ISSN 2220-3230 (online)

World Journal of *Transplantation*

World J Transplant 2021 March 18; 11(3): 16-87

Published by Baishideng Publishing Group Inc

World Journal of Transplantation

Contents

Monthly Volume 11 Number 3 March 18, 2021

World Journal of Transplantation

Monthly Volume 11 Number 3 March 18, 2021

Contents

ABOUT COVER

Editorial Board Member, Tom SJ Darius, MD, PhD, Professor, Surgery and Abdominal Transplant Unit, University Clinics Saint Luc, Université Catholique de Louvain, Brussels 1200, Belgium. tom.darius@uclouvain.be

AIMS AND SCOPE

The primary aim of *World Journal of Transplantation* (*WJT*, *World J Transplant*) is to provide scholars and readers from various fields of transplantation with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJT mainly publishes articles reporting research results obtained in the field of transplantation and covering a wide range of topics including bone transplantation, brain tissue transplantation, corneal transplantation, descemet stripping endothelial keratoplasty, fetal tissue transplantation, heart transplantation, kidney transplantation, liver transplantation, lung transplantation, pancreas transplantation, skin transplantation, *etc*..

INDEXING/ABSTRACTING

The *WJT* is now abstracted and indexed in PubMed, PubMed Central, Scopus, China National Knowledge Infrastructure (CNKI), and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Transplantation

ISSN
ISSN 2220-3230 (online)

LAUNCH DATE
December 24, 2011

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Maurizio Salvadori, Sami Akbulut, Vassilios Papalois

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2220-3230/editorialboard.htm

PUBLICATION DATE
March 18, 2021

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

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Transplantation

Submit a Manuscript: https://www.f6publishing.com

World J Transplant 2021 March 18; 11(3): 16-36

DOI: 10.5500/wjt.v11.i3.16

ISSN 2220-3230 (online)

REVIEW

Microbiota, renal disease and renal transplantation

Maurizio Salvadori, Aris Tsalouchos

ORCID number: Maurizio Salvadori 0000-0003-1503-2681; Aris Tsalouchos 0000-0002-8565-4059.

Author contributions: Salvadori M and Tsalouchos A contributed equally to the manuscript; Salvadori M designed the study, performed the last revision and provided answers to the reviewers; Tsalouchos A collected the data from literature; Salvadori M and Tsalouchos A analyzed the collected data and wrote 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

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

Aris Tsalouchos, Nephrology and Dialysis Unit, Saints Cosmas and Damian Hospital, Pescia 51017, Italy

Corresponding author: Maurizio Salvadori, MD, Professor, Department of Transplantation Renal Unit, Careggi University Hospital, Viale Pieraccini 18, Florence 50139, Italy. maurizio.salvadori1@gmail.com

Abstract

Aim of this frontier review has been to highlight the role of microbiota in healthy subjects and in patients affected by renal diseases with particular reference to renal transplantation. The microbiota has a relevant role in conditioning the healthy status and the diseases. In particular gut microbiota is essential in the metabolism of food and has a relevant role for its relationship with the immune system. The indigenous microbiota in patients with chronic renal failure is completely different than that of the healthy subjects and pathobionts appear. This abnormality in microbiota composition is called dysbiosis and may cause a rapid deterioration of the renal function both for activating the immune system and producing large quantity of uremic toxins. Similarly, after renal transplantation the microbiota changes with the appearance of pathobionts, principally in the first period because of the assumption of immunosuppressive drugs and antibiotics. These changes may deeply interfere with the graft outcome causing acute rejection, renal infections, diarrhea, and renal interstitial fibrosis. In addition, change in the microbiota may modify the metabolism of immunosuppressive drugs causing in some patients the need of modifying the immunosuppressant dosing. The restoration of the indigenous microbiota after transplantation is important, either to avoiding the complications that impair the normal renal graft, and because recent studies have documented the role of an indigenous microbiota in inducing tolerance towards the graft. The use of prebiotics, probiotics, smart bacteria and diet modification may restore the indigenous microbiota, but these studies are just at their beginning and more data are needed to draw definitive conclusions.

Key Words: Gut commensals; Microbioma; Microbiota; Renal disease; Renal transplantation; Transplant outcomes

manuscript

Specialty type: Transplantation

Country/Territory of origin: Italy

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

Received: December 4, 2020
Peer-review started: December 4, 2020
First decision: December 27, 2020
Revised: January 6, 2021
Accepted: February 12, 2021
Article in press: February 12, 2021
Published online: March 18, 2021

P-Reviewer: Sales-Campos H, Zhu Y
S-Editor: Zhang L
L-Editor: A
P-Editor: Yuan YY

Core Tip: Recent studies on the microbiota have documented that a microbiota modification, related to the assumption of immunosuppressive drugs and of antibiotics, as happens in the first period after transplantation may modify the outcomes of the graft. Indeed, dysbiosis may cause acute rejections and reduce the possibility of a tolerance status. In addition, dysbiosis if often the cause of infections and renal fibrosis. Dysbiosis may also cause diarrhea that is a frequent and severe complication in the transplanted patient. Modification of dysbiosis is possible with an appropriate treatment, but studies on this topic are just at their beginning.

Citation: Salvadori M, Tsalouchos A. Microbiota, renal disease and renal transplantation. *World J Transplant* 2021; 11(3): 16-36
URL: https://www.wjgnet.com/2220-3230/full/v11/i3/16.htm
DOI: https://dx.doi.org/10.5500/wjt.v11.i3.16

INTRODUCTION

The microbiota is defined as the micro-organisms that live in the human body without damaging it in healthy conditions. The most important and the best studied is the microbiota of the digestive system. In particular, the urinary microbiota has also been studied in studies concerning renal diseases and renal transplants.

In recent years the function of the microbiota, particularly the gut microbiota has been extensively examined and the relationship between the microbiota and diseases has been elucidated with particular reference to organs such as the kidney. In this frontier review, the definition of the microbiota and its variety will be provided, along with descriptions of its functions and relationship with the immune system. In addition, the relationship between an abnormal microbiota or pathobionts and renal diseases and renal transplantation has been documented in several studies[1-5]. The relationship between the microbiota and its alterations in patients with kidney disease will be elucidated with particular references to the relationship between the microbiota and renal transplantation.

DEFINITIONS

The words microbiota and microbioma are often mutually used, but they have a different meaning.

The term microbiota refers to all the microorganisms inhabiting some specific niches as gut, skin, lungs and other organs and encompasses bacteria, viruses, fungi and archea. In this review the term microbiota refers principally to bacteria even if in general it strictly refers also to other microorganisms. In a recent study the estimated total number of bacteria for a 70 kg man is approximately 3.8×10^{13} and is approximately of the same order of the number of human cells[6]. The gut microbiota is the most important community because of its quantity and its relationship with kidney disease. The gut microbiota is already present within the first few years of life, and its composition should remain stable in adults, where the dominant bacteria are Bacteroides, Firmicutes and Actinobacteria[7-9]. In the healthy subject the resident microbiota is also called indigenous microbiota. When the indigenous microbiota, due to genetic or environmental factors, cause inflammatory disorders or other diseases, is generally called pathobionts and this condition is called dysbiosis. Pathobionts should be distinguished from acquired infectious agents also called pathogens[10]. Due to the relevance of microbiota both in healthy status and diseases, several national and international scholars performed studies of gut microbiota, such as the Canadian Microbioma Initiative, The Human Meta Genome Consortium Japan, the My New Gut Project of the European Union and the International Human Microbioma Consortium[11-13]. The composition of the gut microbiota under standard conditions is shown in Table 1.

As mentioned above, the term microbioma has a different meaning than the microbiota and refers to all the microbiota genes and is approximately 150 times larger than the human genome[14,15]. In healthy subjects the gut microbioma is stable and

Table 1 Distribution of normal gut flora in different parts of intestine

Intestine sections	Function	Normal flora
Stomach	Acid production, pepsin, amylase, CFU < 10^3 /mL	*Lactobacillus; Streptococcus; Helycobacter pylori*
Small intestine: duodenum, jejunum	Pancreatic enzymes, bicarbonate ions, bile salts, CFU: 10^3-10^4/mL	*Lactobacilli; Enterococci; Streptococci; Actinobacteria*
Small intestine: ileum	CFU: 10^3-10^9/mL	*Enterococcus; Bacteroidetes; Lactobacillus; Clostridium; Corynebacteria*
Large intestine: caecum, colon	Mucus and bicarbonate, CFU:10^{10}-10^{12}/mL	*Bacteroidetes; Clostridium; Eubacterium; Ruminococcus; Streptococcus; Enterococcus; Lactobacillus; Fusobacteria*

CFU: Colony forming units.

exerts important functions throughout the body as shown in Table 2.

FUNCTIONS OF THE MICROBIOTA

Metabolic functions

Dietary fibers produce energy when metabolized, but not all dietary fibers are metabolized by digestive enzymes[16]. The gut microbiota of the large intestine contains enzymes that are able to metabolize these fibers and recover additional energy[17,18].

Undigested proteins are degraded into peptides, amino acids and other metabolites in the large intestine. Some of these metabolites are dangerous to the body and could cause diseases as colorectal cancers and kidney dysfunction[19]. The MEROPS database documented that the composition of the large intestine microbiota may contains different proteases responsible for inducing the production of different metabolites[20,21]. The gut microbiota also exerts important actions on lipids, bile salts and polyphenols.

Structural functions

The structural integrity of the intestinal epithelium is essential to avoid a dangerous increase in permeability. The maintenance of structural integrity is essential for the microbiota. In normal conditions, cytokines produced in the gut may back diffuse in small quantities passing through the gut barrier. The barrier function of the tight junction in dysbiosis condition, may be weakened by several endotoxins of some pathogens as *Escherichia coli* (*E. coli*), *Clostridium difficile and Clostridium perfrigens*. In this condition of dysbiosis, the diffusion of citokines such as interleukin 4, interleukin 1 beta, tubular necrosis factor alpha and interferon gamma is increased[22-26].

Protective function

The gastrointestinal tract represents a bidirectional barrier between the gut microbiota and the gut immune system[27]. The barrier is composed of three layers: the mucus layer, the antimicrobial peptides (AMPs) and the IgA system.

Mucin glycoproteins secreted by goblet cells form a layer over the epithelia to restrict bacterial adhesion. This layer prevents the adherence of commensal microbiota to gut epithelial cells, limiting the bacterial adhesion[28]. A second layer is represented by AMPs secreted by epithelial cells. AMPs include α and β defensins secreted by the epithelium and mediated by cytosolic nucleotide-binding oligomerization domain-containing protein 2[29,30]. C-type lectins activate Toll-like receptors to limit bacterial penetration through the gut barrier[31].

The third layer is composed of the IgA system. Dendritic cells (DCs) located beneath the epithelial dome of Peyer's patches take up bacteria, migrate to mesenteric lymph nodes and induce B cells to differentiate into IgA plasma cells that secrete IgA[32,33].

THE MICROBIOTA AND THE IMMUNE SYSTEM

The indigenous microbiota, pathobionts and pathogens promote in the gut the generation of several Th cells among which Th1, Th2, Th17 and Treg. At mucosal sites this may also be due to the production of microbiota metabolites. In particular, the

Table 2 Functional activities of normal gut flora

Protective function	Metabolic function	Structural function
Nutrient competition; Barrier fortification; Innate and adaptive immunity activation; Antimicrobial compounds secretion	Vitamin and amino acid biosynthesis; Bile acid biotransformation; Dietary fiber fermentation; Short chain fatty acids production	Mucus layer properties; Crypt and villi development; Villi microvascularization; Tight junction regulation

Seven division of bacteria (*Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, Verrucomicrobia, Actinobacteria, Cynobacteria*), 300-1000 species.

microbiota stimulate epithelial cells to the generation and accumulation of Treg by increase of TGFβ, stimulate macrophages to induce Th17 cells by increase of interleukin 1 beta, and through DNA methylation can induce proliferation of colonic Treg cells. Other actions on immune cells are due to microbiota metabolites as butyrate. Butyrate down regulates IL-10 production from neutrophils and generates an anti-inflammatory activity. Butyrate, down regulating IL-6 from macrophages, induces increased levels of histone acetylation. On the other hand, butyrate, by inhibition of histone deacetylase, inhibits the activation of NF-kB inducing a Th1 cell response[34,35]. The balance of Treg cells and the effector T cells in the intestinal mucosa is related to the ratio between the indigenous microbiota and the pathobionts. In particular the subset of Th1 and Th2 cells activation is characterized by the expression of proinflammatory cytokines including IFNγ, IL4, IL5 and IL13, and IL23[22]. Th 17 cells are characterized by the synthesis of IL-17, which stimulates cells to express the proinflammatory cytokines as IL-6, IL-8, and Il-22[36,37].

The indigenous microbiota plays a fundamental role in the induction, education and function of the immune system (Figure 1).

The microbiota composition may be modified by several conditions, among which the use of antibiotics, immunosuppressants or diet alterations. In such conditions pathobionts appear and modify the immune system and promote the development of inflammatory diseases[38].

Microbiota-derived Toll-like receptors and NOD ligands and metabolites [such as short-chain fatty acids (SCFAs) and aryl hydrocarbon receptors] may act on local gut cells but also penetrate beyond the mucosa to tune immune cells in peripheral tissues[39].

SCFAs promote DC precursor activation and release into the bloodstream. Microbiota- derived NOD1 Ligands induce mesenchymal cells to produce hemato-poietic growth factors as IL7, stem cell factor (SCF), thrombopoietin, recombinant human flt3-Ligand, IL6[40-42].

In addition, microbiota-derived riboflavin metabolites promote the development of mucosal- associated invariant T cells[43], and commensal bacterial-induced cytokines IL1β and IL23 promote IL17A production from gamma delta T cells[44,45].

Finally, commensal bacterial colonization promotes effector and regulatory T cell responses.

Clostridia colonization promotes retinoic acid receptor-related orphan nuclear receptor gamma (RORγt)[46], and Foxp3+ Treg cell accumulation, which in turn limits colonic Th2 and Th17 cell responses.

Foxp3+ Tregs cells localize in Peyer's patches and promote B class switching and the production of IgA, which fosters a different microbiota and ensures commensal bacteria compartmentalization from the intestinal epithelium[47].

Under healthy conditions, a balance between antigenic stimuli exists due to the microbiota and the immune response.

However, an aggressive immune response due to the appearance of pathobionts or pathogens in some subjects may cause inflammatory diseases, and a weak response may cause the overgrowth and diffusion of the pathobionts themselves.

Commensal bacteria induce CD4+ cells to differentiate into 4 main subtypes: Th1, Th2, Th17 and Treg. The indigenous microbiota contributes to normalizing the ratio of these subtypes.

Additionally, IgA production contributes to controlling excessive microbiota growth and limiting the growth of pathobionts.

In healthy conditions, segmental filamentous bacteria induce the growth and differentiation of Th17 and Th1 cells[48]. In animal studies has been documented that this is impaired in animals treated with antibiotics while is normal in germ free conditions. Still in the animals, in healthy conditions, Clostridia promote the accumulation of Tregs and production of IL10, which exerts anti-inflammatory effects[49].

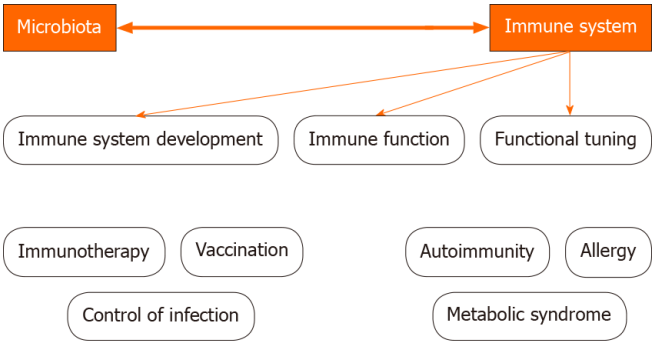


Figure 1 Role of microbiota in the induction, education and function nof the immune system.

Bacterioides fragilis also contributes to maintaining a correct equilibrium between the microbiota and immune system by producing of polysaccharide A and inducing the production of IL10 and Tregs[50].

When the microbiota loses its richness and its correct composition, pathobionts appear and dysbiosis occurs. This change may lead to diseases and kidneys and kidney grafts are among the main targets.

THE INTESTINAL MICROBIOTA AND THE KIDNEY

Communication between the gut and kidney occurs either by the activation of the immune system and by microbiota-derived metabolites.

Several studies have documented that the activation of Th17 cells in the gut by the microbiota leads to activation of Th17 cells in the kidney[51]. Chemokine ligand 20/C-C[52] recruits Th17 cells to the kidney.

In animals, the addition of antibiotics reduces Th17 levels and renal damage[53]. The crucial role of Th17 cells in inducing tissue injury is also evidenced by the high levels of Th17 cells in humans with auto-immune kidney diseases and in glomerulonephritis[54].

This phenomenon is bidirectional because acute kidney injury (AKI) determines intestinal dysbiosis and T helper Th17 cells, neutrophils and M1 macrophages mediate intestinal inflammation, as well as leaky gut with bacterial translocation. On the other hand, dysbiotic microbiota may exert an adverse effect on kidney injury and the depletion of the pathobionts may mitigate kidney injury[55].

Microbiota-derived metabolites may affect kidney and other organ functions. Indeed, the microbiota may interact with a large number of vital functions in the health body *via* several metabolites. The targets are host metabolism and immunity as well as cardiovascular and brain functions. Additionally, the microbiota metabolism utilizes enzymes not encoded by the human genome and generates biological products relevant to the host's health as bile acids, choline, vitamins and SCFAs[56].

SCFAS are among the most relevant metabolites produced by microbiota[57].

SCFAs activate G protein-coupled receptors (GPR) including GPR41, GPR43 and GPR109A.

The binding of SCFAs to their receptors exerts beneficial effects on the kidney. Indeed, this signaling pathway regulates energy homeostasis[58], stimulates glucagon-like peptide 1 secretion[59], and inhibits the progression of atherosclerosis in mice[60]. The binding of SCFAs to another receptor, Olfr78 exerts beneficial effects on blood pressure[61]. These and other data support a beneficial effect of SCFAs on kidney injury (Figure 2).

In addition, SCFAs also regulate cytokine expression in T cells and the generation of Tregs through histone deacetylase inhibition.

Overall, SCFAs exert a beneficial effect on AKI by reducing the production of cytokines and chemokines such as IL1β, IL6, TNFα and monocyte chemoattractant protein 1[62].

In addition, SCFAs have also extraintestinal actions controlling appetite regulation, glucose and lipid metabolism. This is due to the fact that the above mentioned receptors have also been found in cells as adipocytes, neurons and immune and vascular cells[63].

Equol, produced by certain microbiota subtypes has several beneficial effects,

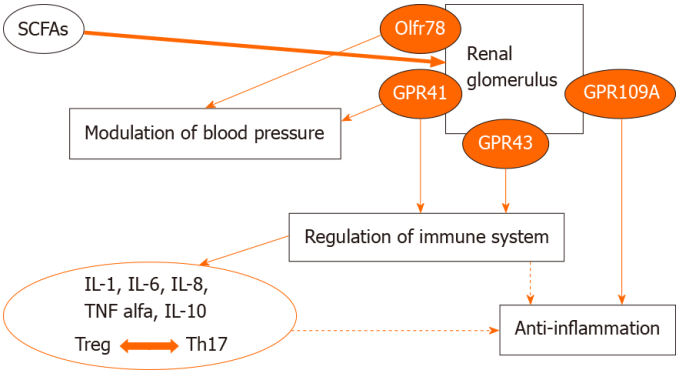


Figure 2 Short-chain fatty acids and the receptors in the kidney. Olfr78: Olfactory receptor 78; GPR41: G protein receptor 41; GPR43: G protein receptor 43; GPR109A: G protein receptor 109A; SCFA: Short chain fatty acid.

including antiapoptosis, antioxidation, and anti-atherosclerosis, the production of nitric oxide in endothelial cells, antiproliferation and/or migration, and promotion of vascular smooth cells relaxation[64].

On the contrary, negative effects on vascularization are exerted by metabolites as indoxylsulfate and trimethylamine N oxide (TMAO).

Indoxylsulfate produced by pathobionts as *E. coli* has deleterious effect on the vascular system. Indoxylsulfate induces apoptosis, senescence, prothrombotic events, proliferation and/or migration and modulation of vascular tone in vascular smooth muscle cells. Similar negative vascular effects are exerted by TMAO.

TMAO is a product of gut bacterial metabolism of choline. Differently from SFCAs it promotes renal interstitial fibrosis[65].

The different effects of these metabolites are shown in Figure 3.

The gut microbiota may also produce uremic toxins that, in the case of dysbiosis, may be produced in high quantities and may damage the kidney[66].

The quorum sensing signals (QS) may be produced either by pathobionts or by indigenous microbiota. Indeed, QS may be divided into two types. Those produced by GRAM$^-$ bacteria such as *Pseudomonas aeruginosa* have negative immune-related processes such as IkK phosphorilation, and activation of mitogen activated protein kinase (MAPK) pathways. These induce NF-kB signaling and chemotaxis. As a result they increase inflammatory genes expression. Differently, the QS signals induced by *Bacillus subtilis,* have beneficial effects through the induction of p38 MAPK on protein kinase B[57].

Dysbiosis may facilitate AKI either by modifying the SCFAs composition or generating higher quantites of TMAO and uremic toxins. This modification may facilitate the transition from AKI to chronic renal disease (CKD). Indeed, a cross-talk between the intestinal microbiota and the kidney has been observed. During experimental AKI, gut pathobionts may modify immune cells and other pathophysiological mediators to alter the course of AKI. AKI may in turn modify the gut bacterial composition[67,68]. This topic has been extensively studied by Vaziri *et al*[68] who observed substantial differences in the gut microbiota composition between patients with end stage renal disease and control patients.

This result has been confirmed by Cigarran Guldris *et al*[69], who substantially found dysbiosis in patients affected by end stage renal disease, due to the presence of pathobionts. Pathobionts modify protein absorption, reduce the utilization of alimentary fibers and are frequently associated with the use of antibiotics[70,71].

In summary, in the healthy subject the indigenous microbiota provides benefits to our health. Indigenous microbiota affects the host by production of metabolites and gut neuropeptides. By sending the informations about the state of inner organs to the brain, they control many important functions as mood, immune response, digestion and heart rate. By this way a bidimensional communication between the gut, its microbioma and the nervous and neuroendocrine systems is established[72].

THE MICROBIOTA AND RENAL TRANSPLANTATION

Different factors, including immunosuppressant and antibiotic therapy, lifestyle and diet, may alter the microbiota and lead to generation of pathobionts and dysbiosis.

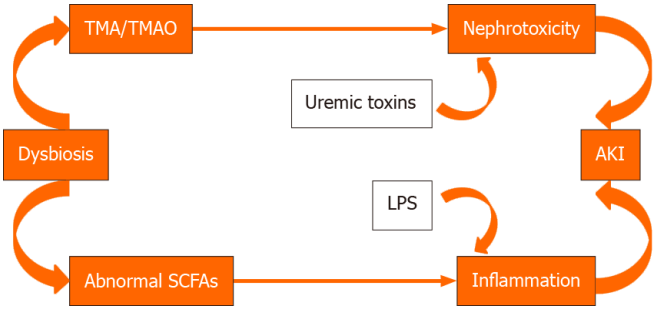


Figure 3 Dysbiosis during acute kidney injury. TMA: Trimethylamine; TMAO: Trimethylamine N oxide; AKI: Acute kidney injury; SCFA: Short chain fatty acid; LPS: Lipopolysaccharide.

Dysbiosis disrupts the gut epithelial barrier, induces a loss of barrier integrity and leads to pathogen overgrowth. The leaky gut and increased permeability facilitate the translocation of bacteria and their components into the inner environment. In this dysbiosis situation, the proinflammatory response triggers the elimination of pathogens by intestinal epithelial cells (IL-1, IL-6 and IL-18 secretion), DCs and macrophages that induce the development of the effector CDE4+ cells, Th1 and Th17. Innate immune responses lead to a systemic and allograft inflammation. Moreover, dysbiosis decreases the number of regulatory T cells and increases the number of effector T cells that activate innate immunity. On the other hand, in the colon and liver, dysbiotic gut-derived uremic toxins are further metabolized to TMAO. The accumulation of p-cresyl sulfate in the kidney generates reactive oxygen species that lead to the production of inflammatory cytokines and profibrotic factors. In addition, indoxylsulfate induces inflammation and nephrotoxicity[73-77].

Characteristics of the microbiota after renal transplantation

Renal transplant patients, in addition to receiving relevant immunosuppressive therapy in the first period after transplantation, receive several antibiotic treatments as a prophylactic measure to avoid infections.

All these drugs extensively modify the human microbiota, principally at the gut and urinary tract levels. Historically, since the initiation of renal transplantation, when very high doses of cyclosporine A were used, gingival overgrowth was observed as an important side effect. This change was related to modifications of the oral microbiota and generation of pathobionts[78].

In a pilot study, Lee *et al*[79], performed polymerase chain reaction in samples from 26 kidney transplant recipients and documented a change in the microbiota between the pre- and posttransplant periods. The results are shown in Table 3.

Firmicutes were the most abundant bacteria detected pre- and posttransplantation, but their quantity posttransplantation was lower than in healthy subjects[80]. The same study reported posttransplantation an increase in the abundance of Bacteroides that included infective pathogens such as *E. coli* and *Klebsiella pneumoniae*[81].

Overall, the study by Lee and colleagues documented a dysbiosis that was later confirmed by other studies. A recent review from Xiao *et al*[82] on microbiota modifications in response to solid organ transplantation highlighted an increase in the abundance of pathogenic Proteobacteria, which might represent the cause of infectious diseases occurring after transplantation.

These data were confirmed by a recent study by Swarte *et al*[83] that confirmed a reduction in the abundance of *Firmicutes* with variability among the species. The most significant reduction was observed for *Streptococcus thermophilus* and *Blautiawexlerae*.

Overall these authors observed an increase in the abundance of *Proteobacteria* (*E. coli*) and a decrease in the abundance of *Actinobacteria* posttransplantation. The increase in *Proteobacteria* has already been proposed as a marker of dysbiosis[84]. Additionally, the same study observed a reduction in SFCAs producing bacteria after transplantation. In particular, reductions in the abundance of *Eubacterium rectale, Coprococcuscatus and Roseburia* were observed. All these bacteria produce SCFAs[85] that exert beneficial effects on the kidney and increase the number of Tregs, reducing systemic inflammation[86,87]. The use of proton pump inhibitors, of MMF and aging were the prevalent determinants of this form of dysbiosis[88,89].

Another study[90] analyzed the gut microbiota in 142 kidney transplant recipients. The authors detected potential pathogens, such as *Clostridium difficile* and *E. coli* in 30% of patients. These pathogens were not associated with diarrhea, as expected.

Table 3 Alterations in the gut microbiota following kidney transplantation according phylum and order

Phylum	Pre Tx cohort	Post Tx cohort
Firmicutes	91.8%	87.7%
Actinobacteria	2.0%	7.6%
Proteobacteria	0.9%	4.1%
Bacteroidetes	2.8%	0.6%
Order		
Clostridiales	64.8%	64.3%
Lactobacillales	19.1%	12.0%
Erysipelotrichales	5.6%	10.2%
Bifidobacteriales	1.6%	6.6%
Enterobacteriales	0.4%	3.9%
Bacteroidales	2.8%	0.6%

A different study[91] observed that major changes in the microbioma occur in the first month after transplantation, with substantial differences among patients. The authors concluded that longitudinal analyses should be performed to provide more information.

In conclusion, dysbiosis after renal transplantation is related to an imbalance between the indigenous microbiota and the pathobionts. This imbalance is related principally to the need for immunosuppressant and prophylactic and therapeutic antimicrobial agents[92].

The metabolic and clinical consequences of dysbiosis are represented by a higher incidence of acute rejections, acute infections, interstitial fibrosis, posttransplant diarrhea, reduced production of protective agents such as SCFAs by the gut microbiota, and modification of immunosuppressant levels in the blood.

Dysbiosis and acute rejection

Several experimental studies conducted in animals have documented en effect of the gut microbiota on immune responses that lead to transplant rejection[93].

Few studies have been conducted in the humans on this topic.

In the aforementioned study by Lee *et al*[79], the differences in the fecal bacteria composition of patients with and without rejection are shown in Table 4.

In one recent study[84], the microbiota was evaluated pre- and posttransplant in 60 patients who received a renal transplant.

Samples from urine, oral swabs, rectal swabs and blood were evaluated for up to 6 mo after transplantation.

In the study, the most relevant changes in the microbiota principally verified in the first month after transplant, when the immunosuppressive treatment was heavier because of the induction therapy. Further modifications in the microbiota were verified in the first six months after transplantation. In urine samples and in oral swab samples, changes were verified principally in the phylum *Proteobacteria*. In the rectal swab samples, *Firmicutes* were the bacteria whose composition changed more frequently.

Significant changes in *Leptotrichia*, *Neisseria* and *Actinobacteria* were observed in five patients who experienced acute rejection. Four patients experienced late acute rejection and displayed significant changes in *Anaerotruncus, Coprobacillus* and *Coprococcus*.

Dysbiosis and infections

The same authors of the study on acute rejection[94] documented that similar changes in the microbiota were also associated with a higher incidence of urinary tract infections.

In particular, in four patients with posttransplant infections, the abundance of the genus *Anaerotruncus* of *Firmicutes* was markedly decreased compared to the other patients.

Several factors may cooperate with dysbiosis to generate infections, as shown in Table 5. This higher incidence of both urinary and gastrointestinal infections was also reported in the aforementioned studies by Lee *et al*[79] and Chan *et al*[95].

In a recent study[96], a transplant patient with recurrent urinary infections recovered

Table 4 Microbial composition of fecal specimens from patients with or without acute rejection, by Philum and Order

Phylum	No AR cohort	AR cohort	*P* value
Firmicutes	91.4%	76.6%	0.40
Actinobacteria	3.7%	8.2%	0.60
Proteobacteria	1.3%	15.2%	0.33
Bacteroidetes	3.1%	0.02%	0.03
Order			
Clostridiales	63.1%	16.9%	0.01
Lactobacillales	12.7%	49.9%	0.04
Erysipelotrichales	13.3%	9.2%	0.32
Bifidobacteriales	3.1%	7.9%	0.44
Enterobacteriales	1.0%	14.7%	0.17
Bacteroidales	3.1%	0.02%	0.03

AR: Acute rejection.

Table 5 Potential transplant associated factors that may lead to changes in the gastrointestinal microbiota and cause infections

Risk factors	Microbiota changes	Consequences	Interventions
Dietary patterns	Increase in bacteria translocation	Gastrointestinal upset *e.g.*, diarrhea	Diet
Changes to colonic and bowel transit time	Increase in metabolic endotoxemia	Urinary tract infections	Prebiotics
Immunosuppression	Increase in gut-derived microbial toxin formation	Other infections not yet explored	Probiotics
Antibiotics			Synbiotics
Lifestyle (sedentary, smoking, alcohol)			

after fecal microbiota transplantation (FMT), which induced a marked decrease in the abundance of *E. coli* in the urinary microbiota.

In conclusion, according to these studies, some microbial species may exert a protective effect on the mucosal surface under normal conditions, and when the microbiota changes, pathobionts and aggressive phenotypes appear to induce renal dysfunction.

Dysbiosis and interstitial fibrosis

The hypothesis that urinary dysbiosis is principally responsible for the development of interstitial fibrosis of the graft was based on the findings that patients affected by interstitial fibrosis/tubular atrophy (IF/TA) had abnormalities in the urinary microbiota with appearance of pathobionts and, consequently, in the immune response. Two studies, conducted in humans[97,98] detected antibodies directed against *E. coli* LPS, a powerful activator of the immune system *via* TLR4 receptor in the biopsies of patients affected by IF/TA.

In a recent study of transplant patients, Modena *et al*[99] collected urine samples from 25 patients at two time points after kidney transplantation (approximately 1 mo and 6 mo after transplantation). All these patients demonstrated developed IF/TA in surveillance biopsies collected 6 mo after transplantation.

These samples were compared with 23 patients with normal surveillance biopsies and stable renal function at 6 mo after transplantation.

At six months after transplantation, patients affected by IF/TA displayed decreased abundances in the *Lactobacillus* and *Streptococcus* genera along with an increase in the abundance of no dominant species.

The authors concluded that the urinary microbiota, modified posttransplantation, may contribute to IF/TA development by altering the host immune response.

IF/TA is associated with a loss of the indigenous dominant resident urinary microbiota and an increase in the abundance of pathobionts or nonresident, pathogenic bacteria.

The phenomenon of IF/TA may be mediated by myofibroblasts, as has already been documented in the gut, where gut dysbiosis potentially leads to intestinal fibrosis[100]. Myofibroblasts may be derived from transdifferentiation processes such as the epithelial to mesenchymal transition or endothelial to mesenchymal transition. These processes may be induced and aggravated by modifications in the indigenous microbiota.

In conclusion, myofibroblasts may play a relevant role in inducing IF/TA either at the gut or renal level, and the indigenous microbiota might have regulatory and protective functions under normal conditions.

Dysbiosis and diarrhea

Diarrhea represents a severe complication after kidney transplantation, affecting approximately 20% of patients[101], and it represents an important cause of graft loss and death[102]. However, its etiology is still being discussed, and a clear diagnosis not available for approximately 85% of transplanted patients affected by diarrhea. With the exception of the few cases that are ascribed to a specific infection and the presence of pathogens, the diarrhea etiology is often ascribed to the use of immunosuppressants, in particular MMF. However, a reduction in the MMF dose is dangerous and may lead to an increased risk of allograft rejection[103].

In the pilot study by Lee *et al*[79], the authors observed a reduction in the commensal indigenous microbiota, such as *Ruminococcus, Dorea* and *Coprococcus*, in 26 renal transplant patients affected by diarrhea. In addition, they did not detect pathogens such as *Clostridium difficile* or norovirus in fecal specimens. These findings prompted the hypothesis that in the majority of patients, gut dysbiosis rather than the presence of pathogens may represent an important cause of posttransplant diarrhea. In a recent study by Lee *et al*[104], fecal specimens from 25 patients presenting diarrhea in the first three months after transplantation were compared with 46 patients who did not develop diarrhea. In the diarrhea group, the abundance of the genera *Eubacterium*, *Anaerostipes*, *Coprococcus, Romboutsia, Ruminococcus, Dorea*, and *Faecalibacterium* were significantly decreased, while the abundance of the genera *Lachnoclostridium*, *Escherichia* and *Enterococcus* were significantly increased. Table 6 provides a detailed description of the data. Many of the bacteria that were present at lower abundance in the diarrhea group belong to the *Lachnospiraceae* and *Ruminococcaceae* families[105] and contribute to metabolic functions essential for gut health[106]. Utilizing the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States Analysis[107], 9 metabolism-related pathways were decreased in the diarrhea group. The decrease in the abundance of these indigenous microbiota bacteria in the subjects affected by diarrhea contributes to the development of an abnormal metabolic status, which might lead to diarrhea.

Interestingly, a similar decrease in the abundance of protective bacteria was also observed in nontransplant patients affected by diarrhea[108].

Notably, the specimens from transplanted patients with diarrhea were negative for known bacterial and protozoan pathogens that cause diarrhea.

Finally, two transplanted patients affected by persistent diarrhea underwent FMT from allogeneic donors. Diarrhea resolved in the first month after FMT, and the abundances of 13 protective bacteria taxa increased with a simultaneous decrease in the abundances of the 3 identified pathobionts or pathogenic bacterial taxa[96,108].

Short Chain Fatty Acids and other metabolites in renal transplantation

SCFAs are produced in the gut by the indigenous microbiota and have a trophyc action on the gut epithelium. In addition, these substances exert an anti-inflammatory effect on the whole body and regulate immune cells.

Ninety-five percent of SFCAs are represented by acetic acid, propionic acid, butyric acid and valeric acid, all of which are derived from saccharolytic fermentation. Under normal conditions with a microbiota producing normal quantities of SCFAs, several beneficial effects have been documented after transplantation both in animals and in humans.

In humans, SCFAs increase the expression of antimicrobial peptides secreted to the external surface by epithelial cells[109]. Studies *in vitro* or in animals documented that SCFAs modulate the production of immune mediators, including IL-18 and other cytokines and chemokines[110], regulate the differentiation, recruitment and activation of immune cells, including neutrophils[111], DCs, macrophages[112] and T lymphocytes[113].

Finally, Wu *et al*[114] documented, in a murine kidney transplantation model, that SCFAs are able to induce donor-specific tolerance by inducing the production of T regulatory cells[114].

Table 6 Most significant genus level composition in the fecal specimens from the diarrhea group and the no diarrhea group

Bacterial Taxonomy Genus	Median relative abundance in the diarrhea group	Median relative abundance in the no diarrhea group	*P* value
Eubacterium	0.002	0.017	1.5E-09
Anaerostipes	0.000	0.005	2.7E-08
Coprococcus	0.000	0.004	3.0E-08
Romboutsia	0.000	0.014	4.2E-06
Ruminococcus	0.007	0.025	8.3E-06
Dorea	0.000	0.007	3.4E-05
Enterococcus	0.002	0.000	1.3E-04
Faecalibacterium	0.000	0.019	1.4E-04
Fusicatenibacter	0.000	0.006	0.001
Oscillibacter	0.001	0.008	0.001
Ruminiclostridium	0.005	0.021	0.002

Andrade-Oliveira *et al*[115] evaluated the effect of SFCAs on a mouse model of ischemia-reperfusion[115].

In the animals, the treatment with SCFAs improved renal function after ischemia-reperfusion injury, reduced the apoptosis, inhibited NFkB activation and nitric oxide production and reactive oxygen species production. All these actions of SCFAs are summarized in Table 7.

In mice, SCFAs decrease the activation of bone marrow-derived DCs and inhibit their function as antigen presenting cells[115].

In conclusion, the authors showed that SFCA supplementation reduces inflammation in their model and improves ischemia-reperfusion injury.

To our knowledge, few studies have been conducted in humans. A recent study by Lee *et al*[116] studied 168 kidney transplant recipients and divided the patients according to whether they had higher levels of butyrate-producing bacteria (BPG) or low levels of BPG. The posttransplant administration of antibiotics was associated with a decrease in BPG levels. These patients have a higher incidence of respiratory tract infections.

For the first time, the clinically beneficial effects of higher butyrate levels and posttransplant-induced dysbiosis were documented in transplanted men and may induce higher infection rates.

Similarly, in another study on transplanted humans, 51 renal transplanted recipients have been followed up to 12 mo after transplantation to study the serum levels of uremic toxins as p cresyl sulfate, p cresyl glucoronide, indoxyl sulfate, TMAO and phenylacetylglutamine. The results were compared with CKD control patients with similar renal function. The study documented that after transplantation the colonic microbiota derived uremic retention solutes decreases. As the urinary excretion is lower in transplanted patients, this fact suggests an independent effect after transplantation on intestinal uptake and a different colonic microbial metabolism and absorption[117].

The microbiota and tolerance

The aforementioned hypothesis that gut microbioma metabolites such as SCFAs could induce donor-specific tolerance through the induction of regulatory T cell differentiations[114], introduces the chapter on the relationship between microbiota and tolerance.

This relationship is well known in the development of immune tolerance in children. Indeed, in the first 1000 d of life, the early exposure of food allergens to indigenous intestinal microbiota induces tolerance through activation of Tregs and subsequent production of TGFβ and IL-10[118].

In a recent study, Colas *et al*[119] examined the urinary microbiota of 86 renal transplant patients. Patients were divided into 3 groups: Normally immuno-suppressed with stable renal function, minimally immunosuppressed, and spontaneously tolerant patients. Differences in microbiota profiles were observed, and a unique and specific urinary microbiota was detected in patients with spontaneous

Table 7 Actions of short-chain fatty acids on a model of ischemia reperfusion syndrome

Actions
SCFAs improve renal function
SCFAs decrease apoptosis and increase tubular proliferating cells
SCFAs decrease activation of bone marrow derived dendritic cells and inhibit their function as antigen presenting cells
SCFAs inhibit NFkB activation and nitric oxide production
SFCAs inhibit ROS production

SCAF: Short chain fatty acid; ROS: Reactive oxygen species; NFkB: Nuclear factor kappa-light-chain-enhancer of activated B cells.

tolerance characterized by a clear *Proteobacteria* profile. The profile was different in patients stratified according to gender (higher in males) and inversely correlated with the quantity of immunosuppressive drugs.

The *Proteobacteria* detected in tolerant subjects included *Janthinobacterium, Clostridia* and *Firmicutes*. *Janthinobacterium* is known to produce an indole-derived peptide with antiproliferative and anti-inflammatory activities[120,121]. Clostridia exert an anti-inflammatory effect by producing SCFAs[122]. *Firmicutes* produce indole derivatives[123] and polyphosphate[124] with anti-inflammatory activities.

In conclusion, the indigenous microbiota may favor the induction of tolerance, but the use of immunosuppressants modifying the microbiota may represent an obstacle to the development of the tolerance state.

Interactions between the microbiota and immunosuppressants

Bilateral actions between the microbiota and immunosuppressive drugs have been identified. On one hand, the microbiota may modify the absorption and the metabolism of immunosuppressants; on the other hand, immunosuppressants may modify the indigenous microbiota.

The vast majority of studies on this issue have been conducted on calcineurine inhibitors.

Several studies have extensively documented that factors such as age, gender, race and CYP3A5 polymorphisms influence the absorption and metabolism of immunosuppressants and account for interindividual variability such that the individual dosing is not the same for all patients.

Recently, the gut indigenous microbiota or the pathobionts have been suspected to exert a powerful effect, justifying the different metabolism from one patient to another and in the same subject.

The assumption of other drugs, such as antibiotics, modifying the indigenous microbiota may account for this variability[125-128].

Lee *et al*[129] examined the microbiota in the fecal specimens of 19 patients who received a kidney transplant and were on tacrolimus (TAC) as the principal immunosuppressive therapy. All patients received the same prophylactic antibiotic therapy to avoid biases. Patients were divided into two groups according to the need to receive increasing TAC doses (Dose Escalation Group) or not (Dose Stable Group). By examining the microbiota, the authors found a significantly higher level of *Faecalibacterium prausnitzii* in patients from the Dose Escalation Group than in patients from the Dose Stable Group. In addition, *Faecalibacterium prausnitzii* was the most significant factor justifying the need to increase the TAC dose. Even if a large quantity of TAC is absorbed by the small intestine, it may also be absorbed in the colon[130]. Although the Lee's study is a pilot one, the results raise the question of the relevance of microbiota and of *Faecalibacterium prausnitzii,* particularly on TAC trough levels, which are also important due to the narrow therapeutic index of TAC.

In a different study, Guo *et al*[131] incubated *Faecalibacterium prausnitzii* cells *in vitro* with TAC. The authors detected a compound named M1 that is a cheto-produced metabolite of TAC with a less powerful immunosuppressant. The authors measured a large quantity of M1 in the stool samples of patients with a larger quantity of *Faecalibacterium prausnitzii* in the stool.

In addition, the same study documented that other bacteria, such as Clostridia and Bacteroidales, are able to convert TAC into M1 metabolites. The authors conclude that several commensal microbiota may metabolize TAC in the gut to less powerful compounds, explaining the differences in TAC exposure in transplant recipients.

On one hand, the microbiota may alter the metabolism of immunosuppressants; on the other hand, immunosuppressants may alter the gut indigenous microbiota. The study by Gibson *et al*[132] reviewed this topic extensively. Unfortunately the vast majority of studies have been conducted on calcineurine inhibitors and very few have examined renal transplantation.

The studies by Zhang *et al*[133] and by Lee *et al*[129] documented the effect of TAC on the gut microbiota in renal transplant recipients. Other studies[134] analyzed the same phenomenon in liver transplant recipients. Zaza *et al*[135] examined the microbiota in patients receiving TAC + MMF or everolimus + MMF, but they did not observe any difference.

In the pilot study by Lee *et al* [79], patients with early corticosteroid withdrawal had fewer *Clostridiales* and *Erysipelotrichaeles* in the microbiota, but the difference was not statistically significant.

Finally, a recent study[136] documenting that encapsulated cyclosporine A does not change the composition of the human indigenous microbiota is worth mentioning.

MICROBIAL THERAPIES IN KIDNEY HEALTHY, DISEASE AND TRANSPLANTATION

The treatment of gut dysbiosis may be divided into probiotics, smart bacteria, prebiotics, a high-fiber diet and fecal microbiota transplantation.

Several of these therapies have been used in patients affected by chronic kidney disease.

Probiotics are defined by the World Health Organization as live organisms that, when administered in adequate amounts, confer a health benefit to the host[137]. Probiotics such as *Lactobacilli* and *Streptococci*[138,139] have been used to treat CKD. They are able to enhance gut barriers, improve mucosal immunity and modulate the host signaling pathways by reducing the activation of NFkB and the MAPK[140,141]. Smart bacteria are genetically modified bacteria that are able to remove toxic molecules in animal studies[142,143].

Prebiotics are nonviable food components that confer health benefits to the host associated with the modulation of the microbiota[144]. A prebiotic must be resistant to gastric acid and digestive enzymes, allowing it to reach the small and the large intestines to stimulate the activity of beneficial microbes. To date, only insulin and trans-galacto-oligosaccharides have these characteristics and may be considered prebiotics[145].

The principal mechanisms of action of prebiotics are to increase the production of SCFAs and to decrease the intestinal pH[146].

Unfortunately, the vast majority of studies using these therapies have been conducted in animal models of CKD.

Few studies have assess probiotics in humans, particularly kidney transplant recipients and most studies were conducted in liver transplant patients[5,95].

Currently, the most effective treatment for renal transplant recipients appears to be FMT, principally in patients affected by infection and/or diarrhea due to resistant *Clostridium difficile* or *E. coli*[79,96].

FUTURE PERSPECTIVES

Two main issues are involved in the search for new perspectives: the search for new therapies and an improved knowledge of gut microbiota and pathobionts.

New therapies: Potential benefits of nutritional and supplementation approaches may target microbiota in CKD patients. In CKD, nutritional management and supplementation, including salt and protein restriction, vegetable intakes, and the use of pro-, pre-, and synbiotics, has several benefits. Modulate gut microbiota dysbiosis, decrease colonic production of proteolytic derived uremic toxins and reduce inflammation and oxidative stress[147].

Strategies targeting the microbial source of immune regulation are also promising. The presence of *Lactobacillales* in the gut microbiota promotes Treg cells and suppresses Th17 in the kidney. The oral administration of *Lacidophilus* ATCC4356 in the animals attenuates atherosclerotic progression[148].

Lubiprostone, a synthetic derivative of prostaglandin, in a rat model of CKD is associated with reduction of kidney inflammation and improvement of microbioma

profile with proliferation of saccarolytic bacteria.

Similarly, the trimethylamine inhibitor 3,3-dimethyl-1-butanol inhibits the atherosclerotic lesions in mice[149].

The identification of causative bacteria in the context of kidney disease and the distinction of indigenous microbioma from pathobionts is a technical challenge.

Sequencing techniques and a wide application of metabolomics allowed us for an improved understanding of microbioma in health and diseases.

The National Institute of Diabetes and Digestive and Kidney Diseases is conducting a study (ClinicalTrials.gov Identifier: NCT02572882)[150] aimed to Characterize the Gut Microbiome of Individuals With End-stage Renal Disease Treated With Maintenance Hemodialysis, and to Explore Effects of P-inulin on the Gut Microbiome.

Future studies should explore the interaction of microbioma with human genoma and how the microbioma should be treated in the case of renal disease and renal transplantation[137].

CONCLUSION

In the last decade, relevant importance in conditioning both the healthy status and several diseases has been assumed by the microbiota. The microbiota is defined as the microorganisms that live in our body.

Gut microbiota has an important function because can metabolize food and produce substances as SCFAs extremely useful for the body. In addition, the microbiota has important relationship with the immune system and, when modified may induce abnormal activation of the immunity that may cause disease.

Renal diseases may be induced by dysbiosis both for the activation of the immune system and for the production of an excess of uremic system.

In several renal diseases and in particular in the case of end stage renal disease the normal microbiota changes with development of pathobionts and the consequent dysbiosis is responsible for the further deterioration of the renal function.

In the case of renal transplantation, the microbiota has a relevant function.

After transplantation, because of the assumption of immunosuppressive drugs and of prophylactic antibiosis, the gut indigenous microbiota profile modifies, particularly in the first month after transplantation. This modification may influence the graft outcomes causing acute rejection, infections, renal fibrosis and modifications of the drug metabolism, immunosuppressants included. It is possible to modify an abnormal microbiota with the use of prebiotics, probiotics and diet modification.

It should be highlighted that all the studies referring to the microbiota in renal transplantation are few, refer to small number of patients, often retrospectives. In addition, many of these studies have been conducted in animals. Because of this fact the microbiota in general and in solid organ transplantation in particular may be considered a new frontier in medical studies.

REFERENCES

1 **Yamaguchi H**, Goto S, Takahashi N, Tsuchida M, Watanabe H, Yamamoto S, Kaneko Y, Higashi K, Mori H, Nakamura Y, Horii A, Kurokawa K, Narita I. Aberrant mucosal immunoreaction to tonsillar microbiota in immunoglobulin A nephropathy. *Nephrol Dial Transplant* 2021; **36**: 75-86 [PMID: 33099625 DOI: 10.1093/ndt/gfaa223]

2 **Coppo R**. The Gut-Renal Connection in IgA Nephropathy. *Semin Nephrol* 2018; **38**: 504-512 [PMID: 30177022 DOI: 10.1016/j.semnephrol.2018.05.020]

3 **Rollino C**. Up-to date of glomerular disease. *J Nephrol* 2016; **29**: 461-462 [PMID: 27372476 DOI: 10.1007/s40620-016-0330-5]

4 **Mu Q**, Zhang H, Liao X, Lin K, Liu H, Edwards MR, Ahmed SA, Yuan R, Li L, Cecere TE, Branson DB, Kirby JL, Goswami P, Leeth CM, Read KA, Oestreich KJ, Vieson MD, Reilly CM, Luo XM. Control of lupus nephritis by changes of gut microbiota. *Microbiome* 2017; **5**: 73 [PMID: 28697806 DOI: 10.1186/s40168-017-0300-8]

5 **Ardalan M**, Vahed SZ. Gut microbiota and renal transplant outcome. *Biomed Pharmacother* 2017; **90**: 229-236 [PMID: 28363168 DOI: 10.1016/j.biopha.2017.02.114]

6 **Sender R**, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol* 2016; **14**: e1002533 [PMID: 27541692 DOI: 10.1371/journal.pbio.1002533]

7 **Scholtens PA**, Oozeer R, Martin R, Amor KB, Knol J. The early settlers: intestinal microbiology in early life. *Annu Rev Food Sci Technol* 2012; **3**: 425-447 [PMID: 22224552 DOI: 10.1146/annurev-food-022811-101120]

8 **Koenig JE**, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE.

Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci* 2011; **108** Suppl 1: 4578-4585 [PMID: 20668239 DOI: 10.1073/pnas.1000081107]

9 **Rajilić-Stojanović M**, Heilig HG, Molenaar D, Kajander K, Surakka A, Smidt H, de Vos WM. Development and application of the human intestinal tract chip, a phylogenetic microarray: analysis of universally conserved phylotypes in the abundant microbiota of young and elderly adults. *Environ Microbiol* 2009; **11**: 1736-1751 [PMID: 19508560 DOI: 10.1111/j.1462-2920.2009.01900.x]

10 **Chow J**, Tang H, Mazmanian SK. Pathobionts of the gastrointestinal microbiota and inflammatory disease. *Curr Opin Immunol* 2011; **23**: 473-480 [PMID: 21856139 DOI: 10.1016/j.coi.2011.07.010]

11 **Human Microbiome Project Consortium**. Structure, function and diversity of the healthy human microbiome. *Nature* 2012; **486**: 207-214 [PMID: 22699609 DOI: 10.1038/nature11234]

12 **Gevers D**, Knight R, Petrosino JF, Huang K, McGuire AL, Birren BW, Nelson KE, White O, Methé BA, Huttenhower C. The Human Microbiome Project: a community resource for the healthy human microbiome. *PLoS Biol* 2012; **10**: e1001377 [PMID: 22904687 DOI: 10.1371/journal.pbio.1001377]

13 **NIH HMP Working Group**. , Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, Bonazzi V, McEwen JE, Wetterstrand KA, Deal C, Baker CC, Di Francesco V, Howcroft TK, Karp RW, Lunsford RD, Wellington CR, Belachew T, Wright M, Giblin C, David H, Mills M, Salomon R, Mullins C, Akolkar B, Begg L, Davis C, Grandison L, Humble M, Khalsa J, Little AR, Peavy H, Pontzer C, Portnoy M, Sayre MH, Starke-Reed P, Zakhari S, Read J, Watson B, Guyer M. The NIH Human Microbiome Project. *Genome Res* 2009; **19**: 2317-2323 [PMID: 19819907 DOI: 10.1101/gr.096651.109]

14 **Qin J**, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Jian M, Zhou Y, Li Y, Zhang X, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J; MetaHIT Consortium, Bork P, Ehrlich SD. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59-65 [PMID: 20203603 DOI: 10.1038/nature08821]

15 **Bäckhed F**, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, Khan MT, Zhang J, Li J, Xiao L, Al-Aama J, Zhang D, Lee YS, Kotowska D, Colding C, Tremaroli V, Yin Y, Bergman S, Xu X, Madsen L, Kristiansen K, Dahlgren J, Wang J. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. *Cell Host Microbe* 2015; **17**: 852 [PMID: 26308884 DOI: 10.1016/j.chom.2015.05.012]

16 **Anderson JW**, Baird P, Davis RH Jr, Ferreri S, Knudtson M, Koraym A, Waters V, Williams CL. Health benefits of dietary fiber. *Nutr Rev* 2009; **67**: 188-205 [PMID: 19335713 DOI: 10.1111/j.1753-4887.2009.00189.x]

17 **Lombard V**, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res* 2014; **42**: D490-D495 [PMID: 24270786 DOI: 10.1093/nar/gkt1178]

18 **Louis P**, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett* 2009; **294**: 1-8 [PMID: 19222573 DOI: 10.1111/j.1574-6968.2009.01514.x]

19 **Nicholson JK**, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. *Science* 2012; **336**: 1262-1267 [PMID: 22674330 DOI: 10.1126/science.1223813]

20 **Rawlings ND**, Barrett AJ, Thomas PD, Huang X, Bateman A, Finn RD. The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. *Nucleic Acids Res* 2018; **46**: D624-D632 [PMID: 29145643 DOI: 10.1093/nar/gkx1134]

21 **Portune KJ**, Benítez-Páez A, Del Pulgar EM, Cerrudo V, Sanz Y. Gut microbiota, diet, and obesity-related disorders-The good, the bad, and the future challenges. *Mol Nutr Food Res* 2017; **61** [PMID: 27287778 DOI: 10.1002/mnfr.201600252]

22 **Adak A**, Khan MR. An insight into gut microbiota and its functionalities. *Cell Mol Life Sci* 2019; **76**: 473-493 [PMID: 30317530 DOI: 10.1007/s00018-018-2943-4]

23 **Saitoh Y**, Suzuki H, Tani K, Nishikawa K, Irie K, Ogura Y, Tamura A, Tsukita S, Fujiyoshi Y. Tight junctions. Structural insight into tight junction disassembly by Clostridium perfringens enterotoxin. *Science* 2015; **347**: 775-778 [PMID: 25678664 DOI: 10.1126/science.1261833]

24 **Yuhan R**, Koutsouris A, Savkovic SD, Hecht G. Enteropathogenic Escherichia coli-induced myosin light chain phosphorylation alters intestinal epithelial permeability. *Gastroenterology* 1997; **113**: 1873-1882 [PMID: 9394726 DOI: 10.1016/s0016-5085(97)70006-4]

25 **Hecht G**, Koutsouris A, Pothoulakis C, LaMont JT, Madara JL. Clostridium difficile toxin B disrupts the barrier function of T84 monolayers. *Gastroenterology* 1992; **102**: 416-423 [PMID: 1732112 DOI: 10.1016/0016-5085(92)90085-d]

26 **Lee SH**. Intestinal permeability regulation by tight junction: implication on inflammatory bowel diseases. *Intest Res* 2015; **13**: 11-18 [PMID: 25691839 DOI: 10.5217/ir.2015.13.1.11]

27 **Round JL**, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009; **9**: 313-323 [PMID: 19343057 DOI: 10.1038/nri2515]

28 **Johansson ME**, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc Natl Acad Sci* 2008; **105**: 15064-15069 [PMID: 18806221 DOI: 10.1073/pnas.0803124105]

29 **Wilson CL**, Ouellette AJ, Satchell DP, Ayabe T, López-Boado YS, Stratman JL, Hultgren SJ, Matrisian LM, Parks WC. Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* 1999; **286**: 113-117 [PMID: 10506557 DOI: 10.1126/science.286.5437.113]

30 **Boneca IG**, Dussurget O, Cabanes D, Nahori MA, Sousa S, Lecuit M, Psylinakis E, Bouriotis V, Hugot JP, Giovannini M, Coyle A, Bertin J, Namane A, Rousselle JC, Cayet N, Prévost MC, Balloy V, Chignard M, Philpott DJ, Cossart P, Girardin SE. A critical role for peptidoglycan N-deacetylation in Listeria evasion from the host innate immune system. *Proc Natl Acad Sci* 2007; **104**: 997-1002 [PMID: 17215377 DOI: 10.1073/pnas.0609672104]

31 **Cash HL**, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* 2006; **313**: 1126-1130 [PMID: 16931762 DOI: 10.1126/science.1127119]

32 **Macpherson AJ**, Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* 2004; **303**: 1662-1665 [PMID: 15016999 DOI: 10.1126/science.1091334]

33 **Rescigno M**, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, Granucci F, Kraehenbuhl JP, Ricciardi-Castagnoli P. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2001; **2**: 361-367 [PMID: 11276208 DOI: 10.1038/86373]

34 **Rahmel T**. [SSC International Guideline 2016 - Management of Sepsis and Septic Shock]. *Anasthesiol Intensivmed Notfallmed Schmerzther* 2018; **53**: 142-148 [PMID: 29426052 DOI: 10.1055/s-0043-114639]

35 **Belkaid Y**, Harrison OJ. Homeostatic Immunity and the Microbiota. *Immunity* 2017; **46**: 562-576 [PMID: 28423337 DOI: 10.1016/j.immuni.2017.04.008]

36 **Hooper LV**, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annu Rev Nutr* 2002; **22**: 283-307 [PMID: 12055347 DOI: 10.1146/annurev.nutr.22.011602.092259]

37 **Ivanov II**, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 2008; **4**: 337-349 [PMID: 18854238 DOI: 10.1016/j.chom.2008.09.009]

38 **Arrieta MC**, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The intestinal microbiome in early life: health and disease. *Front Immunol* 2014; **5**: 427 [PMID: 25250028 DOI: 10.3389/fimmu.2014.00427]

39 **Maslowski KM**, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, Xavier RJ, Teixeira MM, Mackay CR. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 2009; **461**: 1282-1286 [PMID: 19865172 DOI: 10.1038/nature08530]

40 **Rooks MG**, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol* 2016; **16**: 341-352 [PMID: 27231050 DOI: 10.1038/nri.2016.42]

41 **Kim YG**, Udayanga KG, Totsuka N, Weinberg JB, Núñez G, Shibuya A. Gut dysbiosis promotes M2 macrophage polarization and allergic airway inflammation *via* fungi-induced PGE_2. *Cell Host Microbe* 2014; **15**: 95-102 [PMID: 24439901 DOI: 10.1016/j.chom.2013.12.010]

42 **Zhang D**, Chen G, Manwani D, Mortha A, Xu C, Faith JJ, Burk RD, Kunisaki Y, Jang JE, Scheiermann C, Merad M, Frenette PS. Neutrophil ageing is regulated by the microbiome. *Nature* 2015; **525**: 528-532 [PMID: 26374999 DOI: 10.1038/nature15367]

43 **Gensollen T**, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. *Science* 2016; **352**: 539-544 [PMID: 27126036 DOI: 10.1126/science.aad9378]

44 **Duan J**, Chung H, Troy E, Kasper DL. Microbial colonization drives expansion of IL-1 receptor 1-expressing and IL-17-producing gamma/delta T cells. *Cell Host Microbe* 2010; **7**: 140-150 [PMID: 20159619 DOI: 10.1016/j.chom.2010.01.005]

45 **Paget C**, Chow MT, Gherardin NA, Beavis PA, Uldrich AP, Duret H, Hassane M, Souza-Fonseca-Guimaraes F, Mogilenko DA, Staumont-Sallé D, Escalante NK, Hill GR, Neeson P, Ritchie DS, Dombrowicz D, Mallevaey T, Trottein F, Belz GT, Godfrey DI, Smyth MJ. CD3bright signals on γδ T cells identify IL-17A-producing Vγ6Vδ1+ T cells. *Immunol Cell Biol* 2015; **93**: 198-212 [PMID: 25385067 DOI: 10.1038/icb.2014.94]

46 **Bukina Y**, Thyhonovska M, Koval M, Marushchak M, Krynytska I, Kamyshnyi A. The effect of immunoregulatory bacteria on the transcriptional activity of Foxp3 and RORyt genes in the gut-associated lymphoid tissue with *Salmonella*-induced inflammation in the presence of vancomycin and *Bacteroides fragilis*. *Iran J Microbiol* 2020; **12**: 231-241 [PMID: 32685120 DOI: 10.18502/ijm.v12i3.3241]

47 **Yang BH**, Hagemann S, Mamareli P, Lauer U, Hoffmann U, Beckstette M, Föhse L, Prinz I, Pezoldt J, Suerbaum S, Sparwasser T, Hamann A, Floess S, Huehn J, Lochner M. Foxp3(+) T cells expressing RORγt represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. *Mucosal Immunol* 2016; **9**: 444-457 [PMID: 26307665 DOI: 10.1038/mi.2015.74]

48 **Ivanov II**, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. Induction of

intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009; **139**: 485-498 [PMID: 19836068 DOI: 10.1016/j.cell.2009.09.033]

49 **Atarashi K**, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, Taniguchi T, Takeda K, Hori S, Ivanov II, Umesaki Y, Itoh K, Honda K. Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* 2011; **331**: 337-341 [PMID: 21205640 DOI: 10.1126/science.1198469]

50 **Mazmanian SK**, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 2008; **453**: 620-625 [PMID: 18509436 DOI: 10.1038/nature07008]

51 **Kitching AR**, Holdsworth SR. The emergence of TH17 cells as effectors of renal injury. *J Am Soc Nephrol* 2011; **22**: 235-238 [PMID: 21289213 DOI: 10.1681/ASN.2010050536]

52 **Krebs CF**, Paust HJ, Krohn S, Koyro T, Brix SR, Riedel JH, Bartsch P, Wiech T, Meyer-Schwesinger C, Huang J, Fischer N, Busch P, Mittrücker HW, Steinhoff U, Stockinger B, Perez LG, Wenzel UO, Janneck M, Steinmetz OM, Gagliani N, Stahl RAK, Huber S, Turner JE, Panzer U. Autoimmune Renal Disease Is Exacerbated by S1P-Receptor-1-Dependent Intestinal Th17 Cell Migration to the Kidney. *Immunity* 2016; **45**: 1078-1092 [PMID: 27851911 DOI: 10.1016/j.immuni.2016.10.020]

53 **Krebs CF**, Kapffer S, Paust HJ, Schmidt T, Bennstein SB, Peters A, Stege G, Brix SR, Meyer-Schwesinger C, Müller RU, Turner JE, Steinmetz OM, Wolf G, Stahl RA, Panzer U. MicroRNA-155 drives TH17 immune response and tissue injury in experimental crescentic GN. *J Am Soc Nephrol* 2013; **24**: 1955-1965 [PMID: 23949802 DOI: 10.1681/ASN.2013020130]

54 **Velden J**, Paust HJ, Hoxha E, Turner JE, Steinmetz OM, Wolf G, Jabs WJ, Özcan F, Beige J, Heering PJ, Schröder S, Kneißler U, Disteldorf E, Mittrücker HW, Stahl RA, Helmchen U, Panzer U. Renal IL-17 expression in human ANCA-associated glomerulonephritis. *Am J Physiol Renal Physiol* 2012; **302**: F1663-F1673 [PMID: 22442208 DOI: 10.1152/ajprenal.00683.2011]

55 **Yang J**, Kim CJ, Go YS, Lee HY, Kim MG, Oh SW, Cho WY, Im SH, Jo SK. Intestinal microbiota control acute kidney injury severity by immune modulation. *Kidney Int* 2020; **98**: 932-946 [PMID: 32470493 DOI: 10.1016/j.kint.2020.04.048]

56 **Rowland I**, Gibson G, Heinken A, Scott K, Swann J, Thiele I, Tuohy K. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr* 2018; **57**: 1-24 [PMID: 28393285 DOI: 10.1007/s00394-017-1445-8]

57 **Lin L**, Zhang J. Role of intestinal microbiota and metabolites on gut homeostasis and human diseases. *BMC Immunol* 2017; **18**: 2 [PMID: 28061847 DOI: 10.1186/s12865-016-0187-3]

58 **Inoue D**, Tsujimoto G, Kimura I. Regulation of Energy Homeostasis by GPR41. *Front Endocrinol (Lausanne)* 2014; **5**: 81 [PMID: 24904531 DOI: 10.3389/fendo.2014.00081]

59 **Tolhurst G**, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, Cameron J, Grosse J, Reimann F, Gribble FM. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion *via* the G-protein-coupled receptor FFAR2. *Diabetes* 2012; **61**: 364-371 [PMID: 22190648 DOI: 10.2337/db11-1019]

60 **Lukasova M**, Malaval C, Gille A, Kero J, Offermanns S. Nicotinic acid inhibits progression of atherosclerosis in mice through its receptor GPR109A expressed by immune cells. *J Clin Invest* 2011; **121**: 1163-1173 [PMID: 21317532 DOI: 10.1172/JCI41651]

61 **Pluznick J**. A novel SCFA receptor, the microbiota, and blood pressure regulation. *Gut Microbes* 2014; **5**: 202-207 [PMID: 24429443 DOI: 10.4161/gmic.27492]

62 **Singh N**, Thangaraju M, Prasad PD, Martin PM, Lambert NA, Boettger T, Offermanns S, Ganapathy V. Blockade of dendritic cell development by bacterial fermentation products butyrate and propionate through a transporter (Slc5a8)-dependent inhibition of histone deacetylases. *J Biol Chem* 2010; **285**: 27601-27608 [PMID: 20601425 DOI: 10.1074/jbc.M110.102947]

63 **Andrade-Oliveira V**, Foresto-Neto O, Watanabe IKM, Zatz R, Câmara NOS. Inflammation in Renal Diseases: New and Old Players. *Front Pharmacol* 2019; **10**: 1192 [PMID: 31649546 DOI: 10.3389/fphar.2019.01192]

64 **Matsumoto T**, Kojima M, Takayanagi K, Taguchi K, Kobayashi T. Role of S-Equol, Indoxyl Sulfate, and Trimethylamine N-Oxide on Vascular Function. *Am J Hypertens* 2020; **33**: 793-803 [PMID: 32300778 DOI: 10.1093/ajh/hpaa053]

65 **Tang WH**, Wang Z, Kennedy DJ, Wu Y, Buffa JA, Agatisa-Boyle B, Li XS, Levison BS, Hazen SL. Gut microbiota-dependent trimethylamine N-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. *Circ Res* 2015; **116**: 448-455 [PMID: 25599331 DOI: 10.1161/CIRCRESAHA.116.305360]

66 **Satoh M**, Hayashi H, Watanabe M, Ueda K, Yamato H, Yoshioka T, Motojima M. Uremic toxins overload accelerates renal damage in a rat model of chronic renal failure. *Nephron Exp Nephrol* 2003; **95**: e111-e118 [PMID: 14646363 DOI: 10.1159/000074327]

67 **Noel S**, Martina-Lingua MN, Bandapalle S, Pluznick J, Hamad AR, Peterson DA, Rabb H. Intestinal microbiota-kidney cross talk in acute kidney injury and chronic kidney disease. *Nephron Clin Pract* 2014; **127**: 139-143 [PMID: 25343838 DOI: 10.1159/000363209]

68 **Vaziri ND**, Wong J, Pahl M, Piceno YM, Yuan J, DeSantis TZ, Ni Z, Nguyen TH, Andersen GL. Chronic kidney disease alters intestinal microbial flora. *Kidney Int* 2013; **83**: 308-315 [PMID: 22992469 DOI: 10.1038/ki.2012.345]

69 **Cigarran Guldris S**, González Parra E, Cases Amenós A. Gut microbiota in chronic kidney disease. *Nefrologia* 2017; **37**: 9-19 [PMID: 27553986 DOI: 10.1016/j.nefro.2016.05.008]

70 **Aron-Wisnewsky J**, Clément K. The gut microbiome, diet, and links to cardiometabolic and chronic

disorders. *Nat Rev Nephrol* 2016; **12**: 169-181 [PMID: 26616538 DOI: 10.1038/nrneph.2015.191]

71 **Sabatino A**, Regolisti G, Brusasco I, Cabassi A, Morabito S, Fiaccadori E. Alterations of intestinal barrier and microbiota in chronic kidney disease. *Nephrol Dial Transplant* 2015; **30**: 924-933 [PMID: 25190600 DOI: 10.1093/ndt/gfu287]

72 **Guo TL**, Chen Y, Xu HS, McDonough CM, Huang G. Gut microbiome in neuroendocrine and neuroimmune interactions: The case of genistein. *Toxicol Appl Pharmacol* 2020; **402**: 115130 [PMID: 32673657 DOI: 10.1016/j.taap.2020.115130]

73 **Anders HJ**, Andersen K, Stecher B. The intestinal microbiota, a leaky gut, and abnormal immunity in kidney disease. *Kidney Int* 2013; **83**: 1010-1016 [PMID: 23325079 DOI: 10.1038/ki.2012.440]

74 **Ramezani A**, Raj DS. The gut microbiome, kidney disease, and targeted interventions. *J Am Soc Nephrol* 2014; **25**: 657-670 [PMID: 24231662 DOI: 10.1681/ASN.2013080905]

75 **Evenepoel P**, Poesen R, Meijers B. The gut-kidney axis. *Pediatr Nephrol* 2017; **32**: 2005-2014 [PMID: 27848096 DOI: 10.1007/s00467-016-3527-x]

76 **Koppe L**, Mafra D, Fouque D. Probiotics and chronic kidney disease. *Kidney Int* 2015; **88**: 958-966 [PMID: 26376131 DOI: 10.1038/ki.2015.255]

77 **Ramezani A**, Massy ZA, Meijers B, Evenepoel P, Vanholder R, Raj DS. Role of the Gut Microbiome in Uremia: A Potential Therapeutic Target. *Am J Kidney Dis* 2016; **67**: 483-498 [PMID: 26590448 DOI: 10.1053/j.ajkd.2015.09.027]

78 **Rapone B**, Ferrara E, Santacroce L, Cesarano F, Arazzi M, Liberato LD, Scacco S, Grassi R, Grassi FR, Gnoni A, Nardi GM. Periodontal Microbiological Status Influences the Occurrence of Cyclosporine-A and Tacrolimus-Induced Gingival Overgrowth. *Antibiotics (Basel)* 2019; **8** [PMID: 31438651 DOI: 10.3390/antibiotics8030124]

79 **Lee JR**, Muthukumar T, Dadhania D, Toussaint NC, Ling L, Pamer E, Suthanthiran M. Gut microbial community structure and complications after kidney transplantation: a pilot study. *Transplantation* 2014; **98**: 697-705 [PMID: 25289916 DOI: 10.1097/TP.0000000000000370]

80 **Integrative HMP (iHMP) Research Network Consortium**. The Integrative Human Microbiome Project. *Nature* 2019; **569**: 641-648 [PMID: 31142853 DOI: 10.1038/s41586-019-1238-8]

81 **Taur Y**, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, Lee YJ, Dubin KA, Socci ND, Viale A, Perales MA, Jenq RR, van den Brink MR, Pamer EG. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clin Infect Dis* 2012; **55**: 905-914 [PMID: 22718773 DOI: 10.1093/cid/cis580]

82 **Xiao J**, Peng Z, Liao Y, Sun H, Chen W, Chen X, Wei Z, Yang C, Nüssler AK, Liu J, Yang W. Organ transplantation and gut microbiota: current reviews and future challenges. *Am J Transl Res* 2018; **10**: 3330-3344 [PMID: 30662590]

83 **Swarte JC**, Douwes RM, Hu S, Vich Vila A, Eisenga MF, van Londen M, Gomes-Neto AW, Weersma RK, Harmsen HJM, Bakker SJL. Characteristics and Dysbiosis of the Gut Microbiome in Renal Transplant Recipients. *J Clin Med* 2020; **9** [PMID: 32024079 DOI: 10.3390/jcm9020386]

84 **Shin NR**, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol* 2015; **33**: 496-503 [PMID: 26210164 DOI: 10.1016/j.tibtech.2015.06.011]

85 **Flint HJ**, Duncan SH, Scott KP, Louis P. Links between diet, gut microbiota composition and gut metabolism. *Proc Nutr Soc* 2015; **74**: 13-22 [PMID: 25268552 DOI: 10.1017/S0029665114001463]

86 **Furusawa Y**, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013; **504**: 446-450 [PMID: 24226770 DOI: 10.1038/nature12721]

87 **Sampaio-Maia B**, Simões-Silva L, Pestana M, Araujo R, Soares-Silva IJ. The Role of the Gut Microbiome on Chronic Kidney Disease. *Adv Appl Microbiol* 2016; **96**: 65-94 [PMID: 27565581 DOI: 10.1016/bs.aambs.2016.06.002]

88 **Imhann F**, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, Vork L, Tigchelaar EF, Jankipersadsing SA, Cenit MC, Harmsen HJ, Dijkstra G, Franke L, Xavier RJ, Jonkers D, Wijmenga C, Weersma RK, Zhernakova A. Proton pump inhibitors affect the gut microbiome. *Gut* 2016; **65**: 740-748 [PMID: 26657899 DOI: 10.1136/gutjnl-2015-310376]

89 **Spasić A**, Catić-Đorđević A, Veličković-Radovanović R, Stefanović N, Džodić P, Cvetković T. Adverse effects of mycophenolic acid in renal transplant recipients: gender differences. *Int J Clin Pharm* 2019; **41**: 776-784 [PMID: 31028595 DOI: 10.1007/s11096-019-00837-z]

90 **Westblade LF**, Satlin MJ, Albakry S, Botticelli B, Robertson A, Alston T, Magruder M, Zhang LT, Edusei E, Chan K, Lubetzky M, Dadhania DM, Pamer EG, Suthanthiran M, Lee JR. Gastrointestinal pathogen colonization and the microbiome in asymptomatic kidney transplant recipients. *Transpl Infect Dis* 2019; **21**: e13167 [PMID: 31502737 DOI: 10.1111/tid.13167]

91 **Wang W**, Xu S, Ren Z, Jiang J, Zheng S. Gut microbiota and allogeneic transplantation. *J Transl Med* 2015; **13**: 275 [PMID: 26298517 DOI: 10.1186/s12967-015-0640-8]

92 **Ahmad S**, Bromberg JS. Current status of the microbiome in renal transplantation. *Curr Opin Nephrol Hypertens* 2016; **25**: 570-576 [PMID: 27517135 DOI: 10.1097/MNH.0000000000000262]

93 **Lei YM**, Chen L, Wang Y, Stefka AT, Molinero LL, Theriault B, Aquino-Michaels K, Sivan AS, Nagler CR, Gajewski TF, Chong AS, Bartman C, Alegre ML. The composition of the microbiota modulates allograft rejection. *J Clin Invest* 2016; **126**: 2736-2744 [PMID: 27322054 DOI: 10.1172/JCI85295]

94 **Fricke WF**, Maddox C, Song Y, Bromberg JS. Human microbiota characterization in the course of renal transplantation. *Am J Transplant* 2014; **14**: 416-427 [PMID: 24373208 DOI: 10.1111/ajt.12588]

95 **Chan S**, Hawley CM, Campbell KL, Morrison M, Campbell SB, Isbel NM, Francis RS, Playford EG, Johnson DW. Transplant associated infections-The role of the gastrointestinal microbiota and potential therapeutic options. *Nephrology (Carlton)* 2020; **25**: 5-13 [PMID: 31587409 DOI: 10.1111/nep.13670]

96 **Biehl LM**, Cruz Aguilar R, Farowski F, Hahn W, Nowag A, Wisplinghoff H, Vehreschild MJGT. Fecal microbiota transplantation in a kidney transplant recipient with recurrent urinary tract infection. *Infection* 2018; **46**: 871-874 [PMID: 30109576 DOI: 10.1007/s15010-018-1190-9]

97 **Cheng J**, Torkamani A, Grover RK, Jones TM, Ruiz DI, Schork NJ, Quigley MM, Hall FW, Salomon DR, Lerner RA. Ectopic B-cell clusters that infiltrate transplanted human kidneys are clonal. *Proc Natl Acad Sci U S A* 2011; **108**: 5560-5565 [PMID: 21415369 DOI: 10.1073/pnas.1101148108]

98 **Grover RK**, Cheng J, Peng Y, Jones TM, Ruiz DI, Ulevitch RJ, Glass JI, Dennis EA, Salomon DR, Lerner RA. The costimulatory immunogen LPS induces the B-Cell clones that infiltrate transplanted human kidneys. *Proc Natl Acad Sci U S A* 2012; **109**: 6036-6041 [PMID: 22492977 DOI: 10.1073/pnas.1202214109]

99 **Modena BD**, Milam R, Harrison F, Cheeseman JA, Abecassis MM, Friedewald JJ, Kirk AD, Salomon DR. Changes in Urinary Microbiome Populations Correlate in Kidney Transplants With Interstitial Fibrosis and Tubular Atrophy Documented in Early Surveillance Biopsies. *Am J Transplant* 2017; **17**: 712-723 [PMID: 27597148 DOI: 10.1111/ajt.14038]

100 **Rieder F**. The gut microbiome in intestinal fibrosis: environmental protector or provocateur? *Sci Transl Med* 2013; **5**: 190ps10 [PMID: 23785034 DOI: 10.1126/scitranslmed.3004731]

101 mofetil in renal transplantation: 3-year results from the placebo-controlled trial. European Mycophenolate Mofetil Cooperative Study Group. *Transplantation* 1999; **68**: 391-396 [PMID: 10459543 DOI: 10.1097/00007890-199908150-00011]

102 **Bunnapradist S**, Neri L, Wong W, Lentine KL, Burroughs TE, Pinsky BW, Takemoto SK, Schnitzler MA. Incidence and risk factors for diarrhea following kidney transplantation and association with graft loss and mortality. *Am J Kidney Dis* 2008; **51**: 478-486 [PMID: 18295064 DOI: 10.1053/j.ajkd.2007.11.013]

103 **Bunnapradist S**, Lentine KL, Burroughs TE, Pinsky BW, Hardinger KL, Brennan DC, Schnitzler MA. Mycophenolate mofetil dose reductions and discontinuations after gastrointestinal complications are associated with renal transplant graft failure. *Transplantation* 2006; **82**: 102-107 [PMID: 16861948 DOI: 10.1097/01.tp.0000225760.09969.1f]

104 **Lee JR**, Magruder M, Zhang L, Westblade LF, Satlin MJ, Robertson A, Edusei E, Crawford C, Ling L, Taur Y, Schluter J, Lubetzky M, Dadhania D, Pamer E, Suthanthiran M. Gut microbiota dysbiosis and diarrhea in kidney transplant recipients. *Am J Transplant* 2019; **19**: 488-500 [PMID: 29920927 DOI: 10.1111/ajt.14974]

105 **Lopetuso LR**, Scaldaferri F, Petito V, Gasbarrini A. Commensal Clostridia: leading players in the maintenance of gut homeostasis. *Gut Pathog* 2013; **5**: 23 [PMID: 23941657 DOI: 10.1186/1757-4749-5-23]

106 **Rajilić-Stojanović M**, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiol Rev* 2014; **38**: 996-1047 [PMID: 24861948 DOI: 10.1111/1574-6976.12075]

107 **Langille MG**, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol* 2013; **31**: 814-821 [PMID: 23975157 DOI: 10.1038/nbt.2676]

108 **Antharam VC**, Li EC, Ishmael A, Sharma A, Mai V, Rand KH, Wang GP. Intestinal dysbiosis and depletion of butyrogenic bacteria in Clostridium difficile infection and nosocomial diarrhea. *J Clin Microbiol* 2013; **51**: 2884-2892 [PMID: 23804381 DOI: 10.1128/JCM.00845-13]

109 **Raqib R**, Sarker P, Bergman P, Ara G, Lindh M, Sack DA, Nasirul Islam KM, Gudmundsson GH, Andersson J, Agerberth B. Improved outcome in shigellosis associated with butyrate induction of an endogenous peptide antibiotic. *Proc Natl Acad Sci* 2006; **103**: 9178-9183 [PMID: 16740661 DOI: 10.1073/pnas.0602888103]

110 **Kelly CJ**, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, Wilson KE, Glover LE, Kominsky DJ, Magnuson A, Weir TL, Ehrentraut SF, Pickel C, Kuhn KA, Lanis JM, Nguyen V, Taylor CT, Colgan SP. Crosstalk between Microbiota-Derived Short-Chain Fatty Acids and Intestinal Epithelial HIF Augments Tissue Barrier Function. *Cell Host Microbe* 2015; **17**: 662-671 [PMID: 25865369 DOI: 10.1016/j.chom.2015.03.005]

111 **Rodrigues HG**, Takeo Sato F, Curi R, Vinolo MAR. Fatty acids as modulators of neutrophil recruitment, function and survival. *Eur J Pharmacol* 2016; **785**: 50-58 [PMID: 25987417 DOI: 10.1016/j.ejphar.2015.03.098]

112 **Millard AL**, Mertes PM, Ittelet D, Villard F, Jeannesson P, Bernard J. Butyrate affects differentiation, maturation and function of human monocyte-derived dendritic cells and macrophages. *Clin Exp Immunol* 2002; **130**: 245-255 [PMID: 12390312 DOI: 10.1046/j.0009-9104.2002.01977.x]

113 **Park J**, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, Kim CH. Short-chain fatty acids

induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol* 2015; **8**: 80-93 [PMID: 24917457 DOI: 10.1038/mi.2014.44]

114 **Wu H**, Singer J, Kwan TK, Loh YW, Wang C, Tan J, Li YJ, Lai SWC, Macia L, Alexander SI, Chadban SJ. Gut Microbial Metabolites Induce Donor-Specific Tolerance of Kidney Allografts through Induction of T Regulatory Cells by Short-Chain Fatty Acids. *J Am Soc Nephrol* 2020; **31**: 1445-1461 [PMID: 32482686 DOI: 10.1681/ASN.2019080852]

115 **Andrade-Oliveira V**, Amano MT, Correa-Costa M, Castoldi A, Felizardo RJ, de Almeida DC, Bassi EJ, Moraes-Vieira PM, Hiyane MI, Rodas AC, Peron JP, Aguiar CF, Reis MA, Ribeiro WR, Valduga CJ, Curi R, Vinolo MA, Ferreira CM, Câmara NO. Gut Bacteria Products Prevent AKI Induced by Ischemia-Reperfusion. *J Am Soc Nephrol* 2015; **26**: 1877-1888 [PMID: 25589612 DOI: 10.1681/ASN.2014030288]

116 **Lee JR**, Huang J, Magruder M, Zhang LT, Gong C, Sholi AN, Albakry S, Edusei E, Muthukumar T, Lubetzky M, Dadhania DM, Taur Y, Pamer EG, Suthanthiran M. Butyrate-producing gut bacteria and viral infections in kidney transplant recipients: A pilot study. *Transpl Infect Dis* 2019; **21**: e13180 [PMID: 31544324 DOI: 10.1111/tid.13180]

117 **Poesen R**, Evenepoel P, de Loor H, Bammens B, Claes K, Sprangers B, Naesens M, Kuypers D, Augustijns P, Meijers B. The influence of renal transplantation on retained microbial-human co-metabolites. *Nephrol Dial Transplant* 2016; **31**: 1721-1729 [PMID: 26961998 DOI: 10.1093/ndt/gfw009]

118 **Vitetta L**, Vitetta G, Hall S. Immunological Tolerance and Function: Associations Between Intestinal Bacteria, Probiotics, Prebiotics, and Phages. *Front Immunol* 2018; **9**: 2240 [PMID: 30356736 DOI: 10.3389/fimmu.2018.02240]

119 **Colas L**, Mongodin EF, Montassier E, Chesneau M, Guerif P, Hittle L, Giral M, Bromberg JS, Brouard S; DIVAT Consortium. Unique and specific Proteobacteria diversity in urinary microbiota of tolerant kidney transplanted recipients. *Am J Transplant* 2020; **20**: 145-158 [PMID: 31374143 DOI: 10.1111/ajt.15549]

120 **Masuelli L**, Pantanella F, La Regina G, Benvenuto M, Fantini M, Mattera R, Di Stefano E, Mattei M, Silvestri R, Schippa S, Manzari V, Modesti A, Bei R. Violacein, an indole-derived purple-colored natural pigment produced by Janthinobacterium lividum, inhibits the growth of head and neck carcinoma cell lines both *in vitro* and in vivo. *Tumour Biol* 2016; **37**: 3705-3717 [PMID: 26462840 DOI: 10.1007/s13277-015-4207-3]

121 **Verinaud L**, Lopes SC, Prado IC, Zanucoli F, Alves da Costa T, Di Gangi R, Issayama LK, Carvalho AC, Bonfanti AP, Niederauer GF, Duran N, Costa FT, Oliveira AL, Höfling MA, Machado DR, Thomé R. Violacein Treatment Modulates Acute and Chronic Inflammation through the Suppression of Cytokine Production and Induction of Regulatory T Cells. *PLoS One* 2015; **10**: e0125409 [PMID: 25938431 DOI: 10.1371/journal.pone.0125409]

122 **Atarashi K**, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz JV, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, Morita H, Hattori M, Honda K. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 2013; **500**: 232-236 [PMID: 23842501 DOI: 10.1038/nature12331]

123 **Etienne-Mesmin L**, Chassaing B, Gewirtz AT. Tryptophan: A gut microbiota-derived metabolites regulating inflammation. *World J Gastrointest Pharmacol Ther* 2017; **8**: 7-9 [PMID: 28217370 DOI: 10.4292/wjgpt.v8.i1.7]

124 **Segawa S**, Fujiya M, Konishi H, Ueno N, Kobayashi N, Shigyo T, Kohgo Y. Probiotic-derived polyphosphate enhances the epithelial barrier function and maintains intestinal homeostasis through integrin-p38 MAPK pathway. *PLoS One* 2011; **6**: e23278 [PMID: 21858054 DOI: 10.1371/journal.pone.0023278]

125 **Teuteberg JJ**, Shullo MA, Zomak R, Toyoda Y, McNamara DM, Bermudez C, Kormos RL, McCurry KR. Alemtuzumab induction prior to cardiac transplantation with lower intensity maintenance immunosuppression: one-year outcomes. *Am J Transplant* 2010; **10**: 382-388 [PMID: 19889126 DOI: 10.1111/j.1600-6143.2009.02856.x]

126 **Federico S**, Carrano R, Capone D, Gentile A, Palmiero G, Basile V. Pharmacokinetic interaction between levofloxacin and ciclosporin or tacrolimus in kidney transplant recipients: ciclosporin, tacrolimus and levofloxacin in renal transplantation. *Clin Pharmacokinet* 2006; **45**: 169-175 [PMID: 16485913 DOI: 10.2165/00003088-200645020-00003]

127 **Page RL 2nd**, Klem PM, Rogers C. Potential elevation of tacrolimus trough concentrations with concomitant metronidazole therapy. *Ann Pharmacother* 2005; **39**: 1109-1113 [PMID: 15855244 DOI: 10.1345/aph.1E399]

128 **Zheng Y**, Masand A, Wagner M, Kapur S, Dadhania D, Lubetzky M, Lee JR. Identification of Antibiotic Administration as a Potentially Novel Factor Associated With Tacrolimus Trough Variability in Kidney Transplant Recipients: A Preliminary Study. *Transplant Direct* 2019; **5**: e485 [PMID: 31579813 DOI: 10.1097/TXD.0000000000000930]

129 **Lee JR**, Muthukumar T, Dadhania D, Taur Y, Jenq RR, Toussaint NC, Ling L, Pamer E, Suthanthiran M. Gut microbiota and tacrolimus dosing in kidney transplantation. *PLoS One* 2015; **10**: e0122399 [PMID: 25815766 DOI: 10.1371/journal.pone.0122399]

130 **Tsunashima D**, Kawamura A, Murakami M, Sawamoto T, Undre N, Brown M, Groenewoud A, Keirns JJ, Holman J, Connor A, Wylde H, Wilding I, Ogawara K, Sako K, Higaki K, First R.

Assessment of tacrolimus absorption from the human intestinal tract: open-label, randomized, 4-way crossover study. *Clin Ther* 2014; **36**: 748-759 [PMID: 24680768 DOI: 10.1016/j.clinthera.2014.02.021]

131 **Guo Y**, Crnkovic CM, Won KJ, Yang X, Lee JR, Orjala J, Lee H, Jeong H. Commensal Gut Bacteria Convert the Immunosuppressant Tacrolimus to Less Potent Metabolites. *Drug Metab Dispos* 2019; **47**: 194-202 [PMID: 30598508 DOI: 10.1124/dmd.118.084772]

132 **Gibson CM**, Childs-Kean LM, Naziruddin Z, Howell CK. The alteration of the gut microbiome by immunosuppressive agents used in solid organ transplantation. *Transpl Infect Dis*2020: e13397 [PMID: 32609940 DOI: 10.1111/tid.13397]

133 **Zhang Z**, Liu L, Tang H, Jiao W, Zeng S, Xu Y, Zhang Q, Sun Z, Mukherjee A, Zhang X, Hu X. Immunosuppressive effect of the gut microbiome altered by high-dose tacrolimus in mice. *Am J Transplant* 2018; **18**: 1646-1656 [PMID: 29316256 DOI: 10.1111/ajt.14661]

134 **Lu H**, He J, Wu Z, Xu W, Zhang H, Ye P, Yang J, Zhen S, Li L. Assessment of microbiome variation during the perioperative period in liver transplant patients: a retrospective analysis. *Microb Ecol* 2013; **65**: 781-791 [PMID: 23504024 DOI: 10.1007/s00248-013-0211-6]

135 **Zaza G**, Dalla Gassa A, Felis G, Granata S, Torriani S, Lupo A. Impact of maintenance immunosuppressive therapy on the fecal microbiome of renal transplant recipients: Comparison between an everolimus- and a standard tacrolimus-based regimen. *PLoS One* 2017; **12**: e0178228 [PMID: 28542523 DOI: 10.1371/journal.pone.0178228]

136 **O'Reilly C**, O'Sullivan Ó, Cotter PD, O'Connor PM, Shanahan F, Cullen A, Rea MC, Hill C, Coulter I, Ross RP. Encapsulated cyclosporine does not change the composition of the human microbiota when assessed *ex vivo* and *in vivo*. *J Med Microbiol* 2020; **69**: 854-863 [PMID: 31958048 DOI: 10.1099/jmm.0.001130]

137 **Nallu A**, Sharma S, Ramezani A, Muralidharan J, Raj D. Gut microbiome in chronic kidney disease: challenges and opportunities. *Transl Res* 2017; **179**: 24-37 [PMID: 27187743 DOI: 10.1016/j.trsl.2016.04.007]

138 **Konstantinov SR**, Smidt H, de Vos WM, Bruijns SC, Singh SK, Valence F, Molle D, Lortal S, Altermann E, Klaenhammer TR, van Kooyk Y. S layer protein A of Lactobacillus acidophilus NCFM regulates immature dendritic cell and T cell functions. *Proc Natl Acad Sci* 2008; **105**: 19474-19479 [PMID: 19047644 DOI: 10.1073/pnas.0810305105]

139 **van Baarlen P**, Troost FJ, van Hemert S, van der Meer C, de Vos WM, de Groot PJ, Hooiveld GJ, Brummer RJ, Kleerebezem M. Differential NF-kappaB pathways induction by Lactobacillus plantarum in the duodenum of healthy humans correlating with immune tolerance. *Proc Natl Acad Sci* 2009; **106**: 2371-2376 [PMID: 19190178 DOI: 10.1073/pnas.0809919106]

140 **Maudet C**, Mano M, Eulalio A. MicroRNAs in the interaction between host and bacterial pathogens. *FEBS Lett* 2014; **588**: 4140-4147 [PMID: 25128459 DOI: 10.1016/j.febslet.2014.08.002]

141 **Thomas CM**, Versalovic J. Probiotics-host communication: Modulation of signaling pathways in the intestine. *Gut Microbes* 2010; **1**: 148-163 [PMID: 20672012 DOI: 10.4161/gmic.1.3.11712]

142 **Prakash S**, Chang TM. Microencapsulated genetically engineered live E. coli DH5 cells administered orally to maintain normal plasma urea level in uremic rats. *Nat Med* 1996; **2**: 883-887 [PMID: 8705857 DOI: 10.1038/nm0896-883]

143 **Piñero-Lambea C**, Ruano-Gallego D, Fernández LÁ. Engineered bacteria as therapeutic agents. *Curr Opin Biotechnol* 2015; **35**: 94-102 [PMID: 26070111 DOI: 10.1016/j.copbio.2015.05.004]

144 **Roberfroid M**. Prebiotics: the concept revisited. *J Nutr* 2007; **137**: 830S-837S [PMID: 17311983 DOI: 10.1093/jn/137.3.830S]

145 **Slavin J**. Fiber and prebiotics: mechanisms and health benefits. *Nutrients* 2013; **5**: 1417-1435 [PMID: 23609775 DOI: 10.3390/nu5041417]

146 **de Vrese M**, Marteau PR. Probiotics and prebiotics: effects on diarrhea. *J Nutr* 2007; **137**: 803S-811S [PMID: 17311979 DOI: 10.1093/jn/137.3.803S]

147 **Cosola C**, Rocchetti MT, Cupisti A, Gesualdo L. Microbiota metabolites: Pivotal players of cardiovascular damage in chronic kidney disease. *Pharmacol Res* 2018; **130**: 132-142 [PMID: 29518493 DOI: 10.1016/j.phrs.2018.03.003]

148 **Gong J**, Noel S, Pluznick JL, Hamad ARA, Rabb H. Gut Microbiota-Kidney Cross-Talk in Acute Kidney Injury. *Semin Nephrol* 2019; **39**: 107-116 [PMID: 30606403 DOI: 10.1016/j.semnephrol.2018.10.009]

149 **Wang Z**, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, Gu X, Huang Y, Zamanian-Daryoush M, Culley MK, DiDonato AJ, Fu X, Hazen JE, Krajcik D, DiDonato JA, Lusis AJ, Hazen SL. Non-lethal Inhibition of Gut Microbial Trimethylamine Production for the Treatment of Atherosclerosis. *Cell* 2015; **163**: 1585-1595 [PMID: 26687352 DOI: 10.1016/j.cell.2015.11.055]

150 **University of Pennsylvania**. Gut Microbiome and p-Inulin in Hemodialysis. [accessed 2021 January 5]. In: ClinicalTrials. gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT02572882.

World Journal of Transplantation

Submit a Manuscript: https://www.f6publishing.com

World J Transplant 2021 March 18; 11(3): 37-53

DOI: 10.5500/wjt.v11.i3.37

ISSN 2220-3230 (online)

MINIREVIEWS

Noninvasive markers of liver steatosis and fibrosis after liver transplantation – Where do we stand?

Ivana Mikolasevic, Sanja Stojsavljevic, Filip Blazic, Maja Mijic, Delfa Radic-Kristo, Toni Juric, Nadija Skenderevic, Mia Klapan, Andjela Lukic, Tajana Filipec Kanizaj

ORCID number: Ivana Mikolasevic 0000-0001-9676-0642; Sanja Stojsavljevic 0000-0002-1626-3003; Filip Blazic 0000-0002-6190-3233; Maja Mijic 0000-0002-8355-1013; Delfa Radic-Kristo 0000-0002-2827-7808; Toni Juric 0000-0002-7175-6249; Nadija Skenderevic 0000-0003-4296-6375; Mia Klapan 0000-0001-6322-5600; Andjela Lukic 0000-0002-7337-5810; Tajana Filipec Kanizaj 0000-0002-9828-8916.

Author contributions: Mikolasevic I researched the database, wrote the manuscript, and is the guarantor of this work; Stojsavljevic S, Blazic F, Mijic M, Radic-Kristo D, Juric T, Skenderevic N, Klapan M, Lukic A, Filipec Kanizaj T contributed to the discussion and reviewed/edited the manuscript.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Ivana Mikolasevic, Department of Gastroenterology, Clinical Hospital Centre Rijeka, Rijeka, Croatia; Department of Gastroenterology, Clinical hospital Merkur, Zagreb, Croatia; Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Sanja Stojsavljevic, Department of Gastroenterology, University Hospital Center "Sestre Milosrdnice", Zagreb 10000, Croatia

Filip Blazic, Department of Gastroenterology, University Hospital Center Rijeka, Rijeka 51000, Croatia

Maja Mijic, Nadija Skenderevic, Department of Gastroenterology, University Hospital Merkur, Zagreb 10000, Croatia

Delfa Radic-Kristo, Department of Hematology, University Hospital Merkur, Zagreb, Croatia; Faculty of Medicine, University of Zagreb, Zagreb, Croatia

Toni Juric, Mia Klapan, Andjela Lukic, School of Medicine, School of Medicine, Rijeka 51000, Croatia

Tajana Filipec Kanizaj, Department of Gastroenterology, University Hospital Merkur, Zagreb, Croatia; Faculty of Medicine, University of Zagreb, Zagreb, Croatia

Corresponding author: Ivana Mikolasevic, PhD, Assistant Professor, Department of Gastroenterology, Clinical Hospital Centre Rijeka, Rijeka, Croatia; Department of Gastroenterology, Clinical hospital Merkur, Zagreb, Croatia; Faculty of Medicine, University of Rijeka, Rijeka, Croatia. ivana.mikolasevic@gmail.com

Abstract

In the last two decades, advances in immunosuppressive regimens have led to fewer complications of acute rejection crisis and consequently improved short-term graft and patient survival. In parallel with this great success, long-term post-transplantation complications have become a focus of interest of doctors engaged in transplant medicine. Metabolic syndrome (MetS) and its individual components, namely, obesity, dyslipidemia, diabetes, and hypertension, often develop in the post-transplant setting and are associated with immuno-suppressive therapy. Nonalcoholic fatty liver disease (NAFLD) is closely related to MetS and its individual components and is the liver manifestation of MetS. Therefore, it is not surprising that MetS and its individual components are

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: Croatia

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C
Grade D (Fair): D, D
Grade E (Poor): 0

Received: July 21, 2020
Peer-review started: July 21, 2020
First decision: October 21, 2020
Revised: December 10, 2020
Accepted: March 1, 2021
Article in press: March 1, 2021
Published online: March 18, 2021

P-Reviewer: Ferrarese A, Link A, Wang H
S-Editor: Zhang L
L-Editor: A
P-Editor: Yuan YY

associated with recurrent or "*de novo*" NAFLD after liver transplantation (LT). Fibrosis of the graft is one of the main determinants of overall morbidity and mortality in the post-LT period. In the assessment of post-LT steatosis and fibrosis, we have biochemical markers, imaging methods and liver biopsy. Because of the significant economic burden of post-LT steatosis and fibrosis and its potential consequences, there is an unmet need for noninvasive methods that are efficient and cost-effective. Biochemical scores can overestimate fibrosis and are not a good method for fibrosis evaluation in liver transplant recipients due to frequent post-LT thrombocytopenia. Transient elastography with controlled attenuation parameter is a promising noninvasive method for steatosis and fibrosis. In this review, we will specifically focus on the evaluation of steatosis and fibrosis in the post-LT setting in the context of *de novo* or recurrent NAFLD.

Key Words: Steatosis; Fibrosis; Noninvasive methods; Transient elastography; Transplantation; Nonalcoholic fatty liver disease

Core Tip: Fibrosis of the graft is one of the main determinants of overall morbidity and mortality in the post-transplantation period. In the assessment of post-transplantation steatosis and fibrosis, we have biochemical markers, imaging methods and liver biopsy. Because of the significant economic burden of post-transplantation steatosis and fibrosis and the potential consequences, there is an unmet need for noninvasive methods that are efficient and cost-effective.

Citation: Mikolasevic I, Stojsavljevic S, Blazic F, Mijic M, Radic-Kristo D, Juric T, Skenderevic N, Klapan M, Lukic A, Filipec Kanizaj T. Noninvasive markers of liver steatosis and fibrosis after liver transplantation – Where do we stand? *World J Transplant* 2021; 11(3): 37-53
URL: https://www.wjgnet.com/2220-3230/full/v11/i3/37.htm
DOI: https://dx.doi.org/10.5500/wjt.v11.i3.37

INTRODUCTION

The prevalence of metabolic syndrome (MetS) and obesity is increasing; hence, nonalcoholic fatty liver disease (NAFLD)-induced chronic liver disease (CLD) is more frequent[1-4]. NAFLD has become the most common CLD today and has a high socioeconomic impact. This CLD is becoming a focus of interest of many authors in the transplant population because it has multiple impacts on liver transplantation (LT); influencing the number of patients on the waiting list for transplantation, number and quality of organ donors and increasingly important graft and recipient post-transplant outcome[1,2]. NAFLD-related end-stage liver disease (ESLD) is currently assumed to be the second most common cause of LT in the United States[1].Growing prevalence of NAFLD in the West, advancements in hepatitis C virus infection (HCV) therapy, and the aging population, will have NAFLD-driven ESLD emerge as the leading cause for LT in the Western world in the decades to come[5]. Therefore, NAFLD and diagnostic approach in LT setting has been the center-point of LT academic interest and this review[1].

Liver transplantation is the optimal treatment method for most patients with ESLD and for some patients with hepatocellular carcinoma or acute liver failure[6]. In the last two decades, advances in immunosuppressive regimens have led to fewer complications of acute rejection crisis and consequently improved short-term graft and patient survival. In parallel with this great success, long-term post-LT complications have become a focus of interest of doctors engaged in transplant medicine. MetS and its individual components, namely, obesity, dyslipidemia, diabetes, and hypertension are highly present in LT candidates, in addition it often develops *de novo* or deteriorates in the posttransplant setting as a consequence of prescribed immunosuppressive therapy[6,7]. NAFLD is closely related to MetS and its individual components and is the liver manifestation of MetS. Therefore, it is not surprising that

MetS and its individual components are associated with recurrent or "*de novo*" NAFLD after LT. Consequently, MetS and NAFLD after LT potentially impact recipients' post-LT survival[2,6].

As there are no specific or well-validated pharmaceuticals currently available for NAFLD, treatment options are focused on the identification of high-risk patients. It is well known that liver fibrosis is the main driver of CLD as well as the main factor influencing post-LT morbidity and mortality. The gold standard for the diagnosis and staging of all CLD is liver biopsy (LB). However, LB is an invasive procedure. Because of the significant economic burden of post-LT steatosis and fibrosis (*i.e.*, NAFLD) and its potential consequences, there is an unmet need for noninvasive methods that will be efficient and cost-effective[8]. In the last decade, numerous laboratory tests and biomarkers for steatosis, inflammation and fibrosis detection as well as imaging methods have been intensively investigated.

In this review, we will specifically focus on the evaluation of steatosis and fibrosis in the post-LT setting in the context of *de novo* or recurrent NAFLD.

NONALCOHOLIC FATTY LIVER DISEASE AFTER LIVER TRANSPLANTATION

As mentioned, notable development of immunosuppressive treatment and progress of transplant surgery has resulted in improvement in survival rates after LT, with an approximately 90% survival rate at the first year and a survival rate of more than 70% five years after the surgical procedure[2]. With these excellent post-LT survival rates, research interest is now focusing on long-term complications, such as MetS, cardiovascular disease (CVD) and chronic kidney disease (CKD). Immunosuppressive therapy, such as calcineurin inhibitors (CNIs), mTOR inhibitors (sirolimus and everolimus) and steroids that we use today in the transplant setting, promotes the development of MetS and its individual components[6]. Immunomodulatory and steroid therapy post-LT promotes the advancement of preexisting and *de novo* MetS features, such as weight gain (> 90% of all recipients), hypertension (50%-100%), dyslipidemia (45%-69%) and diabetes (10%-40%)[6,9-13]. According to relevant studies, MetS develops in up to 60% of liver recipients and is related to CVD, CKD, NAFLD/fatty allograft disease and progression of recurrent HCV[9-19]. As a liver manifestation of MeS, NAFLD can reoccur in a previously NAFLD/MetS burdened patient, facilitate accelerated progression toward ESLD, leading to possible retransplantation, or appear *de novo* in pre-LT NAFLD naive patients. Recurrent steatosis and steatohepatitisare very common (30%-100%)[7] and were present in 1/3 of the cases at 6 months postoperatively in a study by Bhagat *et al*[11]; specifically, they were present in 33% of the group transplanted for NAFLD *vs* 0% of the group transplanted for alcoholic liver disease, $P < 0.0001$. Most important study data about incidence and outcome of recurrent and *de novo* NAFLD in posttransplant setting are summarized in Table 1[4,12,14,15,19]. Interestingly, in most studies the serum amino-transferase levels did not correlate with NAFLD recurrence or the fibrosis progression rate[12,14].

According to a meta-analysis published a year ago, the recurrence rate of both NAFLD/nonalcoholic steatohepatitis (NASH) and the occurrence rates of new-onset NAFLD/NASH are highly variable across studies[13] due to most studies dealing with the recurrence of NAFLD/NASH being retrospective, single-centered, and lacking a universal post-LT biopsy regimen, standardized histological criteria and consistent study inclusion/exclusion criteria. The authors also found that NAFLD after LT is associated with metabolic risk factors, especially high BMI.

Important point in the context of recurrent or *de novo* NAFLD after LT needs to be addressed. Although NAFLD is very common after LT, there are no clear data regarding whether NAFLD in allografts is histologically the same or different from NAFLD in native livers. The limited data that address histologic findings in *de novo* or recurrent NAFLD after LT did not address that question clearly. Thus, investigations that determine NAFLD in the allograft histologically like NAFLD in native livers are needed[16-18].

The real impact of NAFLD recurrence or *de novo* disease on allograft and patient outcomes is unclear. New-onset NAFLD appears more benign than recurrent NAFLD, with a later onset and favorable clinical course, rarely resulting in NASH. Most of the available knowledge about recurrent or *de novo* NAFLD comes from data that are based on a small number of patients, and in the majority of them, there are no protocolar biopsies, and the follow-up time is short[15,16]. Further prospective research

Table 1 Studies investigating the role of nonalcoholic fatty liver disease in post-liver transplant setting

Ref.	Type of the study	Study population	Follow up	Diagnostic method	Incidence of NAFLD	Major outcomes
Bhagat *et al*[11]	Retrospective	71 NAFLD, 81 alcoholic liver disease	Median 1517-1686 d	43.4% biopsy, 56.6% US	30% NAFLD, 0% alcoholic liver disease	NAFLD recurrence more common than *de novo*; acute cellular rejections more common in NAFLD group; no influence on CVD and overall mortality
Bhati *et al*[12]	Retrospective	103 NAFLD	Median 47-78 mo	90% biopsy or TE	87.5% steatosis (TE), reccurent NAFLD 88.2% (biopsy)	20.6% had bridging fibrosis (TE); advanced fibrosis (> F3) was seen in 26.8% (biopsy)
Seo *et al*[4]	Retrospective	68 non-NAFLD	Median 28 mo		18% *de novo* NAFLD, 9% NASH	Increase in BMI > 10% risk factor for *de novo* NAFLD; ACE-I protective role
Dumortier *et al*[14]	Retrospective	421 non-NAFLD	48 mo	Biopsy	53% had steatosis grade 1, 31% grade 2 and 16% grade 3 steatosis; 29% perisinusidal fibrosis; 3.8% NASH. 2.25% cirrhosis	MetS and its individual components, tacrolimus-based immunosuppressive therapy, alcoholic liver disease as the primary indication for LT and liver graft steatosis were associated with post-LT steatosis
Vallin *et al*[15]	Retrospective	80 *de novo* NAFLD, 11 recurrent NAFLD	5 yr		NASH and severe fibrosis (stages 3 and 4) were more common in recipients with recurrent than in those with *de novo* NAFLD (71.4% *vs* 12.5% and 71.4% *vs* 17.2%, respectively)	Recurrent NAFLD is a more severe disease with an earlier onset; prevalence of diabetes mellitus was higher in patients with recurrent NAFLD
Narayanan *et al*[19]	Retrospective	588 LT recipients; 9.7% NAFLD; 90.3% non-NAFLD	10 yr	41.5% biopsy, other US, CT, MR	Recurrent steatosis developed 77.6% and *de novo* 44.7%	Allograft steatosis did not influence post-LT survival or adverse CVD events, while underlying; NAFLD diagnosis was associated with a 2.04 increased risk of adverse cardiovascular events

LT: Liver transplantation; NAFLD: Nonalcoholic liver fatty disease; NASH: Nonalcoholic stetohepatitis, MeS: Metabolic syndrome; TE: Transient elastography; US: Ultrasound; CT: Computed tomography; BMI: Body mass index; ACE-I: Angiotensin converting enzyme inhibitors; MR: Magnetic resonance; CVD: Cardiovascular disease.

on the matter is warranted as clinical courses of new onset and recurrent NAFLD differ[13,15,16]. According to the available data, one more point in the context of post-LT NAFLD should be addressed: the definition of recurrence *vs de novo* NAFLD requires identification of preexisting NAFLD, which is often difficult to define and thus can be underrecognized. Additionally, we must think about steatosis and even fibrosis that can occur from other secondary etiologies, such as recurrence disease or some drugs; therefore, it should be excluded, although it is often difficult since many etiological factors can overlap in the same patient. Further studies should address this point and may find some biomarker that will truly identify these patients[16].

Finally, there are no proven drugs for NAFLD treatment; thus, the management of post-LT NAFLD is based on the identification of risk factors. The most common risk factors are hypertension, diabetes, dyslipidemia, and weight gain. Other factors, such as immunosuppressive drugs, have not been clearly identified to date. In the general population, the use of steroids relates to MetS and steatosis. However, in the post-LT setting, this effect could be different because most transplant centers taper steroids in the 3-6-mo period after LT. Therefore, the impact of steroids on post-LT NAFLD could be minimal. However, further studies on this topic are needed in the population of patients with liver transplant. On the other hand, CNIs are known to promote insulin resistance and MetS development. Both CNIs are related to hypertension and diabetes mellitus, but tacrolimus is a more diabetogenic medication, and cyclosporin is more related to hypertension development. From the general population, we know that MetS is related to NAFLD development. However, the development of steatosis in relation to CNIs after LT is not well investigated[16-22]. A small retrospective study investigated the posttransplant recurrence of NAFLD as well as outcomes after LT in recipients who underwent LT for NAFLD-related cirrhosis. They analyzed 88 patients. The authors have reported that the choice of CNIs (tacrolimus *vs* cyclosporine) was not significantly different among patients with NAFLD recurrence and those without[17]. On the other hand, Dumortier *et al*[14] reported that steatosis is a frequent complication after LT. In their multivariate analysis, factors that were independently related to post-LT steatosis were diabetes mellitus, post-LT obesity, hypertension, dyslipidemia,

tacrolimus-based regimen, alcoholic cirrhosis as the primary indication for LT, and pretransplant liver graft steatosis[14]. Therefore, this topic requires further long-term prospective studies with protocolar liver biopsies. Additionally, some nonmodifiable risk factors are recognized as potential factors for steatosis development, such as age, sex, and genetics[16]. Studies have shown that the PNPLA-3 non-CC genotype is associated with posttransplant obesity[22]. Additionally, Finkenstedt *et al*[23] found that recipients who carry rs738409-G in PNPLA3 have a risk for hepatic triglyceride accumulation. Interestingly, some other genetic associations, such as the transmembrane gene (TM6SF), are not investigated in the context of LT and should be investigated in upcoming investigations[16].

Another less known factor that is possibly involved in NAFLD pathogenesis and that has attracted much research interest in the general population is the gut microbiome. To the best of our knowledge, no studies have investigated gut dysbiosis in liver transplant recipients in relation to NAFLD recurrence or development. The link with MetS and obesity in the general population requires translation into the liver transplant recipient.

DIAGNOSIS OF STEATOSIS AND FIBROSIS AFTER LIVER TRANSPLANTATION – WHAT IS THE OPTIMAL DIAGNOSTIC METHOD?

Transplanted liver is prone to complications specific to transplant procedures, as well as to liver diseases like the general population. The causes partially depend on the time after LT, but there is no universal prevalence or time distribution of the various causes of graft injury. Most commonly, graft injury is related to vascular, biliary, or infective complications; toxic hepatitis; acute and chronic cellular rejection; preservation injury; or recurrence of previous liver disease. In routine practice, graft dysfunction is suspected by an increase in liver enzymes. Unfortunately, enzyme levels do not correlate with the cause or severity of liver disease. Furthermore, many diseases may be evident by a combination of clinical, microbiological, or serological findings and imaging methods. Nevertheless, in most situations, LB is needed to confirm the diagnosis[21]. Studies on long-term LT recipients and graft outcomes have shown a high prevalence of histological changes in protocolar biopsies even in the absence of abnormal liver enzymes and function tests. Therefore, occasionally, biopsy alterations may be the first sign of graft disease. Since usually more than one risk factor could be related to the development and progression of allograft fibrosis, LB is still the most performed and golden standard procedure. Knowing the challenges related to sampling error, interpretation variability, significant costs and repeatability, the major limitation in the performance of LB is the risk of complications. This allows the opportunity for noninvasive methods as a screening and monitoring method for subclinical changes in liver grafts after LT[21].

Liver allograft fibrosis is one of the main determinants of allograft survival and the need for retransplantation; therefore, early recognition of fibrosis is of great clinical interest in the management of liver transplant recipients[24-26]. Patients with LT can have many risk factors for fibrosis recurrence after LT. For example, until the era of direct anti-viral agents, patients who were transplanted due to end-stage liver disease as a consequence of HCV infection had almost universal recurrence of HCV infection with the development of cirrhosis in up to 30% by 5 years post-LT[24-26]. Furthermore, due to the high incidence of MetS after LT, recurrent or *de novo* NAFLD after LT is an important cause of post-LT recurrent fibrosis. Hepatic fibrosis is likely be more common in recurrent disease and may occur in younger individuals with NAFLD[13]. Except for HCV and NAFLD, there are other factors that may have a negative effect on fibrosis recurrence after LT, such as demographic factors (*i.e.*, recipient and donor age), immunosuppressive therapy and cytomegalovirus infection[24-26]. In the assessment of post-LT steatosis and fibrosis, we have biochemical markers, imaging methods and LB. Liver biopsy is the gold standard for diagnosing and grading all stages of liver disease and the best available standard of reference for fibrosis evaluation. The usefulness of LB is even more pronounced in post liver transplant, where today, there is no single method that can assess steatosis, necroinflammation and fibrosis concurrently in a population at risk for other concomitant causes of liver injury[16]. Knowing the practical challenges and possible complications of LB, in routine clinical practice, even in LT setting, noninvasive markers are needed to assess fat in the liver, as well as inflammation and fibrosis of the liver.

The usefulness of biochemical markers after liver transplantation

In the general population, several algorithms, based on clinical and biochemical factors, have been developed to detect individuals with advanced fibrosis. It is believed that serum fibrosis biomarkers have the potential to reflect dynamic changes in fibrogenesis and thus the ability to assess matrix turnover earlier in the disease process, allowing earlier intervention or closer surveillance. Unfortunately, none of the routinely available serum fibrosis biomarkers were designed to reflect the dynamic process of fibrogenesis, differentiate between adjacent disease stages, diagnose NAFLD, or follow longitudinal changes in fibrosis or disease activity caused by natural history or therapeutic interventions.

Biochemical markers are based on readily available parameters. According to data, few studies have investigated the usefulness of biochemical markers for fibrosis detection in the post-LT setting. The most investigated biomarkers in the post-LT setting are the asparthate-aminotraspherase-to-platelet ratio index (APRI) and the Fibrosis score 4 (FIB-4)[24,25]. Studies that investigated the diagnostic accuracy of the APRI and FIB-4 to predict fibrosis F2-4 in LT recipients are shown in Table 2.

One of the first studies that was published in 2007 included 51 patients who were transplanted due to HCV[27]. In this analysis, the area under the receiver operating characteristic curves (AUROC) of the APRI was better in female than in male recipients (0.871 *vs* 0.753). At the cut-off value of > 1.4, the APRI in women had 91% sensitivity and 75% specificity in detecting a staging score of fibrosis > 2, while in men, the corresponding values were 60% and 77%, respectively[27]. Later, Pissaia *et al*[28] analyzed the APRI and FIB-4 in 50 liver transplant recipients[28]. The primary etiologies of end-stage liver disease were HCV in 23% of cases, hepatitis B virus (HBV) infection in 14%, alcoholic disease in 33%, cholestatic disease in 19%, and others in 11% of recipients. The mean period after LT was 30.7 mo (range, 12-108 mo). The AUROC of the APRI and FIB-4 to predict fibrosis were 0.87 and 0.78, respectively. Kamphues *et al*[29] prospectively analyzed the stage of fibrosis in 135 Liver transplant recipients (94 HCV, 41 alcoholic cirrhosis)[29]. According to this study, both the APRI and FIB-4 failed to assess liver fibrosis with satisfactory accuracy. Furthermore, Pinto *et al*[30] analyzed the accuracy of the APRI score in 30 children/adolescents with LT[30]. The AUROC for significant fibrosis detection was 0.74. However, in multivariate analysis, the APRI failed to be an independent predictor of significant fibrosis. Unfortunately, most of the studies evaluated biochemical markers in LT recipients with diseases other than NAFLD, consequently mora data and validation in NAFLD LT recipients are needed. The NAFLD fibrosis score (NFS) was designed to assess liver fibrosis exclusively in patients with NAFLD and has been well investigated in the general population[31]. It's accuracy in the post-LT setting is not well investigated. Kabbany *et al*[32] investigated 93 LT recipients who were transplanted due to HCV- or NAFLD-related ESLD[32]. In addition to APRI and FIB-4, NFS was also studied. The authors found that the APRI and FIB-4 could not accurately predict advanced fibrosis in LT recipients, while NFS correlated with advanced fibrosis in the graft when the indication of LT was NAFLD[32]. An interesting study was published five years ago by Bhat *et al*[33]. They retrospectively analyzed the usefulness of FIB-4, APRI and NFS in 547 liver transplant recipients in predicting death and graft loss after LT[33]. The authors found that serum fibrosis biomarkers 1 year after LT and changes in serum fibrosis biomarkers predict death and graft loss in LT recipients[33]. Given the encouraging results of the aforementioned studies, further prospective, controlled, multicenter studies in the NAFLD population with protocol biopsies as gold standard are needed. Also, the validation in routine practice is necessary, mainly with the aim of defining its role in assessing the course and outcome of the disease. However, we have to draw attention to the fact that the main limitation of the biomarkers that are well investigated and validated in the pre-LT setting is that all three biomarkers (APRI, FIB-4 and NFS) have thrombocytes in their formulas. According to earlier data, thrombocytopenia can persist after LT even though portal hypertension has reversed following LT. Therefore, these scores can overestimate fibrosis and are not a good method for fibrosis evaluation in LT recipients[16]. Serum biomarkers are well investigated in the pre-LT setting and are recommended by the guidelines of the European Association for the Study of the Liver (EASL). It is recommended that noninvasive methods could substitute for LB when combined in the pretransplant setting[34]. However, due to the abovementioned limitation (*i.e.*, post-LT thrombocytopenia), their use in the post-LT setting possibly could not be as useful as it is in the pre-LT setting.

Various other combinations of cytokines, chemokines, genetic polymorphisms, microRNAs, and post-translationally modified glycoproteins have also been proposed as candidate biomarkers of fibrosis but have not yet been validated or made available outside research laboratories[35]. Their application is difficult given the heterogeneity of

Table 2 Aspartate-aminotraspherase-to-platelet ratio index and fibrosis score 4 for fibrosis detection in liver transplant recipients

Ref.	Study population and etiology of ESLD	Prevalence F2-F4 (%)	Months after LT	Biochemical marker	Cut-off	Se	Sp	AUC	PPV	NPV
Toniutto *et al*[27], 2007	51 patients; HCV	32.4	24	APRI	1.4	76	77	0.80	46	93
Pissaia *et al*[28], 2009	50 patients; various etiologies	28	30.7	APRI	0.5	81	80	0.87	62	91
Kamphues *et al*[29], 2010	135 recipients; 94 HCV, 41 alcoholic cirrhosis	68.1	80.6	APRI	0.48	70	63	0.68	80	80
Pinto *et al*[30], 2014	30; biliary atresia, metabolic disease, other	20	60	APRI	0.4	83	58	0.74	31	94
Crespo *et al*[31], 2016	72; HCV	33	12	APRI	1.36	69	87	0.83	75	83
Pissaia *et al*[28], 2009	50 patients; various etiologies	28	30.7	FIB-4	3.25	31	94	0.78	67	77
Kamphues *et al*[29], 2010	135 recipients; 94 HCV, 41 alcoholic cirrhosis	68.1	80.6	FIB-4	2.8	44	87	0.66	88	42
Crespo *et al*[31], 2016	72; HCV	33	12	FIB-4	3.23	77	80	0.81	69	86

ESLD: End-stage liver disease; F: Fibrosis; Se: Sensitivity; Sp: Specificity; AUC: The area under the curve; PPV: Positive predictive value; NPV: Negative predictive value; HCV: Hepatitis C; APRI: AST-to-platelet ratio index; FIB-4: Fibrosis score 4.

liver diseases, especially regarding the detection of specific histological changes. Recent studies aiming to investigate markers related to the risk of NASH incorporated PNPLA3 I148M and rs738409 polymorphisms as well as other molecules related to inflammation (*e.g.*, K18), lipid metabolism, peptides, gut microbiome, circulating mRNA, DNA methylation, *etc*[35]. Investigations in genomics, epigenomics, metabolomics, lipidomics and proteomics have led to the identification of new markers able to define the type and severity of NAFLD as a long disease course. Before their routine application proof of concept is needed in the clinical field along with further validation.

In conclusion, there is a need to further investigate noninvasive biomarkers to decrease reliance on LB in assessing the progression of fibrosis in LT patients.

ULTRASOUND

Imaging of the liver by ultrasound (US) represents a valuable asset in addressing the characteristics of the liver graft in a pre-transplant setting and helps quickly identify some of the acute post-LT complications concerning vascular structures, especially when paired with contrast enhancement[36]. Ultrasound is noninvasive, widely available, inexpensive and portable method. Hepatic steatosis is seen on liver ultrasound as a hyperechoic (bright) liver compared with parenchyma of the ipsilateral kidney, while in a liver without steatosis, the liver and the renal parenchyma should exhibit similar echogenicity[37,38].

A meta-analysis of forty-nine studies with 4720 participants compared ultrasound with the gold standard LB in detecting liver steatosis. The overall sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio of US for the detection of moderate-severe fatty liver compared to histology were 84.8% (95% confidence interval: 79.5-88.9), 93.6% (87.2-97.0), 13.3 (6.4-27.6), and 0.16 (0.12-0.22), respectively[39]. However, the sensitivity of ultrasound decreases with the decrement of fatty infiltration, so in the presence of a hepatic fat content of 10% to 19%, it had a sensitivity of only 55% shown in a study on 100 Living liver donor candidates[40]. As mentioned earlier, the presence of morbid obesity (BMI greater than 40 kg/m^2) also lowers the sensitivity and specificity of ultrasound in detecting steatosis, which fall to 49% and 75%, respectively, as well as detecting the presence of severe fibrosis[39,41].

Simply classifying liver steatosis by US as mild, moderate or severe is quite dependent on the experience of the sonographist and the image quality, which can be impaired in many circumstances; thus, it amounts to a quite subjective analysis

without proper quantification of liver steatosis. Therefore, to adequately address steatosis by ultrasound and minimize operator and image-dependent bias, several computer-aided approaches have been proposed to quantify the level of liver steatosis[38,42,43]. Studies by Webb *et al*[38] and Mancini *et al*[43] reported that computer-aided measurement of the ultrasound hepatic/renal echo-intensity ratio (H/R) was highly correlated with the liver fat content determined by histology and [1H]-magnetic resonance spectroscopy, respectively. Xia *et al*[42] confirmed those conclusions in their study and added the hepatic/renal intensity ratio and ultrasound hepatic echo-intensity attenuation rate measurement and a tissue-mimicking phantom for standardization to make the results more comparable among different US machines. The optimal cut-off value for liver fat content that is sufficient to diagnose hepatic steatosis by ultrasound was 9.15%, and by using this cutoff, the sensitivity and specificity for quantitative computer-assisted ultrasound to diagnose hepatic steatosis were 95.1% and 100%, respectively, which were better than those of qualitative US, whose sensitivity and specificity were 82.5% and 83.3%, respectively[42].

Several other methods have been proposed to ameliorate the quantitative detection of liver steatosis with US, such as texture analysis by a gray-level co/occurrence matrix algorithm and the implementation of artificial intelligence of convolutional neural networks, which do not require the selection of the region of interest by the sonographer and thus minimize the subjectivity of the procedure[44-46]. Although there are unquestionable advancements in the quantification of liver steatosis by US, the diversity of the mechanisms used and the algorithms as well as the lack of appropriate cut-off levels and implementation of such methods in the post-LT liver graft, the conclusion is that US can be used as a screening modality for detecting hepatic steatosis but not as a quantitative assessment in the LT setting[47].

Since the introduction of fibroelastography in the evaluation of liver fibrosis, basic US has had little or almost a peripheral role. With the introduction of contrast-enhanced US and liver-specific contrasts, there is still hope for US. A recent study on 409 patients with hepatitis C used a liver-specific contrast agent to investigate the associations between the collapse of microbubbles and the progression of liver disease, and the range of bubble destruction was significantly increased according to the progression of fibrosis staging[48].

TRANSIENT ELASTOGRAPHY

In the last decade, clinical attention has been focused on one-dimensional transient elastography (TE), which is an US-based method that uses shear wave velocity to assess tissue (*e.g.*, liver) stiffness[49]. Since 2001, TE has been applied in medical practice under the name FibroScan®[49]. Liver stiffness measurements (LSM) as assessed by TE have been validated in pre-LT patients with various CLDs[50,51]. Initially, TE was developed for the assessment of liver stiffness as a surrogate marker of liver fibrosis; thus, LSM has been present in TE devices from its beginning. LSM values range from 1.5 to 75 kPa, where lower values indicate a more elastic liver[49]. Later, in 2011, a new parameter called the controlled attenuation parameter (CAP) was developed and incorporated into the TE device. CAP has allowed the detection and grading of steatosis by assessing the degree of US attenuation due to liver fat using the TE probe simultaneously with LSM. With this improvement, by use of TE with CAP, we can simultaneously assess both steatosis and fibrosis. The lowest CAP value is 100 and the highest 400 dB/m, where higher numbers indicate more pronounced steatosis[24,49].

Comparison of transient elastography and liver biopsy

In comparison to the LB, TE measures a much larger region of interest. With the help of TE, we can measure a cylindrical liver segment 1 cm wide and 4 cm long at a medium depth of 4.5 cm. This region of the liver parenchyma is approximately 100 times larger than the volume of the liver cylinder obtained by LB. The result of the TE exam is obtained as a median of at least 10 measurements. The drawback is that the information (LSM and CAP) cannot be obtained by a single measurement[24,49].

Effects of probe choice on transient elastography results

Earlier data reported the limitations of the M probe in obese patients in those with an increased skin-to-liver capsular distance. In those patients, if we use the M probe, there is a much higher failure rate. This led to the development of the XL probe that is specially designed for obese people[52]. Additionally, there were some uncertain data regarding the impact of other histological features on LSM; for example, there are

some data that reported that steatosis can influence LSM readings. Similarly, some studies suggested that cut-off values differ according to probe choice, M or XL[52-54]. However, recently, Eddowes *et al*[52] published the largest study about the accuracy of CAP and LSM obtained with the M or XL probe only in a population of patients with NAFLD. An automatic probe selection tool was set in the TE software that recommends the adequate probe depending on the skin-to-liver capsule distance of each patient. According to this study, CAP and LSM are accurate noninvasive tools for assessing liver steatosis and fibrosis in patients with NAFLD. In contrast to some conflicting earlier data, the authors have found that probe type and steatosis did not affect the LSM values, and the only parameter that affects LSM was the histological fibrosis grade[52].

Transient elastography in different liver diseases

The first purpose of TE devices was to assess the fibrosis stage in patients with viral hepatitis to reduce the need for LB. Those studies showed a good association of LSM with liver histology[49,55-59]. According to earlier data, the AUROC for the detection of significant fibrosis in patients with chronic HBV ranges from 0.86 to 0.97, with cut-off values from 5.2 to 8.0 kPa, while chronic HCV ranges from 0.73 to 0.91, with cut-off values from 5.2 to 9.5 kPa. In the case of patients with cirrhosis, the AUROC for identification in HBV ranges from 0.80 to 0.97, with cut-off values from 9.7 to 14.0 kPa, and in chronic HCV, the AUROC for cirrhosis ranges from 0.87 to 0.98, with cut-off values from 11.9 to 14.8 kPa[49,55-59]. Later, few studies investigated the accuracy of LSM in patients with NAFLD. According to these studies, the LSM cut-off value for significant fibrosis (F ≥ 2) ranges from 6.2 to 11 kPa; for F ≥ 3, from 8 to 12 kPa; and for F4, the LSM cut-off values range from 9.5 to 20 kPa[60-65]. The largest study that investigated the accuracy of LSM only in the NAFLD population reported that LSM identified patients with fibrosis with AUROCs of 0.77 (95%CI: 0.72-0.82) for F ≥ F2; 0.80 (95%CI: 0.75-0.84) for F ≥ F3; and 0.89 (95%CI: 0.84-0.93) for F = F4[52]. Furthermore, Youden cut-off values for F ≥ F2, F ≥ F3, and F4 were 8.2 kPa, 9.7 kPa, and 13.6 kPa, respectively[52].

Challenges in transient elastography performance

Taken together, TE with CAP is an adjunctive modality that can replace the gold standard, LB, when clinically warranted[24]. However, it should be mentioned that LSM is not an absolute measure of fibrosis but is instead a component of liver assessment and should be interpreted together with other clinical results, such as underlying liver disease, comorbidity, physical examination, laboratory tests, and other imaging methods[49]. Additionally, we must keep in mind that TE has some limitations. For example, it has been shown that food intake affects LSM values, and it is suggested that a minimum two-hour fast is currently recommended prior to the exam[49,66]. Bardou-Jacquet *et al*[67] reported that active alcohol consumption led to an overestimation of the LSM[67]. In cases of liver inflammation, such as chronic hepatitis with transaminase flare, LSM can also be overestimated. Thus, it is suggested that LSM interpretations in patients with high alanine-aminotraspherase (ALT) levels must be made with caution. Acute hepatitis and extrahepatic cholestasis also increase LSM, as does the case of heart failure in which LSM may be increased due to increased blood volume in the liver. In patients with ascites, TE is not possible because elastic waves do not travel through liquids, and in patients with narrow intercostal spaces, the success rate of TE examination is low (Table 3)[49].

In the post-LT population, data regarding the use of TE with CAP are sparse, especially in the context of *de novo* or recurrent NAFLD.

Usefulness of transient elastography in the post-LT setting

Interesting data regarding the use of TE with CAP in the context of LT were reported for the donor selection process and acute cellular rejection (ACR). One of the key points in successful LT is the determination of graft steatosis. There are differences in the mean of liver graft evaluation for the presence of steatosis between transplant centers, and there is no consensus regarding the need for LB[68]. Mancia *et al*[69] investigated the usefulness of CAP and LSM in the assessment of steatosis and fibrosis in 23 brain-dead potential donors. The authors concluded that CAP and LSM had good prediction of the histological status of steatosis of a potential liver graft[69]. Furthermore, the usefulness of LSM was investigated in the context of ACR because the inflammatory cascade driving ACR could be a cause of increased LSM. Crespo *et al*[70] investigated the usefulness of LSM in the detection and grading of ACR in liver transplant patients. The authors concluded that LSM has good diagnostic accuracy for

Table 3 Factors that influence liver stiffness measurement measurements

Factors	Influence
Food intake	Increase LSM
Active alcohol consumption	Increase LSM
Liver inflammation	Increase LSM
Cholestasis	Increase LSM
Right heart failure	Increase LSM
Ascites	Unreliable measurements
Operator inexperience	High rate of unsuccessful measurements and examinations

LSM: Liver stiffness measurement.

discriminating mild from moderate/severe ACR with an AUROC of 0.924[70]. A cut-off value of 8.5 kPa had a positive predictive value of 100% to diagnose moderate/severe ACR[70]. Before routine performance in this setting, further studies are needed to better define the cut-off points and TE applicability in decision and treatment algorithms.

Data from a previous meta-analysis comparing noninvasive methods for assessment of post-LT graft fibrosis shows that TE performs better than the serum-based biomarkers APRI and FIB 4 TE odds ratio 21.17 (95%CI: 14.10-31.77, APRI: 9.02, 95%CI: 5.79-14.07; and FIB-4 7.08, 95%CI: 4.00-12.55)[25].

In contrast to the investigation of the usefulness of TE with CAP in the pre-LT setting, its rate of investigation and accuracy in the post-LT setting was defined by underlying disease. Numerous studies have confirmed the TE accuracy post-LT in diagnosing patients with significant and advanced fibrosis, but mostly in HCV-positive recipients, even though data for various other etiologies are emerging[71-74]. Studies on the HCV population were performed to discriminate between slow and rapid progressors of graft fibrosis and response to therapy[71]. A study by Rinaldi *et al*[75] revealed that significant changes in LSM are related to the development of clinically significant graft disease (*e.g.*, all cases with a 20% increase in LSM in at least 3 measurements 3 mo apart developed biopsy proven significant graft injury or even cirrhosis).

To the best of our knowledge, only two studies have investigated the accuracy of TE with CAP in diagnosing fatty liver disease in post-LT patients. The first one was published five years ago by Karlas *et al*[76]. The authors evaluated post-LT steatosis by TE with CAP in 204 Liver transplant recipients[76]. Of 204 patients, 50% were transplanted due to alcoholic cirrhosis, and 2% were transplanted due to ESLD because of NAFLD. Since this study was published in 2015, at the time of study, the XL probe was not available, which is probably the reason why only 157 of the cases were able to achieve valid results. According to this study, 44% of recipients had steatosis, with 24% having advanced steatosis[76]. Given that the authors did not have the XL probe, the incidence of steatosis could be even higher. According to LSM, there was a high prevalence of transplant fibrosis (31%, defined by LSM > 7.9 kPa) and cirrhosis (13%, defined by LSM > 12 kPa). Advanced fibrosis (TE > 7.9 kPa) was associated with increased CAP results[76]. The relatively high prevalence of fibrosis and cirrhosis defined by LSM could be a consequence of a higher rate of obese recipients and a longer follow-up interval since LT[76]. The authors did not compare the results of TE with CAP measurements with the LB. However, the authors have shown that the same risk factors for fatty liver disease in the general population were associated with increased CAP; increased BMI and diabetes mellitus, which are specific components of MetS, were associated with an increased risk of advanced steatosis and fibrosis[76]. Interestingly, the authors found a correlation between CAP values and the liver recipient PNPLA3 status[76]. Furthermore, this year, Chayanupatkul *et al*[77] published the second study about the usefulness of TE with CAP in a post-LT setting. They analyzed 150 LT recipients. The presence of steatosis was defined by CAP values of ≥ 222 dB/m, and severe steatosis was defined as ≥ 290 dB/m. Of the 150 analyzed recipients in this study, 70% had steatosis, while 40% of these had severe steatosis. Interestingly, 81.0% of recipients with severe steatosis had normal ALT at the time of TE. In multivariable analyses, age at LT, post-LT obesity and alcoholic liver disease were significant predictors of severe steatosis[77]. Additionally, in this study also, the results of TE with CAP were not investigated in comparison to the LB. In this study,

there was a much higher prevalence of steatosis defined by TE than that in the study published by Karlas *et al*[76]. The authors did not find that steatosis defined by increased CAP values is a risk factor for morbidity and mortality after LT. The median follow-up period after LT was 66.1 mo. There was no difference with respect to the overall death rates and the percentage of recipients with cirrhosis between the severe steatosis and non-severe steatosis groups[77]. As mentioned, it was shown that most recipients with severe steatosis and, more importantly, those with cirrhosis had normal ALT (< 40 U/L). These results are in line with the results of Dumortier *et al*[14], who showed that there was no significant difference in ALT levels between those with and without fibrosis. Moreover, 31% of recipients with LB-proven NASH post-LT had normal ALT. From the data in the pre-LT setting, we know that approximately 50% of patients with NAFLD have normal transaminase levels; thus, ALT is not a good method of NAFLD screening in the post-LT setting[77].

Taken together, the clinical consequences of nonalcoholic fatty liver (NAFL) in the context of the post-LT setting have not yet been completely elucidated. Currently, we know that graft steatosis occurs in a considerable proportion of LT recipients, but there are currently no data about graft steatosis as a risk factor for advanced fibrosis, graft loss or impaired survival after LT. Thus, further imaging-based steatosis and fibrosis investigations are needed using LB comparison in the LT population[16].

OTHER IMAGING METHODS

pSWE/ARFI techniques

Published concordance between TE and SWE findings in the general population ranges from moderate to excellent depending on the study. Studies on the LT population are limited. In a study of Dubois *et al*[78], mean SWE value for patients without significant fibrosis (≤ F1) was 15.90 ± 9.2 kPa *vs* 19.27 ± 7.7 kPa for patients with fibrosis and did not reach statistical significance (P = 0.185). 2D-SWE values were higher in patients with cirrhosis when compared with those without, but there was also no significant difference (24.5 ± 7.3 kPa *vs* 16.0 ± 9 kPa, P = 0.119). The possible explanation of this lack of significant association could be underpowering. Also, it is important to stress out the high rate of liver stiffness of patients with no significant fibrosis, that was significantly higher than those reported in native livers, and possibly influenced by other post-LT specific factors influencing the liver stiffness (*e.g.*, inflammation, congestion, steatosis). A 2D-SWE cutoff value ≥ 17.05 kPa was found optimal for the detection of any grade of significant fibrosis, with an AUROC of 0.657 ± 0.13 (95%CI: 41%-91%), a sensitivity of 71.4% (95%CI: 35%-92%), a specificity of 59.2% (95%CI: 45%-72%), and PPV and NPV of 20% and 94%, respectively. Overall, this cutoff value correctly classified 60.7% patients. A 2D-SWE value below 7.85 kPa rules out the presence of significant fibrosis, resulting in a 100% NPV. A 2D-SWE value above 26.35 kPa ruled in significant fibrosis, with a 33.3% PPV[78].

A study by Perry *et al*[79], revealed no significant difference in mean PSWE measurements in patients with native livers and those with transplanted livers compared to finding of LB. pSWE accurately differentiate between patients with no-to-mild hepatic fibrosis (F0-F1) and moderate-to-severe hepatic fibrosis (≥ F2) with sensitivity of 72% and specificity of 69%.

To conclude the position of pSWE/ARFI in routine practice and evaluation of disease outcome, this method should be fully investigated[79].

MR elastography

MR elastography (MRE) is established as an accurate current non-invasive method for assessment of liver fibrosis. MRI has been found to perform better than US or computed tomography with sensitivity and specificity of 90% and 91% respectively, however still needs further validation[80-83]. Interestingly, and contrary to TE, studies have reported the excellent diagnostic accuracy of MRE in the diagnosis of cirrhosis and fibrosis even in patients with higher BMI or in those with ascites[81-83]. In the general population, comparisons between the accuracy of TE and MR elastography provide conflicting results. In a LT setting MRE can be use alone for fibrosis assessment or combined with standard liver magnetic resonance cholangio-pancreatography protocol to evaluate the graft and biliary tree[83]. The study by Singh *et al*[84] revealed a mean AUROC for significant fibrosis and cirrhosis between 0.69 and 0.96 in LT-setting. A Kamphues *et al*[85] analyzed 25 patients, who had received a liver graft due to HCV. All patients underwent both liver biopsy and MR elastography. They have found that AUROC of MR elastography based on μ for diagnosis of severe

fibrosis (F ≥ 3) was 0.87 and 0.65 for diagnosis of significant fibrosis (F ≥ 2)[85]. Thus authors had found that MR elastography is a good diagnostic tool for the assessment of higher grades of fibrosis in HCV patients after LT[85]. On the other hand, the poor correlation for lower grades of fibrosis was reported[85]. According to available data, MRE appears to demonstrate good diagnostic accuracy in the diagnosis of advanced fibrosis in post-LT setting. We can combine MRE with standard liver MRI/magnetic resonance cholangiopancreatography in order to evaluate liver parenchyma as well as focal graft lesions and finally biliary obstruction. However, its applicability is influenced by availability, cost, and time-related concerns. Before final conclusions about its routine applicability, further studies specifically on LT recipients, are needed[83].

CONCLUSION

Until further data arrive, LB remains the gold standard for establishing a conclusive diagnosis of recurrent NAFLD as well as to rule out competing etiologies. Management of LT recipients is focused on prevention and treatment of any graft diseases. Except for possible acute and chronic rejections, infections, biliary or vascular complications, recipient and graft morbidity and mortality are closely related to the development of various causes of liver fibrosis. Many regular laboratory and morphological evaluations are performed as early as possible to recognize any graft damage, and LB plays a central role in the diagnosis and exclusion of various graft diseases and the detection of fibrosis. TE with CAP in LT recipients has not yet been fully investigated. We strongly believe that this method could be very useful in post-LT settings. An important advantage of noninvasive methods, especially TE with CAP, in the evaluation of liver fibrosis are their noninvasiveness and repeatability, offering insight into dynamic changes in graft disease and the development of fibrosis. As shown in earlier data, fibrosis of liver allografts often occurs with normal transaminase levels. Thus, ALT is not a good marker for the prediction of fibrosis. Per protocol biopsies are not performed in many transplant centers, and as mentioned, many transplant recipients with advanced fibrosis have normal or mildly elevated ALT; therefore, LSM could be a good method for the selection of those who need LB. Given that TE with CAP is a noninvasive and easily obtained method, it is risk free, objective and operator-independent and requires only 5-10 min for the examination, and it is a great method for the follow-up of fibrosis progression in every-day clinical practice. In our opinion, patients with permanently elevated and increasing LSM findings should be scheduled for LB to identify the cause and stage of liver graft disease. Previous meta-analysis shows that TE performs better than the serum-based biomarkers APRI and FIB 4[25]. Still, considering their performance and invasiveness, LB and various noninvasive methods are not exclusive and should be used as complementary procedures.

There is little published experience so far using TE with CAP, especially in the context of *de novo* or recurrent NAFLD. Therefore, prospective, well-designed studies with per protocol biopsies should investigate the usefulness of TE with CAP in the post-LT setting. Additionally, these studies should answer the most important question of the optimal cut-off values of graft fibrosis in comparison to LSM in the post-LT population.

Second, post-LT graft steatosis is becoming an increasingly important issue in the transplant population. Both recurrent and *de novo* NAFLD are common after LT. By longitudinal use of CAP, we could recognize those two conditions. The question arises as to whether TE with CAP can be used to detect and monitor *de novo* NAFLD and recurrent NAFLD. Additionally, the progression of LSM values may be used as a determinant of liver allograft fibrosis severity. To date, there are still no efficient drugs for NAFLD, and the only treatment options for NAFLD generally include lifestyle changes and treatment of obesity, diabetes, hypertension and dyslipidemia. Therefore, the question arises as to whether monitoring the changes in the CAP and LSM could be useful for evaluating the treatment of those MetS components and the effect of treatment of MetS and its components on *de novo* and recurrent NAFLD. Additionally, this could motivate clinicians who manage LT recipients to treat MetS more aggressively and its components. We still do not know much about *de novo* and recurrent NAFLD; some data are connecting them with the poor survival and with a higher incidence of cardiovascular events[86]. These data are not surprising given the data in the pre-LT setting, where it has been shown that NAFLD is not only a liver disease but also a multisystem disease that is mainly connected to diabetes mellitus,

cardiovascular diseases and chronic kidney disease but also to some other chronic diseases, such as colorectal cancer[87]. CAP, as a surrogate marker of NAFLD in the pre-LT setting, showed a correlation with cardiovascular risk[88,89] and CKD[90]. Given this association, the question is whether patients with *de novo* or recurrent NAFLD with both increased CAP and specifically an increased LSM could benefit from much earlier and much stronger screening for CVD and CKD. This is important because CKD and CVD are the main determinants of patient and allograft survival. We are asking whether CAP and LSM could be surrogate markers of subclinical atherosclerosis and consequently markers of increased CVD risk in the post-LT setting.

Finally, cost-effective studies are needed to investigate the usefulness of TE with CAP in the post-LT setting.

REFERENCES

1 **Wong RJ**, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, Ahmed A. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 2015; **148**: 547-555 [PMID: 25461851 DOI: 10.1053/j.gastro.2014.11.039]

2 **Mikolasevic I**, Filipec-Kanizaj T, Mijic M, Jakopcic I, Milic S, Hrstic I, Sobocan N, Stimac D, Burra P. Nonalcoholic fatty liver disease and liver transplantation - Where do we stand? *World J Gastroenterol* 2018; **24**: 1491-1506 [PMID: 29662288 DOI: 10.3748/wjg.v24.i14.1491]

3 **Younossi ZM**, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, Srishord M. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clin Gastroenterol Hepatol* 2011; **9**: 524-530. quiz e60 [PMID: 21440669 DOI: 10.1016/j.cgh.2011.03.020]

4 **Seo S**, Maganti K, Khehra M, Ramsamooj R, Tsodikov A, Bowlus C, McVicar J, Zern M, Torok N. De novo nonalcoholic fatty liver disease after liver transplantation. *Liver Transpl* 2007; **13**: 844-847 [PMID: 17029282 DOI: 10.1002/Lt.20932]

5 **Baffy G**, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. *J Hepatol* 2012; **56**: 1384-1391 [PMID: 22326465 DOI: 10.1016/j.jhep.2011.10.027]

6 **Mikolasevic I**, Orlic L, Hrstic I, Milic S. Metabolic syndrome and non-alcoholic fatty liver disease after liver or kidney transplantation. *Hepatol Res* 2016; **46**: 841-852 [PMID: 26713425 DOI: 10.1111/hepr.12642]

7 **Patel YA**, Berg CL, Moylan CA. Nonalcoholic Fatty Liver Disease: Key Considerations Before and After Liver Transplantation. *Dig Dis Sci* 2016; **61**: 1406-1416 [PMID: 26815171 DOI: 10.1007/s10620-016-4035-3]

8 **Castera L**, Friedrich-Rust M, Loomba R. Noninvasive Assessment of Liver Disease in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2019; **156**: 1264-1281. e4 [PMID: 30660725 DOI: 10.1053/j.gastro.2018.12.036]

9 **Watt KD**, Charlton MR. Metabolic syndrome and liver transplantation: a review and guide to management. *J Hepatol* 2010; **53**: 199-206 [PMID: 20451282 DOI: 10.1016/j.jhep.2010.01.040]

10 **Gitto S**, Villa E. Non-Alcoholic Fatty Liver Disease and Metabolic Syndrome after Liver Transplant. *Int J Mol Sci* 2016; **17**: 490 [PMID: 27049380 DOI: 10.3390/ijms17040490]

11 **Bhagat V**, Mindikoglu AL, Nudo CG, Schiff ER, Tzakis A, Regev A. Outcomes of liver transplantation in patients with cirrhosis due to nonalcoholic steatohepatitis versus patients with cirrhosis due to alcoholic liver disease. *Liver Transpl* 2009; **15**: 1814-1820 [PMID: 19938128 DOI: 10.1002/lt.21927]

12 **Bhati C**, Idowu MO, Sanyal AJ, Rivera M, Driscoll C, Stravitz RT, Kohli DR, Matherly S, Puri P, Gilles H, Cotterell A, Levy M, Sterling RK, Luketic VA, Lee H, Sharma A, Siddiqui MS. Long-term Outcomes in Patients Undergoing Liver Transplantation for Nonalcoholic Steatohepatitis-Related Cirrhosis. *Transplantation* 2017; **101**: 1867-1874 [PMID: 28296807 DOI: 10.1097/TP.0000000000001709]

13 **Saeed N**, Glass L, Sharma P, Shannon C, Sonnenday CJ, Tincopa MA. Incidence and Risks for Nonalcoholic Fatty Liver Disease and Steatohepatitis Post-liver Transplant: Systematic Review and Meta-analysis. *Transplantation* 2019; **103**: e345-e354 [PMID: 31415032 DOI: 10.1097/TP.0000000000002916]

14 **Dumortier J**, Giostra E, Belbouab S, Morard I, Guillaud O, Spahr L, Boillot O, Rubbia-Brandt L, Scoazec JY, Hadengue A. Non-alcoholic fatty liver disease in liver transplant recipients: another story of "seed and soil". *Am J Gastroenterol* 2010; **105**: 613-620 [PMID: 20040915 DOI: 10.1038/ajg.2009.717]

15 **Vallin M**, Guillaud O, Boillot O, Hervieu V, Scoazec JY, Dumortier J. Recurrent or de novo nonalcoholic fatty liver disease after liver transplantation: natural history based on liver biopsy analysis. *Liver Transpl* 2014; **20**: 1064-1071 [PMID: 24961607 DOI: 10.1002/lt.23936]

16 **Germani G**, Laryea M, Rubbia-Brandt L, Egawa H, Burra P, O'Grady J, Watt KD. Management of Recurrent and De Novo NAFLD/NASH After Liver Transplantation. *Transplantation* 2019; **103**: 57-67 [PMID: 30335694 DOI: 10.1097/TP.0000000000002485]

17 **Dureja P**, Mellinger J, Agni R, Chang F, Avey G, Lucey M, Said A. NAFLD recurrence in liver transplant recipients. *Transplantation* 2011; **91**: 684-689 [PMID: 21248661 DOI: 10.1097/TP.0b013e31820b6b84]

18 **Patil DT**, Yerian LM. Evolution of nonalcoholic fatty liver disease recurrence after liver transplantation. *Liver Transpl* 2012; **18**: 1147-1153 [PMID: 22740341 DOI: 10.1002/lt.23499]

19 **Narayanan P**, Mara K, Izzy M, Dierkhising R, Heimbach J, Allen AM, Watt KD. Recurrent or De Novo Allograft Steatosis and Long-term Outcomes After Liver Transplantation. *Transplantation* 2019; **103**: e14-e21 [PMID: 29994981 DOI: 10.1097/TP.0000000000002317]

20 **Sprinzl MF**, Weinmann A, Lohse N, Tönissen H, Koch S, Schattenberg J, Hoppe-Lotichius M, Zimmermann T, Galle PR, Hansen T, Otto G, Schuchmann M. Metabolic syndrome and its association with fatty liver disease after orthotopic liver transplantation. *Transpl Int* 2013; **26**: 67-74 [PMID: 23126674 DOI: 10.1111/j.1432-2277.2012.01576.x]

21 **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]

22 **Watt KD**, Dierkhising R, Fan C, Heimbach JK, Tillman H, Goldstein D, Thompson A, Krishnan A, Charlton MR. Investigation of PNPLA3 and IL28B genotypes on diabetes and obesity after liver transplantation: insight into mechanisms of disease. *Am J Transplant* 2013; **13**: 2450-2457 [PMID: 23859071 DOI: 10.1111/ajt.12355]

23 **Finkenstedt A**, Auer C, Glodny B, Posch U, Steitzer H, Lanzer G, Pratschke J, Biebl M, Steurer M, Graziadei I, Vogel W, Zoller H. Patatin-like phospholipase domain-containing protein 3 rs738409-G in recipients of liver transplants is a risk factor for graft steatosis. *Clin Gastroenterol Hepatol* 2013; **11**: 1667-1672 [PMID: 23872669 DOI: 10.1016/j.cgh.2013.06.025]

24 **Winters AC**, Mittal R, Schiano TD. A review of the use of transient elastography in the assessment of fibrosis and steatosis in the post-liver transplant patient. *Clin Transplant* 2019; **33**: e13700 [PMID: 31441967 DOI: 10.1111/ctr.13700]

25 **Bhat M**, Tazari M, Sebastiani G. Performance of transient elastography and serum fibrosis biomarkers for non-invasive evaluation of recurrent fibrosis after liver transplantation: A meta-analysis. *PLoS One* 2017; **12**: e0185192 [PMID: 28953939 DOI: 10.1371/journal.pone.0185192]

26 **Neumann UP**, Berg T, Bahra M, Seehofer D, Langrehr JM, Neuhaus R, Radke C, Neuhaus P. Fibrosis progression after liver transplantation in patients with recurrent hepatitis C. *J Hepatol* 2004; **41**: 830-836 [PMID: 15519657 DOI: 10.1016/j.jhep.2004.06.029]

27 **Toniutto P**, Fabris C, Bitetto D, Falleti E, Avellini C, Rossi E, Smirne C, Minisini R, Pirisi M. Role of AST to platelet ratio index in the detection of liver fibrosis in patients with recurrent hepatitis C after liver transplantation. *J Gastroenterol Hepatol* 2007; **22**: 1904-1908 [PMID: 17914968 DOI: 10.1111/j.1440-1746.2006.04628.x]

28 **Pissaia A Jr**, Borderie D, Bernard D, Scatton O, Calmus Y, Conti F. APRI and FIB-4 Scores Are Useful After Liver Transplantation Independently of Etiology. *Transplant Proc* 2009; **41**: 679-681 [PMID: 19328955 DOI: 10.1016/j.transproceed.2008.12.014]

29 **Kamphues C**, Lotz K, Röcken C, Berg T, Eurich D, Pratschke J, Neuhaus P, Neumann UP. Chances and limitations of non-invasive tests in the assessment of liver fibrosis in liver transplant patients. *Clin Transplant* 2010; **24**: 652-659 [PMID: 19925459 DOI: 10.1111/j.1399-0012.2009.01152.x]

30 **Pinto J**, Matos H, Nobre S, Cipriano MA, Marques M, Pereira JM, Gonçalves I, Noruegas MJ. Comparison of acoustic radiation force impulse/serum noninvasive markers for fibrosis prediction in liver transplant. *J Pediatr Gastroenterol Nutr* 2014; **58**: 382-386 [PMID: 24164902 DOI: 10.1097/MPG.0000000000000226]

31 **Crespo G**, Gambato M, Millán O, Casals G, Ruiz P, Londoño MC, Mira A, Forns X, Brunet M, Jiménez W, Navasa M. Early non-invasive selection of patients at high risk of severe hepatitis C recurrence after liver transplantation. *Transpl Infect Dis* 2016; **18**: 471-479 [PMID: 26992003 DOI: 10.1111/tid.12526]

32 **Kabbany MN**, Conjeevaram Selvakumar PK, Guirguis J, Rivas J, Akras Z, Lopez R, Hanouneh I, Eghtesad B, Alkhouri N. Accuracy of Noninvasive Fibrosis Scores in Predicting the Presence of Fibrosis in Patients after Liver Transplantation. *Exp Clin Transplant* 2018; **16**: 562-567 [PMID: 28952917 DOI: 10.6002/ect.2016.0340]

33 **Bhat M**, Ghali P, Rollet-Kurhajec KC, Bhat A, Wong P, Deschenes M, Sebastiani G. Serum fibrosis biomarkers predict death and graft loss in liver transplantation recipients. *Liver Transpl* 2015; **21**: 1383-1394 [PMID: 26194602 DOI: 10.1002/lt.24217]

34 **European Association for Study of Liver**. Asociacion Latinoamericana para el Estudio del Higado. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. *J Hepatol* 2015; **63**: 237-264 [PMID: 25911335 DOI: 10.1016/j.jhep.2015.04.006]

35 **Vilar-Gomez E**, Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: Clinical prediction rules and blood-based biomarkers. *J Hepatol* 2018; **68**: 305-315 [PMID: 29154965 DOI: 10.1016/j.jhep.2017.11.013]

36 **Como G**, Montaldo L, Baccarani U, Lorenzin D, Zuiani C, Girometti R. Contrast-enhanced ultrasound applications in liver transplant imaging. *Abdom Radiol (NY)* 2020 [PMID: 31925494 DOI: 10.1007/s00261-020-02402-z]

37 **Quinn SF**, Gosink BB. Characteristic sonographic signs of hepatic fatty infiltration. *AJR Am J Roentgenol* 1985; **145**: 753-755 [PMID: 3898784 DOI: 10.2214/ajr.145.4.753]

38 **Webb M**, Yeshua H, Zelber-Sagi S, Santo E, Brazowski E, Halpern Z, Oren R. Diagnostic value of a computerized hepatorenal index for sonographic quantification of liver steatosis. *AJR Am J*

Roentgenol 2009; **192**: 909-914 [PMID: 19304694 DOI: 10.2214/AJR.07.4016]

39 **Hernaez R**, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology* 2011; **54**: 1082-1090 [PMID: 21618575 DOI: 10.1002/hep.24452]

40 **Ryan CK**, Johnson LA, Germin BI, Marcos A. One hundred consecutive hepatic biopsies in the workup of living donors for right lobe liver transplantation. *Liver Transpl* 2002; **8**: 1114-1122 [PMID: 12474149 DOI: 10.1053/jlts.2002.36740]

41 **Mottin CC**, Moretto M, Padoin AV, Swarowsky AM, Toneto MG, Glock L, Repetto G. The role of ultrasound in the diagnosis of hepatic steatosis in morbidly obese patients. *Obes Surg* 2004; **14**: 635-637 [PMID: 15186630 DOI: 10.1381/096089204323093408]

42 **Xia MF**, Yan HM, He WY, Li XM, Li CL, Yao XZ, Li RK, Zeng MS, Gao X. Standardized ultrasound hepatic/renal ratio and hepatic attenuation rate to quantify liver fat content: an improvement method. *Obesity (Silver Spring)* 2012; **20**: 444-452 [PMID: 22016092 DOI: 10.1038/oby.2011.302]

43 **Mancini M**, Prinster A, Annuzzi G, Liuzzi R, Giacco R, Medagli C, Cremone M, Clemente G, Maurea S, Riccardi G, Rivellese AA, Salvatore M. Sonographic hepatic-renal ratio as indicator of hepatic steatosis: comparison with (1)H magnetic resonance spectroscopy. *Metabolism* 2009; **58**: 1724-1730 [PMID: 19716568 DOI: 10.1016/j.metabol.2009.05.032]

44 **Gaitini D**, Baruch Y, Ghersin E, Veitsman E, Kerner H, Shalem B, Yaniv G, Sarfaty C, Azhari H. Feasibility study of ultrasonic fatty liver biopsy: texture vs. attenuation and backscatter. *Ultrasound Med Biol* 2004; **30**: 1321-1327 [PMID: 15582231 DOI: 10.1016/j.ultrasmedbio.2004.08.001]

45 **Byra M**, Styczynski G, Szmigielski C, Kalinowski P, Michałowski Ł, Paluszkiewicz R, Ziarkiewicz-Wróblewska B, Zieniewicz K, Sobieraj P, Nowicki A. Transfer learning with deep convolutional neural network for liver steatosis assessment in ultrasound images. *Int J Comput Assist Radiol Surg* 2018; **13**: 1895-1903 [PMID: 30094778 DOI: 10.1007/s11548-018-1843-2]

46 **Bharti P**, Mittal D, Ananthasivan R. Computer-aided Characterization and Diagnosis of Diffuse Liver Diseases Based on Ultrasound Imaging: A Review. *Ultrason Imaging* 2017; **39**: 33-61 [PMID: 27097589 DOI: 10.1177/0161734616639875]

47 **Cesaretti M**, Addeo P, Schiavo L, Anty R, Iannelli A. Assessment of Liver Graft Steatosis: Where Do We Stand? *Liver Transpl* 2019; **25**: 500-509 [PMID: 30380197 DOI: 10.1002/lt.25379]

48 **Wakui N**, Nagai H, Yoshimine N, Amanuma M, Kobayashi K, Ogino Y, Matsui D, Mukozu T, Matsukiyo Y, Matsui T, Daido Y, Momiyama K, Shinohara M, Kudo T, Maruyama K, Sumino Y, Igarashi Y. Flash Imaging Used in the Post-vascular Phase of Contrast-Enhanced Ultrasonography is Useful for Assessing the Progression in Patients with Hepatitis C Virus-Related Liver Disease. *Ultrasound Med Biol* 2019; **45**: 1654-1662 [PMID: 31031037 DOI: 10.1016/j.ultrasmedbio.2019.03.005]

49 **Mikolasevic I**, Orlic L, Franjic N, Hauser G, Stimac D, Milic S. Transient elastography (FibroScan(®)) with controlled attenuation parameter in the assessment of liver steatosis and fibrosis in patients with nonalcoholic fatty liver disease - Where do we stand? *World J Gastroenterol* 2016; **22**: 7236-7251 [PMID: 27621571 DOI: 10.3748/wjg.v22.i32.7236]

50 **Ganne-Carrié N**, Ziol M, de Ledinghen V, Douvin C, Marcellin P, Castera L, Dhumeaux D, Trinchet JC, Beaugrand M. Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. *Hepatology* 2006; **44**: 1511-1517 [PMID: 17133503 DOI: 10.1002/hep.21420]

51 **Kuo YH**, Lu SN, Hung CH, Kee KM, Chen CH, Hu TH, Lee CM, Changchien CS, Wang JH. Liver stiffness measurement in the risk assessment of hepatocellular carcinoma for patients with chronic hepatitis. *Hepatol Int* 2010; **4**: 700-706 [PMID: 21286340 DOI: 10.1007/s12072-010-9223-1]

52 **Eddowes PJ**, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, Guha IN, Cobbold JF, Deeks JJ, Paradis V, Bedossa P, Newsome PN. Accuracy of FibroScan Controlled Attenuation Parameter and Liver Stiffness Measurement in Assessing Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2019; **156**: 1717-1730 [PMID: 30689971 DOI: 10.1053/j.gastro.2019.01.042]

53 **Petta S**, Wong VW, Cammà C, Hiriart JB, Wong GL, Marra F, Vergniol J, Chan AW, Di Marco V, Merrouche W, Chan HL, Barbara M, Le-Bail B, Arena U, Craxì A, de Ledinghen V. Improved noninvasive prediction of liver fibrosis by liver stiffness measurement in patients with nonalcoholic fatty liver disease accounting for controlled attenuation parameter values. *Hepatology* 2017; **65**: 1145-1155 [PMID: 27639088 DOI: 10.1002/hep.28843]

54 **Kim SU**, Kim JK, Park YN, Han KH. Discordance between liver biopsy and Fibroscan® in assessing liver fibrosis in chronic hepatitis b: risk factors and influence of necroinflammation. *PLoS One* 2012; **7**: e32233 [PMID: 22384189 DOI: 10.1371/journal.pone.0032233]

55 **Castéra L**, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350 [PMID: 15685546 DOI: 10.1053/j.gastro.2004.11.018]

56 **Lupsor Platon M**, Stefanescu H, Feier D, Maniu A, Badea R. Performance of unidimensional transient elastography in staging chronic hepatitis C. Results from a cohort of 1,202 biopsied patients from one single center. *J Gastrointestin Liver Dis* 2013; **22**: 157-166 [PMID: 23799214]

57 **Wang JH**, Changchien CS, Hung CH, Eng HL, Tung WC, Kee KM, Chen CH, Hu TH, Lee CM, Lu SN. FibroScan and ultrasonography in the prediction of hepatic fibrosis in patients with chronic viral

hepatitis. *J Gastroenterol* 2009; **44**: 439-446 [PMID: 19308312 DOI: 10.1007/s00535-009-0017-y]

58 **Degos F**, Perez P, Roche B, Mahmoudi A, Asselineau J, Voitot H, Bedossa P; FIBROSTIC study group. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: a multicenter prospective study (the FIBROSTIC study). *J Hepatol* 2010; **53**: 1013-1021 [PMID: 20850886 DOI: 10.1016/j.jhep.2010.05.035]

59 **Zarski JP**, Sturm N, Guechot J, Paris A, Zafrani ES, Asselah T, Boisson RC, Bosson JL, Guyader D, Renversez JC, Bronowicki JP, Gelineau MC, Tran A, Trocme C, De Ledinghen V, Lasnier E, Poujol-Robert A, Ziegler F, Bourliere M, Voitot H, Larrey D, Rosenthal-Allieri MA, Fouchard Hubert I, Bailly F, Vaubourdolle M; ANRS HCEP 23 Fibrostar Group. Comparison of nine blood tests and transient elastography for liver fibrosis in chronic hepatitis C: the ANRS HCEP-23 study. *J Hepatol* 2012; **56**: 55-62 [PMID: 21781944 DOI: 10.1016/j.jhep.2011.05.024]

60 **Yoneda M**, Yoneda M, Fujita K, Inamori M, Tamano M, Hiriishi H, Nakajima A. Transient elastography in patients with non-alcoholic fatty liver disease (NAFLD). *Gut* 2007; **56**: 1330-1331 [PMID: 17470477 DOI: 10.1136/gut.2007.126417]

61 **Cassinotto C**, Boursier J, de Lédinghen V, Lebigot J, Lapuyade B, Cales P, Hiriart JB, Michalak S, Bail BL, Cartier V, Mouries A, Oberti F, Fouchard-Hubert I, Vergniol J, Aubé C. Liver stiffness in nonalcoholic fatty liver disease: A comparison of supersonic shear imaging, FibroScan, and ARFI with liver biopsy. *Hepatology* 2016; **63**: 1817-1827 [PMID: 26659452 DOI: 10.1002/hep.28394]

62 **Wong VW**, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, Choi PC, Kowo M, Chan AW, Merrouche W, Sung JJ, de Lédinghen V. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 454-462 [PMID: 20101745 DOI: 10.1002/hep.23312]

63 **Lupsor M**, Badea R, Stefanescu H, Grigorescu M, Serban A, Radu C, Crişan D, Sparchez Z, Iancu S, Maniu A. Performance of unidimensional transient elastography in staging non-alcoholic steatohepatitis. *J Gastrointestin Liver Dis* 2010; **19**: 53-60 [PMID: 20361076]

64 **Yoneda M**, Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, Nozaki Y, Yonemitsu K, Higurashi T, Takahashi H, Kobayashi N, Kirikoshi H, Abe Y, Inamori M, Kubota K, Saito S, Tamano M, Hiraishi H, Maeyama S, Yamaguchi N, Togo S, Nakajima A. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). *Dig Liver Dis* 2008; **40**: 371-378 [PMID: 18083083 DOI: 10.1016/j.dld.2007.10.019]

65 **Kumar R**, Rastogi A, Sharma MK, Bhatia V, Tyagi P, Sharma P, Garg H, Chandan Kumar KN, Bihari C, Sarin SK. Liver stiffness measurements in patients with different stages of nonalcoholic fatty liver disease: diagnostic performance and clinicopathological correlation. *Dig Dis Sci* 2013; **58**: 265-274 [PMID: 22790906 DOI: 10.1007/s10620-012-2306-1]

66 **Lemoine M**, Shimakawa Y, Njie R, Njai HF, Nayagam S, Khalil M, Goldin R, Ingiliz P, Taal M, Nyan O, Corrah T, D'Alessandro U, Thursz M. Food intake increases liver stiffness measurements and hampers reliable values in patients with chronic hepatitis B and healthy controls: the PROLIFICA experience in The Gambia. *Aliment Pharmacol Ther* 2014; **39**: 188-196 [PMID: 24308698 DOI: 10.1111/apt.12561]

67 **Bardou-Jacquet E**, Legros L, Soro D, Latournerie M, Guillygomarc'h A, Le Lan C, Brissot P, Guyader D, Moirand R. Effect of alcohol consumption on liver stiffness measured by transient elastography. *World J Gastroenterol* 2013; **19**: 516-522 [PMID: 23382630 DOI: 10.3748/wjg.v19.i4.516]

68 **Mikolasevic I**, Milic S, Filipec-Kanizaj T. Fatty liver allografts are associated with primary graft non-function and high mortality after transplantation. *Liver Int* 2017; **37**: 1113-1115 [PMID: 28710818 DOI: 10.1111/liv.13453]

69 **Mancia C**, Loustaud-Ratti V, Carrier P, Naudet F, Bellissant E, Labrousse F, Pichon N. Controlled Attenuation Parameter and Liver Stiffness Measurements for Steatosis Assessment in the Liver Transplant of Brain Dead Donors. *Transplantation* 2015; **99**: 1619-1624 [PMID: 25719261 DOI: 10.1097/TP.0000000000000652]

70 **Crespo G**, Castro-Narro G, García-Juárez I, Benítez C, Ruiz P, Sastre L, Colmenero J, Miquel R, Sánchez-Fueyo A, Forns X, Navasa M. Usefulness of liver stiffness measurement during acute cellular rejection in liver transplantation. *Liver Transpl* 2016; **22**: 298-304 [PMID: 26609794 DOI: 10.1002/lt.24376]

71 **Carrión JA**, Torres F, Crespo G, Miquel R, García-Valdecasas JC, Navasa M, Forns X. Liver stiffness identifies two different patterns of fibrosis progression in patients with hepatitis C virus recurrence after liver transplantation. *Hepatology* 2010; **51**: 23-34 [PMID: 19839063 DOI: 10.1002/hep.23240]

72 **Cholongitas E**, Tsochatzis E, Goulis J, Burroughs AK. Noninvasive tests for evaluation of fibrosis in HCV recurrence after liver transplantation: a systematic review. *Transpl Int* 2010; **23**: 861-870 [PMID: 20704691 DOI: 10.1111/j.1432-2277.2010.01142.x]

73 **Barrault C**, Roudot-Thoraval F, Tran Van Nhieu J, Atanasiu C, Kluger MD, Medkour F, Douvin C, Mallat A, Zafrani ES, Cherqui D, Duvoux C. Non-invasive assessment of liver graft fibrosis by transient elastography after liver transplantation. *Clin Res Hepatol Gastroenterol* 2013; **37**: 347-352 [PMID: 23318116 DOI: 10.1016/j.clinre.2012.11.003]

74 **Beckebaum S**, Iacob S, Klein CG, Dechêne A, Varghese J, Baba HA, Sotiropoulos GC, Paul A, Gerken G, Cicinnati VR. Assessment of allograft fibrosis by transient elastography and noninvasive biomarker scoring systems in liver transplant patients. *Transplantation* 2010; **89**: 983-993 [PMID: 20335832 DOI: 10.1097/TP.0b013e3181cc66ca]

75 **Rinaldi L**, Valente G, Piai G. Serial Liver Stiffness Measurements and Monitoring of Liver-Transplanted Patients in a Real-Life Clinical Practice. *Hepat Mon* 2016; **16**: e41162 [PMID: 28123442 DOI: 10.5812/hepatmon.41162]

76 **Karlas T**, Kollmeier J, Böhm S, Müller J, Kovacs P, Tröltzsch M, Weimann A, Bartels M, Rosendahl J, Mössner J, Berg T, Keim V, Wiegand J. Noninvasive characterization of graft steatosis after liver transplantation. *Scand J Gastroenterol* 2015; **50**: 224-232 [PMID: 25429378 DOI: 10.3109/00365521.2014.983156]

77 **Chayanupatkul M**, Dasani DB, Sogaard K, Schiano TD. The Utility of Assessing Liver Allograft Fibrosis and Steatosis Post-Liver Transplantation Using Transient Elastography With Controlled Attenuation Parameter. *Transplant Proc* 2020 [PMID: 32434740 DOI: 10.1016/j.transproceed.2020.02.160]

78 **Dubois M**, Ronot M, Houssel-Debry P, Brun V, Rayar M, Auger M, Beuzit L, Turlin B, Aubé C, Paisant A. Performance of B-mode ratio and 2D shear wave elastography for the detection and quantification of hepatic steatosis and fibrosis after liver transplantation. *Eur J Gastroenterol Hepatol* 2020; **32**: 222-230 [PMID: 31464783 DOI: 10.1097/MEG.0000000000001500]

79 **Perry MT**, Savjani N, Bluth EI, Dornelles A, Therapondos G. Point Shear Wave Elastography in Assessment of Hepatic Fibrosis: Diagnostic Accuracy in Subjects With Native and Transplanted Livers Referred for Percutaneous Biopsy. *Ultrasound Q* 2016; **32**: 201-207 [PMID: 26808171 DOI: 10.1097/RUQ.0000000000000219]

80 **van Werven JR**, Marsman HA, Nederveen AJ, Smits NJ, ten Kate FJ, van Gulik TM, Stoker J. Assessment of hepatic steatosis in patients undergoing liver resection: comparison of US, CT, T1-weighted dual-echo MR imaging, and point-resolved 1H MR spectroscopy. *Radiology* 2010; **256**: 159-168 [PMID: 20574093 DOI: 10.1148/radiol.10091790]

81 **Asbach P**, Klatt D, Schlosser B, Biermer M, Muche M, Rieger A, Loddenkemper C, Somasundaram R, Berg T, Hamm B, Braun J, Sack I. Viscoelasticity-based staging of hepatic fibrosis with multifrequency MR elastography. *Radiology* 2010; **257**: 80-86 [PMID: 20679447 DOI: 10.1148/radiol.10092489]

82 **Chen J**, Talwalkar JA, Yin M, Glaser KJ, Sanderson SO, Ehman RL. Early detection of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease by using MR elastography. *Radiology* 2011; **259**: 749-756 [PMID: 21460032 DOI: 10.1148/radiol.11101942]

83 **Navin PJ**, Olson MC, Knudsen JM, Venkatesh SK. Elastography in the evaluation of liver allograft. *Abdom Radiol (NY)* 2020 [PMID: 31950204 DOI: 10.1007/s00261-019-02400-w]

84 **Singh S**, Venkatesh SK, Keaveny A, Adam S, Miller FH, Asbach P, Godfrey EM, Silva AC, Wang Z, Murad MH, Asrani SK, Lomas DJ, Ehman RL. Diagnostic accuracy of magnetic resonance elastography in liver transplant recipients: A pooled analysis. *Ann Hepatol* 2016; **15**: 363-376 [PMID: 27049490 DOI: 10.5604/16652681.1198808]

85 **Kamphues C**, Klatt D, Bova R, Yahyazadeh A, Bahra M, Braun J, Klauschen F, Neuhaus P, Sack I, Asbach P. Viscoelasticity-based magnetic resonance elastography for the assessment of liver fibrosis in hepatitis C patients after liver transplantation. *Rofo* 2012; **184**: 1013-1019 [PMID: 22893489 DOI: 10.1055/s-0032-1313126]

86 **Gitto S**, de Maria N, di Benedetto F, Tarantino G, Serra V, Maroni L, Cescon M, Pinna AD, Schepis F, Andreone P, Villa E. De-novo nonalcoholic steatohepatitis is associated with long-term increased mortality in liver transplant recipients. *Eur J Gastroenterol Hepatol* 2018; **30**: 766-773 [PMID: 29505475 DOI: 10.1097/MEG.0000000000001105]

87 **Mikolasevic I**, Milic S, Turk Wensveen T, Grgic I, Jakopcic I, Stimac D, Wensveen F, Orlic L. Nonalcoholic fatty liver disease - A multisystem disease? *World J Gastroenterol* 2016; **22**: 9488-9505 [PMID: 27920470 DOI: 10.3748/wjg.v22.i43.9488]

88 **Mikolasevic I**, Orlic L, Milic S, Lukenda V, Racki S, Stimac D, Avdovic E, Zaputovic L. Nonalcoholic fatty liver disease (NAFLD) proven by transient elastography in patients with coronary heart disease. *Wien Klin Wochenschr* 2014; **126**: 474-479 [PMID: 24652022 DOI: 10.1007/s00508-014-0538-0]

89 **Mikolasevic I**, Orlic L, Milic S, Zaputovic L, Lukenda V, Racki S. Non-alcoholic fatty liver disease proven by transient elastography in hemodialysis patients: is it a new risk factor for adverse cardiovascular events? *Blood Purif* 2014; **37**: 259-265 [PMID: 24993140 DOI: 10.1159/000360270]

90 **Mikolasevic I**, Racki S, Bubic I, Jelic I, Stimac D, Orlic L. Chronic kidney disease and nonalcoholic Fatty liver disease proven by transient elastography. *Kidney Blood Press Res* 2013; **37**: 305-310 [PMID: 24029696 DOI: 10.1159/000350158]

World Journal of Transplantation

Submit a Manuscript: https://www.f6publishing.com

World J Transplant 2021 March 18; 11(3): 54-69

DOI: 10.5500/wjt.v11.i3.54

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Risk prediction model for cutaneous squamous cell carcinoma in adult cardiac allograft recipients

Nandini Nair, Zhiyong Hu, Dongping Du, Enrique Gongora

ORCID number: Nandini Nair 0000-0002-1243-4389; Zhiyong Hu 0000-0002-5697-6788; Dongping Du 0000-0001-7095-6946; Enrique Gongora 0000-0002-3149-7553.

Author contributions: Nair N, Du D and Hu Z participated in the data acquisition, research design, data analysis, and the writing of the paper; Gongora E contributed to conception of the research idea.

Supported by National Science Foundation, No. CMMI-1728338.

Institutional review board statement: This is to certify that this study was done on a public database with decoded data and no patient identifiable information. The database was provided by the United Network of Organ Sharing. Hence the study is exempted from the Institutional Review Board review.

Informed consent statement: The study used a decoded database provided by the United Network of Organ sharing with no patient identifiers hence there was no requirement for informed consent. This was a retrospective database analysis.

Conflict-of-interest statement: None of the authors have any conflict of interest with respect to

Nandini Nair, Division of Cardiology, Department of Internal Medicine, Texas Tech Health Sciences Center, Lubbock, TX 79430, United States

Zhiyong Hu, Dongping Du, Department of Industrial, Manufacturing and Systems Engineering, Texas Tech University, Lubbock, TX 79409, United States

Enrique Gongora, Department of Cardiothoracic Surgery, University of Alabama at Birmingham, Birmingham, AL 35233, United States

Corresponding author: Nandini Nair, MD, PhD, Professor, Division of Cardiology, Department of Internal Medicine, Texas Tech Health Sciences Center, 3601 4th Street, Lubbock, TX 79430, United States. nandini.nair@ttuhsc.edu

Abstract

BACKGROUND

Heart transplant recipients are at higher risk of developing skin cancer than the general population due to the long-term immunosuppression treatment. Cancer has been reported as one of the major causes of morbidity and mortality for patients after heart transplantation. Among different types of skin cancers, cutaneous squamous cell carcinoma (cSCC) is the most common one, which requires timely screening and better management.

AIM

To identify risk factors and predict the incidence of cSCC for heart transplant recipients.

METHODS

We retrospectively analyzed adult heart transplant recipients between 2000 and 2015 extracted from the United Network for Organ Sharing registry. The whole dataset was randomly divided into a derivation set (80%) and a validation set (20%). Uni- and multivariate Cox regression were done to identify significant risk factors associated with the development of cSCC. Receiver operating characteristics curves were generated and area under the curve (AUC) was calculated to assess the accuracy of the prediction model. Based on the selected risk factors, a risk scoring system was developed to stratify patients into different risk groups. A cumulative cSCC-free survival curve was generated using the Kaplan-Meier method for each group, and the log-rank test was done to compare the inter-group cSCC rates.

this research work.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

Manuscript source: Unsolicited manuscript

Specialty type: Transplantation

Country/Territory of origin: United States

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

Received: September 6, 2020
Peer-review started: September 6, 2020
First decision: December 1, 2020
Revised: December 25, 2020
Accepted: February 19, 2021
Article in press: February 19, 2021
Published online: March 18, 2021

P-Reviewer: Carbone J, Greenway SC
S-Editor: Gao CC
L-Editor: A
P-Editor: Yuan YY

RESULTS

There were 23736 heart-transplant recipients during the study period, and 1827 of them have been reported with cSCC. Significant predictors of post-transplant cSCC were older age, male sex, white race, recipient and donor human leukocyte antigen (HLA) mismatch level, malignancy at listing, diagnosis with restrictive myopathy or hypertrophic myopathy, heart re-transplant, and induction therapy with OKT3 or daclizumab. The multivariate model was used to predict the 5-, 8- and 10-year incidence of cSCC and respectively provided AUC of 0.79, 0.78 and 0.77 in the derivation set and 0.80, 0.78 and 0.77 in the validation set. The risk scoring system assigned each patient with a risk score within the range of 0-11, based on which they were stratified into 4 different risk groups. The predicted and observed 5-year probability of developing cSCC match well among different risk groups. In addition, the log-rank test indicated significantly different cSCC-free survival across different groups.

CONCLUSION

A risk prediction model for cSCC among heart-transplant recipients has been generated for the first time. It offers a c-statistic of ≥ 0.77 in both derivation and validation sets.

Key Words: Cutaneous squamous cell carcinoma; Heart transplantation; Cox proportional hazard model; Risk assessment; Squamous cell carcinoma; Mortality outcomes

Core Tip: We retrospectively analyzed 23736 heart-transplant recipients between 2010 and 2015. Eight risk factors associated with post-transplant cutaneous squamous cell carcinoma were identified, including older age, male sex, lower human leukocyte antigen mismatch level, white race, malignancy at listing, diagnosis with restrictive myopathy or hypertrophic myopathy, heart re-transplant, induction therapy with OKT3 or daclizumab. A multivariate risk prediction model was developed with c-statistics of ≥ 0.77 in both derivation and validation sets. A risk scoring system was designed to stratify patients into 4 risk groups based on their total risk scores. The predicted and observed 5-year probability of developing cutaneous squamous cell carcinoma match well among different risk groups.

Citation: Nair N, Hu Z, Du D, Gongora E. Risk prediction model for cutaneous squamous cell carcinoma in adult cardiac allograft recipients. *World J Transplant* 2021; 11(3): 54-69
URL: https://www.wjgnet.com/2220-3230/full/v11/i3/54.htm
DOI: https://dx.doi.org/10.5500/wjt.v11.i3.54

INTRODUCTION

Skin cancer has been reported as one of the major causes of morbidity and mortality in heart transplantation recipients[1]. The incidence rate of nonmelanoma and melanoma skin cancers, especially cutaneous squamous cell carcinoma (cSCC), is significantly higher in heart transplant recipients than the general population with equivalent age and gender[2].

Multiple studies have been done to investigate the risk of skin cancer in heart transplant recipients[1], and factors including male gender, older age, white race, greater sunlight exposure were commonly identified to be associated with a high risk of post-transplant skin cancer[3-6]. Although risk factors have been characterized, few stratification models have been developed to predict the incidence of skin cancer after transplantation. Accurately stratifying the risk of skin cancer has been a challenge that prevents the development of evidence-based screening recommendations. In addition, most of the existing studies investigated the risk factors of several skin cancers collectively. The risk of cSCC, the most common skin cancer among heart transplant recipients, has not been exclusively assessed for a large patient population.

In this study, we sought to develop a risk prediction model for cSCC after heart

transplantation using a national organ transplant database, *i.e.*, the United Network for Organ Sharing (UNOS). The model aims to stratify patients into different risk groups regarding the development of cSCC post-transplantation and provides a useful tool for pre-transplant counseling and post-transplant surveillance and management.

MATERIALS AND METHODS

Population

The data consisted of 23736 adults (aged ≥ 18 years) heart transplant recipients between 2000 and 2015 were extracted from the UNOS registry of thoracic organ transplantation database. Patients who were listed for and received multi-organ transplantation were excluded from this study. Information on patient characteristics, cancer history, induction therapy, and other risk predictors were extracted for each transplant event, which includes age, sex, race, primary diagnosis, patient's malignancy status at listing and at transplant, patient's emergency status at transplant, donor's cancer history, the recipient and donor human leukocyte antigen (HLA) Mismatch level, recipient's most recent tests before transplant for panel-reactive antibody (PRA) against Class I and Class II antigens, induction with different types of drugs including thymoglobulin, ATGAM, OKT3, daclizumab, basiliximab, and alemtuzumab. cSCC event was determined by the post-transplant follow-up of malignancy status. Time to cSCC development was calculated as days between transplantation and the first reported incidence of cSCC or the last follow-up.

Statistical analysis

The data was randomly divided into a derivation set (80%) and a validation set (20%). All variables were compared between the derivation and validation sets as well as between the cancer and non-cancer groups (Table 1). Continuous variables were reported as mean (standard deviation), and categorical variables were summarized as percentages. Categorical variables and continuous variables were compared using χ^2 test and Wilcoxon rank-sum test, respectively.

Uni- and multivariate Cox regression analyses were done to assess the association of different risk factors with post-transplant cSCC, and p-values, hazard ratios and their confidence intervals were reported. Variables with small P values (< 0.1) in the univariate analysis were selected as inputs to the multivariate analysis. Stepwise forward selection was done to select the final multivariate model. The multivariate model was used to predict the probability of developing cSCC in 5, 8, and 10 years after heart transplantation. The model accuracy was assessed using receiver operating characteristics (ROC) curves and area under curves (AUCs). Based on the hazard ratio, a risk score was assigned to each significant variable (P value < 0.05), and the sum of all scores predicted the risk of a recipient developing cSCC after heart transplantation. The risk scoring system was validated by comparing the predicted and observed probability of developing cSCC 5 years after transplantation across different risk groups. The cumulative cSCC-free survival curves of different risk groups were derived using the Kaplan-Meier method, and the log-rank test was done to quantitatively assess the difference of cSCC risk. All the analysis was performed using MATLB software from MathWorks, Inc.

RESULTS

Patient characteristics

Table 1 provides the summary of all variables between the derivation and validation cohorts as well as between the cancer and non-cancer groups. No significant differences were observed between the derivation and validation groups for all factors. Within the study population, 1827 recipients (7.70%) developed cSCC whereas 21909 recipients (92.30%) were not reported with the event. Patients in the cSCC positive group were older, had a higher percentage of male sex and white race, had a lower level of recipient and donor HLA mismatch level, had a lower level of PRA against Class I and Class II antigens. The cSCC positive group had a higher percentage of patients who had coronary artery disease at listing, and a lower percentage of patients who had congenital heart defect at listing. More patients in the cSCC positive group had malignancy at listing and at transplantation. Patients in the cSCC positive group were less likely to be in status 1A and more likely in status 1B or status 2. In addition,

Table 1 Patient characteristics and predictive variables

	Total (*n* = 23736)	Derivation group (*n* = 18989)	Validation group (*n* = 4747)	*P* value for derivation *vs* validation groups	cSCC positive (*n* = 1827)	cSCC negative (*n* = 21909)	*P* value for cSCC positive *vs* cSCC negative
Age	52.1 (12.6)[1]	52.1 (12.6)[1]	52.3 (12.6)[1]	0.293	59.1 (7.76)[1]	51.6 (12.7)[1]	< 0.001
Female	24.5	24.7	23.7	0.159	9.58	25.8	< 0.001
HLA mismatch level	4.67 (1.02)[1]	4.67 (1.02)[1]	4.68 (1.01)[1]	0.966	4.59 (1.05)[1]	4.68 (1.01)[1]	< 0.001
PRA against Class I antigens	5.36 (16.3)[1]	5.41 (16.4)[1]	5.16 (15.6)[1]	0.960	3.61 (13.2)[1]	5.51 (16.5)[1]	< 0.001
PRA against Class II antigens	3.95 (14.3)[1]	3.98 (14.4)[1]	3.83 (14.0)[1]	0.404	2.85 (12.2)[1]	4.04 (14.5)[1]	0.003
Race							
White	71.4	71.4	71.6	0.716	97.0	69.3	< 0.001
Black	17.6	17.6	17.5	0.906	0.712	19.0	< 0.001
Hispanic	7.26	7.26	7.27	0.989	1.81	7.72	< 0.001
Other	3.70	3.74	3.54	0.514	0.493	3.97	< 0.001
Diagnosis							
Dilated myopathy	82.1	82.1	82.0	0.884	81.5	82.1	0.515
Restrictive myopathy	2.22	2.27	2.00	0.261	2.24	2.21	0.932
Heart re-transplant	2.63	2.58	2.84	0.301	2.68	2.62	0.883
Coronary artery disease	4.47	4.43	4.61	0.582	6.51	4.30	< 0.001
Hypertrophic myopathy	1.92	1.90	2.00	0.636	1.70	1.94	0.475
Valvular heart disease	2.01	2.10	1.69	0.0716	2.35	1.99	0.282
Congenital heart defect	2.46	2.48	2.42	0.835	0.985	2.59	< 0.001
Other	2.23	2.18	2.44	0.272	2.03	2.25	0.532
Donor cancer history							
No	98.1	98.1	98.0	0.566	98	98.1	0.764
Yes	1.60	1.56	1.73	0.422	1.81	1.58	0.457
Unknown	0.282	0.29	0.253	0.669	0.164	0.292	0.322
Malignancy at listing							
No	92.7	92.8	92.5	0.476	90.3	92.9	< 0.001
Yes	5.83	5.80	5.94	0.708	7.72	5.67	< 0.001
Unknown	1.45	1.42	1.58	0.416	1.97	1.41	0.055
Malignancy at transplant							
No	98.1	98.1	97.8	0.15	97.4	98.1	0.039
Yes	0.421	0.416	0.442	0.802	0.712	0.397	0.046
Unknown	1.51	1.45	1.75	0.136	1.86	1.48	0.204
Donor skin cancer history							
No	97.4	97.4	97.2	0.571	97.6	97.3	0.518

Yes	0.139	0.147	0.105	0.486	0.164	0.137	0.764
Unknown	2.50	2.46	2.65	0.454	2.24	2.52	0.462
Patient status at transplant							
Status 1A	46.4	46.6	45.5	0.213	38.2	47.0	< 0.001
Status 1B	37.6	37.4	38.3	0.268	40.6	37.3	0.006
Status 2	16.0	16.0	16.2	0.817	21.2	15.6	< 0.001
Induction with thymoglobulin	14.6	14.7	14.1	0.335	14.4	14.6	0.819
Induction with ATGAM	5.02	5.11	4.66	0.201	5.15	5.01	0.795
Induction with OKT3	2.32	2.29	2.44	0.517	5.42	2.06	< 0.001
Induction with daclizumab	8.30	8.43	7.77	0.142	12.2	7.98	< 0.001
Induction with basiliximab	17.5	17.4	18.0	0.321	12.6	17.9	< 0.001
Induction with alemtuzumab	1.56	1.56	1.81	0.116	1.48	1.57	0.771

[1]Continuous variables are expressed as mean (SD). The rest of the values are categorical variables expressed as percentages. cSCC: Cutaneous squamous cell carcinoma; HLA: Human leukocyte antigen; PRA: Panel-reactive antibody.

recipients with post-transplant cSCC were more likely to be inducted with OKT3 or daclizumab while less likely to be inducted with basiliximab.

Prediction of cSCC

Table 2 gives a summary of the univariate Cox regression analysis, where 10 variables were significant ($P < 0.05$). These variables include age, sex, race, HLA mismatch level, PRA against Class I antigens, PRA against Class II antigens, diagnosis of coronary artery disease or congenital heart disease, patient's malignancy status at listing, and at transplant, and OKT3. The final multivariate model had 8 variables (Table 3), including age, sex, HLA mismatch level, race, malignancy at listing, diagnosis at listing, and induction with OKT3 or daclizumab. ROC curves for the 5-year, 8-year and 10-year post-transplant cSCC prediction provided AUCs of 0.79, 0.78, 0.77 respectively in the derivation set and 0.80, 0.78, 0.77 respectively in the validation set (Figure 1).

Risk stratification

Table 4 provides the risk scores derived based on the multivariate model to predict the risk of developing cSCC 5 years after heart transplantation. The scoring system can classify patients into 4 risk groups: very low-risk group (score ≤ 5, n = 12383), low-risk group (score = 6, n = 6162), medium-risk group (score = 7, n = 4371), high-risk group (score ≥ 8, n = 820). Figure 2 shows the predicted and observed probabilities of developing cSCC 5 years after heart transplantation, which match well across different riskgroups. Patients in the high-risk group (score ≥ 8) had a higher probability (11-fold higher) of developing cSCC after transplant than patients in the very low-risk group (score ≤ 5).

Figure 3 shows the Kaplan Meier estimator of the cSCC-free survival curve and risk table for each risk group. It shows that the probability of developing cSCC in the very low-risk group is significantly lower than that of the high-risk group, and about 20% of the subjects in the high-risk group developed cSCC 5 years after transplantation. In addition, log-rank test was performed to test the null hypothesis that there was no difference regarding the occurrence probability of cSCC among the four groups. The results in Table 5 show that the risk of developing cSCC in high-risk group is greater than that in the low and medium-risk groups. Significant differences (P value < 0.001) were observed between every two groups. The cSCC risk in the high-risk group is respectively 9.16-fold, 2.18-fold, and 1.28-fold higher than that of the very low-risk, low-risk, and medium-risk group; the risk of the medium-risk group is respectively 7.12-fold and 1.69-fold higher than that of the very low-risk and low-risk group, and the risk of the low-risk group is 4.19-fold higher than that of the very low-risk group.

Table 2 Univariate analysis of predictive variables associated with incidence probability of post-transplant cutaneous squamous cell carcinoma

Covariates	Hazard ratio (95%CI)	*P* value
Age	1.08 (1.07-1.09)	< 0.001
Female	0.310 (0.260-0.370)	< 0.001
HLA mismatch level	0.914 (0.870-0.960)	< 0.001
PRA against Class I antigens	0.994 (0.990-0.998)	0.006
PRA against Class II antigens	0.994 (0.989-0.999)	0.012
Race		
White	1	-
Black	0.0390 (0.0221-0.068)	<0.001
Hispanic	0.178 (0.120-0.265)	<0.001
Other	0.108 (0.0512-0.226)	<0.001
Diagnosis		
Dilated myopathy	1	-
Restrictive myopathy	1.38 (0.985-1.93)	0.061
Heart re-transplant	1.12 (0.807-1.55)	0.500
Coronary artery disease	1.49 (1.22-1.82)	< 0.001
Hypertrophic myopathy	0.923 (0.630-1.35)	0.681
Valvular heart disease	1.16 (0.842-1.59)	0.368
Congenital heart defect	0.393 (0.232-0.666)	0.001
Other	1.01 (0.695-1.47)	0.951
Donor cancer history		
No	1	-
Yes	1.28 (0.883-1.84)	0.195
Unknown	0.997 (0.321-3.10)	0.997
Malignancy at listing		
No	1	-
Yes	1.72 (1.43-2.09)	< 0.001
Unknown	0.983 (0.667-1.45)	0.930
Malignancy at transplant		
No	1	0
Yes	2.55 (1.48-4.41)	0.001
Unknown	0.791 (0.528-1.18)	0.255
Donor skin cancer history		
No	1	-
Yes	1.06 (0.265-4.24)	0.935
Unknown	0.631 (0.439-0.906)	0.013
Patient status at transplant		
Status 1A	1	-
Status 1B	1.07 (0.950-1.20)	0.274
Status 2	0.983 (0.854-1.13)	0.805
Induction with thymoglobulin	1.05 (0.911-1.22)	0.481

Induction with ATGAM	0.980 (0.784-1.22)	0.857
Induction with OKT3	1.59 (1.27-2.01)	< 0.001
Induction with daclizumab	1.16 (0.995-1.36)	0.057
Induction with basiliximab	1.08 (0.927-1.26)	0.322
Induction with alemtuzumab	1.18 (0.773-1.80)	0.444

HLA: Human leukocyte antigen; PRA: Panel-reactive antibody; CI: Confidence interval.

Table 3 Risk factors selected from multivariate analysis

Covariates	Hazard ratio (95%CI)	*P* value
Age	1.068 (1.062-1.075)	< 0.001
Female	0.412 (0.344-0.494)	< 0.001
HLA mismatch level	0.951 (0.905-0.999)	0.043
Race		
White	1	-
Black	0.124 (0.059-0.261)	< 0.001
Hispanic	0.058 (0.033-0.102)	< 0.001
Other	0.229 (0.154-0.340)	< 0.001
Diagnosis		
Dilated myopathy	1	-
Restrictive myopathy	1.869 (1.333-2.619)	< 0.001
Heart re-transplant	1.711 (1.231-2.378)	0.001
Coronary artery disease	1.144 (0.935-1.400)	0.192
Hypertrophic myopathy	1.596 (1.087-2.345)	0.017
Valvular heart disease	1.159 (0.842-1.596)	0.364
Congenital heart defect	1.106 (0.649-1.886)	0.710
Other	1.381 (0.9477-2.012)	0.093
Malignancy at listing		
No	1	-
Yes	1.593 (1.315-1.930)	< 0.001
Unknown	0.982 (0.666-1.448)	0.926
Induction with OKT3	1.380 (1.095-1.739)	0.006
Induction with daclizumab	1.371 (1.173-1.603)	< 0.001

HLA: Human leukocyte antigen; CI: Confidence interval.

Mortality outcomes

Most of the registry data including UNOS database showed that heart-transplant recipients with skin cancer revealed significantly lower overall survival than the recipients with no skin cancer. To demonstrate the consistency of our dataset, the survival experience of these two groups of patients were compared using landmark analysis[7]. Median time from the date of transplantation to cSCC was taken as the landmark time point. Kaplan Meier survival curves of the two groups were displayed in Figure 4. The log-rank test demonstrates a significant difference between the two groups and the mortality risk of the group with skin cancer is 1.51-fold greater than its counterpart.

Table 4 Risk score for the 5-yr development of cutaneous squamous cell carcinoma after transplantation

Covariates	Category	Score
Age	18-40	0
	40-60	1
	> 60	2
Sex	Female	0
	Male	2
HLA mismatch level	> 5	0
	≤ 5	1
Race	White	2
	Other	0
Diagnosis	Restrictive myopathy	1
	Heart re-transplant	1
	Hypertrophic myopathy	1
	Other	0
Malignancy at listing	No	0
	Yes	1
	Unknown	0
Induction with OKT3	No	0
	Yes	1
Induction with daclizumab	No	0
	Yes	1

HLA: Human leukocyte antigen.

Table 5 Log-rank test to compare the cumulative incidence of post-transplant cutaneous squamous cell carcinoma between risk groups

Group	*P* value	Hazard ratio (95%CI)
Low *vs* very low	< 0.001	4.19 (3.66-4.78)
Medium *vs* very low	< 0.001	7.12 (6.18-8.21)
Medium *vs* low	< 0.001	1.69 (1.52-1.88)
High *vs* very low	< 0.001	9.16 (6.23-13.5)
High *vs* low	< 0.001	2.18 (1.74-2.72)
High *vs* medium	0.004	1.28 (1.07-1.54)

CI: Confidence interval.

Prediction of cSCC without OKT3 and daclizumab

Since induction drugs of OKT3 and daclizumab are not used currently, additional analysis without these two drugs was conducted. The analysis followed the same procedure as described in the Statistical Analysis section. The multivariate model excluding OKT3 and daclizumab was given in Table 6, which had six variables, including age, sex, HLA mismatch level, race, diagnosis at listing, and malignancy at listing. None of the rest of the induction drugs were significant and selected in the multivariate model. The AUCs for 5-year, 8-year, and 10-year post-transplant cSCC prediction were 0.79, 0.77, 0.77 respectively in the derivation set and 0.79, 0.76, 0.75 respectively in the validation set (Figure 5). Eliminating OKT3 and daclizumab slightly

Table 6 Risk factors selected from multivariate analysis without OKT3 and daclizumab

Covariates	Hazard ratio (95%CI)	*P* value
Age	1.068 (1.062-1.075)	< 0.001
Female	0.412 (0.344-0.494)	< 0.001
HLA mismatch level	0.948 (0.903-0.996)	0.034
Race		
White	1	-
Black	0.126 (0.060-0.265)	< 0.001
Hispanic	0.058 (0.033-0.102)	< 0.001
Other	0.228 (0.154-0.339)	< 0.001
Diagnosis		
Dilated myopathy	1	-
Restrictive myopathy	1.897 (1.354-2.658)	< 0.001
Heart re-transplant	1.703 (1.226-2.366)	0.002
Coronary artery disease	1.135 (0.927-1.389)	0.219
Hypertrophic myopathy	1.589 (1.082-2.334)	0.018
Valvular heart disease	1.156 (0.840-1.592)	0.373
Congenital heart defect	1.098 (0.645-1.872)	0.730
Other	1.329 (0.913-1.935)	0.138
Malignancy at listing		
No	1	-
Yes	1.589 (1.312-1.925)	< 0.001
Unknown	0.983 (0.666-1.449)	0.930

HLA: Human leukocyte antigen; CI: Confidence interval.

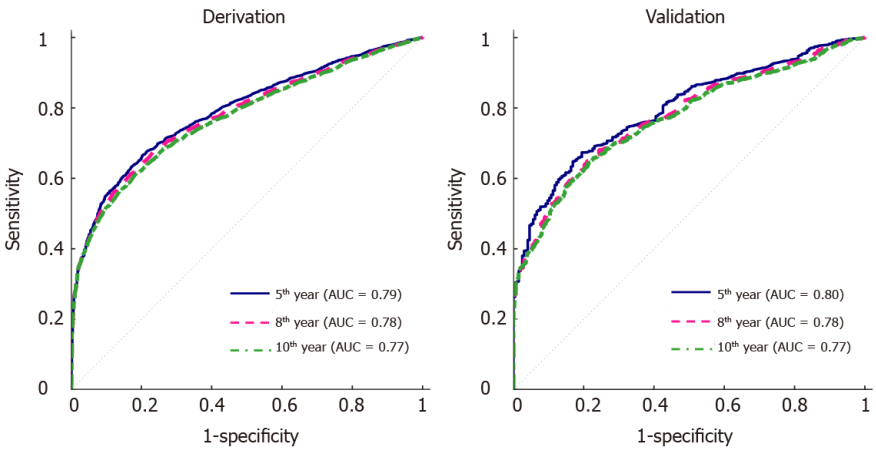


Figure 1 Receiver operating characteristics curves of the multivariate model for the 5-yr, 8-yr and 10-yr post-transplant cutaneous squamous cell carcinoma prediction. A: The derivation set; B: The validation set. AUC: Area under the curve.

affected the AUCs (decreased by 0.01-0.02) in the validation set compared to the model with OKT3 and daclizumab. In addition, a new risk stratification model without OKT3 and daclizumab was developed, and the risk scores were given in Table 7. The scoring system without OKT3 and daclizumab divided patients into 4 risk groups: very low-risk group (score ≤ 5), low-risk group (score = 6), medium-risk group (score = 7), high-risk group (score ≥ 8). The predicted and observed probabilities of developing cSCC 5

Table 7 Risk score without OKT3 and daclizumab for the 5-yr development of cutaneous squamous cell carcinoma after transplantation

Covariates	Category	Score
Age	18-40	0
	40-60	1
	> 60	2
Sex	Female	0
	Male	2
HLA mismatch level	> 5	0
	≤ 5	1
Race	White	2
	Other	0
Diagnosis	Restrictive myopathy	1
	Heart re-transplant	1
	Hypertrophic myopathy	1
	Other	0
Malignancy at listing	No	0
	Yes	1
	Unknown	0

HLA: Human leukocyte antigen.

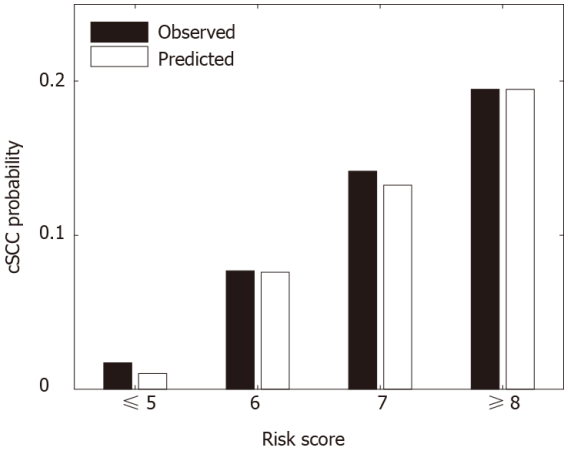


Figure 2 Predicted *vs* observed probabilities of developing cSCC 5 yr after transplant in different risk groups: very low-risk group (score ≤ 5), low-risk group (score = 6), medium-risk group (score = 7), high-risk group (score ≥ 8). cSCC: Cutaneous squamous cell carcinoma.

years after transplant in different risk groups were shown in Figure 6, and the Kaplan Meier estimator of the cSCC-free survival curve was given in Figure 7. Further, log-rank test was done to compare the risk between different groups where patients were divided using the new scoring system, and significant differences were observed between every two groups (Table 8). The new stratification model without induction drugs provided comparable results to the model with OKT3 and daclizumab.

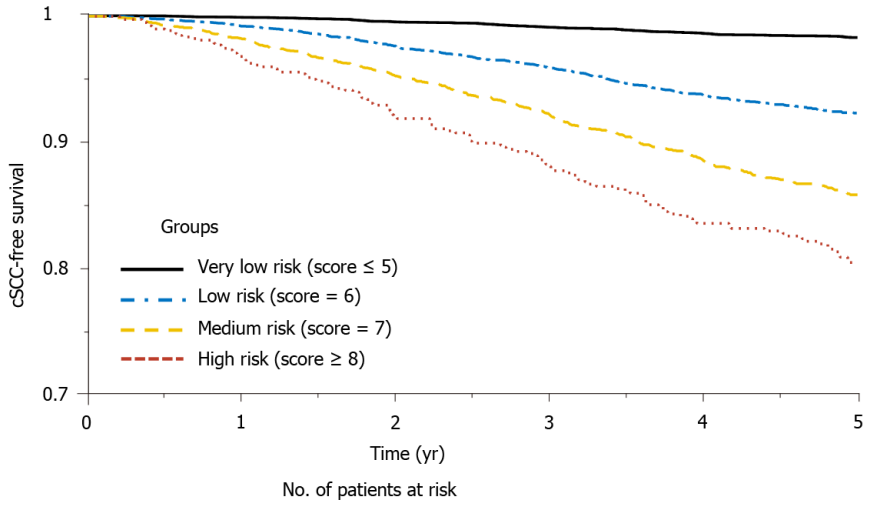
DISCUSSION

cSCC is a predominant skin malignancy among heart transplant recipients. Studies have been done to investigate the risk factors of post-transplant cSCC, but risk stratification and prediction have not been examined in the literature. This study

Table 8 Log-rank test to compare the cumulative incidence of post-transplant cutaneous squamous cell carcinoma between different risk groups where patients were divided using the scoring system without OKT3 and daclizumab

Group	*P* value	Hazard ratio (95%CI)
Low *vs* very low	< 0.001	3.97 (3.51-4.50)
Medium *vs* very low	< 0.001	6.80 (5.86-7.90)
Medium *vs* low	< 0.001	1.70 (1.52-1.90)
High *vs* very low	< 0.001	10.1 (5.41-18.8)
High *vs* low	< 0.001	2.48 (1.78-3.47)
High *vs* medium	0.003	1.41 (1.09-1.83)

CI: Confidence interval.



No. of patients at risk

Very Low	12383	11336	9634	8195	6920	5736
Low	6162	5690	4949	4276	3668	3114
Medium	4371	3955	3348	2872	2405	2005
High	820	730	605	517	436	374

Figure 3 Cumulative cSCC-free survival curves for different risk groups. cSCC: Cutaneous squamous cell carcinoma.

conducted a retrospective study of the post-transplant event of cSCC for a large cohort of heart transplant patients in the UNOS registry and developed a risk score model to stratify patients into different risk groups.

In the univariate analysis, PRA against Class I and Class II antigens were identified as significant factors, but they were not significant in the multivariable analysis. Coronary artery disease was a risk factor in univariate analysis but was not selected in the multivariate model. The univariate analysis also identified congenital heart defect as a protective factor, but the observation did not hold up in multivariate analysis. The possible reason is that these two diseases are strongly correlated with patient age, thus the inclusion of age in the multivariate model eliminated the influence of these two diseases.

Eight predictors, including age, gender, HLA mismatch level, race, patient's malignancy at listing, patient's diagnosis at listing, induction therapy with OKT3 or daclizumab were selected in the final multivariate model. Among these predictors, older age, male sex, and white race have been previously reported as significant risk factors in many studies[3,8,9]. In addition, the multivariate model included the HLA mismatch level as a protective factor for cSCC, which is consistent with the observation in a recent study on the relationship between the HLA antigen mismatch level and the skin cancer incidence after heart and lung transplantation[10]. Heart re-transplant was identified as a significant risk factor as compared to dilated myopathy,

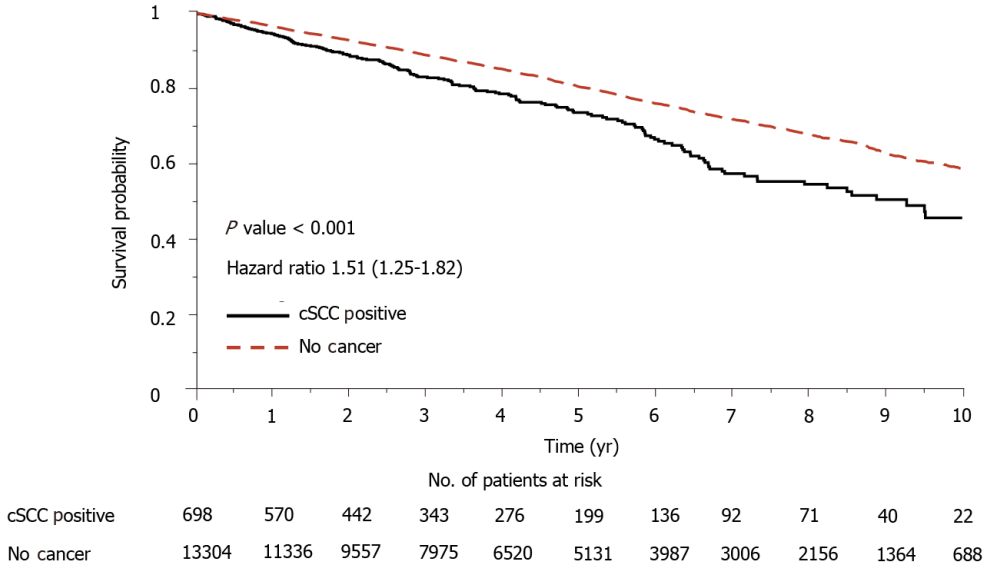


Figure 4 Cumulative survival curves for heart transplant recipients with cSCC and with no cancer. cSCC: Cutaneous squamous cell carcinoma.

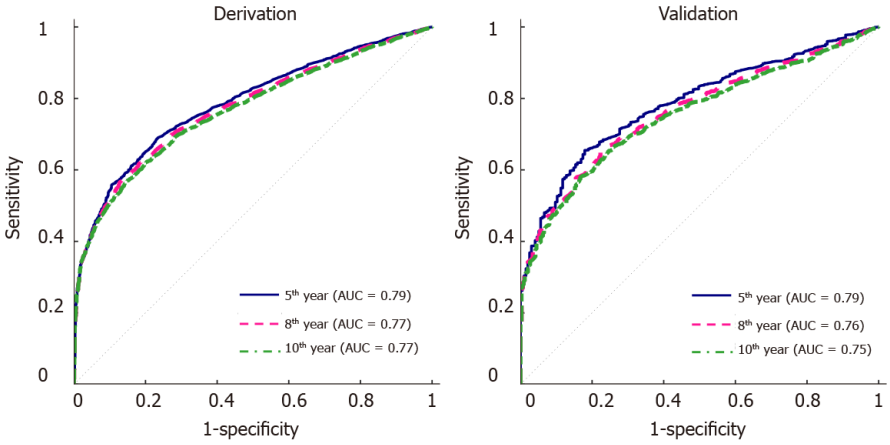


Figure 5 Receiver operating characteristics curves of the multivariate model without OKT3 and daclizumab for the 5-yr, 8-yr and 10-yr post-transplant cutaneous squamous cell carcinoma prediction. A: The derivation set; B: The validation set. AUC: Area under the curve.

which matches with a previous report that suggested re-transplant was a risk factor *vs* cardiomyopathy[11]. The multivariate model also showed that patients diagnosed with restrictive myopathy or hypertrophic myopathy before transplant had a higher risk of developing cSCC than patients who had other types of conditions. Recipients' malignancy status is an indication of patients' cancer history, which has been reported as a risk factor for skin cancer development in various studies[12,13], and was also identified as a risk factor for heart-transplant recipients in this study. In addition, the multivariate analysis revealed that induction therapy with OKT3 resulted in an increased incidence of cSCC, which is consistent with the observation reported in a previous study on a small cohort of heart transplant patients[3]. Our analysis also found that induction with daclizumab significantly (P value < 0.001) increased the risk of post-transplant cSCC.

The risk score separated patients into four risk groups (Figure 2), and the observed and predicted probabilities of developing cSCC 5 years after transplantation in very low-risk, low-risk, medium-risk, and high-risk groups were 0.017 *vs* 0.010, 0.077 *vs* 0.076, 0.142 *vs* 0.133 and 0.195 *vs* 0.195, respectively. The cumulative incidence probability of post-transplant cSCC was compared between different risk groups (Figure 3). For the high-risk group, the cumulative incidence rate increased significantly with respect to time. The one-, three-, and five-year incidence probabilities in the high-risk group were 0.03, 0.12, and 0.19, respectively. The significant differences in the cumulative incidence rates among different risk groups

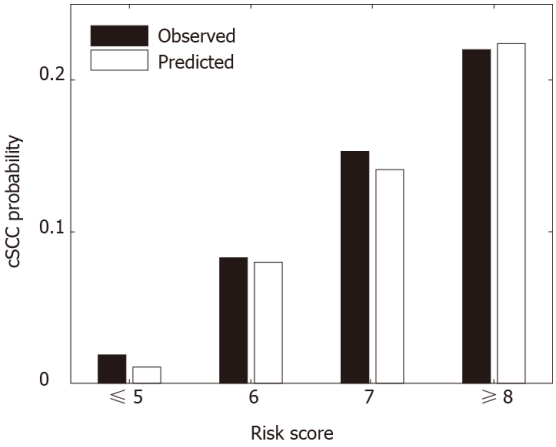
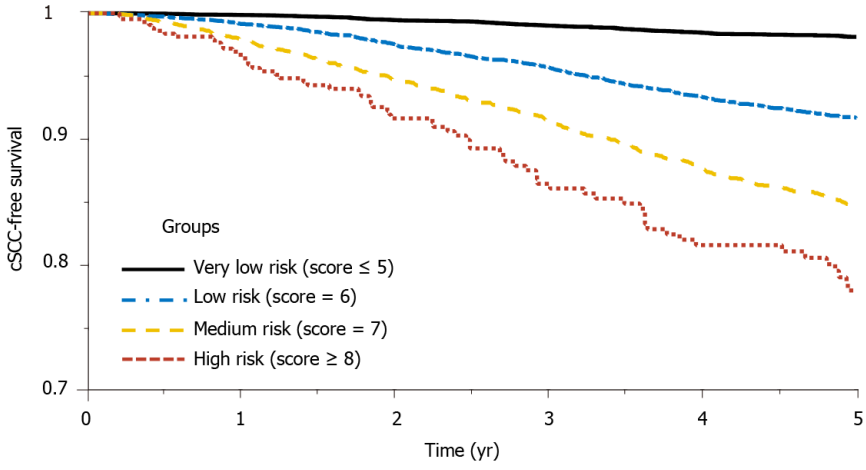


Figure 6 Predicted *vs* observed probabilities of developing cSCC 5 yr after transplant in different risk groups where patients were divided using the scoring system without OKT3 and daclizumab: very low-risk group (score ≤ 5), low-risk group (score = 6), medium-risk group (score = 7), high-risk group (score ≥ 8). cSCC: Cutaneous squamous cell carcinoma.



No. of patients at risk

Very Low	12802	11727	10000	8544	7246	6045
Low	6475	5990	5228	4537	3906	3322
Medium	4018	3609	3009	2545	2096	1715
High	441	385	299	234	181	147

Figure 7 Cumulative cSCC-free survival curves for different risk groups where patients were divided using the scoring system without OKT3 and daclizumab. cSCC: Cutaneous squamous cell carcinoma.

show the effectiveness of the proposed risk stratification model. Furthermore, cSCC greatly increased the mortality after heart transplantation with a hazard ratio of 1.51 (P value < 0.001) (Figure 4), which shows the importance of early screening and identification of cSCC among heart-transplant recipients.

Limits of the study

The study has limitations which are discussed here. Firstly, this is a retrospective study using a single data source for the derivation and the validation cohorts. Missing data and poor data quality are generally recognized as drawbacks of retrospective studies. Thus, the results will need to be replicated in a separate patient population and ideally prospectively. Secondly, sunshine exposure has been identified as a risk factor for skin cancer but was not included in the current study. Ultraviolet exposure information such as latitude, average daily total global solar radiation, or patients' reports of previous sun exposure was used in many studies to assess the risk of ultraviolet exposure on skin cancer. However, it was previously reported that such information was not reliable biomarkers of ultraviolet radiation[9], and these data were

not reported in the UNOS database.

In addition, the UNOS database contains missing and inaccurate reporting. Some posttransplant malignancy forms submitted to the Organ Procurement Transplant Network registry have been reported to be incomplete[9,14]. To minimize the possible bias due to incomplete reports, our analysis only used patient records with a clear indication of post-transplant malignancy status. That is, the records with unknown post-transplant malignancy status were excluded for the analysis.

CONCLUSION

In conclusion, this study developed a risk prediction model for post-transplant cSCC using a group of basic demographic and clinical parameters that can be estimated in every local center. The model provides a simple tool to aid clinical judgment for pre-transplant counseling and post-transplant health management. Identification of high-risk patients can facilitate the diagnosis of skin cancer in an early stage and potentially reduce morbidity and mortality after heart transplantation.

ARTICLE HIGHLIGHTS

Research background

Heart transplant recipients are at higher risk of developing skin cancer than the general population due to the long-term immunosuppression treatment. Cancer has been reported as one of the major causes of morbidity and mortality for patients after heart transplantation.

Research motivation

Cutaneous squamous cell carcinoma (cSCC) is reported as the most common skin cancer in adult heart transplant recipients. This study was initiated to develop a risk stratification model using the United Network for Organ Sharing database in order to identify important risk factors and predict post-transplant incidence of cSCC. Among the different types of skin cancers, cSCC is the most common type of cancer. Timely screening and better management would help in prevention of long-term complications.

Research objectives

To identify risk factors and predict the incidence of cSCC for heart transplant recipients. Develop a risk prediction model for cSCC.

Research methods

The whole dataset was randomly divided into a derivation set (80%) and a validation set (20%). Uni- and multivariate Cox regression were done to identify significant risk factors associated with the development of cSCC. Receiver operating characteristics curves were generated and area under the curve (AUC) was calculated to assess the accuracy of the prediction model.

Research results

Of the 23736 heart-transplant recipients in the database during the study period, 1827 were reported to have cSCC. Significant predictors of post-transplant cSCC were older age, male sex, white race, recipient and donor human leukocyte antigen mismatch level, malignancy at listing, a diagnosis of restrictive myopathy or hypertrophic myopathy, re-transplantation of the heart, and induction therapy with OKT3 or daclizumab. The multivariate model was used to predict the 5-, 8- and 10-year incidence of cSCC and respectively provided AUC of 0.79, 0.78, and 0.77 in the derivation set and 0.80, 0.78, and 0.77 in the validation set. The risk scoring system assigned each patient with a risk score within the range of 0-11. Based on the scores they were stratified into 4 different risk groups. The predicted and observed 5-year probability of developing cSCC match well among different risk groups. In addition, the log-rank test indicated significantly different cSCC-free survival across different groups.

Research conclusions

A risk prediction model for cSCC among heart-transplant recipients has been generated for the first time. It offers a c-statistic of ≥ 0.77 in both derivation and validation sets.

Research perspectives

Using a risk prediction score for screening of adult cardiac allograft recipients for early detection of cSCC can become a reality. The risk prediction score can be further validated in independent data sets in the future. Identification of risk factors is an important step towards the prevention of cSCC in this population.

REFERENCES

1 **Jäämaa-Holmberg S**, Salmela B, Lemström K, Pukkala E, Lommi J. Cancer incidence and mortality after heart transplantation - A population-based national cohort study. *Acta Oncol* 2019; **58**: 859-863 [PMID: 30821595 DOI: 10.1080/0284186X.2019.1580385]

2 **Alam M**, Brown RN, Silber DH, Mullen GM, Feldman DS, Oren RM, Yancy CW; Cardiac Transplant Research Database Group. Increased incidence and mortality associated with skin cancers after cardiac transplant. *Am J Transplant* 2011; **11**: 1488-1497 [PMID: 21718441 DOI: 10.1111/j.1600-6143.2011.03598.x]

3 **Molina BD**, Leiro MG, Pulpón LA, Mirabet S, Yañez JF, Bonet LA, Vilchez FG, Delgado JF, Manito N, Rábago G, Arizón JM, Romero N, Roig E, Blasco T, Pascual D, de la Fuente L, Muñiz J. Incidence and risk factors for nonmelanoma skin cancer after heart transplantation. *Transplant Proc* 2010; **42**: 3001-3005 [PMID: 20970593 DOI: 10.1016/j.transproceed.2010.08.003]

4 **Brewer JD**, Colegio OR, Phillips PK, Roenigk RK, Jacobs MA, Van de Beek D, Dierkhising RA, Kremers WK, McGregor CG, Otley CC. Incidence of and risk factors for skin cancer after heart transplant. *Arch Dermatol* 2009; **145**: 1391-1396 [PMID: 20026847 DOI: 10.1001/archdermatol.2009.276]

5 **Caforio AL**, Fortina AB, Piaserico S, Alaibac M, Tona F, Feltrin G, Pompei E, Testolin L, Gambino A, Volta SD, Thiene G, Casarotto D, Peserico A. Skin cancer in heart transplant recipients: risk factor analysis and relevance of immunosuppressive therapy. *Circulation* 2000; **102**: III222-III227 [PMID: 11082391 DOI: 10.1161/01.cir.102.suppl_3.iii-222]

6 **España A**, Redondo P, Fernández AL, Zabala M, Herreros J, Llorens R, Quintanilla E. Skin cancer in heart transplant recipients. *J Am Acad Dermatol* 1995; **32**: 458-465 [PMID: 7868716 DOI: 10.1016/0190-9622(95)90069-1]

7 **Anderson JR**, Cain KC, Gelber RD. Analysis of survival by tumor response. *J Clin Oncol* 1983; **1**: 710-719 [PMID: 6668489 DOI: 10.1200/JCO.1983.1.11.710]

8 **Alam M**, Silber D, Mullen M, Brown R, Yancy C, Feldman D, Oren R, Kirklin J. Cutaneous malignancies after cardiac transplantation-risk factors and effect on late survival. *J Heart Lung Transplant* 2005; **24**: S46 [DOI: 10.1016/j.healun.2004.11.032]

9 **Garrett GL**, Blanc PD, Boscardin J, Lloyd AA, Ahmed RL, Anthony T, Bibee K, Breithaupt A, Cannon J, Chen A, Cheng JY, Chiesa-Fuxench Z, Colegio OR, Curiel-Lewandrowski C, Del Guzzo CA, Disse M, Dowd M, Eilers R Jr, Ortiz AE, Morris C, Golden SK, Graves MS, Griffin JR, Hopkins RS, Huang CC, Bae GH, Jambusaria A, Jennings TA, Jiang SI, Karia PS, Khetarpal S, Kim C, Klintmalm G, Konicke K, Koyfman SA, Lam C, Lee P, Leitenberger JJ, Loh T, Lowenstein S, Madankumar R, Moreau JF, Nijhawan RI, Ochoa S, Olasz EB, Otchere E, Otley C, Oulton J, Patel PH, Patel VA, Prabhu AV, Pugliano-Mauro M, Schmults CD, Schram S, Shih AF, Shin T, Soon S, Soriano T, Srivastava D, Stein JA, Sternhell-Blackwell K, Taylor S, Vidimos A, Wu P, Zajdel N, Zelac D, Arron ST. Incidence of and Risk Factors for Skin Cancer in Organ Transplant Recipients in the United States. *JAMA Dermatol* 2017; **153**: 296-303 [PMID: 28097368 DOI: 10.1001/jamadermatol.2016.4920]

10 **Gao Y**, Twigg AR, Hirose R, Roll GR, Nowacki AS, Maytin EV, Vidimos AT, Rajalingam R, Arron ST. Association of HLA Antigen Mismatch With Risk of Developing Skin Cancer After Solid-Organ Transplant. *JAMA Dermatol* 2019; **155**: 307-314 [PMID: 30673077 DOI: 10.1001/jamadermatol.2018.4983]

11 **Youn JC**, Stehlik J, Wilk AR, Cherikh W, Kim IC, Park GH, Lund LH, Eisen HJ, Kim DY, Lee SK, Choi SW, Han S, Ryu KH, Kang SM, Kobashigawa JA. Temporal Trends of De Novo Malignancy Development After Heart Transplantation. *J Am Coll Cardiol* 2018; **71**: 40-49 [PMID: 29301626 DOI: 10.1016/j.jacc.2017.10.077]

12 **Kransdorf E**, Patel J, Kittleson M, Czer L, Chang D, Dimbil S, Levine R, Hsu A, Davis T, Norland K, Trento A, Kobashigawa JA. Does a History of Malignancy Prior to Heart-transplant Increase Post-transplant Risk? *J Heart Lung Transplant* 2018; **37**: S421 [DOI: 10.1016/j.healun.2018.01.1091]

13 **Jambusaria-Pahlajani A**, Crow LD, Lowenstein S, Garrett GL, Melcher ML, Chan AW, Boscardin J, Arron ST. Predicting skin cancer in organ transplant recipients: development of the SUNTRAC screening tool using data from a multicenter cohort study. *Transpl Int* 2019; **32**: 1259-1267 [PMID: 31423648 DOI: 10.1111/tri.13493]

14 **Garrett GL**, Yuan JT, Shin TM, Arron ST; Transplant Skin Cancer Network (TSCN). Validity of skin cancer malignancy reporting to the Organ Procurement Transplant Network: A cohort study. *J Am Acad Dermatol* 2018; **78**: 264-269 [PMID: 29031659 DOI: 10.1016/j.jaad.2017.09.003]

World Journal of Transplantation

Submit a Manuscript: https://www.f6publishing.com

World J Transplant 2021 March 18; 11(3): 70-87

DOI: 10.5500/wjt.v11.i3.70

ISSN 2220-3230 (online)

META-ANALYSIS

Belatacept in renal transplantation in comparison to tacrolimus and molecular understanding of resistance pattern: Meta-analysis and systematic review

Jayant Kumar, Isabella Reccia, Francesco Virdis, Mauro Podda, Ajay Kumar Sharma, Ahmed Halawa

ORCID number: Jayant Kumar 0000-0003-0490-7323; Isabella Reccia 0000-0003-1194-8828; Francesco Virdis 0000-0003-1194-8822; Mauro Podda 0000-0001-9941-0883; Ajay Kumar Sharma 0000-0003-4050-6586; Ahmed Halawa 0000-0002-7305-446X.

Author contributions: Halawa A and Sharma AK designed the idea of study; Reccia I and Kumar J contributed to literature review and data collection; Kumar J, Reccia I, Podda M, Halawa A, and Sharma AK contributed to manuscript writing and critical revision.

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.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Open-Access: This article is an open-access article that was selected by an in-house editor and

Jayant Kumar, Isabella Reccia, Department of Cancer and Surgery, Imperial College, London W12 0HS, United Kingdom

Francesco Virdis, Department of Emergency General Surgery, Royal Free Hospital, London NW3 2QG, United Kingdom

Mauro Podda, Department of Surgery, General, Emergency and Robotic Surgical Unit, San Francesco Hospital, Nuoro 08100, Italy

Ajay Kumar Sharma, Department of Transplantation, Royal Liverpool University Hospital, Liverpool L7 8XP, United Kingdom

Ahmed Halawa, Department of Surgery, Sheffield Teaching Hospitals, Sheffield S10 2JF, United Kingdom

Corresponding author: Jayant Kumar, MD, MSc, PhD, Academic Fellow, Attending Doctor, Senior Research Fellow, Department of Cancer and Surgery, Imperial College, DuCane, London W12 0HS, United Kingdom. jkumar@ic.ac.uk

Abstract

BACKGROUND

The T-cell costimulation blocking agent belatacept has been identified as a possible substitute for calcineurin inhibitors, however, no consensus has been established against its use over the standard care agent Tacrolimus.

AIM

To evaluate the effectiveness of belatacept based maintenance immuno-suppressive regimens in comparison to tacrolimus in renal transplantion.

METHODS

We did extensive search of all the available literature comparing the role of belatacept to tacrolimus in renal transplant recipients by searching the PubMed, Embase, Cochrane, Crossref, Scopus, clinical trials registry on October 5, 2020.

RESULTS

The literature search identified four randomized controlled trials (n = 173 participants) comparing belatacept with tacrolimus. There was no significant difference in estimated renal function at 12 mo [mean difference 4.12

Manuscript source: Invited manuscript

Specialty type: Transplantation

Country/Territory of origin: United Kingdom

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

Received: November 23, 2020
Peer-review started: November 23, 2020
First decision: December 21, 2020
Revised: December 23, 2020
Accepted: February 12, 2021
Article in press: February 12, 2021
Published online: March 18, 2021

P-Reviewer: Cantarovich F, Kute VB
S-Editor: Zhang L
L-Editor: A
P-Editor: Yuan YY

mL/min/1.73 m^2, confidence interval (CI): -2.18 to 10.42, P = 0.20]. Further, belatacept group was associated with significant increase in biopsy proven acute rejection [relative risk (RR) = 3.27, CI: 0.88 to 12.11, P = 0.08] and worse 12 mo allograft survival (RR = 4.51, CI: 1.23 to 16.58, P = 0.02). However, incidence of new onset diabetes mellitus was lower with belatacept at 12 mo (RR = 0.26, CI: 0.07 to 0.99, P = 0.05).

CONCLUSION
The evidence reviewed in this meta-analysis suggested that belatacept-based maintenance immunosuppression regimens were associated with an increased risk allograft loss in renal transplant recipients with equivalent renal functioning against standard tacrolimus; however, observed significantly reduced new onset diabetes mellitus after transplantation incidence and lower serum low density lipid profile levels in belatacept group. In addition, the adaptation of belatacept in renal transplantation has been forestalled by increased rates of rejection and resistance owing to development of various effector memory T cells through, parallel differentiation and immunological plasticity.

Key Words: Adverse events; Calcineurin inhibitors; Belatacept; Tacrolimus; Graft failure; Kidney transplantation

Core Tip: This meta-analysis suggested that belatacept-based maintenance immunosuppression regimens were associated with an increased risk allograft loss in renal transplant recipients with equivalent renal functioning against standard tacrolimus.

Citation: Kumar J, Reccia I, Virdis F, Podda M, Sharma AK, Halawa A. Belatacept in renal transplantation in comparison to tacrolimus and molecular understanding of resistance pattern: Meta-analysis and systematic review. *World J Transplant* 2021; 11(3): 70-87
URL: https://www.wjgnet.com/2220-3230/full/v11/i3/70.htm
DOI: https://dx.doi.org/10.5500/wjt.v11.i3.70

INTRODUCTION

The success immunosuppression in kidney transplantation has added a significant number of productive years to the life of chronic kidney disease patients[1]. The calcineurin inhibitors (CNIs), cyclosporine A and tacrolimus (Tac) were introduced in clinical practice in 1980's and form the cornerstone of immunosuppressive therapy in renal transplant recipients. Globally most of the kidney transplant recipients have been initially get treated with a calcineurin inhibitor (usually tacrolimus), an antimetabolite (preferentially mycophenolate), and steroids plus in many instances require an additional agent of induction as basiliximab or thymoglobulin. Various studies including randomized controlled trials (RCT) and meta-analysis reported that these immunosuppressive regimens have been associated with more than 90% one-year graft survival whilst extending a rejection rate of below 15%-20%[2-4].

However, the superlative results of short-term allograft survival have not been maintained for long owing to renal and non-renal toxicities of these drugs which produce slow, steady decline in renal functioning[5]. The non-renal toxicities as cardiovascular adverse events and malignancies are considered to be the most important determinants of death with functioning graft in renal transplant recipients[6]. In addition, CNIs have been associated with development of various cardiovascular risk factors such as hyperlipidemia, hypertension, and new onset diabetes mellitus after transplantation (NODAT)[7,8].

In the given circumstances, it is important to note, that, CNI induced nephrotoxicity as a consequence to interstitial fibrosis and tubular atrophy represents a major obstacle to the long-term success of the renal transplant. The pathophysiology behind CNI induced nephrotoxicity involves increased production of vasoconstrictors, *e.g.*,

thromboxane and endothelin, with limited secretion of the vasodilators, such as nitric oxide, prostaglandin E2, and prostacyclin. The long-term graft failure has been observed in 96.8% of allograft biopsies[9,10]. In addition, the biggest challenge with immunosuppression therapy is to maintain the balance of immunosuppression in order to avert any rejection episode, whilst keeping the check on the toxicities. Studies have shown that a reduction or withdrawal from a CNI can significantly improve renal function[11-14].

In last decade, T-cell costimulation blocking agent belatacept has been identified as possible substitute to CNI therapy and obtained United States Food and Drug Administration approval in 2011 for the prevention of rejection in kidney transplant recipients[15-18]. Belatacept is a human fusion protein, which selectively binds to CD80 and CD86 with higher affinity than CD28. Thus blocks the interaction between CD86-CD28, hence, inhibits the complete activation of T-cells and promotes anergy and apoptosis[19,20] (Figure 1). Additional studies have demonstrated that costimulation blockade modulates T cell mediated immune processes which ought to abridge the dependence on the traditional maintenance immunosuppressive drugs[21].

These distinct immunological properties and limited nephrotoxic potential of belatacept have prevailed clinicians to use them as a surrogate to CNIs; cyclosporine A and Tac[22,23]. Given these findings, clinical trials in humans were undertaken to investigate the possibilities of belatacept as an adjunct to CNI based regimens. A recent, meta-analysis conducted by Talawila *et al*[24], included five trials to better elucidate the usefulness of belatacept in juxtaposition to cyclosporine. The group outlined the potential benefit for belatacept by reducing the risk of CNI toxicity, especially renal function, without any increased evidence of acute rejection at 12 mo.

Indeed, most of the kidney transplant recipients approximately 90% in the United States have been initially managed with a calcineurin inhibitor of which Tac is primarily used agent in 92% whilst cyclosporine is alternative option in 2%. The primary reason behind preferring Tac over cyclosporine includes decreased acute rejection rates, better tolerability, relatively lower requirement of mycophenolate mofetil (MMF)[3,4,25-27]. A meta-analysis conducted by Webster *et al*[3] included 30 studies (4102 patients) comparing tacrolimus and cyclosporine, demonstrated that tacrolimus significantly lowered the risk of graft loss following six months of renal transplantation [relative risk (RR): 0.56, 95% confidence interval (CI): 0.36-0.86]. Further, tacrolimus continued to favour allograft loss and reported 1-year, 2-year and 3-years graft loss of RR: 0.77 (CI: 0.58-1.02), RR: 0.74 (CI: 0.46-1.21) and RR: 0.71 (CI: 0.52-0.96) respectively. Moreover, tacrolimus also decreased the risk of acute rejection at one year (RR: 0.66, 95%CI: 0.6-0.79).

However, it was very unfortunate that till 2016 only one prospective study had been conducted to assess the usefulness of *de novo* belatacept over Tac. However, to bridge this lack of evidence Muduma *et al*[28] performed an "indirect treatment comparison" of belatacept to Tac. Here, they simultaneously conducted two consecutive meta-analyses comparing Tac to cyclosporine and cyclosporine to belatacept respectively and then compared the results of these analyses with each other to generate a direct comparison between Tac to belatacept. However, the review failed to find any conclusive evidence of difference towards the beneficence of belatacept as primary maintenance immunosuppressive agent in place of Tac.

Despite the availability of enormous literature on the applicability of belatacept in renal transplantation, intriguingly many questions are yet to be answered such as what is the true potential of this drug in current practice of renal transplantation with the principle of primum non nocere? Hence, the present study aimed to systematically review and where possible meta-analyze the available data on the clinical effectiveness of *de novo* belatacept as an alternative to Tac in patients undergoing renal transplantation and further highlighted the immunological basis for the development of belatacept-resistant rejection (BRR).

MATERIALS AND METHODS

The present meta-analysis was conducted following completion of registration (CRD42018086032) in PROSPERO an international database of prospectively registered systematic reviews. A detailed literature search was made on National Library of Medicine Database (PubMed), Embase, Cochrane, Crossref, Scopus databases, clinical trial registries on October 5, 2020 to determine the immunosuppressive role of belatacept as an alternative to Tac. The search covered the period 2005 (the year of the first reported use of belatacept) to October 5, 2020[17,29]. The search strategy designed

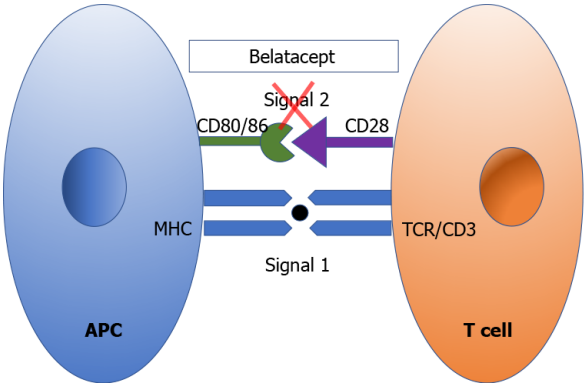


Figure 1 Pictorial depiction of mechanism of action of belatacept. APC: Antigen-presenting cell.

according to the guidelines mentioned in the Cochrane Handbook for Systematic Reviews of Interventions and reported as per the guidelines proposed by Meta-analysis of Observational Studies in Epidemiology. The medical subject headings terms and free text words were searched in various permutations and combinations: "Adverse events", "Calcineurin Inhibitors", "Tacrolimus", "Belatacept", "Graft Rejection", "Graft Survival", "Kidney Transplantation", to complete the analysis. In addition, a manual search was conducted for conference abstracts, bibliographies and citations list of the relevant articles were examined for additional studies.

Inclusion criteria

Only prospectively, systematically and quantitatively done RCT, comparing *de novo* belatacept with Tac in both living and/or deceased kidney transplant recipient were included. All other studies or publications types as retrospective studies, editorials, reviews, posters and letters were excluded. The primary outcome of interest was renal function, estimated glomerular filtration rate, and secondary outcomes were biopsy proven acute rejection (BPAR), patient and graft survival, NODAT, blood pressure, hyperlipidaemia, CMV viremia, and polyomavirus infection (Table 1).

Data extraction

Two separate physician reviewers (Kumar K and Reccia I) employed a two-stage method to conduct study screening independently. At the first stage, titles and abstracts were scrutinized for excluding obviously ineligible studies. At the second stage, the full texts were read carefully for further excluding any ineligible studies. Disagreements were resolved *via* consensus, and matters for which consensus could not be made were settled after much deliberation with senior author. The Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines were used here to complete search strategy and study selection (Figure 2 and 3).

Statistical analysis

The internal validity of pre-specified inclusion and exclusion criteria of the included studies were determined by independently by the authors using the Cochrane Risk of Bias tool. Each study was thoroughly analyzed to evaluate the above mentioned parameters (Table 2).

The Cochrane Collaboration, Review Manager (RevMan) Version 5.3 can analyze minimum of two trials and available continuous and dichotomous trial data. The data formulated as RR for dichotomous data, mean difference for continuous outcomes including 95%CI, heterogeneity between the trials compared and I^2 statistic of more than 30% determined as significant. I^2 statistic of more than 30% was determined to be significant. In the stance of significant heterogeneity, the random effects model assessment was used following the evaluation of forest plot while fixed-effect model was applied in the situation of low heterogeneity. In perspective of significant heterogeneity, the random effects model assessment was done following the evaluation of forest plot of involved trials[30,31]. Publication bias formally assessed through funnel plots but that requires at least 10 trials unfortunately present meta-analysis involved only four trials, so, we couldn't assess publication bias[32].

Table 1 Criteria for the inclusion of studies

Type	
Study design	Prospective cohort design with a well-defined study population
Study group	Post renal transplant
Study size	Any
Length of follow-up	Any
Source	Peer-reviewed journals
Language	English
Outcome measure	Renal function, patient safety, adverse events, and graft functioning and survival

Table 2 Characteristics of included studies

Ref.	Study design	Donor type	Belatacept based (group 1)	Tacrolimus based (group 2)	Belatacept based (group 3)
Ferguson *et al*[33], 2011	Multicentre, prospective, randomized (93 patients, 1 yr)	Living and deceased	Belatacept 10 mg/kg on day 1 and 5, then once every 2 wk through 3 mo, every 4 wk through 6 mo and 5 mg/kg from 7 mo onwards; MMF: 1 mg twice daily; Induction: Thymoglobulin + Corticosterids	Tac 0.2 mg/kg divided into two doses; Tac 0.2 mg/kg divided into two doses; Induction: Thymoglobulin + Corticosterids	Belatacept 10 mg/kg on day 1 and 5, then once every 2 wk through 3 mo, every 4 wk through 6 mo and 5 mg/kg from 7 mo onwards; SRL initiated on day 1 and dose level 7-12 ng/mL. Induction: Thymoglobulin + Corticosterids
de Graav *et al*[34], 2017	Single centre, prospective, randomized (40 patients, 1 yr)	Living	Belatacept 10 mg/kg on day 0, 4, 15, 30, 60, 90 d of transplant, following that 5 mg/kg till 12 mo	Tac 0.2 mg/kg divided into two doses. Target concentration 10 to 15 ng/mL (week 1-2); 8 to 12 ng/mL (week 3-4); 5-10 ng/mL (week > 5)	NA
Newell *et al*[35], 2017	Multicentre, prospective, randomized (19 patients, 1 yr)	Living and deceased	Belatacept 10 mg/kg on day 0 (day of transplant) and then on days 4, 14, 28, 56, and 84. After day 84, participants received a maintenance dose of 5 mg/kg every 4 wk until completion of the trial; MMF: 1 mg twice daily; Induction: Thymoglobulin, rapid methylprednisolone taper	Tac 0.1 mg/kg divided into two doses; Target concentration8 to 12 ng/mL (week 24), then 5 to 8 ng/mL (week > 24); MMF: 1 mg twice daily; Induction: Thymoglobulin, rapid methylprednisolone taper	Belatacept 10 mg/kg on day 0 (day of transplant) and then on days 4, 14, 28, 56, and 84. After day 84, participants received a maintenance dose of 5 mg/kg every 4 wk. Tac 0.1 mg/kg divided into two doses then adjusted to target trough levels: 8-12 ng/mL by Day 29, 5-8 ng/mL by Day 57, 3-5 ng/mL by Day 85 then stopped. MMF: 1 mg twice daily; Tac: 5 to 8 ng/mL (till 24 wk); Induction: Basiliximab + Corticosteroids
Trial 1856257[36], 2017	Multicentre, prospective, randomized (69 patients, 1 yr)	Living and deceased	Belatacept 10 mg/kg on day 1 (24 h of transplant) and then on days 5, 14, 28, 56, and 84. MMF: 1 mg twice daily; Induction: Thymoglobulin + Corticosteroids	Tac started on day 0/1; Target concentration 8 to 12 ng/mL (week 24), then 5 to 8 ng/mL (week > 24); MMF: 1 mg twice daily; Induction: Thymoglobulin + Corticosteroids	Belatacept 10 mg/kg on day 1 (24 h of transplant) and then on days 5, 14, 28, 56, and 84. Tac started on day 0/1; Target concentration 8 to 12 ng/mL (day 1-84) and then decreased by 1/3 at day 84 and by 1/3 at week 16. If trough levels were less than or equal to 3 ng/mL at week 20 then all tac was stopped. Otherwise, the dose was reduced by 1/2 and stopped at week 24. MMF: 1 mg twice daily; Induction: Basiliximab + Corticosteroids + Tac

MMF: Mycofenolate mofetil; NA: Not applicable; SRL: Sirolimus; Tac: Tacrolimus.

RESULTS

Our literature searches yielded a total of 158 manuscripts. After careful evaluation, 154 articles were excluded based on our selection criteria mentioned above. After resolution of differences between reviewers a total of four studies were retrieved for further review and data extraction[33-36].

These include three published papers, and one unpublished data from clinical trial registry (Table 2). In a study conducted by Ferguson *et al*[33] they compared two belatacept based regimen, hence to maintain uniformity we considered analysis regimen including belatacept, and MMF only without sirolimus[33]. Similarly for study by Newel *et al*[35,36] and trial 1856257 we only did analysis with regimen including

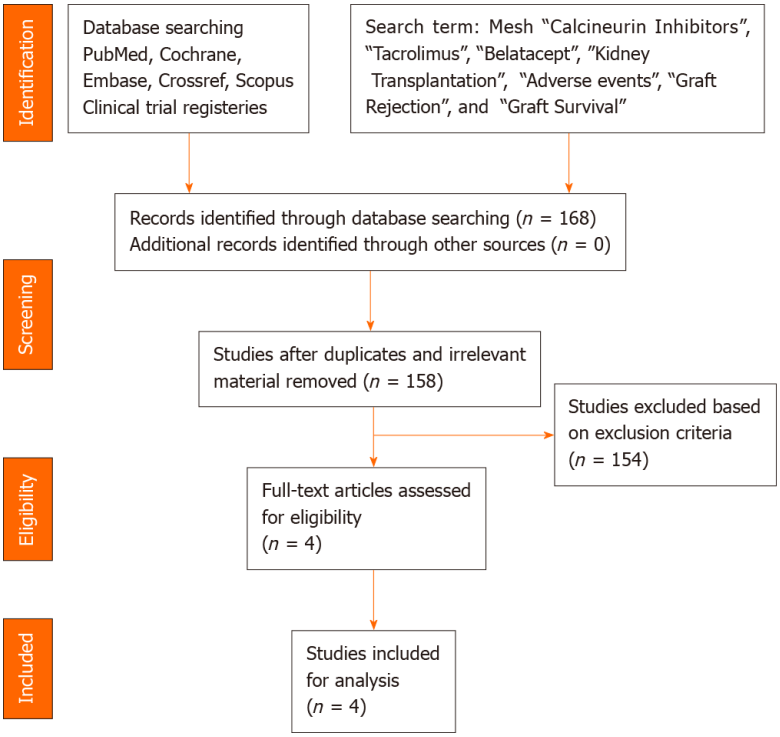


Figure 2 Search strategy and selections strategy applied in this meta-analysis as per PRISMA protocol.

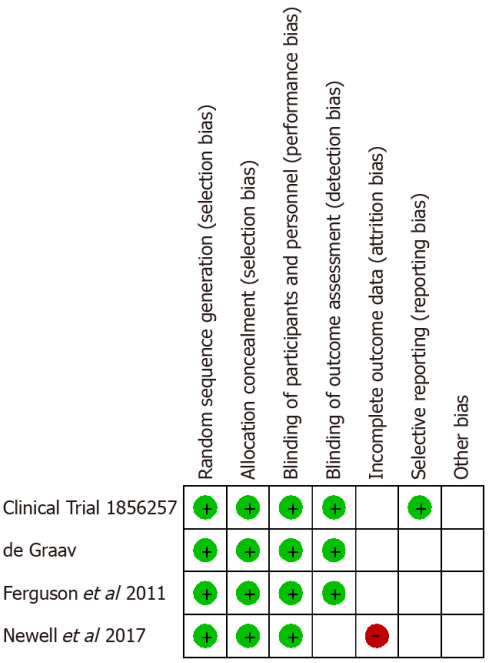


Figure 3 Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

belatacept with MMF only without Tac[33-36]. The detailed data of all the studies related with the renal functioning, BPAR, survival and adverse events were summarized in Tables 3-5. The results of these data analysis were outlined below.

Renal function

There was no significant difference in estimated renal function in the either groups at 12 mo (four trials, 154 patients, mean difference 4.12 mL/ min/1.73 m^2, CI: -2.18 to 10.42, P = 0.20, I^2 = 0%); (Figure 4A).

Table 3 Summary of outcomes in clinical trials

Ref.	Renal function (Gp 1 *vs* Gp 2)	BPAR (Gp 1 *vs* Gp 2)	Adverse event (Gp1 or *vs* Gp 2)	Remarks
Ferguson *et al*[33], 2011	12 mo; Sr. Cr: NA; eGFR: 63.6 ± 27.27 *vs* 54.0 ± 14.95 mL/min; (P = 0.14)	15.2% (5/33) *vs* 3.3% (1/30) (P = 0.24)	SAE/Infection: 57.5% (19/33) *vs* 53.3% (16/30); (P = 0.007); CMV infection: 3.0% (1/33) *vs* 3.3% (1/30) (P = 0.96); BK infection: 6.0% (2/33) *vs* 3.3% (1/30) (P = 0.59); NODAT: 0% (0/33) *vs* 3.3% (1/30) (P = 0.47)	Graft survival: 93.93% (31/33) *vs* 100% (30/30) (P = 0.51); Patient survival 93.93% (31/33) *vs* 100% (30/30) (P = 0.51)
de Graav *et al*[34], 2017	12 mo; Sr. Cr: 133.5 ± 39.26 *vs* 127.5 ± 28.87 μmol/L (P = 0.80); eGFR: 56.25 ± 17.61 *vs* 54.25 ± 14.73 mL/min (P = 0.57)	55% (11/20) *vs* 10% (2/20) (P = 0.006)	SAE/Infection: 10.25 ± 4.18 *vs* 11.90 ± 5.43 (P = 0.41); CMV infection: 10% (2/20) *vs* 5% (1/20) (P = 0.96); BK infection: 5% (1/20) *vs* 3.3% (2/20) (P = 0.54); NODAT: 5% (1/20) *vs* 35% (7/20) (P = 0.04)	Graft survival: 85% (17/20) *vs* 100% (20/20) (P = 0.22); Patient Survival 100% (20/20) *vs* 95% (19/20) (P = 0.31)
Newell *et al*[35], 2017	12 mo; Sr Cr: NA; eGFR: 51.6 ± 23.5 *vs* 55.9 ± 8.9 mL/min (P = 0.74)	33.3% (2/6) *vs* 50% (3/6) (P = 0.55)	SAE/Infection: 33.3% (2/6) *vs* 33.3% (2/6) (P = 1.0); CMV infection: 0% (0/6) *vs* 16.6% (1/6) (P = 0.29); BK infection: 0% (0/6) *vs* 0% (0/6) (P = 1.00); NODAT: 0% (0/6) *vs* 0% (0/6) (P = 1.00)	Graft survival: 50% (3/6) *vs* 83.33% (5/6) (P = 0.85); Patient survival 100% (6/6) *vs* 83.33% (5/6) (P = 0.29)
Clinicaltrial.gov 1856257[36], 2017	12 mo, Sr. Cr: NA, eGFR: 61.5 ± 23.3 *vs* 59.2 ± 19.9 mL/min (P = 0.70)	37.9% (11/29) *vs* 6.8% (2/29) (P = 0.009)	SAE/Infection: 72.41% (21/29) *vs* 65.5% (19/29) (P = 0.77); CMV infection: 20.6% (6/29) *vs* 3.4% (1/29) (P = 1.0); BK infection: 13.7% (4/29) *vs* 0% (0/29) (P = 0.11); NODAT: 3.4% (1/29) *vs* 3.4% (1/29) (P = 1.0)	Graft survival: 93.1% (27/29) *vs* 100% (29/29) (P = 0.49); Patient survival: 93.1% (27/29) *vs* 100% (29/29) (P = 0.49)

CMV: Cytomegalovirus; eGFR: Estimated glomerular filtration rate; Gp: Group; SAE: Serious adverse experiences; Sr Cr: Serum creatinine; NODAT: New onset diabetes mellitus after transplantation.

Biopsy proven acute rejection

The incidence of BPAR was significantly higher in belatacept groups compared to Tac groups (four trials, 173 patients, RR = 3.27, CI: 0.88 to 12.11, P = 0.08, I^2 = 59%) over 12 mo (Figure 4B).

Graft survival

At 12 mo, the rates of graft survival were significantly worse for belatacept groups than Tac groups (four trials, 173 patients, RR = 4.51, CI: 1.23 to 16.58, P = 0.02, I^2 = 0%) (Figure 4C).

Adverse events

Adverse events are summarized in Table 3. Over 12 mo, there was no significant difference in the incidence of serious adverse events/infection between the either groups (three trials, 129 patients, RR = 0.92, CI: 0.71 to 1.21, P = 0.56, I^2 = 0%) (Figure 4D). Four trials reported comparable incidence of BK virus or polyomavirus infection, in both group (Four trials, 173 patients, RR = 2.09, CI: 0.60 to 7.21, P = 0.24, I^2 = 19%) (Figure 4E).

Metabolic outcomes

The metabolic parameters as blood pressure and lipid profile of all four studies are outlined in Table 5. The incidence of NODAT was significantly lower with belatacept over 12 mo (four trials, 173 patients, RR = 0.26, CI: 0.07 to 0.99, P = 0.05, I^2 = 0%) (Figure 4F). Belatacept therapy resulted in no significant changes in systolic (four trials, 150 patients, MD = -3.77 mmHg, CI: -9.29 to 1.75, P = 0.18, I^2 = 0%) (Figure 5A) and diastolic blood pressure (four trials, 150 patients, MD = -1.27 mmHg, CI = -5.90 to 3.37, P = 0.59, I^2 = 35%) at 12 mo (Figure 5B).

There total serum cholesterol level and total triglycerides were comparable in both groups (two trials, 52 patients, MD = -2.85 mg/dL, CI: -23.68 to 17.98, P = 0.79, I^2 = 0%) and (two trials, 52 patients, MD = -6.56 mg/dL, CI: -59.79 to 46.67, P = 0.81, I^2 = 26%) respectively at 12 mo (Figure 5C and D). The serum low density lipoprotein (LDL) levels were lower for belatacept at 12 mo (two trials, 52 patients, MD = -25.68 mg/dL, CI: -48.15 to -3.22, P = 0.03, I^2 = 0%) (Figure 5E).

DISCUSSION

To our knowledge, this is the first meta-analysis assessing the efficacy and safety of

Table 4 Summary of biopsy proven acute rejection in clinical trials

Ref.	IA		IB		IIA		IIB		III		Mixed			
	Gp1 (Belatacept)	Gp2 (Tacrolimus)	Gp1 (Belatacept)	Gp2 (Tacrolimus)	Gp1 (Belatacept)	Gp2 (Tacrolimus)	Gp1 (Belatacept)	Gp2 (Tacrolimus)	Gp1 (Belatacept)	Gp2 (Tacrolimus)	Gp1 (Belatacept)	Gp2 (Tacrolimus)	Gp1 (Belatacept)	Gp2 (Tacrolimus)
Ferguson *et al*[33], 2011	0	0	0	0	3	0	2	1	0	0				
de Graav *et al*[34], 2017	0	0	1	1	2	1	6	0	1	0	1	0		
Newell *et al*[35], 2017	1	0	0	0	0	0	1	0	0	0			1	0
Clinicaltrial.gov 1856257[36], 2017	3	0	1	1	4	0	0	0	2	0			1	1

BPAR: Biopsy proven acute rejection; Gp: Group; AMR: Antibody mediated rejection.

belatacept based immunosuppressive maintenance regimen with Tac in kidney transplant recipients. The meta-analysis demonstrated that belatacept has been associated with an increased risk of allograft loss, following an increased risk of acute rejection in the first year of renal transplantation. These findings are in contrast to the previous notion, where studies have reported better allograft functioning without any significant change in patient and allograft survival over 12 mo' study period for the belatacept *vs* CNI groups, however, almost all of these studies have drawn this conclusion following comparison of belatacept to cyclosporine, not Tac[24]. Further, the above finding could be reflection of limited number available study assessing the role of belatacept in comparison to Tac or benefit could be sought following long duration of therapy.

Owing to the limited number of studies the data regarding the comparative studies of Tac based immunosuppression with belatacept is quite lucid, nevertheless, the outcomes of this meta-analysis will play a crucial role in formulating future studies. The renal function was assessed in all four trials and pooled analysis of data suggested that there is no significant difference present in either group. Along with that, the present meta-analysis also demonstrated a significant rise in BPAR in belatacept group. These outcomes have been further translated in terms of lower allograft and patient survival, and poor outcomes in renal transplant recipients who received belatacept.

Previous studies been shown that cardiovascular disease and its associated underlying risk factors as NODAT, hypertension and dyslipidemia are major cause of

Table 5 Summary of metabolic outcomes in clinical trials

Parameters	Ferguson *et al*[33], 2011 (25)		de Graav *et al*[34], 2017		Newell *et al*[35], 2017 (27)		Clinicaltrial.gov 1856257, 2017 (28)	
	Gp1 (Belatacept)	Gp2 (Tacrolimus)	Gp1 (Belatacept)	Gp2 (Tacrolimus)	Gp1 (Belatacept)	Gp2 (Tacrolimus)	Gp1 (Belatacept)	Gp2 (Tacrolimus)
Total CH, Mean (SD) (mg/dL)	NA	NA	193.34 ± 42.43	187.41 ± 42.28	187.0	156.0 ± 30.4	163.7 ± 38.8	177.1 ± 25.6
Total TG, Mean (SD) (mg/dL)	NA	NA	194.86 ± 51.14	221 ± 127.87	187.0	319.3 ± 294.0	170.0 ± 118.6	125.8 ± 93.0
LDL, Mean (SD) (mg/dL)	NA	NA	64.78 ± 30.20	96.67 ± 55.84	114.0	69.5 ± 38.0	86.3 ± 50.6	102.9 ± 17.7
BP mm/Hg (SBP/DBP) (12 mo)	129.3 ± 19.24/73.3 ± 11.96	138.2 ± 19.50/77.6 ± 10.51	141.25 ± 14.75/74 .25 ± 8.75	142.5 ± 17.31/78.0 ± 13.0	146.7 ± 5.1/92.7 ± 9.8	147.5 ± 18.7/80.8 ± 12.8	133.7 ± 14.7/79.1 ± 10.2	135.0 ± 18.9/77.7 ± 10.9

Lipid parameters represented in mean change from baseline to month 12 post transplantation.

mortality in kidney transplant recipients[37,38]. The reported incidence of NODAT in current literature is approximately 10%-30% in renal transplant recipients following CNI therapy[39-41]. Our finding supports previous literature comparing cyclosporine with belatacept and outlined significantly reduced odds for NODAT at 12 mo following belatacept in contrast to Tac[20,24].

Experimental studies have demonstrated that serum lipids nephrotoxicity play important role in the progression of chronic kidney disease[42]. Sandhu *et al*[43], conducted a meta-analyses involving 26 RCT and outlined that lowering serum LDL cholesterol positively influence the rate of reduction of glomerular filtration by approximately 1 mL/min per year. Our, the data analysis revealed lower LDL level in belatacept treated patients, hence, making it safer drug alternative for maintenance immunosuppression considering the renal and cardiac perspective, however, these benefits are do not outweigh the risks of other associated perils of belatacept based therapy. Further, studies assessed the impact of transition to belatacept during maintenance phase, which have outlined similar metabolic benefits, however, more research is required to elucidate true potential of these immunosuppressive regimen[44,45]. As mentioned in the results, the present meta-analysis did not demonstrate any significant difference in terms of adverse events in the belatacept group compared with the Tac based regimen. Further, it did not show any statistically significant increase in incidence of BK virus infection in the belatacept group (Figure 6).

The outcomes of this meta-analysis were quite dreary to the speculation that belatacept could further enhance the benefits of renal transplantation. However, every cloud has silver lining and the received setbacks provide enormous learning opportunities and open doors for development of newer drugs. Hence, further investigations are required to better elucidate reasons behind the observed outcome with belatacept, including the cipher of BRR. Belatacept binds to CD80 and/or CD86 on antigen-presenting cells (APCs) and fosters T-cell anergy by depriving T-cells with co-stimulatory signal[16,46]. Belatacept's adoption as a mainstay immunosuppressive therapy has been tempered by increased BPAR and resistance to treatment. Further probe into the underlying mechanisms of resistance and rejection has been done not only to enhance the knowledge regarding clinical applicability of belatacept but also to avail the development of tailored immunosuppressive strategies.

However, recent evidence suggests the plausible explanations for the development of resistance to the clinical usefulness and limitations of belatacept based immunosuppression, further in the discussion we have tried to interpret the reason behind the deceptive behaviour of current costimulatory inhibitors through the review of the available literature.

Firstly, an aggressive, T cell-mediated allogeneic responses observed in belatacept treated patients clearly explicate the actions of memory T-cells that are less or not susceptible to co-stimulatory blockade pathway CD28-CD80/86[47-50]. This could be explained by the fact that belatacept inhibits T-cell proliferation in a dose-dependent manner. However, even with the higher dosages of belatacept, the inhibition of T cell proliferation does not exceed more than ± 70%, hence gives a window for residual T

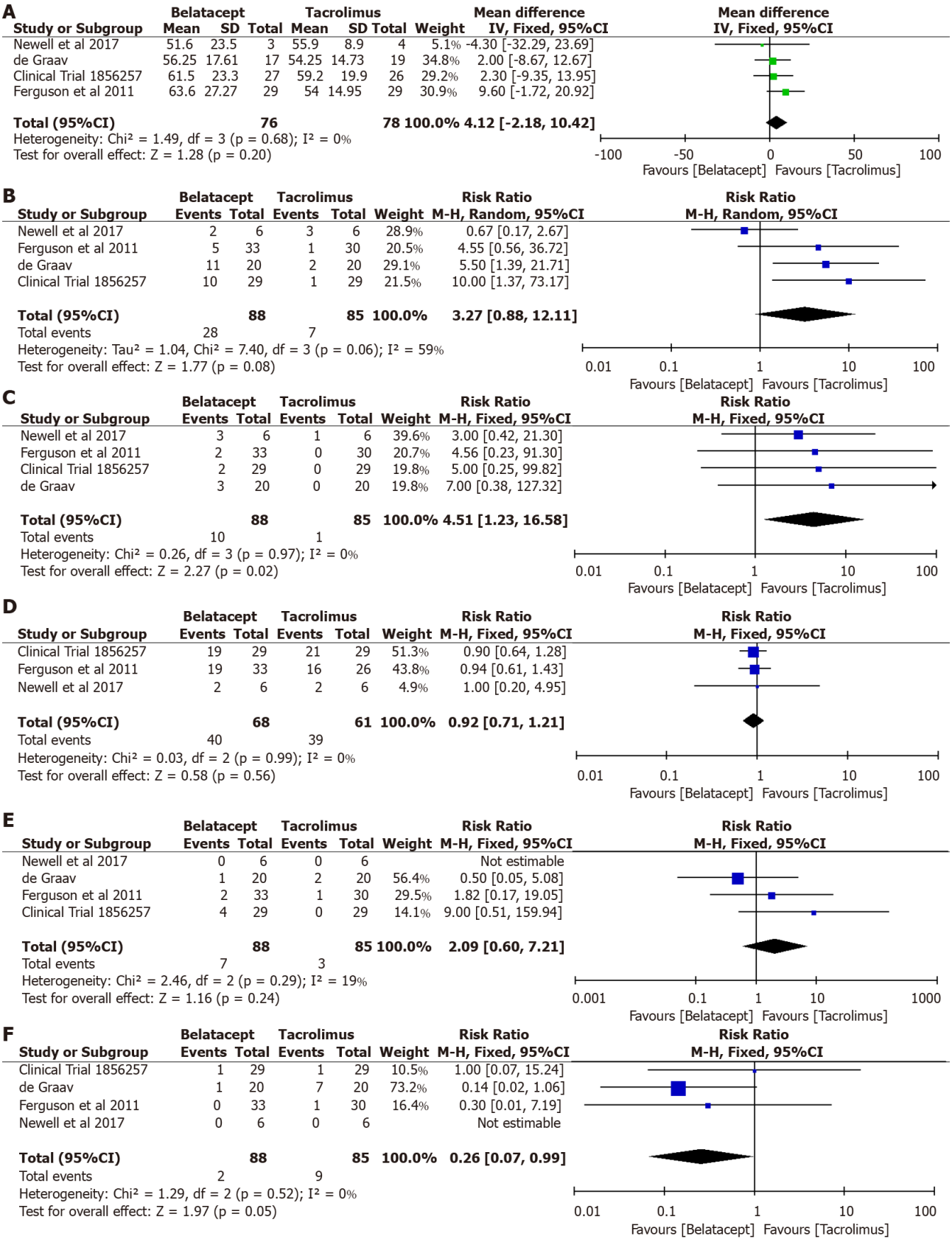


Figure 4 Forest plot represents the changes at 12 mo in kidney transplant recipients when treated with belatacept or tacrolimus. Squares represent size effects of studies, comparing the weight of the study in the meta-analysis. 95 percent confidence intervals represented in horizontal bars. A: The eGFR at 12 mo in kidney transplant recipients; B: The biopsy proven acute rejection over 12 mo in kidney transplant recipients. The diamond shows significant favour towards tacrolimus group following random effect analysis; C: Graft survival over 12 mo in kidney transplant recipients. The diamond shows significant favour towards tacrolimus group following fixed effect analysis; D: The adverse events over 12 mo in kidney transplant recipients. The diamond doesn't suggest any significant difference following fixed effects analysis; E: The BK virus infection over 12 mo in kidney transplant recipients. The diamond doesn't suggest any significant difference

following fixed effects analysis; F: The new onset diabetes mellitus after transplantation over 12 mo in kidney transplant recipients. The diamond suggests significant favour towards belatacept group following fixed effects analysis.

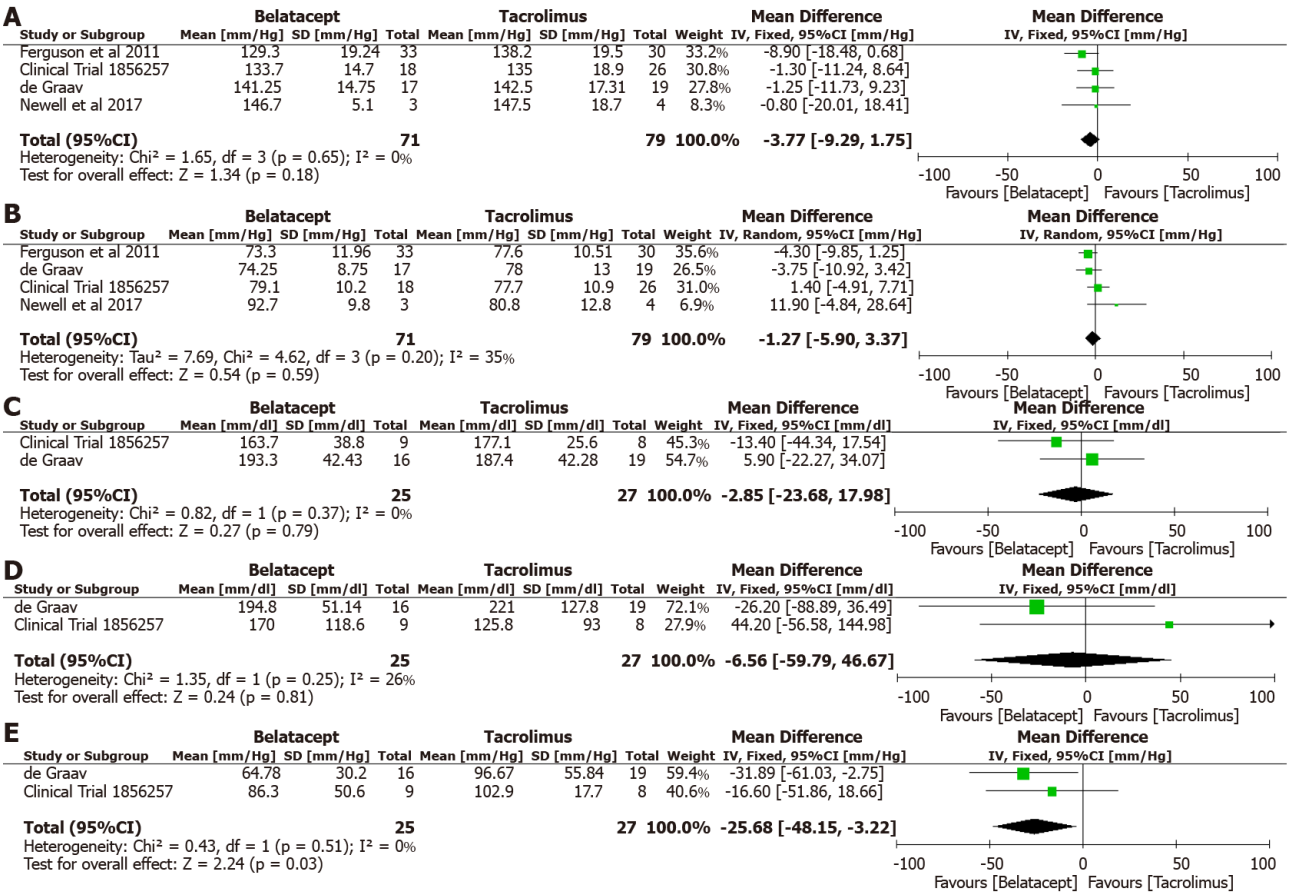


Figure 5 Forest plot represents the changes at 12 mo in kidney transplant recipients when treated with belatacept or tacrolimus. Squares represent size effects of studies, comparing the weight of the study in the meta-analysis. 95 percent confidence intervals represented in horizontal bars. A: The systolic blood pressure at 12 mo in kidney transplant recipients. The diamond doesn't suggest any significant difference following fixed effects analysis; B: The diastolic blood pressure at 12 mo in kidney transplant recipients. The diamond doesn't suggest any significant difference following random effects analysis; C: Serum total cholesterol at 12 mo in kidney transplant recipients. The diamond doesn't suggest any significant difference following fixed effects analysis; D: Serum triglycerides at 12 mo in kidney transplant recipients. The diamond doesn't suggest any significant difference following fixed effects analysis; E: Serum low density lipoprotein at 12 mo in kidney transplant recipients. The diamond suggests favour towards belatacept group following fixed effects analysis.

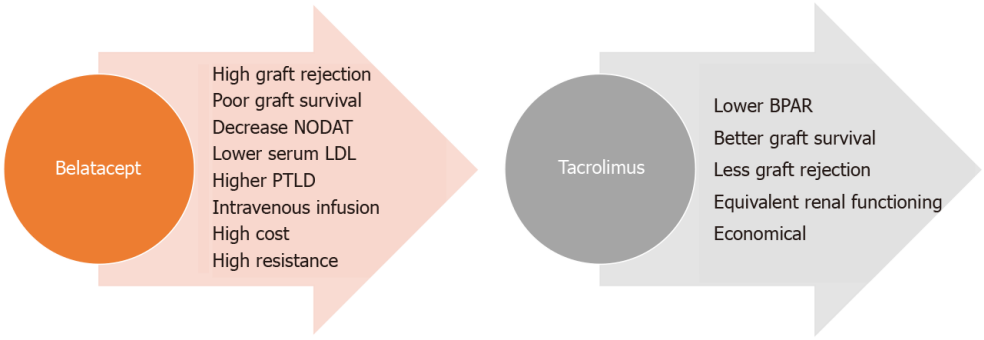


Figure 6 Factors modified by belatacept and tacrolimus based regimen. BPAR: Biopsy proven acute rejection; NODAT: New onset diabetes mellitus after transplantation; Serum LDL: Serum low density lipoprotein.

cells proliferation up to ± 30%[51].

Secondly, the plasticity theory of sequential, parallel differentiation and immunological synapse throws light on the development and maintenance of resistant

effector memory T cell in belatacept treated patients[50,52,53]. This fact broaches a concern that, witnessed resistance to belatacept might be explained by the biological underpinning causing cross-connection between naïve, effector and memory T cells populations. The precise underlying mechanism remains obscure, however, it is possibly conferred by the development of the interaction between the B7 protein on APCs and CD28 (also known as cytotoxic T-lymphocyte-associated protein 4) on T cells[54,55]. Following differentiation, the expression of CD28 is markedly downregulated and the resulting memory T cells are no longer able to reinstate co-stimulation for the secondary immune responses[56,57]. Furthermore, the downregulation persuades T cell migration and extravasation at inflammatory sites through the expression of adhesion molecules over vascular endothelium. The molecules as LFA-1 and VLA-4 bind intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 while CD2 promotes T-cell activation and adhesion by binding to LFA-3 on APCs[58-60]. Hence, the belatacept induced CD28 downregulation not only instigates effector memory cells proliferation but also promotes cellular infiltration into the renal allograft, which disrupts the bridge to achieve adequate immunosuppression in the transplant recipient[61,62].

In addition, an elevated profile of T-cell mediated allogeneic responses with variability in cell surface phenotype are detected following belatacept treatment. The lymphocyte repertoire transforms itself substantially over time as a ramification of environmental pathogen exposure, which forms the basis for the down regulation of the CD28 expression on the membrane of effector-memory T-cells following belatacept treatment. Such CD8+CD28− T cells are highly cytotoxic and bring imperil to the traditional immunosuppressive shield, however, lack in the proliferative capacity[63,64]. Hence, D28-CD80/86 pathway is not the sole explanation of the development of BRR[65,66]. Mou *et al*[66] outlined, the loss of CD28 expression as a major requisite towards the development of BRR, however, it was not sole attribute for the instigation of BRR and highlighted certain other plausible explanations. The study demonstrated increased rejection with the expression of CD57 on the membrane of CD28 negative T cells populations with cytolytic potential. This notion was further supported by demonstrating the infiltration of CD57+ CD4 T cells in renal allograft biopsies in patients developing rejection in spite of being on belatacept. Hence, CD57+CD4+CD28- T cells represent a potential therapeutic target and act as a practical screening tool to identify patients at risk for ACR while on belatacept. However, the identification of such phenotype (CD57+CD4+CD28-) T cells in the peripheral blood of patients awaiting renal transplantation may aid in identifications of recipients' not amenable for belatacept-based therapy.

An another kind of effector memory CD8+CD28++ EMRA T cells that has caught attention as a possible explanation for the development of resistance in belatacept patients[67,68]. However, de Graav *et al*[51] reported that absolute numbers or proportions of pretransplant CD28++ cells within the CD8+ EMRA T cell population did not increase BRR.

Differences in rate and severity of BRR in patients with pre-emptive transplantations lies within the differentiation, immunological synapse and plasticity that helps in modulating the effector memory T cell in belatacept treated renal transplant recipients. Hence at present, we can't rule out the possibility of the presence of any other memory cell or mixed effect of these cells as a possible mechanism for development BRR. The above mentioned facts do not mean that there is a failure of any kind it actually opens the way for instigation of better drugs and modified regimen, which can be used in much-tailored way to preserve the renal allograft functioning for long. The development of humoral response through production *de novo* donor-specific antibodies following renal transplantation is considered as the one of the primary reason for late-onset renal allograft failure.

The precise mechanisms by which belatacept is involved in the control of humoral responses requires thorough investigation. Studies outlined that belatacept minimizes humoral immune response including plasmablast differentiation, immunoglobulin production, and the expression of the intricate transcription factor implicated in the functioning of the plasma cell, activation of the STAT3 transcription factor in functioning B cells and reduced the expression of CD86 and blocked CD28-mediated activation of T helper cells. Lately, Leibler *et al*[69] reasoned these facts as a plausible explanation towards the lesser degree of *de novo* donor-specific antibodies generation in the belatacept treated renal allograft recipients than conventional immunosuppression regimen. Hence, attention is now turning towards the development of target costimulatory molecules which become advantageous in the field of transplantation and autoimmune conditions (Figure 7).

The present meta-analysis has certain limitations, which needs to be acknowledged.

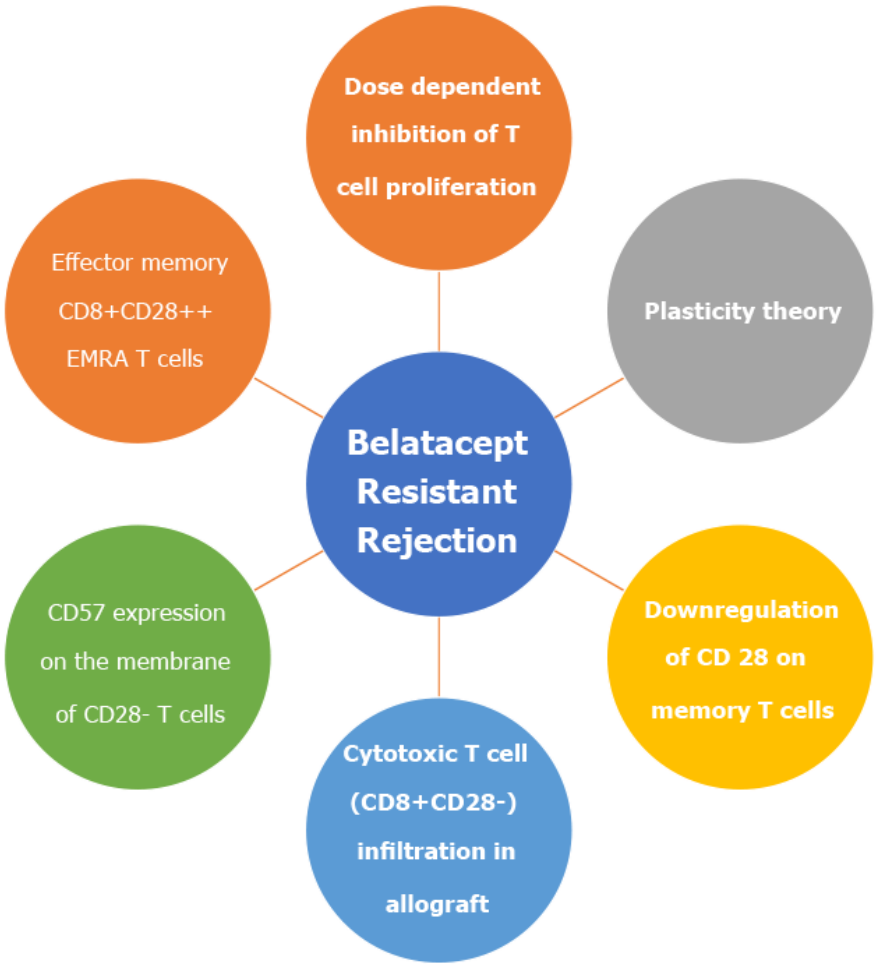


Figure 7 Mechanism of the development of resistance to belatacept.

Here, we only identified four trials and thus further large-scale trials would provide much-needed data to allow firmer conclusions, regarding the use of belatacept. However, considering costs and ethical concerns owing to the increased risk of renal graft loss, conducting such a study is a matter of debate. Second, publication bias can only be tested with formal statistical tests in the case of ≥ 10 included studies. Therefore, we cannot exclude the possibility that the results from meta-analyses involving < 10 studies could be driven by publication bias.

CONCLUSION

The present meta-analysis showed that belatacept-based maintenance immunosuppression regimens were associated with an increased risk allograft loss for renal transplant recipients with equivalent renal functioning when compared to standard of care agent Tac. The widespread adaptation of belatacept in renal transplantation has been limited by increased rates of rejection, which is conferred owing to development of resistance secondary to differentiation into various types of effector memory T cells. Henceforth, the applicability of belatacept should be tailored according to the need of transplant recipients particularly as a transition to belatacept in the maintenance phase of immunosuppression. In light of present evidence the applicability of belatacept does look like foe, however, it still has some explicit potential role, particularly in situations such as Caucasian recipients with two-haplotype identical human leukocyte antigen, living related allografts and obesity. Additional factors ought to be considered are the cardiovascular and hemodynamic complications associated with poor allograft function, along with the immunological risk as role of belatacept is never reported in the recipients with PRA > 30%. Further research are required to assess the safety and efficacy of belatacept in the setting of immunological sensitizationand to better elucidate the mechanism of resistance and development of therapeutic strategies with focus on adhesion molecule blockade or abrogation of memory-specific responses.

ARTICLE HIGHLIGHTS

Research background

The T-cell costimulation blocking agent belatacept is considered as possible substitute for calcineurin inhibitors, however, no consensus has been established against its standard immunusuppressive drug Tacrolimus.

Research motivation

To find the alternative to current immunosuppressive medicine tacrolimus because of its high toxic adverse effects.

Research objectives

To understand the effectiveness of belatacept based maintenance immunosuppressive regimens in comparison to tacrolimus in renal transplantion through meta-analysis.

Research methods

The present meta-analysis was conducted following completion of registration (CRD42018086032) in Prospero an international database of prospectively registered systematic reviews. A detailed literature search was made on National Library of Medicine Database (PubMed), Embase, Cochrane, Crossref, Scopus databases, clinical trial registries on December 5, 2018 to determine the immunosuppressive role of belatacept as an alternative to Tac and analyis of data was performed through The Cochrane Collaboration, Review Manager (RevMan) Version 5.3.

Research results

The literature search revealed four prospective randomized control studies (n = 173 participants) comparing belatacept with tacrolimus. There was no significant difference in estimated renal function at 12 mo [mean difference 4.12 mL/min/1.73 m^2, confidence interval (CI): -2.18 to 10.42, P = 0.20]. Further, belatacept group was associated with significant increase in biopsy proven acute rejection [relative risk (RR) = 3.27, CI: 0.88 to 12.11, P = 0.08] and worse 12 mo allograft survival (RR = 4.51, CI: 1.23 to 16.58, P = 0.02). Although, the incidence of new onset diabetes mellitus was lower with belatacept at 12 mo (RR = 0.26, CI: 0.07 to 0.99, P = 0.05).

Research conclusions

The meta-analysis demonstrated that belatacept-based maintenance immuno-suppression regimens were associated with an increased risk allograft loss in renal transplant recipients with equivalent renal functioning against standard tacrolimus. Further, the inclusion of belatacept as routine immunosuppresive agent in renal transplantation has been thwarted by increased rates of rejection and resistance owing to development of various effector memory T cells through, parallel differentiation and immunological plasticity.

Research perspectives

Study required to determine the safety and efficacy of belatacept in the setting of immunological sensitization and to better elucidate the mechanism of resistance and development of therapeutic strategies with focus on adhesion molecule blockade or abrogation of memory-specific responses.

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 **Webster AC**, Woodroffe RC, Taylor RS, Chapman JR, Craig JC. Tacrolimus *vs* 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]

4 **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]

5 **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]

6 **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]

7 **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]

8 **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]

9 **Cornell LD**, Colvin RB. Chronic allograft nephropathy. *Curr Opin Nephrol Hypertens* 2005; **14**: 229-234 [PMID: 15821415 DOI: 10.1097/01.mnh.0000165888.83125.07]

10 **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]

11 **Hamdy AF**, El-Agroudy AE, Bakr MA, Mostafa A, El-Baz M, El-Shahawy el-M, Ghoneim MA. Comparison of sirolimus with low-dose tacrolimus *vs* sirolimus-based calcineurin inhibitor-free regimen in live donor renal transplantation. *Am J Transplant* 2005; **5**: 2531-2538 [PMID: 16162204 DOI: 10.1111/j.1600-6143.2005.01064.x]

12 **Oberbauer R**, Segoloni G, Campistol JM, Kreis H, Mota A, Lawen J, Russ G, Grinyó JM, Stallone G, Hartmann A, Pinto JR, Chapman J, Burke JT, Brault Y, Neylan JF; Rapamune Maintenance Regimen Study Group. Early cyclosporine withdrawal from a sirolimus-based regimen results in better renal allograft survival and renal function at 48 mo after transplantation. *Transpl Int* 2005; **18**: 22-28 [PMID: 15612979 DOI: 10.1111/j.1432-2277.2004.00052.x]

13 **Kumar J**, Bridson JM, Sharma A, Halawa A. Systematic Review on Role of Mammalian Target of Rapamycin Inhibitors as an Alternative to Calcineurin Inhibitors in Renal Transplant: Challenges and Window to Excel. *Exp Clin Transplant* 2017; **15**: 241-252 [PMID: 27915965 DOI: 10.6002/ect.2016.0270]

14 **Kumar J**, Reccia I, Kusano T. Is Early Conversion to mTOR Inhibitors Represent a Suitable Choice in Renal Transplant Recipients? *Int J Organ Transplant Med* 2017; **8**: 68-76 [PMID: 28828166]

15 **Vincenti F**, Larsen C, Durrbach A, Wekerle T, Nashan B, Blancho G, Lang P, Grinyo J, Halloran PF, Solez K, Hagerty D, Levy E, Zhou W, Natarajan K, Charpentier B; Belatacept Study Group. Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 2005; **353**: 770-781 [PMID: 16120857 DOI: 10.1056/NEJMoa050085]

16 **Larsen CP**, Pearson TC, Adams AB, Tso P, Shirasugi N, Strobert E, Anderson D, Cowan S, Price K, Naemura J, Emswiler J, Greene J, Turk LA, Bajorath J, Townsend R, Hagerty D, Linsley PS, Peach RJ. Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. *Am J Transplant* 2005; **5**: 443-453 [PMID: 15707398 DOI: 10.1111/j.1600-6143.2005.00749.x]

17 **Wekerle T**, Grinyó JM. Belatacept: from rational design to clinical application. *Transpl Int* 2012; **25**: 139-150 [PMID: 22151353 DOI: 10.1111/j.1432-2277.2011.01386.x]

18 **Vincenti F**, Dritselis A, Kirkpatrick P. Belatacept. *Nat Rev Drug Discov* 2011; **10**: 655-656 [PMID: 21878974 DOI: 10.1038/nrd3536]

19 **Grinyó JM**, Budde K, Citterio F, Charpentier B. Belatacept utilization recommendations: an expert position. *Expert Opin Drug Saf* 2013; **12**: 111-122 [PMID: 23206310 DOI: 10.1517/14740338.2013.748747]

20 **Vanrenterghem Y**, Bresnahan B, Campistol J, Durrbach A, Grinyó J, Neumayer HH, Lang P, Larsen CP, Mancilla-Urrea E, Pestana JM, Block A, Duan T, Glicklich A, Gujrathi S, Vincenti F. Belatacept-based regimens are associated with improved cardiovascular and metabolic risk factors compared with cyclosporine in kidney transplant recipients (BENEFIT and BENEFIT-EXT studies). *Transplantation* 2011; **91**: 976-983 [PMID: 21372756 DOI: 10.1097/TP.0b013e31820c10eb]

21 **Garnock-Jones KP**. Belatacept: in adult kidney transplant recipients. *BioDrugs* 2012; **26**: 413-424 [PMID: 22928660 DOI: 10.2165/11208900-000000000-00000]

22 **Masson P**, Henderson L, Chapman JR, Craig JC, Webster AC. Belatacept for kidney transplant recipients. *Cochrane Database Syst Rev*2014: CD010699 [PMID: 25416857 DOI: 10.1002/14651858.CD010699.pub2]

23 **Wéclawiak H**, Kamar N, Ould-Mohamed A, Cardeau-Desangles I, Rostaing L. Biological agents in kidney transplantation: belatacept is entering the field. *Expert Opin Biol Ther* 2010; **10**: 1501-1508 [PMID: 20726688 DOI: 10.1517/14712598.2010.514901]

24 **Talawila N**, Pengel LH. Does belatacept improve outcomes for kidney transplant recipients? *Transpl Int* 2015; **28**: 1251-1264 [PMID 25965549 DOI: 10.1111/tri.12605]

25 **Krämer BK**, Montagnino G, Krüger B, Margreiter R, Olbricht CJ, Marcen R, Sester U, Kunzendorf U, Dietl KH, Rigotti P, Ronco C, Hörsch S, Banas B, Mühlbacher F, Arias M; European Tacrolimus

vs Ciclosporin Microemulsion Renal Transplantation Study Group. Efficacy and safety of tacrolimus compared with ciclosporin-A in renal transplantation: 7-year observational results. *Transpl Int* 2016; **29**: 307-314 [PMID: 26565071 DOI: 10.1111/tri.12716]

26 **Margreiter R**; European Tacrolimus vs Ciclosporin Microemulsion Renal Transplantation Study Group. Efficacy and safety of tacrolimus compared with ciclosporin microemulsion in renal transplantation: a randomised multicentre study. *Lancet* 2002; **359**: 741-746 [PMID: 11888584 DOI: 10.1016/S0140-6736(02)07875-3]

27 **Saudek F**, Malaise J, Boucek P, Adamec M; Euro-SPK Study Group. Efficacy and safety of tacrolimus compared with cyclosporin microemulsion in primary SPK transplantation: 3-year results of the Euro-SPK 001 trial. *Nephrol Dial Transplant* 2005; **20** Suppl 2: ii3-i10, ii62 [PMID: 15814547 DOI: 10.1093/ndt/gfh1076]

28 **Muduma G**, Hart WM, Patel S, Odeyemi AO. Indirect treatment comparison of belatacept *vs* tacrolimus from a systematic review of immunosuppressive therapies for kidney transplant patients. *Curr Med Res Opin* 2016; **32**: 1065-1072 [PMID: 26907083 DOI: 10.1185/03007995.2016.1157463]

29 **McIntyre JA**, Fernández D. Belatacept: Treatment of transplant rejection. *Drugs Future* 2005; **30**: 873-876 [DOI: 10.1358/dof.2005.030.09.936694]

30 **Chootrakool H**, Shi JQ, Yue R. Meta-analysis and sensitivity analysis for multi-arm trials with selection bias. *Stat Med* 2011; **30**: 1183-1198 [PMID: 21538449 DOI: 10.1002/sim.4143]

31 **Mavridis D**, Welton NJ, Sutton A, Salanti G. A selection model for accounting for publication bias in a full network meta-analysis. *Stat Med* 2014; **33**: 5399-5412 [PMID: 25316006 DOI: 10.1002/sim.6321]

32 **Deeks JJ**, Higgins JP, Altman DG. Analysing Data and Undertaking Meta-Analyses. In: Cochrane Handbook for Systematic Reviews of Interventions: Cochrane Book Series; 2008: 243-296 [DOI: 10.1002/9780470712184.ch9]

33 **Ferguson R**, Grinyó J, Vincenti F, Kaufman DB, Woodle ES, Marder BA, Citterio F, Marks WH, Agarwal M, Wu D, Dong Y, Garg P. Immunosuppression with belatacept-based, corticosteroid-avoiding regimens in de novo kidney transplant recipients. *Am J Transplant* 2011; **11**: 66-76 [PMID: 21114656 DOI: 10.1111/j.1600-6143.2010.03338.x]

34 **de Graav GN**, Baan CC, Clahsen-van Groningen MC, Kraaijeveld R, Dieterich M, Verschoor W, von der Thusen JH, Roelen DL, Cadogan M, van de Wetering J, van Rosmalen J, Weimar W, Hesselink DA. A Randomized Controlled Clinical Trial Comparing Belatacept With Tacrolimus After De Novo Kidney Transplantation. *Transplantation* 2017; **101**: 2571-2581 [PMID: 28403127 DOI: 10.1097/TP.0000000000001755]

35 **Newell KA**, Mehta AK, Larsen CP, Stock PG, Farris AB, Mehta SG, Ikle D, Armstrong B, Morrison Y, Bridges N, Robien M, Mannon RB. Lessons Learned: Early Termination of a Randomized Trial of Calcineurin Inhibitor and Corticosteroid Avoidance Using Belatacept. *Am J Transplant* 2017; **17**: 2712-2719 [PMID: 28556519 DOI: 10.1111/ajt.14377]

36 February 12, 2021 Newell K. Open-Label Phase 2 Trial of a Steroid-Free, CNI-Free, Be-latacept-Based Immunosuppressive Regimen, 17 December, 2020 [cited 19 January, 2021]. In: National Institute of Allergy and Infectious Diseases. ClinicalTrials.gov Identifier:NCT01856257 Available from: https://clinicaltrials.gov/ct2/show/results/NCT01856257?cond=01856257&rank=1§=X01256

37 **Meier-Kriesche HU**, Baliga R, Kaplan B. Decreased renal function is a strong risk factor for cardiovascular death after renal transplantation. *Transplantation* 2003; **75**: 1291-1295 [PMID: 12717218 DOI: 10.1097/01.TP.0000061602.03327.E2]

38 **Fellström B**, Jardine AG, Soveri I, Cole E, Neumayer HH, Maes B, Gimpelewicz C, Holdaas H; ALERT Study Group. Renal dysfunction is a strong and independent risk factor for mortality and cardiovascular complications in renal transplantation. *Am J Transplant* 2005; **5**: 1986-1991 [PMID: 15996249 DOI: 10.1111/j.1600-6143.2005.00983.x]

39 **Ojo AO**. Cardiovascular complications after renal transplantation and their prevention. *Transplantation* 2006; **82**: 603-611 [PMID: 16969281 DOI: 10.1097/01.tp.0000235527.81917.fe] **Svensson M**, Jardine

40 A, Fellström B, Holdaas H. Prevention of cardiovascular disease after renal transplantation. *Curr Opin Organ Transplant* 2012; **17**: 393-400 [PMID: 22790074 DOI: 10.1097/MOT.0b013e3283560a3b]

41 **Vanrenterghem YF**, Claes K, Montagnino G, Fieuws S, Maes B, Villa M, Ponticelli C. Risk factors for cardiovascular events after successful renal transplantation. *Transplantation* 2008; **85**: 209-216 [PMID: 18212625 DOI: 10.1097/TP.0b013e318160254f]

42 **Moorhead JF**, Chan MK, El-Nahas M, Varghese Z. Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. *Lancet* 1982; **2**: 1309-1311 [PMID: 6128601 DOI: 10.1016/s0140-6736(82)91513-6]

43 **Sandhu S**, Wiebe N, Fried LF, Tonelli M. Statins for improving renal outcomes: a meta-analysis. *J Am Soc Nephrol* 2006; **17**: 2006-2016 [PMID: 16762986 DOI: 10.1681/ASN.2006010012]

44 **Gupta S**, Rosales I, Wojciechowski D. Pilot Analysis of Late Conversion to Belatacept in Kidney Transplant Recipients for Biopsy-Proven Chronic Tacrolimus Toxicity. *J Transplant* 2018; **2018**: 1968029 [PMID: 29854421 DOI: 10.1155/2018/1968029]

45 **Vincenti F**, Blancho G, Durrbach A, Grannas G, Grinyó J, Meier-Kriesche HU, Polinsky M, Yang L, Larsen CP. Ten-year outcomes in a randomized phase II study of kidney transplant recipients administered belatacept 4-weekly or 8-weekly. *Am J Transplant* 2017; **17**: 3219-3227 [PMID: 28758341 DOI: 10.1111/ajt.14452]

46 **Huurman VA**, Unger WW, Koeleman BP, Oaks MK, Chandraker AK, Terpstra OT, Roep BO. Differential inhibition of autoreactive memory- and alloreactive naive T cell responses by soluble cytotoxic T lymphocyte antigen 4 (sCTLA4), CTLA4Ig and LEA29Y. *Clin Exp Immunol* 2007; **150**: 487-493 [PMID: 17924973 DOI: 10.1111/j.1365-2249.2007.03513.x]

47 **Durrbach A**, Pestana JM, Pearson T, Vincenti F, Garcia VD, Campistol J, Rial Mdel C, Florman S, Block A, Di Russo G, Xing J, Garg P, Grinyó J. A phase III study of belatacept *vs* cyclosporine in kidney transplants from extended criteria donors (BENEFIT-EXT study). *Am J Transplant* 2010; **10**: 547-557 [PMID: 20415898 DOI: 10.1111/j.1600-6143.2010.03016.x]

48 **Weaver TA**, Charafeddine AH, Agarwal A, Turner AP, Russell M, Leopardi FV, Kampen RL, Stempora L, Song M, Larsen CP, Kirk AD. Alefacept promotes co-stimulation blockade based allograft survival in nonhuman primates. *Nat Med* 2009; **15**: 746-749 [PMID: 19584865 DOI: 10.1038/nm.1993]

49 **Lo DJ**, Weaver TA, Stempora L, Mehta AK, Ford ML, Larsen CP, Kirk AD. Selective targeting of human alloresponsive CD8+ effector memory T cells based on CD2 expression. *Am J Transplant* 2011; **11**: 22-33 [PMID: 21070604 DOI: 10.1111/j.1600-6143.2010.03317.x]

50 **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]

51 **de Graav GN**, Hesselink DA, Dieterich M, Kraaijeveld R, Weimar W, Baan CC. Down-Regulation of Surface CD28 under Belatacept Treatment: An Escape Mechanism for Antigen-Reactive T-Cells. *PLoS One* 2016; **11**: e0148604 [PMID: 26919152 DOI: 10.1371/journal.pone.0148604]

52 **Grakoui A**, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML. The immunological synapse: a molecular machine controlling T cell activation. *Science* 1999; **285**: 221-227 [PMID: 10398592 DOI: 10.1126/science.285.5425.221]

53 **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]

54 **Deeths MJ**, Mescher MF. B7-1-dependent co-stimulation results in qualitatively and quantitatively different responses by CD4+ and CD8+ T cells. *Eur J Immunol* 1997; **27**: 598-608 [PMID: 9079798 DOI: 10.1002/eji.1830270305]

55 **Guerder S**, Carding SR, Flavell RA. B7 costimulation is necessary for the activation of the lytic function in cytotoxic T lymphocyte precursors. *J Immunol* 1995; **155**: 5167-5174 [PMID: 7594526]

56 **Shin T**, Yoshimura K, Shin T, Crafton EB, Tsuchiya H, Housseau F, Koseki H, Schulick RD, Chen L, Pardoll DM. In vivo costimulatory role of B7-DC in tuning T helper cell 1 and cytotoxic T lymphocyte responses. *J Exp Med* 2005; **201**: 1531-1541 [PMID: 15897272 DOI: 10.1084/jem.20050072]

57 **Mandelbrot DA**, McAdam AJ, Sharpe AH. B7-1 or B7-2 is required to produce the lymphoproliferative phenotype in mice lacking cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). *J Exp Med* 1999; **189**: 435-440 [PMID: 9892625 DOI: 10.1084/jem.189.2.435]

58 **Selvaraj P**, Plunkett ML, Dustin M, Sanders ME, Shaw S, Springer TA. The T lymphocyte glycoprotein CD2 binds the cell surface ligand LFA-3. *Nature* 1987; **326**: 400-403 [PMID: 2951597 DOI: 10.1038/326400a0]

59 **Bachmann MF**, McKall-Faienza K, Schmits R, Bouchard D, Beach J, Speiser DE, Mak TW, Ohashi PS. Distinct roles for LFA-1 and CD28 during activation of naive T cells: adhesion *vs* costimulation. *Immunity* 1997; **7**: 549-557 [PMID: 9354475 DOI: 10.1016/s1074-7613(00)80376-3]

60 **Lamphear JG**, Stevens KR, Rich RR. Intercellular adhesion molecule-1 and leukocyte function-associated antigen-3 provide costimulation for superantigen-induced T lymphocyte proliferation in the absence of a specific presenting molecule. *J Immunol* 1998; **160**: 615-623 [PMID: 9551895]

61 **Yusuf-Makagiansar H**, Anderson ME, Yakovleva TV, Murray JS, Siahaan TJ. Inhibition of LFA-1/ICAM-1 and VLA-4/VCAM-1 as a therapeutic approach to inflammation and autoimmune diseases. *Med Res Rev* 2002; **22**: 146-167 [PMID: 11857637 DOI: 10.1002/med.10001]

62 **Anderson ME**, Siahaan TJ. Targeting ICAM-1/LFA-1 interaction for controlling autoimmune diseases: designing peptide and small molecule inhibitors. *Peptides* 2003; **24**: 487-501 [PMID: 12732350 DOI: 10.1016/s0196-9781(03)00083-4]

63 **Ford ML**, Adams AB, Pearson TC. Targeting co-stimulatory pathways: transplantation and autoimmunity. *Nat Rev Nephrol* 2014; **10**: 14-24 [PMID: 24100403 DOI: 10.1038/nrneph.2013.183]

64 **Gourley TS**, Wherry EJ, Masopust D, Ahmed R. Generation and maintenance of immunological memory. *Semin Immunol* 2004; **16**: 323-333 [PMID: 15528077 DOI: 10.1016/j.smim.2004.08.013]

65 **Xu H**, Perez SD, Cheeseman J, Mehta AK, Kirk AD. The allo- and viral-specific immunosuppressive effect of belatacept, but not tacrolimus, attenuates with progressive T cell maturation. *Am J Transplant* 2014; **14**: 319-332 [PMID: 24472192 DOI: 10.1111/ajt.12574]

66 **Mou D**, Espinosa JE, Stempora L, Iwakoshi NN, Kirk AD. Viral-induced CD28 Loss evokes costimulation independent alloimmunity. *J Surg Res* 2015; **196**: 241-246 [PMID: 25801976 DOI: 10.1016/j.jss.2015.02.033]

67 **Espinosa J**, Herr F, Tharp G, Bosinger S, Song M, Farris AB 3rd, George R, Cheeseman J, Stempora L, Townsend R, Durrbach A, Kirk AD. CD57(+) CD4 T Cells Underlie Belatacept-Resistant Allograft Rejection. *Am J Transplant* 2016; **16**: 1102-1112 [PMID: 26603381 DOI: 10.1111/ajt.13613]

68 **Mathews DV**, Wakwe WC, Kim SC, Lowe MC, Breeden C, Roberts ME, Farris AB, Strobert EA, Jenkins JB, Larsen CP, Ford ML, Townsend R, Adams AB. Belatacept-Resistant Rejection Is Associated With CD28+ Memory CD8 T Cells. *Am J Transplant* 2017; **17**: 2285-2299 [PMID: 28502128 DOI: 10.1111/ajt.14349]

69 **Leibler C**, Thiolat A, Hénique C, Samson C, Pilon C, Tamagne M, Pirenne F, Vingert B, Cohen JL, Grimbert P. Control of Humoral Response in Renal Transplantation by Belatacept Depends on a Direct Effect on B Cells and Impaired T Follicular Helper-B Cell Crosstalk. *J Am Soc Nephrol* 2018; **29**: 1049-1062 [PMID: 29321143 DOI: 10.1681/ASN.2017060679]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com