cunningham R, Bartlett ST. Morphological spectrum of polyoma virus disease in renal allografts: diagnostic accuracy of urine cytology. *Am J Transplant* 2001; **1**: 373-381 [PMID: 12099383 DOI: 10.1034/j.1600-6143.2001.10414.x]

61 **Sar A**, Worawichawong S, Benediktsson H, Zhang J, Yilmaz S, Trpkov K. Interobserver agreement for Polyomavirus nephropathy grading in renal allografts using the working proposal from the 10th Banff Conference on Allograft Pathology. *Hum Pathol* 2011; **42**: 2018-2024 [PMID: 21733554 DOI: 10.1016/j.humpath.2011.03.008]

62 **Masutani K**, Shapiro R, Basu A, Tan H, Wijkstrom M, Randhawa P. The Banff 2009 Working Proposal for polyomavirus nephropathy: a critical evaluation of its utility as a determinant of clinical outcome. *Am J Transplant* 2012; **12**: 907-918 [PMID: 22390378 DOI: 10.1111/j.1600-6143.2012.03993.x]

63 **Hirsch HH**, Randhawa P; AST Infectious Diseases Community of Practice. BK polyomavirus in solid organ transplantation. *Am J Transplant* 2013; **13** Suppl 4: 179-188 [PMID: 23465010 DOI: 10.1111/ajt.12110]

64 **Drachenberg CB**, Papadimitriou JC, Hirsch HH, Wali R, Crowder C, Nogueira J, Cangro CB, Mendley S, Mian A, Ramos E. Histological patterns of polyomavirus nephropathy: correlation with graft outcome and viral load. *Am J Transplant* 2004; **4**: 2082-2092 [PMID: 15575913 DOI: 10.1046/j.1600-6143.2004.00603.x]

65 **Buehrig CK**, Lager DJ, Stegall MD, Kreps MA, Kremers WK, Gloor JM, Schwab TR, Velosa JA, Fidler ME, Larson TS, Griffin MD. Influence of surveillance renal allograft biopsy on diagnosis and prognosis of polyomavirus-associated nephropathy. *Kidney Int* 2003; **64**: 665-673 [PMID: 12846764 DOI: 10.1046/j.1523-1755.2003.00103.x]

66 **Boan P**, Hewison C, Swaminathan R, Irish A, Warr K, Sinniah R, Pryce TM, Flexman J. Optimal use of plasma and urine BK viral loads for screening and predicting BK nephropathy. *BMC Infect Dis* 2016; **16**: 342 [PMID: 27448566 DOI: 10.1186/s12879-016-1652-6]

67 **Singh HK**, Andreoni KA, Madden V, True K, Detwiler R, Weck K, Nickeleit V. Presence of urinary Haufen accurately predicts polyomavirus nephropathy. *J Am Soc Nephrol* 2009; **20**: 416-427 [PMID: 19158358 DOI: 10.1681/ASN.2008010117]

68 **Ding R**, Medeiros M, Dadhania D, Muthukumar T, Kracker D, Kong JM, Epstein SR, Sharma VK, Seshan SV, Li B, Suthanthiran M. Noninvasive diagnosis of BK virus nephritis by measurement of messenger RNA for BK virus VP1 in urine. *Transplantation* 2002; **74**: 987-994 [PMID: 12394843 DOI: 10.1097/01.TP.0000032151.07990.1E]

69 **Dadhania D**, Snopkowski C, Ding R, Muthukumar T, Lee J, Bang H, Sharma VK, Seshan S, August P, Kapur S, Suthanthiran M. Validation of noninvasive diagnosis of BK virus nephropathy and identification of prognostic biomarkers. *Transplantation* 2010; **90**: 189-197 [PMID: 20526237 DOI: 10.1097/TP.0b013e3181e2a932]

70 **Hussain S**, Orentas R, Walczak J. Prevention of BKV nephritis by monitoring BK viremia in renal transplant recipients: a prospective study. *Graft* 2004; **7**: 28-30

71 **Saad ER**, Bresnahan BA, Cohen EP, Lu N, Orentas RJ, Vasudev B, Hariharan S. Successful treatment of BK viremia using reduction in immunosuppression without antiviral therapy. *Transplantation* 2008; **85**: 850-854 [PMID: 18360267 DOI: 10.1097/TP.0b013e318166cba8]

72 **Eckardt KU**, Kasiske BL. Kidney disease: improving global outcomes. *Nat Rev Nephrol* 2009; **5**: 650-657 [PMID: 19786993 DOI: 10.1038/nrneph.2009.153]

73 **Liacini A**, Seamone ME, Muruve DA, Tibbles LA. Anti-BK virus mechanisms of sirolimus and leflunomide alone and in combination: toward a new therapy for BK virus infection. *Transplantation* 2010; **90**: 1450-1457 [PMID: 21079551 DOI: 10.1097/TP.0b013e3182007be2]

74 **Morath C**, Arns W, Schwenger V, Mehrabi A, Fonouni H, Schmidt J, Zeier M. Sirolimus in renal transplantation. *Nephrol Dial Transplant* 2007; **22** Suppl 8: viii61-viii65 [PMID: 17890266]

75 **Polanco N**, González Monte E, Folgueira MD, Morales E, Gutiérrez Martínez E, Bengoa I, Hernández A, Morales JM, Praga M, Andrés A. Everolimus-based immunosuppression therapy for BK virus nephropathy. *Transplant Proc* 2015; **47**: 57-61 [PMID: 25645770 DOI: 10.1016/j.transproceed.2014.11.008]

76 **Jouve T**, Rostaing L, Malvezzi P. Place of mTOR inhibitors in management of BKV infection after kidney transplantation. *J Nephropathol* 2016; **5**: 1-7 [PMID: 27047803 DOI: 10.15171/jnp.2016.01]

77 **Tohme FA**, Kalil RS, Thomas CP. Conversion to a sirolimus-based regimen is associated with lower incidence of BK viremia in low-risk kidney transplant recipients. *Transpl Infect Dis* 2015; **17**: 66-72 [PMID: 25582442 DOI: 10.1111/tid.12347]

78 **Masutani K**, Shapiro R, Basu A, Tan H, Ninomiya T, Randhawa P. Putative episodes of T-cell-mediated rejection in patients with sustained BK viruria but no viremia. *Transplantation* 2012; **94**: 43-49 [PMID: 22691957 DOI: 10.1097/TP.0b013e318253e7a4]

79 **Babel N**, Fendt J, Karaivanov S, Bold G, Arnold S, Sefrin A, Lieske E, Hoffzimmer M, Dziubianau M, Bethke N, Meisel C, Grütz G, Reinke P. Sustained BK viruria as an early marker for the development of BKV-associated nephropathy: analysis of 4128 urine and serum samples. *Transplantation* 2009; **88**: 89-95 [PMID: 19584686 DOI: 10.1097/TP.0b013e3181aa8f62]

80 **Hardinger KL**, Koch MJ, Bohl DJ, Storch GA, Brennan DC. BK-virus and the impact of pre-emptive immunosuppression reduction: 5-year results. *Am J Transplant* 2010; **10**: 407-415 [PMID: 20055811 DOI: 10.1111/j.1600-6143.2009.02952.x]

81 **Menter T**, Mayr M, Schaub S, Mihatsch MJ, Hirsch HH, Hopfer H. Pathology of resolving polyomavirus-associated nephropathy. *Am J Transplant* 2013; **13**: 1474-1483 [PMID: 23721552 DOI: 10.1111/ajt.12218]

82 **Arroyo D**, Chandran S, Vagefi PA, Wojciechowski D. Adjuvant Ciprofloxacin for Persistent BK Polyomavirus Infection in Kidney Transplant Recipients. *J Transplant* 2014; **2014**: 107459 [PMID: 25349720 DOI: 10.1155/2014/107459]

83 **Knoll GA**, Humar A, Fergusson D, Johnston O, House AA, Kim SJ, Ramsay T, Chassé M, Pang X, Zaltzman J, Cockfield S, Cantarovich M, Karpinski M, Lebel L, Gill JS. Levofloxacin for BK virus prophylaxis following kidney transplantation: a randomized clinical trial. *JAMA* 2014; **312**: 2106-2114 [PMID: 25399012 DOI: 10.1001/jama.2014.14721]

84 **Jung YH**, Moon KC, Ha JW, Kim SJ, Ha IS, Cheong HI, Kang HG. Leflunomide therapy for BK virus allograft nephropathy after pediatric kidney transplantation. *Pediatr Transplant* 2013; **17**: E50-E54 [PMID: 23210794 DOI: 10.1111/petr.12029]

85 **Shah T**, Vu D, Naraghi R, Campbell A, Min D. Efficacy of Intravenous Immunoglobulin in the Treatment of Persistent BK Viremia and BK Virus Nephropathy in Renal Transplant Recipients. *Clin Transpl* 2014: 109-116 [PMID: 26281134]

86 **Kuypers DR**, Vandooren AK, Lerut E, Evenepoel P, Claes K, Snoeck R, Naesens L, Vanrenterghem Y. Adjuvant low-dose cidofovir therapy for BK polyomavirus interstitial nephritis in renal transplant recipients. *Am J Transplant* 2005; **5**: 1997-2004 [PMID: 15996251 DOI: 10.1111/j.1600-6143.2005.00980.x]

87 **Johnston O**, Jaswal D, Gill JS, Doucette S, Fergusson DA, Knoll GA. Treatment of polyomavirus infection in kidney transplant recipients: a systematic review. *Transplantation* 2010; **89**: 1057-1070 [PMID: 20090569 DOI: 10.1097/TP.0b013e3181d0e15e]

88 **Balduzzi A**, Lucchini G, Hirsch HH, Basso S, Cioni M, Rovelli A, Zincone A, Grimaldi M, Corti P, Bonanomi S, Biondi A, Locatelli F, Biagi E, Comoli P. Polyomavirus JC-targeted T-cell therapy for progressive multiple leukoencephalopathy in a hematopoietic cell transplantation recipient. *Bone Marrow Transplant* 2011; **46**: 987-992 [PMID: 20921942 DOI: 10.1038/bmt.2010.221]

P- Reviewer: Esposito P, Friedman EA, Moens U **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Yan JL

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 December 24; 7(6): 339-348

DOI: 10.5500/wjt.v7.i6.339

ISSN 2220-3230 (online)

MINIREVIEWS

Human leukocyte antigen typing and crossmatch: A comprehensive review

Mohammed Mahdi Althaf, Mohsen El Kossi, Jon Kim Jin, Ajay Sharma, Ahmed Mostafa Halawa

Mohammed Mahdi Althaf, Jack Pryor Renal Unit, Norfolk and Norwich University Hospital - NHS Foundation Trust, Norwich NR4 7UY, United Kingdom

Mohammed Mahdi Althaf, Mohsen El Kossi, Jon Kim Jin, Ajay Sharma, Ahmed Mostafa Halawa, Faculty of Health and Science, University of Liverpool, Institute of Learning and Teaching, School of Medicine, Liverpool L69 3GB, United Kingdom

Mohsen El Kossi, Doncaster Royal Infirmary, Doncaster DN2 5LT, United Kingdom

Jon Kim Jin, Nottingham Children Hospital, Nottingham NG7 2UH, United Kingdom

Ajay Sharma, Royal Liverpool University Hospitals, Liverpool L7 8XP, United Kingdom

Ahmed Mostafa Halawa, Department of Transplantation Surgery, Sheffield Teaching Hospitals, Sheffield S5 7AU, United Kingdom

ORCID number: Mohammed Mahdi Althaf (0000-0002-3158-8316); Mohsen El Kossi (0000-0002-2490-2784); Jon Kim Jin (0000-0003-4307-8513); Ajay Sharma (0000-0003-4050-6586); Ahmed Mostafa Halawa (0000-0002-7305-446X).

Author contributions: Althaf MM performed the majority of the writing, prepared the figures and tables; Sharma A performed input to structure, content and wrote the abstract; El Kossi M and Jin JK performed input to content; Halawa AM designed the outline, performed input to content and coordinated the writing of the paper.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other co-authors contributed their efforts in this manuscript.

Manuscript source: Unsolicited manuscript

Correspondence to: Ahmed Mostafa Halawa, MSc, MD, PGCE, MEd (Higher Education), FRCS, FRCS (Gen), Senior Lecturer (Hon), Consultant Transplant Surgeon, Department of Transplantation Surgery, Sheffield Teaching Hospital, Herries Road, Sheffield S5 7AU,
United Kingdom. ahmed.halawa@sth.nhs.uk
Telephone: +44-77-87542128
Fax: +44-11-42714604

Received: July 12, 2017
Peer-review started: July 14, 2017
First decision: August 7, 2017
Revised: August 22, 2017
Accepted: October 17, 2017
Article in press: October 17, 2017
Published online: December 24, 2017

Abstract

Renal transplantation remains the best option for patients suffering from end stage renal disease (ESRD). Given the worldwide shortage of organs and growing population of patients with ESRD, those waitlisted for a transplant is ever expanding. Contemporary crossmatch methods and human leukocyte antigen (HLA) typing play a pivotal role in improving organ allocation and afford better matches to recipients. Understanding crossmatch as well as HLA typing for renal transplantation and applying it in clinical practice is the key step to achieve a successful outcome. Interpretation of crossmatch results can be quite challenging where clinicians have not had formal training in applied transplant immunology. This review aims to provide a worked example using a clinical vignette. Furthermore, each technique is discussed in detail with its pros and cons. The index case is that of a young male with ESRD secondary to Lupus nephritis. He is offered a deceased donor kidney with a 1-0-0 mismatch. His complement dependent cytotoxicity (CDC) crossmatch reported positive for B lymphocyte, but flow cytometry

crossmatch (FCXM) was reported negative for both B and T lymphocytes. Luminex-SAB (single antigen bead) did not identify any donor specific antibodies (DSA). He never had a blood transfusion. The positive CDC-crossmatch result is not concordant with DSA status. These implausible results are due to underlying lupus erythematosus, leading to false-positive B-lymphocyte crossmatch as a result of binding immune complexes to Fc-receptors. False positive report of CDC crossmatch can be caused by the underlying autoimmune diseases such as lupus erythematosus, that may lead to inadvertent refusal of adequate kidney grafts. Detailed study of DSA by molecular technique would prevent wrong exclusion of such donors. Based on these investigations this patient is deemed to have "standard immunological risk" for renal transplantation.

Key words: Human leukocyte antigen typing; Cytotoxic crossmatch; Flow cytometry crossmatch; Virtual crossmatch; Human leukocyte antigen null alleles

Core tip: Understanding crossmatch for renal transplantation and applying it in clinical practice is the fundamental step to achieve a successful outcome. At times, interpreting an ambivalent report of crossmatch can be very challenging for clinicians since they have not been trained formally in applied transplant immunology. While there are several published reviews, this is presented as a worked example and is aimed to discuss immunological risk stratification by using an example of an index case.

Althaf MM, El Kossi M, Jin JK, Sharma A, Halawa AM. Human leukocyte antigen typing and crossmatch: A comprehensive review. *World J Transplant* 2017; 7(6): 339-348 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i6/339.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i6.339

INTRODUCTION

Renal transplantation is the best option in suitable and fit patients who have end stage renal disease (ESRD) as a result of lupus nephritis. Several studies have shown that five- and ten-year allograft survival are similar to that of recipients with other causes of ESRD[1-3]. It is also worth noting that lupus nephritis can recur in the allograft. The risk of recurrence of clinically apparent disease in renal transplantation is between 2%-30% of cases[1,4,5]. Systemic Lupus Erythematosus and its complications predominantly occur in women. However, it is worth noting that clinical manifestations are slightly different in men who have poorer outcomes[6,7]. Understanding crossmatch for renal transplantation and applying it in clinical practice is the key step to achieve a successful outcome. At times, interpreting an ambivalent report of crossmatch can be very challenging for clinicians since they have not been trained formally in applied transplant immunology. The following review is aimed to discuss an immunological risk stratification by using an example of an index case.

CLINICAL VIGNETTE

We are presented with a 30-year-old male patient who had been on maintenance haemodialysis for five years. His primary disease was Systemic Lupus Erythematosus which was complicated with lupus nephritis which eventually progressed to end-stage renal disease. He was offered a kidney from a deceased donor with a 1-0-0 mismatch. His complement dependent cytotoxicity (CDC) crossmatch reported positive for B lymphocyte, and flow cytometry crossmatch (FCXM) was reported negative for both B and T lymphocytes. His Luminex-SAB did not identify any donor specific antibodies (DSA). He never had a blood transfusion.

HLA TYPING

HLA typing is a crucial step in renal transplantation, as recognition of foreign HLA by recipient T lymphocytes would trigger an immune response. T lymphocyte activation initiates a cascade of mediators that direct the immune system against the allograft[8]. HLA laboratories currently perform serologic as well as molecular typing methods.

Serological typing

In this approach, a tray containing sera with antibodies to a multitude of known HLA alleles is used. These are commercially available. For typing, recipient lymphocytes are introduced into the tray wells contacting sera, complement and dye. In tray wells where antibodies can bind to the antigens on the surface of lymphocytes; complement is activated. This results in complement pathways triggered resulting in cell death, ultimately allowing the dye to enter the cell. Tray wells with significant cell death are then identified under phase contrast microscopy. Through a process of comparison and elimination of positive wells the HLA type is assigned. The key benefit of serologic typing is that results are available in a short period. This is particularly important in deceased donor renal transplantation. Quick results mean less cold ischemia times. This method also offers the ability to differentiate HLA alleles that have identifiable DNA sequences with molecular typing but with no cell surface antigen expression. These alleles termed "null" HLA alleles are of less immunological significance[9]. The downside of this method is the lack of sera with antibody specificities that are capable of identifying the ever-growing number of HLA alleles[10]. The HLA-Cw, DQ, and DP antigen may have clinically significant effects on the outcomes of allografts. However, serologic assays are scarce for these loci. Furthermore, serologic methods do not readily detect differences in HLA protein small amino

acids. These may be antigenic enough to trigger potent immunological responses[11,12]. With more advanced methods of typing currently available serological typing has fallen into disuse.

Molecular typing

Sequence-specific primer polymerase chain reaction: In this approach extracted DNA from the subject is amplified in several wells. Each well has primers that are complementary to specific HLA alleles. In wells where DNA probes are complementary to the specific sequence of the HLA molecule, an amplification product is formed. This is then instilled into an agarose gel and undergoes electrophoresis where they appear as a band. HLA typing is then allocated by matching the primers of the amplification product to DNA sequences of several candidate alleles.

Sequence specific oligonucleotide probes: Amplified DNA is mixed with oligonucleotide probes that are complementary to specific segments of the DNA of different alleles. Unique HLA alleles are then identified using fluorescent tags. For a particular gene of interest, the precise order of nucleotides is determined through sequencing. HLA type is then assigned using available HLA allele sequences[10].

Direct DNA sequencing: This method determines the precise order of nucleotides in the gene of interest. Using published HLA allele sequences, HLA type is subsequently assigned by comparison.

Molecular typing regardless of the method can clearly identify differences in HLA antigen between donor and recipient. Often with detail to the amino acid level that can provide insight to the risk accompanying mismatched donor-recipient antigens, epitopes and amino acid[12,13]. HLA typing based on polymerase chain reaction (PCR) is highly specific where specific alleles are identified with no cross-reactivity. However, a gene may occur in two or more forms called alleles. Cross-reactivity is the identification of an allele which is essentially similar to the allele of interest. While this feature is a key advantage of this method its acts as a double-edged sword. The disadvantage it poses is that new alleles not currently on the HLA sequence databank will fail to be identified. Primers used in HLA-typing are constructed on an HLA sequence databank that contains alleles available when the databank was designed[14].

HLA typing of the donor kidney and our patient revealed a 1-0-0-mismatch that corresponds to the pair of alleles mismatched, respectively, at HLA-A, HLA-B and HLA-DR. These three antigens are the considered as the most important ones in kidney transplantation. Logically the fewer the mismatches; the better the match between donor and recipient resulting in a successful transplant outcome. The dissimilarity in the HLA antigen reflects the alloimmune burden that a donor kidney presents to the recipient. In this case, there is 1 HLA mismatch which is that of HLA-A. Mismatch for different HLA antigens does not have equal weight. We know from the initial Collaborative Transplant Study (CTS) analysis that HLA-DR and HLA-B antigens offer the most alloimmune burden with less so from HLA-A[15]. Eurotransplant and old United Kingdom transplant data suggest that HLA-DR matching has a far greater effect than HLA-A or HLA-B[16,17]. Interestingly one study demonstrated that the influence of HLA-DR mismatching had the most effect during the first six months post-transplant while the maximal effect of HLA-B mismatching occurred two years post-transplant[18]. Data from the United Network for Organ Sharing (UNOS) registry further highlighted the significance of paying attention to having the least number of mismatches. They looked at quantifying the risk of transplant failure with HLA mismatch in patients who had their first adult kidney allografts from deceased donors. This study revealed that having six HLA mismatches translated to a 64% higher risk while the risk was down to 13% with just one HLA mismatch. Furthermore, these results were independent of locus[19]. Another study identified seven specific HLA mismatch combinations that were associated with decreased renal allograft survival. These were termed "taboo mismatches". A taboo mismatch translated to 81% one-year survival and 50% five-year survival[20].

In recent times, the HLA mismatching in deceased donor kidney transplants is of lesser significance due to the use of more potent immunosuppression and better identification of non-immunological determinants of transplantation[21]. Nonetheless, HLA matching continues to have a significant impact on allograft survival.

HLA ANTIBODY SCREENING

Almost a third of patients who are waitlisted for transplantation may have a degree of anti-HLA antibodies detected. The usual route for sensitisation towards HLA antigens occurs in three instances; pregnancy, post blood transfusion and prior transplantation. Preformed antibodies increase the chances of immunological failure of the allograft by causing positive crossmatches and, thereby, result in the exclusion of donors[9]. The index patient did not have a history of prior transplantation or blood transfusions. Both sensitive and specific detection of anti-HLA antibodies is crucial. Where crossmatch is negative, even low titres of DSA can lead to early as well as late antibody mediated rejection[22,23]. For sensitised patients, successful transplantation is possible by employing strategies such as desensitisation, paired exchange and acceptable mismatching[13,24,25]. There are different methods used for HLA antibody screening as shown below.

Cytotoxic (cell-based) antibody screening

A set of cell donors are randomly selected to be representative of a population. This should be representative of the population of potential deceased donors. Each panel consists of around 30 to 40 different donor lymphocytes. The method is similar to that of serologic typing however

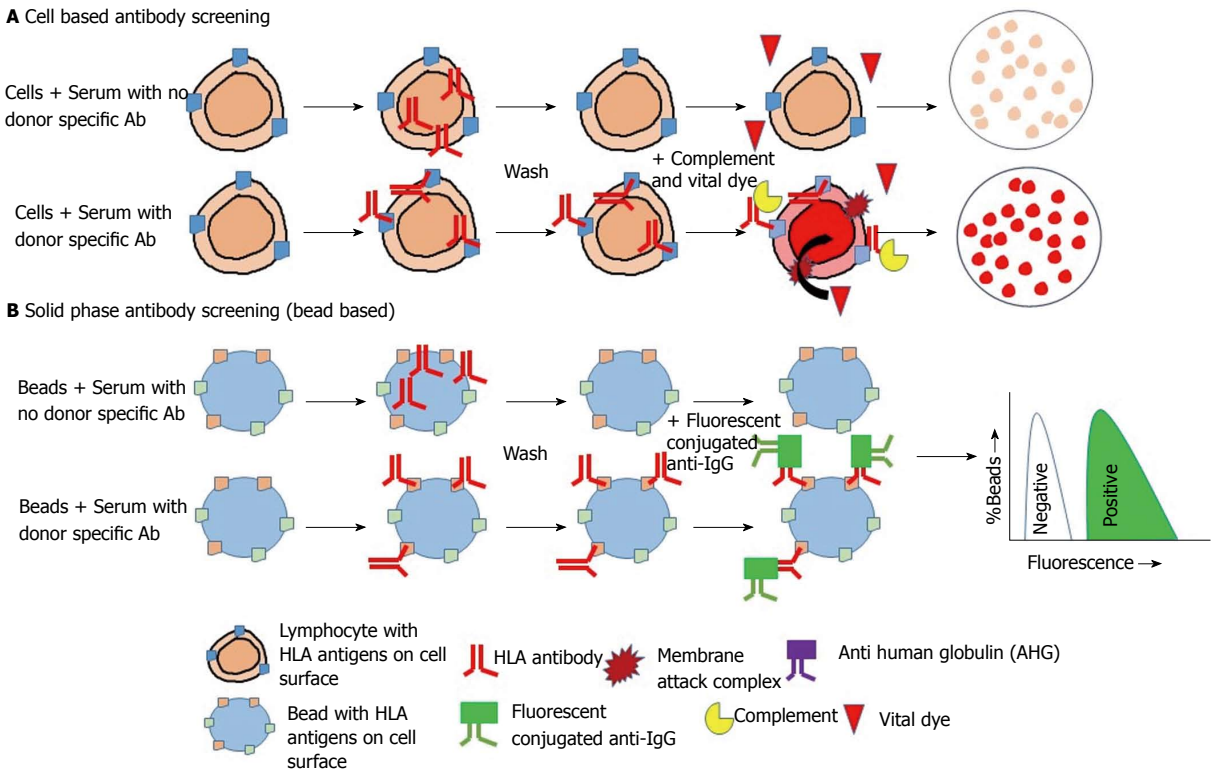


Figure 1 Schematic diagram of lymphocyte based antibody screening and solid phase (bead based) antibody screening. A: Cell based antibody screening; B: Solid phase (bead based) antibody screening.

here recipient serum is mixed with "cell donor" lymphocytes in individual wells along with complement and dye. Where the serum contains antibodies that bind to the cell surface with adequate density complement pathways are activated which results in cell death and uptake of the dye (Figure 1A). The degree of cytotoxicity is expressed as percentage PRA (panel reactive antibody). It is a tool that can be employed to approximate the risk of a given recipient of having a positive crossmatch. This is to a likely organ donor taken from a similar population.

The limitations of this method are that PRA percent can be different numerically without a corresponding change in the type or amount of antibody. This largely depends on the cell panel used which are commercially produced and may not truly represent the population. HLA frequencies and racial differences need to be factored in but cannot be done. Moreover, significant false positive results can be produced due to non-HLA antibodies, autoantibodies and nonspecific IgM antibodies. Similarly, false negative results are possible as this is purely complement dependent that requires higher antibody titres to be activated[26-28]. The lack of a complement activation simply due to low titres allows a true antibody to be hidden[29]. Precise, complete lists of antibody specificities and unacceptable antigens cannot be identified using this method as there are several antigens in each well[9].

Solid phase antibody screening

This method employs soluble or recombinant HLA molecules instead of lymphocytes targets - as lymphocytes present both HLA as well as non-HLA molecules (Figure 1B). The variants of these methods are:

Enzyme-linked immunosorbent assay platform: In this method, purified HLA molecules are applied to enzyme-linked immunosorbent assay (ELISA) platforms and will bind individually to HLA antibody after the addition of recipient serum[30,31]. Enzyme conjugated antibodies to IgG (human) is then added to detect the presence of HLA antibody in the serum which is bound to the antigen. Detection is performed by optical density reading.

Microbead platform/single-antigen beads: Pooled panel beads with several different class Ⅰ or Ⅱ HLA antigens on a bead yield a positive or negative result and are utilised for screening[32]. The phenotype or also known as ID beads are individually coated with class Ⅰ or Ⅱ HLA antigens of an individual patient-derived cell line. Microbead that is fluorescent dye conjugated is then added to detect the presence of HLA antibody in the serum which is bound to the antigen. Fluorescence detection can be done traditionally using a flow cytometer (Flow PRA®) or *via* the single-antigen beads (SAB) Luminex® platform. These estimate PRA by the proportion of positive beads. SAB are individually coated with a single HLA antigen and yield a list of distinct antibody specificities[33]. Specificities are subsequently compared with HLA frequencies in the donor population

to determine the calculated panel-reactive antibody (cPRA)[34]. This yields the best estimate of the likelihood of a positive crossmatch/donor specific antibody to a randomly selected donor[35,36].

It is important to understand the difference between PRA and cPRA. A high traditional PRA value translated to a high probability of a positive crossmatch. cPRA is based on unacceptable HLA antigens - those that the patient has been sensitized to. Furthermore, if these were present in a donor, would represent an unacceptable risk to the potential recipient or organ transplantation program. cPRA is calculated from HLA antigen frequencies among approximately twelve thousand kidney donors in the United States during the period between 2003 and 2005. This, therefore, represents the proportion of actual organ donors who express one or more of the unacceptable HLA antigens[36]. cPRA is useful in the allocation of kidney and pancreas transplants. cPRA estimates the proportion of donors with whom a particular recipient would be incompatible. An offer for a recipient with a high cPRA is a high probability of a positive crossmatch. Formerly, the same highly sensitised potential recipient would be higher on the list of each match run for donors with their blood group. Renal transplant programs were hesitant to set up final crossmatches for more highly sensitised patients for fear of not allocating the kidneys[37-39].

We are able to better discriminate immunologically relevant positive crossmatches from false-positive results when traditional cell based methods are complemented with solid phase assays[40]. Microbead assays (both Flow PRA® and Luminex®) are ten percent more sensitive for lower titre antibody than ELISA. ELISA is ten percent more sensitive compared to anti-human globulin (AHG) enhanced cytotoxicity based assays. Being in control of the antigens places on the beads, these assays are specific for anti-HLA antibodies. SAB assays are rapid with results available in 3-4 h. The assay is also quite efficient in a single reaction chamber up to one hundred unique antigen beads can be tested. Its additional multiplexing ability permits testing many patients simultaneously[41]. Results from SAB enable virtual crossmatching (VXM) to identify DSA pre-transplant, thereby enabling organ allocation and risk stratification[37]. SAB assays permit identification of anti-HLA antibodies for all common and numerous rare antigens and alleles. Its range of identification is up to eleven HLA loci[33].

Despite the fact that solid phase antibody screening addresses most of the short comings with cellular assays they have limitations as well. They detect both complement and non-complement binding simultaneously. Being too sensitive they can detect antibody that is below the threshold associated with a positive crossmatch. The detected antibody may not always have clinical implications but can preclude a potential donor. Non-HLA antibodies are also increasingly being recognised as clinically relevant predictors, and these cannot be accounted for utilising this method solely[42,43]. With the ever-growing list of HLA alleles, the complete spectrum of unique HLA antigens cannot be fully presented on solid phase assays.

The SAB - Luminex® assay has been shown to be susceptible to an artefact known as the prozone phenomenon[44]. This phenomenon is recognised when sera with high titer anti-HLA antibodies give negative results when tested neat, however, react strongly positive after 1:10 dilution[45,46]. The complement-mediated prozone effect is most likely caused by complement component 1 (C1) by competitively displacing the detection antibodies in the confined spaces between antibodies bound to HLA molecules. This in turn prevents HLA antibody binding to the HLA antigen on the bead. A similar scenario can arise with the binding of IgM antibodies or other serum factors to the beads. This can be resolved by treatment with dithiothreitol (DTT) and serum dilution. It is worth noting that nonspecific binding by serum proteins as well as drugs such as intravenous immunoglobulin (IVIG) could also interfere with the specific binding of anti-HLA antibodies to the HLA antigens on beads. Another cause for a false negative result is epitope sharing. Different HLA antigens on different beads share mutual antibody binding epitopes leading to the binding of an anti-HLA antibody to more than one bead. This leads to a reduction in the mean fluorescence intensity (MFI) on a single bead[41].

CROSSMATCHING (XM)

The cytotoxic assay was implemented as the requisite test prior to transplantation when it was shown that recipients with DSA had significantly higher rates of allograft failure due to hyperacute rejection as well as primary failure[47,48]. The presence of donor-specific cytotoxic antibodies depicted as a positive crossmatch was a contraindication to transplantation. With PRA that identifies several antibodies to a potential cluster of donors, the crossmatch will identify if a recipient had antibodies to a specific donor of interest. Despite the obvious benefits of testing the T cell cytotoxic crossmatch had a twenty percent false positive rate and a four percent false negative rate. Therefore, it is insufficient to identify all relevant antibodies, and in addition to that, it may needlessly exclude patients from transplant. The solid-phase antibody test should be used together with crossmatch results to identify those that are immunologically relevant[49].

Complement-dependent cytotoxicity crossmatch

Similar to cytotoxic assay the complement-dependent cytotoxicity crossmatch is interpreted as positive if a considerable number of lymphocytes are destroyed after the incorporation of complement (Figure 2A). This suggests that a significant DSA has been bound to the cell surface. Complement-dependent cytotoxicity crossmatch (CDC-XM) can be done for B and T lymphocytes. Sensitivity is limited if the relevant antibody is in low titres, but this can be overcome by increasing the incubation

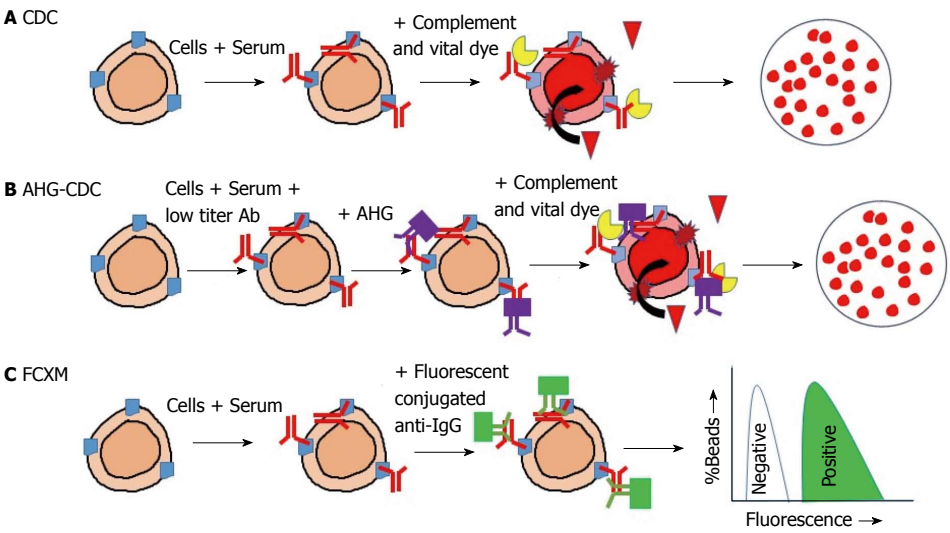


Figure 2 Schematic diagram of complement-dependent cytotoxicity crossmatch antibody to human immunoglobulin complement-dependent cytotoxicity crossmatch flow cytometry crossmatch. A: CDC; B: AHG-CDC; C: FCXM. CDC: Complement-dependent cytotoxicity; AHG: Antibody to human immunoglobulin; FCXM: Flow cytometry crossmatch.

time, the use of the AHG-enhanced method as well as additional wash steps[26,27]. The complement fixing antibody to anti-human immunoglobulin (AHG) will bind to any DSA present on lymphocytes (Figure 2B). This increases the chances of activating complement and thus raises the sensitivity of the test.

The antibodies that are present in lower titres are clinically significant as a negative test has an 18% graft loss in 1 year compared to a positive test that is associated with 36%[28]. Similar to cytotoxic PRA this method could miss low titre antibody resulting in false negatives. CDC-XM can also give false positives by detecting autoantibody, IgM/IgG HLA or non-HLA.

Flow cytometry crossmatch

Flow cytometry crossmatch (FCXM) detects DSA independent of complement fixation. It precisely detects the presence or lack of IgG DSA on donor lymphocytes. In this method, recipient serum is mixed with donor lymphocytes and then tagged with a fluorochrome-conjugated anti-IgG antibody. Several antibodies with separate fluorochromes particular to B and T lymphocyte surface proteins can be added (Figure 2C). With the use of flow-cytometry, B and T lymphocytes can be readily identified and have their DSA individually interrogated. Compared to complement-dependent cytotoxicity crossmatch this offers greater sensitivity[9]. Different laboratories use different methods, and this can result in a difference in the results between them[50]. However, this approach is not widely available, and its role in assessing immunological risk is still unclear.

Virtual crossmatching

In virtual crossmatch (VXM), both donor HLA typing and solid phase antibody screening are utilised together. It is not precisely a crossmatch in the sense of mixing serum and lymphocytes. The data is used to forecast the actual *in vitro* crossmatch results by "mixing" identified antibody specificities of recipient serum with donor HLA antigens[9]. The use of VXM can lead to shorter wait times and improved outcomes for sensitised transplant recipients. The speed of results generated allows a VXM to be performed at the time of donor identification owing to the fact that there is progressively sensitive and specific flow cytometry technology. VXM permits transplant physicians to consider donor organs that would not otherwise be available by means of a prospective crossmatch strategy, and thereby, allows to consider a potentially positive crossmatch a risk factor for donor selection[51,52].

Titres, specificities, and presence or absence of antibodies could significantly vary over time. Thus, the use of antibody specificity from historical serum sample (earlier than six months) could not predict a crossmatch with certainty. Other factors that can influence antibody specificities should be considered, and these include pregnancies, transplants and blood transfusions. The VXM should, therefore, be done considering all available serum results including at least one recent within less than 3-6 mo for a given patient. False positive results of VXM may arise where there are significantly low titre and/or non-complement binding antibodies, thereby, resulting in the wrong exclusion of potential donors[9]. The VXM can also give false negative results due to the fact that the list of all potential HLA donor antigens have been classed differently and, therefore, can not be correctly represented[53]. The results from VXM are not a hundred percent accurate and current practice mandates an actual crossmatch be performed as well[37]. Furthermore, VXM does not identify the HLA "Null" alleles. Null HLA alleles are ones have identifiable DNA sequences with molecular typing but do not express HLA products on the cell surface. In excess of 190 null alleles have been identified across HLA class Ⅰ and Ⅱ. There is a significant risk where a null allele is misidentified for its fully expressed

Table 1 Summary of the pre-transplant risk assessment of immunological challenge

Donor crossmatch result	Crossmatch method	Current or historical	Antibody screening results	Interpretation of immunological risk
Positive T and B lymphocyte	CDC (DTT)	C	IgG HLA class Ⅰ DSA	High risk[1] Hyperacute rejection (veto to transplantation)
Positive B lymphocyte	CDC (DTT)	C	IgG HLA class Ⅱ DSA	High risk[1]
Positive B lymphocyte	CDC (DTT)	C	Weak IgG HLA class I DSA	Intermediate risk[2]
Positive T and B lymphocyte	FCXM (CDC neg)	C	IgG HLA class Ⅰ DSA	Intermediate risk[2]
Positive B lymphocyte	FCXM (CDC neg)	C	IgG HLA class Ⅱ DSA	Intermediate risk[2]
Positive T and B lymphocyte	CDC (DTT)	H	IgG HLA class Ⅰ DSA	High risk[3]
Positive B cell	CDC (DTT)	H	IgG HLA class Ⅱ DSA	High risk[3]
Positive B lymphocyte	CDC (DTT)	H	Weak IgG HLA class I DSA	Intermediate risk[2]
Positive T and B lymphocyte	FCXM (CDC neg)	H	IgG HLA class Ⅰ DSA	Intermediate risk[2]
Positive B lymphocyte	FCXM (CDC neg)	H	IgG HLA class Ⅱ DSA	Intermediate risk[2]
Positive T and B lymphocyte	CDC (neg DTT)	C or H	IgM HLA class Ⅰ DSA	Standard risk
Positive B lymphocyte	CDC (neg DTT)	C or H	IgM HLA class Ⅱ DSA	Standard risk
Positive T and B lymphocyte	CDC (neg DTT)	C or H	IgM non-HLA (often autoreactive)	Standard risk
Positive B lymphocyte	CDC (neg DTT)	C or H	IgM non-HLA (often autoreactive)	Standard risk
Negative T and B lymphocyte	FCXM	C or H	IgG HLA class Ⅰ or Ⅱ DSA (detected by Luminex SAB alone)	Standard risk
Positive T and/or B lymphocyte	CDC and/or FCXM	C or H	Negative (Luminex Ab detection and/or SAB)	Standard risk (IgM/IgG non-HLA, often showing *in vitro* autoreactivity)
Positive T; Nnegative B lymphocyte	CDC and/or FCXM	C or H	Positive (Luminex SAB-not donor-specific) or negative	Standard risk (results suggest antibody is not HLA-specific)
Negative T and B lymphocyte	FCXM	C or H	Positive (Luminex SAB) not donor HLA-specific	Standard risk
Negative T and B lymphocyte	CDC and/or FCXM	C or H	Negative (Luminex Ab detection and/or SAB)	Standard risk
Donor crossmatch result	Crossmatch method	Current or historical	Antibody screening results	Interpretation of immunological risk
Positive T and B lymphocyte	CDC (DTT)	C	IgG HLA class Ⅰ DSA	High risk[1] Hyperacute rejection (veto to transplantation)
Positive B lymphocyte	CDC (DTT)	C	IgG HLA class Ⅱ DSA	High risk[1]
Positive B lymphocyte	CDC (DTT)	C	Weak IgG HLA class Ⅰ DSA	Intermediate risk[2]
Positive T and B lymphocyte	FCXM (CDC neg)	C	IgG HLA class Ⅰ DSA	Intermediate risk[2]
Positive B lymphocyte	FCXM (CDC neg)	C	IgG HLA class Ⅱ DSA	Intermediate risk[2]
Positive T and B lymphocyte	CDC (DTT)	H	IgG HLA class Ⅰ DSA	High risk[3]
Positive B lymphocyte	CDC (DTT)	H	IgG HLA class Ⅱ DSA	High risk[3]
Positive B lymphocyte	CDC (DTT)	H	Weak IgG HLA class I DSA	Intermediate risk[2]
Positive T and B lymphocyte	FCXM (CDC neg)	H	IgG HLA class Ⅰ DSA	Intermediate risk2
Positive B lymphocyte	FCXM (CDC neg)	H	IgG HLA class Ⅱ DSA	Intermediate risk2
Positive T and B lymphocyte	CDC (neg DTT)	C or H	IgM HLA class Ⅰ DSA	Standard risk
Positive B lymphocyte	CDC (neg DTT)	C or H	IgM HLA class Ⅱ DSA	Standard risk
Positive T and B lymphocyte	CDC (neg DTT)	C or H	IgM non-HLA (often autoreactive)	Standard risk
Positive B lymphocyte	CDC (neg DTT)	C or H	IgM non-HLA (often autoreactive)	Standard risk
Negative T and B lymphocyte	FCXM	C or H	IgG HLA class Ⅰ or Ⅱ DSA (detected by Luminex SAB alone)	Standard risk
Positive T and/or B lymphocyte	CDC and/or FCXM	C or H	Negative (Luminex Ab detection and/or SAB)	Standard risk (IgM/IgG non-HLA, often showing *in vitro* autoreactivity)
Positive T; negative B lymphocyte	CDC and/or FCXM	C or H	Positive (Luminex SAB-not donor-specific) or negative	Standard risk (results suggest antibody is not HLA-specific)
Negative T and B lymphocyte	FCXM	C or H	Positive (Luminex SAB) not donor HLA-specific	Standard risk
Negative T and B lymphocyte	CDC and/or FCXM	C or H	Negative (Luminex Ab detection and/or SAB)	Standard risk

[1]High immunological risk: Hyperacute rejection is unlikely (reported only in cases with very high titre HLA-DR antibodies) but donor-specific HLA class II antibodies are increasingly recognised as being associated with refractory humoral rejection and poor transplant prognosis; [2]Intermediate immunological risk: Transplantation should be avoided if reasonably possible (*i.e.*, short waiting time, easy to avoid unacceptable mismatches) but may be undertaken with appropriate clinical caution; consideration for enhanced immunosuppression, proactive use of clinical intervention strategies and post-transplant antibody monitoring; [3]Risk of anamnestic secondary T and/or B lymphocyte response: Need to consider high risk immunosuppression strategy, the duration, titre and priming source of antibody and repeat mismatches (pregnancy or regraft). Historical positive crossmatches caused by cross-reactive alloantibodies (avoiding the main specificity and priming stimulus) constitute intermediate immunological risk and are less likely to be associated with refractory T or B lymphocyte responses. CDC: Complement dependent cytotoxicity; DTT: Dithiothreitol; HLA: Human leukocyte antigen; DSA: Donor specific antibodies; FCXM: Flow cytometry crossmatch; SAB: Single-antigen beads.

counterpart in stem cell transplantation. However, the risk is slightly lower in solid organ transplantation. A recipient will have the risk of developing DSA for the mismatch where the null allele is misidentified as a fully expressed product and, therefore, transplanted with a donor bearing the expressed antigen. This mismatch is not life threatening but can affect future transplantation. In contrast, where a donor null allele is misidentified as a fully expressed product and subsequently transplanted into a recipient bearing the expressed antigen results in no humoral rejection and is well tolerated[54].

DEFINING RISK

Gebel *et al*[49] stratified the prospective renal transplant patients into various categories according to immunological risk in renal transplantations. On the basis of this with further additions the principles of risk assessment are as follows:

High immunological risk

At the time of transplantation, there are high titres of circulating antibodies specific for mismatched donor HLA (DSA). This can lead to hyperacute rejection. The presence of DSA precludes transplantation. However, there are reports of innovative pre-transplant desensitisation regimens to reduce this risk.

Intermediate immunological risk

At the time of transplantation, there is a low titer of DSA, and historic DSA is not detectable. It may be acceptable to consider intensified immunosuppression as well as immunological monitoring in the post-transplant period.

Standard immunological risk

Where there is no evidence of donor directed sensitisation to HLA. Refer to Table 1 that gives a summary of the immunological risk assessment pre-transplant based on donor crossmatch and antibody screening outcomes[55].

RISK ASSESSMENT OF OUR CLINICAL VIGNETTE

Our patient had CDC-XM reported positive for B and T lymphocytes but FCXM was reported negative for both B and T lymphocytes. His Luminex-SAB did not identify any DSA. These results can be risk stratified as "standard immunological risk", and we can proceed with transplantation. Positive CDC-XM result is not in accordance with DSA status. These implausible results are due to underlying lupus erythematosus, leading to false-positive B- lymphocyte crossmatches as a result of binding immune complexes to Fc-receptors.

CONCLUSION

Interpretation and clinical application of transplant immunology are crucial steps to a successful outcome. Understanding of crossmatch results and the caveats of individual tests can be quite challenging where clinicians have not had formal training in applied transplant immunology. This case illustrated a common scenario and detailed the approach to testing and its interpretation. If we were to rely simply on the CDC-XM, we would have made an erroneous conclusion. It is crucial to realise that false positive report of CDC-XM can be due to autoimmune diseases where type Ⅲ hypersensitivity occurs such as in Systemic Lupus Erythematosus. The false-positive B-lymphocyte crossmatch result from immune complexes binding to Fc-receptors[56,57]. Such a result may lead to inadvertent refusal of adequate kidney grafts. It has been previously reported that false positive CDC-XM could also be a result of medications such as Isoniazid and Hydralazine[58,59]. Detailed study of DSA by molecular technique would prevent erroneous exclusion of such donors. This can eventually lead to improved organ allocation and shorter waiting times in transplant lists.

REFERENCES

1 **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]

2 **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]

3 **Ponticelli C**, Moroni G. Renal transplantation in lupus nephritis. *Lupus* 2005; **14**: 95-98 [PMID: 15732296 DOI: 10.1191/0961203305lu2067oa]

4 **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]

5 **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]

6 **Costenbader KH**, Feskanich D, Stampfer MJ, Karlson EW. Reproductive and menopausal factors and risk of systemic lupus erythematosus in women. *Arthritis Rheum* 2007; **56**: 1251-1262 [PMID: 17393454 DOI: 10.1002/art.22510]

7 **Gilbert EL**, Ryan MJ. Estrogen in cardiovascular disease during systemic lupus erythematosus. *Clin Ther* 2014; **36**: 1901-1912 [PMID: 25194860 DOI: 10.1016/j.clinthera.2014.07.021]

8 **von Andrian UH**, Mackay CR. T-cell function and migration. Two sides of the same coin. *N Engl J Med* 2000; **343**: 1020-1034 [PMID: 11018170 DOI: 10.1056/nejm200010053431407]

9 **Tinckam KJ**. Basic histocompatibility testing methods. In: Chandraker A, editor. Core concepts in renal transplantation. New York: Springer Science+Business Media, LLC, 2012: 21-42

10 **Robinson J**, Waller MJ, Parham P, de Groot N, Bontrop R, Kennedy LJ, Stoehr P, Marsh SG. IMGT/HLA and IMGT/MHC: sequence databases for the study of the major histocompatibility complex. *Nucleic Acids Res* 2003; **31**: 311-314 [PMID: 12520010 DOI: 10.1093/nar/gkg070]

11 **Claas FH**, Witvliet MD, Duquesnoy RJ, Persijn GG, Doxiadis II. The acceptable mismatch program as a fast tool for highly sensitized patients awaiting a cadaveric kidney transplantation: short waiting time and excellent graft outcome. *Transplantation* 2004; **78**: 190-193 [PMID: 15280676 DOI: 10.1097/01.TP.0000129260.86766.67]

12 **Duquesnoy RJ**, Claas FH. Is the application of HLAMatchmaker relevant in kidney transplantation? *Transplantation* 2005; **79**: 250-251 [PMID: 15665779 DOI: 10.1097/01.TP.0000144327.92898.A6]

13 **Claas FH**, Roelen DL, Oudshoorn M, Doxiadis II. Future HLA matching strategies in clinical transplantation. *Dev Ophthalmol* 2003; **36**: 62-73 [PMID: 12494682 DOI: 10.1159/000067657]

14 **Gyllensten U**, Allen M. PCR-based HLA class II typing. *PCR Methods Appl* 1991; **1**: 91-98 [PMID: 1842935 DOI: 10.1101/gr.1.2.91]

15 **Opelz G**. Correlation of HLA matching with kidney graft survival in patients with or without cyclosporine treatment. *Transplantation* 1985; **40**: 240-243 [PMID: 3898488 DOI: 10.1097/00007890-198509000-00003]

16 **Doxiadis II**, de Fijter JW, Mallat MJ, Haasnoot GW, Ringers J, Persijn GG, Claas FH. Simpler and equitable allocation of kidneys from postmortem donors primarily based on full HLA-DR compatibility. *Transplantation* 2007; **83**: 1207-1213 [PMID: 17496537 DOI: 10.1097/01.tp.0000261108.27421.bc]

17 **Gilks WR**, Bradley BA, Gore SM, Klouda PT. Substantial benefits of tissue matching in renal transplantation. *Transplantation* 1987; **43**: 669-674 [PMID: 3554659 DOI: 10.1097/00007890-198705000-00013]

18 **Danovitch GM**, Nast C. Dialysis and Transplantation. In: Owen WF, Pereira BJ, Sayegh MH, ed. Philadelphia: W.B.Saunders, 2000: 504

19 **Williams RC**, Opelz G, McGarvey CJ, Weil EJ, Chakkera HA. The Risk of Transplant Failure With HLA Mismatch in First Adult Kidney Allografts From Deceased Donors. *Transplantation* 2016; **100**: 1094-1102 [PMID: 26901078 DOI: 10.1097/tp.0000000000001115]

20 **Doxiadis II**, Smits JM, Schreuder GM, Persijn GG, van Houwelingen HC, van Rood JJ, Claas FH. Association between specific HLA combinations and probability of kidney allograft loss: the taboo concept. *Lancet* 1996; **348**: 850-853 [PMID: 8826810 DOI: 10.1016/S0140-6736(96)02296-9]

21 **Su X**, Zenios SA, Chakkera H, Milford EL, Chertow GM. Diminishing significance of HLA matching in kidney transplantation. *Am J Transplant* 2004; **4**: 1501-1508 [PMID: 15307838 DOI: 10.1111/j.1600-6143.2004.00535.x]

22 **van den Berg-Loonen EM**, Billen EV, Voorter CE, van Heurn LW, Claas FH, van Hooff JP, Christiaans MH. Clinical relevance of pretransplant donor-directed antibodies detected by single antigen beads in highly sensitized renal transplant patients. *Transplantation* 2008; **85**: 1086-1090 [PMID: 18431226 DOI: 10.1097/TP.0b013e31816b3ed1]

23 **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]

24 **Stegall MD**, Gloor J, Winters JL, Moore SB, Degoey S. A comparison of plasmapheresis versus high-dose IVIG desensitization in renal allograft recipients with high levels of donor specific alloantibody. *Am J Transplant* 2006; **6**: 346-351 [PMID: 16426319 DOI: 10.1111/j.1600-6143.2005.01178.x]

25 **Gentry SE**, Segev DL, Simmerling M, Montgomery RA. Expanding kidney paired donation through participation by compatible pairs. *Am J Transplant* 2007; **7**: 2361-2370 [PMID: 17845570 DOI: 10.1111/j.1600-6143.2007.01935.x]

26 **Amos DB**, Cohen I, Klein WJ Jr. Mechanisms of immunologic enhancement. *Transplant Proc* 1970; **2**: 68-75 [PMID: 4107294]

27 **Fuller TC**, Fuller AA, Golden M, Rodey GE. HLA alloantibodies and the mechanism of the antiglobulin-augmented lymphocytotoxicity procedure. *Hum Immunol* 1997; **56**: 94-105 [PMID: 9455498 DOI: 10.1016/S0198-8859(97)00174-2]

28 **Kerman RH**, Kimball PM, Van Buren CT, Lewis RM, DeVera V, Baghdahsarian V, Heydari A, Kahan BD. AHG and DTE/AHG procedure identification of crossmatch-appropriate donor-recipient pairings that result in improved graft survival. *Transplantation* 1991; **51**: 316-320 [PMID: 1994522 DOI: 10.1097/00007890-199102000-00008]

29 **Gebel HM**, Bray RA. Sensitization and sensitivity: defining the unsensitized patient. *Transplantation* 2000; **69**: 1370-1374 [PMID: 10798756 DOI: 10.1097/00007890-200004150-00027]

30 **Zachary AA**, Ratner LE, Graziani JA, Lucas DP, Delaney NL, Leffell MS. Characterization of HLA class I specific antibodies by ELISA using solubilized antigen targets: II. Clinical relevance. *Hum Immunol* 2001; **62**: 236-246 [PMID: 11250041 DOI: 10.1016/S0198-8859(00)00253-6]

31 **Zachary AA**, Delaney NL, Lucas DP, Leffell MS. Characterization of HLA class I specific antibodies by ELISA using solubilized antigen targets: I. Evaluation of the GTI QuikID assay and analysis of antibody patterns. *Hum Immunol* 2001; **62**: 228-235 [PMID: 11250040 DOI: 10.1016/S0198-8859(00)00254-8]

32 **Pei R**, Wang G, Tarsitani C, Rojo S, Chen T, Takemura S, Liu A, Lee J. Simultaneous HLA Class I and Class II antibodies screening with flow cytometry. *Hum Immunol* 1998; **59**: 313-322 [PMID: 9619770 DOI: 10.1016/S0198-8859(98)00020-2]

33 **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: 10.1097/01.tp.0000040431.80510.98]

34 **Zachary AA**, Braun WE. Calculation of a predictive value for transplantation. *Transplantation* 1985; **39**: 316-318 [PMID: 3975995 DOI: 10.1097/00007890-198503000-00024]

35 **Cecka JM**, Kucheryavaya AY, Reinsmoen NL, Leffell MS. Calculated PRA: initial results show benefits for sensitized patients and a reduction in positive crossmatches. *Am J Transplant* 2011; **11**: 719-724 [PMID: 21114658 DOI: 10.1111/j.1600-6143.2010.03340.x]

36 **Cecka JM**. Calculated PRA (CPRA): the new measure of sensitization for transplant candidates. *Am J Transplant* 2010; **10**: 26-29 [PMID: 19958328 DOI: 10.1111/j.1600-6143.2009.02927.x]

37 **Bray RA**, Nolen JD, Larsen C, Pearson T, Newell KA, Kokko K, Guasch A, Tso P, Mendel JB, Gebel HM. Transplanting the highly sensitized patient: The emory algorithm. *Am J Transplant* 2006; **6**: 2307-2315 [PMID: 16939516 DOI: 10.1111/j.1600-6143.2006.01521.x]

38 **Tambur AR**, Leventhal J, Kaufman DB, Friedewald J, Miller J, Abecassis MM. Tailoring antibody testing and how to use it in the calculated panel reactive antibody era: the Northwestern University experience. *Transplantation* 2008; **86**: 1052-1059 [PMID: 18946342 DOI: 10.1097/TP.0b013e3181874b06]

39 **Bingaman AW**, Murphey CL, Palma-Vargas J, Wright F. A virtual crossmatch protocol significantly increases access of highly sensitized patients to deceased donor kidney transplantation. *Transplantation* 2008; **86**: 1864-1868 [PMID: 19104435 DOI: 10.1097/TP.0b013e318191404c]

40 **Bray RA**, Nickerson PW, Kerman RH, Gebel HM. Evolution of HLA antibody detection: technology emulating biology. *Immunol Res* 2004; **29**: 41-54 [PMID: 15181269 DOI: 10.1385/ir:29:1-3:041]

41 **Konvalinka A**, Tinckam K. Utility of HLA Antibody Testing in Kidney Transplantation. *J Am Soc Nephrol* 2015; **26**: 1489-1502 [PMID: 25804279 DOI: 10.1681/asn.2014080837]

42 **Sumitran-Holgersson S**. Relevance of MICA and other non-HLA antibodies in clinical transplantation. *Curr Opin Immunol* 2008; **20**: 607-613 [PMID: 18675346 DOI: 10.1016/j.coi.2008.07.005]

43 **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]

44 **Weinstock C**, Schnaidt M. The complement-mediated prozone effect in the Luminex single-antigen bead assay and its impact on HLA antibody determination in patient sera. *Int J Immunogenet* 2013; **40**: 171-177 [PMID: 22913810 DOI: 10.1111/j.1744-313X.2012.01147.x]

45 **Lowe D**. The high-dose hook effect in the detection and monitoring

of HLA specific antibody by Luminex assay. *Int J Immunogenetics* 2007; **34**: 288

46 **Stenzel A**, Bringmann G, Koch K, Strathmann K, Zeiler T. Prozone phenomenon of anti-HLA-A2 in Lifecodes Luminex single antigen class I, Tepnel (LSA Class I). 42nd Annual Meeting of German Society for Transfusion Medicine and Immunohematology (DGTI). *Transfus Med Hemotherapy* 2009; **36** (Suppl 1): 24 [DOI: 10.1159/000242471]

47 **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]

48 **Stiller CR**, Sinclair NR, Abrahams S, Ulan RA, Fung M, Wallace AC. Lymphocyte-dependent antibody and renal graft rejection. *Lancet* 1975; **1**: 953-954 [PMID: 48125 DOI: 10.1016/S0140-6736(75)92010-3]

49 **Gebel HM**, Bray RA, Nickerson P. Pre-transplant assessment of donor-reactive, HLA-specific antibodies in renal transplantation: contraindication vs. risk. *Am J Transplant* 2003; **3**: 1488-1500 [PMID: 14629279 DOI: 10.1046/j.1600-6135.2003.00273.x]

50 **Scornik JC**, Bray RA, Pollack MS, Cook DJ, Marrari M, Duquesnoy R, Langley JW. Multicenter evaluation of the flow cytometry T-cell crossmatch: results from the American Society of Histocompatibility and Immunogenetics-College of American Pathologists proficiency testing program. *Transplantation* 1997; **63**: 1440-1445 [PMID: 9175808 DOI: 10.1097/00007890-199705270-00013]

51 **Pescovitz MD**. B cells: a rational target in alloantibody-mediated solid organ transplantation rejection. *Clin Transplant* 2006; **20**: 48-54 [PMID: 16556153 DOI: 10.1111/j.1399-0012.2005.00439.x]

52 **Zangwill S**, Ellis T, Stendahl G, Zahn A, Berger S, Tweddell J. Practical application of the virtual crossmatch. *Pediatr Transplant* 2007; **11**: 650-654 [PMID: 17663689 DOI: 10.1111/j.1399-3046.2007.00746.x]

53 **Schreuder GM**, Hurley CK, Marsh SG, Lau M, Fernandez-Vina M, Noreen HJ, Setterholm M, Maiers M; World Marrow Donor Associations Quality Assurance and IT Working Groups; WHO Nomenclature Committee for Factors of the HLA system; 13th International Histocompatibility Workshop Serology Component; International Cell Exchange, UCLA; US National Marrow Donor Program. The HLA Dictionary 2004: a summary of HLA-A, -B, -C, -DRB1/3/4/5 and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR and -DQ antigens. *Tissue Antigens* 2005; **65**: 1-55 [PMID: 15663741 DOI: 10.1111/j.1399-0039.2005.00350.x]

54 **Kallon D**. Histocompatibility Immunogenetics. A collection of brief revision notes. 2001: 38. Available from: URL: http://www.histocompatibilityandimmunogenetics.com/

55 **Guidelines BTS**. Detection and characterisation of clinically relevant antibodies in allotransplantation (joint with BSHI). British Society for Histocompatibility Immunogenetics and British Transplantation Society, 2016. Available from: URL: https://bts.org.uk/guidelines-standards/

56 **Schlaf G**, Mauz-Körholz C, Ott U, Leike S, Altermann W. General insufficiency of the classical CDC-based crossmatch to detect donor-specific anti-HLA antibodies leading to invalid results under recipients' medical treatment or underlying diseases. *Histol Histopathol* 2012; **27**: 31-38 [PMID: 22127594 DOI: 10.14670/hh-27.31]

57 **Eggleton P**. Hypersensitivity: Immune Complex Mediated (Type III). John Wiley Sons, Ltd, 2001: 1-14 [DOI: 10.1038/npg.els.0001138]

58 **Poli F**, Innocente A, Cagni N, Brambilla C, Crespiatico L, Colombo MB, Scalamogna M. Isoniazid in patient plasma may cause a false-positive result on the complement-dependent cytotoxicity test. *Hum Immunol* 2009; **70**: 758-759 [PMID: 19539003 DOI: 10.1016/j.humimm.2009.06.012]

59 **Kucharz EJ**, Goodwin JS. Hydralazine causes nonspecific binding of antibodies to human lymphocytes in vitro. *Immunopharmacology* 1990; **19**: 1-4 [PMID: 2307578 DOI: 10.1016/0162-3109(90)90020-F]

P- Reviewer: Nagata T, Sanchez-Zapardiel E **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Yan JL

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 December 24; 7(6): 349-358

DOI: 10.5500/wjt.v7.i6.349

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Risk factors and clinical indicators for the development of biliary strictures post liver transplant: Significance of bilirubin

Elizabeth Ann Forrest, Janske Reiling, Geraldine Lipka, Jonathan Fawcett

Elizabeth Ann Forrest, Janske Reiling, Geraldine Lipka, Jonathan Fawcett, Queensland Liver Transplant Service, Princess Alexandra Hospital, Brisbane, Queensland 4102, Australia

Elizabeth Ann Forrest, Department of Surgery, Gold Coast Hospital and Health Service, Gold Coast, Queensland 4215, Australia

Janske Reiling, Jonathan Fawcett, Faculty of Medicine, the University of Queensland, Brisbane, Queensland 4006, Australia

Janske Reiling, Gallipoli Medical Research Institute, Greenslopes Private Hospital, Brisbane, Queensland 4120, Australia

Janske Reiling, Department of Surgery, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, AZ Maastricht 6202, The Netherlands

Janske Reiling, PA Research Foundation, Princess Alexandra Hospital, Brisbane, Queensland 4102, Australia

ORCID number: Elizabeth Ann Forrest (0000-0002-2905-1384); Janske Reiling (0000-0001-6621-6582); Geraldine Lipka (0000-0002-7473-0722); Jonathan Fawcett (0000-0003-2982-7530).

Author contributions: Forrest EA, Reiling J and Fawcett J designed the research; Forrest EA, Reiling J and Lipka G performed the research; Forrest EA and Reiling J analyzed the data; Forrest EA wrote the paper; Reiling J, Lipka G and Fawcett J revised the manuscript prior to submission.

Institutional review board statement: The study was reviewed and approved by the Princess Alexandra Hospital (HREC/13/QPAH/382) and University of Queensland (UQ: 2015001248) Human Research Ethics Committee.

Informed consent statement: Approval was obtained from the Queensland Government to access confidential patient information, held by Queensland Health, for the Purpose of Research under the provision of section 280 of the Public Health Act 2005.

Conflict-of-interest statement: The authors of this study have no conflict of interests to disclose.

Manuscript source: Unsolicited manuscript

Correspondence to: Elizabeth Ann Forrest, MD, BSc BA, Resident Medical Officer, Department of Surgery, Gold Coast Hospital and Health Service, 1 Hospital Boulevard, Southport, Gold Coast, Queensland 4215,
Australia. elizabeth.forrest3@health.qld.gov.au
Telephone: +61-7-56870000
Fax: +61-7-56870098

Received: August 13, 2017
Peer-review started: August 14, 2017
First decision: September 4, 2017
Revised: September 18, 2017
Accepted: November 1, 2017
Article in press: November 2, 2017
Published online: December 24, 2017

Abstract

AIM

To identify risk factors associated with the formation of biliary strictures post liver transplantation over a period of 10-year in Queensland.

METHODS

Data on liver donors and recipients in Queensland between 2005 and 2014 was obtained from an electronic patient data system. In addition, intra-operative and

post-operative characteristics were collected and a logistical regression analysis was performed to evaluate their association with the development of biliary strictures.

RESULTS

Of 296 liver transplants performed, 285 (96.3%) were from brain dead donors. Biliary strictures developed in 45 (15.2%) recipients. Anastomotic stricture formation (n = 25, 48.1%) was the commonest complication, with 14 (58.3%) of these occurred within 6-mo of transplant. A percutaneous approach or endoscopic retrograde cholangiography was used to treat 17 (37.8%) patients with biliary strictures. Biliary reconstruction was initially or ultimately required in 22 (48.9%) patients. In recipients developing biliary strictures, bilirubin was significantly increased within the first post-operative week (Day 7 total bilirubin 74 μmol/L *vs* 49 μmol/L, P = 0.012). In both univariate and multivariate regression analysis, Day 7 total bilirubin > 55 μmol/L was associated with the development of biliary stricture formation. In addition, hepatic artery thrombosis and primary sclerosing cholangitis were identified as independent risk factors.

CONCLUSION

In addition to known risk factors, bilirubin levels in the early post-operative period could be used as a clinical indicator for biliary stricture formation.

Key words: Biliary stricture; Liver transplantation; Bilirubin; Anastomotic stricture; Ischemic type biliary lesion; Magnetic resonance cholangiopancreatography

Core tip: Biliary stricture formation post liver transplantation is a frequent cause for patient morbidity and mortality and is referred to as the Achilles' Heel of transplant. Strictures can be anastomotic or non-anastomotic depending on their number and anatomical location. Early stricture identification is key to providing successful treatment options. Known risk factors for biliary stricture formation include surgical technique, bile leak, hepatic artery thrombosis, primary sclerosing cholangitis, donation after circulatory death donors and increased cold ischemic time. This study identifies risk factors and clinical indicators for the development of biliary strictures post liver transplantation. It also discusses the importance of bilirubin and its potential role when implementing surveillance tools for biliary stricture formation post-transplant.

Forrest EA, Reiling J, Lipka G, Fawcett J. Risk factors and clinical indicators for the development of biliary strictures post liver transplant: Significance of bilirubin. *World J Transplant* 2017; 7(6): 349-358 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i6/349.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i6.349

INTRODUCTION

Orthotopic liver transplantation is currently the gold-standard treatment for patients with end-stage liver disease[1,2]. Post-operative biliary complications, in particular biliary stricture formation, are a frequent cause for patient morbidity and mortality and is often referred to as the Achilles' heel of liver transplantation. Hospital re-admissions and clinical interventions used to treat biliary complications post-transplant are also a significant cost to health systems[3]. Despite advances in treatment techniques for biliary strictures post liver transplant, including non-surgical methods, formation is still observed in approximately 5%-32% of recipients[2,4]. Biliary tract complications post liver transplant include anastomotic strictures (AS), non-anastomotic strictures (NAS), bile leaks, stone formation, sludge and sphincter of Oddi dysfunction. It is important to note however, that biliary complications are often sub-clinical and studies have showed approximately 19% of the total number are clinically relevant[4].

Usually manifesting 5-8 mo post-transplant, AS occur when there is a narrowing of the anastomosis between the donor and the recipient bile ducts[4,5]. With a reported incidence of 4%-9%, the development of AS are thought to be associated with biliary ischemia, provoking a localized fibrotic response[5]. In symptomatic patients, treatment options include balloon dilatation or stenting using endoscopic retrograde cholangiography (ERCP) or placement of a biliary drain using percutaneous trans-hepatic cholangiography (PTC). In 16%-32% of patients, surgical interventions including re-operation of the biliary anastomosis or re-transplant are used[6,7]. Published literature indicates that risk factors for AS formation are mainly due to suboptimal surgical technique or the presence of a bile leak in the post-operative period[8].

In contrast, NAS are a narrowing of the biliary duct system at any site outside of the biliary tree and proximal to the biliary anastomosis, this can be both extra-hepatic and intra-hepatic. The pathophysiology remains largely unknown, however, fibrosis following injury to the biliary epithelium is the proposed pathological process for the development of NAS. Macroangiopathy and microangiopathy are two proposed etiologies of NAS. Those NAS occurring within the first year of transplant are thought to be associated with hepatic artery thrombosis (HAT), those that occur without HAT are often referred to as ischemic type biliary lesions. The incidence of NAS is varied, with 1%-20% incidence reported in the literature[9]. NAS are complex due to their location, often occurring in multiples and are longer in length. Due to complicated management issues, morbidity and mortality related to NAS is higher compared with AS[10]. Known risk factors for NAS formation include hepatic artery thrombosis, chronic ductopenic rejection, ABO incompatibility, primary sclerosing cholangitis (primary pathology), donation after circulatory death donors (DCD), prolonged use of vasopressors, older age of

donor, preservation injury and prolonged cold and warm ischemia times[5,11]. Endoscopic treatment methods, including ERCP with balloon dilatation and stenting, are also used to treat NAS, however, patients often require multiple treatments with a reported 50%-75% success rate[5,12]. Secondary graft loss is common with up to 50% of patients experiencing graft loss and either requiring re-transplant or succumb to their illness whilst waiting for a life-saving re-transplant[13-15].

The aim of this study was to identify risk factors and clinical indicators associated with the formation of biliary strictures post orthotopic liver transplantation in the state of Queensland, Australia over a 10-year period. In addition to this, the study aimed to investigate potential post-transplant surveillance methods that could be used to identify patients at risk of biliary stricture formation.

MATERIALS AND METHODS

Study population

We retrospectively analyzed all adult liver transplant recipients in the state of Queensland, Australia between 1st January 2005 and 31st December 2014 with studied follow up until 30th June 2015. Transplants analyzed consisted of varied graft types, including whole liver, right split and heart-liver-lung (HLL). HLL graft types were excluded from this study as these transplants were performed and followed up at a different transplant center within the state of Queensland (n = 5). Of the transplants studied, 25 were repeat liver transplants. De-identified donor and recipient data was collected from internal hospital records. The study protocol was approved by the Human Research Ethics Committee for the state of Queensland, Australia (HREC/13/QPAH/382) as well as the University of Queensland Ethics Committee (2015001248). In addition, approval was obtained from the Queensland Government to access confidential patient information, held by Queensland Health, for the Purpose of Research under the provision of section 280 of the Public Health Act 2005.

Organ retrieval process

To prevent coagulopathy, the organ retrieval process routinely involved a 25000 IU flush of Heparin into the donor. The dopamine antagonist Chlorpromazine was used in addition to Heparin as per the discretion of the retrieval surgeon. Rapid cooling of organs was achieved by the instigation of a 2 L cold saline flush, followed by University of Wisconsin cold storage solution (UW Solution) through aortic and portal vein cannulas. Organs were transported in static cold storage prior to transplantation.

Transplant procedure and post-operative care

Orthotopic transplants at our center was exclusively performed using the piggyback technique. Right split grafts were transplanted using either the piggyback or venovenous bypass technique if indicated. A 1 L saline flush of the liver was infused during the inferior vena cava (IVC) anastomosis. Prior to reperfusion, 500 mL of blood was vented from the IVC. Hepatic artery anastomosis was performed after reperfusion had occurred. Right split liver transplant was performed using the piggy back. Venous-arterial extension grafts were used when required for both whole and right split liver transplantation. The biliary anastomosis was performed using one of two methods, Roux-en-Y hepaticojejunostomy or end-to-end choledochocholedochostomy. Use of each method was based on consideration of the patient's past medical history and surgeon preference. Early in our cohort, T-tubes were routinely inserted to drain bile in transplant recipients, however, these were later replaced by silicon stents, used at the surgeons' discretion. The macrolide calcineurin inhibitor, Tacrolimus, was used in conjunction with oral corticosteroids and Azathioprine post-transplant. Tacrolimus dose was titrated based on blood levels, a therapeutic level of > 8 but < 10 μg/L was considered optimal. Patients were initially followed up daily in an outpatient clinic post hospital inpatient discharge, following this twice weekly if three week's post-transplant, weekly if two months post-transplant, monthly if three months post-transplant and finally with third monthly blood tests if 12-mo post-transplant.

Data collection

Donor and recipient demographic data associated with the formation of biliary strictures was collected for this study. For recipients, this included age, gender, body mass index, reason for transplant, previous transplant and follow-up period. Donor demographic data included age, gender, body mass index, cause of death, donor type and cause of death. In addition to these parameters, intraoperative data was collected, including cold ischemic time (CIT), warm ischemic time (WIT), hepatic artery warm ischemic time, time of portal vein anastomosis, type of biliary anastomosis performed and the use of *T*-drains. Post-operative Day 0 to Day 7 liver functions tests, including total bilirubin were also collected.

Complications, treatments and outcomes

Biliary stricture formation and the time frame that this occurred post-transplant were classified into the following categories; anastomotic stricture, ischemic type biliary stricture (ITBS) and recurrence of primary sclerosing cholangitis. For continuity of diagnosis, biliary stricture identification was made by an experienced transplant surgeon (JF) in our center using patient records and visualization of radiological imaging and reports. A stricture was defined as a narrowing of the bile duct with dilatation of the proximal biliary duct. No strict diameter cut-offs were used to define the structure. Routine post-operative magnetic resonance cholangiopancreatography (MRCP) was not performed at our center. Instead, imaging is guided by patient symptomatology. For the purposes of this study, a

Table 1 Baseline donor and recipient characteristics *n* (%)

Characteristics	Overall (*n* = 296)	Biliary strictures (*n* = 45)	Nil biliary strictures (*n* = 251)	*P* value
Donor characteristics				
Age (yr)	42 (28-54)	48 (40-57)	42 (27-54)	NS
Gender (male)	165 (55.7)	25 (55.6)	140 (55.8)	NS
BMI (kg/m^2)				NS
< 18.5	5 (1.7)	0 (0.0)	5 (2.0)	
18.5-24.9	137 (46.3)	18 (40.0)	119 (47.4)	
25-29.9	117 (39.5)	20 (44.4)	97 (38.6)	
> 30	37 (12.5)	7 (15.5)	30 (12.0)	
Donor type				NS
Donation after brain death	285 (96.3)	44 (97.8)	241 (96.0)	
Donation after circulatory death	11 (3.7)	1 (2.2)	10 (4.0)	
Cause of death				NS
Stroke	154 (52.0)	29 (64.4)	125 (49.8)	
Hypoxia	43 (14.5)	6 (13.3)	37 (14.7)	
Accident	37 (12.5)	3 (6.7)	34 (13.5)	
Other	62 (20.9)	7 (15.6)	55 (21.9)	
Recipient characteristics				
Age at transplant (yr)	52 (45-57)	53 (40-58)	52 (45-57)	NS
Gender (male)	207 (69.9)	31 (68.8)	176 (70.1)	NS
BMI (kg/m^2) (*n* = 267)	267	44	223	NS
Underweight (⩽ 18.5)	5 (1.9)	0 (0.0)	5 (2.0)	
Normal weight (18.5-24.9)	94 (35.2)	17 (38.6)	77 (30.7)	
Overweight (25-29.9)	93 (34.8)	15 (34.1)	78 (31.1)	
Obese ⩾ 30	75 (28.1)	12 (27.3)	63 (25.1)	
Reason for transplant				NS
Viral hepatitis (B, C) ± hepatocellular carcinoma	124 (41.9)	19 (42.2)	105 (41.8)	
Hepatocellular carcinoma without hepatitis	22 (7.4)	1 (2.2)	21 (8.4)	
Alcohol	33 (11.1)	6 (13.3)	27 (10.8)	
Biliary[1]	29 (9.8)	8 (17.8)	21 (8.4)	
Non-alcoholic steatohepatitis	14 (4.7)	3 (6.7)	11 (4.4)	
Acute/fulminant liver failure	12 (4.1)	2 (4.4)	10 (4.0)	
Complications first transplant	21 (7.1)	3 (6.7)	18 (7.2)	
Other	41 (13.8)	3 (6.7)	38 (15.1)	
Previous transplant	25 (8.5)	5 (11.1)	20 (8.0)	NS

[1]Biliary - Primary sclerosing cholangitis, primary biliary cirrhosis. BMI: Body mass index; NS: Not significant.

biliary stricture was considered a true stricture only in those requiring intervention. The requirement for post-operative ERCP and percutaneous drainage was recorded. In addition to this, we examined the number of patients who required re-transplantation and those who ultimately died as a result of biliary complications. The incidence of hepatic artery thrombosis was also established.

Statistical analysis

All continuous variables are expressed as median (interquartile range) and all categorical variables as frequency (percentage)[16]. Multivariable logistical regression analysis was performed to determine the risk factors for biliary strictures following transplantation[17]. All factors with a *P*-value of 0.1 or less in univariable regression were included in the model. A *P*-value of < 0.05 was considered statistically significant. Statistical analysis was performed using IMB SPSS Statistics for Macintosh, Version 23.0 (IBM Corp. IMB SPSS statistics, Armonk, NY). No external statistical review was obtained.

RESULTS

Population characteristics

Demographic data on donors and recipients are shown in Table 1. Between 1st January 2005 and 31st December 2014, a total of 296 patients underwent liver transplantation (Table 1). The average age of liver donors that developed biliary complications was higher than those that did not (48 and 42 years, *P* = 0.10). Most donors either had a normal BMI or were overweight, 137 (46.3%) and 117 (39.5%) respectively. Of the 11 DCD donors in the cohort, the majority did not develop biliary strictures (*n* = 10). Stroke was the leading cause of death in both groups, but was more substantial in the biliary stricture group (64.4% *vs* 49.8%, *P* = 0.28).

Liver transplant recipients had a median age of 52 years, the majority were male. Overweight or obese patients accounted for 93 (34.8%) and 75 (28.1%) of the recipient population respectively. The indication for transplantation did not differ between the two groups, with viral hepatitis B, C with or without hepatocellular carcinoma accounting for approximately

Table 2 Transplant procedure characteristics *n* (%)

Characteristic	Total (*n* = 296)	Biliary strictures (*n* = 52)	Nil biliary strictures (*n* = 244)	*P* value
Cold ischemic time (min)	415 (308-520)	414 (319-530)	415 (307-520)	NS
Warm ischemic time (min)	27 (23-32)	28 (23-33)	27 (23-32)	NS
Time until hepatic artery anastomosis (min)	74 (61-88)	70 (60-86)	74 (61-89)	NS
Time between portal vein and hepatic artery anastomosis (min)	47 (35-60)	41 (36-57)	48 (35-60)	NS
Anastomosis used			246	NS
Duct to duct	213 (72.0)	29 (64.4)	184 (73.3)	
Roux-en-Y	78 (26.4)	16 (35.6)	62 (24.7)	
T-drain used (*n* = 231)	23 (7.8)	3 (6.7)	19 (8.0)	NS

NS: Not significant.

Table 3 Interventions required per type of biliary stricture *n* (%)

Complication	Total frequency (*n* = 45)[1]	Requiring intervention (ERCP/PTC) (*n* = 29)[1]	Reoperation of biliary anastomosis (*n* = 22)[1]	Retransplant (*n* = 12)[1]
Anastomotic stricture	25 (55.6)	17 (58.6)	15 (68.2)	2 (16.7)
< 6 mo	15 (33.3)	10 (34.4)	8 (36.4)	1 (8.3)
> 6 mo	10 (22.2)	7 (24.1)	7 (31.8)	1 (8.3)
Right split graft	3 (6.7)	1 (3.4)	2 (9.1)	1 (8.3)
Ischemic type biliary stricture[2]	10 (22.2)	7 (24.1)	6 (27.3)	6 (50.0)
< 6 mo	8 (17.8)	5 (17.2)	5 (22.7)	5 (41.7)
> 6 mo	2 (4.4)	2 (6.9)	1 (4.5)	1 (8.3)
DCD donor	1 (2.2)	1 (3.4)	1 (4.5)	1 (8.3)
Right split graft	3 (6.7)	2 (6.9)	1 (4.5)	1 (8.3)
PSC recurrence	8 (17.8)	3 (10.3)	2 (9.1)	1 (8.3)
Ischemic cholangiopathy due to HAT	3 (6.7)	3 (10.3)	0 (0.0)	3 (25.0)
Total patients[1]	46 (100.0)	0 (100.0)[3]	23 (100.0)	12 (100.0)

[1]One patient had two complications; [2]Excluding hepatic artery thrombosis, including primary sclerosing cholangitis and ischemic type biliary stricture with anastomotic stricture; [3]Some patients were represented more than once as they underwent two interventions for biliary stricture formation. PSC: Primary sclerosing cholangitis; HAT: Hepatic artery thrombosis; DCD: Donation after circulatory death; ERCP: Endoscopic retrograde cholangiography; PTC: Percutaneous trans-hepatic cholangiography.

40% of transplants. Recipients demographic data overall did not differ when comparing those with or without biliary complications. Of the parameters analyzed for this study, 98.5% of the data was available.

Transplant procedure characteristics

Table 2 presents the data on the transplant procedure characteristics, comparing those with and without biliary complications. Overall, no significant difference were found between the two groups.

Biliary stricture formation and treatment

A total of 45 (15.2%) recipients developed biliary strictures throughout the study period. One patient developed two complications. Anastomotic stricture formation was the commonest complication with 15 (33.3%) of these occurring within 6-mo of transplantation (Table 3). Anastomotic stricture formation was the leading cause for intervention with ERCP/PTC and reoperation of the biliary anastomosis (17, 58.6% and 15, 68.2% respectively) (Table 3). The development of ITBS accounted for 22.2% of all biliary stricture formation. ITBS were the primary indication with five (41.7%) patients undergoing an additional transplant within the first six-months of initial graft placement. Some patients were represented more than once as they underwent two interventions for biliary stricture formation. Serum Tacrolimus levels on post-operative Days 1-7 were found not to be significantly associated with the development of biliary strictures.

Risk factors for biliary stricture formation

In recipients developing biliary strictures, total bilirubin was significantly increased within the first post-operative week (Day 7 total bilirubin 74 μmol/L *vs* 49 μmol/L, P = 0.012) (Figure 1). In both univariate and multivariate regression analysis, Day 7 total bilirubin > 55 μmol/L was associated with the development of biliary stricture formation (Table 4) with an odds ratio of 2.54 (1.22-5.29), P = 0.013. In addition, hepatic artery thrombosis and primary sclerosing cholangitis were identified as independent risk factors for biliary stricture formation (OR = 25.23, $P \leqslant 0.001$ and OR = 3.10, P = 0.028, respectively). The sensitivity and specificity

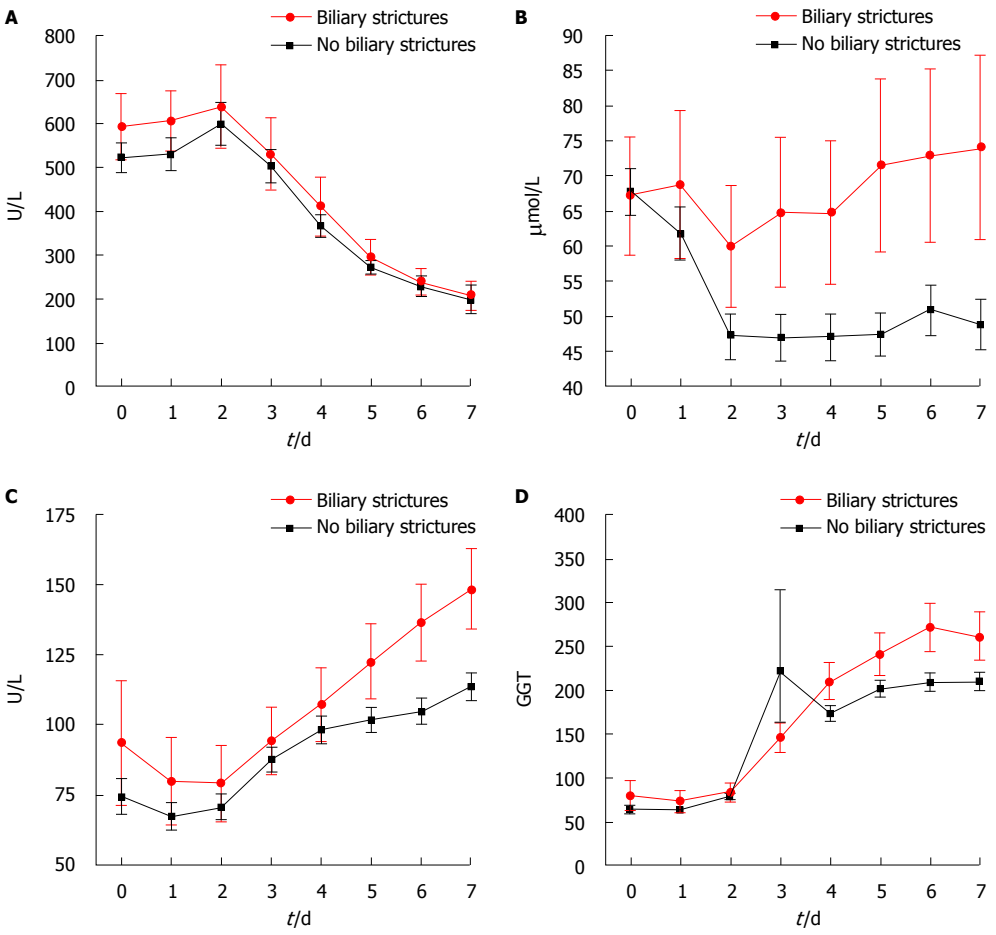


Figure 1 Post-transplant Days 0-7 liver function tests and serum total bilirubin. This graph demonstrates the trends in A: Serum alanine transaminase; B: Serum total bilirubin; C: Serum alkaline phosphatase; D: Serum γ-Glutamyl transferase (biliary strictures: Day 7 total bilirubin 74 μmol/L and nil biliary stricture: 49 μmol/L, P = 0.012) in recipients with biliary strictures (red line) and recipients without biliary strictures (black line) over post liver transplant Day 0-7.

Table 4 Risk factors associated with biliary stricture formation

Characteristic	Univariate regression		Multivariate regression	
	Odds ratio (95%CI)	*P* value	Odds ratio (95%CI)	*P* value
Biliary strictures (n = 45)[1]				
Donor age > 55 yr	1.54 (0.80-3.00)	NS		
Cause of death - stroke	1.88 (1.00-3.52)	0.049	1.49 (0.72-3.08)	NS
Donation after circulatory death	0.55 (0.07-4.39)	NS		
Split *vs* whole graft	1.09 (0.43-2.79)	NS		
Primary sclerosing cholangitis as primary indication for transplant	2.40 (0.82-7.07)	0.11	3.10 (1.13-8.51)	0.028
Hepatic artery thrombosis	22.93 (4.59-114.52)	< 0.001	25.23 (4.73-143.64)	< 0.001
⩾ 2 prior transplants	1.44 (0.51-4.07)	NS		
Day 7 total bilirubin > 55 μmol/L	2.19 (1.11-4.30)	0.024	2.54 (1.22-5.29)	0.013

[1]Inclusive of anastomotic, ischemic type biliary strictures, primary sclerosing cholangitis and ischemic cholangiopathy due to hepatic artery thrombosis. NS: Not significant.

for Day 7 total bilirubin > 55 μmol/L was 38.6% and 77.6%, respectively.

DISCUSSION

In this study, we analyzed the risk factors associated with biliary stricture formation post liver transplant in an Australian cohort. Total bilirubin in the direct post-operative period was found to be associated with the development of biliary strictures, especially at post-operative Day 7.

The development of post-operative biliary strictures is still regarded as the Achilles' heel of liver transplantation, causing significant patient morbidity and mortality.

This study supports this statement, as it demonstrated an overall incidence of biliary stricture formation post deceased liver donor transplantation in an Australian center at 15%. This is comparable to other reported literature globally, with a 5%-15% incidence reported in deceased donors and a 28%-32% incidence reported in living liver donors[18]. These comparable incidence rates of biliary stricture formation are of interest, as unlike other overseas hospitals, our center does not routinely screen for biliary stricture formation with modalities such as MRCP. Instead, regular ultrasounds are used in the immediate inpatient setting. After this period, our center only images patients that are symptomatic or in those in which a stricture is suspected.

It is difficult to predict the formation of biliary strictures in adult patients post liver transplant. MRCP is a non-invasive technique that enables detailed visualization of the biliary tree, however is a limited and expensive resource[19]. Through analyzing post-operative recipient blood work, total bilirubin was found to be elevated in those that developed biliary strictures, in particular, a total bilirubin level > 55 μmol/L on post-operative Day 7. These findings suggest that total bilirubin levels could be used as an inexpensive tool to identify those patients more at risk of biliary stricture formation and these patients could potentially benefit from a surveillance MRCP.

It is important to note that bilirubin has previously been recognized as a significant marker in the identification of liver graft dysfunction and graft survival. This is demonstrated in the currently accepted definition of early allograft dysfunction which includes one or more of the following variables: (1) total bilirubin ⩾ 171 μmol/L on post-operative day 7; (2) INR ⩾ 1.6 on post-operative Day 7; and (3) an aminotransferase level (ALT or AST) ⩾ 2000 IU/mL within the first seven post-operative days[20]. To support this, a study conducted by Wagener *et al*[21] concluded that elevated total bilirubin levels on post-operative Day 0-7 significantly correlated with graft dysfunction within the first 90 day post-operatively. This study went on further to report post-operative Days 1-2 bilirubin > 112 μmol/L should warn clinicians of potential EAD[21]. On the other hand, Olthoff *et al*[20] contradicts this statement suggesting that elevated total bilirubin levels on post-operative Days 1-3 should be excluded when predicting EAD as these values may reflect the pre-transplant status and not graft functionality.

The underlying mechanism of the association between elevated total bilirubin levels at Day 7 post-transplantation and biliary stricture formation remains to be determined. Previous studies have identified a more toxic bile composition in recipients developing non-anastomotic biliary strictures[22,23]. Furthermore, prolonged graft ischaemia was found to cause an unparalleled impairment of bile acid transporter expression in cholangiocytes leading to prolonged biliary transit time of bile acids inducing apoptosis[24,25]. Although not formally assessed in this study, increased total bilirubin levels at Day 7 post transplantation might be the results of impaired bile transporter function post transplantation and associated increased in bile toxicity resulting in stricture formation.

Currently, the literature reports biliary stricture formation presents as a later complication, between 5-8 mo post-transplant[18]. This is dependent on the type of stricture that has formed, with non-anastomotic stricture presenting between 3.3-5.9 mo[26,27]. Our study demonstrated AS formation as the most common complication (55.6%), with 33.3% of these forming within the first six-months post-transplant. Therefore, the proposed surveillance MRCP should be completed within the first three to six-months post-transplant.

Risk factors for biliary stricture formation have been well documented in the literature and are multifactorial including factors related to the recipient, donor and operative characteristics[5,11,28]. In line with previous findings, the results of our study identified stroke as donor cause of death, hepatic artery thrombosis (OR = 22.93) and Day 7 total bilirubin > 55 μmol/L as significant risk factors for biliary stricture formation on univariate regression. Upon multivariate analysis, PSC as primary indication for transplantation, HAT and Day 7 total bilirubin > 55 μmol/L were all significant risk factors. Specific donor characteristics, such as increased donor age (> 55 years) and DCD donor type were not found not to be a significant risk factor in the formation of biliary strictures in this study which could be due to our small cohort size and relative underrepresentation of DCD donor grafts.

In our cohort, we had a low percentage of ITBS compared to previous reports in the literature[29]. It was found that radiologically, it is difficult to distinguish between the development of PSC recurrence and ITBS formation[29]. For the purposes of this study, the investigator classified these questionable lesions as PSC recurrence, this in turn could have underestimated the presence of ITBS on our data set. Another point to note is that as DCD donation is infrequently used in our transplant center and therefore we were unable to assess this as a risk factor for stricture formation. Previously, the risk of biliary complications and ischemic cholangiopathy has been found to be significantly increased in DCD donors by Foley *et al*[19]. As immunological factors have been associated with biliary stricture formation, post-operative Days 1-7 serum Tacrolimus levels were measured by found not to be significantly associated with the development of biliary strictures.

Currently, there is no clear consensus as to which anastomotic reconstruction technique (duct-to-duct *vs* Roux-en-Y) of the biliary system is superior regarding biliary stricture formation. It is important to note that the surgical technique used if often dependent on the indication for transplant (*e.g.,* PSC with previous diseased bile duct) or split liver graft and is usually weighed up against the need to restore original anatomy[11]. In saying this, a running suture, without a bile tube has been proven to be of benefit in preventing early

biliary complications[30]. Although a greater percentage of patients that underwent Roux-en-Y anastomosis developed biliary complications compared to those in the end to end anastomosis group, Roux-en-Y was not found to be a significant risk factor for biliary stricture formation. Our study demonstrated a higher incidence of biliary stricture formation in the Roux-en-Y technique, with this being the less common method used at our center (26.4%). T-tube use was not associated with biliary stricture formation.

In addition, recipient WIT has been identified as a risk factor for non-anastomotic biliary stricture formation[31]. It has previously been found that there is a 2.64 ($P \leqslant 0.01$) relative risk of developing non-anastomotic biliary strictures with every hour increase of WIT[14]. Our study did not identify CIT or WIT as a risk factor for biliary stricture formation, with the average CIT and WIT being comparable. Again, the smaller cohort represented in our study may have accounted for this finding.

Our study was limited by the fact that it consisted of a relatively small cohort and because data was collected retrospectively collected and some cases were not documented appropriately or contained missing data. Overall 98.5% of parameters included in the total dataset were available for analysis. Furthermore, due to the limited number of patients in our cohort that received right split grafts (n = 37, 12.5%), we were unable to draw comparisons between whole and split liver grafts on risk of biliary stricture formation. Wan *et al*[32] demonstrated an OR = 0.64 favoring right split grafts compared to whole grafts in the formation of biliary strictures post liver transplant in adults. Similarly, only 11 patients received a DCD liver graft in out cohort. In conclusion, the incidence of biliary stricture formation post liver transplant in our center was 15%. Serum total bilirubin levels > 55 μmol/L at Day 7 post-operatively were associated with an increased risk of stricture formation, suggesting that bilirubin could be used to identify those that need closer surveillance following liver transplantation.

ARTICLE HIGHLIGHTS

Research background

Liver transplantation is a lifesaving surgical procedure available to those eligible with end-stage liver failure. Biliary strictures can cause a disruption in the flow of bile and formation post liver transplantation is a frequent cause for patient morbidity and mortality. Due to the significant burden of disease biliary strictures cause, those patients with biliary strictures often require either endoscopic intervention, surgical re-do of the anastomosis or even re-transplantation.

Research motivation

Biliary strictures post liver transplantation can be classified into two categories, non-anastomotic stricture and anastomotic stricture. Non-anastomotic strictures are often difficult to treat. They are associated with worse outcomes as they often present in numbers and are situated anatomically in a difficult to access location outside the biliary tree. Earlier identification and subsequent treatment of biliary strictures post liver transplant have been associated with improved patient outcomes and decrease the need for re-transplant. Current identified risk factors for biliary stricture formation post liver transplant include sub-optimal surgical technique, the presence of bile leak, hepatic artery thrombosis, primary sclerosing cholangitis, donation after circulatory death donors and prolonged cold or warm ischemic time. Identifying risk factors and clinical indicators for the development of biliary strictures would allow clinicians to identify at risk patients and potentially predict stricture formation. This would allow for earlier treatment of strictures, improving clinical patient care and allograft survival.

Research objectives

This study investigates the risk factors and clinical indicators associated with biliary stricture formation post liver transplantation. In order to translate these findings clinically, this study also aimed to describe potential surveillances method for biliary strictures formation post liver transplantation. These clinical tools would allow for the early identification and treatment of biliary strictures, with the aim of improving patient outcomes.

Research methods

Electronic data for this study was collected retrospectively on all liver donors and recipients in the state of Queensland between 2005 and 2014. Within this data set we analyzed demographic, intra-operative and post-operative characteristics of each procedure. In addition, post-operative liver function tests, serum bilirubin and Tacrolimus levels were collected from post-operative Days 0 to 7. Biliary stricture formation post-operatively was recorded, the interventions used to treat and their timing was also identified. This study was unique in that is used logistical regression to identify potential risk factors and clinical indicators for biliary stricture formation.

Research results

This study demonstrated the incidence of biliary strictures post liver transplantation at our center at 15%. Significant risk factors for the formation of biliary strictures post-transplant included primary sclerosing cholangitis as the primary indication for transplant and the presence of hepatic artery thrombosis. As a clinical indicator, Day 7 total serum bilirubin > 55 μmol/L was found to be associated with an increased risk of stricture formation. Investigation into potential mechanisms explaining this rise in bilirubin in patients with strictures would be beneficial.

Research conclusions

As well as known risk factors for biliary stricture formation, this study identified Day 7 total serum bilirubin > 55 μmol/L as a significant clinical indicator for the development of biliary strictures post liver transplant. As biliary strictures pose a significant burden of morbidity and mortality on patients post liver transplantation, identifying clinical indicators such as elevated total serum bilirubin for stricture formation is a useful tool to enable clinicians to provide early and more successful care to those transplant recipients more at risk. This study identified previously known risk factors for biliary stricture formation post transplantation including primary sclerosing cholangitis are the primary indication for transplant and the presence of hepatic artery thrombosis. Previous studies have identified elevated bilirubin in the post-operative period as a risk factor for biliary stricture formation. This study adds to this body of evidence as it proposes a specific measure of total serum bilirubin (> 55 μmol/L) that is associated with biliary stricture formation post liver transplant. The results of this study can be translated into clinical practice by applying a clinical algorithm to patients that are considered at higher risk of biliary stricture formation post-transplant. The authors suggest focused surveillance of these patients for biliary stricture formation within the immediate three to six-month post-operative period with a magnetic resonance cholangiopancreatography scan.

ACKNOWLEDGMENTS

The authors would like to acknowledge Cheryl Fourie and Jade Carey from Donate Life and the Australia and New Zealand Organ Donor Registry.

REFERENCES

1 **Boraschi P**, Donati F. Complications of orthotopic liver

transplantation: imaging findings. *Abdom Imaging* 2004; **29**: 189-202 [PMID: 15290945 DOI: 10.1007/s00261-003-0109-8]

2 **Ryu CH**, Lee SK. Biliary strictures after liver transplantation. *Gut Liver* 2011; **5**: 133-142 [PMID: 21814591 DOI: 10.5009/gnl.2011.5.2.133]

3 **Axelrod DA**, Dzebisashvili N, Lentine KL, Xiao H, Schnitzler M, Tuttle-Newhall JE, Segev DL. Variation in biliary complication rates following liver transplantation: implications for cost and outcome. *Am J Transplant* 2015; **15**: 170-179 [PMID: 25534447 DOI: 10.1111/ajt.12970]

4 **Kienlein S**, Schoening W, Andert A, Kroy D, Neumann UP, Schmeding M. Biliary complications in liver transplantation: Impact of anastomotic technique and ischemic time on short- and long-term outcome. *World J Transplant* 2015; **5**: 300-309 [PMID: 26722658 DOI: 10.5500/wjt.v5.i4.300]

5 **Sharma S**, Gurakar A, Jabbour N. Biliary strictures following liver transplantation: past, present and preventive strategies. *Liver Transpl* 2008; **14**: 759-769 [PMID: 18508368 DOI: 10.1002/lt.21509]

6 **Verdonk RC**, Buis CI, Porte RJ, van der Jagt EJ, Limburg AJ, van den Berg AP, Slooff MJ, Peeters PM, de Jong KP, Kleibeuker JH, Haagsma EB. Anastomotic biliary strictures after liver transplantation: causes and consequences. *Liver Transpl* 2006; **12**: 726-735 [PMID: 16628689 DOI: 10.1002/lt.20714]

7 **Holt AP**, Thorburn D, Mirza D, Gunson B, Wong T, Haydon G. A prospective study of standardized nonsurgical therapy in the management of biliary anastomotic strictures complicating liver transplantation. *Transplantation* 2007; **84**: 857-863 [PMID: 17984838 DOI: 10.1097/01.tp.0000282805.33658.ce]

8 **Kitazono MT**, Qayyum A, Yeh BM, Chard PS, Ostroff JW, Coakley FV. Magnetic resonance cholangiography of biliary strictures after liver transplantation: a prospective double-blind study. *J Magn Reson Imaging* 2007; **25**: 1168-1173 [PMID: 17520726 DOI: 10.1002/jmri.20927]

9 **Thethy S**, Thomson BNj, Pleass H, Wigmore SJ, Madhavan K, Akyol M, Forsythe JL, James Garden O. Management of biliary tract complications after orthotopic liver transplantation. *Clin Transplant* 2004; **18**: 647-653 [PMID: 15516238 DOI: 10.1111/j.1399-0012.2004.00254.x]

10 **Verdonk RC**, Buis CI, Porte RJ, Haagsma EB. Biliary complications after liver transplantation: a review. *Scand J Gastroenterol Suppl* 2006; (**243**): 89-101 [PMID: 16782628 DOI: 10.1080/00365520600664375]

11 **Nemes B**, Gámán G, Doros A. Biliary complications after liver transplantation. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 447-466 [PMID: 25331256 DOI: 10.1586/17474124.2015.967761]

12 **Seehofer D**, Eurich D, Veltzke-Schlieker W, Neuhaus P. Biliary complications after liver transplantation: old problems and new challenges. *Am J Transplant* 2013; **13**: 253-265 [PMID: 23331505 DOI: 10.1111/ajt.12034]

13 **Krok KL**, Cárdenas A, Thuluvath PJ. Endoscopic management of biliary complications after liver transplantation. *Clin Liver Dis* 2010; **14**: 359-371 [PMID: 20682241 DOI: 10.1016/j.cld.2010.03.008]

14 **Guichelaar MM**, Benson JT, Malinchoc M, Krom RA, Wiesner RH, Charlton MR. Risk factors for and clinical course of non-anastomotic biliary strictures after liver transplantation. *Am J Transplant* 2003; **3**: 885-890 [PMID: 12814481 DOI: 10.1034/j.1600-6143.2003.00165.x]

15 **Ward EM**, Kiely MJ, Maus TP, Wiesner RH, Krom RA. Hilar biliary strictures after liver transplantation: cholangiography and percutaneous treatment. *Radiology* 1990; **177**: 259-263 [PMID: 2399328 DOI: 10.1148/radiology.177.1.2399328]

16 **Zhang Z**. Univariate description and bivariate statistical inference: the first step delving into data. *Ann Transl Med* 2016; **4**: 91 [PMID: 27047950 DOI: 10.21037/atm.2016.02.11]

17 **Zhang Z**. Model building strategy for logistic regression: purposeful selection. *Ann Transl Med* 2016; **4**: 111 [PMID: 27127764 DOI: 10.21037/atm.2016.02.15]

18 **Cerný V**, Daniel M. Occurrence of the tick Ixodes ricinus in a landscape strongly influenced by human activities. *Wiad Parazytol* 1986; **32**: 351-353 [PMID: 3660810]

19 **Foley DP**, Fernandez LA, Leverson G, Anderson M, Mezrich J, Sollinger HW, D'Alessandro A. Biliary complications after liver transplantation from donation after cardiac death donors: an analysis of risk factors and long-term outcomes from a single center. *Ann Surg* 2011; **253**: 817-825 [PMID: 21475025 DOI: 10.1097/SLA.0b013e3182104784]

20 **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]

21 **Wagener G**, Raffel B, Young AT, Minhaz M, Emond J. Predicting early allograft failure and mortality after liver transplantation: the role of the postoperative model for end-stage liver disease score. *Liver Transpl* 2013; **19**: 534-542 [PMID: 23576469 DOI: 10.1002/lt.23634]

22 **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]

23 **Op den Dries S**, Sutton ME, Lisman T, Porte RJ. Protection of bile ducts in liver transplantation: looking beyond ischemia. *Transplantation* 2011; **92**: 373-379 [PMID: 21629175 DOI: 10.1097/TP.0b013e318223a384]

24 **Cheng J**, Zhou L, Jiang JW, Qin YS, Xie HY, Feng XW, Gao F, Zheng SS. Proteomic analysis of differentially expressed proteins in rat liver allografts developed acute rejection. *Eur Surg Res* 2010; **44**: 43-51 [PMID: 19996597 DOI: 10.1159/000264602]

25 **Cheng L**, Tian F, Tian F, Tang L, Chen G, Luo Z, Ren J, Wang S. Repression of Farnesoid X receptor contributes to biliary injuries of liver grafts through disturbing cholangiocyte bile acid transport. *Am J Transplant* 2013; **13**: 3094-3102 [PMID: 24266967 DOI: 10.1111/ajt.12479]

26 **Rerknimitr R**, Sherman S, Fogel EL, Kalayci C, Lumeng L, Chalasani N, Kwo P, Lehman GA. Biliary tract complications after orthotopic liver transplantation with choledochocholedochostomy anastomosis: endoscopic findings and results of therapy. *Gastrointest Endosc* 2002; **55**: 224-231 [PMID: 11818927 DOI: 10.1067/mge.2002.120813]

27 **Pfau PR**, Kochman ML, Lewis JD, Long WB, Lucey MR, Olthoff K, Shaked A, Ginsberg GG. Endoscopic management of postoperative biliary complications in orthotopic liver transplantation. *Gastrointest Endosc* 2000; **52**: 55-63 [PMID: 10882963 DOI: 10.1067/mge.2000.106687]

28 **Lemmer ER**, Spearman CW, Krige JE, Millar AJ, Bornman PC, Terblanche J, Kahn D. The management of biliary complications following orthotopic liver transplantation. *S Afr J Surg* 1997; **35**: 77-81 [PMID: 9267176]

29 **Buis CI**, Hoekstra H, Verdonk RC, Porte RJ. Causes and consequences of ischemic-type biliary lesions after liver transplantation. *J Hepatobiliary Pancreat Surg* 2006; **13**: 517-524 [PMID: 17139425 DOI: 10.1007/s00534-005-1080-2]

30 **Wojcicki M**, Lubikowski J, Klek R, Post M, Jarosz K, Białek A, Wunch M, Czuprynska M. Reduction of biliary complication rate using continuous suture and no biliary drainage for duct-to-duct anastomosis in whole-organ liver transplantation. *Transplant Proc* 2009; **41**: 3126-3130 [PMID: 19857693 DOI: 10.1016/j.transproceed.2009.07.091]

31 **Koneru B**, Sterling MJ, Bahramipour PF. Bile duct strictures after liver transplantation: a changing landscape of the Achilles' heel. *Liver Transpl* 2006; **12**: 702-704 [PMID: 16628684 DOI: 10.1002/lt.20753]

32 **Wan P**, Li Q, Zhang J, Xia Q. Right lobe split liver transplantation versus whole liver transplantation in adult recipients: A systematic review and meta-analysis. *Liver Transpl* 2015; **21**: 928-943 [PMID: 25832308 DOI: 10.1002/lt.24135]

P- Reviewer: Guo JS, Hua YP, Sugawara Y, Tsoulfas G, Zhang ZH
S- Editor: Cui LJ **L- Editor**: A **E- Editor**: Yan JL

World Journal of Transplantation

Submit a Manuscript: http://www.f6publishing.com

World J Transplant 2017 December 24; 7(6): 359-363

DOI: 10.5500/wjt.v7.i6.359

ISSN 2220-3230 (online)

CASE REPORT

Mucocele mimicking a gallbladder in a transplanted liver: A case report and review of the literature

Thomas Chaly, Jeffrey Campsen, Ryan O'Hara, Rulon Hardman, Juan F Gallegos-Orozco, Heather Thiesset, Robin D Kim

Thomas Chaly, Jeffrey Campsen, Robin D Kim, Division of Transplantation and Advanced Hepatobiliary Surgery, University of Utah, School of Medicine, Salt Lake City, UT 84132, United States

Ryan O'Hara, Rulon Hardman, Division of Radiology, University of Utah, School of Medicine, Salt Lake City, UT 84132, United States

Juan F Gallegos-Orozco, Division of Hepatology, University of Utah, School of Medicine, Salt Lake City, UT 84132, United States

Heather Thiesset, Department of Surgery, University of Utah, School of Medicine, Salt Lake City, UT 84132, United States

ORCID number: Thomas Chaly (0000-0002-5254-5606); Jeffrey Campsen (0000-0002-4112-585X); Ryan O'Hara (0000-0001-7042-0535); Rulon Hardman (0000-0003-4213-0084); Juan F Gallegos-Orozco (0000-0001-7042-0471); Heather Thiesset (0000-0002-7820-8229); Robin D Kim (0000-0002-7820-8229).

Author contributions: All authors listed have contributed to this manuscript as described by the criteria in the authorship guidelines of the International Committee of Medical Journal Editors (ICJME).

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: The authors declare that there is no conflict of interest regarding the publication of this article.

Manuscript source: Unsolicited manuscript

Correspondence to: Thomas Chaly, MD, Division of Transplantation and Advanced Hepatobiliary Surgery, University of Utah, School of Medicine, 30 N. 1900 E. 3B110, Salt Lake City, UT 84132, United States. thomas.chaly@hsc.utah.edu
Telephone: +1-801-5852708

Received: July 15, 2017
Peer-review started: July 20, 2017
First decision: September 5, 2017
Revised: November 30, 2017
Accepted: December 5, 2017
Article in press: December 6, 2017
Published online: December 24, 2017

Abstract

Biliary mucoceles after deceased donor liver transplantation are a rarity, and mucoceles mimicking a gallbladder from the recipient remnant cystic duct have not been described until this case. We describe a 48-year-old male who presented with right upper quadrant pain and was found to have a recipient cystic duct mucocele 3 mo after receiving a deceased donor liver transplant. We describe the clinical presentation, laboratory and imaging findings (including the appearance of a gallbladder), multidisciplinary approach and surgical resolution of this mucocele originating from the recipient cystic duct, and a review of the literature.

Key words: Liver; Transplantation; Mucocele; Complications post-transplant

Core tip: Biliary mucoceles after deceased donor liver transplantation are a rarity, and mucoceles mimicking a gallbladder from the recipient remnant cystic duct have not been described until this case. We describe a 48-year-old male who presented with right upper quadrant pain and was found to have a recipient cystic

duct mucocele 3 mo after receiving a deceased donor liver transplant. We describe the clinical presentation, laboratory and imaging findings (including the appearance of a gallbladder), multidisciplinary approach and surgical resolution of this mucocele originating from the recipient cystic duct, and a review of the literature.

Chaly T, Campsen J, O'Hara R, Hardman R, Gallegos-Orozco JF, Thiesset H, Kim RD. Mucocele mimicking a gallbladder in a transplanted liver: A Case report and review of the literature. *World J Transplant* 2017; 7(6): 359-363 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i6/359.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i6.359

INTRODUCTION

Orthotopic deceased donor liver transplantation has become the standard of care for patients with end stage liver disease secondary to hepatitis C virus (HCV) and hepatocellular cancer (HCC). The standard biliary anastomosis is a duct-to-duct anastomosis performed in either a running or interrupted fashion using absorbable suture. In addition, a donor cholecystectomy is routinely performed after reperfusion and in instances of a long recipient cystic duct, this duct is either opened or over sewn. We present a case of a 48-year-old male who underwent an uncomplicated deceased donor liver transplant and was found to have a mucocele which mimicked a gallbladder 3 mo post-operatively. A biliary mucocele is a complication after deceased donor liver transplantation that has not been well described in the literature. Furthermore, mucoceles from the recipient *vs* donor remnant cystic duct have not yet been described. Such a biliary abnormality can be difficult for the surgeon to both diagnose and treat. It can be extremely difficult to make the diagnosis of a recipient duct *vs* a donor duct mucocele preoperatively even with excellent imaging.

CASE REPROT

A 48-year-old man with end stage liver disease secondary to HCV and HCC received a liver transplant from a deceased donor, and per routine, a graft cholecystectomy was performed and confirmed by pathology. The donor cystic duct was ligated with a 2-0 silk tie, and the recipient cystic duct was over sewn with a 4-0 proline suture. The patient's post-operative course was unremarkable, and the patient was subsequently discharged on post-operative day 8. The patient did well in the weeks after the transplant with his liver functions tests (LFTs) indicating excellent graft function.

Three months after the transplant, the patient complained of right upper quadrant pain. He was found to have abnormal LFTs, specifically an elevated gamma-glutamyltransferase (GGT), alkaline phosphatase (alk phos), and total bilirubin (T.bili). A liver biopsy showed no signs of rejection or recurrent HCV. A magnetic resonance cholangiopancreatography (MRCP) showed an anastomotic biliary stricture. An endoscopic retrograde cholangiopancreatography (ERCP) demonstrated an anastomotic stricture, a covered stent was placed, and the biliary duct then displayed free flowing contrast through the biliary system (Figure 1). The alk phos, GGT and T.bili normalized.

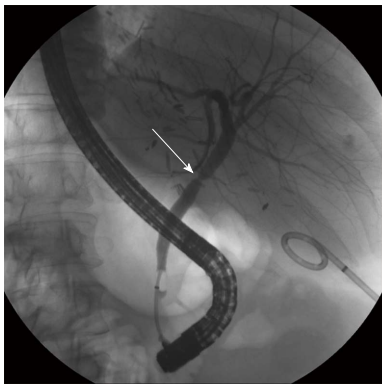


Figure 1 Endoscopic retrograde cholangiopancreatography which showed biliary stricture.

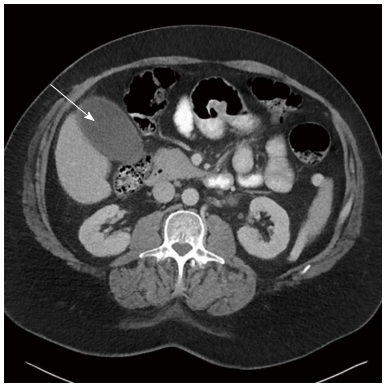


Figure 2 Collection inferior to segment 5 in the donor gall bladder fossa mimicking a gallbladder.

In the 4 wk following the procedures, the patient complained of persistent right upper quadrant pain with associated nausea, vomiting, and intermittent elevated temperatures. A CT scan showed what appeared to be a fluid collection mimicking a gallbladder (Figure 2). The suspected collection was presumed to be either a hematoma or biloma. Given the patients clinical symptoms of right upper quadrant pain, nausea, and vomiting, it was decided to place a drain in the collection *via* interventional radiology.

Following drain placement, bile tinged fluid was extracted, and a biloma was presumed. The patient was asymptomatic following drain placement, and his liver graft function was normal. The patient was seen for a follow up visit in clinic, during which the drain exhibited signs of minimal output and was subsequently removed.

Three weeks following drain removal the patient had a recurrence of his symptoms. His right upper quadrant pain was now increased in caliber, which forced in-

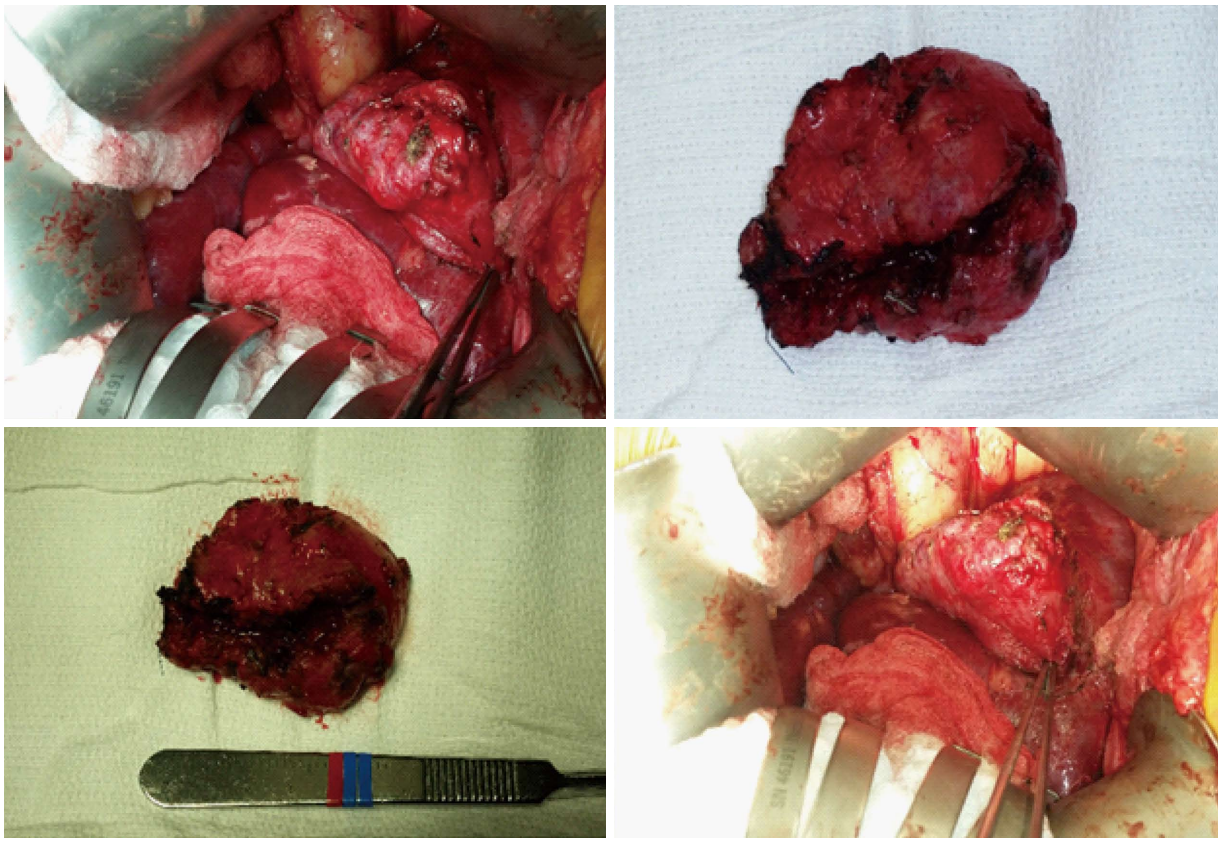


Figure 3 Intraoperative views and gross images of cystic duct mucocele.

patient admission. The patient clinically appeared to have cholangitis. On repeat imaging, the collection had now re-appeared inferior to the liver. A drain was once again placed and had evidence of purulence and bile tinged fluid. In addition to the interventional radiology procedure, the patient had an ERCP for stent removal, which showed resolution of his previous biliary stricture.

A diagnosis of cystic duct remnant mucocele was made given its appearance and recurrence, and ablation with sclerosing agents (ETOH) was attempted by interventional radiology on three separate occasions but was of little benefit. The patient continued to have recurrent symptoms with drain removal and a re-appearance of the mucocele.

After repeated attempts at minimally invasive techniques, 12 mo after transplant, the patient underwent an exploratory laparotomy. Abdominal exploration revealed a mucocele originating from the remnant of the recipient's cystic duct (Figure 3). This was subsequently surgically excised, and the defect was closed with proline suture. The patient did well after the operation with complete resolution of his symptoms. Furthermore, the patient's liver graft function remained excellent, with normalization of his alk phos, GGT and T.bili. The final pathology report revealed a cystic duct mucocele with signs of cholangitis and scarring. The mucosal wall had biliary mucosa (Figure 4).

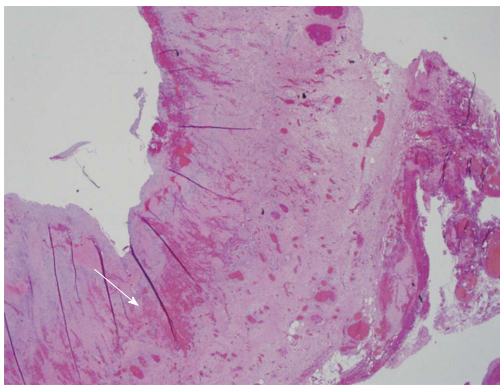


Figure 4 Section shows chronic cholangitis with prominent fibrosis, granulation tissue formation through mucosa, muscularis, and adventitia.

DISCUSSION

Orthotopic liver transplant is an accepted therapy for patients with end stage liver disease. Technical complications remain a significant cause of morbidity and mortality with biliary complications accounting for approximately 13% to 19% of the morbidities. Anastomotic biliary strictures and bile leaks account for most of these[1,2].

It is well known that the junction of the cystic and common hepatic ducts can be variable. In approximately 20% of cases, the cystic duct descends for a considerable distance, either along the right side of, or posterior to, the common duct before joining the common duct[2-4].

The presence of a recipient cystic duct mucocele after deceased donor liver transplant is a very rare anatomical complication. The reported cases in the literature are

few, and they all describe instances in which the donor cystic duct remained long to avoid damage to the donor common bile duct with resultant cystic duct mucocele. Chaterjee *et al*[5] describe a case in which there was high ligation of the donor cystic duct and incorporation of the cystic duct into the anastomosis to facilitate drainage but resulted in a donor duct mucocele nonetheless. Caputo *et al*[2] describe 13 patients in a 13-year history of 283 liver transplants, who developed donor duct mucoceles without encountering a recipient duct mucocele. Most of the reported cases demonstrate a mucocele derived from the donor anatomy as opposed to the recipient anatomy as described here[6-8]. In our case, it is believed that the recipient cystic duct was not adequately oversewn or ligated at the time of the initial liver transplantation. It is also possible that the ERCP stent had inappropriately obstructed the recipient cystic duct causing this mucocele; however, once removed, the mucocele persisted, making this unlikely.

The diagnosis of a mucocele, whether recipient or donor in origin, can be difficult but a rigorous diagnostic evaluation can aid in the diagnosis. With elevated liver enzymes after liver transplantation one must first exclude a vascular insult such as hepatic artery thrombosis or portal vein thrombosis, and an ultrasound with doppler of the liver transplant is necessary. In addition, an ultrasound may provide information on the biliary tree and whether there is intrahepatic biliary dilation indicating a possible biliary stricture. With a normal ultrasound examination, a liver biopsy is an appropriate next step to rule out rejection. With vascular compromise and rejection ruled out, the pathway may proceed in a variety of ways. A cholescintigraphy (HIDA) scan will provide the clinician with information in regard to the uptake and excretion of bile through the intrahepatic and extrahepatic biliary system. In rare instances, it may also reveal a possible mucocele. With a normal HIDA scan, a CT scan of the abdomen will give a better understanding of the hilar anatomy and assist in diagnosing any fluid collections, bilomas, or potential mucoceles. In addition, an MRCP can also aid in this diagnosis and exclude any biliary strictures. Given the condensed area of the hepatic hilum, it is difficult to distinguish the actual origin of the mucocele. If the mucocele were small and/or compressing the common duct, similar to Mirizzi's syndrome, this would point to the diagnosis of a donor duct mucocele. If there was no compression on the common duct and scans gave the impression of a remnant gall bladder, this may point to a recipient duct origin. From a clinical standpoint, however, the constellation of symptoms between a recipient duct and donor duct mucocele are very similar. Therefore, while imaging criteria may suggest a certain origin, the final diagnosis may not be achieved until operative intervention.

Treatment of cystic duct mucoceles, whether donor or recipient in nature, can be achieved by either interventional radiology (IR) or surgery. The IR approach is safe, effective, and can be achieved by percutaneous drain placement and ethanol ablation. Surgically, if the mucocele is donor duct in origin, excision of the mucocele followed by roux-en-y hepaticojejunostomy has been described as an accepted treatment method[2,5]. In the instance of a recipient duct mucocele, one would expect, depending on the recipient variation of cystic duct anatomy, to have a well-drained cystic duct either into the recipient common duct or directly into the duodenum. Regardless of the anatomy, our patient developed a mucocele from the recipient remnant cystic duct, and only required simple excision.

When a transected cystic duct is encountered during preparation for liver transplant, it should be excised completely, even if it is close to the common duct. If this is not possible, the cystic duct should be excised as much as possible, rather than creating a blind mucosa-lined sac with the potential of enlarging and creating obstruction. However, this should be done under the discretion of the surgeon, as injury to the right hepatic artery, the common bile duct and its branches, and the biliary tract blood supply are possibilities. If excision of the cystic duct is thought too dangerous, its distal end can be incorporated in the anastomotic suture line to allow drainage of the cystic duct stump. However, wherever the valves of Heister are left to drain a blind biliary pouch that produces mucous, the risk of mucocele remains.

The diagnosis of cystic duct mucocele in the appropriate setting is usually made by radiologic modalities. Demonstration of fluid collection at the porta hepatis is a nonspecific finding. However, a combination of a well-defined, round, fluid collection adjacent to the common hepatic duct would confirm evidence. In some cases, the mucocele appears as a gallbladder and immediately calls into question whether a graft cholecystectomy was performed.

A recipient cystic duct mucocele is a rarity after deceased donor liver transplantation. It is a recognized complication to observe a donor duct mucocele that may compress the common bile duct. However, we describe for the first time a patient with a more ambiguous clinical picture found to have a recipient duct mucocele, showing the importance of the consideration of this complication in the post-transplant patient.

ARTICLE HIGHLIGHTS

Case characteristics

A 48-year-old male who presented with right upper quadrant pain and was found to have a recipient cystic duct mucocele 3 mo after receiving a deceased donor liver transplant.

Clinical diagnosis

A diagnosis of cystic duct remnant mucocele was made given its appearance and recurrence, and ablation with sclerosing agents (ETOH) was attempted by interventional radiology on three separate occasions but was of little benefit.

Differential diagnosis

Biloma, anastomotic bile leak, pancreatic cyst, pancreatic pseudo cysts, and cystic duct mucocele after transplantation.

Laboratory diagnosis

Liver function test revealed elevated canallicullar enzymes, complete blood count

showed elevated white blood cells, and blood cultures were unremarkable.

Imaging diagnosis

Abdominal X-ray (nonspecific), abdominal CT (mass or fluid collection at porta hepatis), abdominal MRI (mass or fluid collection at porta hepatis), MRCP (showed filling of the fluid collection), and ERCP (showed filling of the fluid collection).

Pathological diagnosis

Resection and histologic analysis of the fluid filled mucocele revealed chronic cholangitis with prominent fibrosis, granulation tissue formation through mucosa, muscularis, and adventitia.

Treatment

Surgical resection of the mass, and perioperative antibiotics.

Related reports

See the reference list: Ref. [2,4,7].

Term explanation

Mucocele: A swelling like a sac that is due to distension of a hollow organ or cavity with mucus; MRCP: Magnetic resonance cholangiopancreatography: A special type of magnetic resonance imaging (MRI) exam that produces detailed images of the hepatobiliary and pancreatic systems, including the liver, gallbladder, bile ducts, pancreas and pancreatic duct; ERCP: Endoscopic retrograde cholangiopancreatography: A technique that combines the use of endoscopy and fluoroscopy to diagnose and treat certain problems of the biliary or pancreatic ductal systems; Gamma-glutamyltransferase: A transferase (a type of enzyme) that catalyzes the transfer of gamma-glutamyl functional groups from molecules such as glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate); Mirizzi's Syndrome: A rare complication in which a gallstone becomes impacted in the cystic duct or neck of the gallbladder causing compression of the common bile duct or common hepatic duct, resulting in obstruction and jaundice; Hepaticojejunostomy: Biliary-enteric anastomosis is usually to smaller ducts, which can be multiple if the injury or stricture is above the bifurcation of the right and left ducts.

Experiences and lessons

Cystic duct mucoceles after transplantation require a high index of suspicion requiring h and p, lab tests (liver function test, complete blood count, blood cultures), and imaging studies (abdominal X-ray, abdominal CT, abdominal MRI, ERCP) with resolution through surgical resection.

REFERENCES

1 **Caputo M**, Spagnol A, Minguzzi MT, Papa S, Calculli L, Soro A, Grazi GL, Morganti M, Gavelli G. Incidence of dilatation of residual cystic duct in liver transplantation. Our experience with 152 cases. *Radiol Med* 1995; **89**: 647-650 [PMID: 7617905]

2 **Caputo M**, Piolanti M, Riccioli LA, Pazienza L, Fabbro E, Gruppioni F, Grazi G, Gavelli G. Nonobstructive residual mucocele of the cystic duct. Reassessment of complications in our 13 years' experience with liver transplantation. *Radiol Med* 2000; **100**: 354-356 [PMID: 11213414]

3 **Liang TB**, Zhao ZC, Jia CK, Zheng SS. Mucocele formation of cystic bile duct remnant after orthotopic liver transplantation. *Chin Med J* (Engl) 2007; **120**: 254-256 [PMID: 17355834]

4 **Koneru B**, Zajko AB, Sher L, Marsh JW, Tzakis AG, Iwatsuki S, Starzl TE. Obstructing mucocele of the cystic duct after transplantation of the liver. *Surg Gynecol Obstet* 1989; **168**: 394-396 [PMID: 2652346]

5 **Chatterjee S**, Das D, Hudson M, Bassendine MF, Scott J, Oppong KE, Sen G, French JJ. Mucocele of the cystic duct remnant after orthotopic liver transplant: a problem revisited. *Exp Clin Transplant* 2011; **9**: 214-216 [PMID: 21649573]

6 **Piardi T**, Panaro F, Gheza F, Wolf P. 'Cystic' lesion in the porta hepatis after liver transplantation. *Liver Int* 2011; **31**: 859 [PMID: 21645217 DOI: 10.1111/j.1478-3231.2011.02460.x]

7 **Abcarian PW**, Emond JC, Ring EJ. Cystic duct remnant mucocele in a liver transplant recipient. *J Vasc Interv Radiol* 1994; **5**: 127-130 [PMID: 8136589 DOI: 10.1016/S1051-0443(94)71468-8]

8 **Zajko AB**, Bennett MJ, Campbell WL, Koneru B. Mucocele of the cystic duct remnant in eight liver transplant recipients: findings at cholangiography, CT, and US. *Radiology* 1990; **177**: 691-693 [PMID: 2243970 DOI: 10.1148/radiology.177.3.2243970]

P- Reviewer: Coelho JCU, Kai K, Zhang CW **S- Editor**: Kong JX
L- Editor: A **E- Editor**: Yan JL

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com