

# World Journal of *Transplantation*

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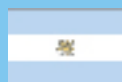
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## Organ assessment and repair centers: The future of transplantation is near

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### Abstract

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

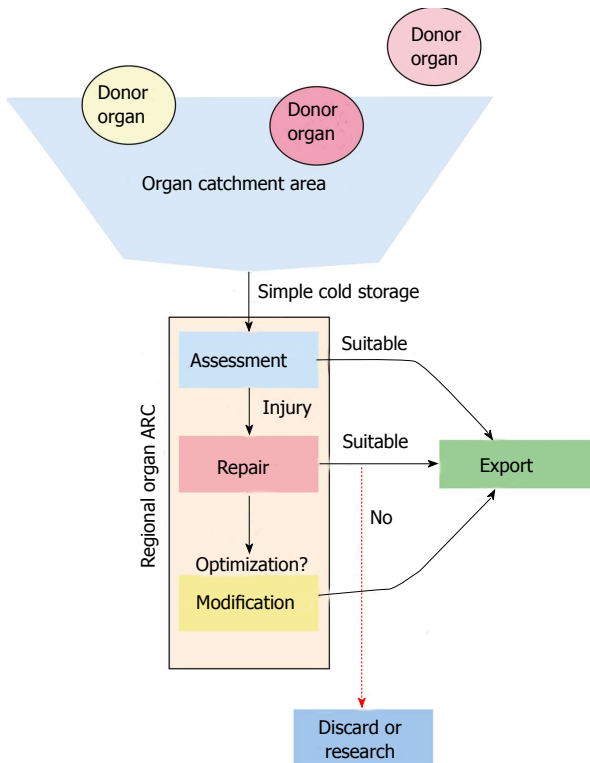
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**Key words:** Organ transplantation; *Ex vivo* organ perfusion; Lung; Liver; Kidney; Heart

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

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

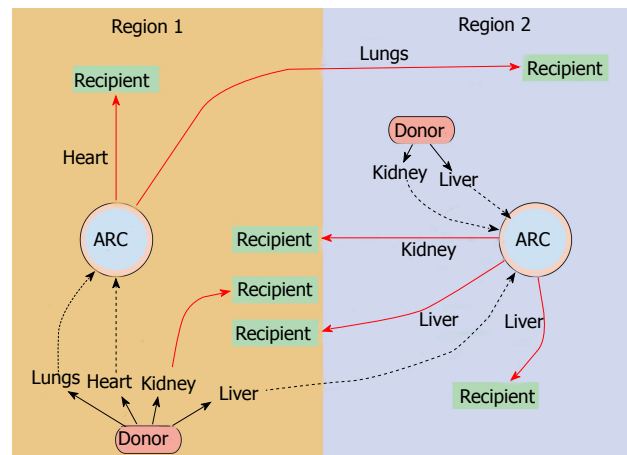
Regional organ ARCs build upon normo-thermic *ex vivo* organ perfusion (EVOP) technology, which in turn provides a potential platform to assess, repair and eventually modify donor organs. Currently, normo-thermic EVOP allows for assessment of organ function prior to transplantation and is largely focused clinically on lung transplantation. Normo-thermic EVOP allows for potential donor organs to have their function evaluated and



**Figure 1** A conceptualized Organ assessment and repair centers would receive donated organs (traditional and marginal donor quality) and assess their function. During the assessment, the quality, viability, and function of the evaluated organ would be determined. There may be subspecialization of assessment and repair center with local expertise in one or two organ types. ARC: Assessment and repair center.

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

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



**Figure 2** Through the use of *ex vivo* organ perfusion, preservation times would be extended. With this extension of total ischemic times, organs would have the opportunity to truly be matched with the recipient who would most benefit from that particular organ at that particular time. Occasionally, the allocation may be the current, traditional approach, where the allocation directly bypasses the ex-vivo assessment at the assessment and repair center and proceed directly to the recipient. (Adapted from Black and Whitson, 2013)<sup>[8]</sup>. ARC: Assessment and repair center.

izing a regional organ ARC approach.

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

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

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

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

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

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

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

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### Abstract

Selection of the appropriate donor is essential to a successful allograft recipient outcome for solid organ transplantation. Multiple infectious diseases have been transmitted from the donor to the recipient *via* transplantation. Donor-transmitted infections cause increased morbidity and mortality to the recipient. In recent years, a series of high-profile transmissions of infections have occurred in organ recipients prompt-

ing increased attention on the process of improving the selection of an appropriate donor that balances the shortage of needed allografts with an approach that mitigates the risk of donor-transmitted infection to the recipient. Important advances focused on improving donor screening diagnostics, using previously excluded high-risk donors, and individualizing the selection of allografts to recipients based on their prior infection history are serving to increase the donor pool and improve outcomes after transplant. This article serves to review the relevant literature surrounding this topic and to provide a suggested approach to the selection of an appropriate solid organ transplant donor.

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**Key words:** Donor selection; Infection; Transplantation; Mass screening; Treatment outcome

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

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



## INTRODUCTION

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

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

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

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

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

## BACTERIAL INFECTIONS

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

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

**Table 1** Mortality figures by type of transplant for 2010 according to the Scientific Registry of Transplant Recipients 2011 Annual Report<sup>1</sup>

Organ transplanted <sup>2</sup>	Waiting list mortality incidence density <sup>3</sup> (deaths per 1000 patient-years)	1 yr posttransplant mortality incidence density (deaths per 1000 patient-years)
Kidney	56.5	34.9
Liver	115.6	123.7
Intestine	71.6	193.5
Heart	115.8	91.8
Lung	154.1	164.2

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

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

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

**Table 2** Approach to selecting suitable donors for solid organ transplantation

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

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

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

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



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

Bacterial meningitis and syphilis may be present in a potential organ donor and, as such, may be transmitted to the allograft recipient. The disparity between available allografts and those awaiting transplantation has grown, such that, these two conditions are no longer deemed absolute contraindications for organ donation. Multiple cases of donor-transmitted syphilis have been reported<sup>[46-48]</sup>. The estimated prevalence of syphilis among potential organ donors based on the incidence in the general population is 0.15%<sup>[49]</sup>. Transmission of syphilis is a rare event, but if a donor tests positive for this organism additional consent from the recipient and/or family should be obtained. Most experts agree that if the organ is accepted the recipient should be treated with a

regimen for late latent syphilis consisting of benzathine penicillin 2.4 million units intramuscularly every week for a total of 3 wk<sup>[1]</sup>. Syphilis IgG of the recipient should be assessed at time of transplant and at 1, 3, 6, and 12 mo. Patients with documented bacterial meningitis are also no longer considered to be excluded from organ donation, provided that pathogen-directed treatment has been initiated. Several instances of successful allograft procurement have been reported in the literature from donors with microbiologically proven bacterial meningitis<sup>[50-54]</sup>. Guidelines now recommend accepting an organ if the etiology of the meningitis is *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Escherichia coli*, or group B streptococcus. Meningitis must be confirmed as the sole site of infection in the donor and acceptance of donor allografts infected with highly virulent organisms such as *Listeria* species should be rejected. Ideally, the donor should be receiving appropriate therapy for 48 h prior to procurement with signs of clinical improvement. Additional consent from the recipient and/or family should be obtained and pathogen-directed therapy of the recipient should be continued for at least 2 wk<sup>[1]</sup>.

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

## TUBERCULOSIS

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

**Table 3** Suggested approach to donor-transmitted *Mycobacterium tuberculosis*

Deceased donors							
<sup>1</sup> TB Risk	<sup>2</sup> Suggestive radiology	<sup>3</sup> Donor testing	<sup>4</sup> Donor treated	Accept allograft	Additional consent	<sup>5</sup> Recipient treatment	Additional recipient testing
Low	No	Negative	N/A	Yes	None	None	None
Low	Yes	Negative	No/Yes	Yes	Yes	Chemoprophylaxis	None
Low	Yes	Pending	No/Yes	No	N/A	N/A	N/A
Elevated	No	Negative	No/Yes	Yes	Yes	Chemoprophylaxis	None
Elevated	Yes	Negative	No/Yes	Yes	Yes	Chemoprophylaxis	None
Elevated	Yes	Pending	No/Yes	No	N/A	N/A	N/A
Elevated	Yes	Positive	No/Yes	No	N/A	N/A	N/A
Prior active TB	Yes	Negative	Yes	Yes	Yes	Chemoprophylaxis	None
Prior active TB	Yes	Pending	Yes	Yes	Yes	Chemoprophylaxis	None
Prior active TB	Yes	Positive	No/Yes	No	N/A	N/A	N/A
Prior active TB	Yes	Positive	No	No	N/A	N/A	N/A
Active TB	Yes	Positive	No/Yes	No	N/A	N/A	N/A
Living donors							
Low	No	Negative	N/A	Yes	No	None	None
LTBI	No	Positive	Yes	No	Yes	None	None
Active TB	No	Positive	No	No	N/A	N/A	N/A
Elevated	Yes	Negative	No/Yes	Yes	Yes	Chemoprophylaxis	None

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

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

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

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

## VIRAL INFECTIONS

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

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

**Table 4 Factors associated with increased risk for human immunodeficiency virus, hepatitis B virus, hepatitis C virus infection and potential donor transmission**

People who have had sex with person known or suspected to have HIV, HBV, or HCV in the preceding 12 mo
MSM in the preceding 12 mo
Women who have had sex with a man with a history of MSM in the preceding 12 mo
People who have had sex in exchange for money or drugs in the preceding 12 mo
People who have had sex with a person who has had sex in exchange for money or drugs in the preceding 12 mo
People who have had sex with a person who has injected drugs for nonmedical reasons in the preceding 12 mo
A child who is $\leq 18$ mo of age and born to a mother known to be infected with, or at risk for HIV, HBV or HCV infection
A child who has been breastfed within the preceding 12 mo and the mother is known to be infected with, or at risk for HIV, HBV or HCV infection
People who have injected drugs for nonmedical reasons in the preceding 12 mo
People who have been in lockup, jail, prison or a juvenile correctional facility for $\geq 72$ consecutive hours in the preceding 12 h
People who have been newly diagnosed with, or have been treated for, syphilis, gonorrhea, <i>Chlamydia</i> or genital ulcers in the preceding 12 mo
People who have been on hemodialysis in the preceding 12 mo (HCV only)

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

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

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

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

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

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

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

## FUNGAL INFECTIONS

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

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

infections, despite treatment of both donor and recipient<sup>[127]</sup>. Renal allografts from donors with candiduria and lung allografts from donors with bronchial cultures positive for *Candida* species can be used with appropriate treatment. Recipients of lung allograft from a donor with documented *Candida* colonization of the airways have been shown to benefit from universal prophylaxis with an echinocandin for the prevention of early posttransplant infections; including empyema<sup>[127,129]</sup>. Treatment of renal allograft recipients from donors with candiduria should consist of a tailored antifungal agent for urinary tract involvement. Urinary levels of fluconazole exceed minimum inhibitory concentration values for most *Candida* species and can be used in most cases. Therapy should be continued for up to 6 wk depending on whether there is vascular involvement of the urinary tract<sup>[62,127,130]</sup>. After lung transplant, treatment should be continued until bronchoscopic evaluation confirms the integrity of the bronchial anastomosis<sup>[127]</sup>.

Cryptococcosis can occur in up to 5% of SOT recipients<sup>[131]</sup>. Most infections after transplant represent reactivation of recipient latent infection, but DTIs do occur in a subset of patients<sup>[132,133]</sup>. The potential for cryptococcal DTI should be considered when a donor presents with undiagnosed neurological illness, unrecognized meningo-encephalitis, or pulmonary nodules in the setting of risk factors for cryptococcosis, such as prior hematologic malignancy, steroid treatment, sarcoidosis, or other cell-mediated immune dysfunction<sup>[134]</sup>. Cerebrospinal fluid cryptococcal antigen and serum cryptococcal antigen should be obtained from donors who meet these clinical risk factors. Donors with active cryptococcal disease should be excluded from donation. Recovery of *Cryptococcus* in the recipient should not be treated as contamination or colonization, but should prompt initiation of therapeutic antifungal treatment<sup>[135]</sup>.

Endemic fungal infection should be considered as a potential DTI when donors reside in endemic areas or travel frequently to areas with high incidence of histoplasmosis, blastomycosis, or coccidioidomycosis. These areas include the Ohio and Mississippi river valleys, the Great Lakes region, and Southwestern United States, respectively. Since histoplasmosis occurs in only 0.5% of SOT recipients residing in endemic regions, routine laboratory screening of all donors is not warranted<sup>[136]</sup>. Donors should be evaluated for a prior history or signs and symptoms compatible with active histoplasmosis. If current concerns or prior history exist, an assessment consisting of agar gel immunodiffusion, complement fixation antibody titers, and urine *Histoplasma* antigen should be undertaken. The presence of antigenuria, H precipitin bands, or complement fixation antibody titers  $\geq 1:32$  should lead to rejection of the donor allograft. Coccidioidomycosis is a dimorphic fungus that is endemic in the Southwestern United States, Mexico, Central and South America. Approximately 150000 infections occur annually in the US, with an estimated 1.4%-6.9% of transplant recipients becoming infected<sup>[137]</sup>. Reactivation of latent infection is the most common mode of



posttransplant infection, but multiple cases of DTI have been documented in patients from both endemic and non-endemic areas<sup>[138,139]</sup>. Patients with active coccidioidomycosis should not be permitted to donate an organ for transplantation. In donors with prior history of coccidioidomycosis, an evaluation should be undertaken to document clearance of infection; including history documenting the resolution of symptoms, resolution of radiographic abnormalities, and at least a 4-fold decrease in antibody titer<sup>[140]</sup>. Fluconazole or itraconazole can be used for the prevention of DTI in the event that a recipient receives an organ from a donor who in retrospect had evidence of remote infection<sup>[141]</sup>. Lifelong prophylaxis is indicated following treatment doses for at least one year. Fluconazole at an average daily dose of 200-400 mg can be used depending on whether prophylaxis is primary or secondary<sup>[137]</sup>.

## PARASITIC INFECTIONS

With increase in international travel and immigration, potential organ donors have greater risk for parasitic infections not endemic to the United States. Transmission of Chagas disease, schistosomiasis, and *Strongyloides* has been reported<sup>[142-144]</sup>.

The optimal screening procedure for schistosomiasis in donors from endemic areas has not yet been established. Screening of living donors from endemic areas with fecal parasitological analysis paired with blood *Schistosoma* antibody detection assay is a reasonable starting point. This can be followed with a stepwise approach including rectal biopsy, liver biopsy, or both depending on the results of the initial screening tests. If stool analysis shows *Schistosoma* eggs, liver biopsy should be performed regardless of the result of *Schistosoma* serology. In the situation where *Schistosoma* eggs are not detected in the stools but the donor is noted to be seropositive for *Schistosoma*, further investigation with a rectal biopsy is indicated. If rectal biopsy demonstrates *Schistosoma* eggs, all allografts from this donor should be rejected. If eggs are found on initial screening, living donor treatment with praziquantel should be initiated followed by repeat testing of stools for *Schistosoma* eggs. Only if repeat stool testing is negative, should the patient be accepted to donate<sup>[145]</sup>.

Screening of both donors and recipients for strongyloidiasis in the pretransplant period is recommended for those at epidemiologic risk and should include both serology and stool studies<sup>[146]</sup>. A donor with documented strongyloidiasis should not be precluded from donation, but additional consent from the recipient should be obtained. Recipients of organs from such donor should be prophylactically treated with ivermectin.

Chagas disease is an infection caused by the parasite *Trypanosoma cruzi*. It is endemic to Mexico, Central, and South America but has the potential to cause DTI in the setting of transplantation from a donor from an endemic region to a recipient in a non-endemic country<sup>[147]</sup>. Most posttransplant infections occurring in recipients from endemic regions occur due to reactivation of latent infec-

tion as a result of iatrogenic immunosuppression. Transmission rates from seropositive donors to seronegative recipients are approximately 20% for kidney transplant recipients and 30% for liver transplant recipients. Screening for Chagas disease should be performed on donors who were born or spent significant time living in an endemic country<sup>[148]</sup>. Donors who have a history of treated Chagas disease should also be screened using the Ortho enzyme immunoassay test (Ortho-Clinical Diagnostics, Inc.; Raritan, New Jersey) and the Abbot Prism Chagas test (Abbott Laboratories; Abbott Park, Illinois). If the initial screening of a living donor is positive, a second confirmatory test should be sent to the CDC; using the radioimmune precipitation assay. Deceased donor testing should also be performed but this information may not be available at the time of transplantation<sup>[149]</sup>. No allograft should be accepted from a donor who died from acute Chagas disease. When a donor has positive serology for Chagas disease or has a history of treated Chagas disease, organs other than the heart or intestine may be suitable for transplantation with additional consent and posttransplant screening of the recipient. Testing should include *T. cruzi* PCR and microscopy of blood peripheral smears at predetermined time intervals, or in the event of fever, and when rejection is present. Treatment is only indicated if surveillance testing of the recipient is consistent with *T. cruzi* infection. Heart or intestinal transplantation from a donor with a positive history or serology for *T. cruzi* is thought to represent too high of a potential risk for DTI to be acceptable<sup>[146,150-152]</sup>.

## CONCLUSION

The demand for allografts for the treatment of end-stage disease processes continues to grow. The need for a thoughtful and thorough approach to donor selection has never been more important in balancing unnecessarily discarding potentially lifesaving organs with reducing infectious complications for the recipient after transplant. Decisions regarding donor acceptability should be made in conjunction with a clinician who has special training and experience in dealing with infections related to transplantation. Donor history and physical examination should be meticulous with an emphasis on documenting current or latent infections that can be transmitted to the recipient. Screening using molecular and microbiological testing should be attempted, as time permits, prior to organ procurement in order to allow for rejection of an unacceptable allograft, or to allow for monitoring and treatment in the recipient. As the need for organs continues to rise, special attention will be focused on ways to expand the donor pool.

Multiple HIV-infected patients die each year awaiting organs that could be provided from living or deceased HIV-infected donors. Approximately 500 HIV positive deceased donors are not currently being utilized to donate organs to HIV-positive recipients<sup>[153]</sup>. Improvements in antiretroviral therapy and report of successful kidney transplantation from a donor with HIV infection in

South Africa make this an interesting, albeit complicated, area for future evaluation and research. A key advancement has recently occurred with the passage of the HIV Organ Policy Equity Act (HOPE Act) on 11/21/2013.

Improved development of NAAT in conjunction with defined and validated algorithms of application may allow for faster and more accurate testing of donor specimens enabling previously excluded donors to be accepted for donation. Focused efforts to reassess the risk of using high-risk donors should be undertaken, and methods for decreasing recipient risk of DTI are imperative. Finally, it is important to continue to build on the substantial contributions to quality and safety made by the DTAC in recent years. Providers should be strongly encouraged to report any possible donor-transmitted event in real time. Critical infrastructure is now in place to investigate potential DTI, to take appropriate action in the treatment of potential recipients at risk, and to analyze indispensable data in the pursuit of evidence-based decision making essential to improving outcomes in this unique patient population.

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## Calcineurin inhibitor sparing strategies in renal transplantation, part one: Late sparing strategies

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### Abstract

Kidney transplantation improves quality of life and reduces the risk of mortality. A majority of the success of kidney transplantation is attributable to the calcineurin inhibitors (CNIs), cyclosporine and tacrolimus, and their ability to reduce acute rejection rates. However, long-term graft survival rates have not improved over time, and although controversial, evidence does suggest a role of chronic CNI toxicity in this failure to improve outcomes. Consequently, there is interest in reducing or removing CNIs from immunosuppressive regimens in an attempt to improve outcomes. Several strategies exist to spare calcineurin inhibitors, including use of agents such as mycophenolate mofetil (MMF), mycophenolate sodium (MPS), sirolimus, everolimus or belatacept to facilitate late calcineurin inhibitor withdrawal, beyond 6 mo post-transplant; or using these agents to plan early withdrawal within 6 mo; or to avoid the CNIs all together using CNI-free regimens. Although numerous reviews have been written on this topic, practice varies significantly between centers. This review organizes the

data based on patient characteristics (*i.e.*, the baseline immunosuppressive regimen) as a means to aid the practicing clinician in caring for their patients, by matching up their situation with the relevant literature. The current review, the first in a series of two, examines the potential of immunosuppressive agents to facilitate late CNI withdrawal beyond 6 mo post-transplant, and has demonstrated that the strongest evidence resides with MMF/MPS. MMF or MPS can be successfully introduced/maintained to facilitate late CNI withdrawal and improve renal function in the setting of graft deterioration, albeit with an increased risk of acute rejection and infection. Additional benefits may include improved blood pressure, lipid profile and serum glucose. Sirolimus has less data directly comparing CNI withdrawal to an active CNI-containing regimen, but modest improvement in short-term renal function is possible, with an increased risk of proteinuria, especially in the setting of baseline renal dysfunction and/or proteinuria. Renal outcomes may be improved when sirolimus is used in combination with MMF. Although data with everolimus is less robust, results appear similar to those observed with sirolimus.

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**Key words:** Kidney transplantation; Calcineurin inhibitor; Withdrawal; Sparing; Cyclosporine; Tacrolimus; Renal function; Graft survival

**Core tip:** Mycophenolic acid derivatives have been used successfully to facilitate late calcineurin inhibitor withdrawal to improve short-term renal function in kidney transplantation. The benefit carries an increased risk of acute cellular rejection. Sirolimus and everolimus are also options, but have comparatively less evidence and carry an increased risk of proteinuria, which is dependent on baseline renal function.

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## INTRODUCTION

Compared with hemodialysis, kidney transplantation improves quality of life and reduces of mortality risk<sup>[1-3]</sup>. The survival benefit of kidney transplantation over hemodialysis applies even to the use of marginal donor kidneys<sup>[4]</sup>. Much of this success has been attributed to calcineurin inhibitors, cyclosporine and tacrolimus, and their ability to reduce acute rejection rates. However, despite dramatic reductions in acute rejection rates over time, long-term graft survival rates have not improved to an appreciable extent<sup>[5,6]</sup>. A number of factors have been postulated that contribute to the lack of improvement in graft survival, including donor factors, recipient factors, human leukocyte antigen matching, death with a functioning allograft, delayed graft function, calcineurin inhibitor toxicity, chronic allograft nephropathy, and infectious nephropathy (BK virus)<sup>[6]</sup>.

Calcineurin inhibitor nephrotoxicity was recognized early after the use of cyclosporine began, and it comes in many forms<sup>[7]</sup>. Calcineurin inhibitors cause acute and chronic nephrotoxicity. The acute forms include arteriopathy, tubular vacuolization and thrombotic microangiopathy. Chronic forms of nephrotoxicity include interstitial fibrosis and tubular atrophy, medial arteriolar hyalinosis, glomerular capsular fibrosis, global glomerulosclerosis, focal segmental glomerulosclerosis, juxtaglomerular apparatus hyperplasia, and tubular microcalcifications, many of which can be caused by other factors and tend to be nonspecific findings on post-transplant biopsy<sup>[7]</sup>. Because of the known contribution of calcineurin inhibitors to nephrotoxicity, there has been much interest in finding the optimal agent and/or regimen<sup>[8-14]</sup>. While many studies demonstrated improved renal function with reduced dose calcineurin inhibitor use, or an early benefit on renal function with tacrolimus use when compared to cyclosporine, improvements in long-term graft function were not demonstrated<sup>[9-14]</sup>. Additionally, there are numerous differences in the adverse event profile of cyclosporine and tacrolimus. Many outside factors differentiate the calcineurin inhibitors and influence their contribution to nephrotoxicity, including therapeutic drug monitoring strategy, dosing strategy, drug-drug interaction, pharmacogenetics, and non-adherence<sup>[15-20]</sup>. These medication-related variables make nephrotoxicity and decline in allograft function very difficult to predict in practice. The lack of surveillance biopsies also makes differentiation of outcomes related to calcineurin inhibitor use and non-medication related factors difficult in practice<sup>[21-25]</sup>.

A long-term biopsy study helped determine the true consequence of calcineurin inhibitors on chronic allograft nephropathy and graft failure<sup>[26]</sup>. In a well-

designed study, Nankivell *et al*<sup>[26]</sup> demonstrated the natural history of chronic allograft nephropathy in 120 type 1 diabetics who underwent kidney-pancreas transplant followed by routine biopsies over a 10-year period. The initial phase (year 1) in the development of chronic allograft nephropathy was characterized by early tubulointerstitial damage from ischemic injury, prior severe rejection, and subclinical rejection, where these findings were present in 94.2% of patients. Beyond year 1, chronic allograft nephropathy was characterized by microvascular and glomerular injury and chronic rejection, defined as subclinical rejection for two or more years, and was uncommon (5.8%). Progressive high-grade arteriolar hyalinosis with luminal narrowing, increasing glomerulosclerosis, and tubulointerstitial damage were linked to the calcineurin inhibitors, and were irreversible. Despite dose reductions of both cyclosporine and tacrolimus, calcineurin inhibitor nephrotoxicity was nearly universal by 10 years, and was found to be the chief cause of late injury and renal function decline<sup>[26]</sup>.

The data from Nankivell *et al*<sup>[26]</sup> suggested that chronic allograft nephropathy was primarily a function of calcineurin inhibitor nephrotoxicity. This has been interpreted with controversy, but the data surrounding the definition and pathophysiology of chronic allograft nephropathy have always been controversial, due to varied definitions utilized in both practice and research<sup>[27,28]</sup>. In addition, many believe that calcineurin inhibitor nephrotoxicity is a non-specific finding<sup>[7,22]</sup>. Still, the evidence from Nankivell<sup>[26]</sup> is the among the most robust long-term evidence available on calcineurin inhibitors. It should also be mentioned that objective assessment is superior to clinical assessment, to determine the presence of chronic allograft nephropathy and calcineurin inhibitor nephrotoxicity, because clinicians underestimate the chronic renal toxicity<sup>[29,30]</sup>. Despite underestimation, clinicians have many ways of dealing with perceived medication toxicity. Commonly, when adverse effects are noted, adjustments are made in the regimen of the individual patient<sup>[31]</sup>. This may result in unintended consequences, such as acute rejection and graft loss<sup>[32-34]</sup>.

Collectively, protocols have been developed to assess the conversion between calcineurin inhibitors, or to select a preferable one, in order to avoid certain toxicities, or promote renal function improvements or short-term graft survival<sup>[9-14,35]</sup>. However, in a paired kidney analysis from a database with 5-year follow-up, no difference could be determined between cyclosporine and tacrolimus with respect to allograft survival, despite superior renal function in the tacrolimus group<sup>[36]</sup>. These results were similar in a prospective study with mean 2.8 years follow-up, and supported a 5-year histologic study that determined similar development of moderate to severe arteriolar hyalinosis with cyclosporine or tacrolimus<sup>[37,38]</sup>. When patients are switched between the two calcineurin inhibitors, or one is used in preference to the other, the basic tenet that calcineurin inhibitors are the primary contributors to graft decline is ignored<sup>[30]</sup>. In addition, the graft decline appears to occur primarily between 5 and 10

years post transplant<sup>[26]</sup>. It must also be emphasized that switching agents off-protocol in an uncontrolled way may have harmful effects, and is inconsistent with evidence-based practice<sup>[32]</sup>.

In recent years, various schools of thought have emerged with the introduction of newer agents and experience gained through research. The main strategies are based on personalization, corticosteroid minimization, and calcineurin inhibitor sparing<sup>[39,40]</sup>. It is too soon for personalized medicine, although the foundation has been laid<sup>[17-19,39]</sup>. Steroid avoidance strategies have been generally disappointing. They focus on minimizing adverse effects, and usually require calcineurin inhibitor persistence for successful outcome<sup>[40-47]</sup>. Calcineurin inhibitor sparing strategies also aim to reduce adverse effects, but also seek to improve graft survival<sup>[43-66]</sup>. Understanding the different treatment options may lead to improvement in long-term care.

Although the calcineurin inhibitor sparing strategies have been extensively reviewed, we aim to provide a unique approach to the issue. Since many transplant centers have set protocols for their specific populations, and clinical trial results or experiences of other centers may not be generalizable, we aim to review the literature according to general age groups (adult and pediatric) and therapeutic approaches (de novo, early or late) based on the specific baseline regimens used. We will analyze calcineurin inhibitor withdrawal and avoidance, and only touch on minimization when directly compared since exposure appears to lead to chronic toxicity and follow-up was usually inadequate to determine the true consequence on chronic allograft nephropathy<sup>[26,54]</sup>.

Due to the expanse of the issue, we will divide the topic into two manuscripts. The first, herein, will cover late calcineurin inhibitor withdrawal, beyond 6 mo post-transplant, and the second will cover early withdrawal and de novo avoidance. We will focus primarily on renal function and graft survival as the main outcomes of interest, and make recommendations based on the available evidence for each clinical subgroup since data on predicting or monitoring the outcome of changes in immunosuppression are still lacking<sup>[67-78]</sup>. The intent of the article is to aid the practicing clinician in identifying studies relevant to their practice to assist in clinical decision making. The clinician may have to refer the cited articles to find more specific information, such as the countries where the analysis was performed, ethnic breakdown of the population, transplant characteristics, *etc.*

## STRATEGIES

Three basic strategies are available for calcineurin-sparing, “Avoidance”, and “Early” and “Late” reduction or withdrawal. Late, defined as calcineurin inhibitor reduction withdrawal or elimination beyond 6 mo ( $> 6$  mo) after the kidney transplant, is a strategy that has been frequently used when patients are faced with diminishing renal function, possibly related to established toxicity, and is the focus of this manuscript. Early, defined as calci-

neurin inhibitor withdrawal or reduction within the first 6 mo ( $\leq 6$  mo) after the kidney transplant, is generally done to prevent anticipated calcineurin inhibitor toxicity or in response to early evidence of diminished renal function. Calcineurin inhibitor avoidance or calcineurin inhibitor-free regimens are typically a proactive strategy in response to the concerns about the potential toxicity of the calcineurin inhibitors and their failure to promote long-term graft survival, despite dramatic reduction in the risk of acute cellular rejection. Early and de novo are the focus of a second manuscript in this series.

Our search strategy involved PubMed database for all years until August 2013 for articles involving kidney or renal transplantation with the search terms calcineurin inhibitor “reduction”, “withdrawal”, “elimination”, “avoidance”, “minimization”, “sparing” and “free”. References of identified articles were reviewed to identify additional articles of interest. Articles were separated according to the post-transplant time period when the intervention took place, according to the three categories (avoidance, early, and late), and then arranged according to population and baseline regimen. Based on the assumption that most long-term calcineurin inhibitor nephrotoxicity is irreversible and progressive, and minimization articles were only included if they directly compared with avoidance or withdrawal/elimination regimens. The remainder of the article will summarize the available evidence by patient type, intervention and baseline regimen.

## ADULT PATIENTS AT VARIABLE TIME POST-TRANSPLANT

### *Regimens utilizing older agents to eliminate calcineurin inhibitors*

**Baseline calcineurin inhibitor and corticosteroid with or without azathioprine:** A meta-analysis by Kasiske *et al.*<sup>[79]</sup> evaluated early studies of calcineurin inhibitor withdrawal in patients on a baseline regimen of azathioprine, cyclosporine and corticosteroid, and compared calcineurin inhibitor withdrawal with continuation (part 1), and calcineurin inhibitor withdrawal with patients who never received calcineurin inhibition (part 2)<sup>[79]</sup>. In part 1 of the meta-analysis, 17 studies were included, with 9 of them including patients withdrawn during the first 6 mo after the transplant. The mean duration of follow-up of the studies was  $26.6 \pm 7.5$  mo. It should be noted that the meta-analysis included mixed populations, containing patients withdrawn due to toxicity of cyclosporine (3 studies), patients with stable renal function and/or without recent rejection (10 studies), recipients of living donor kidneys (6 studies), and patients with first transplant (4 studies). In part 1, there was a higher rate of acute rejection episodes per patient in the cyclosporine withdrawal group (0.126; 95%CI: 0.085-0.167;  $P < 0.001$ ), but no difference in graft loss per patient per year (-0.009; 95%CI: -0.022-0.004,  $P = 0.19$ ) or deaths per patient per year (-0.005; 95%CI: -0.016-0.006,  $P = 0.4$ ). The authors noted a trend toward higher serum creatinine in the con-

trol group who continued cyclosporine relative to those who discontinued the agent ( $1.84 \pm 0.29$  mg/dL *vs*  $1.63 \pm 0.28$  mg/dL;  $P = 0.17$ ). In part 2 of the meta-analysis, consisting of 6 studies, 3 included stable patients, none involved withdrawal due to toxicity, 3 studies included living donor kidneys, and 2 studies included only the first allograft, and 5 were performed in the first 6 mo after the transplant. The mean duration of follow-up was  $28.8 \pm 11.6$  mo. When the six studies were analyzed together, the rate of graft loss per patient per years was similar ( $-0.02$ ; 95%CI:  $-0.022$ - $0.003$ ,  $P = 0.08$ ), but when only the 3 randomized trials were considered, graft survival was better among those withdrawn from cyclosporine ( $0.0382$ ; 95%CI:  $0.0002$ - $0.0762$ ,  $P = 0.049$ ). The deaths per patient per year were similar ( $0.001$ ; 95%CI:  $-0.006$ - $0.008$ ,  $P = 0.87$ ) and the serum creatinine was non significantly higher in the group who never received calcineurin inhibitors ( $1.71 \pm 0.36$  *vs*  $1.50 \pm 0.18$  mg/dL;  $P = 0.2$ ). The authors noted that none of the outcomes were affected by the timing (before or after 6 mo) or method (slow or rapid taper) of cyclosporine withdrawal<sup>[79]</sup>.

This meta-analysis demonstrated that cyclosporine withdrawal resulted in an early increase in the risk of acute cellular rejection, but similar graft function, graft survival and patient survival at about 2-year follow-up to patients retained on cyclosporine or who never received cyclosporine<sup>[79]</sup>. Despite promising results, azathioprine as an antiproliferative has been largely replaced in practice with newer agents that are considered more potent immunosuppressants. Another study evaluated withdrawal of cyclosporine using azathioprine versus mycophenolate mofetil in patients 1 year post-transplant. The primary endpoint was development of donor-specific antibodies (DSAs), measured by complement-dependent cytotoxicity assay, enzyme-linked immunosorbent assay (ELISA) and flow-cytometry crossmatch with donor spleen cells. DSAs, by three methods were not detected during cyclosporine treatment or during acute rejection treatment while on cyclosporine, but after conversion to azathioprine, 3 of 8 (37.5%) had DSAs in the presence of acute rejection, while none (0 of 6) of the mycophenolate mofetil patients had DSAs during rejection. These results highlight the potential benefits of mycophenolic acid over azathioprine, which have been described previously<sup>[80-82]</sup>.

## ADULT PATIENTS 6 OR MORE MONTHS POST-TRANSPLANT

### *Regimens utilizing mycophenolic acid to eliminate calcineurin inhibitors*

**Baseline calcineurin inhibitor and corticosteroid:** At least two studies<sup>[83,84]</sup> evaluated patients withdrawn late from a calcineurin inhibitor with a baseline regimen of calcineurin inhibitor and corticosteroid (Table 1)<sup>[83-93]</sup>. One study was designed to prospectively evaluate arterial distensibility and endothelial function before and after removal of cyclosporine in a population with biopsy-

proven CAN and deteriorating renal function. MMF was introduced at 500 mg per day and increased to a target dose of 2000 mg per day over 4 wk. The mean daily dose of MMF was 1700 mg at the end of the trial. Half the patients were randomized to withdrawal (tapered to off over 4 wk) and half to cyclosporine continuation. At 6 mo, serum creatinine increased slightly in both groups, but to a numerically greater extent on the control group who remained on cyclosporine. Though blood pressure improved from baseline in the intervention group, but not in the control group, there was no significant effect on brachial artery endothelial-dependent vasodilation. Acute rejection was not reported<sup>[83]</sup>. Another study performed by the same investigators also evaluated patients with biopsy-proven CAN, serum creatinine less than 4 mg/dL, and deteriorating renal function. That study introduced MMF more aggressively, at 1 g/d, and titrated to 2 g/d over 3 wk, and then patients were randomized to withdrawal or continuation of the calcineurin inhibitor. In patients randomized to withdrawal, the calcineurin inhibitor was reduced by 33% every 2 wk. The primary endpoint of slope of reciprocal serum creatinine per month at week 35 was positive and higher ( $0.00585 \pm 0.01122$ ) in the dual therapy group than the triple therapy group ( $-0.00728 \pm 0.01105$ ). Additional findings were the degree of proteinuria ( $P = 0.01$ ), diastolic blood pressure ( $P = 0.04$ ) and mean arterial pressure ( $P = 0.04$ ), which were lower in the dual therapy group at follow-up. No episodes of acute rejection were reported<sup>[84]</sup>. These results provide modest evidence that late withdrawal of calcineurin inhibitor with replacement by MMF may improve renal function, or at least reduce the rate of deterioration of renal function, and improve blood pressure relative to calcineurin inhibitor continuation.

**Baseline calcineurin inhibitor, corticosteroid and azathioprine:** A prospective, single-center randomized trial randomized patients on cyclosporine, azathioprine and corticosteroid, with biopsy-proven CAN and deteriorating renal function to MMF or tacrolimus. In patients randomized to cyclosporine, it was discontinued 24 h before tacrolimus was initiated. In patients randomized to MMF, MMF was introduced at 500 mg twice daily and then titrated up over 2-4 wk to 2 g/d. After 6 wk, cyclosporine was incrementally reduced to achieve withdrawal by 14 wk. Azathioprine was discontinued at conversion. At 6-mo, measured glomerular filtration rate (GFR) and serum creatinine were not improved in the tacrolimus group, but in the MMF group, GFR ( $P < 0.001$ ) and serum creatinine ( $P < 0.001$ ) were improved. In contrast, total cholesterol and triglycerides improved from baseline in the tacrolimus group, but not in the MMF group, and systolic and diastolic blood pressure improved in the MMF group, but not the tacrolimus. There were no reported rejection episodes<sup>[85]</sup>. Another study evaluated consecutive patients converted from cyclosporine, azathioprine and corticosteroid to MMF plus corticosteroid for CAN. Azathioprine was immediately stopped and MMF was introduced over 1 wk, with target dose of

**Table 1** renal transplant studies utilizing mycophenolic acid to withdraw calcineurin inhibitor beyond 6 mo post-transplant ("Late")<sup>[83-93]</sup>

Ref.	Design	Population (n)	Baseline Regimen	n	Strategy	Follow-up	Renal function	Acute rejection	Graft survival	Patient survival
Kosch <i>et al</i> <sup>[83]</sup>	Prospective, randomized, single-center	6-mo of deteriorating renal function, BP-CAN	CsA, Prednisolone	12	MMF added, target 2 g per day; CsA withdrawn over 4 wk	6 mo	SCr + 0.03 mg/dL vs baseline ( $P = NS$ )	NA	NA	NA
				12	MMF added, target 2 g; CsA continued		SCr + 0.07 mg/dL vs baseline ( $P = NS$ )	NA	NA	NA
Suwelack <i>et al</i> <sup>[84]</sup>	Prospective, randomized, single-center	> 1-yr post transplant, SCr < 4 mg/dL, BP-CAN, deteriorating renal function	CsA or TAC, Prednisolone	18	MMF added, target 2 g; CsA withdrawn over 6 wk	35 wk	Slope 1/SCr 0.00585 ± 0.01122; 67% responders; Proteinuria 0.5 ± 0.55 g/24 h	0%	100%	NA
				20	MMF added, target 2 g; CsA continued		Slope 1/SCr -0.00728 ± 0.01105 ( $P = 0.0018$ ); 25% responders ( $P = 0.021$ ); Proteinuria 1.5 ± 0.48 g/24 h ( $P = 0.01$ )	0%	85%	NA
McGrath <i>et al</i> <sup>[85]</sup>	Prospective, randomized, single-center	> 1-yr post transplant, BP-CAN, deteriorating renal function	CsA, azathioprine, prednisolone	15	MMF added, target 2 g; CsA withdrawn by 14 wk	6 mo	SCr - 58 µmol/L vs baseline ( $P < 0.001$ ); isotope GFR + 8.5 mL/min vs baseline ( $P < 0.01$ )	0%	NA	NA
				15	CsA switch to TAC		SCr + 15 µmol/L vs baseline ( $P = NS$ ); isotope GFR -2.1 mL/min vs baseline ( $P = NS$ )	0%	NA	NA
Hanvesakul <i>et al</i> <sup>[86]</sup>	Retrospective, consecutive patients, single-center	> 1-yr post transplant, CAN	CsA or TAC, azathioprine, prednisolone	30	MMF added, target 1.5-2 g; azathioprine stopped; CNI withdrawn over 4 wk	1 yr	eGFR + 2 mL/min vs baseline	3.30%	86.70%	96.70%
Dudley <i>et al</i> <sup>[87]</sup>	Randomized, open, multicenter	> 6-mo post transplant, deteriorating renal function, no recent ACR	CsA monotherapy, or CsA/corticosteroid, or CsA/azathioprine/corticosteroids	73	MMF added, target 2 g; azathioprine discontinued, if applicable; CsA withdrawn over 6 wk, if needed corticosteroid added	1 yr	Response rate (6 mo): 58% stabilized or reduced SCr; Response rate (1 yr): 48%; Least squares mean SCr -24.9 µmol/L; Least squares mean CrCL +5 mL/min	0%	93.20%	95.90%
			CsA monotherapy, or CsA/corticosteroid, or CsA/azathioprine/corticosteroids	70	Continued regimen		Response rate (6 mo): 32% stabilized or reduced SCr ( $P = 0.006$ ); Response rate (1 yr): 35% ( $P = 0.1885$ ); Least squares mean SCr +22.2 µmol/L ( $P < 0.01$ ); Least squares mean CrCL -0.7 mL/min ( $P < 0.01$ )	0%	94.3%	100%



Weir <i>et al</i> <sup>[88]</sup>	Prospective, non-randomized, single-center	Mean 853.3 d post transplant, BP-CAN, deteriorating renal function, no ACR	CsA or TAC, prednisone, azathioprine or MMF	18	Azathioprine stopped; MMF added, target 2 g; CNI withdrawn	Mean 651 d	Response rate: 91.7% improved or lack of deterioration in renal function using least squares method slope 1/SCr ( $P = 0.038$ )	NCR	100%	NA
			CsA, prednisone, azathioprine or MMF	67	CsA dose reduced approximately 50%; azathioprine withdrawn; MMF added, target 2 g		Response rate: 51.7% improved or lack of deterioration	NCR	100%	NA
			TAC, prednisone, azathioprine or MMF	33	TAC dose reduced approximately 50%; azathioprine withdrawn; MMF added, target 2 g		59.3% improved or lack of deterioration	NCR	100%	NA
Weir <i>et al</i> <sup>[89]</sup>	Continuation of above trial			13	CNI withdrawn	76 mo	2.7 ± 0.2 mg/dL	7.7%	92.3%	100%
				64	CsA dose reduced	54 mo	3 ± 0.1 mg/dL	4.7%	62.5%	92.2%
				28	TAC dose reduced	42 mo	3 ± 0.2 mg/dL	7.1%	67.8%	100%
Abramowicz <i>et al</i> <sup>[90]</sup>	Randomized, controlled, multicenter	No recent ACR, ≤ 1 ACR overall, 12 to 30 mo post-transplant, stable renal function	CsA, prednisone, ± azathioprine or MMF	85	MMF added over 3 mo, target 2 g; CsA withdrawn over 3 mo	12 mo	CrCL improved 10% in 46%; SCr -1 µmol/L; CrCL + 4.5 mL/min <i>vs</i> control group ( $P = 0.16$ ), eGFR + 2.3 mL/min <i>vs</i> control group ( $P = 0.24$ )	10.6%	100%	NA
				85	MMF added over 3 mo, target 2 g; continued triple therapy		SCr + 4 µmol/L	2.4% ( $P = 0.03$ )	100%	
Abramowicz <i>et al</i> <sup>[91]</sup>	Continuation of above trial			74	CsA withdrawn	60 mo	CrCL 67.4 mL/min	10%	88%	93%
				77	Triple therapy		CrCL 61.7 mL/min ( $P = 0.05$ )	1% ( $P = 0.028$ )	92%	95%
Heeg <i>et al</i> <sup>[92]</sup>	Retrospective	BP-CNI toxicity, deteriorating renal function, mean 11.2 mo post-transplant	CsA or TAC, Prednisolone, ± MMF or MPS	17	MPS added; CNI withdrawn; MMF withdrawn	48 mo	All <i>vs</i> Baseline. SCr at 6 mo -0.5 mg/dL ( $P < 0.05$ ); eGFR at 6 mo + 11 mL/min; SCr at 36 mo -0.5 mg/dL ( $P = 0.063$ ); eGFR at 36 mo +11 mL/min $P = 0.022$ ; SCr at 48 mo + 0.6 mg/dL ( $P = 0.27$ ); eGFR at 48 mo +1 mL/min ( $P = 0.91$ )	NA	NA	NA
Mourer <i>et al</i> <sup>[93]</sup>	Prospective, randomized, single-center	No recent ACR, ≤ 2 ACR overall, at least 12 mo post-transplant, stable renal function	CsA or TAC, Prednisone, MMF	79	CNI withdrawn, MMF concentration controlled	36 mo	eGFR 59.5 ± 2.1 mL/min	5.1%	98.7%	94.9%
				79	MMF withdrawn, CNI concentration controlled		eGFR 51.1 ± 2.1 mL/min ( $P = 0.006$ )	2.5%	98.7%	92.4%

ACR: Acute cellular rejection; BP-CAN: Biopsy-proven chronic allograft nephropathy; CAN: Chronic allograft nephropathy; CNI: Calcineurin inhibitor; CsA: Cyclosporine; eGFR: Estimated glomerular filtration rate; GFR: Glomerular filtration rate; MMF: Mycophenolate mofetil; MPS: Mycophenolate sodium; NA: not assessed/applicable; NCR: Not clearly reported by group; NS: Not significant; SCr: Serum creatinine; TAC: Tacrolimus.

1500 to 2000 mg per day. Calcineurin inhibitor was withdrawn over 4 wk by 25% reduction. Estimated GFR improved from the time of conversion to 1-year follow-up

by 2 mL/min, but the authors cautioned that there was a dramatic increase in the risk of infection in the patients converted to MMF<sup>[86]</sup>.

**Baseline calcineurin inhibitor monotherapy, calcineurin inhibitor with corticosteroid, calcineurin inhibitor with azathioprine, or calcineurin inhibitor, corticosteroid and azathioprine:** The “Creeping Creatinine” study<sup>[87]</sup> evaluated patients on various calcineurin inhibitor-based regimen who did not receive MMF at baseline. In the open, randomized, multicentered trial, patients had a negative slope of reciprocal serum creatinine, baseline serum creatinine of 100 to 400  $\mu\text{mol/L}$  and a calculated creatinine clearance of at least 20 mL/min. A biopsy had to show absence of transplant glomerulopathy, recurrent renal disease, de novo renal disease, obstruction, renal artery stenosis, acute rejection, or acute rejection within 3 mo. Patients were randomized to MMF or maintenance of cyclosporine according to normal practice. Those randomized to MMF had the drug introduced incrementally over 4 wk to a target dose of 2 g/d, and corticosteroids were introduced if not previously used. Cyclosporine was reduced in three steps over 6 wk to off. Patients randomized to maintain cyclosporine were continued as per usual practice with a permitted reduction of cyclosporine to a trough not less than 80 ng/mL. Baseline biopsies documented CAN in 78% of the MMF group and 77% of the cyclosporine group. A responder, defined as an improvement in the slope of 1/SCr with no change in the randomized treatment and no graft loss occurred in 58% of the MMF group and 32% of the control group ( $P = 0.006$ ) at 6 mo and 48% of the MMF group and 35% of the control group ( $P = 0.1185$ ) at 1 year. At 12-mo the least squares mean (LSM) creatinine clearance was +5 mL/min in the MMF group and -0.7 mL/min in the cyclosporine group ( $P < 0.01$ ). LSM serum creatinine and serum cholesterol were lower in the MMF group at follow-up, and platelet count was higher, but triglycerides, hemoglobin, white blood cell count, systolic blood pressure and diastolic blood pressure were not significantly different. There were no acute rejection episodes in either group. The incidence of diarrhea, abdominal pain and opportunistic infections were numerically higher in the MMF group<sup>[87]</sup>.

**Baseline calcineurin inhibitor and corticosteroid with or without azathioprine or MMF:** A study evaluated patients on calcineurin inhibitor and corticosteroid, with or without azathioprine or MMF, in a prospective non-randomized, single-centered fashion where decision to reduce or withdrawal CNI was arbitrary<sup>[88]</sup>. Patients with deteriorating renal function and CAN on biopsy were started on MMF (target 2 g/d) if it was not previously given, and azathioprine was stopped. Patients were analyzed in three groups, those who had CNI withdrawn ( $n = 18$ ), those with a 50% reduction in cyclosporine after MMF introduction ( $n = 67$ ), and those with 50% reduction in tacrolimus after MMF was introduced ( $n = 33$ ). At mean 651 d follow-up, 91.7% of the withdrawal group, 51.7% of the reduced dose cyclosporine group, and 59.3% of the reduced dose tacrolimus group had improved or lack of deterioration in the LS 1/SCr ( $P = 0.038$ ). The withdrawal group also had lower serum

glucose ( $P < 0.05$ ) and total cholesterol ( $P < 0.05$ ), but not systolic or diastolic blood pressure. It should be noted that patients selected for CNI withdrawal had a lower incidence of acute rejection prior to the intervention, but the nadir serum creatinine was similar in all three groups<sup>[88]</sup>. A continuation of the trial, out to 76 mo demonstrated that about one third of the CNI reduction patients and only 7.7% of the withdrawal group lost their graft during follow-up ( $P = 0.05$ ). The serum creatinine at follow-up was 2.7 mg/dL in the withdrawal group and 3 mg/dL in the CNI reduction groups<sup>[89]</sup>. A randomized, controlled, multicenter trial also evaluated patients on cyclosporine and corticosteroid, with or without azathioprine or MMF. Patients were selected if they had a first or second cadaveric or living transplant, were between 12-30 mo post-transplant and maintained on a cyclosporine-based regimen. Patients had to have had no more than one acute rejection episode, with none in the last 3 mo, and a SCr less than 300  $\mu\text{mol/L}$  for at least 3 mo. All patients had MMF introduced to a target of 2 g/d over 3 mo. Patients randomized to cyclosporine withdrawal had it tapered over 3 mo ( $n = 85$ ) and those randomized to remain on cyclosporine ( $n = 85$ ), continued on triple-drug therapy. Creatinine clearance improved by 10% in 46% of the withdrawal group, and the creatinine clearance difference was 4.5 mL/min higher in the withdrawal group 9 mo after randomization ( $P = 0.16$ ). Serum creatinine improved by decreasing 1  $\mu\text{mol/L}$  in the withdrawal group, and increased 4  $\mu\text{mol/L}$  in the continuation group, creating a net effect of 5  $\mu\text{mol/L}$  in favor of the withdrawal group ( $P = 0.34$ ). Withdrawal improved the total ( $P = 0.02$ ) and low density lipoprotein (LDL) cholesterol ( $P = 0.015$ ), but blood pressure did not differ significantly. Acute rejection (10.6% vs 2.4%;  $P = 0.03$ ) and diarrhea were more common in the withdrawal group<sup>[90]</sup>. A five-year follow-up publication demonstrated a creatinine clearance of 67.4 mL/min in the withdrawal group and 61.7 mL/min ( $P = 0.05$ ) in the continuation group, but graft loss due to chronic rejection occurred in 12% of the withdrawal group and 8% of the continuation group, due to a respective acute rejection rate of 10% and 1% ( $P = 0.028$ )<sup>[91]</sup>.

**Baseline calcineurin inhibitor with or without corticosteroid and with or without MMF or mycophenolate sodium:** One retrospective study analyzed 17 patients approximately 11 years post-transplant for 4 years before and after conversion to mycophenolate sodium (MPS) for biopsy-proven CNI toxicity ( $n = 7$ ) or clinical deterioration of GFR and exclusion for other reasons for graft dysfunction. Patients on CNI and corticosteroid were converted to MPS and prednisolone, patients on CNI monotherapy were converted to MPS alone, and patients on triple therapy were converted to MPS with prednisolone. At conversion, GFR was  $43 \pm 15$  mL/min. After conversion, graft function, as determined by GFR, improved within one month, and peaked at  $55.7 \pm 21.7$  mL/min at one year ( $P = 0.00362$ ), but then declined to near-baseline ( $44 \pm 27$  mL/min;  $P = 0.91$ ) by four years,



indicating a slowing of progression. However, the overall slope of the regression line for GFR did not change significantly ( $P = 0.116$ ). Three patients discontinued MPS due to infection ( $n = 2$ ) and lost to follow-up ( $n = 1$ )<sup>[92]</sup>. A randomized trial compared CNI withdrawal ( $n = 79$ ) with MMF withdrawal ( $n = 79$ ) in patients who were on CNI/MMF/corticosteroid triple therapy. This trial used concentration controlled area-under-the-curve (AUC) monitoring for the CNIs (3250 ng/mL per hour for cyclosporine, 120 ng/mL per hour for tacrolimus) and MMF (75 µg/mL per hour). Estimated GFR was significantly better in the CNI withdrawal group at 6 wk ( $63.1 \pm 1.9$  mL/min *vs*  $55.2 \pm 1.9$  mL/min;  $P = 0.004$ ), 1-year ( $61.1 \pm 1.8$  mL/min *vs*  $52.9 \pm 1.8$  mL/min;  $P = 0.002$ ), and 3-year ( $59.5 \pm 2.1$  mL/min *vs*  $51.1 \pm 2.1$  mL/min;  $P = 0.006$ ). By 6 mo, 1.3% of the MMF withdrawal group and 3.8% of the CNI withdrawal group had biopsy-proven acute rejection. None were high immunologic risk. Three year graft survival did not differ. Blood pressure, lipid values, proteinuria and infections did not differ between the groups. Anemia was more frequent in the CNI withdrawal group<sup>[93]</sup>.

#### Summary of MMF and mycophenolic acid studies:

These studies suggest that MMF or MPS can be introduced or maintained to facilitate late (beyond 6-mo post-transplant) CNI withdrawal after kidney transplantation in the setting of graft deterioration and BP-CAN. Withdrawal of CNI using MMF or MPS appears to improve serum creatinine and creatinine clearance/GFR in a majority of patients, without an increased risk of proteinuria. The studies also demonstrate a potential for this strategy to improve blood pressure, lipid profile and serum glucose<sup>[94]</sup>. Benefits of mycophenolic acid derivatives may be offset by increased risk of acute rejection and infection, so patients should be carefully selected. It appears that concentration-controlling the administration may limit the occurrence of these adverse events and possibly explain differences in adverse effects, such as diarrhea<sup>[93,95-97]</sup>. Taken individually, these studies were too small and too limited in follow-up to determine an improvement in graft survival, but a meta-analysis did demonstrate a trend toward improvement in graft survival (OR = 0.72, 95%CI: 0.52-1.01,  $P = 0.06$ ) with CNI withdrawal using MMF in a mixed population that was not limited to late withdrawal<sup>[98]</sup>. Generally speaking, our findings are in line with other recent reviews and meta-analyses, and support a potential role of late CNI elimination with mycophenolic acid derivatives<sup>[98-101]</sup>.

#### Regimens utilizing sirolimus to eliminate calcineurin inhibitors

**Baseline regimen not specified:** The mammalian target of rapamycin inhibitor (mTOR), sirolimus, has also been used to eliminate CNIs. A study<sup>[102]</sup> evaluated patients more than one year post-transplant with chronic allograft dysfunction according to baseline proteinuria stratification in 3 groups, and either withdrew CNI with addition of sirolimus or reduced the dose of CNI with addition

of sirolimus as shown in Table 2<sup>[102-118]</sup>. As shown, the patients who had sirolimus added demonstrated a statistically significant increase in proteinuria when CNI was withdrawn, but not when CNI was reduced. The post-conversion increase in proteinuria was greater, when the baseline proteinuria value was higher. In addition, when analyzed overall (both withdrawal and continuation combined based on baseline proteinuria) the group with negative baseline proteinuria had a mean 10.4 mL/min ( $P = 0.05$ ) improvement in CrCL over about 2 years, while the group with baseline proteinuria 0.3-0.8 g/d had a mean 7 mL/min ( $P = \text{NS}$ ) improvement in CrCL, and the group with baseline proteinuria > 0.8 g/d had a 5.5mL/min ( $P = 0.05$ ) decline in CrCL. Taken together these results suggested that use of sirolimus to facilitate CNI withdrawal beyond 1 year had the potential for an adverse impact on renal function that was dependent on the baseline level of proteinuria. Another retrospective study<sup>[103]</sup> examined 30 patients with unspecified baseline regimen and with about 2 years of follow-up based on indication for switching from CNI to sirolimus, as shown in Table 2. They concluded that sirolimus was associated with an improvement in CrCL and an increase in proteinuria, but that the benefits were achieved only when the conversion occurred within the first year after the transplant<sup>[103]</sup>.

#### Baseline corticosteroid and either azathioprine or calcineurin inhibitor:

A cohort study evaluated 19 patients who had sirolimus added and CNI withdrawn by 3 mo for progressive CAN. At 6-mo follow-up, 36% demonstrated improvement in renal function, 21% exhibited stabilization, and 43% resulted in continued worsening. Patients who demonstrated improvement in renal function had lower baseline SCr ( $2.6 \pm 0.9$  mg/dL *vs*  $3.3 \pm 0.7$  mg/dL)<sup>[104]</sup>.

#### Baseline calcineurin inhibitor and corticosteroid with or without mycophenolate mofetil:

A retrospective study<sup>[105]</sup> of patients more than 1 year post-transplant with CAN examined 32 patients for 8.5 mo who had sirolimus added to their regimen and CNI reduced. Only 3 patients had improved SCr (9.4%) and 13 (40.6%) had stable SCr, suggesting that 50% of the population achieved a benefit from the strategy of CNI dose reduction with sirolimus introduction. The authors suggested that the benefit was greater when the baseline SCr was less than 3 mg/dL.

#### Baseline tacrolimus and mycophenolate mofetil with or without corticosteroids:

A prospective, randomized study of 200 patients more than 1 year post-transplant, with about 3.5 years follow-up, examined sirolimus addition with trough target 5-8 ng/mL and tacrolimus withdrawal by week 2 ( $n = 123$ ) or continuation of the current regimen with target tacrolimus trough of 6-8 ng/mL. As shown in Table 2, the GFR decreased, and proteinuria increased to a similar degree in both groups during follow-up, with similar acute cellular rejection (ACR) and graft survival, suggesting no tangible benefit to the late switch<sup>[106]</sup>. In contrast, a cohort study analyzed

Table 2 Renal transplant studies utilizing sirolimus to withdraw calcineurin inhibitor beyond 6 mo post-transplant

Ref.	Design	Population (n)	Baseline regimen	n	Strategy	Follow-up	Renal function	Acute rejection	Graft survival	Patient survival
Gutierrez <i>et al</i> <sup>[102]</sup>	Cohort	> 1-yr post transplant, chronic allograft dysfunction, no proteinuria	Not specified	8	SRL added, CNI dose reduced 50%	24.6 mo	Proteinuria = +0.56 g/d <i>vs</i> baseline ( <i>P</i> = NS)	NA	90.50%	85.70%
				13	SRL added, CNI withdrawn		Proteinuria = + 0.67 g/d <i>vs</i> baseline ( <i>P</i> = 0.02)			
		> 1-yr post transplant, chronic allograft dysfunction, proteinuria = 0.3-0.8 g/d		10	SRL added, CNI dose reduced 50%	23.2 mo	Proteinuria = +0.5 g/d <i>vs</i> baseline ( <i>P</i> = NS)	NA	83.30%	94.40%
				8	SRL added, CNI withdrawn		Proteinuria = +1.1 g/d <i>vs</i> baseline ( <i>P</i> = 0.05)			
		> 1-yr post transplant, chronic allograft dysfunction, proteinuria > 0.8 g/d		14	SRL added, CNI dose reduced 50%	25.9 mo	Proteinuria = -0.1 g/d <i>vs</i> baseline (NS)	NA	79.20%	87.50%
Maharaj <i>et al</i> <sup>[103]</sup>	Retrospective cohort		Not specified				Proteinuria = +2.3 g/d <i>vs</i> baseline ( <i>P</i> = 0.01)			
		> 1-yr post transplant, CsA-induced biochemical toxicity		6	SRL added, CNI withdrawn	25 mo	Proteinuria = +0.06 g/d <i>vs</i> baseline eGFR = +12.2 mL/min <i>vs</i> baseline	NA	NA	NA
		> 1-yr post transplant, CAN		6			Proteinuria = +0.85 g/d <i>vs</i> baseline eGFR = -9.7 mL/min <i>vs</i> baseline	NA	NA	NA
		> 1-yr post transplant, Severe gum hypertrophy		9			Proteinuria = +0.99 g/d <i>vs</i> baseline eGFR = -1.0 mL/min <i>vs</i> baseline	NA	NA	NA
		4.5 mo post transplant, Posttransplant diabetes		4			Proteinuria = -0.22 g/d <i>vs</i> baseline eGFR = +13.3 mL/min <i>vs</i> baseline	NA	NA	NA
		5.5 mo post transplant, CNI induced histological nephrotoxicity		2			Proteinuria = +0.63 g/d <i>vs</i> baseline eGFR = -10.0 mL/min <i>vs</i> baseline	NA	NA	NA
		> 1-yr post transplant, CNI associated malignancy		3			Proteinuria = +0.09 g/d <i>vs</i> baseline eGFR = +7.0 mL/min <i>vs</i> baseline	NA	NA	NA
Citterlo <i>et al</i> <sup>[104]</sup>	Cohort	> 6-mo post transplant, deteriorating renal function, sCr 2-4.5 mg/dL, proteinuria > 500 mg/d, biopsy confirmed fibrosis, tubular atrophy and intimal hyperplasia	CsA or TAC or azathioprine with corticosteroid	19	SRL added to target trough 8-12 ng/mL, CNI withdrawn by 3 mo	6 mo	Response rate: 57% improved or lacked deterioration in renal function	0%	NA	100%

Wu <i>et al</i> <sup>[105]</sup>	Retrospective cohort	> 1-yr post transplant, CAN	CsA or TAC/ corticosteroids or CsA or TAC/ corticosteroids/ MMF	32	SRL added with CNI dose reduced	8.5 mo	Response rate: 50% improved or lacked deterioration in renal function	3.10%	87.50%	NA
Chhabra <i>et al</i> <sup>[106]</sup>	Randomized, prospective, open-label, single-center	> 1-yr post transplant	TAC, MMF	123	SRL added to target trough 5-8 ng/mL, TAC withdrawn by week 2	41.1 mo	eGFR = -3.3 mL/min per 1.73 m <sup>2</sup> vs baseline proteinuria > 1 g/d = + 4.7% vs baseline	5.70% (ACR) 4.1% (AHR)	97.60%	97%
				64	Continue TAC to target trough 6-8 ng/mL	40.7 mo	eGFR = -8.7 mL/min per 1.73 m <sup>2</sup> vs baseline, proteinuria > 1 g/d = + 7.4% vs baseline	6.40% (ACR) 3.1% (AHR)	97%	100%
Wali <i>et al</i> <sup>[107]</sup>	Cohort	Renal dysfunction and biopsy confirmed CAN	TAC/MMF or TAC/MMF/ corticosteroids	159	SRL added, target trough 8-10 ng/mL, TAC withdrawn after second dose of SRL	24 mo	sCr = -1.1 mg/dL vs baseline ( $P < 0.0001$ ) eGFR = +21 mg/dL vs baseline ( $P < 0.0001$ )	9.60%	65%	90%
Diekmann <i>et al</i> <sup>[108]</sup>	Cohort	> 1-yr post transplant, biopsy confirmed CNI toxicity	CsA or TAC/ corticosteroids, or CsA or TAC/ corticosteroids/ azathioprine, or CsA or TAC/ corticosteroids/ MMF, or CsA or TAC/ MMF, or TAC/MMF/ corticosteroids CsA or TAC/ azathioprine	22	SRL added, target trough 8-12 ng/mL, CsA or TAC reduced by 50% immediately then further reduced 10%-20% weekly	6 mo	sCr = -0.7 mg/dL vs baseline (% = NS), Response rate: 59.1% improved or lacked deterioration in renal function	NA	86%	100%
Bumbea <i>et al</i> <sup>[109]</sup>	Prospective, single-center cohort	>6-mo post transplant, chronic allograft dysfunction or recurrent cutaneous cancer	CsA or TAC/ corticosteroids, or CsA or TAC/ corticosteroids/ azathioprine or CsA or TAC/ corticosteroids/ MMF	43	SRL added, target trough = 8-12 ng/mL, CNI withdrawn abruptly or by week 3	27 mo	sCr = -17.8 μmol/L vs baseline ( $P = NS$ ) CrCL = +2.3 mL/min vs baseline ( $P = NS$ ) Proteinuria (> 1g/d): 20.6% at 2 yr ( $P = 0.01$ )	0%	93%	95.30%
Boratynska <i>et al</i> <sup>[110]</sup>	Cohort	> 1-yr post transplant, biopsy confirmed CAN	CsA, prednisone, azathioprine	5	SRL added, target trough 10-18 ng/mL, CsA withdrawn immediately. After 5 mo, SRL withdrawn and CsA reinitiated	3 mo	sCr = +1.6 mg/dL and proteinuria = +461 mg/dL after 3 mo SRL vs baseline sCr = +1.1 mg/dL and proteinuria = +6 mg/dL 6 mo after reconversion to CsA vs baseline sCr = -0.5 mg/dL and proteinuria = -455 mg/dL after reconversion to CsA vs SRL	0%	40%	100%
Martínez-Mier <i>et al</i> <sup>[111]</sup>	Retrospective cohort	> 6-mo post transplant, > 20% sCr increase in 6 mo or sCr 2-4.5 mg/dL	CsA, prednisone, MMF	15	SRL added, target trough 8-12 ng/mL, CsA withdrawn immediately	6 mo	sCr = -0.78 mg/dL vs baseline ( $P = 0.003$ ) BUN = - 9.84 mg/dL vs baseline ( $P = NS$ )	0%	100%	100%

Kamar <i>et al</i> <sup>[112]</sup>	Prospective, multicenter, noncomparative, open-label cohort	> 1-yr post transplant, moderate renal insufficiency, sCr 160-265 $\mu\text{mol/L}$	CsA or TAC, corticosteroids, MMF	44	SRL added to target trough 6-10 ng/mL, CNI withdrawn	6 mo	GFR = +7.09 mL/min <i>vs</i> baseline ( $P = 0.03$ ) Proteinuria = +0.57 g/d	2.30%	100%	100%
Chen <i>et al</i> <sup>[113]</sup>	Cohort	> 6-mo post transplant, biopsy confirmed CAN	CsA or TAC, prednisone, MMF	16	SRL added, target trough 5-8 ng/mL, CNI withdrawn	12 mo	Response rate: 43.8% improved or lacked deterioration in renal function	0%	88%	100%
Stallone <i>et al</i> <sup>[114]</sup>	Prospective, open-label, single-center	> 1-yr post transplant, Scr 1-3 mg/dL	CsA or TAC, corticosteroids, MMF	50	40% CNI dose reduction	24 mo	sCr = -0.02 mg/dL <i>vs</i> baseline ( $P = \text{NS}$ ) CrCL -3.0 mL/min <i>vs</i> baseline ( $P = \text{NS}$ ) Proteinuria = +0.17 <i>vs</i> baseline ( $P = \text{NS}$ ) Follow-up biopsy: worsened CAN score, increased $\alpha$ -SMA	0%	84%	100%
				34	SRL added, CNI immediately withdrawn		sCr = -0.14 mg/dL <i>vs</i> baseline ( $P = \text{NS}$ ) CrCL = +3.0 mg/dL <i>vs</i> baseline ( $P = \text{NS}$ ) Proteinuria = +0.37 g/d <i>vs</i> baseline ( $P = \text{NS}$ ) Follow-up biopsy: stable CAN score, improved $\alpha$ -SMA	0%	97% ( $P = 0.04$ )	100%
Paoletti <i>et al</i> <sup>[115]</sup>	Cohort	> 6-mo post transplant, biopsy confirmed renal allograft dysfunction	CsA or TAC, corticosteroids, MMF	13	SRL added, target trough 4-8 ng/mL, CNI withdrawn	3 yr	sCr = -0.3 mg/dL <i>vs</i> baseline ( $P = 0.016$ ) eGFR = +5.5 mg/dL <i>vs</i> baseline ( $P = 0.011$ ) Proteinuria = +0.21 g/d <i>vs</i> baseline ( $P = 0.83$ )	8%	100%	100%
		> 6-mo post transplant with stable graft function		26	Continued regimen		sCr = +0.3 mg/dL <i>vs</i> baseline ( $P = 0.016$ ) eGFR = -6.4 mg/dL <i>vs</i> baseline ( $P = 0.011$ ) Proteinuria = +0.17 g/d <i>vs</i> baseline ( $P = 0.83$ )	4%	96%	96%
Alarrayed <i>et al</i> <sup>[116]</sup>	Retrospective, Observational, single-center	> 1-yr post transplant, sCr < 140 $\mu\text{mol/L}$	CsA or TAC, corticosteroids, azathioprine or MMF	45	SRL added to target trough 5-8 ng/mL, CNI withdrawn immediately	72.8 mo	sCr = -6 $\mu\text{mol/L}$ <i>vs</i> baseline ( $P = 0.001$ ) Proteinuria = +0.2 g/d <i>vs</i> baseline ( $P = \text{NS}$ )	0%	100%	NA
		> 1-yr post transplant, sCr $\geq 140$ $\mu\text{mol/L}$		19			sCr = -13 $\mu\text{mol/L}$ <i>vs</i> baseline ( $P = 0.01$ ) Proteinuria = +0.6 g/d <i>vs</i> baseline ( $P = 0.001$ )	36.40%	72.70%	NA



Fischereder <i>et al</i> <sup>[117]</sup>	Prospective cohort	> 1-yr post transplant, deteriorating renal function, Scr 1.8-4 mg/dL	CsA or TAC, corticosteroids, azathioprine or MMF	12	SRL added, target trough = 10-20 ng/mL, CNI withheld by 4 wk	12 mo	sCr = -0.3 mg/dL <i>vs</i> baseline ( <i>P</i> = 0.198) CrCL = +5.8 mL/min ( <i>P</i> = 0.0368) Proteinuria = +735 mg/g creatinine <i>vs</i> baseline ( <i>P</i> = 0.13)	0%	100%	100%
Schena <i>et al</i> <sup>[118]</sup>	Randomized, prospective, open-label, multicenter, blinded, comparative trial	> 6-mo post transplant, baseline GFR > 40 mL/min	CsA or TAC, corticosteroids, azathioprine or MMF	497	SRL added, target trough 8-20 ng/mL, CNI withdrawn in 1 d, MMF or azathioprine dose reduced or withdrawn	24 mo	GFR = +1.3 mL/min in patients converted to SRL as compared with patients continued on CNI at 12 mo ( <i>P</i> = NS) GFR = +1.3 mL/min <i>vs</i> baseline, UPr/Cr = -84 <i>vs</i> baseline	7.80%	92.40%	95.60%
		> 6-mo post transplant, baseline GFR 20-40 mL/min		58			GFR = +3.8 mL/min in patients converted to SRL as compared with patients continued on CNI at 24 mo ( <i>P</i> = NS)	8.60%	65.50%	82.80%
		> 6-mo post transplant, baseline GFR > 40 mL/min		246	Continue regimen		GFR = -1.8 mL/min <i>vs</i> baseline, UPr/Cr = -31 <i>vs</i> baseline	6.50%	93.90%	96.30%
		> 6-mo post transplant, baseline GFR 20-40 mL/min		29			GFR = +2.6 mL/min in patients continued on CNI as compared with patients converted to SRL at 12 mo ( <i>P</i> = NS)	10.30%	62.10%	89.70%

$\alpha$ -SMA: A-smooth muscle actin; AHR: Acute humoral rejection; CAN: Chronic allograft nephropathy; CNI: Calcineurin inhibitor; CsA: Cyclosporine; GFR: Glomerular filtration rate; MMF: Mycophenolate mofetil; NS: Not significant; SCR: Serum creatinine; TAC: Tacrolimus.

patients on tacrolimus/MMF or tacrolimus/MMF/corticosteroids with biopsy-proven CAN and progressive renal dysfunction when tacrolimus was converted to sirolimus (10 mg per day for 3 d, then 5 mg/d targeting trough levels 8-10 ng/mL<sup>[107]</sup>). Overall, SCr decreased and GFR improved, as shown in Table 2. About 1/3 of the patients were non-responders. Although first ACR was about 10%, it was less than the rate observed prior to the conversion (17%). Follow-up biopsies demonstrated significant improvement in interstitial fibrosis and tubular atrophy relative to baseline. It is important to note that this study only included patients who tolerated 90 d of sirolimus therapy<sup>[107]</sup>.

**Baseline CNI with corticosteroids, or CNI with azathioprine, or CNI with mycophenolate mofetil, or CNI with corticosteroids and azathioprine or mycophenolate mofetil:** Two studies evaluated patients with wide variability in baseline regimens<sup>[108,109]</sup>. One study evaluated patients more than one year post-transplant with biopsy proven CNI toxicity (*n* = 22) and demonstrated a modest decrease in SCr and a 59.1% response rate of improved or lack of progression in renal func-

tion deterioration at 6 mo after CNI conversion to sirolimus<sup>[108]</sup>. The other study evaluated patients more than 6 mo post-transplant with chronic allograft dysfunction or recurrent cancer and demonstrated a modest, non-significant reduction in SCr and increase in CrCL at 27 mo follow-up. However, proteinuria greater than 1 g/d occurred in 20.6% of the population at 2 years<sup>[109]</sup>. Neither study reported any episodes of ACR<sup>[108,109]</sup>.

**Baseline calcineurin inhibitor, corticosteroid and azathioprine:** A 5-patient cohort with BP-CAN explored conversion from cyclosporine to sirolimus. After 3 mo, SCr nearly doubled and proteinuria increased, at which time patients were converted back to CNI and proteinuria decreased, but SCr continued to rise, and 3 (60%) patients returned to dialysis<sup>[110]</sup>.

**Baseline calcineurin inhibitor, corticosteroid and azathioprine or mycophenolate mofetil:** A retrospective study of patients more than 6 mo post-transplant, with a 20% increase in SCr in 6 mo or a current SCr 2-4.5 mg/dL were converted to sirolimus with CNI withdrawn immediately. At 6 mo, there was a significant reduction in

SCr versus baseline, and no evidence of ACR, as shown in Table 2<sup>[111]</sup>. A prospective, multicentered study of 44 patients more than one year post-transplant with moderate renal insufficiency demonstrated a 7 mL/min ( $P = 0.03$ ) improvement in GFR with a 0.57 g/d increase in proteinuria ( $P = 0.002$ ). Adverse effects observed included an increase in triglycerides, total cholesterol and LDL cholesterol, and a decrease in hemoglobin levels, and one episode of mild ACR<sup>[112]</sup>. In a cohort of 16 patients with sirolimus added and CNI withdrawn for biopsy proven -chronic allograft nephropathy (BP-CAN), 43.8% demonstrated improved, or lack of deterioration in SCr, without an increased risk of ACR. Patients with SCr at baseline < 2.48 mg/dL were more likely to achieve improvement in SCr after the conversion, and patients with higher SCr or C4d deposition in peritubular capillaries were less likely to achieve success<sup>[113]</sup>.

A prospective open-label single-center study conducted by Stallone and colleagues<sup>[114]</sup> compared a 40% dose reduction in CNI ( $n = 50$ ) with sirolimus addition and CNI elimination ( $n = 34$ ) at greater than 1 year post-transplant<sup>[114]</sup>. Compared with baseline, CNI reduction resulted in no significant change in SCr, CrCL or proteinuria versus baseline at 2 years follow-up. To a similar degree, SCr, CrCL or proteinuria were similar to baseline in the CNI withdrawal group, although graft survival was improved (84% *vs* 97%,  $P = 0.04$ ). On follow-up biopsies, CAN grade and  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) protein expression worsened in the CNI reduction group, and  $\alpha$ -SMA decreased ( $P = 0.005$ ) and CAN grade remained stable in the sirolimus group<sup>[114]</sup>. Another study compared sirolimus addition and CNI elimination ( $n = 13$ ) in patients with BP-CAN versus CNI continuation ( $n = 26$ ) in patients with stable renal function, at least 6 mo post-transplant followed patients for 3 years<sup>[115]</sup>. In that study, sirolimus resulted in an improvement in SCr and GFR, with a statistically significant increase in proteinuria relative to baseline, while CNI continuation resulted in worsening of SCr and GFR and a similar degree of proteinuria. There were more cardiovascular events ( $P = 0.024$ ) in the CNI continuation group, although patient survival was similar. The 3-year change in GFR was the only significant predictor of event-free survival by Cox regression analysis (HR = 0.96, 95%CI: 0.93-0.99,  $P = 0.017$ ), and sirolimus was the strongest predictor of GFR<sup>[115]</sup>.

One retrospective study compared the effects of sirolimus addition and CNI elimination relative to baseline SCr ( $\geq 140 \mu\text{mol/L}$  *vs* < 140  $\mu\text{mol/L}$ ) and found that patients with more baseline renal dysfunction had a larger decline in SCr relative to baseline, but also developed more proteinuria and had a higher rate of ACR (36.4% *vs* 0%)<sup>[116]</sup>. Another prospective study targeted sirolimus trough 10-20 ng/mL and CNI elimination over 4 wk, in patients more than 1 year post-transplant, and demonstrated a 5.8 mL/min improvement in CrCL along with a non-significant increase in proteinuria at 12 mo<sup>[117]</sup>.

A randomized, prospective, open-label multicentered comparative trial (CONVERT) evaluated sirolimus to facilitate CNI withdrawal in the setting of concurrent

azathioprine or MMF reduction or withdrawal versus continuation of the CNI-based regimen, according to baseline GFR (20-40 mL/min *vs* > 40 mL/min) in patients more than 6 mo post-transplant<sup>[118]</sup>. As shown in Table 2, patients with GFR > 40 mL/min and converted to sirolimus had a non-significant improvement in GFR relative to baseline and relative to CNI continuation at 24 mo. Patients with baseline GFR 20-40 mL/min had a slightly higher, but still non-significant improvement in GFR at 24 mo relative to CNI continuation. Graft survival was poor in all patients with baseline GFR 20-40 mL/min regardless of regimen (62%-66%). A post-hoc analysis revealed that patients with GFR > 40 mL/min who had a baseline urinary protein-to-creatinine ratio ( $U_{P/Cr}$ ) less than or equal to 0.11 had more favorable outcome with sirolimus conversion<sup>[118]</sup>.

**Summary of sirolimus studies:** There appears to be less data comparing sirolimus-facilitated late CNI withdrawal to an active CNI-containing regimen than was found for mycophenolic acid (MPA)-facilitated CNI withdrawal. Sirolimus has the potential to support late CNI withdrawal, through a modest improvement in short-term renal function, which has been corroborated in a systematic review<sup>[119]</sup>. However, the benefit of sirolimus is somewhat limited by an increased risk of proteinuria, especially in the setting of baseline renal dysfunction and/or proteinuria and high rate of discontinuation for adverse effects which ranges from 17% in nonrandomized trials to 28% of randomized trials<sup>[119-121]</sup>. Adverse effects of sirolimus on renal function were confirmed in a trial which evaluated late sirolimus withdrawal using MMF and found improvement in the slope 1/SCr in 15 of 17 (88%) patients<sup>[122]</sup>. Renal function results associated with use of sirolimus appear to be improved to a relatively greater degree when sirolimus is used in combination with mycophenolate mofetil<sup>[123]</sup>. This combination may increase the risk of MMF adverse effects, in part due to a drug-drug interaction<sup>[123,124]</sup>. It should also be noted that use of reduced dose CNI in conjunction with sirolimus may also suffer from a pharmacokinetic interaction, which potentiates each's nephrotoxicity<sup>[125]</sup>.

### **Regimens utilizing everolimus to eliminate calcineurin inhibitors**

**Baseline calcineurin inhibitor and unspecified adjunctive agents:** A second mTOR inhibitor, everolimus has also generated evidence on late CNI withdrawal in renal transplantation. As shown in Table 3<sup>[126-135]</sup>, a small case series evaluated 21 Hispanic first renal transplant patients (15 cadaveric), including 5 children, who were undergoing conversion from CNI to everolimus with MPA at a mean 8 mo post-transplant, due to CAN or CNI toxicity. Over 10-mo follow-up there was no mortality or graft loss and a slight mean decline of SCr of 0.2 mg/dL, but ACR rate was 17%<sup>[126]</sup>. Another case series of 78 patients converted CNI to everolimus at a mean 77 mo post-transplant, without manipulation or addition of MPA, and noted a statistically significant mean increase

**Table 3 Renal transplant studies utilizing everolimus to withdraw calcineurin inhibitor beyond 6 mo post-transplant**

Ref.	Design	Population (n)	Baseline regimen	n	Strategy	Follow-up	Renal function	Acute rejection	Graft survival	Patient survival
Giron <i>et al</i> <sup>[126]</sup>	Case series	Conversion due to unspecified reasons in Hispanic renal transplant patients (15 from cadaveric donors), mean conversion 8 mo post-transplant	CsA or TAC, and unspecified regimen	21	Everolimus added with MPS or MMF with complete suspension of CNI	10 mo (range, 2 to 22)	Mean SCr showed a trend to decline: preconversion 1.7 mg/dL; post-conversion 1.5 mg/dL	17%	100%	100%
Sánchez Fructuoso <i>et al</i> <sup>[127]</sup>	Case series, prospective, open	CAN or other reasons, stable renal function, mean 77 mo post-transplant	CNI and unspecified regimen	78	Switched to everolimus with complete and quick elimination of the CNI: An initial dose of 3 mg/d was adequate to obtain the recommended trough levels between 5 and 10 ng/mL	12 mo	Baseline CrCL = 51.9 ± 2.7 mL/min, and 3 mo = 55.7 ± 3.2 ( <i>P</i> = 0.02). 12-mo CrCL not stated. Proteinuria = increased at 3 mo ( <i>P</i> < 0.001), decreased between 3 to 6 mo ( <i>P</i> = 0.001), but remained higher than basal levels ( <i>P</i> = 0.002). Everolimus stopped in 13 patients (16.7%)	NA	NA	NA
Ruiz <i>et al</i> <sup>[128]</sup>	Case Series	CAN with deteriorating renal function	CsA or TAC, and unspecified regimen; triple drug (41%), double-drug (52%), monotherapy (7%)	32	Everolimus added, to eliminate CNI	6 mo	Baseline SCr 1.93 ± 0.13 mg/dL vs 1.86 ± 0.14, <i>P</i> = 0.07. Proteinuria = 1.62 ± 0.62 g/d vs 2.11 ± 0.73 ( <i>P</i> = 0.11)	NA	NA	NA
Fernández <i>et al</i> <sup>[129]</sup>	Case series	Cadaveric renal transplant patients with CAN, at a mean 123.8 ± 74.2 mo post-transplant	CsA or TAC, ± MMF or azathioprine, corticosteroid not specified	17	Converted to everolimus with complete suspension of CNI	24 mo	Baseline SCr of 1.8 ± 0.4; after a year, 1.62 ± 0.49; and after 2 yr, 1.56 ± 0.49 mg/dL ( <i>P</i> < 0.05). Proteinuria was baseline 0.30 ± 0.13 mg/mg, 1 yr = 0.63 ± 0.68 ( <i>P</i> < 0.05), and 2 yr = 0.48 ± 0.34. Protein/creatinine quotient was: baseline 0.30 ± 0.13; one year 0.63 ± 0.68; and 2 yr 0.48 ± 0.34. CrCL was baseline 37.1 ± 11.14 mL/min and 2 yr = 46.6 ± 14.6 ( <i>P</i> < 0.05)	NA	NA	100%
		Cadaveric renal transplant patients treated with non-CAN diagnosis at a mean 123.8 ± 74.2 mo post-transplant	CsA or TAC, ± MMF or azathioprine, corticosteroid not specified	10	Converted to everolimus with complete suspension of CNI	24 mo	Baseline SCr of 1.1 ± 0.32 mg/dL; 1 yr 0.97 ± 0.15, and 2 yr 0.97 ± 0.15. Proteinuria at baseline 0.12 ± 0.07 mg/mg, 1 yr = 0.46 ± 0.68 ( <i>P</i> < 0.05), and 2 yr = 0.32 ± 0.17 ( <i>P</i> < 0.05). Protein/creatinine quotient was: baseline 0.2 ± 0.07, 1 yr = 0.73 ± 0.7, and 2 yr = 0.32 ± 0.17. CrCL was baseline 68.81 ± 19 mL/min and 2 yr 74.56 ± 12.3	NA	NA	50%, due to tumors

Kamar <i>et al</i> <sup>[130]</sup>	Retrospective case-control	DSA-free kidney transplant patients with CNI toxicity, CAN or other diagnosis	CsA or TAC or belatacept, ± MPA or azathioprine, ± corticosteroids	61	Converted to everolimus-based regimen without CNIs	36 ± 25 mo	SCr (mmol/L) baseline 135 ± 37 to 141 ± 54 ( <i>P</i> = NS). aMDRD GFR (mL/min) 54 ± 18 to 56 ± 22 ( <i>P</i> = NS)	NA	NA	NA
			CsA or TAC, ± MPA or azathioprine, ± corticosteroids	61	Matched control patients on CNI		SCr (mmol/L) baseline 133 ± 51 to 131 ± 45 ( <i>P</i> = NS). aMDRD GFR (mL/min) 65.7 ± 25 to 62 ± 24 ( <i>P</i> = NS)			
Morales <i>et al</i> <sup>[131]</sup>	Case series	1 <sup>st</sup> or 2 <sup>nd</sup> transplant, converted due to CAN, nephrotoxicity or malignancy, mean 5 yr post-transplant	CsA or TAC, ± MMF or azathioprine, ± corticosteroid	8	Everolimus added to replace ( <i>n</i> = 6) or decrease (30% reduction) CNI dose ( <i>n</i> = 2) Antiproliferative dose reduced.	1-16 mo	Mean baseline SCr was 1.96 ± 0.69 mg/dL <i>vs</i> 1.59 ± 0.52. Mean CrCL = 51 ± 34.6 mL/min <i>vs</i> 56.5 ± 25.5. Mean Proteinuria:creatinine ratio = 1.34 ± 2.17 <i>vs</i> 1.28 ± 1.19 mg/g.	NA	NA	NA
Holdaas <i>et al</i> <sup>[132]</sup>	Prospective, randomized, open-label, multi-center. ASCERTAIN study	> 6-mopost transplant, renal impairment, no recent ACR < 3 mo	CsA or TAC, ± MPA or azathioprine, ± corticosteroids	127	Everolimus added, target 8-12 ng/mL; to eliminate CNI	24 mo	Mean measured GFR at month 24, 48 ± 22 mL/min per 1.73 m <sup>2</sup> Difference <i>vs</i> control was 1.12 mL/min per 1.73 m <sup>2</sup> , 95%CI : -3.51-5.76 ( <i>P</i> = 0.63). Urine protein: creatinine (mg/mmol) median increased from baseline 16.6 (3.5-413.7) to 32.6 (4.1-665.9; <i>P</i> = 0.007 <i>vs</i> control)	5.50%	94.50%	97.60%
				144	Everolimus added, target 3-8 ng/mL; to decrease CNI dose		Mean measured GFR at month 24, 46.6 ± 21.1 mL/min per 1.73 m <sup>2</sup> . Difference <i>vs</i> control was 0.59 mL/min per 1.73 m <sup>2</sup> , 95%CI: -3.88-5.07 ( <i>P</i> = 0.79). Urine protein: creatinine (mg/mmol) median increased from baseline 13.5 (2.4-319.4) to 22.4 (5.1-513.5; <i>P</i> = 0.54 <i>vs</i> control)	5.60%	92.40%	97.90%
				123	Controls maintained current CNI-based regimen		Mean measure GFR at month 24 46 ± 20.4 mL/min. Urine protein:creatinine (mg/mmol) median remained stable from baseline 14.3 (3.3-431.9) to 19.3 (3.3-431.9)	2.40%	95.10%	100%
Inza <i>et al</i> <sup>[133]</sup>	Case series	Cadaveric kidney allograft, SCr > 2 mg/dL, proteinuria < 1 g/ 24 h	CsA or TAC, ± MPA or sirolimus, corticosteroids	22	Switched CNI to Everolimus, mean starting dose 1.4 mg/d.	24 mo	Baseline CrCL 29.31 ± 10.15 mL/min to 3-mo 37.99 ± 14.44 ( <i>P</i> = 0.0076). No results specified for 24 mo, but authors stated CrCL trended to decline ( <i>P</i> = 0.6). Proteinuria (mg/24 h) increased from baseline 384 ± 26.13 to one month, 958 ± 1019.38 ( <i>P</i> = 0.05), to month 12, 1295 ± 1200.83 ( <i>P</i> = 0.0106)	4.50%	90.50%	100%



Cataneo-Dávila <i>et al.</i> <sup>[134]</sup>	Prospective, randomized, open pilot	> 6-mo post transplant, stable renal function, Banff grade I or II CAN within 6 mo, without ACR or grade III CAN in last 3 mo	CsA or TAC, MMF or azathioprine, corticosteroids	10	MMF or azathioprine were withdrawn and Everolimus added to decrease CNI dose by 80%.	12 mo	Baseline and end-of-study data were as follows: SCr, 1.27 ± 0.35 mg/dL vs 1.24 ± 0.4 mg/dL; estimated GFR = 72.4 ± 19.86 mL/min vs 76.26 ± 22.69 mL/min ( <i>P</i> = NS); microalbuminuria 0 mg/g (range 0-50) vs 0 (range 0-609; <i>P</i> = NS)	10%	NA	NA
			CsA or TAC, MMF or azathioprine, corticosteroids	10	Everolimus added to eliminate CNI gradually. MMF or azathioprine withdrawn, then re-introduced at CNI elimination		Baseline and end-of-study data were as follows: SCr 1.27 ± 0.36 mg/dL vs 1.25 ± 0.3 mg/dL; estimated GFR 66.2 ± 12.95 mL/min vs 66.2 ± 13.73 mL/min ( <i>P</i> = NS); microalbuminuria 0 mg/g (range 0-60) vs 0 (range 0-34; <i>P</i> = NS)	0%	NA	NA
Albano <i>et al.</i> <sup>[135]</sup>	Prospective, randomized, open-label, multi-center. FOREVER trial	Completion of CALLISTO study of patients at risk for DGF, from transplantation to month 12, with proteinuria < 1 g/24 h at month 12	Low-exposure CsA, everolimus, corticosteroids	15	Switch CsA to mycophenolate sodium 720 mg/d, increase everolimus, target trough goal 6-10 ng/mL	12 mo	Median (range) mGFR was 54 (21-87) mL/min at baseline ( <i>P</i> = 0.053 vs CNI at baseline) vs 56 (18-126) mL/min at month 12 ( <i>P</i> = 0.007 vs CNI continuation; <i>P</i> = 0.3 vs baseline). Difference in mGFR (SE) was +10.3 mL/min (4.8) vs baseline. SCr (SE) = 24 μmol/mL (27). Proteinuria least squares mean change from baseline (SE) = 0.16 g/24 h (0.2)	0%	100%	100%
				15	Continue CsA and everolimus unchanged, trough goal 3-8 ng/mL		Median (range) mGFR was 37 (range 18-69) mL/min at baseline ( <i>P</i> = 0.053) vs 32 (12-63) mL/min at month 12 ( <i>P</i> = 0.007). Difference in mGFR (SE) was -4.1 mL/min (5) vs baseline. Proteinuria least squares mean change from baseline (SE) = 0.08 g/24 h (0.23)	6.67%	100%	93.3%

ACR: Acute cellular rejection; aMDRD: Abbreviated modified diet in renal disease; BP-CAN: Biopsy-proven chronic allograft nephropathy; CAN: Chronic allograft nephropathy; CNI: Calcineurin inhibitor; CsA: Cyclosporine; DGF: Delayed graft function; DSA: Donor specific antibody; eGFR: Estimated glomerular filtration rate; GFR: Glomerular filtration rate; MMF: Mycophenolate mofetil; MPA: Mycophenolic acid (includes MMF and MPS); MPS: Mycophenolate sodium; NA: Not assessed/applicable; NCR: Not clearly reported by group; NS: Not significant; SCr: Serum creatinine; TAC: Tacrolimus.

in CrCL of 3.8 mL/min at 3 mo post-conversion, but 12-mo CrCL was not stated. It should be noted that proteinuria increased from baseline at all time points studied, and 16.7% of patients stopped everolimus due to worsening renal function (*n* = 5), dermal eruptions (*n* = 3), or other reasons (*n* = 5)<sup>[127]</sup>. A case series of 32 patients took patients with deteriorating renal function in the face of CAN and added everolimus to eliminate CNI. At 6-mo, SCr decreased slightly, but not significantly (*P* = 0.07), and proteinuria trended toward an increase (*P* = 0.11)<sup>[128]</sup>. Of particular interest, a small study retrospectively com-

pared 17 patients with CAN converted to everolimus with 10 patients being converted to everolimus for other reasons. In the CAN group, SCr was higher and CrCL lower at baseline relative to the non-CAN group. SCr in the CAN group decreased steadily out to 2 years follow-up (*P* < 0.05), and CrCL improved significantly, with 100% patient survival. In contrast, the non-CAN group did not demonstrate a significant improvement in SCr or CrCL, and had a 50% mortality rate due to malignancy present at the time of the switch. An increase in proteinuria was observed in both groups<sup>[129]</sup>.

**Baseline calcineurin inhibitor or belatacept with or without mycophenolic acid or azathioprine or corticosteroids:** A retrospective case-control study evaluated patients on a CNI or belatacept with or without MPA or azathioprine or corticosteroids ( $n = 61$ ) converted to everolimus, and another 61 matched patients maintained on CNI-based regimen to determine if DSAs developed after conversion. At mean 36 mo follow-up there was no changes from baseline or between the groups in SCr or CrCL. None of the patients had DSAs at baseline, but the everolimus group had a follow-up incidence of 9.8% ( $P = 0.03$ ) and the CNI continuation group had an incidence of 5% ( $P = \text{NS}$ ). The only factor independently associated with DSA development was higher age at transplantation, associated with less DSA formation. Overall, 33% of everolimus patients withdrew from everolimus treatment at a mean 32 mo, due to DSA formation ( $n = 5$ ), lymphedema ( $n = 4$ ), proteinuria ( $n = 3$ ), and other reasons. None of the patients switched back to CNI developed DSAs<sup>[130]</sup>. Another case series examined 8 patients converted from CNI to everolimus at approximately 5 years post-transplant for CAN or malignancy. Everolimus replaced the CNI in 6 patients and was used to lower the CNI dose 30% in 2 patients. At 1-16 mo, SCr reduced slightly, CrCL improved slightly, and proteinuria:creatinine ratio decreased slightly. Three of the 8 patients developed serious infections<sup>[131]</sup>. A more robust study, the ASCERTAIN study<sup>[132]</sup>, was a prospective, randomized, open-label, multicenter study with 24 mo follow-up. Patients enrolled were at least 6 mo post-transplant (mean 5.6 years), with renal impairment, and without ACR within 3 mo. The study compared addition of everolimus to eliminate CNI ( $n = 127$ ), addition of everolimus to decreased CNI dose ( $n = 144$ ) and controls maintained on CNI ( $n = 123$ ). Overall, at 24-mo follow-up, ACR rates, graft survival and patient survival were similar. The primary endpoint of the study, CrCL at 24 mo, was not met, because CrCL was similar in all the groups at baseline and at follow-up. Proteinuria increased from baseline and relative to control in the CNI elimination group. Post-hoc analysis showed that patients with a baseline CrCL  $> 50$  mL/min had a larger improvement in CrCL after CNI elimination<sup>[132]</sup>.

**Baseline calcineurin inhibitor with mycophenolic acid or azathioprine and corticosteroids:** In cadaveric recipients on a CNI with MPA or azathioprine and corticosteroids and a SCr  $> 2$  mg/dL with proteinuria less than 1 g/24 h, everolimus was used to withdraw CNI. CrCL improved from baseline to 3 mo, but no results for 24 mo were presented, although the authors noted a trend toward decline. Proteinuria increased by one month ( $P = 0.05$ ) and more than 3-fold by month 12 ( $P = 0.0106$ ). Two of the 22 patients lost their grafts due to nephrotic syndrome and increasing SCr, and one patient developed ACR<sup>[133]</sup>. Another study compared 10 patients managed with everolimus to facilitate an 80% CNI dose reduction versus 10 patients with gradual complete CNI elimination. MMF or azathioprine were withdrawn when

everolimus was introduced in both groups, but were reintroduced only when the CNI was eliminated. At 12 mo, both groups had similar follow-up SCr, GFR and microalbuminuria, as well as similar changes from baseline. ACR occurred in 10% of the CNI reduction group and none of the CNI elimination group. It is interesting to note that in this study, many of the patients received angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARBs), which could have impacted the degree of proteinuria. Triglycerides and total cholesterol increased due to everolimus<sup>[134]</sup>.

**Baseline calcineurin inhibitor with everolimus and corticosteroids:** The FOREVER trial<sup>[135]</sup> examined patients previously enrolled in another trial of CsA with everolimus who either switched CsA to MPS and increased everolimus ( $n = 15$ ) or continued CsA and everolimus. This study, although prospective and randomized, suffered from differences in baseline GFR between the groups that impacted the interpretation of the results. The median (range) baseline measured GFR was 54 (21-87) mL/min in the CsA withdrawal group, and 37 (18-69) mL/min in the CsA continuation group ( $P = 0.053$ ). The difference at follow-up in GFR was -14.4 mL/min for the CsA continuation group, which did not meet statistical significance. Study drugs were discontinued in 7% of the CNI-free patients and 20% of the CNI-treated patients. Adverse event rates were similar, except aphthous stomatitis and pyrexia were more common in the CNI-free group, and hypertension, proteinuria, acute renal failure and urinary tract infection were more common in the CNI-treated patients<sup>[135]</sup>.

**Summary of everolimus studies:** Although the majority of evidence was from small, low-quality studies, there was clear evidence of an increase in proteinuria with conversion to everolimus from a CNI-based regimen, similar to what has been observed with sirolimus<sup>[119-121,127-129]</sup>. It is interesting to speculate that this may be manageable with ACEI or ARBs<sup>[134]</sup>. As expected, everolimus also had an adverse effect profile similar to sirolimus<sup>[112,122,134,135]</sup>. Here was evidence of a modest short-term improvement in renal function after CNI elimination with use of everolimus, and like sirolimus, combination of the mTOR inhibitor and the CNI resulted in enhanced adverse effect profile<sup>[122,125,135]</sup>. Also, like sirolimus, there was little evidence comparing late CNI withdrawal to an active CNI-containing regimen.

### **Regimens utilizing other agents to eliminate calcineurin inhibitors**

**Calcineurin inhibitor and variable adjunctive agents:** A randomized, open label phase II trial<sup>[136]</sup> evaluated the T cell costimulation blocker, belatacept for comparison with continued CNI in patients 6-36 mo post-transplant. Patients were randomized to switch to belatacept ( $n = 84$ ) intermittent therapy (5 mg/kg on days 1, 15, 29, 43 and 57, followed by every 28 d thereafter), or to continue the current regimen, which consisted of CNI and the current

regimen (80.7% corticosteroid, 3.4% azathioprine, and 94.3% MMF or MPA). Patients randomized to belatacept underwent a progressive taper to eliminate CNI by day 29. The primary endpoint was renal function over 12 mo as determined by calculated GFR, and the belatacept group improved  $7 \pm 11.99$  mL/min and the CNI group improved  $2.1 \pm 10.34$  mL/min from baseline ( $P = 0.0058$  for comparison at follow-up). Patients in the belatacept group with a baseline CrCL 45-60 mL/min exhibited the greatest numeric improvement ( $10 \pm 13.41$  mL/min). Belatacept patients with baseline CrCL  $< 45$  mL/min improved  $3.7 \pm 11.01$  mL/min and patients with CrCL  $> 60$  mL/min improved  $5.7 \pm 10.17$  mL/min. In contrast, patients remaining on CNI exhibited similar CrCL change according to baseline CrCL stratification, ranging from 1.9-2.8 mL/min. Mild to moderate ACR occurred in 6 patients in the belatacept group, all within the first 6 mo. Four of these patients were on belatacept therapy and 2 had discontinued belatacept. SCr returned to baseline in 4 of the 6 patients. No ACR episodes were reported in the CNI continuation group. Proteinuria occurred in one patient in each group. No grafts were lost in either group in the first 12 mo. One patient in the CNI group died with a functioning graft on day 142. Serious adverse event occurred in 24% of the belatacept group and 19% of the CNI continuation group. The biggest discrepancy in the adverse effects, pyrexia occurred in 4% of the belatacept group and 0% of the CNI group<sup>[136]</sup>. A 2-year follow-up to this study demonstrated 1 additional graft loss in each group, no additional ACR, and a mean change in CrCL from baseline 8.8 mL/min in the belatacept group and 0.3 mL/min in the CNI continuation group. Serious adverse events occurred in 37% of the belatacept group and 33% of the CNI group<sup>[137]</sup>.

## PEDIATRIC PATIENTS 6 OR MORE MONTHS POST-TRANSPLANT

Pediatric renal transplant patients also commonly receive CNIs and are at risk for potential CNI nephrotoxicity. Based on a comparison with adult kidney transplant recipients, pediatric patients have similar graft survival at 10 years ( $P = 0.4325$ ), with similar rates of delayed graft function and SCr levels. However, acute rejections were more common in pediatric patients, and 10-year patient survival tends to be lower in the pediatric transplant group (90.3% *vs* 76.8%;  $P < 0.02$ )<sup>[138]</sup>. Consequently, pediatric patients are at similar or greater risks as adult patients, depending on the endpoint studied, and thus may be considered for immunosuppression changes from CNIs over time<sup>[139]</sup>.

### **Regimens using mycophenolic acid or sirolimus to eliminate CNIs**

**CNI and variable regimen:** Weintraub and colleagues retrospectively evaluated 17 patients on a baseline regimen of CNI plus either sirolimus, MMF or azathioprine, with or without corticosteroids who were being switched

to sirolimus or MMF for CNI toxicity ( $n = 9$ ), CAN ( $n = 6$ ) or diabetes mellitus ( $n = 2$ ) at a mean 5.9 years post-transplant. Mean CrCL actually decreased from baseline after the switch at 6 mo ( $P = 0.04$ ) and 12 mo ( $P = 0.02$ ), and 41% of patient developed ACR. Risk of ACR was predicted by prior AR history, which was present in 9 of 17 patients, lower sirolimus trough levels, and lower calcineurin inhibitor toxicity scores. Graft loss occurred in 24% of patients and was associated with worse CrCL, proteinuria, and histologic chronicity. Proteinuria increased in a manner unrelated to sirolimus use. Four patients returned to a CNI-base regimen based on adverse effects. The authors suggested that worsened graft function and graft loss after conversion could be minimized by selecting patients with high CNI toxicity scores and low chronicity scores on biopsy, and excluding patients with a history of ACR<sup>[140]</sup>.

### **Regimens using mycophenolic acid to eliminate CNIs**

**Baseline CNI, corticosteroid and azathioprine :** In another study of patients averaging 40 mo post-transplant, but at least 3 mo post-transplant, conversion from CNI, azathioprine and corticosteroid to MMF plus corticosteroid ( $n = 29$ ) or addition of MMF and elimination of azathioprine, without CNI withdrawal ( $n = 9$ ) resulted in overall patient survival of 100% and graft survival of 94% at approximately 5-year follow-up. There was no significant difference in ACR or proteinuria between the groups. Introduction of MMF resulted in improvement in GFR over 2 year regardless of which group was evaluated, but the patients with CNI withdrawn had a numerically increased GFR<sup>[141]</sup>.

### **Regimens using sirolimus and MMF to eliminate CNI**

**Baseline calcineurin inhibitor, corticosteroid and azathioprine:** A group retrospectively analyzed addition of sirolimus and MMF to eliminate CNI, and compared the strategy to CNI minimization (39% dose reduction), MMF and corticosteroid. One year after conversion, the sirolimus group had a  $10.3 \pm 3$  mL/min improvement in CrCL ( $P < 0.05$ ) versus baseline, while the CNI minimization group had a  $17.7 \pm 7.1$  mL/min ( $P < 0.05$ ) improvement in CrCL. No patient experienced ACR in either group. The authors concluded that sirolimus and MMF introduction had similar benefit to MMF introduction with CNI minimization<sup>[142]</sup>.

**Summary of pediatric studies:** Data is currently very limited on late CNI withdrawal to improve renal function and further study is required. Patient characteristics may impact the success of selected regimens.

## CONCLUSION

This manuscript presents available evidence on late conversion, beyond 6 mo, from CNIs to alternative regimens as a means to aid practicing clinicians in determining therapeutic options for patients exhibiting CNI toxicity

or CAN. Although recent evidence suggests that CNI toxicity and CAN are non-specific findings, and graft dysfunction may alternatively or additionally be a function of C4d and DSA, it has been shown that 5-year graft survival is not independently predicted by DSA and C4d, suggesting that clinicians will still modify regimens based on the presence of CAN and CNI toxicity on biopsy<sup>[143-146]</sup>. These studies provide moderate-level evidence of a short-term improvement in renal function, that is not without regimen-specific risks, such as increased infection rate with MPA or proteinuria with mTOR inhibitors. There appears to be a “point of no return” after which kidney damage is irreversible and the patient stands to benefit less from withdrawal of CNI<sup>[103-105,132]</sup>. Since the benefit of late withdrawal appears to be modest and dependent on baseline renal function, the second manuscript in this series will evaluate the data surrounding early conversion and de novo CNI avoidance.

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## Pre-and-post transplant considerations in patients with nonalcoholic fatty liver disease

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### Abstract

Non-alcoholic fatty liver disease (NAFLD) is currently the third most common indication for liver transplantation in the United States. With the growing incidence of obesity, NAFLD is expected to become the most common indication for liver transplantation over the next few decades. As the number of patients who have undergone transplantation for NAFLD increases, unique challenges have emerged in the management and long-term outcomes in patients. Risk factors such as obesity, hypertension, diabetes, and hyperlipidemia continue to play an important role in the pathogenesis of the disease and its recurrence. Patients who undergo liver transplantation for NAFLD have similar long-term survival as patients who undergo liver transplantation for other indications. Research shows that post-transplantation recurrence of NAFLD is commonplace with some patients progressing to recurrent non-alcoholic steatohepatitis and cirrhosis. While treatment of comorbidities is important, there is no consensus on the management of modifiable risk factors or the role of pharmacotherapy and immunosuppression in patients who develop recurrent or *de novo* NAFLD post-transplant.

This review provides an outline of NAFLD as indication for liver transplantation with a focus on the epidemiology, pathophysiology and risk factors associated with this disease. It also provides a brief review on the pre-transplant considerations and post-transplant factors including patient characteristics, role of obesity and metabolic syndrome, recurrence and *de novo* NAFLD, outcomes post-liver transplantation, choice of medications, and options for immunosuppression.

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**Key words:** Liver transplantation; Non-alcoholic fatty liver disease; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Nonalcoholic steatohepatitis; Cirrhosis; Obesity

**Core tip:** Non-alcoholic fatty liver disease (NAFLD) is a major cause of chronic liver disease and one of the leading indication for liver transplantation (LT) nowadays. Although, it remains the third most common indication for LT in the United States, it is projected to become the most common indication by 2025. It presents a unique challenge for the transplant community in terms of management and long-term outcomes. Many risk factors for NAFLD pre-transplant such as obesity, hypertension, hyperlipidemia, diabetes continue to play an important role in the pathogenesis of post-transplant NAFLD. In addition to therapy focused on prevention and management of coexisting medical conditions, physicians must weight the benefits and harms of both medical and surgical options in patients undergoing LT.

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## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is increasingly recognized as a major etiology leading to chronic liver disease since its first description by Ludwig *et al*<sup>[1]</sup> in 1980. NAFLD has become an umbrella term to describe the pathologic picture of alcohol induced liver injury that occurs in the absence of alcohol abuse<sup>[2]</sup>. Histologically, NAFLD ranges from simple or bland steatosis to nonalcoholic steatohepatitis (NASH) and can progress to end-stage liver disease including fibrosis and cirrhosis. The pathologic definition of NASH is based on findings of macro vesicular steatosis, nuclear glycogenation, lobular and portal inflammation, and Mallory hyaline<sup>[1]</sup>. Progression of NASH to advanced fibrosis and cirrhosis is thought to be secondary to chronic inflammation and fibrosis<sup>[3]</sup>. Obesity has been strongly associated with NAFLD and NASH with some authors suggesting that NAFLD is the hepatic manifestation of metabolic syndrome<sup>[4]</sup>. With the global epidemic of obesity on the rise, there has been a consistent increase in NAFLD and NASH cases leading to increasing frequency of liver transplantation (LT) for this indication. According to the Scientific Registry of Transplant Recipients database (SRTR), NASH now represents the third most common indication for LT in the United States, surpassed only by hepatitis C and alcohol induced liver disease<sup>[5,6]</sup>. Furthermore, LT secondary to NASH is the only indication that has increased in frequency from 1.2% to 9.7% in less than a decade (from 2001-2009)<sup>[6]</sup>. Based on this data, end-stage liver failure secondary to NAFLD is estimated to become the most common indication for LT within the next two decades<sup>[5,6]</sup>.

In this manuscript, we provide an overview of NAFLD in the context of LT. First, we review the epidemiology, pathophysiology and risk factors for NAFLD and how obesity and metabolic syndrome play a role in the development of the disease. We then explore the pre-transplant factors affecting this patient population such as patient characteristics and availability of livers available for transplantation. Finally, we discuss the post-transplant considerations such as recurrence and de-novo NAFLD, outcomes, pharmacotherapy and immunosuppression. The goal of this review is to educate and assist in the management of unique challenges for patients with NAFLD both pre- and post LT.

## DEFINITION OF NAFLD AND NASH

An early diagnosis of NAFLD is often difficult as many patients remain asymptomatic until the disease has progressed to fibrosis and cirrhosis. Biochemically, there are no reliable serum biomarkers for NAFLD at the present time. Patients may have elevated serum transaminase levels; however, normal transaminases do not exclude the diagnosis. Per the United States Third National Health and Nutrition Examination Survey (NHANES III), the prevalence of NAFLD with and without elevated transaminases was found to be 3.1% and 16.4% respectively<sup>[7]</sup>.

**Table 1 Non-alcoholic fatty liver disease Activity Score**

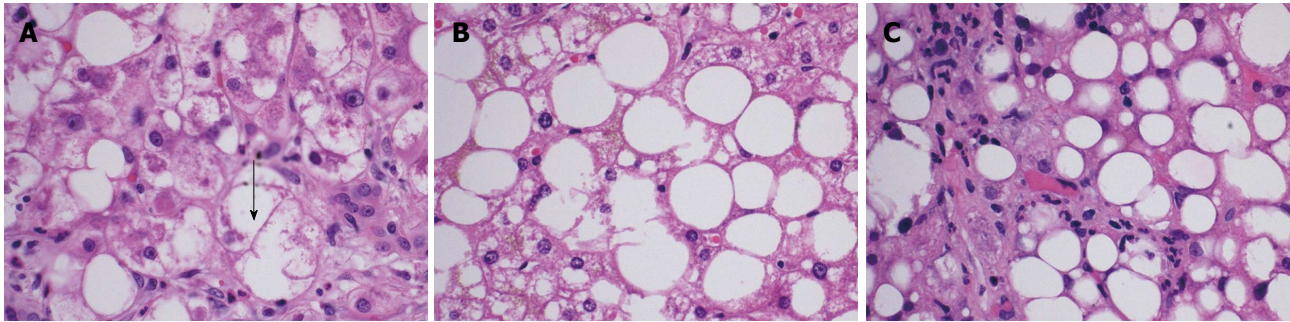
Component	Score
Steatosis grade	
< 5%	0
5%-33%	1
33%-66%	2
> 66%	3
Lobular inflammation	
No foci	0
< 2 foci per 200 × field	1
2-4 foci per 200 × field	2
> 4 foci per 200 × field	3
Ballooning	
None	0
Few balloon cells	1
Prominent/many cells	2

Scoring system assigns a score for steatosis (0-3), lobular inflammation (0-3) and hepatocyte ballooning (0-2) and sum of the scores is correlated with a score of greater than or equal to five as "definite NASH" and a score of less than or equal to three as "not NASH"<sup>[6]</sup>. Adapted from Tanaka *et al*<sup>[6]</sup>.

When elevated, aspartate aminotransferase and alanine aminotransferase are seldom greater than four times the upper limit of normal<sup>[8]</sup>. Therefore, the diagnosis of NAFLD remains a diagnosis of exclusion requiring elimination of other causes of abnormal liver function tests in presence of imaging or biopsy suggestive of steatosis. Liver biopsy remains the gold standard for its diagnosis. On biopsy, NAFLD must have histologic findings of macro vesicular steatosis in greater than 5% of hepatocytes<sup>[9]</sup>. For the diagnosis of NASH, most experts require additional findings suggestive of active inflammatory process including hepatocyte swelling, ballooning and degeneration with lobular inflammation<sup>[10]</sup>. The Nonalcoholic Steatohepatitis Clinical Research Network has designed and validated a histologic scoring system for NAFLD, called the NAFLD Activity Score that allows for evaluation of steatosis, inflammation and ballooning scores<sup>[11]</sup>. This scoring system assigns a score for steatosis (0-3), lobular inflammation (0-3) and hepatocyte ballooning (0-2) and sum of the scores if greater than or equal to five is defined as "definite NASH" and a score of less than or equal to three as "not NASH" (Table 1). In general, the diagnosis of both NAFLD and NASH requires the presence of hepatic steatosis, no significant alcohol consumption and no other etiology to explain liver disease<sup>[12,13]</sup>. Figure 1 illustrates the microscopic findings in biopsies of patients suspected of having NAFLD and depicts hepatocyte ballooning (Figure 1A), steatosis (Figure 1B) and lobular inflammation (Figure 1C).

## EPIDEMIOLOGY AND RISK FACTORS IN NAFLD PATIENTS

Although the prevalence of NAFLD is unknown, its incidence is estimated to be on the rise with the concurrent obesity epidemic. According to the National Center for Health Statistics, the prevalence of obesity in the United States in 2009-2010 is estimated to be 35.5% of



**Figure 1** Microscopic findings in biopsies of patients' suspected of having non-alcoholic fatty liver disease/nonalcoholic steatohepatitis. A: H and E stained liver tissue at  $\times 40$  showing ballooning degeneration of a hepatocyte (marked with black arrow); B: H and E stained liver tissue at  $\times 40$  showing steatosis without steatohepatitis. C: H and E stained liver tissue at  $\times 40$  showing inflammation (neutrophilic inflammation surrounding fatty hepatocytes).

the male population and 35.8% of the female population<sup>[14]</sup>. A recent cross-sectional study in the setting of outpatient general internal medicine clinic in Texas shows the prevalence of NAFLD to be 46%, with findings of NASH in 12.2% of patients<sup>[15]</sup>. The projection from this study reports the anticipated prevalence of NASH in the US to be anywhere between three and eight million<sup>[15]</sup>. Despite these estimates, the frequency of progression from NAFLD to end-stage liver disease is unknown. In case series reports, transition from NASH to fibrosis are reported as high as a third of patients<sup>[16-18]</sup>. The rate of progression to decompensated cirrhosis and need for LT remains uncertain, however; this is the only indication for LT that has been steadily increasing<sup>[6]</sup>. Additionally, it is suggested that a high percentage of cases initially classified as cryptogenic cirrhosis may represent progression from NAFLD to cirrhosis<sup>[19]</sup>. As fibrosis distorts a fatty liver into a cirrhotic one, various histologic components such as steatosis and inflammatory changes become less evident and may even disappear<sup>[5]</sup>. Therefore, end-stage liver disease secondary to NAFLD is projected to become the most common indication for LT by 2025<sup>[6]</sup> given its increasing incidence and the steady decrease in frequency of hepatitis C infection and alcohol induced liver disease.

## **PATHOPHYSIOLOGY OF NAFLD AND NASH**

NAFLD accounts for two types of fatty infiltration of the liver: simple steatosis and non-alcoholic steatohepatitis (NASH). Simple fatty liver infiltration, also called bland hepatic steatosis is a benign condition in which liver function tests are within normal limits or maybe slightly elevated. In this condition, liver biopsy shows liver tissue that is essentially normal except for fatty infiltration in hepatocytes. On the other hand, NASH is defined by the presence of inflammatory changes. The development of inflammation and subsequently NASH from hepatic steatosis is thought to be a complex mechanism involving insulin resistance, oxidative stress, and inflammatory cascade. Several models have been described in the literature to suggest the interplay between these

processes and how simple steatosis is transformed into steatohepatitis, including the “two-hit hypothesis”. First described by Day *et al*<sup>[20]</sup>, insulin resistance is the “first hit” that leads to steatosis in hepatocytes. During states of insulin resistance, both muscle and adipose tissues preferentially oxidize lipids, resulting in release of free-fatty acids. The liver incorporates these free fatty acids into triglycerides, and remaining free-fatty acids undergo oxidation in the mitochondria, peroxisomes or microsomes<sup>[21]</sup>. Then a “second hit” that occurs in the form of oxidative stress leads to inflammation and fibrosis<sup>[22]</sup>. Figure 2 summarizes the multiple factors that play a role in the development of NASH from steatosis. Others have also described a change in lipid metabolism through elevated peripheral fatty acids and *de novo* synthesis leading to an increase in fatty deposition in the liver. In patients with NAFLD, Donnelly *et al*<sup>[23]</sup> noted that the majority (60%) of the triacylglycerol in the liver arises from free fatty acids while 26% and 15% are attributable to *de novo* lipogenesis and diet, respectively<sup>[23,24]</sup>. Insulin resistance at the level of adipose tissue leads to an increased release of free fatty acids leading to an increased activation of macrophages and other immune cells. The entry of these free fatty acids in the liver also leads to the activation of intracellular inflammatory pathways causing hepatic inflammation and consequently fibrosis<sup>[25,26]</sup>. Furthermore, insulin resistance leads to hyperglycemia which in turn triggers stellate cell activation leading to fibrosis<sup>[27]</sup>. Genes also play an integral role in the development of NASH as evidenced by ethnic-specific allele frequencies and certain genotypes that purport a greater lipid content, more aggressive disease, and increase in serum aminotransferase levels<sup>[28]</sup>.

Several studies have shown an increased prevalence of risk factors in the form of hypertension, diabetes, obesity and hyperlipidemia - all components of metabolic syndrome in patients' who have undergone LT<sup>[29]</sup>. In these patients, studies have also shown an increase in pro-steatotic cytokines such as leptin<sup>[30]</sup> and decrease in anti-steatotic cytokines such as adiponectin<sup>[31]</sup>. Additionally, the advanced age of the donors may exacerbate the effects of insulin resistance post-transplant due to accelerated fibrosis<sup>[32]</sup>.



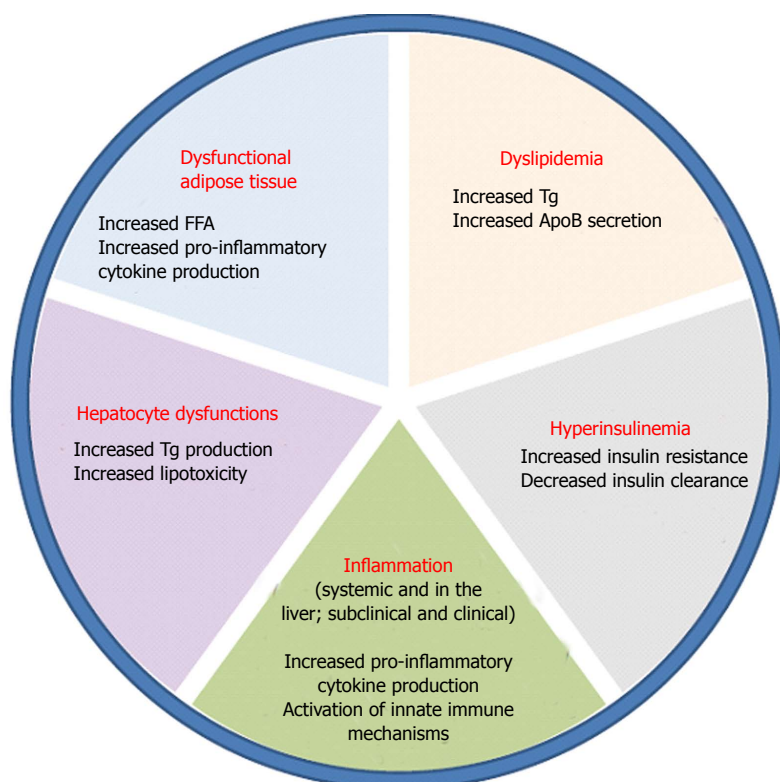


Figure 2 Multiple factors that play a role in the progression of steatosis to nonalcoholic steatohepatitis.

## METABOLIC SYNDROME, OBESITY AND NAFLD

A large proportion of patients diagnosed with NAFLD have been identified to have the phenotype associated with metabolic syndrome. Although many organizations have defined the term “metabolic syndrome” differently, all definitions include risk factors for cardiovascular disease and type 2 diabetes such as hypertension, dyslipidemia (elevated triglycerides and lower high-density lipoprotein cholesterol), raised fasting glucose and central obesity<sup>[33]</sup>. Liver biopsies from patients who meet the strict definition of metabolic syndrome shows more advanced histologic changes and a high risk of severe fibrosis<sup>[34]</sup>. Additionally, obesity itself has been independently shown to be a predictor of advanced fibrosis in the liver. A study conducted by Dixon *et al*<sup>[35]</sup> showed that in 105 consecutive patients who underwent laparoscopic obesity surgery and had liver biopsies taken, there were findings of NASH in 25% with nearly half demonstrating findings of advanced fibrosis. Colicchio *et al*<sup>[36]</sup> also found severe steatosis to be uniformly present in non-diabetic patients with body mass index (BMI) greater than 39.9 kg/m<sup>2</sup> (grade III obesity) when evaluated using liver ultrasound. It is however, the central or visceral obesity that is associated with the development of NAFLD independent of overall obesity<sup>[37,38]</sup>. Dyslipidemia and diabetes have also been shown to have an independent association with NAFLD. One study by Assy *et al*<sup>[38]</sup> showed that in patients with hypertriglyceridemia, there is a significantly higher risk of fatty infiltration than in patients with other forms of dyslipidemia, further supporting the association between metabolic syndrome and NAFLD.

## PRE-TRANSPLANT CONSIDERATIONS

### Patient characteristics

Obesity and insulin resistance have been implicated as the key pathogenic factors associated with NAFLD<sup>[39]</sup>. The risk factors associated with the histological severity of NASH in the non-transplant population include male sex, higher BMI, insulin resistance, hypertension, and presence of type II diabetes<sup>[18,40,41]</sup>. Analysis of the SRTR database by Charlton *et al*<sup>[6]</sup> showed that the people who underwent LT for NASH cirrhosis were older, had larger BMI, were more likely to be female, had a greater prevalence of diabetes and hypertension, and a lower incidence of hepatocellular carcinoma compared with other patients in the transplant cohort. Hence, prior to undergoing LT, optimization of modifiable factors in patients is essential for improved outcomes. In addition to medical optimization such as improved blood pressure and glycemic control, patients should strongly be encouraged to undergo supervised weight loss. A study by Nair *et al*<sup>[42]</sup> measured graft and patient survival in obese patients receiving LT in the United States. This study concluded that patients with morbid obesity (BMI > 40 kg/m<sup>2</sup>) had significantly higher rates of primary graft non-function and significantly increased immediate, one and two year mortality. Five year mortality rates were also significantly higher in severely obese (BMI between 35.1 and 40 kg/m<sup>2</sup>) and morbidly obese patients, secondary to increased cardiovascular mortality. Based on these findings, the American Association for the Study of Liver Disease (AASLD) considers morbid obesity a contraindication to LT<sup>[43]</sup>, and recommends weight loss in all patients awaiting LT, especially if the patient's BMI is greater than 35

kg/m<sup>2</sup>. Additionally, weight loss has been shown to help with improvement in the severity of steatosis and NASH prior to transplant. Meta-analysis by Mummadi *et al*<sup>[44]</sup> in the non-LT population who underwent bariatric surgery shows that a 19%-41% reduction in BMI was associated with improvement of steatosis in 91.6%, steatohepatitis in 81.3%, fibrosis in 65.5% and complete resolution of NASH in 69.5% of patient's post-bariatric surgery.

Concurrent bariatric surgery and LT has also been evaluated in obese patients. A recent study analyzed thirty-seven patients referred for LT with BMI > 35 who had achieved weight loss prior to transplant and underwent LT alone and compared them with seven patients who underwent LT with sleeve gastrectomy<sup>[45]</sup>. This study reported that in patients with LT alone, there was a higher frequency of weight gain, steatosis, post-transplant diabetes, graft loss and death when compared with the sleeve gastrectomy group. This small study suggests that although bariatric surgery may play a promising role in patients undergoing transplant, more studies are needed to evaluate long-term survival in these patients and it may be appropriate for some patients who have persistent obesity and fail non-invasive management.

#### **Availability of livers for transplant in the NAFLD population**

The increasing prevalence of obesity has led to further increases in hepatic steatosis in potential donors, which has reduced the number of transplantable livers available for any indication. The use of steatotic livers for transplant depends on the level of fatty infiltration. Donor livers with greater than 60% steatosis are deemed non-transplantable whereas those with less than 30% are deemed useable with good function. Even though livers with 30%-60% steatosis are potentially used for patients, they have been associated with poor results due to decreased function, graft survival and decreased patient survival<sup>[46]</sup>. The biggest concern remains primary non-function of the graft which has been reported as high as 13% in donor livers with greater than 30% steatosis compared with < 3% in those with no steatosis on biopsy prior to transplant<sup>[47,48]</sup>. More recent studies show the rate of primary non-function of the graft to be less than 5% in those undergoing LT with steatosis of less than 30%<sup>[49-51]</sup>. Increased hepatic graft steatosis has also been associated with intrahepatic cholestasis and transient hyperbilirubinemia during regeneration after living donor transplant but the mechanism remains elusive<sup>[52]</sup>.

The use of living donors for LT also has its challenges. Although the maximum percentage of steatosis in living donors is unknown for LT, most centers are reluctant to transplant grafts with greater than 30% steatosis given the increased risk of primary non-function of the graft<sup>[53]</sup>. With the growing incidence of obesity, finding grafts with less than 10% steatosis (preferred by most centers) is difficult<sup>[54]</sup>. Studies report that one third to one half of potential living donors have steatosis on liver biopsies and in these studies more than one-third of biopsies showed steatosis greater than 10%<sup>[55,56]</sup>. The need for

liver biopsy in living transplant donors is also not without risk, given that the sensitivity of imaging modalities is low for small amounts of steatosis and improves with increasing steatosis<sup>[55]</sup>.

## **POST-TRANSPLANT CONSIDERATIONS**

### **Recurrence of NAFLD and NASH**

The development of steatosis post-LT in patients is common with some observational studies reporting prevalence as high as 100%<sup>[57]</sup>. One study of post-liver transplant patients by Maor-Kendler *et al*<sup>[58]</sup>, showed the incidence of grade 2 steatosis or higher in 38% of recipients with pre-transplant diagnosis of NASH/cryptogenic cirrhosis when compared to 6% in cholestatic disease, 16% in alcoholic disease and 9% in patients with HCV cirrhosis. Table 2 summarizes several studies that evaluated the incidence of NAFLD, NASH and cirrhosis post LT<sup>[57,59-66]</sup>. A recent study by Dureja *et al*<sup>[59]</sup> analyzed post-transplant data in eighty-eight patients who underwent transplant for NAFLD and report prevalence of recurrent NAFLD to be 39%, recurrent NASH to be 28.4% and fibrosis (stage 3 and 4) to be 3.4% respectively. Moreover, according to Contos *et al*<sup>[57]</sup> when comparing the cases of cryptogenic cirrhosis with those transplanted for alcoholic liver disease, primary biliary cirrhosis and primary sclerosing cholangitis, the rates of steatosis and subsequent NASH were significantly higher in the cryptogenic cirrhosis group. Similarly, Bhagat *et al*<sup>[61]</sup> reported the recurrence of NASH in 33% of the patients who were transplanted for cryptogenic cirrhosis with NASH phenotype compared with those transplanted for alcohol related cirrhosis at six months post-LT. Tanaka *et al*<sup>[66]</sup> recently reported recurrence of NASH in one patient who underwent living donor LT for NAFLD; however, this study is limited by small sample size and had only seven patients who were transplanted for this indication. Based on the studies (summarized in Table 2), the recurrence of steatosis, NASH and cirrhosis in patients transplanted for NAFLD is clearly possible and further studies are needed to determine the risk of recurrence in patients' post-LT.

### **De novo NAFLD/NASH**

Little is known about the prevalence of *de novo* NAFLD and NASH in patients who undergo liver transplantation for non-NASH cirrhosis and have been transplanted a donor graft free of steatosis. Report by Seo *et al*<sup>[63]</sup> who evaluated sixty-eight liver transplant patients with various causes of liver cirrhosis using pre-transplant and post-transplant biopsies, noted the prevalence of *de novo* steatosis in twelve patients (18%) with prevalence of *de novo* NASH in six patients (9%). In another study that evaluated thirty patients with mostly infectious cirrhosis from HBV and HCV, incidence of steatosis and NASH were 40% and 13% respectively, although it is unclear how much of this was *de novo*<sup>[62]</sup>. In another case series in which patients underwent transplantation for HCV and alcohol cirrhosis, four patients developed *de novo* NAFLD post-transplant in the absence of graft steatosis<sup>[67]</sup>. Thus,

**Table 2** Various studies examining the incidence/recurrence of non-alcoholic fatty liver disease (*de novo* or recurrent), non-alcoholic steatohepatitis and Cirrhosis in the post-liver transplant population *n* (%)

Ref.	Year of publication	Indication of transplant	Number of patients	Findings of NAFLD post-transplant	Findings of NASH post-transplant	Findings of cirrhosis post-transplant	Mean follow-up duration
Tanaka <i>et al</i> <sup>[66]</sup>	2013	Living donor transplant for NAFLD	7	0 (0)	1 (14)	None	5.3 yr
Dureja <i>et al</i> <sup>[59]</sup>	2011	NAFLD	88	34 (39)	25 (28.4)	3 (3.4) (reported as fibrosis grade 3/4)	82 mo
Dumortier <i>et al</i> <sup>[60]</sup>	2010	Several indication	599	131 (31.1)	5 (3.8)	3 (2.25)	40 mo
Bhagat <i>et al</i> <sup>[61]</sup>	2009	Cryptogenic/NASH Cirrhosis <i>vs</i> alcoholic cirrhosis	71	N/A	31 (33)	None	1517 d
Lim <i>et al</i> <sup>[62]</sup>	2007	Non-NAFLD indication (18 HBV, 7 HCV, 5 others)	30	12 (40)	4 (13)	None	44 mo
Seo <i>et al</i> <sup>[63]</sup>	2007	68 various causes, 84% HCV	68	12 <sup>1</sup> (18)	6 <sup>1</sup> (9)	None	28 mo
Ong <i>et al</i> <sup>[64]</sup>	2001	Cryptogenic cirrhosis	51	13 (25.4)	8 (15.7)	None	26 mo
Contos <i>et al</i> <sup>[57]</sup>	2001	Cryptogenic/NASH cirrhosis	30	30 (100)	3 (10)	None	3.5 yr
Charlton <i>et al</i> <sup>[65]</sup>	2001	NASH cirrhosis	16	9 (60)	5 (33)	2 (12.5)	28.1 mo

<sup>1</sup>*De novo*. HCV: Hepatitis C virus; HBV: Hepatitis B virus; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

the incidence, prevalence and the mechanism of *de novo* NAFLD or NASH remains unclear and there is an emerging need for studies in this area.

#### **Influence of NAFLD/NASH on outcomes after liver transplantation**

Data suggests that the outcome of LT in patients who undergo transplant for most common causes of cirrhosis in the United States, including cholestatic liver disease (primary biliary cirrhosis, primary sclerosing cholangitis), alcoholic liver disease, and HCV are excellent, with one year survival rates of 85%-90% and five year survival rates of 70%-80% respectively<sup>[6,68]</sup>. Review of literature for patients undergoing LT for NASH cirrhosis shows mortality after transplant to be similar at five years when compared with patients undergoing transplant for other indications, however the one and three year mortality in NASH cirrhosis patients were significantly higher<sup>[68]</sup>. Malik *et al*<sup>[68]</sup> reported a higher one year mortality in NASH patients with age  $\geq 60$  years and BMI  $\geq 30$  kg/m<sup>2</sup> with diabetes and hypertension. A more recent review of transplant patients by Charlton *et al*<sup>[6]</sup> however reports survival at one year and three years after LT for NASH to be 84% and 78%, respectively and similar for other indications. They also report that patient and graft survival was similar to values for other indications when adjusted for age, sex, BMI and serum creatinine. There is, however, a higher incidence of cardiac events following LT in a subset of patients with higher BMI, elevated serum creatinine, diabetes, systolic blood pressure elevation, hypercholesterolemia, and these may represent to some extent the cause of poor outcomes in LT patients with NASH cirrhosis<sup>[69]</sup>. Malik *et al*<sup>[68]</sup> reported statistically significant differences in infection as the cause of death is NASH cirrhosis patients post-LT when compared with other indications and explain the likely cause to be elevated hyperglycemia and diabetes which may predispose these

patients' to increased risk of infection. With the growing number of NAFLD and NASH patients' post-LT, it is expected that more studies would emerge in the upcoming years that would be high-powered to provide further details on these issues.

#### **Management of NAFLD patients after liver transplant**

Little data exists for the treatment of NAFLD patients' post-LT. All recommendations for management of NAFLD post-transplant are a reflection of studies done on the non-LT population and can be divided into three broad categories: Lifestyle modifications, Pharmacotherapy and Bariatric Surgery.

**Lifestyle modifications:** The mainstay of medical management includes weight reduction through physical activity and diet modification and pharmacological management of medical co-morbidities such as hypertension, hypercholesterolemia and diabetes<sup>[4]</sup>. A low-carbohydrate (< 60 g of carbs/d) low caloric diet when compared with high carbohydrate (> 180 g of carbs/d) low caloric diet has been shown to lead to a more pronounced reduction in intrahepatic triglyceride content and improves insulin sensitivity<sup>[70]</sup>. Weight loss has also been shown to improve hepatic steatosis and inflammation with weight loss of 3%-5% showing improvement in steatosis and 7%-10% weight loss showing improvement in the level of steatohepatitis<sup>[13]</sup>. Physical activity has an important effect on the level of NAFLD and should be encouraged in patients. Moderate and vigorous activity was compared with controls that were generally inactive. This study showed that vigorous activity was beneficial in preventing progression to fibrosis in NAFLD patients over moderate activity<sup>[71]</sup> and thus should be encouraged. The role of caffeine in coffee has also been evaluated in patients with NAFLD. Molloy *et al*<sup>[72]</sup> showed that when comparing 4 different groups (controls, bland steatosis/not-NASH,



NASH stage 0-1, and NASH stage 2-4), there was a significant reduction in the risk of fibrosis among patients with higher coffee consumption per day.

**Pharmacotherapy:** The use of insulin sensitizing medications including metformin and thiazolidinedione has been evaluated in patients with NAFLD and NASH. Although metformin use had been associated with normalization of aminotransferases and improvement in liver echographic findings in prior studies<sup>[73,74]</sup>, pooled results from meta-analysis have found no significant improvement on steatosis, inflammation or fibrosis in metformin treated patients with NASH<sup>[75]</sup>. The study concluded that in patients without diabetes, targeted lifestyle interventions might be at least as beneficial as metformin and there is little evidence to suggest benefit of metformin in patients with NAFLD without pre-existing glucose intolerance regardless of the dose. Thiazolidinediones (TZDs), including rosiglitazone and pioglitazone, have been evaluated in multiple studies on its benefit in NASH patients. Rosiglitazone has however been shown to be associated with increased rate of myocardial infarction<sup>[76]</sup> and has been removed from European markets and highly restricted in the United States. Given the risk factors for NASH also mirror risk factors for coronary artery disease, rosiglitazone is likely not an optimal treatment option in patients. Pioglitazone was evaluated in a large multicenter study<sup>[77]</sup> for 96 wk at doses of 30 mg/d and compared with Vitamin E 800 IU/d or placebo in patients without diabetes with NASH. This study concluded that both treatment groups (Vitamin E and Pioglitazone) demonstrated improvement in hepatic steatosis, ballooning and inflammation, although only Vitamin E was associated with statistically significant improvements. Neither treatment had an effect on fibrosis but both Vitamin E and pioglitazone led to improvement in aminotransferase levels. Although Vitamin E may have a role in the treatment of NAFLD patients without diabetes, it is important to note that Vitamin E use has been associated with increased all-cause mortality and prostate cancer, especially at doses of 400 IU/d or higher<sup>[78,79]</sup>. Other small randomized control trials have also shown similar benefit of pioglitazone at 30-45 mg/d in NASH patients with or without diabetes demonstrating improvements in aminotransferase levels, hepatic steatosis, improved insulin sensitivity and inflammation<sup>[80,81]</sup> however no improvement in fibrosis were noted. Additionally, unlike rosiglitazone that has been associated with increased cardiovascular mortality<sup>[76]</sup>, pioglitazone has only been associated with having a slightly positive or neutral effect on the cardiovascular system<sup>[82]</sup>. Based on this data, pioglitazone at doses of 30 mg/d and titrated up for glycemic control if necessary, may be recommended for patients with NAFLD, however should be used with caution in patients with history of heart failure and bladder cancer<sup>[82]</sup>.

The use of statins has been investigated in small pilot studies for the treatment of NAFLD, although there have been mixed results. Rosuvastatin at dose of 10 mg/d given to NAFLD patients without diabetes, showed

normalization of aminotransferase and cholesterol levels after follow-up for eight months<sup>[83]</sup> whereas another trial in NASH patients receiving simvastatin 40 mg/d demonstrated no significant differences in hepatocellular structure and aminotransferase levels when compared with placebo over a duration of one year<sup>[84]</sup>. Based on conflicting reports, AASLD has recommended against the use of statins in the treatment of NASH until more randomized clinical control trials can demonstrate its efficacy<sup>[13]</sup>.

Ursodiol or ursodeoxycholic acid, approved for the treatment of primary biliary cirrhosis, has also been evaluated for NASH patients and trials thus far have not demonstrated significant differences in overall histology<sup>[85,86]</sup>.

Pentoxifylline, a drug that inhibits the synthesis of TNF- $\alpha$  which is thought to be associated with possible progression to fibrosis<sup>[87]</sup> in NAFLD patients has also been studied for the treatment of NASH. A recent randomized control trial evaluated pentoxifylline 1200 mg/d compared to placebo in biopsy-confirmed NASH patients over a course of one year and found improvements in aminotransferase levels and histologic features from baseline but these were not significant when compared to placebo<sup>[88]</sup>.

Use of pharmacological intervention to augment weight loss in NASH and NAFLD patients with orlistat has also shown improvement in steatosis and aminotransferase levels<sup>[89]</sup>, however it is most likely the observed changes were associated with weight loss rather than the drug itself.

**Role of bariatric surgery:** As in the non-transplant population, weight loss has its own challenges in the post-LT population. In addition to obesity pre-transplant, many recipients experience rapid weight gain post-transplant that leads to recurrence and *de novo* steatosis in the graft liver<sup>[60]</sup>. Weight gain can partially be attributed to immunosuppressive medication such as steroids and calcineurin inhibitors taken to suppress the immune system post-LT. Few studies exist on the benefit of bariatric surgery post-OLT, mostly in the form of case reports and case series<sup>[90-93]</sup>. Duchini *et al*<sup>[92]</sup> reported Roux-en-Y bypass as a successful procedure in two NAFLD patients post-LT with morbid obesity demonstrating significant weight reduction, normalization of liver function and metabolic parameters, including lipid profile and hyperglycemia. A recent study from the University of Minnesota identified seven patients who underwent Roux-en-Y gastric bypass post-LT between 2001 and 2009<sup>[93]</sup>, and reported therapeutic weight loss, improved glycemic control, and improved high-density lipoprotein in the presence of continued dyslipidemia. More studies however, are needed for consideration of bariatric surgery in post-LT patients before definite recommendations could be made.

### Choice of Immunosuppression in NAFLD patients

Many immunosuppressive regimens used in the treatment



of post-LT patients are associated with diabetes, hypertension, hyperlipidemia, obesity and increased risk of infection<sup>[94]</sup>. Patients who undergo LT for NASH often have metabolic syndrome and are at increased risk for the development of major vascular events<sup>[68]</sup>. Some studies have shown an increased risk of recurrence of hepatocellular carcinoma<sup>[95]</sup> in addition to other known adverse effects from steroids including diabetes, osteoporosis and obesity. Given that steroids have been linked to much adverse effects, they should be withdrawn from maintenance therapy within three months post-LT. Moving away from a steroid based immunosuppressive regimen in LT patients was evaluated by Segev *et al*<sup>[94]</sup> in their meta-analysis of thirty publications, including nineteen randomized control trials which showed there was no difference in death, graft loss and infection rates in patients who were on steroid-free regimens when compared with steroid-based immunosuppression. Additionally, the analysis showed a trend towards reduced hypertension and statistically significant decrease in CMV infection and cholesterol levels in steroid-free regimens. The authors also reported that if the steroids were replaced by another immunosuppression medication, there is a reduced risk of diabetes, rejection and severe rejection. This would advocate for the role of avoidance of steroids post-LT for immunosuppression, especially in patients with NASH cirrhosis.

Calcineurin inhibitors include tacrolimus (FK506) and cyclosporine and act by inhibiting T-cell activation. Although these drugs are commonly used, studies have shown acute and chronic nephrotoxicity as a major adverse effect of both tacrolimus and cyclosporine, occurring in up to 20% of patients depending on the organ transplanted<sup>[96]</sup>. Due to these outcomes, studies have advocated for conversion to sirolimus therapy in patients who develop renal insufficiency due to calcineurin inhibitors<sup>[97]</sup>, however their complete avoidance has been associated with higher rejection rates<sup>[98]</sup>. Additionally, tacrolimus has been associated with neurotoxicity and development of de-novo diabetes, while cyclosporine has been associated with hypertension and hyperlipidemia<sup>[99,100]</sup>.

Mycophenolic acid and Azathioprine are two other medications commonly used post-LT however require close monitoring due to the risk of bone marrow suppression<sup>[101]</sup> and their experience in NASH-related LT is limited. The decision on the type of immunosuppression regimen to be used should be based on maintaining a balance between drug toxicity and efficacy and dictated by patient factors such as age, ethnicity and etiology of their liver disease.

## CONCLUSION

NAFLD is increasingly recognized as a major etiology leading to chronic liver disease and remains the only indication for LT that has steadily and steeply increased in frequency over the past decades. As the third most common indication for LT in the United States after

HCV and alcoholic liver disease, NAFLD is projected to become the most common indication by 2025. The increasing prevalence of NAFLD both pre- and post-transplant presents unique challenges for the transplant community in terms of management and long-term outcomes. Many risk factors for NAFLD pre-transplant such as obesity, hypertension, hyperlipidemia, diabetes continue to play an important role in the pathogenesis of post-transplant NAFLD. In addition to prevention and management of coexisting medical conditions, physicians must weigh the benefits and harms of both medical and surgical therapies in patients undergoing LT. New research in pharmacotherapy such as insulin sensitizing drugs, statins, metformin and others continues to emerge, yet more research is needed to help identify methods to reduce and possibly reverse progression to fibrosis in these patients. The recommendation on avoidance of steroids and minimization of calcineurin inhibitors in this patient population would likely be beneficial in decreasing the risk factors associated with post-transplant NAFLD and should be considered. Further research is still needed to better understand the issues that affect this unique patient population.

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## Coronary microvasculopathy in heart transplantation: Consequences and therapeutic implications

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### Abstract

Despite the progress made in the prevention and treatment of rejection of the transplanted heart, cardiac allograft vasculopathy (CAV) remains the main cause of death in late survival transplanted patients. CAV consists of a progressive diffuse intimal hyperplasia and the proliferation of vascular smooth muscle cells, ending in wall thickening of epicardial vessels, intramyocardial arteries (50-20  $\mu$ m), arterioles (20-10  $\mu$ m), and capillaries (< 10  $\mu$ m). The etiology of CAV remains unclear; both immunologic and non-immunologic mechanisms contribute to endothelial damage with a sustained inflammatory response. The immunological factors involved are Human Leukocyte Antigen compatibility between donor and recipient, alloreactive T cells and the humoral immune system. The non-immunological factors are older donor age, ischemia-reperfusion time, hyperlipidemia and CMV infections. Diagnostic techniques that are able to assess microvascular function are lacking. Intravascular ultrasound and fractional flow reserve, when performed during coronary angiography, are able to detect epicardial coronary artery disease but are not sensitive enough to assess microvascular changes. Some authors have proposed an index of microcircula-

tory resistance during maximal hyperemia, which is calculated by dividing pressure by flow (distal pressure multiplied by the hyperemic mean transit time). Non-invasive methods to assess coronary physiology are stress echocardiography, coronary flow reserve by transthoracic Doppler echocardiography, single photon emission computed tomography, and perfusion cardiac magnetic resonance. In this review, we intend to analyze the mechanisms, consequences and therapeutic implications of microvascular dysfunction, including an extended citation of relevant literature data.

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**Key words:** Heart transplantation; Cardiac allograft vasculopathy; Microvascular function; Coronary flow reserve; Endothelial dysfunction

**Core tip:** In this review, we intend to analyze the mechanisms, consequences and therapeutic implications of microvascular dysfunction in heart transplantation recipients, including an extended citation of relevant data from the literature. We think that this manuscript could be of interest for many research workers and physicians working in the field of cardiovascular surgery, cardiology and transplant medicine.

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### INTRODUCTION

Heart transplantation (HT) is the most effective treatment for patients with end-stage heart failure. Recently, early survival after HT has been improved through the

use of immunosuppressive therapy and updated surgical procedures. Unfortunately, late survival is still limited by the onset of malignancies and cardiac allograft vasculopathy (CAV). CAV is a specific form of coronary artery disease that affects heart transplanted patients and is characterized by an early, diffuse intimal proliferation of both the epicardial and microvascular vessels, resulting in epicardial coronary artery stenosis and small vessel occlusion<sup>[1]</sup>. The 29<sup>th</sup> Official Adult Heart Transplant Report, edited by the Registry of Heart and Lung Transplantation, noted a relatively small decrease in the cumulative incidence of CAV: at 7 years after transplant, 37% of the patients transplanted between 2003 and June 2010 had CAV, compared with 42% of those transplanted between April 1994 and 2002. In fact, CAV affects 8% by year 1, 30% by year 5 and 50% by year 10 after transplant<sup>[2]</sup>. This decrease seems to be related to newer approaches to CAV treatment, such as targeting lower low-density lipoproteins (LDL)-cholesterol levels or the use of mammalian target of rapamycin (mTOR) inhibitors or drug-eluting coronary stents<sup>[3]</sup>. The 1-year survival rate after HT is 81%, and the 5-year survival rate is 69%, with a median survival of 11 years for all HT patients and 13 years for those surviving the first year. CAV causes approximately 10%-15% of the deaths between years 1 and 3 after HT and contributes to potentially more deaths resulting from graft dysfunction<sup>[4]</sup>. Epicardial coronary artery disease is detectable by intravascular ultrasound (IVUS) during coronary angiography. Coronary microvascular function can be assessed by transthoracic Doppler echocardiography (TDE) measuring coronary flow reserve (CFR)<sup>[5]</sup>. Understanding the physiopathology of endothelial and microvascular dysfunction in CAV plays a crucial role in the development of new therapies.

## THE ROLE OF ENDOTHELIAL FUNCTION

Coronary endothelial vasodilator dysfunction is a common finding in HT recipients and is an early marker for the development of intimal thickening and graft atherosclerosis. Since 1988, a paradoxical coronary vasoconstriction to acetylcholine in allograft recipients with and without angiographic evidence of CAV has been observed<sup>[6]</sup>. Subsequently, other investigators have observed abnormal responses (vasoconstriction and/or impairment in coronary blood flow response) to serotonin, substance P, cold-pressor testing, and exercise<sup>[7-10]</sup>. The impairment of endothelial function is time-dependent. Endothelial dysfunction is caused by both immunological and non-immunological risk factors<sup>[11]</sup>. The immunological response is the principal initiating stimulus and results in endothelial injury and dysfunction and altered endothelial permeability, with consequent myo-intimal hyperplasia and extracellular matrix synthesis. Non-immunological events, including ischemia/reperfusion time, donor age, donor brain death, infections (*i.e.*, Cytomegalovirus, CMV) and traditional risk factors such as hypertension, dyslipidemia and diabetes, contribute to maintaining inflammatory responses and to extend vessel damage<sup>[12-14]</sup>.

## Immunological response

Alloimmune injury is initiated when donor major histocompatibility antigens expressed on the surface of graft endothelial cells interact with recipient dendritic cells, resulting in a chronic immune response<sup>[15]</sup>. Recipient CD4<sup>+</sup> lymphocytes recognize donor major histocompatibility complex (MHC) class II antigens on the cell's surface (HLA-DR, DP and DQ) and are activated. This process leads to a cascade of cytokines, such as Interleukin-2 (IL-2), IL-4, IL-5, IL-6, interferon- $\gamma$  (IFN- $\gamma$ ), and tumor necrosis factor  $\alpha$  and  $\beta$  (TNF- $\alpha$ , TNF- $\beta$ ), which promote the proliferation of alloreactive T cells and stimulate the expression of other cytokines and adhesion molecules (*i.e.*, intercellular adhesion molecule-1, ICAM, and vascular cell adhesion molecule-1, VCAM) by the endothelium with leukocyte adhesion to the vessel wall. As a result, the activated macrophages and lymphocytes in the intima of the artery secrete platelet-derived growth factor and transforming growth factor, which stimulate the proliferation of smooth muscle cells (SMCs) and vascular remodeling<sup>[16]</sup>. Non-human leukocyte antigen (HLA) allo- and auto-antibodies are an increasingly recognized component of the immune response. They are often directed against angiotensin type-1 receptor and the endothelin-1 type A receptor and may alone induce endothelial activation, trigger proinflammatory, and both proliferative and profibrotic responses<sup>[17-19]</sup>.

## Nitric oxide pathway

Cytokines and growth factors lead to coronary endothelial vasodilator dysfunction through the dysregulation of the L-arginine-nitric oxide pathway, resulting in the reduced synthesis and bioactivity of the vasodilators in favor of endothelium-derived vasoconstrictors such as endothelin (ET) and thromboxane. Endothelium-derived nitric oxide (NO) is the most potent endogenous vasodilator known. It induces vasodilatation by stimulating soluble guanylate cyclase to produce cyclic guanosine monophosphate and inhibits platelet and leukocyte adherence to the vessel wall. IFN- $\gamma$  is the determinant mediator, linking endothelial dysfunction to structural changes in transplanted human arteries through the down-regulation of endothelial NO synthase (eNOS) expression, inducible-NOS (iNOS) activation and potentiating growth-factor-induced SMC mitogenesis. The iNOS is not a normal constituent of quiescent healthy cells but is expressed in a wide variety of cell types that have been exposed to bacterial endotoxin or combinations of inflammatory cytokines. Under conditions of reduced availability of L-arginine (the NO precursor), the product of iNOS is the superoxide anion, which can increase local oxidative stress and exacerbate the inflammatory process<sup>[10,20,21]</sup>. The increased production of reactive oxygen species (ROS) is considered a major determinant of reduced levels of NO<sup>[22]</sup>. In human cardiac allografts, enhanced endomyocardial iNOS mRNA expression is accompanied by the expression of nitrotyrosine protein, suggesting peroxynitrite-mediated vessel damage. Importantly, dietary L-arginine has been shown to attenuate the structural changes of CAV *in vivo*

and has been associated with the down-regulation of insulin-like growth factor- I and IL-6<sup>[10]</sup>. Recently, great importance has been attributed to the ratio of L-arginine/asymmetric dimethylarginine (ADMA), which is an endogenous NO synthase inhibitor. ADMA is normally produced by the hydrolysis of proteins and degraded by the oxidant-sensitive enzyme dimethylarginine dimethylaminohydrolase (DDAH)<sup>[23]</sup>. An increase in the ADMA levels of HT patients has been observed due to an oxidative impairment of the DDAH. The loss of endothelium-derived NO permits the increased activity of the pro-inflammatory transcription nuclear factor kappa B (NF- $\kappa$ B), resulting in the expression of leukocyte adhesion molecules<sup>[22]</sup>.

### Non-immunological mechanisms

Non-immunological risk factors for endothelial dysfunction are the same as those observed in non-transplanted patients, such as CMV infections, diabetes and dyslipidemia. CMV infection of seronegative HT recipients plays an important role in CAV development. It increases the ADMA levels, generates ROS and, through NF- $\kappa$ B activation and TNF- $\alpha$  production, induces proinflammatory cytokines and destabilizes the mRNA message for eNOS<sup>[24]</sup>. Donor- or recipient-related factors (*e.g.*, age/gender, pre-transplant diagnosis) and factors related to surgery (*e.g.*, ischemia-reperfusion injury) also increase the risk of CAV<sup>[25,26]</sup>. Diabetes mellitus is present in 28% of recipients at 1 year after HT and in 40% of patients at 5 years after HT<sup>[4]</sup>. Risk factors for new-onset diabetes include pre-transplant blood glucose of  $> 5.6$  mmol/L, a family history of diabetes, being overweight, and the pre-transplant use of immunosuppressive drugs, particularly calcineurin inhibitors and corticosteroids<sup>[27]</sup>.

Insulin resistance impedes the removal of triglycerides (TG) from very-low-density lipoproteins (VLDL) that are in circulation, resulting in hypertriglyceridemia and high VLDL concentrations. This impedance increases the transfer of cholesterol from high-density lipoproteins (HDL), thus decreasing the HDL concentrations and forming small cholesterol-depleted LDL<sup>[28]</sup>. These small dense LDL particles are rich in TG but contain relatively little cholesterol and are not readily cleared by the physiological LDL receptor; these particles are highly atherogenic<sup>[29]</sup>. Markers of metabolic syndrome such as a TG/HDL ratio of  $\geq 3$  and levels of C-reactive protein (CRP)  $> 3$  mg/L are considered markers of insulin resistance and may lead to endothelial dysfunction and the development of CAV<sup>[28]</sup>. Hyperlipidemia occurs frequently in HT recipients, with pre-existing or similar conditions to treatment with calcineurin inhibitors and corticosteroids. Hyperlipidemia leads to an increased intimal thickening, but there is only limited evidence that shows its direct association with CAV development<sup>[28]</sup>. Importantly, the benefits from statin therapy are well documented. Early treatment has been reported to be beneficial to first-year survival and has helped reduce severe rejection, thereby decreasing the development of CAV<sup>[30]</sup>. Statins inhibit MHC II induction by IFN- $\gamma$  on primary human endo-

thelial cells and monocytes-macrophages and may exert a dampening effect on MHC II-mediated T lymphocyte activation<sup>[31]</sup>.

## HISTOPATHOLOGICAL FEATURES

The precise interaction between host and donor endothelium remains unclear, but there is a significant amount of data showing a partial re-endothelization from recipient-derived cells, possibly as a response to allogenic stimuli causing vascular injury<sup>[32-34]</sup>. Endothelial chimerism (the coexistence of both donor and recipient endothelial cells) has been shown to be much higher in the microcirculation than in larger vessels, with a predilection for small epicardial and intramyocardial vessels, which had a notable 3- to 5-fold-greater chimerism than their larger counterparts. The high degree of endothelial chimerism may have immune implications for myocardial rejection or graft vasculopathy<sup>[33-37]</sup>. It has been hypothesized that this replacement could lead to a decrease in alloreactivity with a positive influence on graft outcome, but further studies are needed<sup>[38]</sup>.

A study conducted by our group investigated the correlation between levels of human endothelial circulating progenitor cells (EPCs) and microvascular dysfunction, as evaluated by CFR. We demonstrated that EPCs in both the circulation and the graft decrease significantly in HT recipients with microvascular damage. A possible explanation for this may involve humoral factors that occur in a chronic low-grade rejection and influence mobilization, migration, and cell survival<sup>[39,40]</sup>.

Hiemann *et al*<sup>[41]</sup> established a grading system of microvasculopathy in post-transplantation biopsies by light microscopy. The endothelial layer was defined as the mono-cell layer at the inner part of the blood vessel wall. The presence of a thin layer of cells whose diameter was less than the diameter of the endothelial cell cores was considered normal. Endothelial cells were graded as thickened if the diameter of the cell layer was at least as thick as the endothelial cell cores. The wall layer (media) was defined as the poly-cell layer adjacent to the endothelium. The wall was graded as normal if its diameter was less than the luminal radius. Wall thickening was classified as non-stenotic if the ratio of the luminal radius to wall thickness was  $< 3$  but  $\geq 1$ , and stenotic wall thickening was graded if this ratio was  $< 1$  (Table 1). Stenotic microvasculopathy was diagnosed if there was evidence of microvascular stenosis due to either endothelial thickening or wall thickening in at least one blood vessel per field of view on endomyocardial biopsies<sup>[41]</sup>.

## MICROVASCULOPATHY: DIAGNOSTIC TOOLS

Microvascular disease can be detected in HT recipients using both invasive and non-invasive techniques. The international society of heart and lung transplantation (ISHLT) guidelines has suggested CFR during coronary



**Table 1** Different definitions of microvasculopathy

Author	Microvessels diameter ( $\mu\text{m}$ )	Microvasculopathy assessment
Drakos <i>et al</i> <sup>[97]</sup>	< 60	Microvascular density (number of microvessels/total tissue analysis area)
Escaned <i>et al</i> <sup>[96]</sup>	< 100	Arteriolar density, capillary and arteriolar obliteration index
Hiemann <i>et al</i> <sup>[41]</sup>	10-20	Luminal radius/medial thickness < 1

angiography as an option for detecting microvascular disease in HT recipients who are suspected of having CAV, but its routine use has not yet been widely instituted<sup>[31,42]</sup>. CFR is the ratio of the maximum stress flow (during intravenous adenosine vasodilator stress) to the rest flow for a given arterial distribution with or without a stenosis or diffuse narrowing, and it could be performed in more quickly and less expensively using TDE<sup>[43,44]</sup>. Our group demonstrated that microvascular dysfunction, as evaluated by CFR measured in the distal portion of the left anterior descending coronary artery (LAD), correlates with intimal hyperplasia measured by IVUS in patients with physiologically normal epicardial coronary arteries<sup>[45-47]</sup>.

Dobutamine stress echocardiography (DSE) is a useful technique for HT recipients unable to undergo an angiogram for CAV detection. For CAV detection, the sensitivity and specificity of DSE have been shown in different studies to vary from 67% to 95% and from 55% to 91%, respectively<sup>[48-50]</sup>. However, its ability in detecting microvascular graft disease is still uncertain<sup>[51]</sup>.

Another noninvasive test is dual-source computed tomography, which showed a sensitivity of 100%, a specificity of 92%, a positive predictive value of 50%, a negative predictive value of 100%, and a global accuracy of 93% in detecting CAV. Similar to DSE, its predictive value in microvascular dysfunction is not well established<sup>[52]</sup>.

Magnetic resonance perfusion imaging with myocardial perfusion reserve (MPR) analysis showed a significant correlation with CFR when invasively evaluated.

Muehling and colleagues analyzed the resting endomyocardial/epimyocardial perfusion ratio (Endo/Epi ratio), which is decreased in impaired coronary circulation. CAV can be excluded by an MPR of > 2.3 with a sensitivity and specificity of 100% and 85%, respectively, and an Endo/Epi ratio of > 1.3 with a sensitivity and specificity of 100% and 80%, respectively<sup>[53,54]</sup>.

## MEDICAL TREATMENT

CAV prevention requires a combination of immunosuppressant agents, the prevention of CMV infection and a reduction in common cardiovascular risk factors<sup>[25,42,55]</sup>.

Endothelial dysfunction is an early marker and contributes to the development of CAV<sup>[6,56-58]</sup>. Standard immunosuppression after cardiac transplantation includes a calcineurin inhibitor (CNIs, such as cyclosporin or

tacrolimus) in combination with an antiproliferative agent [mycophenolate mofetil (MMF) or azathioprine (AZA)] with or without corticosteroids<sup>[59]</sup>. Cyclosporin (Cy-A) was the first immunosuppressive drug that had an important impact on the result of clinical HT by reducing the incidence and severity of rejection. Cy-A is known to impair endothelial function by increasing the release and response to vasoconstrictors, impairing the synthesis of NO, and generating free radicals. It may also result in increased ET levels and an impaired vascular response to NO<sup>[60-63]</sup>. Kobashigawa *et al*<sup>[64]</sup> showed that the five-year survival and incidence of angiographic CAV were similar between groups treated with microemulsion Cy-A- or tacrolimus. In a study by Meiser *et al*<sup>[65]</sup>, a more pronounced intimal proliferation was detected in the group treated with Cy-A and MMF than in the tacrolimus-MMF-treated group. Moreover, microvascular endothelial function deteriorates more in Cy-A-treated patients than in tacrolimus-treated patients, a finding that correlates with the enhanced ET-1 concentration and reduced vascular remodeling<sup>[65-67]</sup>. The progression of CAV is slower in patients randomized to receive MMF instead of AZA. The combination of Cy-A and MMF was associated with a 35% reduction in 3-year mortality or graft loss compared with patients treated with Cy-A and AZA<sup>[68]</sup>. MMF-treated HT patients, when compared to AZA-treated patients, both treated concurrently on Cy-A and corticosteroids, have significantly less progression of first-year intimal thickening<sup>[69]</sup>. In terms of CAV prevention, MMF is superior to AZA in both combinations. A trend toward improved survival in MMF patients was noted. The lower number of rejection episodes in the MMF groups may have contributed to these results.

MMF is associated with the reduction of leukocyte adhesion to the graft endothelium and inhibition of the proliferation of SMCs<sup>[70-72]</sup>. Rapamycin therapy has been associated with improved coronary artery physiology at the level of both the epicardial artery and the microvasculature soon after HT<sup>[73]</sup>. Proliferation signal inhibitors (PSIs), *e.g.*, sirolimus and everolimus, may have the potential to reduce the incidence of microvasculopathy and, later, of CAV. In a 2-year randomized clinical trial, the use of sirolimus was associated with fewer acute rejection episodes and a significant absence of the progression of intimal plus medial proliferation compared with the use of AZA<sup>[74,75]</sup>. These drugs were also associated with a lower rate of CMV infection<sup>[76,77]</sup>. The occurrence of malignancies after HT is a well-described consequence of immunosuppression that affects the long-term prognosis of HT recipients. Patients on mTOR inhibitors, a class of drugs that has been experimentally proven to have both immunosuppressive and potent antitumor effects, developed significantly fewer malignancies, as expected due to the drug's mechanism of action<sup>[78]</sup>. In a recent retrospective study, Fröhlich *et al*<sup>[79]</sup> demonstrated that statin use is also protective against malignancies. Hypercholesterolemia and hypertriglyceridemia may occur in HT recipients who are treated with sirolimus, but the presence of these side effects did not appear to impair its ability to slow the progression of CAV<sup>[80]</sup>. Everolimus is an analog

of sirolimus. Several studies demonstrated a decreased severity and incidence of CAV in HT recipients receiving immunosuppressive therapy with everolimus. It was compared with AZA in the largest trial conducted thus far for HT, which randomized 634 patients. This study showed that both average intimal thickening by IVUS and the incidence of acute rejection at 6 mo after HT were significantly lower in patients receiving everolimus<sup>[74,81,82]</sup>. Prophylaxis consisting of CMV hyperimmune globulin plus ganciclovir has been associated with decreased intimal thickening and reduced coronary artery disease<sup>[83]</sup>.

Of the recommendations made by the ISHLT regarding CAV management, only statin therapy had a level of evidence A<sup>[42]</sup>. In several studies, cholesterol and TG have been proven to directly correlate with the development and progression of CAV<sup>[84]</sup>. It is currently advocated that statins should be given soon after HT, when the most rapid expansion of intimal hyperplasia occurs. Different statins have been associated with the reduced progression of CAV. Simvastatin improved the 8-year survival in HT recipients<sup>[85]</sup>. A one-year trial in 92 patients randomized to pravastatin or no 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor showed not only lower mean cholesterol levels but also less intimal thickening by IVUS as well as less frequent high-grade acute rejections and rejections with hemodynamic compromise<sup>[86]</sup>.

The vasculoprotective effects of statins are likely mediated by multiple immunogenic effects. The immunomodulating effects of statins in the presence of Cy-A include the suppression of T-cell responses<sup>[87]</sup>, the reduction of chemokine synthesis by mononuclear cells in the peripheral bloodstream, and the inhibition of the expression of *MHC-II* genes<sup>[88]</sup>. Simvastatin inhibits the proliferation of SMCs, which is an important process in the pathogenesis of the atherosclerotic lesion. Moreover, simvastatin has been shown to have a direct influence on the gene expression of ET-1 in cultivated endothelial cells, leading to improved endothelial function and thus protecting against atherosclerosis and microvasculopathy<sup>[89]</sup>. Another direct positive effect of simvastatin in the atherogenesis process is that it reduces monocyte adhesion to endothelial cells, which is one of the initial steps in the development of atherosclerotic plaques<sup>[90]</sup>.

The use of calcium channel blockers or angiotensin-converting enzyme inhibitors (ACE-Is) decrease the incidence of CAV detected by IVUS<sup>[91]</sup>. Additionally, the use of calcium channel blockers decreases angiographically detected CAV 2-years after HT<sup>[92]</sup>. ACE-Is partially improve allograft microvascular endothelial dysfunction, reduce oxidative stress, and down-regulate endothelial ET-1 release<sup>[93]</sup>, and their use has been associated with plaque regression<sup>[94]</sup> and improved graft survival<sup>[30]</sup>. The combined use of an ACE-I and a calcium-antagonist is more effective than the individual use of either drug alone on CAV development. Large randomized clinical trials are warranted to evaluate the possibility of this synergistic efficacy<sup>[95]</sup>.

## CONCLUSION

Coronary microvascular function has an impact on long-term graft survival after HT. Microvascular vessel disease has been demonstrated by histological findings of stenotic microvasculopathy and evaluated by non-invasive CFR<sup>[41,45,96]</sup>. The potential influence of combined immunosuppressive regimens, lipid-lowering agents, or ACE-Is and/or calcium-antagonists on microvessel response is therefore of major interest. More trials are needed for microvasculopathy prevention and/or CFR preservation and to reduce the negative prognostic impact on the survival of HT recipients.

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## Transplant options for patients with type 2 diabetes and chronic kidney disease

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### Abstract

Chronic kidney disease (CKD) has become a real epidemic around the world, mainly due to ageing and diabetic nephropathy. Although diabetic nephropathy due to type 1 diabetes mellitus (T1DM) has been studied more extensively, the vast majority of the diabetic CKD patients suffer from type 2 diabetes mellitus (T2DM). Renal transplantation has been established as a first line treatment for diabetic nephropathy unless there are major contraindications and provides not only a better quality of life, but also a significant survival advantage over dialysis. However, T2DM patients are less likely to be referred for renal transplantation as they are usually older, obese and present significant comorbidities. As pre-emptive renal transplantation presents a clear survival advantage over dialysis, all T2DM patients with CKD should be referred for early evaluation by a transplant center. The transplant center should have enough time in order to examine their eligibility focusing on special issues related with diabetic nephropathy and explore the best options for each patient. Living donor kidney transplantation should always be considered as the first line treatment. Otherwise, the patient should be listed for deceased donor kidney transplantation. Recent progress in transplantation medicine has improved the "transplant menu" for T2DM patients with diabetic nephropathy and there is an ongoing discussion about

the place of simultaneous pancreas kidney (SPK) transplantation in well selected patients. The initial hesitations about the different pathophysiology of T2DM have been forgotten due to the almost similar short- and long-term results with T1DM patients. However, there is still a long way and a lot of ethical and logistical issues before establishing SPK transplantation as an ordinary treatment for T2DM patients. In addition recent advances in bariatric surgery may offer new options for severely obese T2DM patients with CKD. Nevertheless, the existing data for T2DM patients with advanced CKD are rather scarce and bariatric surgery should not be considered as a cure for diabetic nephropathy, but only as a bridge for renal transplantation.

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**Key words:** Bariatric surgery; Cardiovascular complications; Diabetes; Renal transplantation; Pancreas transplantation

**Core tip:** Kidney transplantation has been established as a first line treatment for patients with type 2 diabetes mellitus (T2DM) and diabetic nephropathy, as it is accompanied with a significant survival advantage over dialysis. Pre-emptive living donor kidney transplantation should be the ultimate goal unless there are obvious contraindications and all patients should be referred for early evaluation by a transplant center. There is an ongoing debate about the exact role of simultaneous pancreas kidney transplantation. At the moment it should be offered only in well selected T2DM patients. Bariatric surgery may serve as a bridge for renal transplantation for severely obese T2DM patients with chronic kidney disease.

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## INTRODUCTION

Chronic kidney disease (CKD) has become a real epidemic around the world, mainly due to ageing and diabetic nephropathy<sup>[1-3]</sup>. Although diabetic nephropathy due to type 1 diabetes mellitus (T1DM) has been studied more extensively, the vast majority (90%-95%) of the diabetic CKD patients suffer from type 2 diabetes mellitus (T2DM). Currently, about 40%-45% of the dialysis (hemodialysis or peritoneal dialysis) population is diabetics and present increased morbidity and mortality compared with other causes of CKD<sup>[1-6]</sup>. In addition diabetic patients comprise almost 40% of the transplant waiting lists nowadays<sup>[7]</sup>.

Diabetic CKD patients undergoing dialysis present excessive morbidity and mortality mainly due to cardiovascular complications<sup>[3-6]</sup>. Several years ago, diabetic nephropathy was considered as a relative or absolute contraindication for renal transplantation, due to increased rates of cardiovascular and infectious complications and unacceptable morbidity and mortality. However, the landmark study of Wolfe *et al*<sup>[8]</sup> has shown that renal transplantation provided a clear survival advantage for diabetics with end-stage renal disease (ESRD) and reduced mortality by 73% compared with patients remaining on the waiting list. The projected life expectancy was more pronounced for younger diabetics (presumably T1DM) reaching a gain of 17 years, whereas the gain was also significant even for patients older than 60 years (presumably T2DM).

Pancreas transplantation and especially simultaneous pancreas kidney (SPK) transplantation outcomes have seen a dramatic improvement regarding both allograft and patient survival, mainly due to advances in immunosuppression and surgical techniques<sup>[9-12]</sup>. Historically, pancreas transplantation was considered as a relative if not absolute contraindication for T2DM<sup>[7]</sup> but current data provide evidence that it can also be offered to well selected T2DM patients with CKD with comparable outcomes<sup>[7,12-14]</sup>. So, the transplant menu for T2DM patients has been expanded, but the best transplant option is still uncertain<sup>[15]</sup>.

This is an update regarding current trends in transplant medicine for T2DM patients with CKD and is based on the studies published in details in peer-reviewed journals, several previous review articles<sup>[4-7,16-18]</sup> and novel data<sup>[14,19-21]</sup> which may change our attitudes and policies regarding the management of this frail CKD population.

## PRE-TRANSPLANT EVALUATION

There is hard evidence that pre-emptive renal transplantation presents a clear survival advantage over dialysis and all T2DM patients with CKD should be referred for early evaluation by a transplant center<sup>[6]</sup>. The goal of the pre-transplant risk evaluation is to determine whether the T2DM candidate is eligible for transplantation and discuss all the potential transplant options which may include: (1) kidney transplantation. The kidney allograft may origin from a deceased donor (DDKT) or by a living donor (LDKT). If the operation takes place before

the need of dialysis it is referred as pre-emptive KT<sup>[22]</sup>, (2) simultaneous pancreas kidney (SPK) transplantation, where there is a combined transplantation of both organs, coming usually from the same donor, during a single operation. The origin of the grafts is usually from deceased donors, but there are also reports of segmental pancreatic grafts from living donors<sup>[23]</sup>.

The pros and the cons of both options will be discussed in details below in separate sections of this review.

The contraindications include the general contraindications for any organ transplantation, such as the presence of malignancy, active infection, psychiatric disease, drug/alcohol dependence, morbid obesity and untreated or end-stage organ damage with special emphasis on cardiovascular comorbidities<sup>[24,25]</sup>. Age should not be considered as an absolute contraindication for renal transplantation<sup>[8]</sup> but the increased rates of medical and surgical complications and the lower graft survival rates<sup>[5,6,8,25]</sup>, should be clearly explained in elderly diabetic candidates although some other studies did not confirm these results. Most transplant centers do not accept diabetic patients older than 45-50 years for SPK<sup>[13,15]</sup> although there are reports of SPK transplantation in patients over this limit. In 2010 the international pancreas transplant registry (IPTR) reported that 2% of pancreas transplant recipients were older than 60 years at the time of transplantation<sup>[12]</sup>.

Obesity [body mass index (BMI) > 30-35 kg/m<sup>2</sup>] has also been considered as a relative contraindication for transplantation in diabetic patients as it is accompanied with inferior outcomes for both KT<sup>[26,27]</sup> and SPK<sup>[28]</sup> mainly due to surgical complications. However, only morbid obesity (BMI > 40 kg/m<sup>2</sup>) should be considered as an absolute contraindication. Recent advances in bariatric surgery can ameliorate this contraindication and make even obese T2DM patients eligible for transplantation<sup>[29,30]</sup>. This important issue will be discussed in the end of this review.

As T2DM patients with diabetic nephropathy present increased cardiovascular morbidity and mortality, the pre-transplant evaluation should focus on the presence and the severity of coronary and peripheral artery disease. Although, there is no consensus regarding the optimal protocol for cardiovascular risk stratification, most transplant centers refer the candidates for cardiac stress testing and/or coronary angiography, especially in ages older than 55-60 years as well as dyslipidemia, history of smoking and presence of cerebrovascular or peripheral vascular disease<sup>[6,24]</sup>. However, the provocative study of Patel *et al*<sup>[31]</sup> has challenged this approach reporting that aggressive pre-transplant testing and coronary interventions did not translate into better outcomes post transplantation in high risk patients.

Peripheral arterial occlusive disease and carotid arteries stenosis examination by ultrasound examination are also mandatory in the pre-transplant evaluation. Many centers suggest a more thorough examination in high risk patients by CT or MR angiography or even intra-arterial angiography<sup>[6,24]</sup>.

All possible advantages of transplantation should be



carefully balanced against the potential complications of the surgical procedure and the long-term side-effects of immunosuppression<sup>[15]</sup>. A state of the art approach is to refer the patient with advanced diabetic nephropathy to the transplant center early, when his estimated glomerular filtration rate is about 25-30 mL/min in order to provide enough time for evaluation of both the transplant candidate and any potential living donors<sup>[6]</sup>. However, most T2DM patients with CKD are not referred early even to a nephrologist and the above policy remains rather elusive. Nevertheless, by an early referral, the transplant team can evaluate more thoroughly the diabetic candidate and order more complicated investigations such as coronary angiography, without increasing the risk of premature start of dialysis<sup>[6]</sup>. In addition, early referral will also provide time to search for LDKT with the most suitable donor or even alternative options for pre-emptive transplantation in cases of immunologically incompatible but still qualified donors, such as kidney paired donation<sup>[32]</sup>.

As SPK transplantation may also be an option for selected T2DM patients with CKD, all available data should be discussed with the transplant candidate. It should be emphasized that SPK transplantation is surgically more challenging compared with kidney transplantation, is accompanied with increased rates of complications and the short- and long-term outcomes should be reported in an unbiased way. However, it is not a standard procedure for most transplant centers and the patient may need to be referred to a more experienced center.

Regarding T2DM patients eligible for transplantation the United Network for Organ Sharing (UNOS) has defined the following criteria for SPK: (1) insulin therapy and C-peptide level < 2 ng/mL; or (2) insulin therapy with C-peptide level > 2 ng/mL and BMI < 28 kg/m<sup>2</sup><sup>[13,14,19,33]</sup>. The initial concern regarding pancreas transplantation in T2DM patients was insulin resistance that prevails in this type of DM and may result in lower pancreas allograft survival due to  $\beta$  cell exhaustion from the increased insulin demands<sup>[15,21]</sup>. These concerns and the discussion about the pros and the cons of SPK transplantation are discussed later in this review.

## KIDNEY TRANSPLANTATION FOR T2DM PATIENTS WITH CKD

Kidney transplantation is not a “panacea” for T2DM patients with CKD. Although pre-emptive renal transplantation<sup>[34]</sup>, offers a significant survival advantage for all (diabetics and non diabetics) CKD patients, diabetic CKD patients present inferior survival rates compared with other populations. Becker *et al*<sup>[22]</sup> have reported that the patient survival benefits of pre-emptive transplantation are more pronounced in LDKT than in DDKT (RR = 0.685;  $P = 0.001$ ). However renal graft survival did not present significant differences in pre-emptive transplantation except LDKT (RR = 0.81,  $P = 0.09$ )<sup>[22]</sup>. The main reason for these poor outcomes is the accumulated cardiovascular burden during the era before reaching

ESRD. Cosio *et al*<sup>[35]</sup> has shown that diabetic patients who have undergone renal transplantation have significantly increased rates of post-transplant cardiovascular events, cardiovascular mortality and all-cause mortality. It is also noteworthy that most cardiovascular events or deaths usually appear during the first three post-transplant months when the most important complications such as rejection or infections present a peak<sup>[8,35,36]</sup>. All these data highlight the importance of a thorough pre-transplant evaluation, which may detect early and potentially reversible abnormalities. In addition elderly T2DM patients with advanced CKD may present significantly decreased survival after renal transplantation rising ethical issues regarding allocation policies in an era of graft shortage and increased demand around the world.

Immunosuppressive regimens for T2DM patients do not show any difference compared with other populations. However, there is a current trend for steroid free or steroid avoidance protocols which may not aggravate glycemic control. These policies have not yet been translated into better long-term outcomes.

Hypertension and hyperlipidemia are also highly prevalent in diabetic patients post-transplantation and they should be treated aggressively. However, diabetic transplant recipients present higher rates of hyperkalemia after renin angiotensin system inhibition<sup>[4,6]</sup>.

Glycemic control should also be intensified as hyperglycemia has been associated with worse outcome. However, optimal targets for renal transplantation have not been set yet and transplant physicians usually follow the guidelines for the general population.

## SPK TRANSPLANTATION FOR T2DM PATIENTS WITH CKD

By the end of 2010 more than 35000 pancreas transplantations had been reported to the IPTR with the vast majority (24000) performed in the United States<sup>[12]</sup>.

Historically, pancreas transplantation was considered as a relative if not absolute contraindication for T2DM<sup>[7]</sup>. This concept relied on the pathophysiology of T2DM where insulin resistance has been considered as the prevailing disorder and these patients do not seem to need extra insulin but a better responsiveness of the peripheral tissues to it. However, the classification of diabetes is not always so simple and many patients present with overlapping clinical syndromes. In addition, even in the long-run, not a few T2DM patients may become dependent on exogenous insulin due to pancreatic b-cells exhaustion. Although the classical phenotype of T2DM with CKD is characterized by advanced age and obesity, there are many patients who do not fit on this model and may be seen as candidates for pancreas transplantation.

Initial reports about SPK transplantation in T2DM were based on cases of “unrecognized” T2DM<sup>[7]</sup>. The IPTR started to be record data about the type of diabetes since 1994. The overall rate of pancreas transplantation in T2DM patients has shown an increase from 2% in 1995 up to 7%

**Table 1** Selected data from pancreas transplantation single-center and database studies in type 2 diabetes mellitus patients

Ref.	PTx Era	Number of PTx (n)	Age at PTx (yr)	BMI (kg/m <sup>2</sup> )	Follow-up (yr)	Pancreas survival rates (yr)	Patient survival rates (yr)
Light <i>et al</i> <sup>[37]</sup>	1989-1999	30 SPK	40 ± 9.3 <sup>1</sup> 41.7 ± 6.6 <sup>2</sup>	24.8 ± 5.4 <sup>1</sup> 25.5 ± 4 <sup>2</sup>	3.8	82% (1) <sup>1</sup> 95% (1) <sup>2</sup> 82% (5) 95% (5)	82% (1) <sup>1</sup> 100% (1) <sup>2</sup> 82% (5) 95% (5)
Light <i>et al</i> <sup>[38]</sup>	1989-2004	38 SPK	40 ± 9.3 <sup>1</sup> 37.9 ± 8.7 <sup>2</sup>	24.8 ± 5.4 <sup>1</sup> 23 ± 4.5 <sup>2</sup>	> 10	67% (5) <sup>1</sup> 56% (10) <sup>2</sup>	73% (5) <sup>1</sup> 70% (10) <sup>2</sup>
Light <i>et al</i> <sup>[20]</sup>	1989-2008	58 SPK	42.8 ± 8.4	26.1 ± 4.4	> 15-20	58.60% (> 10)	75.8% (> 10)
Nath <i>et al</i> <sup>[39]</sup>	1994-2002	7 SPK 4 PAK 6 PTA	52.5 ± 8.4	27.2 ± 5	4.3	65% (3.3)	94% (1) 71% (3.3)
Singh <i>et al</i> <sup>[40]</sup>	2002-2007	7 SPK	51 ± 2.9	ND	3.3	71% (3.3)	86% (1) 71% (3.3)
Chakkerla <i>et al</i> <sup>[41]</sup>	2003-2008	10 SPK	51.9 ± 9	27 ± 3	1.3	100% (1)	100% (1)
Margreiter <i>et al</i> <sup>[21]</sup>	2000-2009	21 SPK	53.6 ± 5.9	25.1 ± 3.3	7.3 ± 3	81.8 (1) 75.9 (5)	90.5 (1) 80.1 (5)
Sampaio <i>et al</i> <sup>[14]</sup>	2000-2007	582 SPK	47 (40-52)	< 18.5 = 2.8% 18.5 to 25 = 43.9% 25 to 30 = 36.2% > 30 = 17.15	3.7	-	67% (5)
Wiseman <i>et al</i> <sup>[19]</sup>	2000-2008	424 SPK	18-34 = 6.1% 35-49 = 54% 50-59 = 39.9%	24.7 ± 2.8	5	87.7 (1) 83.6 (5)	82% (5)

<sup>1</sup>Data for non African-Americans; <sup>2</sup>Data for African-Americans. PTx: Pancreas transplantation; PAK: Pancreas after kidney; PTA: Pancreas transplant alone; SPK: Simultaneous pancreas kidney.

in 2010. According to the same database, in 2010 approximately 8% of SPK, 5% of pancreas after kidney (PAK) and 1% of pancreas transplant alone (PTA) were performed in T2DM patients. T2DM patients who underwent PAK or SPK were older than T1DM patients, whereas there were no age differences between the two groups for PTA. As expected, T2DM patients had a longer duration of DM (22 ± 8 years) and significantly higher BMI<sup>[12]</sup>.

The usefulness of SPK in T2DM patients with CKD can not be justified by evidence from randomized controlled studies and is based on several single center<sup>[20,21,37-41]</sup> and two recent database studies<sup>[14,19]</sup> which will be analyzed in details (Table 1). The main problem of all these studies is that they rely on different approaches regarding the classification of diabetes, which are based on several clinical or laboratorial criteria not validated in CKD and different demographics. There is an ongoing debate about the usefulness of C-peptide for the diagnosis of diabetes as there is evidence that not a few T1DM patients may present measurable serum levels<sup>[42]</sup> and many T2DM patients may also present with undetectable serum levels after many years post-diagnosis. Covic *et al*<sup>[43]</sup> have confirmed these data in CKD patients making the situation even more complicated.

In addition, traditional exclusion criteria for SPK such as age > 50 years and BMI > 30 kg/m<sup>2</sup> which were applied in the first studies, tend to be ignored in the more recent reports, making the interpretation of the short and long-term outcomes not so easy.

### Single-center studies

Light *et al*<sup>[37]</sup> were the first who attempted to publish

pooled data about outcomes of T2DM patients who underwent SPK transplantation. In 2001 they presented data for 30 patients classified as T2DM according to C-peptide levels > 0.8 ng/mL and compared them with a group of 89 patients with lower C-peptide levels over a 10 years period<sup>[37]</sup>. C-peptide levels were not crucial for the decision to proceed with SPK transplantation in their center. There were no differences between the two groups regarding patient and graft survival rates, although T2DM patients tended to be older and heavier (not statistically significant differences). In 2005 the same group extended their follow-up period and reported outcomes in 38 SPK recipients<sup>[38]</sup>. Outcomes at 5 and 10 years post transplant did not show significant differences and the authors suggested that decisions about SPK transplants should not be based on C-peptide levels, but on general acceptance criteria. In 2013 they reported a 20 years experience of SPK transplantation based on data from 173 patients<sup>[20]</sup>. The T2DM group included 58 patients who underwent transplantation from 1989 through 2008 with the same inclusion criteria (C-peptide levels > 0.8 ng/mL). According to this analysis T2DM patients presented better pancreatic graft survival ( $P = 0.064$ ) but lower patient survival (0.019) during the extended follow-up period. There are no definite explanations for these results, but it is noteworthy that T2DM patients presented lower rejection rates. Moreover, the T2DM group included more African-American and was older, heavier and had a shorter duration of insulin dependence. The authors concluded that C-peptide should not be a marker for SPK candidacy and transplant centers should base their decisions on general criteria which prove whether the diabetic

patient can tolerate the surgical procedure and adhere to the complex follow-up post-transplant.

Nath *et al*<sup>[39]</sup> reported a cohort of 17 T2DM patients who underwent pancreas transplantation from 1994 through 2002. Seven patients underwent SPK, 4 patients PAK and 6 patients PTA. The authors adopted the american diabetes association and World Health Organization criteria for T1DM and T2DM and did not rely on C-peptide levels<sup>[39]</sup>. Three patients were on oral hypoglycemic agents at the time of transplantation. Although 1 patient died during the peri-operative period (aspiration pneumonia) the other pancreas recipients presented excellent graft survival rates (94%). Long-term follow up (4.3 years) showed a patient survival rate of 71% and a pancreas survival rate of 63%.

Singh *et al*<sup>[40]</sup> stratified a cohort of 74 SPK transplants from 2002 through 2007 into two groups according to C-peptide cut-off levels of 2 ng/mL. They wisely did not use the terms T1DM or T2DM but they isolated a subgroup of SPK recipients of “insulin requiring diabetic patients with C-peptide production” for further analysis. So, they reported short- and long-term outcomes in 67 patients with “no” C-peptide (mean  $0.2 \pm 0.4$ , range 0-1.9 ng/mL) and 7 patients with C-peptide production (mean  $5.7 \pm 2.7$ , range 2.5-9.5 ng/mL). Their selection criteria for SPK transplantation included insulin requirement for at least 5 years, daily dose  $< 1$  U/kg, age  $< 60$  years, and absence of severe comorbid conditions, but not C-peptide levels. Patient survival was better in the “no” C-peptide group at 3 mo, 1 year and last follow-up (40 mo), whereas death-censored kidney and pancreas graft survivals did not present significant differences between the two groups. However, there were significant differences between the two groups before SPK transplantation, which have definitely influenced outcomes. The group with the C-peptide production included more African-Americans, was older, heavier and had a shorter duration of diabetes and a longer dialysis vintage.

Chakkerla *et al*<sup>[41]</sup> reported a cohort study of 80 patients who underwent SPK transplantation from 2003 until 2008. Among them, 10 patients were identified as T2DM patients according to a composite metric which included clinical criteria (absence of ketoacidosis and use of oral antidiabetics), presence of measurable C peptide levels and negative glutamic acid decarboxylase antibodies (anti-GAD65). Patients were eligible for SPK, if BMI was lower than  $30 \text{ kg/m}^2$  and needed  $< 1$  U/kg of insulin per day. T2DM patients presented excellent (100%) 1 year pancreas survival as well as T1DM patients (96%) and equal renal graft survival rates after a 16 mo follow-up period. The authors also commented on the usual value of the C-peptide cutoffs in the diagnosis of T1DM ( $< 0.8$  ng/mL) and highlighted that there was a significant overlap of C-peptide levels among T1DM (almost 15% had detectable levels and 8%  $> 0.8$  ng/mL), whereas 30% of the T2DM patients presented low C-peptide levels ( $< 2$  ng/mL) and could be misclassified as T1DM.

Margreiter *et al*<sup>[21]</sup> have recently reported their experience from 195 T1DM and 21 T2DM patients who un-

derwent SPK transplantation during a nine years period (2000-2009) in Austria. The vast majority (30/32) of the T2DM patients were on exogenous insulin therapy and had a history of oral antidiabetic agents for at least 6 months. Only 2 patients were receiving oral antidiabetics at the time of transplantation. The main criteria for the diagnosis of T2DM were measurable fasting C peptide levels and absence of autoantibodies for diabetes. All patients presented a low cardiovascular risk profile and were eligible for SPK if BMI was lower than  $32 \text{ kg/m}^2$ . The authors compared outcomes with T1DM patients who underwent SPK transplantation ( $n = 195$ ) and T2DM patients who underwent DDKT alone ( $n = 32$ ) during the same period. Although pancreas allograft survival was lower in T2DM patients, it did not reach statistical significance. In a univariate analysis, the T1DM group presented better patient and kidney survival compared with the other groups. However, in a multivariate analysis model the statistical significance was lost, when data were adjusted for various important confounding variables such as donor and recipient age, secondary complications of diabetes, waiting time, delayed graft function etc.

Selected data for comparison from all these studies are shown in Table 1.

### Database studies

Sampaio *et al*<sup>[14]</sup> studied outcomes of SPK transplantation during the period between 2000 and 2007 using data from the UNOS database. Among 6756 SPK transplants there were 582 T2DM cases (8.6%). T2DM patients presented higher rates of delayed kidney graft function and primary kidney non function and inferior rates of 5 year overall (73.5% *vs* 77.8%,  $P = 0.007$ ) and death censored kidney graft survival 82.9% *vs* 85.3%,  $P = 0.04$ ) compared with T1DM patients. However, this group included more African-American and Hispanics and the patients were older at diabetes onset and at the time of transplantation, were more often obese and had a higher pre-transplant dialysis time. All these parameters are known to impact transplant outcomes and when data were analyzed after adjustment for confounders, diabetes type could not be identified as a risk factor for all outcomes. In details, hazard ratios were 1.10 (95%CI: 0.86-1.42) for patient death, 1.08 (95%CI: 0.91-1.28) for pancreas allograft failure and 1.16 (95%CI: 0.95-1.39) for kidney allograft failure with T1DM values as reference. Further analysis revealed that increased recipients' age, time spend on dialysis pre-transplant and higher BMI were associated with worse outcomes in T2DM patients. However, the study carried a significant limitation regarding the definition of diabetes type which relied mainly on clinical history data and not specified criteria.

Wiseman *et al*<sup>[19]</sup> analyzed data from 424 SPK transplants in T2DM from 2000 through 2008, using the Scientific Registry of Transplant Recipients database and compared outcomes with patients who underwent LDKT or DDKT. They included in their analysis only recipients aged from 18 to 59 years with a BMI index ranging from  $18\text{-}30 \text{ kg/m}^2$ . Although there were no reliable definitions

of diabetes type in this study, the selection criteria have probably eliminated the percentage of misclassification. In this study the authors reported several very interesting and important results. Although SPK outcomes were excellent even after 5 years post-transplant and looked superior to DDKT, this difference was not due to the pancreas allograft *per se* but to other important factors such as younger allograft kidney donors, younger recipient age and less waiting time for transplantation. In addition the analysis provided a clear 5 year survival advantage in favor of LDKT over SPK. However, the authors acknowledge that the possible advantages of SPK (euglycemia) regarding patient and kidney survival may become clearer after a longer follow-up and patients who undergo SPK may represent a special and probably pre-selected population of T2DM patients. In addition quality of life issues (insulin injections, hypoglycemia, *etc.*) may be more important for several T2DM patients with ESRD than survival. Nevertheless, these data provide clear evidence that LDKT should be considered as a first choice treatment for T2DM patients with CKD and SPK should be seen as a second choice for well selected patients.

### Data overview

The results from all these single center and database studies do not provide a clear message about the pros and the cons of SPK in T2DM with CKD and many physicians remain skeptic about its definite role, as it carries significant surgical challenges and it is not an immediately life saving procedure<sup>[13,17]</sup>. The recently applied UNOS criteria for eligibility of T2DM patients for SPK include only C-peptide levels cut-offs and BMI values (see above), although there is no solid data about this policy<sup>[15]</sup>. Theoretically, T2DM patients who are eligible for listing for both DDKT and SKT transplantation may be transplanted faster if listed for SPK according to the priority criteria for kidney and pancreas allocation. Nevertheless, it should be emphasized that this theoretical concern may not be proven correct in the real clinical practice, as SPK transplantation is performed only in selected transplant centers and its rates tend to fall over the last years<sup>[12]</sup>.

## ISLET TRANSPLANTATION AND T2DM

Islet Transplantation refers to the transplantation of isolated pancreatic islets, which have been harvested from one or more deceased donors. It is not a classic surgical procedure and the islets are infused percutaneously into the portal vein<sup>[44]</sup>.

Allogeneic islet transplantation in humans become popular after the landmark study of the Edmonton group in 2000<sup>[44]</sup> which showed insulin independence in seven T1DM patients with a steroid free regimen. Nevertheless, these first encouraging results could not be fully reproduced by other centers and patients needed multiple islet transfusions with a long-term success below 10%<sup>[45-47]</sup>. In addition, the immunosuppressive protocols are potentially nephrotoxic and may be accompanied with a deterioration of the renal function<sup>[48,49]</sup> whereas the

failed islet grafts may lead to recipients' alloimmunization (sensitization) by the production of *de novo* anti-HLA antibodies in titers ranging between 10.8%-31%<sup>[48-50]</sup>. These poor results have raised skepticism in the transplant community<sup>[51]</sup> and today only a few centers continue islet transplants on a regular basis in T1DM patients<sup>[46,47]</sup>. Although the ultimate goal of islet transplantation would be to achieve insulin independence, this remains an exemption and the current goals focus mainly on protection from hypoglycemia, reduction of the daily dose of insulin and correction of HbA1c<sup>[47]</sup>.

Islet transplantation has not been widely applied in T2DM patients. In the literature there is only one report regarding islet transplantation in 5 insulin treated T2DM patients<sup>[52]</sup>. However these patients were undergoing liver transplantation and islet were given as a possible treatment for coexisting T2DM. Three of them presented normalization of HbA1c and no need for insulin therapy. However, although hypothetical, if clinical data for T1DM patients improve in the future, it would not be a surprise to see islet transplantation applied in T2DM patients, following the example of SPK<sup>[17,19]</sup>.

## BARIATRIC SURGERY FOR T2DM PATIENTS WITH CKD

The term "diabesity" has been introduced in the current literature in order to describe the frequent co-existence of T2DM and obesity<sup>[53]</sup>. Although bariatric surgery procedures tend to increase around the world, there is a debate about its place in the treatment of diabetes<sup>[53,54]</sup>. Current standards suggest that it has a role in patients with BMI > 35 kg/m<sup>2</sup> with one at least comorbid condition including T2DM<sup>[54]</sup>. Its theoretical advantages for T2DM patients with lower BMI values remain unproven<sup>[53-55]</sup>. In addition, there is an ongoing interest regarding the impact of obesity on the pathogenesis and the progression of CKD<sup>[56]</sup>. However, there is no solid data regarding the beneficial effects of bariatric surgery in CKD, except some small observational single-center studies focusing mainly on the regression of micro- or macro-albuminuria<sup>[56,57]</sup>.

As most transplant centers include obesity (BMI > 30-35 kg/m<sup>2</sup>) in the contraindications for renal or SPK transplantation due to excessive surgical complications, many obese T2DM patients may not qualify. So, bariatric surgery has been recently introduced, not as a cure for diabetic nephropathy *per se*, but as a "bridge" for transplantation. There are a few reports about this alternative in patients with advanced CKD, but the complication rates were substantially higher than in non CKD patients<sup>[18,58,59]</sup>. However these data came from open surgical procedures and currently applied laparoscopic approaches may reduce complications and improve outcomes. Nevertheless, although promising, bariatric surgery in CKD patients or more especially in T2DM patients with CKD has not been studied in depth and should be still considered as experimental<sup>[56,60]</sup>. If applied, this must be done in specialized and experienced centers under a multidisciplinary



approach.

Nevertheless, a recent analysis of the United States Renal Data System has questioned the current BMI thresholds, as it has shown that even obese diabetic renal transplant recipients may show a survival benefit compared to treatment with dialysis, except patients with BMI > 40 kg/m<sup>2</sup> and obese African Americans<sup>[61]</sup>.

## CONCLUSION

Although during the first era of transplant medicine T2DM patients with CKD were considered non eligible for kidney transplantation, recent progress in transplantation medicine has improved their “transplant menu”. As pre-emptive kidney transplantation provides a clear survival advantage over dialysis, all patients with no obvious contraindications, should be referred for early evaluation by a transplant center.

There are data that SPK transplantation may be offered in T2DM patients with acceptable long-term outcomes, but it should be noted that the decision is not so easy, as these results come from retrospective studies from very experienced centers and these patients carry particular characteristics (younger ages, no obesity, minimal cardiovascular risk, etc.) that may not apply to the average T2DM patient with CKD.

Bariatric surgery may also be considered as a “bridge” to transplantation for very obese T2DM candidates, but at the moment there are no clear data about its outcomes and possible complication rates in this population. Prospective multi-center studies are warranted in order to clarify all these issues. Until then, the most appropriate transplant option for T2DM patients with diabetic nephropathy should always be individualized, taking under consideration the patient's wills, his overall medical condition and the transplant center's experience with all these procedures.

The transplant menu looks delicious, but we must be a bit more patient.

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## Human amniotic membrane transplantation: Different modalities of its use in ophthalmology

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### Abstract

The amniotic membrane (AM) is the inner layer of the fetal membranes and consist of 3 different layers: the epithelium, basement membrane and stroma which further consists of three contiguous but distinct layers: the inner *compact layer*, middle *fibroblast layer* and the outermost *spongy layer*. The AM has been shown to have anti-inflammatory, anti-fibrotic, anti-angiogenic as well as anti-microbial properties. Also because of its transparent structure, lack of immunogenicity and the ability to provide an excellent substrate for growth, migration and adhesion of epithelial corneal and conjunctival cells, it is being used increasingly for ocular surface reconstruction in a variety of ocular pathologies including corneal disorders associated with limbal stem cell deficiency, surgeries for conjunctival reconstruction, as a carrier for *ex vivo* expansion of limbal epithelial cells, glaucoma surgeries and scleral melts and perforations. However indiscriminate use of human AM needs to be discouraged as complications though infrequent can occur. These include risk of transmission of bacterial, viral or fungal infections to the recipient if the donors are not adequately screened for communicable diseases, if the membrane is not processed under sterile condi-

tions or if storage is improper. Optimal outcomes can be achieved only with meticulous case selection. This review explores the ever expanding ophthalmological indications for the use of human AM.

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**Key words:** Human amniotic membrane; Limbus; Stem cells; Ocular surface; Cornea

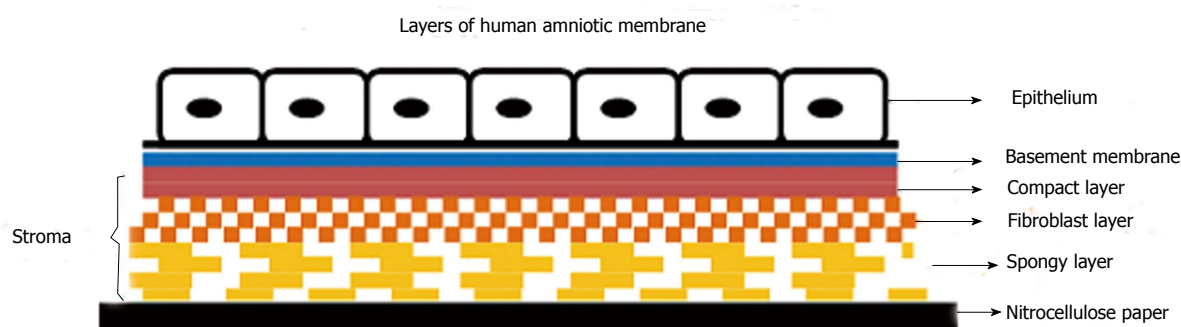
**Core tip:** Amniotic membrane transplantation is a very useful armamentarium in the hands of the ophthalmic surgeons for treating a variety of ocular surface disorders. Because of its transparent structure, anti-inflammatory, anti-fibrotic and anti-angiogenic properties and ability to provide a substrate for growth of corneal and conjunctival epithelial cells, it forms an ideal material for ocular surface reconstruction.

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### INTRODUCTION

The ocular surface is an extremely sensitive and dynamic structure, the health of which is crucial for the optimal functioning of the eye. Any mechanical or chemical insult to it either from exogenous sources, *i.e.*, chemical injuries by substances like acids and alkalis, or from endogenous factors, *i.e.*, change in the amount and composition of the tear film due to severe dry eye states associated with conditions like Stevens Johnson syndrome (SJS), rheumatoid arthritis and other collagen vascular diseases, can result in anatomic, physiologic and optical dysfunction of the eye as a whole.





**Figure 1** Line diagram showing layers of cryopreserved human amniotic membrane, oriented with the stromal side in contact with the nitrocellulose filter paper and epithelial side facing up.

Various biological tissues have been attempted to be used as donor tissue to repair and reconstruct the ocular surface or to decrease the inflammation in instances where the conjunctiva and cornea get significantly damaged. These include among others oral, labial and vaginal mucous membranes and rabbit peritoneum. Amniotic membrane (AM) was first used therapeutically by Davis for skin transplantation in 1910<sup>[1]</sup>. De Roth however is the first person credited with having used fetal membranes in ophthalmic surgery in an attempt to reconstruct the ocular surface in patients with symblepharon<sup>[2]</sup>. The initial enthusiasm for use of this tissue however disappeared from documented ophthalmic literature, till the early nineties, when Batlle *et al*<sup>[3]</sup> used it to repair conjunctival defects and reconstruct the fornices.

## STRUCTURE OF THE FETAL MEMBRANES

The fetal membranes consist of two layers: the outer chorion which is vascular and in contact with the uterine wall, and the amnion which is avascular, lies inner to the chorion and is in contact with amniotic fluid. The AM is 0.02-0.05 mm thick and is classically considered to be composed of three layers (Figure 1).

### Epithelium

Which is a monolayer of metabolically active cuboidal cells with microvilli present on its apical surface.

### Basement membrane

Made up of type IV, V and VII collagen (also found in conjunctival and corneal basement membranes) in addition to fibronectin and laminin<sup>[4]</sup>. It is one of the thickest membranes in the human body and can withstand current cryopreservation techniques.

### Stroma

This is further divided into three contiguous but distinct layers: the inner *compact layer* which is in contact with the basement membrane and contributes to the tensile strength of the membrane, middle *fibroblast layer* which is thick and made up of a loose fibroblast network and the outermost *spongy layer*.

## MECHANISM OF ACTION

Several mechanisms of action are attributed to the AM's ability to help in healing and reconstruction of the ocular surface.

### Mechanical

The AM acts as a biological bandage and shields the regenerating epithelium from the frictional forces generated by the blinking movements of the eyelids<sup>[5]</sup>. This is especially of significance in cases where entropion, trichiasis, keratinization of lid margin/palpebral conjunctiva or other such lid pathology exists which can damage the fragile epithelium, *e.g.*, trachoma, SJS, ocular cicatricial pemphigoid (OCP), *etc.* Use of the AM in addition to tilting the balance of the ocular surface towards healing, also dramatically decreases the subjective symptoms of pain and discomfort experienced by these patients, especially when implanted on deepithelized areas of the cornea. This has been attributed to a purely mechanical effect and not because of the biological mediators present in the membrane, as elegantly demonstrated by Lee *et al*<sup>[6]</sup> in experimental studies on rabbits where application of amniotic fluid to denuded corneas (created by subjecting the animals to excimer laser photo keratectomy) increased the corneal sensitivity and upregulated regeneration of nerves.

### Promotion of epithelialization

The basement membrane of the AM closely resembles that of the conjunctiva and cornea especially with regards to its collagen composition. It thus serves as a substrate on which epithelial cells can grow easily. Four main effects on the regenerating corneal epithelium have been described: (1) facilitation of epithelial cell migration<sup>[7,8]</sup>; (2) reinforcement of basal epithelial cell adhesion<sup>[9-11]</sup>; (3) promotion of epithelial cell differentiation<sup>[12-14]</sup>; and (4) Prevention of apoptosis<sup>[15,16]</sup>. These properties render it suitable for use in cases of nonhealing or persistent epithelial defects of the ocular surface, especially that of the cornea.

### Anti-fibrotic and anti-inflammatory properties

Fetal hyaluronic acid is an important constituent of the

stromal matrix of the AM. This helps to suppress TGF  $\beta$  signaling with reduced expression of TGF  $\beta$ -1,  $\beta$ -2, and  $\beta$ -3 isoforms in addition to reduced expression of TGF-Receptor II. This inhibits proliferation of corneal, limbal and conjunctival fibroblasts. Differentiation of fibroblasts into myofibroblasts is also inhibited, thus reducing scarring after pterygium surgery and ocular surface reconstruction<sup>[17]</sup>. Anti-inflammatory effect of AM is driven by inhibition of expression of pro inflammatory cytokines from the damaged ocular surface, *e.g.*, interleukin (IL) 1a, IL-2, IL-8, interferon- $\gamma$ , tumor necrosis factor- $\beta$ , basic fibroblast growth factor and platelet derived growth factor<sup>[18]</sup>. In addition to the chemically mediated anti-inflammatory effect, Shimmura *et al*<sup>[19]</sup> also demonstrated a more mechanical effect by showing that inflammatory cells get trapped and undergo apoptosis in the matrix of the AM.

### Anti-angiogenic properties

In addition to the anti-inflammatory properties which retard new vessel proliferation, a specific anti-angiogenic effect has also been ascribed to the AM. This has been demonstrated to be due to the production of several potent anti angiogenic chemicals including thrombospondin -1, endostatin and all four tissue inhibitors of metalloproteases (TIMP-1, 2, 3 and 4)<sup>[20]</sup>. Though beneficial in most situations the anti-angiogenic effect of AM needs to be kept in mind and balanced against its other potential benefits when using it in limbal stem cell deficiency associated with limbal ischaemia, *i.e.*, in chemical injuries of the ocular surface.

### Anti- microbial properties

A literature review reveals conflicting reports about the anti-microbial properties of AM. Burn patients treated with AM have been shown to have decreased bacterial counts and control of infections<sup>[21,22]</sup>. Antibacterial effects have been demonstrated against both gram positive cocci including streptococci and *Staphylococcus aureus* as well as gram negative bacilli including *Escherichia coli* and *Pseudomonas aeruginosa*<sup>[23,24]</sup>. These antibacterial effects have been attributed to the presence of several anti-microbial factors in the amniotic fluid including bacitracin, beta-lysin, lysozyme, transferrin and 7S immunoglobulin<sup>[25,26]</sup>. Other investigators however believe that the AM does not per se contain any chemical antimicrobial substances, but rather just constitutes an effective physical barrier against infection because of its ability to adhere closely to the underlying surface<sup>[24,27]</sup>.

In addition to the above properties another important characteristic of the human AM is a lack of expression of the major histocompatibility antigens HLA-A, B, or DR antigens<sup>[28,29]</sup>. Hence immunological rejection after its transplantation does not occur and obviates the need for any immune suppression. This feature along with the transparent structure and ability to be preserved for prolonged periods make the AM an ideal substrate for ocular surface transplantation.

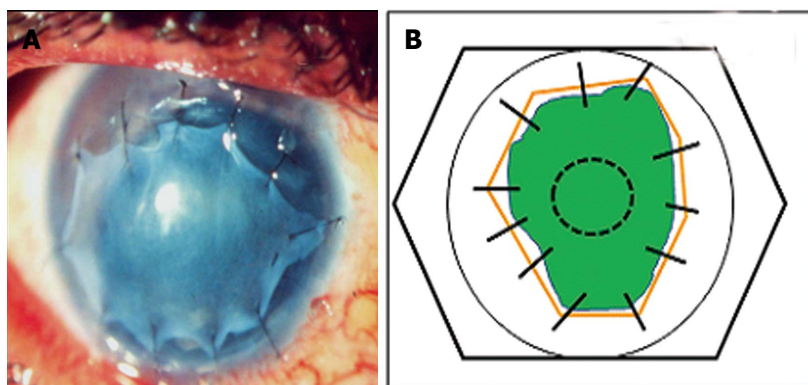
## PROCURING, PROCESSING AND PRESERVING THE AM

AM is retrieved under strict aseptic conditions from donors undergoing elective cesarean section and who have been previously screened serologically for potentially communicable diseases including human immunodeficiency virus, hepatitis B and C viruses and syphilis. Placenta obtained after vaginal delivery are not used for this purpose because of the potential for contamination with bacteria from the vagina. It is recommended that the maternal donor should undergo repeat serological screening after 6 mo (to cover the window period for transmission of communicable diseases) before the AM is released for use<sup>[30]</sup>. Tissue is used for transplantation only when both the samples are negative.

Antibiotics covering both gram positive and gram negative bacteria as well as fungi (50  $\mu$ g/mL penicillin, 50  $\mu$ g/mL streptomycin, 100  $\mu$ g/mL of neomycin, 2.5  $\mu$ g/mL of amphotericin B) are used to wash the placenta under sterile conditions. Blunt dissection is then used to separate the amnion from the chorion. The AM may be preserved by means of cryopreservation (cryopreserved human amniotic membrane, CHAM) or in a dry deepithelialized form (dry human amniotic membrane, DHAM). To prepare CHAM Kim *et al*<sup>[31,32]</sup> and Lee *et al*<sup>[33]</sup> recommended using 50% glycerol in Dulbecco's modified Eagle Medium (DMEM) in a ratio of 1:1 to store the membrane. The membrane is cut into multiple pieces and placed on nitrocellulose paper strips with epithelial side up. It is then placed in vials containing the glycerol/DMEM storage medium and cryopreserved at -80 °C. Just prior to use the membrane should be taken out and warmed to room temperature for 10 min. CHAM stored in glycerol may be safely and effectively used for over a year with the added advantage of antiviral and antibacterial properties of glycerol<sup>[34]</sup>. One drawback with using CHAM is the need of a -80 °C refrigerator. This precludes its use outside big institutions.

DHAM does not require to be attached to nitrocellulose paper and is free standing. DHAM is prepared by subjecting the amniotic membrane to sterilization using low energy electron beam radiation and then preserving it using low heat and air vacuum. DHAM can be stored at room temperature for upto 2-5 years and is rehydrated prior to use. It is usually 35-40 microns thick but a third generation, 110- $\mu$ m-thick, freeze dried, and freestanding human AM allograft (*Ambio 5; IOP Inc, Costa Mesa, California*) is commercially available. This has an additional thick layer of retained collagen from the chorionic membrane, which makes it thicker and confers greater tectonic function.

Both fresh and preserved AM have been found to be equally effective when transplanted onto the ocular surface<sup>[35]</sup>. Use of freshly acquired AM however is associated with certain disadvantages including the risk of transmission of communicable diseases as the donor cannot undergo repeat serological testing, and wastage of



**Figure 2** (A) Amniotic membrane used as an inlay graft (B) line diagram showing amniotic membrane (solid orange lines) used as an inlay graft for a non-healing epithelial defect (green). The membrane is trimmed to fit the size of the underlying defect and sutured to the cornea using interrupted 10-0 nylon monofilament sutures.

unused tissue (with preserved AM, up to 30 grafts can be prepared with one placenta). Preservation of the AM by any means has been shown to adversely affect the viability and proliferative capacity of its epithelial cells<sup>[36,37]</sup>. Kruse *et al.*<sup>[36]</sup> proposed that AM grafts function primarily as a matrix and not by virtue of transplanted functional cells. Other literature on the subject also supports the view that viability of cellular components of the AM is not essential for its biological effectiveness<sup>[38]</sup>.

## SURGICAL PRINCIPLES AND METHODS OF IMPLANTATION

### **Rationale for determining orientation of AM on ocular surface**

This is important as the indication for which the AM is being used and the endpoint desired determines the preferred orientation with which it is used on the ocular surface. Histopathological analysis has revealed that after application of AM the re-epithelialization of the ocular surface by the host epithelium (*i.e.*, by the host corneal or conjunctival epithelium) occurs preferentially on the basement membrane side of the epithelium<sup>[39]</sup>, though Seitz *et al.*<sup>[40]</sup> have also demonstrated that corneal epithelial cells do possess the ability to grow on the stromal side of the membrane. Hence where the membrane is used with the aim of providing conjunctival or corneal cells a substrate to grow on, the AM is used epithelial/basement side up. The stromal matrix of the AM on the other hand has the ability to trap inflammatory cells and induce their apoptosis, thus down regulating the inflammatory response<sup>[38]</sup>. Thus in the presence of acute inflammation, the membrane may be used to protect the ocular surface from the deleterious effects of the pro inflammatory cells and mediators- here it is used epithelial side down, so that the stromal side faces the palpebral aperture.

### **Determining the orientation of the AM**

The AM supplied on the nitrocellulose filter paper is usually oriented epithelial side up, with the stromal side in direct contact with the paper. The stromal surface can be identified by the presence of vitreous-like strands that can be raised by a sponge or a fine forceps. This may need to be performed at a few points for confirmation.

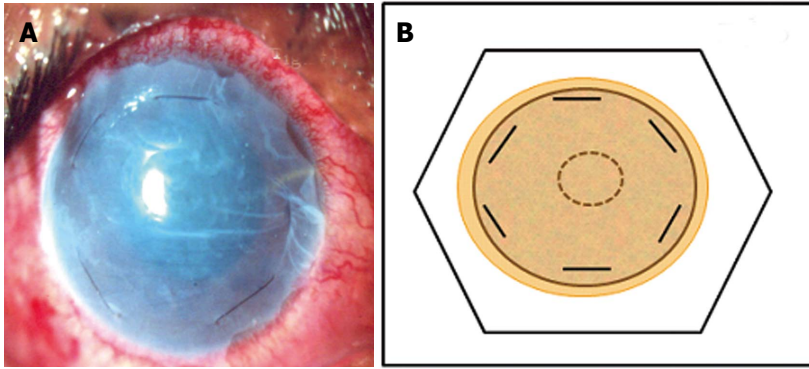
Depending on the indication for which it is used there

are three surgical techniques by which the AM can be used over the ocular surface.

**Graft or inlay technique:** In this technique the AM is intended to act as a substrate or scaffold for epithelial cells to grow. The AM is placed epithelial/basement membrane side up and is trimmed to fit the size of the underlying epithelial or stromal defect. It is usually sutured to the cornea using non absorbable 10-0 nylon sutures and to the episclera and conjunctiva using 9-0 or 10-0 vicryl (Figure 2). It is preferred to keep the epithelial/basement membrane side up in this technique because the basement membrane of the amnion acts as an excellent substrate for growth of the progenitor epithelial cells by prolonging their lifespan, maintaining clonigenecity and preventing apoptosis<sup>[41]</sup>. The surrounding 1-2 mm of the host corneal epithelium is debrided. This ensures that the regenerating epithelium grows over the basement membrane of the AM, and consequently the AM stroma gets incorporated into the host tissue ("graft"). Depending on the depth of the underlying defect this technique may be used as a *single layer graft inlay* where a single layer of AM is used, or *multilayer graft inlay* where multiple layers of the AM are placed into the ground of the ulcer, which is filled without sutures before a superficial graft is sutured to the periphery of the ulcer, again after de-epithelialization of a ring-shaped area around the cornea ulcer. The epithelium is expected to grow over the uppermost layer of this multilayer graft<sup>[40]</sup>. This is also referred to as the *layered or fill in technique*. The layering may be done either by cutting the AM into multiple pieces and placing them one on top of one another or by using a larger piece of AM which is repeatedly folded (*blanket fold*) upon itself.

**Patch or overlay technique:** Here the AM is sutured to the ocular surface keeping it larger than the underlying defect so that host epithelium is present below the membrane. The membrane is sutured to the surrounding conjunctiva or episclera using 9-0 vicryl suture. An additional 10-0 nylon suture may be applied to the peripheral cornea in a purse string manner to ensure prolonged retention (Figure 3). The AM may be used epithelial side up or stromal side up as the host epithelium is expected to grow under the membrane which basically acts only as a "biological bandage contact lens" to protect the fragile





**Figure 3 Amniotic membrane used as an overlay patch.** A: Clinical photograph; B: Line diagram. The amniotic membrane is trimmed to cover the whole of the corneal surface and fixed by 10-0 nylon monofilament sutures at the corneal periphery parallel to the limbus.

new epithelium from the frictional forces generated due to eyelid movements. In this situation the AM is expected to fall off or be removed after a certain time.

**Combined (inlay and overlay) technique:** Two or more layers of AM are used in this technique, with the inner smaller layer/layers acting as a graft and the outer larger layer acting as a patch. Also known as the “*sandwich technique*” a single-layer (Figure 4A) or multilayer inlay (Figure 4B) is combined with an onlay<sup>[40]</sup>. The epithelium is expected to grow under the patch but over the uppermost inlay graft.

The availability of fibrin glue for ophthalmic use has in many cases supplanted the use of sutures, and the AM may be adhered to the ocular surface using the recombinant fibrin glue. This reduces the surgical time and also increases patient comfort.

## OPHTHALMOLOGICAL INDICATIONS FOR USE OF AM

The list of indications for which AM is used in ophthalmology is expanding with each passing year. Broadly its use can be classified into (1) corneal surface disorders, without limbal stem cell deficiency (LSCD); (2) corneal surface disorders with associated LSCD; (3) conjunctival surface reconstruction, *e.g.*, pterygium removal, after removal of large lesions other than pterygium, after symblepharon lysis; (4) as a carrier for ex vivo expansion of corneal epithelial cells; (5) glaucoma; (6) treatment of sclera melts and perforations; and (7) other miscellaneous indications.

### Persistent epithelial defects and Non Healing corneal ulcers

Persistent epithelial defects (PED's) may occur due to a variety of mechanisms including innervations deficits of the cornea (*e.g.*, neurotrophic keratopathy following Herpes Zoster keratitis, after penetrating keratoplasty), chronic inflammation or mechanical factors. These factors may act individually or in synergy, and lead to epithelial defects which are unresponsive to conventional management strategies, *e.g.*, lubrication, bandage contact lenses, tarsorrhaphy, *etc.* Untreated these PED's can progress to stromal collagenolysis, ulceration, perforation or scarring.

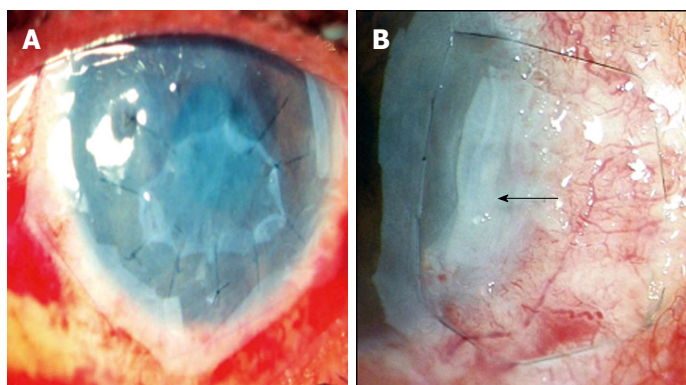
In these situations AM may be used as a single layer or multilayer graft (inlay) depending on the depth of the lesion providing a substrate for the epithelial cells to migrate and adhere to the basement membrane. The inlay may also be combined with an epithelial side down onlay patch graft especially if significant ocular surface inflammation co-exists as stromal surface of the onlay graft will help mop up the inflammatory cells and mediators on the palpebral surface. Success rates of using AM for PED's have been reported as varying from 64% to 91%<sup>[33,42]</sup>. Early detachment of the membrane however remains a major problem despite the use of multiple sutures or a protective bandage contact lens (BCL)<sup>[42]</sup>.

AM has also been used successfully in nonhealing infective ulcers due to bacteria, fungi, viruses and protozoa. The nonhealing of the ulcer in spite of adequate antimicrobial therapy in these situations may be because of release of proinflammatory mediators, proteolytic enzymes and collagenases by the microorganisms, stromal keratocytes and polymorphonuclear cells<sup>[43,44]</sup>. Single or multilayer AM has an inhibitory effect on these proteolytic enzymes and also provides a healthy basement membrane, thus tilting the ocular surface milieu in favour of rapid healing.

### Corneal perforations and descemetocoeles

Corneal perforations and descemetocoeles are globe and sight threatening complications associated with loss of tectonic strength of corneal stroma as well as associated underlying inflammation. A majority of the methods used to treat these conditions including tissue adhesives, lamellar keratoplasty, penetrating keratoplasty, bandage contact lenses and conjunctival flaps provide tectonic support, but do not directly address the inflammatory component. Multilayer AM has been used to treat non traumatic microperforations and descemetocoeles with upto 72.7% to 82.3% success rate being reported<sup>[45,46]</sup>. AM in this situation provides tectonic support, collagen substitution for corneal stroma and anti-inflammatory and anti-fibrotic actions which halt progressive tissue degradation. Depending on the underlying severity and extent of the disease process it may be used as a permanent surgical therapy or as a temporizing measure till a more definitive surgical procedure can be performed. One of the authors of this review (AKJ) has reported successful management of a case of perforated peripheral corneal ulcer in





**Figure 4** Single layer inlay covered by a larger “patch” or “onlay” (A), multi-layer inlay ( amniotic membrane folded upon itself in form of a blanket fold- black arrow) covered by a larger “patch” which is fixed to underlying episclera by a purse string suture in a case of deep, non-resolving peripheral ulcerative keratitis (B).

a patient of acne rosacea with amniotic membrane after three applications of cyanoacrylate glue with bandage contact lens failed to seal the perforation successfully<sup>[47]</sup>. A final best corrected visual acuity of 6/6 was achieved in this patient. Though used most commonly for small perforations, Kim *et al*<sup>[48]</sup> have reported successful outcomes even in patients with perforations > 2 mm in size using fibrin glue to augment the thickness of the AM-a procedure they termed “fibrin glue assisted augmented AMT”.

### **Symptomatic bullous keratopathy**

In symptomatic patients with good visual potential and intolerant to a BCL, AM may be used as a temporizing measure till a definitive treatment for the bullous keratopathy, *i.e.*, endothelial or penetrating keratoplasty is undertaken. It may also be used as an alternative to anterior stromal puncture in patients with a poor visual potential with the objective of providing longer pain free periods. Espana *et al*<sup>[49]</sup> have reported a mean follow up of 25 mo and noted that 88 % patients were able to obtain a pain free status.

### **Band keratopathy**

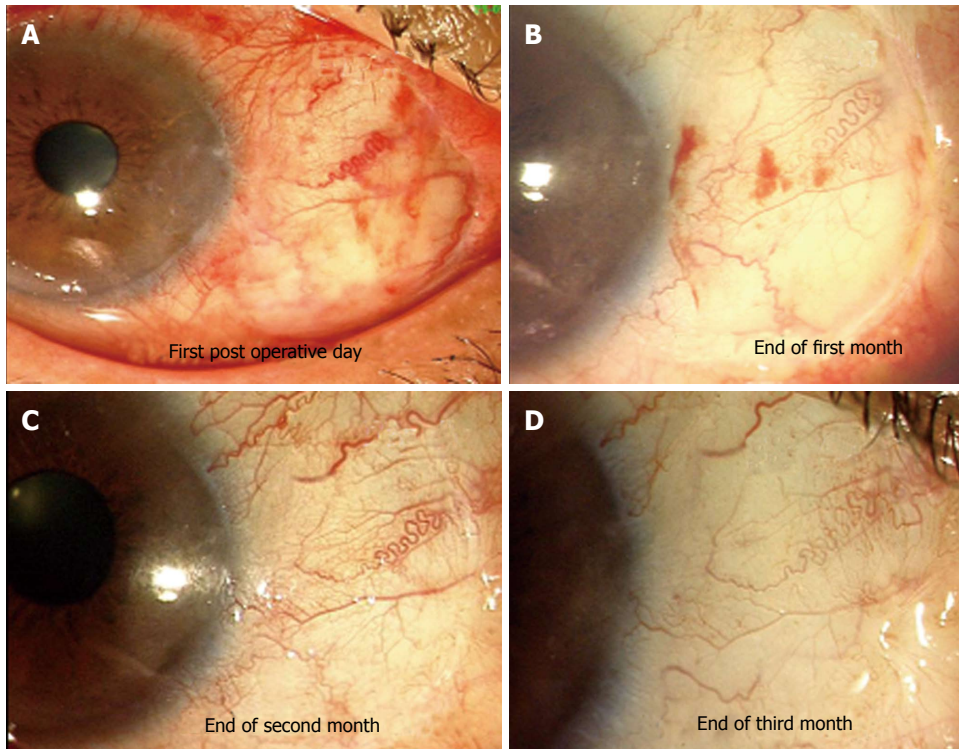
Band keratopathy due to abnormal deposition of calcium on the corneal surface results in ocular irritation and epithelial surface breakdown. Primary treatment involves removal of calcium deposits by ethylene diamine tetra acetic acid chelation or superficial keratectomy. AM transplantation has been used as an adjunct after primary surgical treatment in band keratopathy with pain relief being reported in 93% cases and visual acuity improvement in 44% of sighted eyes<sup>[50]</sup>.

**Corneal disorders with associated LSCD:** Any acute or chronic insult to the the limbal epithelial stem cells can lead to a state of partial or total LSCD which may manifest as conjunctivalization of the cornea, neovascularization, PED's and chronic inflammation. This may be seen after thermal or chemical burns, cicatrizing disorders like SJS and OCP, aniridia, chronic contact lens wear, untreated vernal keratoconjunctivitis (VKC) and multiple surgeries involving the limbal area. Successful long term outcome in these eyes after lamellar or penetrating keratoplasty requires prior optimization of the ocular surface

and restoration of the stem cell population. Success of AM in these scenarios depends on the severity of the LSCD. In cases of partial LSCD amniotic membrane alone can be an effective therapy to restore the ocular surface as by promoting epithelialization and reducing inflammation it restores a normal stroma which maximizes functioning of the remaining limbal stem cells<sup>[51,52]</sup>. In cases of total LSCD however AM transplantation has only an adjunct role to limbal stem cell transplantation which is the definitive modality of treatment, as AM can only optimize functioning of existent limbal stem cells (LSC's) as is seen in partial LSCD, but it cannot cause repopulation of the affected eye with LSC's in cases of advanced total LSCD. In these situations use of de-epithelized AM as a carrier for ex vivo expansion of limbal autologous or allolimbal stem cells is another good option as it combines the advantages of both techniques, *i.e.*, simultaneous optimization of the ocular surface by the AM and replacement of the stem cells.

**Conjunctival reconstruction:** AM can be used for reconstruction of the conjunctival surface as a substitute for conjunctival grafts in situations where availability of autologous conjunctival tissue is limited, *i.e.*, after removal of large conjunctival lesions, patients having undergone repeated conjunctival surgery leading to a scarred conjunctiva, or where the conjunctiva needs to be preserved, *i.e.*, patients with glaucoma who may require filtering surgery in the future. Use of AM in these situations is helpful as in addition to providing a healthy basement membrane for growth of conjunctival epithelial cells it also helps in maintaining the normal goblet cell containing phenotype of these cells<sup>[53]</sup>. However as the AM is only a temporary substitute, to provide long term reepithelialization of the conjunctival surface, the surrounding conjunctival tissue must be healthy with an intact vascular bed.

**Pterygium:** Use of AM as an alternative to autologous conjunctival grafts was described by Prabhasawat *et al*<sup>[54]</sup>. They reported higher recurrence rates (10.9%) for primary pterygia with the use of AM as compared to recurrence with use of autologous conjunctival autografts (2.6%). However later studies which emphasized extensive removal of fibrovascular tissue adjacent to the pterygium have reported that recurrence rates with use of AM



**Figure 5** Amniotic membrane used to cover bare sclera after excision of primary pterygium. A-D: Serial photographs showing appearance of amniotic membrane graft. At end of 3 mo excellent integration and cosmetic appearance was achieved.

transplantation (3%-3.8% in primary pterygia and 9.5% in recurrent pterygia) were similar to those reported after conjunctival autografting and intra operative mitomycin C use<sup>[55,56]</sup>. Jain *et al*<sup>[57]</sup> have described the use of AM transplantation using fibrin glue in primary pterygia using a 'tuck in technique' where the edges of the AM graft were tucked underneath the adjacent free margin of conjunctiva on 3 sides and reported no recurrences in 11 out of 12 patients after a follow up of one year (Figure 5).

However conjunctival autograft is still considered to be the gold standard for treatment of primary pterygia and AM may be a reserved as a reasonable option in cases with diffuse conjunctival involvement, *i.e.*, primary extensive biheaded pterygia, in previous multiple failed surgeries and in patients in whom the bulbar conjunctiva must be preserved for a prospective glaucoma filtering procedure<sup>[58]</sup>.

**Conjunctival tumours and ocular surface squamous neoplasias:** AM has been used for conjunctival reconstruction after excision of both benign and malignant tumours including ocular surface squamous neoplasias (OSSN), melanomas, lymphomas and complex choristomas. The AM provides a substrate for migration of the conjunctival epithelial cells. Advantages of using amniotic membrane as compared to conjunctival autografts in these situations include a lack of donor site morbidity and the ability to clinically monitor local tumour recurrence beneath the transparent AM graft<sup>[59]</sup>.

**After symblepharon lysis:** AM can be used both in the prevention as well as treatment of symblepharon. In the

acute phases of chemical injury the AM can be used as a patch to cover the entire ocular surface and sutured to the fornices through the eyelids to prevent symblepharon formation as well as simultaneously reduce ocular surface inflammation. The AM should be a continuous sheet devoid of buttonholes. A large sheet is placed on the ocular surface and it is first anchored to the inner surface of the everted lower lid close to the lid margin using multiple interrupted 10-0 vicryl sutures. To anchor the sheet to the fornices two sets of double armed 4-0 chromic gut sutures on a cutting needle are used and the needles are passed from the AM surface through the inferior fornix, *via* the full-thickness of the eyelid and are made to exit through the eyelid skin. The two needles of each of the two sets of sutures are passed through two segments of an encircling band and then tied<sup>[60]</sup>. A sutureless amniotic patch (ProKera; Bio-Tissue Inc., Miami, Florida) is also available for this purpose. Another modification suggested by Rahman *et al*<sup>[61]</sup> is the use of a conformer on which the AM is sutured and placed on the ocular surface, with the AM acting as a patch and the conformer maintaining the fornices because of its rigidity. Though the AM has also been used for symblephera associated with SJS and OCP the outcomes are usually not as successful as compared to stable non progressive cicatrization because of the chronic ongoing inflammation associated with these diseases<sup>[62]</sup>.

**As a carrier for *ex vivo* expansion of epithelial cells:** Progenitor stem cells for the conjunctiva and cornea have been established to reside in the conjunctival fornices

and limbal area respectively. In the eye, *i.e.*, under in- vivo conditions these migrate onto the ocular surface and differentiate into daughter cells to continuously regenerate the conjunctival and corneal surface epithelia<sup>[63]</sup>. Expansion of these cell populations on basement membrane side of AM (with amniotic epithelium intact or de-epithelialized AM) as well as on the stromal side of AM have been demonstrated previously. The corneal epithelial cells have been shown to migrate rapidly when limbal explants are placed on AM denuded of amniotic epithelial cells but with the basement membrane intact, relatively slowly when the amniotic epithelium is left behind and slowest when the membrane has been flipped over and the cells are grown on the stromal surface. Culturing the explants on an intact AM with devitalized epithelium favors expansion of an epithelial phenotype that closely resembles limbal stem cells<sup>[38]</sup>.

Clinically in cases of LSCD, limbal biopsies can be used to harvest corneal epithelial stem cells for *ex vivo* expansion on AM, which can then be transplanted onto the eye after appropriate preparation of the host bed by resecting the vascularized pannus or any other procedure which may be required. The main advantage of this approach of expanding corneal epithelial cells *ex vivo* on AM is that only a small amount of limbal tissue is required to be harvested from the contralateral eye as compared to conventional limbal allografts which require up to 12-clock hours of limbal tissue and have the potential risk for limbal deficiency developing in the donor eye. Another advantage is that the AM is a natural substrate and when transplanted onto the corneal surface gets integrated into it. Excellent outcomes have been reported after transplantation of cultivated limbal stem cell on denuded AM for LSCD<sup>[64-66]</sup>.

**Glaucoma:** AM has been used in glaucoma to reduce scarring at the time of filtering surgery, to repair early or late leaks, and act as a cover for valve procedures. Fujishima *et al*<sup>[67]</sup> attempted to reduce scarring in filtering surgery by incorporating a layer of amnion between the scleral flap and bed to prevent an adhesion between the two layers, but achieved only limited success. Use of AM to repair bleb leaks is controversial with some authors reporting good results<sup>[68]</sup> while others have reported it as being ineffective<sup>[69]</sup>.

**Treatment of corneo scleral melts and perforations:** Small sclera perforations or melts can be treated by multilayered AM alone while for larger scleral defects AM has been used over the sclera patch, basement side up so as to facilitate epithelialization of the scleral patch graft as well as to reduce the inflammation<sup>[45,70]</sup>. It has been used with success for both infectious scleral ulcerations after appropriate antimicrobial therapy<sup>[71]</sup>, as well as after non-infectious scleral melts. Tay *et al*<sup>[72]</sup> have reported using a double layer of freeze dried AM (*Ambio 5*; IOP Inc, Costa Mesa, California) in a crescentic manner to manage a case of carrier graft melt in a patient with Boston Keratoprosthesis Type 1.

**Miscellaneous indications:** Severe shield ulcers due to VKC which do not heal with conservative management respond well to surgical debridement of the mucous plaque and debris followed by using the AM as a patch to promote epithelialization. Using this technique Sridhar *et al*<sup>[73]</sup> achieved a success rate of 94.7% with shield ulcers. AM has been occasionally in oculoplasty for lid reconstruction, for treatment of punctal occlusion by applying it as a patch over the denuded punctual orifice<sup>[74]</sup> as a cover for ocular prosthesis at the time of insertion and to cover the tarsal plate in lid split procedure for correction of cicatricial entropion<sup>[75]</sup>.

**Complications and limitations of am use:** Though the AM is finding ever expanding uses in ophthalmology, it must not be used indiscriminately as complications though infrequent can occur. Risk of transmission of bacterial, viral or fungal infections to the recipient exists if the donors are not adequately screened for communicable diseases, if the membrane is not processed under sterile conditions or if storage is improper. Incidence rates of 1.6%-8.0% have been reported post AM transplantation with gram positive isolates being reported most frequently<sup>[76-78]</sup>. Premature degradation of the membrane and cheese wiring may need frequent repeat transplantations. Occasionally, a residual subepithelial membrane may persist in some cases and inadvertently opacify the visual axis<sup>[38]</sup>.

## CONCLUSION

The AM is proving to be a very versatile tool in the hands of the ophthalmologist, and the indications for its use are rapidly expanding as there is a better understanding of its properties. However a judicious use and appropriate patient selection is important for achieving optimal outcomes.

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## Multiple indications for everolimus after liver transplantation in current clinical practice

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### Abstract

**AIM:** To assess our experience with the use and management of everolimus-based regimens post-liver transplantation and to redefine the potential role of this drug in current clinical practice.

**METHODS:** From October 1988 to December 2012, 1023 liver transplantations were performed in 955 patients in our Unit. Seventy-four patients (7.74%) received immunosuppression with everolimus at some time post-transplantation. Demographic characteristics, everolimus indication, time elapsed from transplantation to the introduction of everolimus, doses and levels administered, efficacy, side effects, discontinuation and

post-conversion survival were analyzed.

**RESULTS:** Mean age at the time of conversion to everolimus was  $57.7 \pm 10$  years. Indications for conversion were: refractory rejection 31.1%, extended hepatocellular carcinoma in explanted liver 19%, post-transplant hepatocellular carcinoma recurrence 8.1%, *de novo* tumour 17.6%, renal insufficiency 8.1%, severe neurotoxicity 10.8%, and others 5.4%. Median time from transplantation to introduction of everolimus was 6 mo (range: 0.10-192). Mean follow-up post-conversion was  $22 \pm 19$  mo (range: 0.50-74). The event for which the drug was indicated was resolved in 60.8% of patients, with the best results in cases of refractory rejection, renal insufficiency and neurotoxicity. Results in patients with cancer were similar to those of a historical cohort treated with other immunosuppressants. The main side effects were dyslipidemia and infections. Post-conversion acute rejection occurred in 14.9% of cases. The drug was discontinued in 28.4% of patients.

**CONCLUSION:** Everolimus at low doses in combination with tacrolimus is a safe immunosuppressant with multiple early and late indications post-liver transplantation.

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**Key words:** Everolimus; Liver transplantation; Indications; Off-protocol; Outcome

**Core tip:** Everolimus has a completely different mechanism of action to that of current basal calcineurine inhibitors used worldwide in liver transplantation. This immunosuppressant has a good profile for patients with pre- and post transplant renal dysfunction, one of the main concerns nowadays. It has also a promising role in cancer patients which is common in liver transplantation, either as an underlying disease (hepatocarcinoma in cirrhosis), or as *de novo* developing tumors. We

present our off-protocol experience with partial/total and early/late conversion to everolimus, highlighting its efficacy and safety in fitting with the different emerging scenarios after liver transplantation.

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## INTRODUCTION

Over the last thirty years, immunosuppression protocols in liver transplant patients have been based on calcineurine inhibitors (CNI) - cyclosporine in the eighties and tacrolimus in the nineties. Both were administered in combination with steroids. In the late nineties, monoclonal antibodies and mycophenolate mofetil (MMF), an antimetabolite with a different mechanism of action, were widely used. In the year 2000, sirolimus was the first inhibitor of the mammalian target of rapamycin (mTORi) launched into clinical practice as a primary immunosuppressant to replace the widespread use of CNI. However, its use declined due to severe adverse events and the warning issued on the risk of arterial thrombosis<sup>[1]</sup>. A few years later, everolimus (EVER) another mTORi was approved for use after acute rejection in heart<sup>[2]</sup> and kidney<sup>[3]</sup> transplantation. In 2012, EVER was approved for liver transplantation<sup>[4]</sup> by the EMA. In Spain, EVER was also approved for liver transplantation and obtained full registration at the end of 2012. In non-transplant areas, it has been approved for the treatment of advanced renal cell carcinoma<sup>[5]</sup>.

mTORi reduce the expression of vascular endothelial growth factor and transforming growth factor- $\beta$ , which are associated with tumour angiogenesis<sup>[6,7]</sup>. In solid organ transplantation, efficacy and safety can be achieved by targeting EVER trough levels at 3-8 ng/mL in combination with CNI. EVER is dosed twice daily and yields a steady state by day four.

The use of EVER is gaining acceptance in adult<sup>[8-10]</sup> and paediatric<sup>[11]</sup> liver transplant recipients. It has been used as maintenance<sup>[12-14]</sup>, in *de novo* liver transplant patients<sup>[15]</sup>, in cases of renal dysfunction as a CNI-sparing regimen<sup>[16-18]</sup>, and in recipients with cancer<sup>[19-21]</sup>. The most common adverse events are leucopenia, hyperlipidemia, gastrointestinal disorders, delayed wound healing, stomatitis, angioneurotic oedema, proteinuria and interstitial lung disease<sup>[22-24]</sup>.

EVER was introduced into clinical practice at our centre in 2005, when some of the medical community had lost confidence in mTORi and had relegated the drug to compassionate use and to sporadic and desperate cases when other drugs failed. However, experience with sirolimus, especially the weak points of the drug,

prompted us to use EVER in order to optimise and re-define the true role of mTORi. The principal aim of this single-centre retrospective study was to study the current indications for total or partial conversion to EVER in liver transplant patients treated off-protocol.

## MATERIALS AND METHODS

From October 1988 to October 2012, 1023 liver transplants were performed in 955 patients in our centre. We reviewed the prospectively collected data bases and medical records of these patients, focusing on the patients who received EVER for immunosuppression at some point post-transplantation. We recorded the demographic characteristics of these patients, the causes of conversion to EVER, the pre- and post-conversion immunosuppression regimens, the time elapsed between liver transplantation and the start of EVER treatment, doses and trough levels, efficacy, side effects, causes of discontinuation and mean follow-up post-conversion. Efficacy was assessed overall and according to the time elapsed from liver transplantation to the introduction of EVER. All patients receiving EVER gave their signed informed consent and met all the requirements for compassionate use of the drug.

### Demographic characteristics

The following information on the demographic characteristics of the patients was obtained: age at time of transplantation and at time of conversion; gender; hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) status; indication for transplantation; Child and United Network for Organ Sharing classification status; body mass index (BMI) > 30; presence or absence of hypertension, diabetes mellitus (DM) and renal dysfunction at time of transplant; donor age; donor cause of death; donor time spent in the intensive care unit; presence or absence of graft steatosis > 20%; type of graft; presence or absence of portal thrombosis; type of biliary anastomosis; mean intraoperative red blood cells; and mean cold ischemia time. Renal dysfunction at time of transplant was defined as serum creatinine > 1.5 mg/dL or hepato-renal syndrome or need for dialysis.

### Definition of the causes of conversion

Refractory rejection was defined as an incomplete response to treatment with steroid pulses with or without MMF. Patients outside the Milan criteria and/or with macro- or microvascular invasion in the explanted liver were considered advanced hepatocellular carcinoma (HCC). HCC recurrence was defined as tumour recurrence at any time during the follow-up period after liver transplantation. Diagnosis was based on radiologic images and assessed by a pathologist in either hepatic or extrahepatic specimens. *De novo* tumour was defined as the development of a malignant tumour (excluding HCC) during post-transplantation follow-up. Post-transplant neurological disorders were diagnosed by a neurologist



based on clinical symptoms, electroencephalograms, cranoencephalic computed tomography, cerebral magnetic resonance imaging, lumbar punctation and viral serological testing. Renal dysfunction was defined as the presence of serum creatinine  $> 1.5$  mg/dL. Amelioration of renal function was defined as a statistically significant ( $P < 0.05$ ) difference between mean serum creatinine levels at two different points of follow-up.

### Doses and trough levels

Doses and trough levels of EVER were assessed on the day of conversion and at 15 d and 1, 3, 6 and 12 mo post-conversion. Tacrolimus levels were also assessed at the same times.

### Assessment of efficacy

The variables analysed at the time of conversion and thereafter were: total bilirubin and transaminases; serum creatinine; amelioration or resolution of neurotoxicity or other causes for which EVER was introduced. Serum creatinine was assessed on the day of conversion and at 3, 6 and 12 mo post-conversion. Acute rejection post-conversion was suspected based on enzymatic alteration of liver function, assessed by liver biopsy, and defined according to the Banff criteria.

Patients converted to prevent HCC recurrence were compared with a historical cohort not receiving EVER and matched for MELD status, year of transplantation  $\pm 18$  mo, presence or absence of vascular invasion, tumour type and size. We found appropriate matches for all the variables except vascular invasion due to a scarcity of receptors. Efficacy was assessed by comparing patient survival and the time elapsed from liver transplant to recurrence in the patients receiving EVER and those in the historical cohort.

Patients with HCC recurrence after transplantation were also compared with a historical cohort not receiving EVER and matched for the time elapsed from liver transplantation to tumour recurrence, site of recurrence, and Milan criteria. Efficacy was assessed by comparing patient survival post-recurrence for patients receiving EVER and those in the historical cohort.

Patients who developed *de novo* tumours were compared with a historical cohort of patients not receiving EVER and matched for tumour histology, time elapsed from liver transplantation to tumour, and type of treatment post-diagnosis. Efficacy was assessed by comparing patient survival post-recurrence for patients receiving EVER and those in the historical cohort.

Other efficacy variables were glucose levels and the need for anti-diabetic therapy post-conversion and blood pressure and the need for antihypertensive drugs. These variables were evaluated qualitatively as “amelioration or resolution”, “worsening” and “no change”.

### Time elapsed from liver transplantation to conversion

Early conversion was defined as conversion during the first year post-transplantation, and late conversion as conversion after the first year post-transplantation.

### Side effects and discontinuation

Possible side effects assessed were: hematologic toxicities; diarrhoea; proteinuria (though not assessed in all patients); levels of serum cholesterol and triglycerides and the need for hypolipidemic therapy; infections; and any other post-conversion adverse event.

Discontinuation was defined as stopping the drug when the patient was alive. The reason for EVER discontinuation was recorded.

### Survival post-conversion

All patients were followed up until December 2012, death or drug withdrawal. Patient survival post-conversion and cause of death were analyzed according to the reason for conversion and EVER-related deaths.

### Statistical analysis

The student's *t*-test or the Mann-Whitney *U* test were used for quantitative data and Pearson's  $\chi^2$  or Fisher's exact test for categorical data. Significance was set at  $P < 0.05$ . Data are expressed as mean  $\pm$  SD, or as percentages. The Kaplan-Meier method was used for survival analysis. All analyses were performed with SPSS version 15.0 (SPSS Inc., Chicago, IL, United States).

## RESULTS

Data on the demographic characteristics of recipients, donors and surgery are shown in Table 1. Mean patient age at the time of conversion was  $57.7 \pm 10$  years and median age was 60 years (range: 27-74); nine patients (12.2%) were over 70 years of age.

Reasons for conversion to EVER are shown in Table 2. Pre-conversion therapy was based on tacrolimus in 69 patients, neoral cyclosporine (CyA) in four, and MMF in one. Post-conversion therapy consisted of: tacrolimus in 54 patients, CyA in three, and a CNI-free regimen in 17. Pre- and post-conversion drug combinations are specified in Table 3.

Median time between transplantation and introduction of EVER was 6 mo (range: 0.10-192 mo). Forty-two patients (56.8%) were converted during the first year post-transplantation and the remaining 32 patients (43.2%) after the first year. Median time between event onset and conversion was 1 mo (range: 0.1-19) (Table 1).

### Doses and trough levels

Conversion to EVER was managed differently according to the reason for conversion; however, loading doses were never used. In cases of refractory rejection, EVER was administered at an initial dose of 1 mg every 12 h, with subsequent doses adjusted to obtain trough levels between 3 and 5 ng/mL. At the same time, CNI was maintained at high doses. When the reason for conversion was CNI-related adverse events, EVER was started at 0.5 mg once or twice a day and the CNI dose was reduced to half or completely withdrawn, depending on the severity of the adverse events. When the reason for conversion was cancer (extended tumour in the explant, can-

**Table 1** Characteristics of recipients, donors, surgery and post-transplant evolution in 74 patients receiving everolimus *n* (%)

Recipient	Mean age (yr)	55.5 ± 9 r (25-69)
	Patients > 65 yr	10 (13.5)
	Male/female	55 (74.3)/19 (25.7)
Diagnosis		
	HCC with cirrhosis	35 (47.2)
	Alcoholic cirrhosis	18 (24.3)
	HCV cirrhosis	16 (21.6)
	Cholostatic cirrhosis	3 (4.1)
	Liver insufficiency	2 (2.8)
	HCV - HBV	40 (54)-3 (4)
	ETOH	38 (51.4)
	HIV	4 (5.4)
	Child-Pugh A/B/C (%)	35-30-35
	UNOS (home/Hosp/ICU) (%)	90.5-6.8-2.7
Pre-LT associated disease		
	Renal insufficiency	11 (14.9)
	Diabetes mellitus	18 (24.3)
	Arterial hypertension	14 (18.9)
	Cardiopathy	3 (4.1%)
	Previous surgery	15 (20.3)
Donor	Mean age (yr)	48 ± 19 r (14-81)
	Patients > 70 yr	14 (19)
	Male/female (%)	49 (66)/25 (34)
	Graft steatosis > 20%	11 (15)
	Death (CET, CVA, Other) (%)	43-43-14
Surgery	E-E/E-E + Kehr/C-Y (%)	84-8-8
	Previous portal thrombosis	10 (13.6)
	Median RBC units	4 (r: 0-40)
	Cold ischaemia time (min)	378 ± 97
Post-transplant evolution	Ischaemia-reperfusion injury (ALT > 1000 IU, Quick < 60%)	14 (19)
	Biliary complication	7 (9.5)
	Postoperative arterial complication	2 (2.7)
Median time from event to conversion		1 mo (r: 0.1-19)
Median time from LT to conversion		6 mo (r: 0.1-192)
Early/late conversion	< 1 yr/≥ 1 yr	42 (56.8)/32 (43.2)
Mean follow-up post-conversion		22 ± 19 mo (r: 0.5-74)
Median follow-up post-conversion		17.5 mo

HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; HBV: Hepatitis B virus; ETOH: Cirrhosis due to alcohol; HIV: Human immunodeficiency virus; UNOS: United Network for Organ Sharing classification; ICU: Intensive care unit; LT: Liver transplantation; CET: Cranioencephalic trauma; CVA: Cerebrovascular accident; E-E: End-to-end choledoco-choledostomy; E-E + K: End-to-end choledoco-choledostomy + kehr; C-Y: Choledoco-jejunostomy; RBC: Red blood cells; IU: International units; r: Range.

cer recurrence during follow-up, or *de novo* tumor), EVER was introduced at a dose of 0.5 mg/d, with trough levels adjusted to under 3 ng/mL, and CNIs were drastically reduced to half or completely withdrawn. Doses and levels of EVER for the entire series of patients and tacrolimus levels for patients receiving this drug post-conversion are shown in Figure 1.

### Efficacy

The cause of conversion to EVER was resolved in 60.8%

**Table 2** Causes of conversion and other comorbidities at the time of conversion to everolimus in 74 liver transplant patients *n* (%)

Cause of conversion			
Refractory rejection	23 (31.1)	Resolution	17 (73.9)
Extended HCC in explanted liver	14 (19)	Prevention of recurrence	7 (50)
HCC recurrence during follow-up	6 (8.1)	Stabilization	0 (0)
<i>De novo</i> tumour	13 (17.6)	Prevention of recurrence	8 (61.5)
CNI-related neurotoxicity	8 (10.8)	Resolution or Stabilization	8 (100)
Renal dysfunction	6 (8.1)	Resolution or Amelioration	3 (50)
Other causes	4 (5.4)	Resolution	2 (50)
Comorbidity at time of conversion			
Chronic renal insufficiency	22 (29.8)	Resolution or Amelioration	15 (68.2)
Diabetes mellitus	21 (28.4)	Resolution or Amelioration	8 (38)
Arterial hypertension	25 (33.8)	Resolution or Amelioration	3 (12)
Dyslipidemia	30 (40.5)	Resolution or Amelioration	2 (6.7)

Outcome to everolimus shown as resolution, stabilization or amelioration of the cause or comorbidity. In 45 of 74 patients (60.8%), the cause was resolved, stabilized or ameliorated. HCC: Hepatocellular carcinoma; CNI: Calcineurin inhibitors. Other causes include: 1 chronic biliary cirrhosis recurrence plus chronic rejection, 1 sinusoidal hepatic fibrosis, 1 graft-versus-host disease, 1 chronic cholestatic liver dysfunction in the postoperative period.

of patients.

**Refractory rejection:** When EVER was used to treat refractory rejection (*n* = 23), the event was resolved correctly in 17 patients (73.9%) (Table 2). The remaining six patients failed to respond: four progressed to severe chronic refractory rejection finally requiring retransplantation and two died, one due to sepsis and one from concomitant severe hepatitis C recurrence.

**Prevention of HCC recurrence:** When EVER was indicated for prevention of HCC recurrence (*n* = 14), seven patients (50%) remained recurrence-free for a mean post-conversion follow-up of 33.8 ± 27 mo (Table 2). Three patients suffered recurrence at a mean post-conversion follow-up of 33.7 ± 33 mo, and four patients died due to HCC recurrence at a mean post-conversion follow-up of 15.1 ± 11 mo. When these 14 patients were compared with the historical cohort matched for MELD status, year of transplantation, and some pathological characteristics of the explanted liver, no differences either in survival or in time to recurrence were observed between the two groups (Table 4).

**Patients with HCC recurrence:** Six patients were converted to EVER due to HCC recurrence after liver transplantation. Types of post-transplant recurrences were: intra-abdominal at 122 mo; pulmonary at 6 mo; bone metastasis at 42 mo; liver recurrence at 46 mo; brain metastasis at 10 mo, and peritoneum-pulmonary metastasis

**Table 3** Type of immunosuppression pre- and post-conversion to everolimus

Pre-conversion	n = 74	Post-conversion	n = 74
FK + MMF + ST	16	FK + EVER	38
FK + MMF	20	FK + EVER + MMF	1
FK + ST	12	FK + EVER + ST	11
FK	21	FK + EVER + MMF + ST	4
CyA + MMF + ST	1	CyA + EVER	3
CyA + MMF	1		
CyA	2	EVER	2
		EVER + ST	5
MMF + ST	1	EVER + MMF	2
		EVER + MMF + ST	8

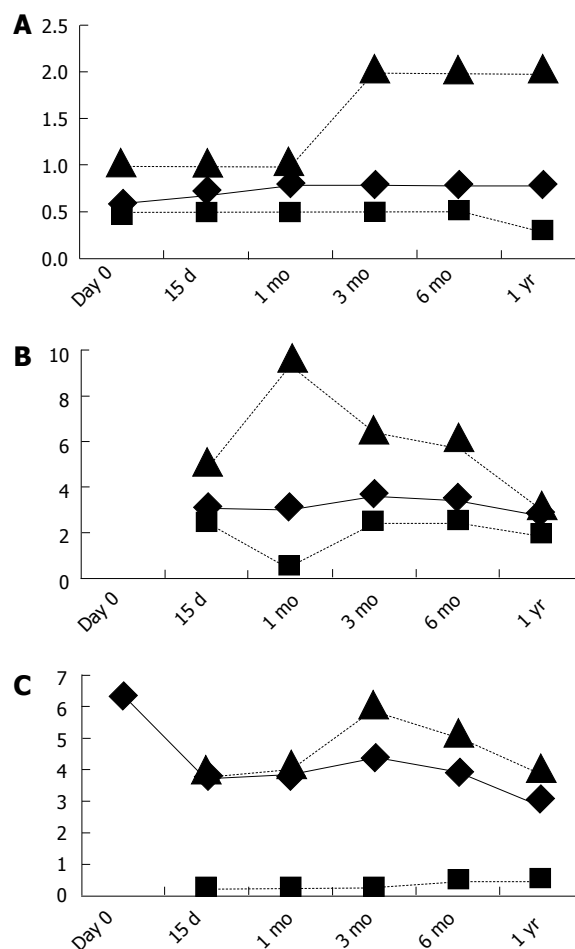
FK: Tacrolimus; MMF: Mycophenolate mofetil; ST: Steroids; CyA: Neoral cyclosporine; EVER: Everolimus.

at 3 mo. Two patients were within the Milan criteria and four outside. All died at a mean time post-conversion of  $14 \pm 10.9$  mo (3-31). When these six patients were compared with the historical cohort matched for recurrence site (1 suprarenal, 2 lung, 1 liver, 1 brain, 1 bone), time to recurrence and Milan criteria, survival post-recurrence was similar in those receiving EVER and those receiving other, non-mTORi immunosuppressants (Table 4).

**Patients with *de novo* tumour:** In thirteen patients, the reason for conversion to EVER was the appearance of a *de novo* tumor: 4 colon, 2 prostate, 2 esophagus, 2 larynx, 1 lung, 1 anus, and 1 breast. After onco surgical treatment of the tumor, eight patients remained alive and tumor-free at a mean follow-up post-tumor treatment of  $37.7 \pm 14.5$  mo, four died at a mean follow-up post-tumor treatment of  $21.5 \pm 12.3$  mo, and one (with colon cancer) is alive but with liver metastasis at 40 mo post-tumor treatment. In patients undergoing surgery, EVER was introduced as soon as healing was completed - 2-4 wk post-surgery. When these 13 patients were compared with the historical cohort matched for tumor type, time to development of the *de novo* tumor, and type of treatment, survival post-tumor treatment was similar in those receiving EVER and in those receiving other, non-mTORi immunosuppressants (Table 5).

**Neurotoxicity:** EVER was indicated in three patients with seizures, two with akinetic mutism, one with a cerebrovascular stroke plus multifocal progressive leukoencephalopathy, one with Guillain-Barré syndrome, and one with generalized tremor. Accompanying symptoms were different levels of speech disorders, including dysarthria, expressive dysphasia and apraxia. In all patients, EVER-based immunosuppression allowed a CNI-free period of time to reverse or ameliorate neurotoxicity.

**Renal dysfunction:** In the six patients in whom EVER was indicated due to renal insufficiency, serum creatinine changed from  $2.54 \pm 1.11$  mg/dL pre-conversion to  $1.63 \pm 0.86$  mg/dL at 3 mo post-conversion,  $1.69 \pm 0.91$  mg/dL at 6 mo post-conversion, and  $2 \pm 1.45$  mg/dL at 12



**Figure 1** (A) Doses and (B) trough levels of everolimus for the entire series and (C) trough levels of tacrolimus for patients receiving this drug in the post-conversion regimen. Mean values and range (minimum-maximum). A: Doses of everolimus (ng/d); B: Trough levels of everolimus (ng/mL); C: Trough levels of tacrolimu (ng/mL).

mo post-conversion. In the three patients who converted within the first year post-transplantation, renal function ameliorated, while two patients with established chronic renal insufficiency for more than five years post-transplantation remained unchanged, and one patient with an episode of acute renal insufficiency in the immediate postoperative period failed to improve. If we consider all the patients suffering from renal insufficiency at the time of EVER introduction, whatever the reason for conversion, the improvement was statistically significant: serum creatinine was  $2.5 \pm 1.01$  pre-conversion,  $1.59 \pm 0.62$  at 3 mo post-conversion,  $1.62 \pm 0.56$  at 6 mo post-conversion, and  $1.74 \pm 0.76$  at 12 mo post-conversion.

**Other causes:** One patient was converted to EVER owing to liver dysfunction with cholestasis starting at 7 mo post-transplantation and progressing to severe cholestasis three months later (Table 2). Two liver biopsies at 8 and 10 mo post-transplantation revealed sinusoidal fibrosis and undetermined hepatitis. After conversion, liver function was completely restored within 1 mo. Another patient with a similar cholestatic syndrome one year af-

**Table 4** Comparison between patients with hepatocellular carcinoma outside Milan criteria in the explanted liver receiving everolimus and a historical cohort not receiving mTOR inhibitors, and liver-transplanted patients with recurrence of hepatocellular carcinoma receiving everolimus and a historical cohort not receiving mammalian target of rapamycin inhibitors *n* (%)

HCC outside Milan criteria in explanted livers	Patients receiving everolimus <i>n</i> = 14	Historical controls without mTORi <i>n</i> = 14	<i>P</i>
Recipient age at transplant (yr)	55.5 ± 11.3	56.38 ± 7.1	NS
Recipient sex (male-female) (%)	86-14	79 - 21	NS
Child-Pugh status	6.7 ± 1.8	6.5 ± 1.4	NS
MELD score	13.6 ± 5	11.4 ± 3.4	NS
Size of largest tumour on pathologic exam	3.43 ± 1.50	3.152 ± 1.05	NS
N <sup>o</sup> of tumours at pathologic exam	2.70 ± 1.7	2.74 ± 1.7	NS
Microvascular invasion	10 (78)	4 (29)	0.02
Macrovascular invasion	5 (39)	0	0.01
Satellitosis	7 (50)	3 (21.4)	NS
Well-moderately differentiated tumour (%)	31-69	50-50	NS
Mean alpha-fetoprotein	366 ± 771	55 ± 125	NS
Median alpha-fetoprotein	12 (3-2571)	8 (2-445)	NS
HCC treatment while on waiting list	9 (64.3)	8 (57)	NS
Mean donor age in years	59 ± 14.9	58 ± 12.6	NS
Mean and median patient survival post-LT (mo)	56 ± 8.5 (59)	67 ± 11 (54)	NS
Recurrence of HCC in the follow-up	6	6	
HCC recurrence in post-LT follow-up			
Recipient age at transplant (yr)	53.6 ± 10	46.5 ± 13	NS
Recipient sex (male-female) (%)	100-0	83-17	NS
Milan criteria in explanted liver (yes-no) (%)	33-67	33-67	NS
Mean donor age (yr)	52.1 ± 16	41 ± 12.8	NS
Months from LT to recurrence	37.9 ± 45	28.5 ± 30	NS
Immunosuppression at recurrence (CyA-FK) (%)	17-83	17-83	NS
Type of recurrence (intra-extrahepatic) (%)	17-83	17-83	NS
Survival after recurrence (mo)	14.1 ± 11	16.6 ± 12.5	NS

HCC: Hepatocellular carcinoma; mTORi: Mammalian target of rapamycin inhibitors; LT: Liver transplantation; CyA: Neoral cyclosporine; FK: Tacrolimus; NS: No significant.

ter transplantation did not improve and finally died. A third patient converted to EVER due to graft-versus-host disease one month post-transplantation. Immunosuppression was changed from tacrolimus to low doses of EVER to reduce any hypersensitivity to tacrolimus and counterbalance the steroid bolus administered. This patient was maintained on EVER monotherapy at 2-3 ng/mL and did well for two months but finally died from sepsis due to bone marrow aplasia as progression of his graft-versus-host disease. A fourth patient converted to EVER suffered long-lasting primary dysfunction of the liver. Two liver biopsies confirmed cholestatic preservation injury. Total bilirubin was normalized after introduction of EVER in combination with tacrolimus and steroids.

**Efficacy for other comorbidities:** Although EVER was never indicated for arterial hypertension and dia-

**Table 5** Comparison between liver-transplanted patients with *de novo* tumour receiving everolimus and a historical cohort not receiving mammalian target of rapamycin inhibitors

	Patients receiving everolimus <i>n</i> = 13	Historical controls without mTORi <i>n</i> = 13	<i>P</i>
Recipient age at transplant (yr)	60.8 ± 5.8	59.5 ± 6.6	NS
Recipient sex (male-female) (%)	77-23	75-25	NS
Indication for LT (%)			NS
Postnecrotic-HCC in cirrhosis	68%	70%	NS
Mean time from LT to diagnosis of <i>de novo</i> tumour (mo)	67 ± 50	65.9 ± 37	NS
Tumour site and histology			NS
Colon ADK	4	4	
Prostate ADK	2	2	
Lung SCC	1	1	
Larynx SCC (4)	2	2	
Esophagus SCC(3) + ADK(1)	2	2	
Anus SCC	1	1	
Breast IDC	1	1	
Type of treatment			NS
Surgery ± QT ± RT	10	10	
QT ± RT	3	3	NS
Immunosuppression at diagnosis			
Cyclosporine-tacrolimus (%)	8-92	24-76	NS
Mean patient survival from diagnosis of tumour (mo)	32.9 ± 15	30.7 ± 20.6	NS

mTORi: Mammalian target of rapamycin inhibitors; LT: Liver transplantation; HCC: Hepatocellular carcinoma; ADK: Adenocarcinoma; SCC: Squamous cell carcinoma; IDC: Infiltrative ductal carcinoma; QT: Chemotherapy; RT: Radiotherapy; NS: No significant.

betes mellitus, 25 patients had high blood pressure at the time of conversion and 21 had insulin-dependent diabetes mellitus (Table 2). Blood pressure improved in three patients (12%), as shown by lower blood pressure or by a reduced need for antihypertensive drugs. One of them was converted to CNI free regimen (EVER and steroids). Glucose values or insulin doses improved in eight patients (38%). Three of them were converted to CNI free regimen (EVER and mycophenolate mofetil). Dyslipidemia was present in 30 patients and serum values improved in only two (6.7%), whose regimen were CNI low dose and EVER.

### **Efficacy according to the time elapsed between transplantation and conversion to EVER**

In general, conversion to EVER was successful in a greater percentage of patients when the conversion occurred during the first year post-transplantation (Table 6). Success rates in cases of early conversion were higher than in those of late conversion, especially in cases of refractory rejection (84.6% *vs* 60%), neurotoxicity (100%) and renal dysfunction (75% *vs* 0%).

### **Side effects and discontinuation**

Liver graft function after conversion was well preserved in all cases except in 11 patients (14.9%) who presented acute cellular rejection (4 moderate and 7 mild) requiring the reintroduction of CNI (Table 7). Ten of these



**Table 6** Efficacy in cases of early (within one year post-transplantation) and late (after one year post-transplantation) conversion to everolimus *n* (%)

Early conversion		
Cause of conversion	42 (56.8)	Resolution/stabilization or prevention of recurrence in 29 patients (69)
Refractory rejection	13 (17.6)	Resolution in 11 (84.6)
Advanced HCC in explanted liver	12 (16.3)	Prevention of recurrence in 6 (50)
HCC recurrence during follow-up	3 (4.1)	-
<i>De novo</i> tumour	0	-
CNI-related neurotoxicity	8 (10.8)	Resolution or amelioration in 8 (100)
Renal dysfunction	4 (5.4)	Resolution in 3 (75)
Other causes	2 (2.6)	Resolution in 1 (50)
Late conversion		
Cause of conversion	32 (43.2)	Resolution/stabilization or prevention of recurrence in 16 patients (50)
Refractory rejection	10 (13.5)	Resolution in 6 (60)
Advanced HCC in explanted liver	2 (2.7)	Prevention of recurrence in 1 (50)
HCC recurrence during follow-up	3 (4.1)	-
<i>De novo</i> tumour	13 (17.6)	Prevention of recurrence in 8 (61.5)
CNI-related neurotoxicity	0	-
Renal dysfunction	2 (2.7)	Resolution in none (0)
Other causes	2 (2.7)	Resolution in 1 (50)

HCC: Hepatocellular carcinoma; CNI: Calcineurine inhibitors.

patients experiencing acute rejection had converted to EVER without CNI within the first year post-transplant.

EVER-related side effects occurred in 27 patients (36.5%), some of whom experienced more than one (Table 7). Dyslipidemia was managed with the introduction of hypolipemic drugs. Infections included severe hepatitis C recurrence in four cases, bacterial pneumonia in two, pulmonary tuberculosis in one, CMV infection, pulmonary aspergillosis and sepsis in graft-versus-host disease in one, and bacteremia in one. Infections were treated according to the cause and by reducing the total amount of immunosuppression. Twenty-one patients (28.4%) stopped taking EVER (Table 7): six owing to resolution of the cause (acute rejection in four, convulsions in one, renal dysfunction in one); six because of inefficacy in resolving chronic rejection; five due to adverse events (infections in four, proteinuria in one); and four due to intercurrent surgery, with reintroduction of EVER two to three weeks after surgery.

#### Patient survival and follow-up

Mean follow-up post-conversion for the entire series was  $22 \pm 19.33$  mo (range: 0.5-74), with a median of 17.5 mo. Actuarial patient survival post-conversion was 54%, 46% and 23% at 1, 3 and 5 years, respectively. Mean and median follow-up differed according to the reason for conversion: refractory rejection,  $15.10 \pm 15.96$  mo (range: 0.5-54) and 9 mo; HCC outside Milan criteria,  $29.10 \pm$

**Table 7** Adverse events, causes of discontinuation and mortality *n* (%)

Patients receiving everolimus ( <i>n</i> = 74)	
Adverse events	27 (36.5)
Dyslipidemia	27 (36.5)
Infections	9 (12.2)
Mucositis	3 (4.1)
Diarrhoea	1 (1.4)
Proteinuria	1 (1.4)
Acute rejections post-conversion	11 (14.9)
Causes of discontinuation	21 (28.4)
Resolution of the cause of conversion	6 (8.1)
Non-responding rejection and retransplantation	6 (8.1)
Drug-related adverse events	5 (6.7)
Intercurrent surgery	4 (5.5)
Causes of mortality	25 (33)
HCC recurrence during follow-up	10
<i>De novo</i> tumour	4
HCV recurrence	4
Chronic rejection	4
Sepsis	1
Graft- <i>vs</i> -host disease	1
Other causes	1

HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus.

24.72 mo (range: 6-74) and 21.50 mo; post-transplant HCC recurrence,  $14.16 \pm 10.94$  mo (range: 3-31) and 13 mo; *de novo* tumor,  $32.92 \pm 15$  mo (range: 5-54) and 32 mo; renal dysfunction,  $14.85 \pm 13.58$  (range: 0.5-41) and 18 mo; and other CNI-related adverse events,  $25.87 \pm 21.53$  mo. Causes of death are shown in Table 7. There were no EVER-related deaths.

## DISCUSSION

The principal aim of this retrospective study was to study the real use and management of EVER in patients treated off-protocol and help redefine the true role of mTORi in clinical practice. In the field of liver transplantation, we are faced with clear challenges for the 21<sup>st</sup> century, one of which is establishing patient profiles for individualising immunosuppression strategies. Sirolimus, the first mTORi introduced into clinical practice some years ago, was largely unsuccessful<sup>[1]</sup>, but it has provided sufficient experience to help improve the use and management of EVER, another mTORi.

#### Reasons for introducing EVER

The most frequent indication for introducing EVER in our series was a high risk of tumour recurrence. So, our first experience with EVER was at low doses within a dual regimen while minimizing CNI. This experience provided evidence of the safety and efficacy of EVER, and we were able to avoid the adverse events associated with high doses of sirolimus. Furthermore, the pharmacokinetic differences between EVER and sirolimus permitted a 12-h administration and offered the possibility of providing much greater accuracy in trough levels and dose calculation.

We have used EVER in all types of transplant patients, regardless of age, sex, cause, the severity of liver disease, or concomitant diseases. Advanced age, co-infection with HCV and HIV viruses, diabetes mellitus, arterial hypertension, obesity, renal insufficiency, or even dyslipidemia did not constitute a contraindication for the use of EVER.

### Cancer patients

Cancer patients deserve special mention, since the mTOR pathway is necessary for tumour cells to grow<sup>[25]</sup>. There are three potential profiles of cancer patients. Firstly, in patients transplanted for HCC outside the Milan criteria and/or with macro- or microvascular invasion in the explanted liver, EVER would be used as prophylaxis and would be introduced in the early post-transplantation period<sup>[10]</sup>. Secondly, in patients transplanted for HCC with recurrence of the original tumour during follow-up, EVER would be used as treatment<sup>[26]</sup>. Finally, in transplanted patients who develop a *de novo* tumour during follow-up, EVER would also be used as treatment<sup>[27,28]</sup>.

In our study, in patients whose tumours were outside the Milan criteria in the explanted liver, either EVER or CNI was administered at low doses between six and twelve weeks post-transplant. We had difficulty finding appropriate historical matches for this subgroup of patients. Although macro- and microvascular invasion was greater in the EVER group, there was also a trend towards longer survival. This trend did not, however, reach statistical significance - probably due to the low number of patients. To date, no published randomized study has demonstrated the beneficial effect of the use of mTORi as prophylaxis, but we believe that EVER provides a benefit since it is the least pro-carcinogenic immunosuppressant and allows doses of known pro-carcinogenic immunosuppressants to be reduced. We await the results of a future randomized prospective study<sup>[29]</sup>.

One of the main late indications in our study was the development of a *de novo* tumour or recurrence of the original HCC. Again, survival in the EVER group was longer but did not reach statistical significance compared to our historical cohort, probably due to the low number of patients included. Taking into account that the anti-tumour properties of mTORi are at doses much higher than those used in clinical practice<sup>[30]</sup>, we agree with other authors<sup>[20,21]</sup> that EVER appears to be effective at reducing tumour recurrence.

### Patients with acute rejection

The second most frequent indication for the introduction of EVER was to reinforce the immunosuppressive regimen in cases of severe or refractory acute rejection. In this situation, EVER could be safely administered together with CNI and steroids as triple therapy or with the addition of MMF as quadruple therapy as early as 10 d post-transplant, once healing was complete. The initial doses and trough levels reached were the highest. The phase II trial<sup>[31]</sup> comparing three doses of EVER showed

that freedom from rejection correlated with trough blood levels of 3 ng/mL or more. Six patients with chronic rejection did not benefit from the introduction of EVER and were finally retransplanted, suggesting that the drug has the greatest effect during the early post-transplantation period and that there is little or no benefit from EVER in the case of chronic rejection.

### Neurotoxicity and other CNI-induced toxicities

Our experience with EVER without CNI was in patients with severe neurotoxicity or other severe adverse effects triggered by CNI, especially in the early post-transplantation period, although some cases were observed during the late post-transplantation period. Initial doses and trough levels were high, in the same range as for patients with refractory rejection. Our findings, consistent with other authors<sup>[32]</sup>, indicate that EVER-based immunosuppression - either with or without other non-CNI drugs - is a feasible and effective option, at least for the time required for CNI-induced neurological complications to disappear. However, the risk of acute rejection during the first year post-transplantation indicates a need for caution. Therefore, we do not believe that regimens based on EVER without CNI should be the principal use of this drug, at least during the first year post-transplantation.

The improvement achieved in some patients with diabetes and arterial hypertension was probably due to the parallel decrease in CNI levels and/or steroids. None of these co-morbidities were indications for conversion and they were evaluated in a qualitative and global way that makes difficult to explain the real cause of improvement. However, we believe that regimens based on EVER and low levels of CNI could play a role in patients with metabolic syndrome<sup>[33]</sup>, although further studies are required to ascertain their ability to modify the risk of cardiovascular disease<sup>[34]</sup>.

### Early and late renal dysfunction

In our study, an overall improvement in serum creatinine levels was observed in patients whose indication for receiving EVER was renal dysfunction. However, when we specifically analyzed the six patients converted for renal insufficiency, the maximum benefit was attained in those converted within the first year post-transplantation. Several liver studies and multicentre randomised trials<sup>[35,36]</sup> introducing EVER at one month post-transplantation have reported an amelioration in the glomerular filtration rate at 12 and 24 mo post-transplantation in patients receiving tacrolimus plus EVER and minimizing CNI compared to those receiving standard tacrolimus and steroids.

### Adverse events and discontinuation at low doses

No life-threatening adverse events were observed. The main adverse event was dyslipidemia, which was easily controlled by reducing the EVER dose and adding a statin. None of our patients presented EVER-associated interstitial pneumonitis or severe sepsis, as had previously been reported in other studies<sup>[37]</sup>, and drug-related deaths

**Table 8 Future challenges in liver transplantation and the potential role of everolimus**

Future challenges	Potential role of everolimus
More marginal donors	Renal function protection
Recipients with more serious disease, selected by MELD	Renal function protection
Recipients with more serious disease, with metabolic syndrome	Prevention of cardiovascular events
Less HCV cirrhosis but more aggressive strains	Antifibrotic effect
More NASH	Prevention of cardiovascular events
More metabolic syndrome during follow-up	Prevention of cardiovascular events
More HCC recurrence	Antiproliferative effect
More <i>de novo</i> tumours	Antiproliferative effect
CNI-related neurotoxicity	Good neurological profile

MELD: Model for end-stage liver disease; HCV: Hepatitis C virus; NASH: Non-alcoholic steato hepatitis; HCC: Hepatocellular carcinoma; CNI: Calcineurine inhibitors.

did not occur. This was probably due to the low doses of EVER (Figure 1) and the lessons learned from our previous experience with sirolimus<sup>[38]</sup>.

A good percentage of failure or discontinuation of the drug is probably related to the timing of the introduction of EVER in critical and irreversible situations where other immunosuppressants have failed. A real problem in the long-term management of mTORi is wound complications, which would render EVER inadvisable in stable patients with good liver function who must undergo some type of intercurrent surgery. In such cases, we would recommend withdrawal of the drug and its reintroduction, if necessary, four weeks after surgery.

### Challenges in the 21<sup>st</sup> century and the potential role of EVER

According to our study, there are several potential indications for the use of EVER after liver transplantation. During the early post-transplantation period, EVER can be used as one component of a triple therapy for refractory rejection, as one component of a double therapy with CNI (both at half the normal dose) in cases of extended tumours in the explanted liver, and at low doses without CNI in cases of severe CNI-related adverse effects. During the late post-transplantation period, EVER can be used at low doses in patients with CNI-related adverse effects and in those with HCC recurrence or *de novo* tumours. In general, we recommend EVER at low doses and as a support immunosuppressant. In this scenario, the rate of adverse events, discontinuations and drug-related deaths will be acceptable.

The future of liver transplantation presents the following scenario (Table 8): (1) increasing acceptance of marginal donors to increase the pool of grafts; (2) recipients with more severe liver disease according to the MELD criteria<sup>[39]</sup>; (3) a higher frequency of recipients with metabolic syndrome as a comorbidity; (4) less HCV cirrhosis and more NASH as the reason for transplantation<sup>[40]</sup>; and (5) longer patient survival but with increased

HCV and HCC recurrence, *de novo* tumours and cardiovascular events. Looking at this scenario, we can imagine more renal dysfunction, more metabolic syndrome and cardiovascular events, and more cases of cancer. Marginal donors would increase the incidence of primary liver dysfunction and resultant renal dysfunction. The use of the MELD score to select patients for transplantation would increase the incidence of post-transplant renal dysfunction. The incidence of metabolic syndrome is increasing both in candidates for liver transplantation and in recipients during the post-transplantation period, as well as in the general non-transplanted population, which in turn would increase the risk of cardiovascular events in the long term. The new antiviral therapies for hepatitis C may affect the need for liver transplantation; however, the HCV in the small number of patients not responding to the new drugs will be more selected and perhaps more aggressive. The incidence of HCC secondary to HCV cirrhosis would decrease, but HCC secondary to NASH would increase. Improved post-transplantation management of patients would mean longer patient survival and thus a greater probability of tumour recurrence or a *de novo* tumour (Table 8). We urgently need an immunosuppressant that will meet all the requirements. EVER is a drug with a good profile for renal dysfunction, a certain antifibrotic effect, an ability to inhibit the mTOR pathway used by cancer cells, and a good degree of effectiveness in reducing cardiovascular risk events. Future trials will demonstrate if EVER is the immunosuppressant we need.

## COMMENTS

### Background

Calcineurine inhibitors (CNI) are the most powerful immunosuppressants used in liver transplantation, however the long-term survival and quality of life are partly overshadowed by the appearance of adverse effects of its chronic use, such as renal dysfunction, metabolic syndrome, cardiovascular complications, *de novo* tumor and recurrence of underlying disease. Previous attempts to overcome these complications with the use of mammalian target of rapamycin (mTOR) inhibitors (an immunosuppressant with a different way of action), did not succeed. However, everolimus seems to cope with them and to partially contribute to search their role.

### Research frontiers

New emerging immunosuppressants must be powerful enough to avoid rejection in the same way as calcineurine inhibitors, but at the same time must avoid calcineurine inhibitors-related adverse events. The association of tacrolimus and everolimus could represent the best regimen to cope with the different profiles of patients after liver transplantation.

### Innovations and breakthroughs

Recent multicentre trials have highlighted the important of everolimus introduction at one month post-transplant together with low dose tacrolimus to protect early and long term renal function after liver transplantation. In this single-centre study, the authors report other off-protocol indications for everolimus, that could fit into the various profiles of patients that most concern to medical teams, cancer patients and patients with co-morbidities derived from calcineurine inhibitors.

### Applications

Due to the lack of new immunosuppressants, optimization of treatment regimens is of great value to increase patient and graft survival after liver transplantation. In the near future two facts will be relevant. First, survival will continue to increase over time, to the same extent that the need for calcineurine inhibitors sparing protocols. Second, the authors probably will see a change in the indications for liver transplantation, from hepatitis C liver cirrhosis toward cancer



patients. This article could serve as a starting point to be explored with further studies.

### Terminology

Everolimus is an orally administered mTOR inhibitor, a proliferation signal employed by many mammalian cells, especially those with a high level of turnover (skin, intestinal and hematological cells), but also many cancer cells and T-cells implied in the second phase of the alloantigenic response. The same pathway used by different cells of the human body, explains the dual characteristic of this mTOR inhibitor as immunosuppressant and as antineoplastic.

### Peer review

This an interesting review, it can be usefull for the readers.

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## Everolimus immunosuppression reduces the serum expression of fibrosis markers in liver transplant recipients

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### Abstract

**AIM:** To evaluate the expression of serum fibrosis markers in liver transplantation (LT) recipients on everolimus monotherapy compared to patients on an anti-calceinurin regimen.

**METHODS:** This cross-sectional case-control study included LT patients on everolimus monotherapy (cases) (E) ( $n = 30$ ) and matched controls on an anti-calceinurin regimen (calceinurin inhibitors, CNI), paired by etiology of liver disease and time since LT ( $n = 30$ ). Clinical characteristics, blood tests and elastography were collected. Serum levels of transforming growth factor- $\beta$  (TGF- $\beta$ ), angiotensin-1, tumor necrosis factor (TNF), platelet derived growth factor, amino-terminal propeptide of type III procollagen (P $\text{III}$ NP), hyaluronic

acid (HA), VCM-1 (ng/mL), interleukin (IL)-10, interferon-inducible protein 10 (IP-10), vascular endothelial growth factor and hepatocyte growth factor (HGF) (pg/mL) were determined by enzyme-linked immunosorbent assay. Expression of these markers between E and CNI was compared. Stratified analysis was done according to factors that may influence liver fibrosis. Variables are described with medians (interquartile range) or percentages.

**RESULTS:** A total of 60 patients [age: 59 (49-64), hepatitis C virus (HCV):  $n = 21$  (35%), time from LT: 73 mo (16-105)] were included. Patients had been on everolimus for a median of 15 mo. No differences in inflammatory activity, APRI test or liver elastography were found between the groups. No significant differences were observed between the groups in serum levels of P $\text{III}$ NP, metalloproteinase type = 1, angiotensin, HGF, IP-10, TNF- $\alpha$ , IL-10 and vascular cell adhesion molecule. Patients on E had a lower expression of TGF- $\beta$  [E: 12.7 (3.7-133.6), CNI: 152.5 (14.4-333.2),  $P = 0.009$ ] and HA [E: 702.89 (329.4-838.2), CNI: 1513.6 (691.9-1951.4),  $P = 0.001$ ] than those on CNI. This difference was maintained in the stratified analysis when recipient age is more than 50 years (TGF- $\beta$ 1:  $P = 0.06$ ; HA:  $P = 0.005$ ), in patients without active neoplasia (TGF- $\beta$ 1,  $P = 0.009$ ; HA:  $P = 0.01$ ), according to time since LT (> than 5 years, TGF- $\beta$ 1:  $P = 0.001$ ; HA:  $P = 0.002$ ), related to previous history of biliary complications (HA:  $P = 0.01$ ) and HCV recurrence (HA:  $P = 0.004$ ). Liver transplant recipients with everolimus monotherapy had less serum expression of TGF- $\beta$  y HA than matched patients with anti-calceinurins. This difference remains when classifying patients according to donor age and time since LT. Due to the small sample size, when examining patients with a prior history of biliary complications or recurrent HCV, the difference was non-significant but trends towards the lower expression of TGF- $\beta$ 1 in the everolimus group. Mammalian target of rapamycin (mTOR) plays a role in the transformation of quiescent hepatocellular stellate cell to their active

profibrotic state, and experimental models have demonstrated the potential activity of mTOR inhibition in attenuating fibrogenesis.

**CONCLUSION:** This study supports a possible role of everolimus in liver fibrosis modulation after LT in a clinical setting and suggests that tailoring immunosuppression could avoid fibrosis progression in the allograft.

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**Key words:** Everolimus; Rapamycin; Liver fibrosis; Mammalian target of rapamycin; Transplantation

**Core tip:** This study tries to approach the possible antifibrotic effect of everolimus, a mammalian target of a rapamycin inhibitor, in the clinical setting. Some studies in animal models suggest that it could also have an antifibrotic effect. The main conclusion of this study is that liver transplantation recipients with everolimus monotherapy had less serum expression of transforming growth factor- $\beta$  and hyaluronic acid than matched patients with anti-calceinurins that play an important role in liver fibrosis. The study offers the rationale for much needed future randomized controlled trials that evaluate the modulation of post-transplant fibrosis.

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## INTRODUCTION

Liver transplantation (LT) is the definitive treatment for end-stage liver disease. However, the outcome of a liver transplant can be compromised by allograft dysfunction due to fibrosis, which can even lead to cirrhosis. Approximately 75% of liver biopsies conducted in long-term LT survivors in whom liver tests are anomalous show significant histopathological abnormalities<sup>[1,2]</sup>. Fibrosis in the graft may be due to the recurrence of native disease [especially recurrent hepatitis C virus (HCV)], hepatotoxicity, *de novo* disease, non-alcoholic steatohepatitis, chronic rejection and/or vascular and biliary complications.

Strategies designed to prevent the progression of fibrosis in the allograft include the specific treatment of native disease<sup>[3,4]</sup> and/or stricter control of factors that can accelerate this fibrosis<sup>[5]</sup>. In addition, tailoring the immunosuppressive regime has been proposed as a strategy to regulate fibrogenesis in the post-transplant period. In HCV patients, measures such as avoiding the use of adjuvant pulse steroids for acute rejection and slow withdrawal of low-dose steroids beyond 12 mo have been proposed to avoid any immune-mediated graft injury that could induce an inflammatory and fibrogenic response<sup>[6-8]</sup>.

However, the results of a meta-analysis indicate no differences in mortality, graft survival, rejection, fibrosing cholestatic hepatitis or severe fibrosis related to the use of the calcineurin inhibitors, cyclosporine and tacrolimus at 1 year of follow-up<sup>[9]</sup>.

For prophylaxis against rejection in kidney transplant patients, new immunosuppressors known as mammalian target of rapamycin (mTOR) inhibitors (sirolimus and everolimus) have been recently introduced<sup>[10]</sup>. Small observational studies have described their use<sup>[11]</sup>, particularly in patients with renal failure<sup>[12-14]</sup> and in those who develop post-transplant neoplasia<sup>[15,16]</sup>. mTOR is a serine/threonine kinase that plays an important role in cell proliferation, stellate cell activation, protein synthesis [synthesis of interleukins interleukin (ILs) and transforming growth factor- $\beta$  (TGF- $\beta$ )]<sup>[17,18]</sup>, angiogenesis<sup>[19]</sup> and cell metabolism (hypoxia inducible factor)<sup>[20]</sup>.

Due to the role played by mTOR in key steps of fibrogenesis, mainly reducing proliferation and activating hepatic stellate cell (HSC) and portal fibroblasts<sup>[21]</sup>, it has been proposed that inhibition of this molecule could alleviate liver fibrosis in the graft. In effect, a recent study conducted on bile duct-ligated (BDL) cirrhotic rats showed that the mTOR inhibitors, sirolimus and everolimus, reduced liver fibrosis compared to the effects of calcineurin inhibitors (CNI) after 5 wk of treatment<sup>[22]</sup>.

The aim of this study was to compare serum levels of mediators of liver fibrosis in liver transplant patients under immunosuppressive regimes based on everolimus (E) with those based on calcineurin inhibitors.

## MATERIALS AND METHODS

This cross-sectional study was conducted over the period April to October 2010. All consecutive patients who underwent liver transplantation between 1995 and 2010 under everolimus immunosuppression alive at the time of the study were enrolled. Patients were matched with control LT patients undergoing calcineurin inhibitor treatment according to liver disease etiology and time since LT. Exclusion criteria for cases and control patients were acute rejection in the previous 6 mo, uncontrolled infection or antiviral treatment, or unresolved biliary complications.

Everolimus (Certican®, Novartis Pharma Schweiz AG, Bern, Switzerland) is approved for prophylaxis against rejection in *de novo* renal transplant recipients<sup>[10]</sup>, for management of malignancy (chemotherapy resistant kidney cancer, subependymal giant cell astrocytoma and neuroendocrine neoplasm)<sup>[20]</sup> and for use in drug-eluting coronary stents<sup>[23]</sup>. However, this drug has also been used off-label in liver and lung transplantation patients<sup>[24-27]</sup>. At our center, the use of everolimus in LT recipients is approved in situations such as renal dysfunction or adverse events like neurotoxicity due to CNI, development of *de novo* malignancies, recurrence of hepatocellular carcinoma, and the presence of predictors of a high risk of hepatocellular carcinoma recurrence in the explanted liver (satellitosis, vascular infiltration and multinodularity disease)<sup>[28,29]</sup>. Con-

traindications for the use of everolimus are a prior history of hepatic artery thrombosis, proteinuria greater than 800 mg/d and/or surgery in the previous 4 wk<sup>[29,30]</sup>.

### Everolimus dosing and switching

An initial dose of 0.5-0.75 mg bid E was administered and then increased 0.5 mg weekly to obtain a trough level of 3-8 ng/mL. Tacrolimus and cyclosporine were tapered by 15%-25% of the usual dose every 2 wk until complete withdrawal. The overlap period between both drugs in the E group treatment was a median of 1 or 2 mo<sup>[31-33]</sup> before monotherapy was achieved. In patients who were started on everolimus, steroids were given according to the usual schedule, and then progressively tapered and withdrawn by month 12 after liver transplantation. Trough levels of everolimus, hematological and lipid profiles, renal and liver function tests and proteinuria were monitored weekly until stable levels of the drug were achieved<sup>[34]</sup>.

### Clinical and laboratory variables

Information was compiled on patient demographics, etiology of cirrhosis, LT surgical variables, postoperative period and laboratory data. The immunosuppression regime data recorded were present dose and blood levels, time of administration, and combined treatment with corticosteroids and/or mycophenolate.

Laboratory tests were performed to determine transaminases, cholestasis enzymes and simple validated fibrosis scores (APRI)<sup>[35-37]</sup>. Additionally, 20 mL of blood were obtained to determine serum biomarkers of fibrosis that had been identified in previous studies<sup>[38-42]</sup>. We determined serum markers that could be correlated with late liver fibrosis by enzyme-linked immunosorbent assay, including those linked to matrix deposition such as hyaluronic acid (Echelos, Bioscience Inc.), amino-terminal propeptide of type III procollagen (P3NP; Cusabio, Bionova), those linked to matrix degradation such as tissue inhibitor of matrix metalloproteinase type 1 (TIMP-1; Ray-Biotech, Bionova), growth factors like angiopoietin (Ray-Biotech, Bionova), hepatocyte growth factor (HGF; Ray-Biotech, Bionova), platelet derived growth factor (PDGF; RayBiotech, Bionova) and finally, inflammatory markers that participate in the fibrogenesis-like TGF- $\beta$ 1 (Dialclone, Bionova), adiponectin and leptin, IP-10 (interferon-inducible protein 10 calcineurin inhibitor; Dialclone, Bionova), tumor necrosis factor alpha (TNF- $\alpha$ ; Dialclone, Bionova), interleukin 10 (IL-10; Dialclone, Bionova) and vascular cell adhesion molecule (VCAM; Cusabio, Bionova).

Liver stiffness was measured by a trained nurse or physician by transient elastography using a Fibroscan instrument (Echosens, Paris, France). Measurements in which 10 acquisitions were achieved, with a success rate of at least 60% and an interquartile range lower than 30 were considered valid<sup>[43,44]</sup>.

### Definitions

The following definitions were made: (1) early allograft dysfunction<sup>[45]</sup>: one or more of the following postop-

erative findings: bilirubin > 10 mg/dL, INR > 1.6 on postoperative day or alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2000 IU/mL within the first 7 postoperative days; (2) previous acute rejection: a histological picture compatible with rejection or in cases of an abnormal liver test, conversion to a normal test result after reaching optimal serum levels of immunosuppressants<sup>[1]</sup>; (3) chronic rejection: a compatible histological picture<sup>[1]</sup>; (4) biliary tract disease: anastomotic and non-anastomotic biliary strictures detected on imaging showing biochemical expression. Resolution of strictures was defined as a non-requirement for endoscopic, radiological or surgical treatment of the stricture in the 6 months before inclusion; (5) uncontrolled neoplasia: a remission time under 2 years; (6) HCV recurrence: histological indicators of inflammation or fibrosis in patients with HCV viremia detected in protocol biopsies at 6 and 12 mo; and (7) arterial hypertension, diabetes mellitus or dyslipidemia: defined according to the criteria established by the European Society of Hypertension and the International Diabetes Federation<sup>[46,47]</sup>.

## RESULTS

Sixty LT patients were recruited for the study (30 on everolimus and 30 on CNI). The demographic characteristics of the participants are provided in Table 1. Patients were predominantly men of median age 60 (49-64) years in the everolimus group and 54 (46-60) years in the control CNI group. The most common cause of liver disease that led to transplantation was alcoholic liver cirrhosis. Median time since LT was approximately 6 years (IQR 16.7-106.4 mo) for both groups. No difference in donor age or in the proportion of patients with early allograft dysfunction was observed.

The median time of everolimus treatment was 15 (5-29) mo and the median time of the initial dose of everolimus given from the time of LT was 2.7 years (0.7-8.3). This is because the main indication to use everolimus in our center was developing neoplasia *de novo* (85.71%). Monotherapy with everolimus was achieved in 25 patients (83.3%). Of the 5 patients on combination therapy (everolimus plus CNI), 1 patient was under cyclosporine treatment and 4 patients received tacrolimus. These patients did not tolerate monotherapy with everolimus immunosuppression. Most patients in the CNI group were receiving tacrolimus (24 patients) and had been under CNI treatment for a median time of 72 (17-108) mo. Approximately 25% of patients in the CNI group were given concomitant mycophenolate mofetil to minimize adverse effects linked to CNI treatment, while only one patient in the everolimus group received this drug. No differences in the proportions of patients under concomitant steroid treatment were observed between the two groups (Table 2).

No differences between treatment groups were detected in: HCV recurrence, previous episodes of acute, chronic rejection and biliary complications, proportion of patients with diabetes mellitus, arterial hypertension,



**Table 1** Demographic and clinical characteristics of patients before liver transplantation

	Everolimus patients ( <i>n</i> = 30)	CNI patients ( <i>n</i> = 30)	<i>P</i> value
Male	24 (80)	24 (80)	1.000
Age (yr)	46 (44-60)	51 (44-59)	0.760
Etiology of liver disease			
EtOH	16 (53.3)	13 (43.3)	0.541
HCV	11 (36.7)	10 (33.3)	
HBV	0	2 (6.7)	
Autoimmune	1 (3.3)	2 (6.7)	
Hemochromatosis	1 (3.3)	2 (6.7)	
Cholestatic disorders	1 (3.3)	0	
Cryptogenic	0	1 (3.3)	
Time from LT (mo)	75 (16-113)	72 (17-108)	0.859
Indication for LT			
HCC	2 (6.7)	4 (13.3)	0.041
Decompensated cirrhosis	16 (53.3)	21 (70)	
Decompensated cirrhosis and HCC	12 (40)	4 (13.3)	
Acute liver Failure	0	1 (3.3)	
Donor age (yr)	55 (34-72)	49 (31-63)	0.521
Early allograft dysfunction	5 (16.7)	2 (6.7)	0.212

Categorical variables are expressed as absolute *n* (%), continuous variables are expressed as medians and interquartilic range. CNI: Calcineurin inhibitors; HCV: Hepatitis C virus; HBV: Hepatitis B virus; LT: Liver transplantation; HCC: Hepatocarcinoma.

obesity or dyslipidemia. Patients in the everolimus group had higher serum levels of cholesterol, a well-known side effect of the drug. As expected, given the accepted local indications for everolimus treatment, patients in this group had a greater proportion of neoplasms and hepatocellular carcinoma outside the Milan criteria (data not shown) at the time of the study.

Although bilirubin levels were higher in the CNI group (*P* = 0.002), no differences were observed in transaminase levels (AST and ALT), GGT or in the proportion of patients with hyperbilirubinemia. Similarly, no differences in APRI or elastography were detected between the groups.

No significant differences were observed between the groups in serum levels of PIIIINP, TIMP-1, angiopoietin, HGF, IP-10, TNF- $\alpha$ , IL-10 and VCAM (Table 3). Interestingly, patients on everolimus showed a markedly lower expression of TGF- $\beta$ 1 [12.7 (3.7-133.6) ng/mL *vs* 152.5 (IQR 14.4-333.2) ng/mL; *P* = 0.009] (Figure 1A). TGF- $\beta$ 1 is the most potent stimulus for hepatic fibrogenesis through activation of hepatic stellate cells<sup>[48]</sup>. Furthermore, patients on everolimus showed the lower expression of hyaluronic acid [702.89 (329.4-838.2) ng/mL *vs* 1513.6 (691.9-1951.4) ng/mL; *P* = 0.001] (Figure 1B), an essential component of the extracellular matrix (ECM) mostly synthesized by hepatic stellate cells<sup>[49]</sup>.

To determine whether the results could be influenced by other factors, markers were compared in different patient subgroups by univariate analysis (Table 4). First of all, we examined the expression of fibrosis markers in patients without active neoplasia given the uneven distribution of neoplasia between groups. Other patient

**Table 2** Clinical characteristics of patients after liver transplantation

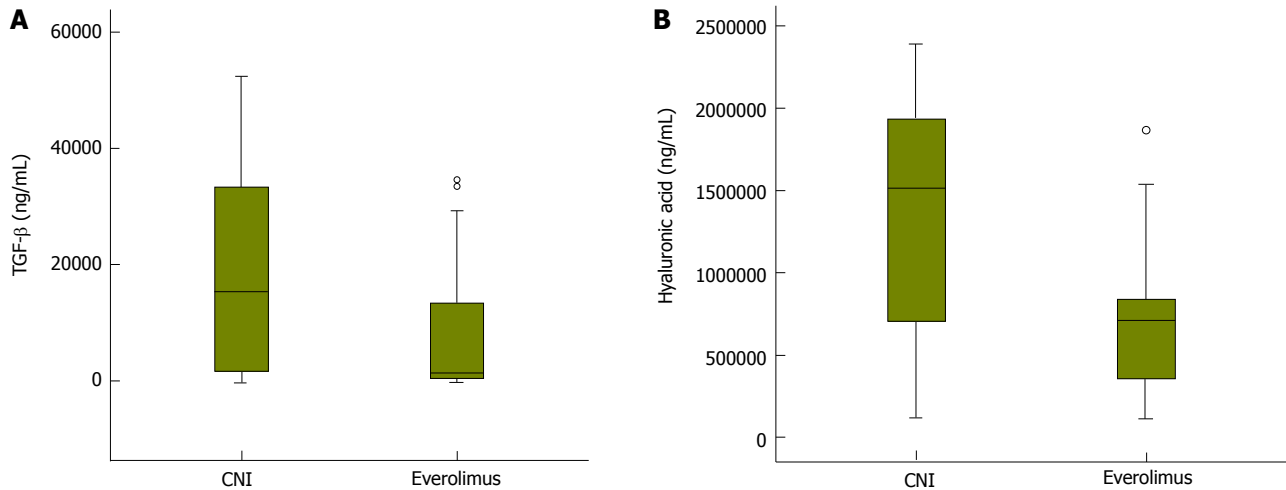
	Everolimus patients ( <i>n</i> = 30)	CNI patients ( <i>n</i> = 30)	<i>P</i> value
Age	60 (49-64.5)	54 (46.5- 60.5)	0.756
BMI (kg/m <sup>2</sup> )	26.2 (23.3-28.2)	27.9 (25.2-31.3)	0.211
Dyslipemia	10 (33.3)	12 (40)	0.789
Diabetes mellitus	9 (30)	13 (43.3)	0.284
HTA	18 (60)	18 (60)	1.000
Neoplasia			
HCC	8 (26.7)	1 (3.3)	0.026
Solid non-hepatic neoplasia	2 (6.7)	0	
Skin neoplasia	4 (13.3)	1 (3.3)	
Hematological neoplasia	2 (6.7)	0	
Recurrent HCV	11 (36.7)	10 (33.3)	1.000
AST (IU/L)	23 (18-53)	35 (25-66)	0.081
ALT (IU/L)	43 (18- 65)	24 (18-62)	0.260
Bilirubin (mg/ dL)	0.5 (0.37-0.7)	0.85 (0.5-1.1)	0.002
Cholesterol (mg/ dL)	188.5 (167-220.75)	158 (141.25-178.25)	0.002
Acute rejection	7 (23.3)	9 (30)	0.771
Chronic rejection	2 (6.7)	1 (3.3)	1.000
Biliary complications	10 (33.3)	12 (40)	0.789
APRI	0.74 (0.48-2)	0.47 (0.34-1.4)	0.135
Liver stiffness (kPa)	7.6 (5.1-8.6)	8.4 (5.6-10.7)	0.134
Concomitant steroids	6 (20)	6 (20)	1.000
Concomitant Mycophenolate mofetil	1 (3.3)	8 (26.7)	0.026

Categorical variables are expressed as absolute *n* (%), continuous variables are expressed as medians and interquartilic range. HCV: Hepatitis C virus; HBV: Hepatitis B virus; LT: Liver transplantation; HCC: Hepatocarcinoma; HTA: Arterial hypertension; APRI: APRI score.

subsets were established according to time since LT (> than 5 years), recipient age (> 50 years), previous history of biliary complications and HCV recurrence. The differences observed between TGF- $\beta$ 1 and hyaluronic acid expression in the main everolimus and CNI groups persisted in our analysis by subgroups. This difference was statistically significant when classifying patients according to donor age and time since LT. However, due to the small sample size, when examining patients with a prior history of biliary complications or recurrent HCV, the difference emerged as a non-significant trend towards the lower expression of TGF- $\beta$ 1 in the everolimus group. Although there were differences in the use of mycophenolate mofetil among both groups, the results described before remained when we compared both groups excluding patients who were receiving mycophenolate mofetil.

## DISCUSSION

In this study we show that LT patients on everolimus therapy have lower serum levels of TGF- $\beta$ 1 and hyaluronic acid than patients matched for disease etiology and time since LT receiving CNI. TGF- $\beta$ 1 is the most significant inducer of the synthesis of extracellular matrix proteins (collagen and glycosaminoglycans such as



**Figure 1** Box plots of serum transforming growth factor- $\beta$  (A) and serum Hyaluronic acid (B) in patients under calcineurin inhibitors or everolimus regime. TGF- $\beta$ : Transforming growth factor- $\beta$ ; CNI: Calcineurin inhibitors.

**Table 3** Serum levels of liver fibrosis mediators

	Everolimus patients (n = 30)	CNI patients (n = 30)	P value
VCAM (ng/mL)	68.25 (25.98-135.17)	58.88 (35.30-115.52)	0.668
P11NP (ng/mL)	172.4 (119.75-1195.90)	879.40 (140.10-1555.15)	0.193
IP-10 (pg/mL)	86.01 (51.10-210.91)	79.61 (59.2-172.64)	0.669
HGF (pg/mL)	225.17 (163.30-320.17)	205.53 (152.59-297.86)	0.363
Angiopoietin (ng/mL)	26.97 (19.58-32.25)	30.108 (24.60-38.8)	0.122
TNF- $\alpha$ (ng/mL)	41.12 (38.35-44.8)	42.70 (40.20-45.07)	0.435
IL-10 (pg/mL)	8.52 (6.07-9.23)	8.66 (6.95-9.40)	0.856
TGF- $\beta$ (ng/mL)	12.7 (3.7-133.6)	152.5 (14.4-333.2)	0.009
HA (ng/mL)	702.89 (329.4-838.2)	1513.6 (691.9-1951.4)	0.001
PDGF (ng/mL)	1.5630 (1.4663-1.6369)	1.5630 (1.4616-1.6369)	0.720

Variables are expressed as medians and interquartilic range. VCAM: Vascular cell adhesion molecule; P11NP: Amino-terminal propeptide of type III procollagen; IP10: Interferon-inducible protein 10 calcineurin inhibitor; TNF- $\alpha$ : Transforming necrosis factor alpha; TGF: Tissue growth factor; HGF: Hepatocyte growth factor; HA: Hyaluronic acid; PDGF: Platelet derived growth factor.

hyaluronic acid) by hepatic stellate cells, and also regulates many proteins involved in their turnover including matrix metalloproteinases (MMP) and their inhibitors (TIMP)<sup>[48,50-52]</sup>.

MTOR signaling includes several steps in the transformation of quiescent HSC to their active profibrotic state<sup>[53]</sup>. Although some studies have addressed the modulation of liver fibrosis in patients on CNI, no study has assessed the role of mTOR inhibitors in fibrogenesis in a clinical setting.

The potential role of mTOR inhibition in attenuating fibrogenic pathways has been examined in experimental models of cirrhosis. After bile duct ligation- and thioacetamide induced cirrhosis, low dose rapamycin led to

**Table 4** Stratified analysis according to factors that could influence liver fibrosis

	TGF- $\beta$ (ng/mL)	P value	HA (ng/mL)	P value
All patients				
E (n = 30)	12.7 (3.7-133.6)	0.009	702.89 (329.4-838.2)	0.001
CNI (n = 30)	152.5 (14.4-333.2)		1513.6 (691.9-1951.4)	
Free of neoplasia				
E (n = 29)	11.1 (3.2-22.4)	0.005	754.8 (351.3-837)	0.030
CNI (n = 22)	137.5 (14.4-333.2)		1296.7 (703.7-1936.1)	
Time from LT > 5 yr				
E (n = 17)	16.5 (7.6-264.6)	0.010	462.0 (351.3-770.3)	0.002
CNI (n = 16)	296.8 (125.4-337.1)		1084.7 (523.7-1665.8)	
Donor age > 50 yr				
E (n = 12)	14.0 (6.0-67.1)	0.06	910.0 (589.2-1510.8)	0.005
CNI (n = 15)	96.0 (14.5-297.1)		1897.0 (1519-2136.5)	
Biliary complications				
E (n = 12)	20.6 (7.6-265)	0.110	516.75 (235.6-1079.4)	0.010
CNI (n = 10)	272.3 (16.5-403.4)		1545.37 (1085.8-1888.7)	
Recurrent HCV				
E (n = 10)	6.5 (1.6-15.3)	0.260	914.55 (768.8-1513.6)	0.410
CNI (n = 11)	14.5 (6.1-225)		1991.17 (1532.4-2168.9)	

Variables are expressed as medians and interquartilic range. TGF- $\beta$ : Transforming growth factor- $\beta$ ; HA: Hyaluronic acid; E: Everolimus; CNI: Calcineurin inhibitors.

the reduced accumulation of ECM-producing cells, ECM components, reduced interstitial MMP-2 activity and a reduced spleen weight as an indicator of portal hypertension than in vehicle-treated cirrhotic rats<sup>[54]</sup>. Higher doses of rapamycin in the BDL rats gave rise to a reduction in HSC activation and proliferation as well as a reduced capacity of other cells to transition to myofibroblasts<sup>[21]</sup>. Lastly, mTOR inhibitors have been noted to reduce liver fibrosis up to 70% and portal pressure up to 50% in BDL rats compared to CNI-treated rats. Furthermore, in mTOR inhibitor-treated rats, the clinical manifestation of portal hypertension was lessened, as indicated by factors such as the development of ascites.

In the context of LT, one of the main causes of fibrosis is recurrent hepatitis C. The activation of HSC has been correlated not only with the fibrosis stage, but

also with the rate of liver fibrosis progression<sup>[55]</sup>. In a retrospective clinical study, the use of sirolimus compared to CNIs was associated with a trend towards diminished disease activity and fibrosis in serial biopsies, although no differences were observed in incidence and time to recurrence of HCV<sup>[56]</sup>.

No differences in the extent of fibrosis as measured by transient elastography and APRI score were detected, although our study was not designed to assess this factor. Elastography has not been validated in long-term liver grafts and its sensitivity to determine fibrosis is probably not comparable to the use of direct molecular markers of fibrogenesis. The limitations of our study include those inherent to its cross-sectional design, which precludes establishing a temporal relationship between drug initiation and serum levels of fibrosis markers. In addition, it has been well established that different etiologies of liver disease produce different fibrosis patterns. Unfortunately, our sample size was insufficient to determine the effect of everolimus according to the etiology of liver disease. Also, serum biomarker expression could be influenced by factors secondary to the inflammatory response or to other forms of chronic visceral damage. To avoid this bias, patients with acute conditions were not included and the influence of other chronic conditions was assessed by examining different patient subgroups.

In conclusion, patients under everolimus therapy show reduced serum expression of fibrosis markers such as TGF- $\beta$ 1 and hyaluronic acid compared to patients matched for LT etiology and time since LT under a CNI immunosuppressive regimen. The results of this study provide direction for future studies designed to address the issue of modulating post-transplant fibrosis using individualized immunosuppression strategies.

## COMMENTS

### Background

The outcome of liver transplant may be conditioned by allograft dysfunction associated with the development of fibrosis which can even lead to cirrhosis. Tailoring immunosuppression has been postulated to have a role in fibrosis progression. Mammalian target of rapamycin (mTOR) inhibitors (sirolimus and everolimus) have been introduced for prophylaxis against rejection in transplant patients and, because of their antiangiogenic, antiproliferative and antifibrotic properties, it has been postulated that they could modulate liver fibrosis in liver transplant (LT) grafts.

### Research frontiers

Low dose rapamycin can reduce accumulation of extracellular matrix (ECM)-producing cells (extracellular matrix), ECM components, interstitial matrix metalloproteinases (MMP)-2 activity (metalloproteinases) and a reduced spleen weight as an indicator of portal hypertension in cirrhotic rats. Experimental models have demonstrated the potential activity of mTOR inhibition in attenuating fibrosis but there is no evidence in a clinical setting. The hotspot of this article is the study about the impact of everolimus immunosuppression in serum levels of liver mediator fibrosis expression in a clinical practice.

### Innovations and breakthroughs

mTOR signaling includes several steps in the transformation of quiescent hepatic stellate cell (HSC) to their active profibrotic state. Higher doses of rapamycin in rats give rise to a reduction in HSC activation and proliferation as well as a reduced capacity of other cells to transition to myofibroblasts. These rats had reduced liver fibrosis up to 70% and portal pressure up to 50% compared to calcineurin inhibitors-treated rats. Clinical manifestation of portal hypertension

like ascites development was lessened in mTOR inhibitor-treated. Due to the potential role mTOR in key steps of fibrogenesis, mainly reducing proliferation and activating hepatic stellate cell and portal fibroblasts, it has been proposed that inhibition of this molecule could alleviate liver fibrosis in the graft. In this study we show that liver transplant patients on everolimus therapy have lower serum levels of transforming growth factor- $\beta$  (TGF- $\beta$ ) 1 and hyaluronic acid than patients matched for disease etiology and time since LT receiving calcineurin inhibitors. TGF- $\beta$ 1 is the most significant inducer of the synthesis of extracellular matrix proteins (collagen and glycosaminoglycans such as hyaluronic acid) by hepatic stellate cells, and also regulates many proteins involved in their turnover including MMP and their inhibitors (TIMP).

### Applications

The study results suggest that mTOR inhibitors could modulate fibrosis progression in liver grafts. Although this is not a prospective study, the results support the need to investigate the role of an immunosuppression regime in fibrosis development after liver transplantation.

### Terminology

MTOR: MTOR is a serine/threonine kinase that plays an important role in cell proliferation, stellate cell activation, protein synthesis (synthesis of interleukins and transforming growth factor beta), angiogenesis and cell metabolism (hypoxia inducible factor).

### Peer review

This is a good descriptive study in which the authors compare serum liver fibrosis expression between both immunosuppression regimes (anti-calcineurin vs everolimus). The results of this study are interesting and provide direction for future studies designed to address the issue of modulating post-transplant fibrosis using individualized immunosuppression strategies in clinical practice.

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## Impact of transplant nephrectomy on peak PRA levels and outcome after kidney re-transplantation

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**RESULTS:** Peak PRA levels between the first and the second transplantation were higher in patients undergoing graft nephrectomy ( $P = 0.098$ ), whereas the last PRA levels before the second kidney transplantation did not differ between the groups. Age adjusted survival for the second kidney graft, censored for death with functioning graft, were comparable in both groups. Waiting time between first and second transplantation did not influence the graft survival significantly in the group that underwent nephrectomy. In contrast, patients without nephrectomy experienced better graft survival rates when re-transplantation was performed within one year after graft loss ( $P = 0.033$ ). Age adjusted patient survival rates at 1 and 5 years were 94.1% and 86.3% vs 83.1% and 75.4% group NE+ and NE-, respectively ( $P < 0.01$ ).

**CONCLUSION:** Transplant nephrectomy leads to a temporary increase in PRA levels that normalize before kidney re-transplantation. In patients without nephrectomy of a non-viable kidney graft timing of re-transplantation significantly influences graft survival after a second transplantation. Most importantly, transplant nephrectomy is associated with a significantly longer patient survival.

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**Key words:** Kidney re-transplantation; Graft nephrectomy; Panel reactive antibodies, Patient and graft survival

**Core tip:** In our paper, presented as "poster of distinction" at the ATC, we show that graft nephrectomy of a first non-functioning kidney graft leads to an increase in peak panel reactive antibody that normalizes before re-transplantation. In 305 low-risk patients who underwent re-transplantation, graft survival did not differ between those with or without prior nephrectomy. Interestingly, patient survival was significantly better in patients with nephrectomy. This supports the find-

### Abstract

**AIM:** To determine the impact of transplant nephrectomy on peak panel reactive antibody (PRA) levels, patient and graft survival in kidney re-transplants.

**METHODS:** From 1969 to 2006, a total of 609 kidney re-transplantations were performed at the University of Freiburg and the Campus Benjamin Franklin of the University of Berlin. Patients with PRA levels above (5%) before first kidney transplantation were excluded from further analysis ( $n = 304$ ). Patients with graft nephrectomy ( $n = 245$ , NE+) were retrospectively compared to 60 kidney re-transplants without prior graft nephrectomy (NE-).

ings of Ayus *et al.*, who investigated patients staying on maintenance dialysis after graft failure. Therefore graft nephrectomy should be considered in patients returning to dialysis after failure of a kidney transplant.

Tittelbach-Helmrich D, Pisarski P, Offermann G, Geyer M, Thomusch O, Hopt UT, Drogitz O. Impact of transplant nephrectomy on peak PRA levels and outcome after kidney re-transplantation. *World J Transplant* 2014; 4(2): 141-147 Available from: URL: <http://www.wjgnet.com/2220-3230/full/v4/i2/141.htm> DOI: <http://dx.doi.org/10.5500/wjt.v4.i2.141>

## INTRODUCTION

Kidney transplantation is the therapy of choice for patients suffering from end-stage renal failure. Due to improvements in immunosuppressive therapy and operative technique, contemporary graft survival rates in first deceased donor transplants have reached 90% after one year and 68% after five years, respectively<sup>[1]</sup>. Patients returning to dialysis after failure of the primary graft have a significantly higher mortality rate compared to patients awaiting their first kidney graft<sup>[2]</sup>. Repeat kidney transplantation has been shown to offer a significant survival benefit in these cases<sup>[3,4]</sup>. However, the outcome of repeat kidney transplantation is known to be inferior to primary transplantation<sup>[1]</sup>. In 2005 18.7% of patients on the waiting list in the United States had been transplanted previously (OPTN/SRTR Annual report 1995-2004) and recent research indicates that the number of patients undergoing kidney retransplantation is growing rapidly<sup>[1]</sup>.

The indication and timing of primary allograft nephrectomy in patients awaiting a secondary renal transplant are still a matter of debate<sup>[5]</sup>. Graft nephrectomy is a safe procedure in experienced centers. It is associated with perioperative morbidity that depends on the surgical technique used (*e.g.*, extra- *vs* intracapsular) and the indication for nephrectomy. Morbidity ranges from 4% to 48% and encompasses bleeding, infection or, less frequently, injury of iliac vessels<sup>[6,7]</sup>. Due to perioperative complications some authors recommend not to remove the non-functional kidney until graft associated complications occur<sup>[8-11]</sup>. However, others advise the routine removal of the failed graft to avoid infection, bleeding, hypertension or erythropoietin resistance due to chronic inflammation<sup>[10,11]</sup>. The most common practice seems to be nephrectomy after early graft loss, while in patients with graft failure after more than one year, nephrectomy is often exclusively reserved for cases experiencing complications<sup>[12-15]</sup>.

The impact of a non-functioning kidney graft left in situ or graft nephrectomy on antibody production and outcome after secondary renal transplantation remains unclear, although PRA levels in patients undergoing nephrectomy seem to be higher than in patients in which the graft is not removed<sup>[16,17]</sup>.

The aim of this study was to determine the influence

**Table 1 Pretransplant demographic data of all patients**

Characteristics	NE+	NE	P
n	245	60	
Sex (M/F)	158/87	41/19	0.650
Age at 1. Tx (yr; mean)	35.5 ± 13.9	39.2 ± 12.9	0.056
Age at 2. Tx (yr; mean)	41.6 ± 13.3	47.2 ± 13.3	0.004
Date of 1. Tx	09/1969-03/2005	10/1979-09/2002	
Date of 2. Tx	09/1981-12/2005	04/1991-09/2006	

M: Male; F: Female.

of nephrectomy on PRA levels and the outcome after secondary renal transplantations.

## MATERIALS AND METHODS

### Patients

The records of all retransplant renal allograft recipients at the University of Freiburg and the University of Berlin, Campus Benjamin Franklin, between 1969 and 2006 were reviewed.

In total 609 re-transplantations were performed, of which 305 (50.1%) were included in our study. Inclusion criteria were as follows: second renal transplantation (third or fourth transplantations were excluded from analysis), PRA prior to first kidney transplantation ≤ 5%, available data on nephrectomy and a minimum of three documented PRA values (before first, between first and second and immediately before second transplantation). Of 305 patients meeting these criteria, 245 patients underwent nephrectomy (NE+) and 60 patients retained their failed first graft (NE-).

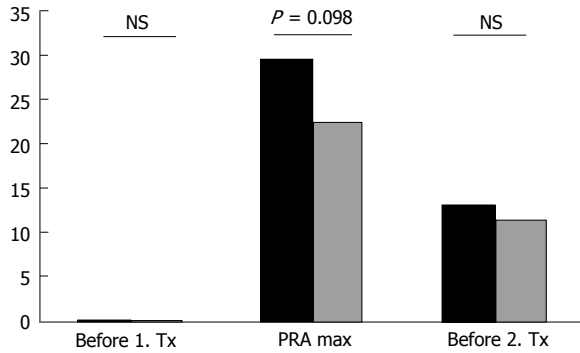
The mean age at the time of the first kidney transplantation was 35.5 ± 13.9 years and 39.3 ± 12.8 years for NE+ and NE- patients, respectively (*P* = 0.056). At the time of second transplantation patients were 41.6 ± 13.3 years old in group NE+ and 47.2 ± 13.3 years in the group NE- (*P* = 0.004). Demographic data of patients are shown in Table 1.

The immunosuppressive regimen included steroids plus cyclosporin A (CsA; *n* = 175), CsA plus azathioprine or mycophenolate mofetil (*n* = 106) or other regimens containing tacrolimus or an induction therapy with antibodies (*n* = 22). All patients in the group NE- received CsA for maintenance therapy.

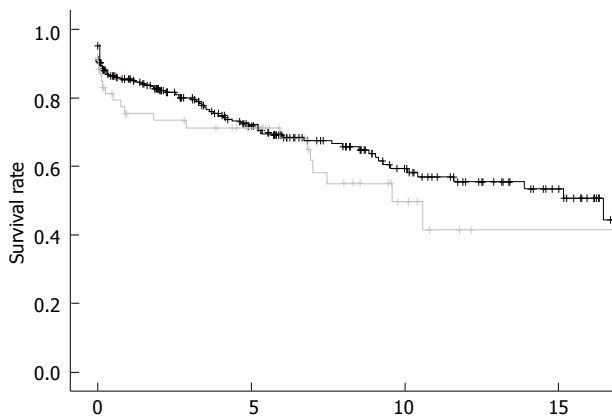
Graft failure was defined as the irreversible loss of graft function with the need to resume dialysis. Immunosuppression (prednisone 5 mg per day) was continued as long as diuresis exceeded 500 mL/d. If urine production fell below 500 mL/d, immunosuppression was discontinued. In group NE-, the non-functioning kidney graft remained in situ, unless patients developed complications (*e.g.*, infections, bleeding or hypertension). Patients in the group NE+ underwent nephrectomy soon after resuming dialysis. Transplant nephrectomy was performed according to the technique described by Rosenthal *et al*<sup>[6]</sup>.

### Statistical analysis

Perioperative and follow-up data of patients were gained



**Figure 1** Levels of panel reactive antibodies before the first transplantation, peak panel reactive antibodies between first and second transplantation and before second transplantation in the groups NE+ (black) and NE- (grey).



**Figure 2** Kidney graft survival of a second renal allograft in patients with (black) or without (grey) prior nephrectomy of a first non-functioning kidney graft, censored for "death with functioning graft".

retrospectively from electronic health care records or from Eurotransplant Network Information System. Statistical analysis was performed using SPSS. Values are expressed as mean  $\pm$  SD unless otherwise stated. Patient and graft survival rates were calculated according to the Kaplan-Meier method. Survival rates among both groups were compared using the univariate log-rank analysis. Group comparisons were calculated by independent Students *t* tests. *P* values of  $< 0.05$  were considered significant. Non-significant differences are indicated as ns.

## RESULTS

Follow-up data were available for all patients. Mean follow-up was 7.9 years (range 0.3–22.8 years) in the group NE+ and 6.2 years (range 0.4–19.3 years) in the group NE-. Mean waiting time from graft loss to re-transplantation was  $3.44 \pm 2.68$  years in the group NE+ and  $2.55 \pm 2.55$  years in the group NE- ( $P = 0.021$ ). In the group NE+, nephrectomy was performed  $0.53 \pm 1.47$  years after graft loss and  $3.05 \pm 2.57$  years before second transplantation.

The last recorded PRA levels before second transplantation did not differ between groups (Figure 1). In

**Table 2** Multivariate Cox regression analysis for graft survival after second renal transplantation

	OR	95%CI	P
NE+/-	1.06	0.71-1.56	0.79
PRA before 1. Tx	1.59	1.11-2.30	0.01
PRA max	0.99	0.98-1.00	0.18
PRA before 2. Tx	1.02	1.00-1.04	0.01
Time from 1. Tx to graft loss	0.96	0.88-1.05	0.37
Time from graft loss to nephrectomy	0.89	0.76-1.07	0.22
Time from nephrectomy to 2. Tx	0.89	0.79-1.02	0.09
Time from graft loss to 2. Tx	0.98	0.91-1.06	0.65
Age at 1. Tx	1.01	0.99-1.03	0.45
Age at 2. Tx	1.01	0.99-1.03	0.13

PRA: Peak panel reactive antibody.

contrast, the mean maximum PRA levels were higher in the group NE+ than in the group NE- (29.7% *vs* 22.5%), although this difference did not reach statistical significance ( $P = 0.09$ ). When comparing the median, the difference in maximum PRA levels reached statistical significance (18.5 in NE+ *vs* 9 in NE-;  $P = 0.038$ ). The maximum PRA level was detected  $1.6 \pm 1.9$  years (NE+) and  $0.5 \pm 2.9$  years (NE-) after graft loss and  $2.2 \pm 2.3$  years (NE+) *vs*  $2.1 \pm 3.4$  years (NE-) before re-transplantation. Maximum PRA levels in the group NE+ were observed at an average of one year after nephrectomy ( $1.0 \pm 2.2$  years).

The uni- and multivariate analysis of potential risk factors show that PRA levels measured directly before transplantations were the only factor being associated with a significantly higher risk of graft loss (Table 2).

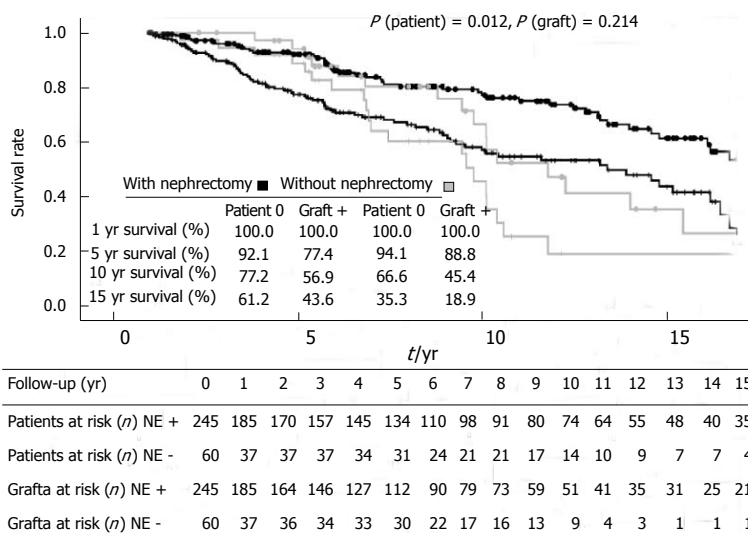
Graft survival for the entire cohort differed significantly with 1, 5 and 10-year graft survival rates of 81.4%, 62.4% and 46.3% *vs* 66.8%, 59.0% and 30.2% for patients of the groups NE+ and NE- ( $P = 0.01$ ), respectively. However, this advantage disappeared when the analysis was censored for death with a functioning graft (Figure 2).

Graft survival rates after the second kidney transplantation did not differ between patients with early failure of the first graft (within 6 mo) and patients with graft loss occurring later than 6 mo.

To further exclude potential confounding variables, any failure of the second graft within one year after re-transplantation, which is mainly related to technical or early immunological complications, was censored (Figure 3). Graft survival rates at 5 and 10 years did not differ and were 77.4% and 56.9% in the group NE+ and 88.8% and 45.4% in group NE- ( $P = 0.214$ ).

In addition, we evaluated the influence of center-specific factors on graft survival rates due to different immunosuppressive regimens. According to our data, patients on triple immunosuppressive regimens using calcineurin inhibitors (mainly CsA) and azathioprine or MMF and steroids experienced significantly better graft survival rates if compared to patients using only CsA and steroids. The graft survival rates of patients in the groups NE+ and NE-, respectively, receiving the same immunosuppressive regimen did not differ between the two centers.





**Figure 3** Patient and graft survival, censored for graft loss within 1 year, in second kidney transplants with prior nephrectomy of a non-functioning first kidney graft (black: group NE+) compared to controls without nephrectomy (grey: NE-).

Interestingly, in patients undergoing nephrectomy prior to re-transplantation (NE+) the timing of second kidney transplantation (within one year after graft loss *vs* later than one year) did not significantly influence the outcome. In contrast, patients without nephrectomy experienced better graft survival rates when re-transplantation was performed within one year after graft loss ( $P = 0.033$ ) (Figure 4).

Patient survival rates according to the Kaplan-Meier method at 1, 5 and 10 years were 94.1%, 86.3%, 72.2% and 79.2%, 73.1%, 44.1% in the group NE+ and in the group NE-, respectively ( $P < 0.01$ ). Since patients of the group NE- were significantly older than patients in the group NE+, patient survival may have been influenced by differences in age at the time of second transplantation. However, Log-rank analysis of age-adjusted patient survival rates after the exclusion of all patients older than 65 years at time of second transplantation, still revealed a significant survival benefit for patients in the nephrectomy group, compared to patients without nephrectomy (94.1% and 86.3% *vs* 83.1% and 75.4% at 1 and 5 years;  $P = 0$ ,  $< 0.01$ ).

## DISCUSSION

Therapeutic strategies for patients having lost a primary kidney graft and awaiting re-transplantation differ from center to center. Until now, there is no consensus regarding the indication and timing of the removal of a nonviable graft.

It is known that graft and patient survival is worse after second kidney transplantation compared to the first transplantation<sup>[1]</sup>. Several factors may contribute to this finding: Kidney re-transplants acquire additional waiting time on dialysis after failure of the first transplant which in itself is well known to increase morbidity and mortality after re-transplantation<sup>[2,18]</sup>. Moreover, patients who undergo repeat renal transplantation are older than at the time of first transplantation and often receive grafts from extended-criteria donors<sup>[19-22]</sup>.

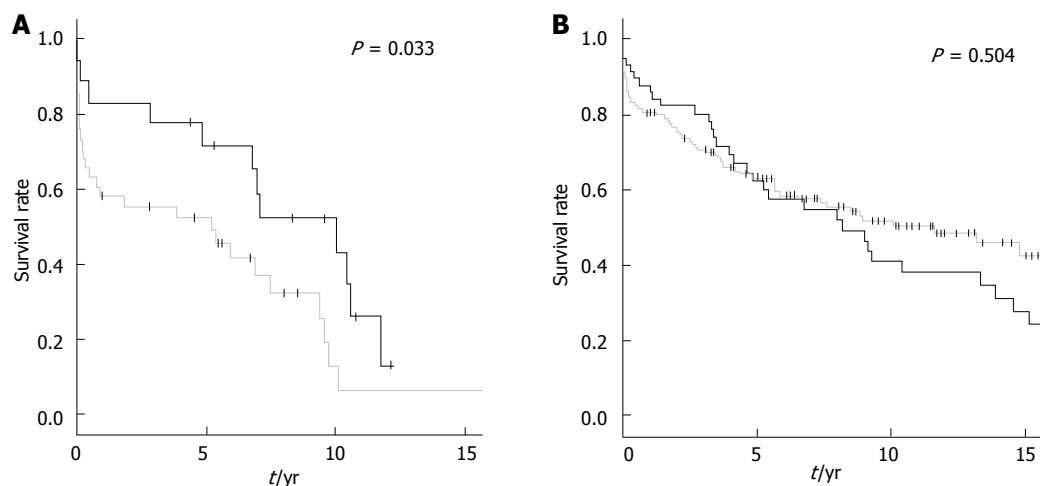
The main finding of our study was a significantly in-

creased patient survival in those second graft recipients who had undergone nephrectomy of their first nonviable graft before receiving a repeat transplantation. This striking effect was observed despite a lack of difference in second kidney graft survival rates between patients who had their first transplant removed before re-transplantation and those who retained their failed graft.

The reasons for the improved survival of repeat transplant candidates who had undergone prior nephrectomy are unclear. However, patients staying on maintenance dialysis after failure of a first kidney graft also show improved survival after graft nephrectomy<sup>[23]</sup>. The residual non-functioning graft in patients not undergoing nephrectomy may thus be a source of complications in itself or through the need for continued immunosuppressive therapy (*e.g.*, infections or a chronic inflammatory condition).

By analyzing graft survival rates censored for death with functioning graft or graft loss within one year, our results revealed no differences for patients with or without nephrectomy, which is in accordance with recent literature<sup>[24]</sup>. Therefore, nephrectomy of the failed first kidney graft does not influence survival of the second graft.

Patients considered for re-transplantation are often immunized or even highly immunized due to the development of HLA-specific antibodies to previous transplant antigens. Yong Won Cho showed that panel reactive antibodies are observed more often after graft loss than after blood transfusions or prior pregnancies<sup>[25]</sup>. Therefore, even with negative complement dependent cytotoxicity crossmatch, these patients are more likely to develop acute humoral rejection episodes<sup>[25-27]</sup>. This correlates with our findings that higher PRA values before first and second transplantation are associated with an increased risk of graft loss. The impact of the elevated PRA levels in the group NE+ remains unclear but was also observed in other studies<sup>[5,28,29]</sup>. Our study design precluded information on presensitized patients. Schleicher *et al*<sup>[29]</sup> could show that in their collective patients undergoing nephrectomy had significantly higher PRA levels at the time of



**Figure 4** Graft survival of a second renal allograft in patients without (A) or with (B) prior nephrectomy of a first non-functioning kidney graft and retransplantation within (black curve) or later than one year after nephrectomy (grey curve).

retransplantation, which led to a worse graft survival in that group. Especially a PRA level > 70% was an independent risk factor for graft loss. In our study, graft survival did not differ between the groups. This may be due to similar PRA levels before retransplantation. Lucarelli *et al*<sup>[30]</sup> also did not find a difference in second graft survival in patients with or without prior nephrectomy. They also observed comparable PRA levels in both groups at the time of retransplantation<sup>[30]</sup>.

Intensified immunosuppression may therefore improve graft and patient survival in patients with elevated PRA after a first graft nephrectomy and can also be found in our data considering the different immunosuppressive regimens.

Other authors state that the rise in HLA antibodies after nephrectomy is an expression of the capacity of even a nonfunctional graft to absorb donor specific antibodies or mount an immune response to the donor's MHC antigens. This may protect a second renal graft<sup>[31,32]</sup>. The graft intolerance syndrome, which leads to chronic inflammatory disease that can be treated by embolization of nonfunctioning renal allografts<sup>[33-35]</sup>, favors the aforementioned hypothesis. However, neither murine and nor human studies could proof these findings<sup>[36]</sup>.

By analyzing graft survival rates censored for death with functioning graft or graft loss within one year, we observed no difference for patients with or without nephrectomy. Therefore, nephrectomy of the failed first kidney graft does not influence survival of the second graft.

Although we observed no influence of prior graft survival, we could confirm the importance of waiting time to retransplantation. In patients undergoing nephrectomy prior to re-transplantation, no difference was evident. In contrast, in patients without nephrectomy, a survival benefit was evident when re-transplantation was performed within one year after graft loss. In our patient group waiting time to retransplantation was about two to three years; in the United States waiting times of more than five years are common<sup>[35]</sup>. This also needs to be taken into account when considering a graft nephrectomy

with its associated perioperative risk.

This study is limited by its retrospective design and the long timeframe in which patients have undergone transplantation. It still offers novel insights into the advantages of graft nephrectomy on the outcome of secondary kidney transplantation.

In a conclusion, Nephrectomy of a nonfunctioning kidney graft prior to re-transplantation is a save procedure in experienced centers that, despite a temporary increase in PRA levels, results in significantly better patient survival. Therefore transplant nephrectomy should be considered in all patients awaiting a kidney re-transplantation.

## COMMENTS

### Background

Kidney transplantation is the treatment of choice for patients with end-stage renal disease. Despite excellent results, the half-life after deceased or living donor transplantation was 8.8 and 11.9 years after transplantation in 2005 in the United States, the management of patients with graft failure is still under debate. Some authors favor removal of the non viable kidney to prevent complications such as infection or chronic inflammatory response, others recommend to leave the nonfunctioning kidney in order to prevent surgery associated complications and a rise in panel reactive antibodies.

### Research frontiers

There are many studies showing that panel reactive antibodies rise after the removal of a non viable kidney transplant. The long-term outcome concerning morbidity and mortality of patients as well as the outcome of a second kidney transplant after graft nephrectomy remains unclear. Prior studies demonstrated controversial results regarding complication rates and mortality with or without nephrectomy in patients staying on dialysis after graft failure or undergoing secondary renal transplantation.

### Innovations and breakthroughs

Nephrectomy of a non-viable kidney graft leads to a temporary increase in panel reactive antibodies (PRA) level which equalizes before the time of retransplantation. Graft survival after a second kidney transplantation is not influenced by nephrectomy of the first graft. If nephrectomy is not performed, re-transplantation should be undertaken within one year after graft loss due to significantly better graft survival rates. Most importantly, patient survival one or five years after a second kidney transplantation is significantly better in patients undergoing nephrectomy of the first failed graft.

### Applications

The study results suggest that in patients with graft failure nephrectomy should be

considered due to a better patient survival after a second renal transplantation.

### Terminology

Kidney or renal transplantation is the process of transferring a kidney of a deceased or living donor to a patient with end-stage renal disease, including not only the surgical procedure but also the immunological management. Graft survival is the rate of kidney transplants that remains with good function after a certain time period. PRA are pre-existing antibodies against cell proteins, which present, if elevated, a risk factor for rejection after organ transplantation.

### Peer review

The article aims to determine the impact of transplant nephrectomy on peak panel reactive antibody levels and patient and graft survival in kidney re-transplants. It is conducted as a retrospective study in a large patient cohort and with a long follow-up. The article is very interesting for anybody involved in the care of renal transplant patients since it offers new insights into the dilemma of management of patients with graft loss and the usefulness of transplant nephrectomy prior to re-transplantation.

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## Intra-abdominal desmoid tumor after liver transplantation: A case report

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short gut post radical resection of aggressive fibromatosis, only rare recurrences were seen. No connection of tumor development with immunosuppression or need to decrease immunosuppressant treatment has been demonstrated in these patients. Our case and the literature show the risk of this tumor presenting in the post-transplantation patient and the need for a high index of suspicion in patients who present with a complex mass after transplantation to prevent progression of the disease beyond a resectable lesion. Results of a thorough search of the literature are detailed and the medical and surgical management of both resectable and unresectable lesions is reviewed.

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**Key words:** Desmoid; Intra-abdominal fibromatosis; Immunosuppression; Liver transplantation; Solid organ transplantation; Recurrence

**Core tip:** Desmoid tumor is a soft tissue tumor seen primarily after surgical resection. A high index of suspicion is necessary as delayed diagnosis can cause significant morbidity with resection. This case presents the first observed desmoid after liver transplantation as well as a literature search detailing the observed desmoid presentations in the context of immunosuppression.

### Abstract

We are reporting the first documented case of an abdominal desmoid tumor presenting primarily after liver transplantation. This tumor, well described in the literature as occurring both in conjunction with familial adenomatous polyposis as well as in the post-surgical patient, has never been noted after solid organ transplantation and was therefore not included in our differential upon presentation. Definitive diagnosis required the patient to undergo surgical excision and immunochemical staining of the mass for confirmation. A review of the literature showed no primary tumors after transplantation. In a population of patients who received a small bowel transplant after they developed

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### INTRODUCTION

We present the first documented case of desmoid tumor appearing after solid organ transplantation. Desmoid



Figure 1 Desmoid tumor at initial presentation.

tumors are a rare malignancy characterized by benign histology and aggressive local recurrence. Incidence and recurrence of desmoids in patients who have undergone transplantation and effects of immunosuppression on desmoid development have not yet been studied.

## CASE REPORT

A 60-year-old male presented to our clinic with a three day history of right upper quadrant pain. He noted two months of fatigue and a recent history of diarrhea, resolved at admission. He denied nausea, vomiting, fevers, chills, or weight loss. Medical history was notable Hepatitis C cirrhosis status post orthotopic liver transplant approximately six months prior, as well as type II diabetes mellitus. Postoperatively, he received basiliximab on the day of transplant and on postoperative day 4 for induction along with a methylprednisolone taper in the days immediately post transplantation. He was maintained on cyclosporine and had previously shown no signs of graft rejection with historically appropriate levels of immunosuppressive therapy. He denied alcohol or drug abuse.

Physical exam was remarkable for tenderness to palpation over the right upper quadrant with a new marked right upper quadrant mass. Laboratory measurements revealed a white blood cell count of  $4.32 \times 10^9/L$  (reference range  $1.5-10.5 \times 10^9/L$ ) with a normal differential, hemoglobin of 11.1 g/dL (reference range 13.5-17.5 g/dL), and creatinine of 1.29 mg/dL (reference range 0.75-1.2 mg/dL). Alkaline phosphatase was elevated at 281 mmol/L (reference range 30-125 mmol/L) and the transaminases and total bilirubin were within normal limits. Cyclosporine levels were within therapeutic ranges (100-150 ng/mL). He had received a colonoscopy at an outside institution two years prior significant only for benign polyps. Alfa-fetoprotein measurement was within normal limits ( $< 10$  ng/mL). Computed tomography imaging showed a 13 cm multi-loculated heterogeneous fluid collection with hyperdense and hypodense components inferior to right hepatic tip with multiple cystic locules (Figure 1). The final radiologic report was read as "large complex multiloculated subhepatic peritoneal collection(s), likely multiloculated hematoma(s) as well as

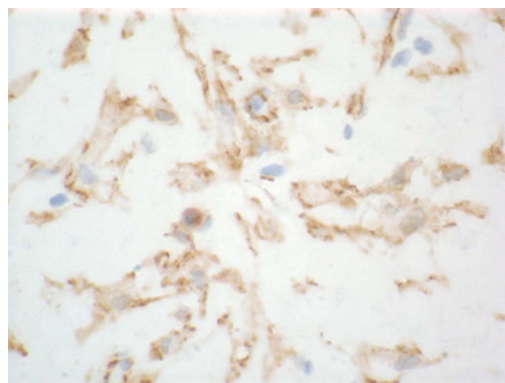


Figure 2 Immunostained slide of tumor histology, demonstrating low cellularity tumor in a myxoid matrix with positive beta-catenin staining.

adjacent loculated hemoperitoneum. The adjacent mesentery now demonstrates amorphous enhancement and therefore superimposed infection/phlegmon cannot be excluded. These collection(s) are essentially new since the prior study (previously mild hemoperitoneum was present in the subhepatic region and both paracolic gutters).

Based on the concern for infected hematoma, the patient was sent for placement of an interventional radiology drain into loculated fluid collection. Fluid pathology and cultures were nondiagnostic. The drain was placed under imaging guidance but was clearly unable to access loculated areas due to solid components interfering with catheter passage. As his symptoms were persistent and mass was well visualized with computed tomography, no further imaging appeared warranted; he was taken to the operating room, where exploratory laparotomy was performed. Intra-operatively the infrahepatic mass was noted to be white, inflamed, and fibrotic. Ten centimeters of small bowel and the mesentery were noted to be inseparably adhered to mass and inflamed, and they were resected en bloc with the mass. Grossly negative margins at abdominal wall and the involved small bowel were achieved but microscopic positivity was confirmed by frozen section in the posterior portion of the mass adjacent to the retroperitoneum. Final pathology returned with a low cellularity tumor with myofibroblasts in disarray in a myxoid matrix, beta-catenin stain positive (Figure 2), consistent with desmoid fibromatosis.

Our patient tolerated the procedure well and was discharged home at prior functional status within ten days. Repeat imaging has been negative for signs of recurrence (Figure 3) in the 23 mo in which he has followed up since resection. He has been started on treatment for chronic hepatitis C with sobosfuvir and simeprevir therapy.

## DISCUSSION

Desmoid tumors are rare tumors which fall into two types: sporadic and those associated with familial adenomatous polyposis. The incidence of desmoid is less than three percent of soft tissue sarcomas and about 0.03% of all malignancies<sup>[1]</sup>. They appear between 15 and 60 years



**Figure 3** Imaging 18 mo after excision.

with a peak age of appearance at 30 years<sup>[2]</sup>. Both types are characterized by monoclonal, fibroblastic proliferation with 80% rate of positive nuclear and cytoplasmic staining for beta-catenin<sup>[3]</sup>. They are histologically benign and do not metastasize but are frequently locally invasive and highly recurrent. Only 5% of sporadic desmoid tumors, in contrast with 80% of FAP-associated desmoids, occur intra-abdominally; other locations include extremities and trunk<sup>[1]</sup>. Both sporadic and FAP-associated desmoids have been well described in the surgical literature as recurring both within the surgical field and intra-compartmentally outside the surgical field. Differential diagnosis encompasses both fibroblastic sarcomas and other reactive fibroblastic process, but these can be distinguished from desmoids as the latter tend to occur with a diagnosis of FAP, nuclear staining for beta-catenin - present in roughly 80% of cases - and screening for mutations of beta-catenin gene, found in approximately 85% of sporadic cases<sup>[3]</sup>. Factors which prognosticate recurrence have been suggested to be sex<sup>[2]</sup> and mutations of the beta-catenin gene, the latter of which were found to be associated with significantly higher rates of local recurrence<sup>[4]</sup>.

Treatment for these tumors includes a wide variety of approaches. As these tumors have no metastatic potential, treatment is usually dictated by rapidity of growth and functional considerations such as pain or local obstruction. Surgical management has historically been the first-line treatment of desmoids<sup>[5]</sup>. Negative margins are generally the goal of surgical intervention; however, for intra-abdominal type especially, morbidity associated with surgery may prevent definitive excision with negative margins. Results have been mixed on whether negative margins were predictive of lower recurrence rate<sup>[6]</sup>. In a case series including 56 patients with either intra- or extra-abdominal primary disease, microscopically positive margins were associated with an almost fourfold increase in local recurrence compared to microscopically negative margins, but no difference in overall survival was observed<sup>[7]</sup>. A review of multiple studies addressing margin status concluded that no definitive conclusion could be reached based on available evidence and that negative

margins should be strove for if they did not compromise functional status<sup>[8]</sup>.

Adjunctive radiotherapy in patients with positive margins has been explored in depth and shown to result in decreased relapse rates but significant complications, including tissue fibrosis<sup>[9]</sup>. Radiotherapy has not been strongly evaluated in patients with negative surgical margins, as many patients with negative margins elect not to undergo radiation therapy. In a comparative review of 22 cases, radiation therapy alone demonstrated a local control rate of 78% as opposed to 61% with surgery alone<sup>[10]</sup>. However, multiple complications were noted and, given the accompanying tissue damage and peak occurrence of desmoids in young patients, it is generally recommended to use radiation therapy only as an adjunct to surgery or for unresectable disease<sup>[11]</sup>.

For unresectable disease, a wide variety of medical treatments have been used, although few have been systematically evaluated. A systematic review of the literature addressed the different strategies noted below<sup>[12]</sup>. As most desmoid tumors express nuclear estrogen receptor-B, tamoxifen and other anti-estrogens have been used with some anecdotal reports of response; however, this has not been evaluated in a larger series. Non-steroidal anti-inflammatory drugs have also been tested and have demonstrated activity against tumors with partial or complete response. In cases of rapidly growing or symptomatic tumor, cytotoxic agents such as methotrexate and vinblastine have been studied; however, these were evaluated largely in the pediatric population and are associated with high, although tolerable, levels of toxicity<sup>[6]</sup>. Imatinib is another agent which is currently under study and has shown promise in multiple low-powered studies<sup>[13,14]</sup> but has not yet been licensed for this indication<sup>[6]</sup>.

No incidences of primary desmoid tumor development have yet been documented in patients who have undergone transplantation of liver or other solid organs. Recurrence of pre-transplant desmoids in patients known to be on immunosuppression, in this case for intestinal transplant, is only addressed in one series. Fourteen patients with desmoid tumors underwent intestinal transplantation, of which three recurred; time interval to recurrence was 15, 17 and 69 mo. Of these patients, eleven were maintained on immunosuppression<sup>[15]</sup>. In a European study of both intra- and extra-abdominal fibromatosis, recurrence was seen at between 0 and 204 mo in 37 patients with a mean time of 14 to 17 mo<sup>[7]</sup>. Although these studies show similar time to recurrence, the power of the study addressing the immunosuppressed patient is so low that it is difficult to draw conclusions on the impact of immunosuppressive therapy on recurrence.

Our patient presented with a sporadic primary tumor. We were able to achieve a grossly negative resection but pathology revealed microscopic disease at the margins; he received no adjunctive therapy but has not recurred at 23 mo, suggesting that his immunosuppression has not caused rapid growth or recurrence.



In conclusion, desmoid tumors are a rare disease for which the primary standard of care differs between surgical excision or watchful waiting, depending on extent of involvement of surrounding structures and postoperative morbidity. We presented a hitherto undocumented case of sporadic desmoid tumor after liver transplantation. The patient has no personal or family history of familial adenomatous polyposis. The primary manifestation was treated with surgical excision. No incidences of primary desmoid tumor development have yet been documented in patients who have undergone transplantation of liver or other solid organs. Influence of immunosuppression on the development of desmoids is unknown; on recurrence, poorly studied. Further study would be helpful to elaborate the effect of immunosuppression on development of desmoids and the rates of recurrence after solid organ transplant.

## COMMENTS

### Case characteristics

A 60-year-old male with a history of liver transplantation presented with right upper quadrant pain.

### Clinical diagnosis

Clinical findings were a palpable tender mass in right upper quadrant.

### Differential diagnosis

Differential diagnosis includes abscess, malignancy, and recurrent hepatitis.

### Laboratory diagnosis

Alkaline phosphatase 281 mmol/L, hemoglobin 11.1 g/dL, and creatinine of 1.29 mg/dL with otherwise normal complete metabolic panel and blood counts.

### Imaging diagnosis

CT showed multiloculated, heterogeneous fluid collection with varying densities and cystic components inferior to and distinct from right hepatic tip; scattered periaortic lymph nodes were seen.

### Pathological diagnosis

Pathology showed a low cellularity tumor with myofibroblasts in disarray in a myxoid matrix with positive beta-catenin staining.

### Treatment

The patient was treated with surgical resection; no adjunctive radiotherapy or chemotherapy was used.

### Related reports

This is the first case of desmoid tumor occurring primarily after organ transplantation; one previous case series addressing viability of small bowel transplantation after resection of desmoid tumor addressed recurrence of fibromatosis on immunosuppression but was insufficiently powered to reach a conclusion.

### Term explanation

Nuclear beta catenin is a protein which regulates cell-cell adhesion and gene transcription encoded by the *CTNNB1* gene; positive staining has been noted with desmoid tumors as well as with colorectal cancer, hepatocellular carcinoma, and lung cancer.

### Experiences and lessons

This case reports the first noted desmoid tumor occurring after solid organ transplantation and presents methods of treatment for both resectable and unresectable lesions as well as describing the literature available on desmoid tumors in immunosuppressed patients.

### Peer review

The manuscript entitled, "Intra-abdominal desmoid tumor after liver transplantation: a case report" by Fleetwood *et al.*, reported a case of intra-abdominal desmoid tumor developing in a post liver transplant recipient. This is the first case presentation of intraabdominal desmoid tumor after liver transplantation, and worth for publication.

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### Acknowledgments

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

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

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

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

*In press*

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

*Organization as author*

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

*Both personal authors and an organization as author*

- 5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European

men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

*No author given*

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

*Volume with supplement*

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

*Issue with no volume*

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

*No volume or issue*

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

### Books

*Personal author(s)*

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

*Chapter in a book (list all authors)*

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

*Author(s) and editor(s)*

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

*Conference proceedings*

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

*Conference paper*

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

**Electronic journal** (list all authors)

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

**Patent** (list all authors)

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

### Statistical data

Write as mean  $\pm$  SD or mean  $\pm$  SE.

### Statistical expression

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

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Use SI units. For example: body mass, *m* (B) = 78 kg; blood pressure, *p* (B) = 16.2/12.3 kPa; incubation time, *t* (incubation) = 96 h; blood glucose concentration, *c* (glucose) 6.4  $\pm$  2.1 mmol/L; blood

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