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REVIEW

Importance of physical capacity and the effects of exercise in heart transplant recipients

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Abstract

One of the most important prognostic factors in heart failure patients is physical capacity. Patients with very poor physical performance and otherwise eligible, may be listed as candidates for heart transplantation (HTx). After such surgery, life-long immunosuppression therapy is needed to prevent rejection of the new heart. The dark side of immunosuppression is the increased risk of infections, kidney failure, cancer and advanced atherosclerosis (cardiac allograft vasculopathy), with the two latter conditions as the main causes of later mortality. In a worldwide perspective, 50% of the HTx patients survive past 10 years. Poor aerobic capacity prior to graft deterioration is not only limited to the failing heart, but also caused by peripheral factors, such as limited function in the skeletal muscles and in the blood vessels walls. Exercise rehabilitation after HTx is of major importance in order to improve physical capacity and prognosis. Effects of high-intensity interval training (HIT) in HTx recipients is a growing field of research attracting worldwide focus and interest. Accumulating evidence has shown that HIT is safe and efficient in maintenance HTx recipients; with superior effects on physical capacity compared to conventional moderate exercise. This article generates further evidence to the field by summarizing results from a decade of research performed at our center supported by a broad, but not strict formal, literature review. In short, this article demonstrates a strong association between physical capacity measured after HTx and long-term survival. It describes the possible "HIT-effect" with increased levels of inflammatory mediators of angiogenesis. It also describes long-term effects of HIT; showing a positive effect in development of anxiety symptoms despite that the improved physical capacity was not sustained, due to downregulation of

exercise and intensity. Finally, our results are linked to the ongoing HITTS study, which investigates safety and efficiency of HIT in *de novo* HTx recipients. Together with previous results, this study may have the potential to change existing guidelines and contribute to a better prognosis for the HTx population as a whole.

Key words: High-intensity interval training; Peak oxygen uptake; Heart transplantation; Survival; Prognosis; Angiogenesis; Inflammation; Physical capacity; Exercise

Core tip: Despite the positive effects of regular exercise after heart transplantation (HTx), HTx recipients' physical capacity remains subnormal, and a strong association between physical capacity and survival has been demonstrated. Thus, the positive effects of high-intensity interval training (HIT) are a growing field of research, attracting worldwide focus and interest. Although the "HIT-effect" is not fully understood, a possible contributing factor is the increased levels of inflammatory mediators of angiogenesis generated during exercise. More high-quality research is strongly warranted, but ongoing studies already have the potential to change existing guidelines and contribute to a better prognosis for the HTx population.

Yardley M, Gullestad L, Nytrøen K. Importance of physical capacity and the effects of exercise in heart transplant recipients. *World J Transplant* 2018; 8(1): 1-12 Available from: URL: http://www.wjgnet.com/2220-3230/full/v8/i1/1.htm DOI: http://dx.doi.org/10.5500/wjt.v8.i1.1

INTRODUCTION

Heart transplantation

For patients with heart failure (HF) the 5-year mortality rates are 62% for women and 75% for men[1], with even higher rates in patients with end-stage HF[2]. Although these are old references, recent findings conclude that survival in HF patients has hardly changed since the 90's[3]. Heart transplantation (HTx) is an established treatment to improve survival in selected patients with end-stage HF. From 1983 and to date, more than 920 HTx have been performed at Oslo University Hospital in Norway.

After HTx, the patients require lifelong immunosuppression to prevent rejection of the graft. These drugs have a potential to give adverse complications such as diabetes, gout, hypertension and osteoporosis, and serious side effects, such as higher risk of infections, renal failure and cancer. These side effects are the leading causes of death in the long-term, together with an advanced HTx-specific process of atherosclerosis, called coronary allograft vasculopathy (CAV)[4].

According to the 2012 ISHLT registry, the median survival for all HTx patients is 10 years, but if surviving the first year, the survival rates are higher and show a 63% survival past 10 years[4]. Increased knowledge about CAV and immunosuppression has resulted in further improved survival. However, the HTx recipients still have a shorter estimated length of survival than the general population.

Physical capacity after HTx

The dynamics of physical capacity after HTx is illustrated in Figures 1 and 2. Physical capacity increases significantly after HTx as a result of therapy, as shown by peak oxygen uptake (VO_{2peak}) levels above 12 mL/kg per minute in published studies (Figures 1 and 2). Osada *et al*[5] and these two figures show that the highest rate of increase is found within the first years. In nearly 70% of the studies, regardless of time after HTx, VO_{2peak} is below 20 mL/kg per minute, also classified as Weber function class B-C[6]. Patients within function class B and C are shown to be similar to coronary artery disease (CAD) and HF patients referred to rehabilitation programs[7]. VO_{2peak} is often used as the primary outcome measure in exercise intervention studies after HTx[8].

Physical capacity as a prognostic variable

The gold standard measurement of physical capacity is VO_{2peak}, and is defined as "the maximum ability of the cardiovascular system to deliver oxygen to exercising muscles and of the exercising muscle to extract oxygen from the blood"[9]. VO_{2peak} is shown to be a strong predictor of survival in general populations[10,11], among patients with CAD[12], and in patients with severe HF[13]. Limited exercise capacity is the cardinal symptom in HF. The HF patients with VO_{2peak} < 12 mL/kg per minute are considered to have the worst prognosis, despite optimal medical therapy, and can be appropriate candidates listed for HTx[14]. These patients are most likely men > 50 years of age[4]. When evaluating younger patients and women, it is found reasonable to include age and gender adjusted levels of exercise capacity, and values ⩽ 50% percent of predicted VO_{2peak} differentiate better in these populations[14].

However, studies addressing the relation between VO_{2peak} and survival after HTx are currently lacking, although a number of other predictors have been identified through register-data analyses. These predictors are: Non-ischemic cardiomyopathy as the primary diagnosis, younger recipient age, younger donor-graft age and shorter allograft ischemic time; all associated with a better long-term prognosis[4,15,16]. The mortality beyond one-year after HTx has remained relatively constant, and Stehlik *et al*[4] predict that interventions resulting in a reduction of mortal events in the long-term are needed to achieve further improvements in survival after HTx.

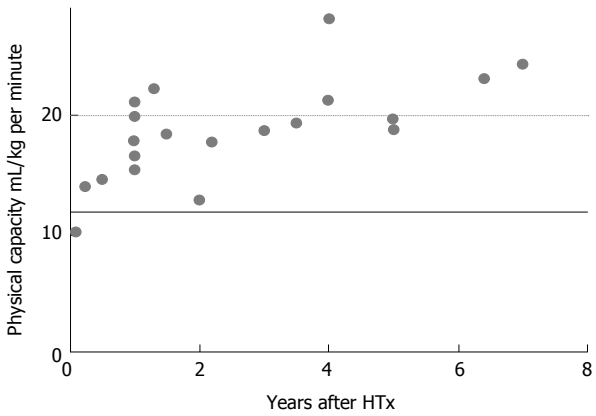

Figure 1 Physical capacity, as assessed by VO_{2peak} after heart transplantation from published studies, illustrated by years after surgery. Black line at 12 mL/kg per minute, show the threshold to be candidates for HTx, dotted line at 20 mL/min per kilogram, show the start of Weber function class A, representing good physical condition. The measurements are carried out from exercise tests from; Bernandi *et al*[85], Carter *et al*[86], Dall *et al*[35], Ewert *et al*[87], Givertz *et al*[88], Gullestad *et al*[89], Habedank *et al*[90], Haykowski *et al*[91], Hermann *et al*[36], Hognestad *et al*[92], Karpolat *et al*[93], Kavanagh *et al*[94], Kemp *et al*[95], Kobashigawa *et al*[96], Nytrøen *et al*[37], Osada *et al*[5], Renlund *et al*[97], Schwaiblmair *et al*[98], Squires *et al*[99], Tegtbur *et al*[100], Wu *et al*[77]. HTx: Heart transplantation.

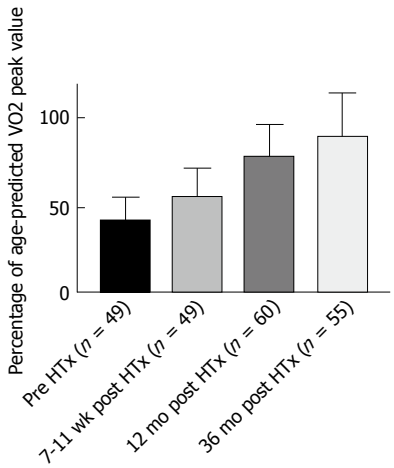

Figure 2 Illustration of the increase in peak VO_2 from pre heart transplantation to 36 mo post heart transplantation. The data presented in the figure is from the unpublished Schedule trial.

In a recent retrospective study from our center, investigating survival in two different HTx populations (n = 178, n = 133), we found that VO_{2peak} and SF-36 physical function (PF) sum-score were strong predictors for survival in each population, respectively[17]. In the "VO_{2peak} cohort" (n = 178), the mean age was 52 years, mean age after HTx was 2.5 years, mean VO_{2peak} was 19.6 mL/kg per minute, 88% were men and mean observation time was 11 years. The most important predictors (analyzed by multiple Cox regression) for survival in this population were VO_{2peak} (HR = 0.917, P < 0.001) age at time of test (HR = 1.045, P < 0.001) and CAV development (HR = 1.968, P = 0.001), and the group above the median VO_{2peak} had an increased survival of four years. Similar results were found in the "SF-36 cohort" where the mean age was 54 years, mean time after HTx was 4.5 years, mean SF-36 physical function (PF) score was 90 and mean observation time was 10 years. The most important predictors (analyzed by multiple Cox regression) for survival in this population were the PF score (HR = 0.983, P < 0.001), age (HR = 1.077, P <0.001), smoking history (HR = 1.077, P = 0.016) and CAV development (HR = 1.674, P = 0.039), and the group above the median PF score value had an increased survival of four years.

Other well-known predictors of HTx survival such as diagnosis prior to HTx, ischemic time, donor age, measurements of cardiac output and kidney function by creatinine did not add any additional explanation to the regression models.

The relationship between physical health and long-term survival

Earlier studies addressing survival, have estimated how physical performance *pre* HTx is related to survival after HTx. Physical capacity (measured by VO_{2peak}) in this population is well known to predict survival and supports the clinicians in the selection of HTx candidates[14]. Our study documented that also VO_{2peak} measured after HTx is a strong predictor for long-term survival[17], and this result is in line with the only study we found that demonstrated a relationship between physical performance (measured by VE/VCO_2 slope) and survival in a small sample of HTx patients (n = 49)[18]. Other related studies on this topic describe how VO_{2peak} is related to soft end-points; how a beneficial VO_{2peak} correlates with NYHA class 1-2 after HTx[19] and how the pre-transplant VO_{2peak}, together with age, predict the gain in physical capacity post HTx[5]. Succeeding our study on survival, Rosenbaum *et al*[20] published new knowledge in this field, with a study investigating the effect of early rehabilitation on survival: They concluded that early cardiac rehabilitation participation after HTx could predict survival time.

The measurement of physical capacity requires CPET equipment and test personnel, and thus, is quite costly. Although VO_{2peak} is the gold standard to examine exercise performance, there are other physical tests with limited costs that can be useful in the follow-up, found to correlate with CPET results. Such physical tests are the 6-min walk test and the shuttle walking test[21], but if these test are associated with prognosis remains to be determined. If resources are limited, we also found that the self-reported physical health (PF-score) showed a similar effect on long-term survival in the HTx population[17]. Research in general populations underscore the importance of physical activity and report a dose-response effect on survival rates[22,23], as well as a strong dose-response relation on self-reported health[24]. As shown in another of our studies[25], physical performance measured as VO_{2peak} is highly correlated with SF-36 PF sum-scores, and both were

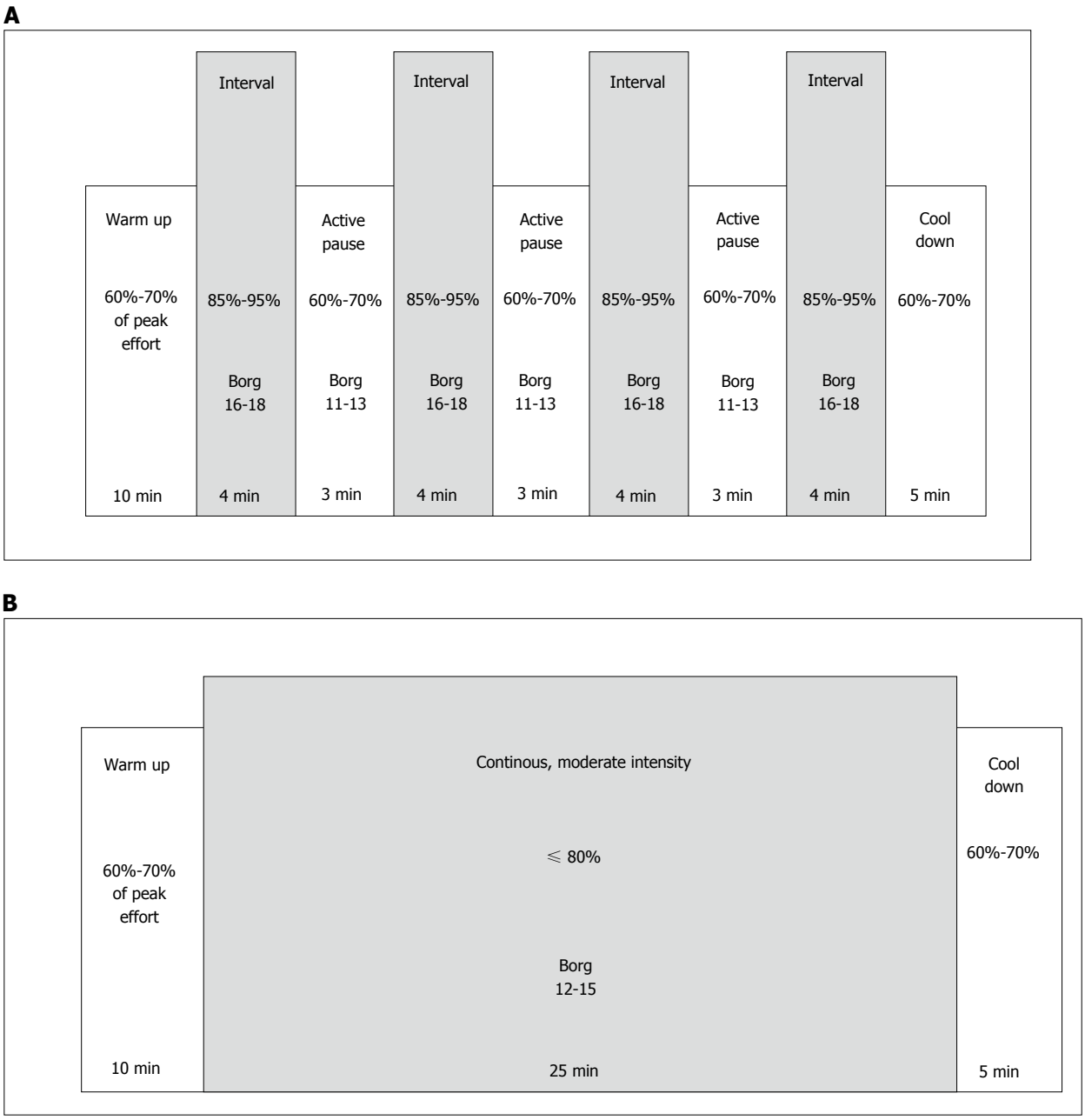

Figure 3 Illustration of two different exercise modalities. A: Illustration of a session with high-intensity interval training (HIT). HIT is an exercise strategy with alternating short periods of intense endurance exercise with less-intense recovery periods. A usual HIT session may include 4 × 4 min periods with high intensity (85%-95% of maximal capacity), with active recovery periods of 3min between each interval (with 60%-70% of maximal capacity); B: Illustration of a session with moderate intensity continuous training (MICT). MICT is an exercise strategy with moderate intensity (60%-70% of maximal capacity) of endurance exercise in periods for usually 25-30 min, with no recovery periods.

found to be highly associated with prognosis in our survival analysis[17]. Accordingly, we suggest that such measures should be more frequently used after HTx to identify patients at higher risk for complications.

Exercise after HTx - the past and the future

To increase physical capacity and prevent long-term complications such as hypertension and diabetes, aerobic exercise after HTx has a positive effect, but HTx recipients' physical capacity still remains subnormal in most studies[26]. High-intensity interval training (HIT) is proven to be a more efficient exercise modality than moderate-intensity continuous training (MICT) in order to increase VO_{2peak}, shown in patients with HF[27], CAD[28], metabolic disease[29], as well as in healthy individuals[30]. The new knowledge has had a great impact on how general cardiac rehabilitation programs are organized today. These two different exercise modalities are illustrated in Figure 3. HIT corresponds to an intensity of 16-18 on Borg's rated perceived exertion (RPE) 6-20 scale[31,32], and MICT to Borg 12-15.

Rehabilitation after HTx has traditionally had, and still has, a more conservative approach, with MICT as traditionally recommended, mainly due to uncertainty

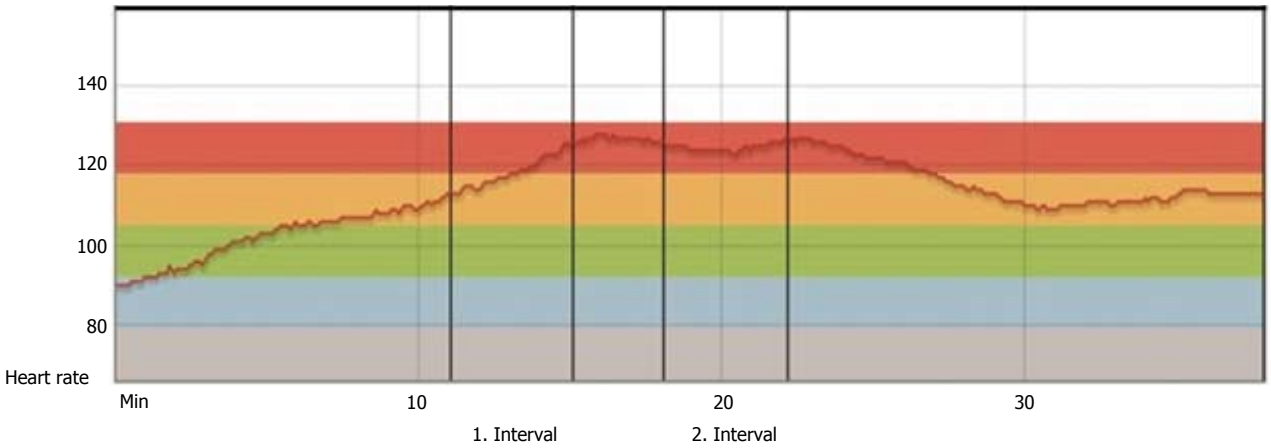

Figure 4 Heart rate during exercise 3 mo post heart transplantation. Patient from our hospital, 3 mo post-HTx: HR curve during warm-up, two high-intensity intervals divided by one recovery period and cool-down. The curve shows a typical pattern of impaired HR responses in the early stage after HTx. HTx: Heart transplantation.

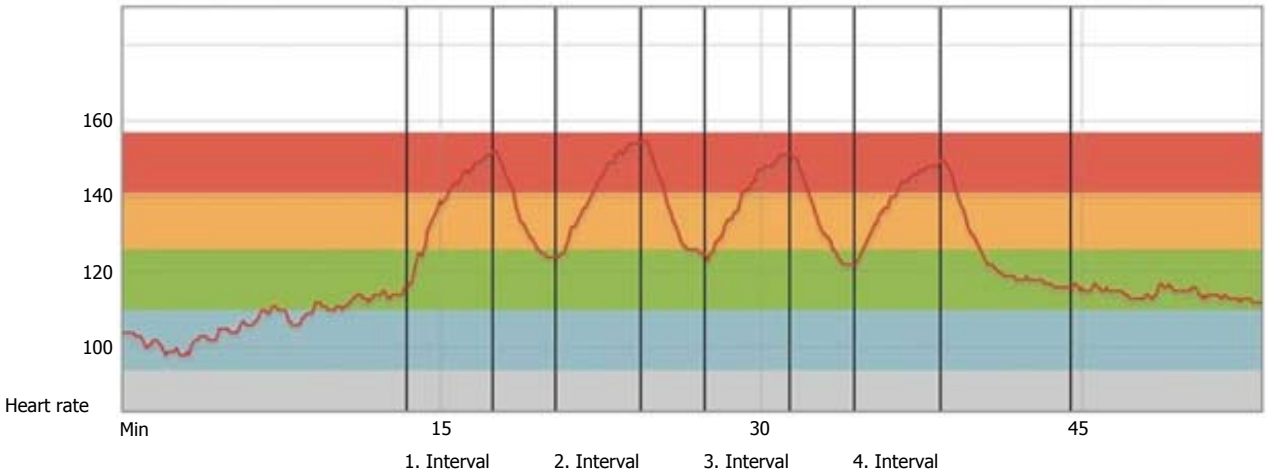

Figure 5 Heart rate during exercise 12 mo post heart transplantation. The same patient 12 mo post-HTx: Heart rate curve during warm-up four high-intensity intervals divided by 3 recovery periods and cool-down. The curve shows a largely normalized HR, with immediate HR adaptions to exercise intensity. HTx: Heart transplantation.

and concerns regarding denervation with consequently chronotropic incompetence and parasympathetic impairment[33]. The heart rate (HR) will typically be higher at rest, with a slower increase during exercise, a lower maximum HR at peak exercise, and a slower HR decrease after exercise cessation (Figure 4).

The chronotropic incompetence is most prominent the first months after HTx and tends to be largely normalized in the majority of patients after 12 mo[34], as illustrated in Figure 5. Recent randomized controlled trials (RCTs), have investigated the effect of HIT in maintenance HTx recipients and have to a large extend overruled the traditional, conservative approach with MICT[35-37]. These studies showed that HIT increased VO_{2peak} significantly compared to the control groups, and that a HIT intervention was safe and well tolerated. References to some of these results are mentioned in the most recent recommendations for cardiac rehabilitation from 2013[38]. The mechanisms of effect are probably multifactorial and might involve improved chronotropic response (CRI)[35,37], endothelial function[36] and less development of CAV after a long-term exercise intervention[39].

So far there are no studies on the effect of HIT in the novo patients, but a similar HIT intervention study is currently ongoing in Scandinavia[40]. One of the goals in this study is to update, optimize and implement new exercise prescriptions also in this group.

Meta-analyses in HF populations[41,42] find a possible long-term effect of exercise-based rehabilitation (MICT protocols) on survival and health related quality of life (HRQoL), and most importantly; a significantly decrease in re-hospitalization. Knowledge about exercise-based rehabilitation and the effect on mortality and hospital admissions in HTx recipients are currently missing, as recently stated in a 2017 Cochrane review on the effectiveness and safety of exercise-based rehabilitation in HTx recipients[43]. The lack of research regarding possible long-term benefits of exercise was also pointed out in the published meeting report from

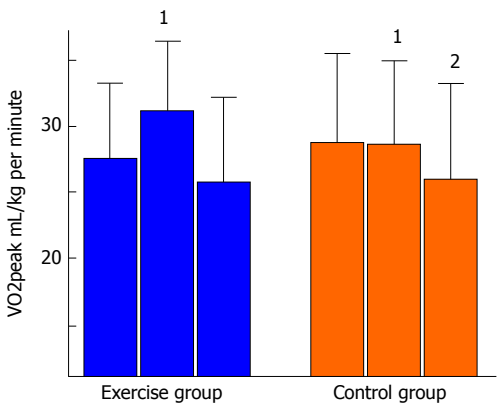

Figure 6 Measurements of VO_{2peak} at baseline, 1-year and 5-year follow-up. [1]Significant changes between groups; [2]Significant changes from baseline to 5-year follow-up within group.

2014: "Consensus recommendations for a research agenda in exercise in solid organ transplantation"[44].

HIT intervention and long-term effects

Regarding long-term effects of exercise in HTx recipients, we have conducted a 5-year follow-up study of a previous RCT investigating the effects of a HIT intervention[25]. Forty-eight maintenance HTx patients, mean four years after HTx were randomized to HIT intervention or control with 12 mo duration[37]. The study demonstrated a significant improved VO_{2peak} (mean difference between groups: 3.6 mL/kg per minute), increased muscular capacity and less development of CAV compared to the control group[37,39]. However, at the 5-year follow-up (n = 41), the HIT group had not sustained the exercise intensity over time, and although the decline in VO_{2peak} from baseline to 5-year follow-up was numerically lower in the HIT-group, there were no significant differences between the groups for the parameters described above (Figure 6). These findings were explained by the similar amount of daily (moderate) activity in both the HIT and the control group, measured at the 5-year follow-up. Our results differ from a study by Moholdt *et al*[45] who investigated long-term effects of a HIT intervention after myocardial infarction (MI). These MI-patients still had a significantly higher aerobic performance at the 30 mo follow-up compared to the control group, explained by more frequent exercise in the HIT group. Although the initial 1-year gain in physical capacity in the HIT group was not sustained and the mean difference between groups at the 5-year follow-up was non-significant, only the control group had a significant decrease within group from baseline to the 5-year follow-up. This significant decrease, corresponding to a 9% decline in mL/kg per minute, could mostly be explained by an expected age-related decrease in VO_{2peak}. Healthy young adults show a decline of 3%-6% each decade, and this decline is shown to accelerate with age; a decline of 15% is found normal and corresponds to the age group of the TEX population[46]. This age related VO_2-decline is related to decreasing maximal stroke volume, decreasing blood flow to skeletal muscles and mitochondrial dysfunction[47]. As for the HIT group, the decrease from baseline to the 5-year follow-up in VO_{2peak} was less pronounced (-6%), and could possibly indicate a hidden long-term effect of the intervention. In contrast, the development of anxiety symptoms was significantly different between the groups; the exercise group showed decreased symptoms of anxiety, whereas the control group had an increased anxiety symptom score. This beneficial trend in anxiety development together with no negative trends in other secondary end points, support the statements of HIT as a safe exercise modality in HF patients[48], and in maintenance HTx patients[35-37].

Nevertheless, more research is still needed regarding long-term effects of exercise, and to optimize the rehabilitation regimes and improve the HTx recipients' future prognosis[43,44].

HIT intervention in de novo HTx recipients

While HIT already is an established exercise modality in patients with HF[27] and CAD[28], and more recently in maintenance HTx[35-37], the upcoming results from the HITTS study[40] will contribute to fill the gap of knowledge related to the effect of HIT among *de novo* HTx recipients. In addition to exercise capacity measurements, other important secondary outcomes are: development of CAV, improvements in chronotropic response and changes in cardiac and endothelial function. The results from the HITTS study will make a strong contribution to improve and increase the knowledge-base about how early HTx-rehabilitation should be organized in order to gain the most optimal results. The study is followed closely by our dedicated HTx-staffs in Scandinavia, and one of our main goals is to document knowledge about safety and effects of HIT, and thereby initiate an update of the current guidelines. If HIT is found to be safe (and with potentially beneficial effects) also among *de novo* HTx patients, the patients will have the possibility to participate in established cardiac rehabilitation programs, which usually combines both MICT and HIT exercise. These rehabilitation programs are usually group based, rather than only consistent individual physiotherapy, thus demanding less government resources.

Mechanisms behind the "HIT-effect"

As described previously, the effects of HIT interventions are so far mostly studied in healthy individuals, CAD and HF patients. The main mechanisms behind the increase in exercise capacity are shown to be through central factors, induced by a prominent improvement in cardiac output (CO)[27,49]. However, the "HIT-effect" in maintenance HTx recipients show different results, seemingly with peripheral factors as the main mechanisms; by improvement in skeletal muscle exercise capacity[37], endothelial function and

Table 1 A simplified illustration of the ANOVA results: The response in markers of inflammation and angiogenesis during high-intensity interval training and moderate intensity continuous training sessions

	MICT	HIT
General inflammation		
CRP	→	→
sTNFr-1	↑	↑
Vascular inflammation		
vWFd	↓	↑
VCAM	→	→
Blood platelets		
PDGF	↑	↑
sCD40L	↑	↑
DKK-1	↑	↑
Angiogenesis		
VEGF-1	↑	↑↑
Ang2	↑	↑↑
Tie-2	→	→
Endostatin	→	→
Cardiokine/myokine		
GDF-15	↑	↓[1]
ST2	→	→
SPARC	↑	↑

[1]The decrease is found in the recovery period (0-2 h) after the exercise-session. Horizontal arrows illustrate non-significant response during exercise. Arrows pointing up illustrate a significant increase with exercise, regardless of intensity, and two arrows illustrate a significant increase by increasing intensity (HIT). An arrow pointing down, illustrates a significant decrease in response during exercise. HIT: High-intensity interval training; MICT: Moderate intensity continuous training.

vasodilatation[36], rather than an increased CO[50]. The underlying triggers behind these peripheral effects are poorly understood, and the potential of inflammatory signaling pathways are not explored in detail. Markers of inflammation have been studied as an additional effect of exercise through long-term steady state levels (before and after exercise intervention), showing mostly neutral results[36,39,51].

We hypothesized that investigation of immediate exercise effects in inflammatory signaling pathways during HIT could contribute to further explain the "HIT-effect" in the HTx recipients, and recently we performed such an exploratory study[52]. Fourteen patients were included in the randomized cross-over study, comparing HIT to MICT. Blood samples were drawn before, during and after exercise. The main results from the enzyme immunoassays analyses were that exercise, regardless of intensity, induced a significant immediate response in several vascular, angiogenetic and particularly in platelet derived inflammatory mediators in HTx recipients shown in Table 1. HIT showed trends to induce an increased response in von willebrand factor (vWF), vascular endothelial growth factor 1 (VEGF-1) and Angiopoetin-2 (Ang-2), and a decreased response in growth derived factor 15 (GDF-15), compared to MICT (Table 1).

HIT and the immediate responses in markers of inflammation and angiogenesis

Exercise training, regardless of intensity, led to an increase in multiple systemic, angiogenetic and platelet derived inflammatory mediators[52]. These results are in line with published research showing the pro-coagulation state during exercise, with blood platelet activation potentially reflecting the increase in catecholamines and shear stress[53], promotion of NO production from activated endothelial cells[54,55], and regulation of the growth and repair of blood vessels[56]. The activation of the endothelium and thereby induction of capillary growth in skeletal muscle through pro-angiogenetic mechanisms may play an important role in the beneficial effects of HIT. When we compared the response in inflammatory mediators during the HIT and MICT sessions, we observed a higher response in both Ang-2 and VEGF-1 with increased intensity. Kilian *et al*[57] have previously shown an increase in mRNA for VEGF in whole blood during HIT in healthy children. VEGF is dominantly secreted by working skeletal muscles, an essential factor to increase capillary density, oxygen delivery and thereby exercise performance[58-60]. Based on our previous results showing improved muscular exercise capacity after HIT[37], and now the finding of an increased VEGF response, we suggest that this mechanism is of high importance also in the HTx recipients. The fact that HIT markedly increased mediators of angiogenesis and neovascularization, may contribute to explain the different trigger mechanisms behind the two different exercise modalities.

CAV

CAV is characterized by intimal thickening and a more diffuse narrowing of the coronary arteries' lumen than conventional atherosclerosis[61]. The mechanisms of development are described as both immunological and non-immunological, possibly modifiable factors[62]. It can be detected by coronary angiography, but intravascular ultrasound (IVUS) is now more frequently used, and is a superior diagnostic tool to detect early changes in intimal thickening (early CAV)[63]. The early CAV has been validated as a reliable surrogate marker for subsequent mortality, nonfatal major adverse cardiac events, and development of angiographic CAV following HTx[64,65]. CAV progression is a highly prioritized field of research among HTx clinicians and researchers, to further improve HTx prognosis. As a result, Kobashigawa *et al*[66] introduced statin therapy that showed to have beneficial effects on one-year survival and the incidence of CAV. Statins became routine therapy after HTx at our center from 1997. More recently, a Scandinavian multicenter RCT (The Schedule-study) has shown that early everolimus initiation with calcineurin inhibitor withdrawal reduces the progression of CAV in *de-novo* HTx recipients[67,68].

HIT and the effect on CAV

The effect of non-medical prevention strategies, such as HIT interventions, has also been studied by IVUS and have shown less progression of atherosclerosis both in mice[69] and in patients after MI[70]. We found

the same trend in maintenance HTx recipients after a HIT intervention[39], but the positive effects were not sustained in the long-term as shown in the 5-year follow-up study[25]. Furthermore, exercise is shown to have a positive influence on the endothelium through increased nitric oxide production, and by reduction of inflammation[71,72]. This effect could possibly be enhanced through higher shear stress triggered with higher exercise intensity. A gain in endothelial function following a HIT intervention is found in CAD patients[73]. However, a relatively small sample size in the 1- and 5-year follow-up studies[25,39] limits our conclusion in the HTx population, and the effect of HIT on CAV should be examined in a larger sample and include a second intervention arm with MICT. It has been explored how early medical therapy can influence CAV progression in the long-term, and studies with everolimus are found to have positive impact on CAV severity in *de novo* HTx patients, whereas no effect is seen if everolimus is introduced later on[74]. The effect on CAV severity by an early initiation was also sustained in the long-term[67,68]. This illustrates an "opportunity window" during the first year after HTx. Knowing that the CAV development is most pronounced the first year after HTx, we anticipate that similar mechanisms may be seen with an early initiation of HIT. Results from the HITTS study[40] will contribute to a better understanding of the relationship between exercise and CAV development.

Health related quality of life

The HRQoL after HTx has been reported to increase significantly, with high levels of satisfaction in overall HRQoL; also stable over a 5-year period (measured from 5 to 10 years after HTx)[75]. Although, when HTx patients are compared with the general population, the HRQoL remains beneath normal values[76]. To improve HRQoL, and especially physical health, exercise interventions have shown to be successful and this is in contrast to the more neutral results reported in control groups[77,78]. Research on HRQoL after HTx regarding the effect of HIT (compared to MICT) is very limited, and the existing studies show mixed results; some studies show similar effects on HRQoL[51], while we and others have shown a beneficial effect with a significant increase after HIT[37,79].

Mental health, anxiety and depression

In the post-transplant stage the prevalence of significant depression and anxiety remains substantially above the general populations, and it tends to increase over time[80,81]. As it is found that depressed HTx recipients have a higher risk of mortality, screening for depressive symptoms during follow-up is recommended[81-83]. As an approach to increase mental health, the effect of exercise and HIT has been studied. The results showed that exercise decreases the burden of depression and anxiety, with HIT showing significant positive effects compared with usual care[79]. Additionally, the results align with the correlation between higher physical capacity and less depression and anxiety rates[25,83,84].

In our 5-year follow-up study after a HIT intervention[25], we measured physical and mental health as well as measures of physical capacity at each study visit, and at the 5-year follow-up there was significantly less development of anxiety symptoms in the HIT group compared to the control group. The long-term difference in anxiety between the HIT group and the control group is considered a valuable finding, as anxiety is a frequent health issue after HTx, especially in the long-term follow-up[80]. Overall, there was a positive correlation between the measured VO2 peak and the self-reported physical health (SF-36 PF sum-score). These findings might suggest that a 1-year "heavy" exercise intervention has a long-term value when it comes to self-confidence and trust regarding what your heart (and body) actually can tolerate of exertion, strain and physical work.

CONCLUDING REMARKS

Our findings, supported by a review of the existing literature, suggest that measures of physical health should be included frequently also after HTx, as they predict prognosis and survival in the long-term. A dose-response effect of physical capacity on survival was also found in the HTx population.

HIT is a feasible and efficient modality of exercise among maintenance HTx recipients, but the mechanisms behind this effect is poorly understood. Our results suggest that the beneficial effects seen in HTx recipients differ from CAD and HF patients, with more prominent peripheral effects from HIT exercise, rather than central adaptations with increased CO. We have showed that HIT significantly increased levels of inflammatory mediators of angiogenesis, suggesting that HIT can regulate and stimulate blood vessel formation in skeletal muscles and thus increase physical capacity.

Considering exercise prescription and future guidelines, our findings suggest that moderate levels of exercise and intensity are insufficient to maintain the improved VO_{2peak} achieved after a HIT intervention. Thus, intermittent periods of HIT are likely to be necessary. Also, the number and length of HIT intervals needed in a HIT session should be further investigated. If a modified HIT protocol with shorter and fewer intervals has comparable effect to a 4 × 4 protocol, it could probably increase the patients' motivation and adherence to exercise in the long-term. When considering other long-term effects, the benefit from a tough and intense HIT-intervention showed a positive effect on the development of anxiety symptoms. The exercise prescription in *de novo* HTx recipients is still conservative, consisting mainly of MICT exercise, but this traditional guideline might change when the ongoing HITTS study is completed. Existing gaps in knowledge are briefly mentioned in Table 2, and the results from the HITTS study will contribute to fill

Table 2 What is known and unknown in this field?

What is known in this field
A proper rehabilitation program including exercise training is recommended in all HTx patients
Good physical fitness is associated with improved outcome in HTx patients
The effect of HIT is superior to the effect of moderate training in general as well as for patients with coronary heart disease and heart failure
Accumulating evidence has shown that this is true also for HTx recipients 1-8 yr after HTx
Gaps in knowledge
There is no consensus on how, when and at which intensity exercise should be performed and organized after HTx
Because newly transplanted patients are totally denervated (without functional nerve supply resulting in impaired heart rate response), the effect of HIT has never been evaluated in this population, and the effect of HIT in *de-novo* HTx patients' needs to be investigated
The effect of HIT on late complications after HTx as CAV, diabetes mellitus, gout, renal function and graft survival needs to be explored
Data on whether a HIT intervention should be carried out decentralized or in cooperation with the primary health care services as well as the safety and cost-effectiveness are scarce
How to optimize ways to maintain exercise training during long-term follow up needs to be investigated

HIT: High-intensity interval training; HTx: Heart transplantation; CAV: Coronary allograft vasculopathy.

some of these gaps, and may also have the potential to update, optimize and possibly include HIT as a safe exercise modality in future guidelines.

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ORIGINAL ARTICLE

Clinical Trials Study

Renal function and physical fitness after 12-mo supervised training in kidney transplant recipients

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Abstract

AIM

To evaluate the effect of a 12-mo supervised aerobic and resistance training, on renal function and exercise capacity compared to usual care recommendations.

METHODS

Ninety-nine kidney transplant recipients (KTRs) were assigned to interventional exercise (Group A; n = 52) and a usual care cohort (Group B; n = 47). Blood and urine chemistry, exercise capacity, muscular strength, anthropometric measures and health-related quality of life (HRQoL) were assessed at baseline, and after 6 and 12 mo. Group A underwent a supervised training three times per week for 12 mo. Group B received only general recommendations about home-based physical activities.

RESULTS

Eighty-five KTRs completed the study (Group A, n = 44; Group B, n = 41). After 12 mo, renal function remained stable in both groups. Group A significantly increased maximum workload (+13 W, P = 0.0003), $V'O_2$ peak (+3.1 mL/kg per minute, P = 0.0099), muscular strength in plantar flexor (+12 kg, P = 0.0368), height in the countermovement jump (+1.9 cm, P = 0.0293) and decreased in Body Mass Index (-0.5 kg/m^2, P = 0.0013). HRQoL significantly improved in physical function (P = 0.0019), physical-role limitations (P = 0.0321) and social functioning scales (P = 0.0346). No improvements were found in Group B.

CONCLUSION

Twelve-month of supervised aerobic and resistance training improves the physiological variables related to physical fitness and cardiovascular risks without consequences on renal function. Recommendations alone are not sufficient to induce changes in exercise capacity of KTRs. Our study is an example of collaborative working between transplant centres, sports medicine and exercise facilities.

Key words: Kidney transplant recipients; Renal function; Supervised exercise; Aerobic exercise; Muscle strength

Core tip: This paper shows that developing a supervised exercise protocol for kidney transplant recipients is a useful and safe non-pharmacologic contribution to usual after-transplant treatments, which can improve the physiological variables related to physical fitness and cardiovascular risks without consequences on renal function. Our study is an example of collaborative working between transplant centres, sports medicine and exercise facilities, aimed to apply the concepts of "exercise is medicine".

Roi GS, Mosconi G, Totti V, Angelini ML, Brugin E, Sarto P, Merlo L, Sgarzi S, Stancari M, Todeschini P, La Manna G, Ermolao A, Tripi F, Andreoli L, Sella G, Anedda A, Stefani L, Galanti G, Di Michele R, Merni F, Trerotola M, Storani D, Nanni Costa A. Renal function and physical fitness after 12-mo supervised training in kidney transplant recipients. *World J Transplant* 2018; 8(1): 13-22 Available from: URL: http://www.wjgnet.com/2220-3230/full/v8/i1/13.htm DOI: http://dx.doi.org/10.5500/wjt.v8.i1.13

INTRODUCTION

Kidney transplantation is considered the gold standard of treatment for most patients with end-stage renal disease, nevertheless kidney transplant recipients (KTRs) are characterized by long-term clinical complications and high risk of cardio-vascular disease (CVD).

In addition to the traditional CVD risk factors (*e.g.*, hypertension, dyslipidaemia, diabetes mellitus) other non-traditional factors influence the high incidence of cardiovascular events (*e.g.*, duration of prior dialysis, graft function after transplantation, elevated inflammatory markers, proteinuria, toxic effects of immunosuppressant drugs, bone mineral metabolism abnormalities and vascular calcifications). However, among all these risk factors, the lack of physical exercise and a sedentary lifestyle seem to play crucial roles[1].

There is mounting evidence that physical exercise reduces the risk of all-cause mortality[2,3] and it is

effective in the primary and secondary prevention of CVD in the general population[4]. Physical activity is also considered a key element in the prevention and management of chronic diseases[5], including Chronic Kidney Disease (CKD).

After transplantation, patients are expected to be more active than before because their uremic syndrome is corrected and they do not have to do haemodialysis treatment[6]. However, their cardiorespiratory fitness remains reduced by 30% in comparison with matched control subjects[7]. Only in selected cases they can achieve results comparable to a healthy population[8], but not all patients increase their physical activity after transplantation; thus, the majority of KTRs maintain a sedentary lifestyle, often associated with an increase in body fat and weight gain[9].

Whether exercise can positively affect outcomes in KTRs has only been addressed in few studies[4], with a small number of subjects and with different types, intensity and durations of interventions lasting almost always not more than six months[10,11]. In some studies, exercise was carried out tightly at home without direct supervision and with a partial adherence to the intervention[6]. Furthermore, few studies have investigated the effect of a combined aerobic and resistance training[4], and the effect of these protocols on kidney function is rather unknown.

In this paper, we present some clinical and fitness outcomes of a 12-mo study conducted on KTRs, with the aim to evaluate the potential effects of supervised exercise combining aerobic and resistance training.

MATERIALS AND METHODS

Organisational model

We introduced a project, based on a model of cooperation among: (1) Transplantation specialists (surgeons and nephrologists), who selected patients suitable for physical activity; (2) sports physicians who prescribed a personalised exercise programme based on the results of functional assessment tests; (3) exercise specialists who supervised the patients performing the prescribed programme. This organisation aims to check the patients from clinical and functional perspectives and to identify facilities in their home districts where patients can easily perform their training programmes under supervision[12,13].

Study design

This is a multicentre, controlled, prospective, non-randomised study that considered the enrolment of KTRs patients with clinical and functional stabilities.

Inclusion criteria were the 18-60 years age range, and at least six months after organ transplantation; exclusion criteria were orthopaedic limitations, psychiatric or neurological disorders, proteinuria within nephrotic range, poor compliance to treatment and any cardiovascular contraindication to exercise testing and training.

Patients were divided into an interventional exercise group (Group A), in which personalised training was supervised, and a usual care group (Group B), in which some exercise indications were given without a specific prescription and supervision. All subjects received individualised counselling by the transplant centre regarding the protocol, and the inclusion in Group B was based on logistic and organisational grounds (patients living in regions not taking part in the project or living in areas without sports medical centres or an accessible gyms). This is the practical reason why we adopted a non-randomised design of our study.

Blood chemistry and urinalysis, complete blood count, and a cardiac evaluation were performed by the transplantation centres to assess the exclusion criteria. After the administration of the SF-36 questionnaire to evaluate Health-Related Quality of Life (HRQoL), the patients were sent to the sports medicine centre to perform the functional assessment tests for exercise capacity, muscle strength, and body composition.

Based on the results of these tests, the sports physicians prescribed a tailored training programme only for Group A. Then, patients in Group A were sent to a certified gym to start the prescribed training under the supervision of exercise specialists, while patients in Group B, as usual, were provided general information to encourage regular physical activities at home but no specific prescription and supervision were given.

Both groups were checked at baseline (T_0), six (T_6) and 12 mo (T_{12}) from the enrolment. The trial did not envisage any change in the immunosuppressive treatment (Table 1).

Written informed consent was obtained by the patients before inclusion, according to the procedures approved by the Ethics Committee. This trial was registered in the ISRCTN registry (Trial ID: ISRCTN66295470) and was conducted in compliance with the ICH Guidelines for Good Clinical Practice, the Helsinki Declaration and national rules regarding clinical trial management.

Supervised training intervention (Group A)

The exercise prescription included sessions of aerobic and resistance training. The total duration of each session was one hour, with a frequency of 3 times per week for 12 mo. In every session, the aerobic training was performed on a stationary bike and was administered with an intensity corresponding to the lactate aerobic threshold[14], previously assessed by the incremental cycling test at T_0 for the first six months and at T_6, for the subsequent period. The intensity was continuously monitored by heart rate monitors (Polar, Finland) allowing the patients to maintain a constant heart rate (HR) corresponding to the aerobic threshold during the aerobic training.

In the same session, the subsequent resistance training consisted of two sets of 20 repetitions at 35% of one Repetition Maximum (1RM) for each muscle

Table 1 Pathologies leading to renal disease and transplantation

Underlying disease	Group A (n = 44)	Group B (n = 41)
Glomerulonephritis	10	6
Nephroangiosclerosis	6	7
Polycystic kidney disease	8	10
End-stage kidney disease	10	5
Alport syndrome	2	2
IgA nephropathy	6	0
Nephrotic syndrome	2	0
Multicystic renal dysplasia	0	4
Interstitial nephritis	0	2
Haemolytic uraemic syndrome	0	4
Vasculitis polyangiitis	0	1

group of the upper (elbow flexors, elbow extensors, shoulder abductors) and lower limbs (knee extensors, plantar flexors). The training intensity at 35% of 1RM was chosen to increase local muscle endurance considering that KTRs are novice individuals for strength training where learning proper form and technique is paramount[15,16]. Resistance training was not performed with the upper limb with arterio-venous fistula. Warm-up, cool-down and stretching exercises were included in all training sessions. The intensities of aerobic and strength trainings were adjusted after the T_6 assessment.

Non-supervised home-based exercise intervention (Group B)

At T_0 and T_6 patients in Group B were provided general information to encourage regular physical activities at home, as usual by the transplant centre, but no specific prescription and supervision were given.

The International Physical Activity Questionnaire (IPAQ) short-version[17] was administered only to Group B at the three-time points to evaluate the level of physical activity through nine items that provide information on the time spent walking, in vigorous- and moderate-intensity activity and in sedentary activity. This questionnaire assessed the actual level of daily physical activity and thus reduce the bias between the two groups.

Primary outcomes

Renal function, lipid values and blood chemistry: In both groups, creatinine (mg/dL) using the Jaffè method, estimated glomerular filtration rate (eGFR) using the chronic kidney disease epidemiology collaboration (CKD-EPI) equation, proteinuria (mg/1000 mL) using the turbidimetry method reported in g/24 h calculating 24-h urine collection were collected to check the renal function at T_0, T_6 and T_{12}.

Total cholesterol and triglycerides were measured from venous blood sample using flow cytometry and light-scattering methods to evaluate lipid metabolism. Haemoglobin and glycaemia values were also measured.

Exercise capacity: Exercise capacity was assessed by an incremental cycling exercise starting from a 5-min unloaded cycle and increasing by 20 W every four minutes until the subject was unable to continue. A 12-lead electrocardiogram was monitored continuously throughout the test. At each step a capillary blood sample from the earlobe was taken to measure blood lactate concentration (YSI 1500-Sport; Yellow Springs, United States) to estimate the workload corresponding to aerobic and anaerobic thresholds, conventionally declared at 2 and 4 mmol/L of lactate, respectively[14]. Systolic and diastolic blood pressures were measured with sphygmomanometers at rest, at each step and at the third minute of recovery.

Oxygen uptake ($V'O_2$) was determined continuously using an open-circuit spirometry system (Sensor Medics, Anaheim, United States), and the $V'O_2$ at the highest tolerated workload was determined and was referred to as $V'O_2$ peak (mL O_2/kg per minute).

Muscular strength and power: A leg press (Technogym, Cesena, Italy) and free weights were utilised to assess the dynamic muscular strength of the lower and upper limbs (knee extensors, plantar flexors, elbow flexors, elbow extensors and shoulder abductors). The 1RM strength was calculated using an indirect method consisting of 7 to 12 repetitions with submaximal loads[18].

The general strength was measured using a handgrip dynamometer (Lafayette, IN, United States).

The power of the lower limbs was measured indirectly from the fly time of a countermovement jump (CMJ) and was expressed as maximum displacement (m) of the centre of mass during fly (Optojump, Microgate, Italy).

Secondary outcomes

BMI and body composition: Body mass index (BMI) was calculated using the ratio between weight and square height (kg/m^2).

Fat mass (FM) percentage was determined using the Jackson and Pollock body density equation considering seven skinfolds in both men and women (abdominal, thigh, triceps, bicep, subscapular, suprailiac, chest) measured with a Harpenden calliper[19] at T_0, T_6 and T_{12}.

Health-related quality of life: The 36-Item Short Form Health Survey (SF-36, Medical Outcomes Trust) was used to evaluate self-reported domains of health status[20] completed by the patients independently at T_0, T_6 and T_{12}.

Statistical analysis

The sample size to assess eventual differences in exercise capacity, muscular strength, renal function, BMI and HRQoL was determined using the Software G-Power (version 3.1.9.2) with an alpha level of 0.01 and a power of 0.90. All descriptive data are presented as the mean ± standard deviation (SD). Linear mixed

Table 2 Immunosuppressive and other therapies in both groups

	Tacrolimus	Cyclosporine	Steroid therapy	Purine synthesis inhibitors	(mTOR) inhibitors	Anti-hypertensive therapy	Beta-blockers	Insulin therapy	Statin	Ezetimibe
Group A, n = 44	28 (64%)	12 (27%)	35 (80%)	33 (75%)	7 (16%)	33 (75%)	18 (14%)	1 (2%)	14 (34%)	2 (5%)
Group B, n = 41	27 (66%)	10 (24%)	30 (73%)	34 (83%)	5 (12%)	30 (73%)	18 (44%)	2 (5%)	18 (41%)	2 (5%)

models were used to assess the effects of time and group on dependent variables, with T_0 and Group B set as the base categories. Random intercepts were used for individual subjects. Significance was set at P < 0.05, and the raw coefficients for the fixed effects and interactions are reported with 95%CI. The statistical analysis was performed using R software for Windows (v. 3.2.3).

RESULTS

Subjects

Ninety-nine KTRs were recruited by nine transplant centres between January 2011 and June 2015. Fifty-two patients were included in Group A, and 47 in Group B. Eight patients from Group A decided to withdraw and were considered dropouts. The causes were economic problems and lack of motivation (n = 4) or work conflicts (n = 4). In Group B, six patients did not show up to the functional assessments during the follow-up.

Forty-four KTRs from Group A (21 female and 23 males, mean ± SD age 47 ± 12 years, mass 69 ± 14 kg, BMI 24.1 ± 4.3 kg/m^2, time from transplant 5.5 ± 7.1 years, dialysis vintage 36 ± 35 mo, range 1-156) and 41 KTRs from Group B (13 female and 28 males, age 49 ± 9 years, weight 75 ± 13 kg, BMI 25.5 ± 4.4 kg/m^2, time from transplant 3.6 ± 4.0 years, dialysis vintage 33 ± 34 mo, range 1-144) were analysed. There were no significant differences between groups regarding: age (P = 0.35), BMI (P = 0.16), time from transplant (P = 0.11), and dialysis vintage (P = 0.42). The only significant difference was found for body mass (P = 0.02).

Pathologies leading to renal disease and immunosuppressive therapies of the patients are shown in Tables 1 and 2 respectively.

Exercise program adherence

In Group A, the exercise program adherence, defined as a total number of exercise sessions completed as proportion of total possible number of session (144 sessions) during the 12-mo period was 93% ± 6%. None adverse events were reported.

Primary outcomes

Creatinine tended to decrease in Group A at T_{12} and increase in Group B at the same time, but the results were not significant. No significant changes were found in eGFR or proteinuria in either group. Average triglyceride and cholesterol levels showed slight changes at T_{12} in both groups which were not significant (Table 3).

Only three patients (one in group A and two in group B) were diabetic under insulin therapy (Table 2). In both groups, glucose values were always < 126 mg/dL without significant changes between the three-time points.

Diastolic and systolic blood pressures were similar (P > 0.05) in the two groups at rest, at the maximum workload and after three minutes of recovery, at T_0, T_6 and T_{12} (Table 3). The only significant difference was found between groups for systolic blood pressure at the third minute of recovery, that was always lower in Group A (P = 0.0489).

Group A showed a significant average improvement in maximum workload and $V'O_2$ peak at T_6 (P = 0.0010, P = 0.0370), and the levels continued to increase at T_{12} (P = 0.0003, P = 0.0099) compared to Group B (Table 3).

The maximum HR, anaerobic threshold workload and corresponding HR significantly increased at T_{12} (P < 0.05) in Group A compared to Group B. In Group B, we found a significant decrease in the anaerobic threshold HR from T_0 to T_{12} (P = 0.0434). No additional significant differences were found in Group B at T_6 and T_{12} in any variables (Table 3).

Group A showed a significant average improvement in lower limb strength and power expressed by an increase in plantar flexor muscle strength (P = 0.0368) and CMJ (P = 0.0293) at T_{12} compared to Group B. No significant differences were found in Group B at T_{12} in any variable (Table 3).

Group A showed a significant improvement in the handgrip test at T_{12} (P < 0.05) compared to Group B (Table 3).

Group B showed a significant increase in elbow flexor, elbow extensor and shoulder abductor strength (respectively, P < 0.05) at T_6, but the levels remained below the values of Group A (Table 3).

No changes were found in Group B in the level of daily physical activity assessed by IPAQ, which remained at a low level (< 600 MET per minute per week) at the 12 mo follow-up. Theoretical IPAQ calculated from the exercise protocol performed by Group A was < 600 METper minute per week at baseline, and > 600 METper minute (range 1215-1413 MET per minute) per week at T_6 and T_{12} (P < 0.01).

Secondary outcomes

Group A showed a significant decrease in BMI at T_{12} (P = 0.0013) and fat mass percentage at T_6 (P = 0.05)

Table 3 Mean ± SD of exercise capacity and blood chemistry

	Group A (n = 44)			Group B (n = 41)		
	T_0	T_6	T_{12}	T_0	T_6	T_{12}
Maximum workload (W)	95 ± 36	107 ± 38[a]	108 ± 41[c]	102 ± 32	102 ± 30	98 ± 34
V'O_2 peak (mL/kg per minute)	22.8 ± 8.3	25.6 ± 9.0[a]	25.9 ± 7.5[c]	21.6 ± 6.8	22.6 ± 6.6	21.5 ± 6.4
HR max (bpm)	142 ± 24	142 ± 22	145 ± 22[c]	133 ± 22	134 ± 23	131 ± 22
Diastolic BP at rest (mmHg)	80 ± 8	80 ± 8	78 ± 6	81 ± 9	82 ± 8	82 ± 8
Diastolic BP at V'O_2 peak (mmHg)	85 ± 11	83 ± 12	82 ± 13	84 ± 10	81 ± 13	80 ± 11
Diastolic BP at 3' recovery (mmHg)	75 ± 9	76 ± 8	75 ± 9	78 ± 10	79 ± 9	78 ± 8
Systolic BP at rest (mmHg)	126 ± 14	126 ± 12	125 ± 11	130 ± 16	126 ± 15	127 ± 13
Systolic BP at V'O_2 peak (mmHg)	183 ± 26	183 ± 21	185 ± 25	181 ± 26	181 ± 27	178 ± 30
Systolic BP at 3' recovery (mmHg)	128 ± 17	129 ± 14	131 ± 16	136 ± 16	136 ± 21	135 ± 20
Body mass index (kg/m^2)	24.1 ± 4.3	24.0 ± 4.3	23.6 ± 4.5[c]	25.5 ± 4.4	25.3 ± 4.0	25.8 ± 4.5
Fat mass (%)	21.1 ± 9.0	19.8 ± 8.3[a]	20.7 ± 7.9	20.0 ± 7.8	18.9 ± 6.5	19.8 ± 8.1
Aerobic threshold workload (W)	53 ± 23	60 ± 29	60 ± 27	53 ± 21	62 ± 27	57 ± 25
Aerobic threshold HR (bpm)	113 ± 20	108 ± 19	112 ± 18	103 ± 16	103 ± 19	103 ± 16
Anaerobic threshold workload (W)	84 ± 30	94 ± 37	91 ± 31[c]	89 ± 32	97 ± 38	88 ± 36
Anaerobic threshold HR (bpm)	131 ± 21	130 ± 23	134 ± 18[c]	125 ± 20	125 ± 21	120 ± 19[e]
Knee extensors right (kg)	87 ± 38	93 ± 40	98 ± 39	55 ± 27	61 ± 26	60 ± 24
Knee extensors left (kg)	80 ± 36	93 ± 40	95 ± 42	51 ± 23	60 ± 25	58 ± 23
Plantar flexors right (kg)	70 ± 34	76 ± 29	82 ± 27[c]	62 ± 35	69 ± 27	65 ± 23
Plantar flexors left (kg)	70 ± 33	77 ± 29	79 ± 28	64 ± 34	71 ± 24	67 ± 24
Counter movement jump (cm)	24.0 ± 10.0	26.4 ± 10.2	25.9 ± 9.3[c]	21.5 ± 9.4	22.9 ± 10.2	20.9 ± 10.2
Handgrip right (kg)	30.8 ± 13.1	33.2 ± 12.2	32.3 ± 11.9[c]	36.3 ± 9.5	35.9 ± 9.8	34.2 ± 9.6
Handgrip left (kg)	29.3 ± 13.6	30.7 ± 11.7	30.6 ± 11.7[c]	35.1 ± 9.3	34.4 ± 9.7	32.7 ± 10.2
Elbow flexors Right (kg)	8.8 ± 2.7	9.4 ± 2.8	9.7 ± 2.8	8.0 ± 3.4	9.4 ± 3.1[e]	9.4 ± 3.5
Elbow flexors Left (kg)	8.7 ± 3.2	9.6 ± 3.4	9.6 ± 3.1	7.3 ± 3.4	8.6 ± 2.7[e]	8.7 ± 3.5
Elbow extensors right (kg)	5.8 ± 2.0	6.5 ± 2.3	6.9 ± 2.3	5.1 ± 2.2	6.1 ± 2.1[e]	6.0 ± 2.1
Elbow extensors left (kg)	5.7 ± 2.0	6.5 ± 2.4	6.8 ± 2.4	4.9 ± 2.3	5.6 ± 1.8[e]	5.7 ± 1.9
Shoulder abductors (kg)	5.3 ± 2.2	6.3 ± 2.5	6.4 ± 2.3	4.2 ± 2.7	5.2 ± 2.1[e]	5.4 ± 2.7
Creatinine (mg/dL)	1.26 ± 0.38	1.27 ± 0.41	1.21 ± 0.29	1.37 ± 0.48	1.32 ± 0.50	1.42 ± 0.47
eGFR (mL/min per 1.73 m^2)	59.4 ± 19.3	58.0 ± 19.6	62.6 ± 21.8	56.3 ± 21.2	58.1 ± 17.8	52.9 ± 17.4
Proteinuria (g/24 h)	0.41 ± 0.51	0.34 ± 0.46	0.52 ± 0.63	0.45 ± 0.57	0.48 ± 0.59	0.61 ± 0.44
Haemoglobin (g/dL)	12.8 ± 1.8	12.3 ± 1.7	12.6 ± 1.6	12.1 ± 1.8	12.5 ± 1.9	12.8 ± 1.5
Triglycerides (mg/dL)	122 ± 42	117 ± 41	117 ± 47	138 ± 69	131 ± 57	132 ± 59
Cholesterol (mg/dL)	196 ± 37	186 ± 52	200 ± 43	195 ± 33	193 ± 31	188 ± 34

In Group A: [a]P < 0.05 between T_0 and T_6, [c]P < 0.05 between T_0 and T_{12}; In Group B: [e]P < 0.05 between T_0 and T_{12}. BP: Blood pressure; HR: Heart rate; eGFR: Estimated glomerular filtration rate.

compared to Group B (Table 3).

In HRQoL, significant improvements were found in Group A in physical function scale at T_6 (P = 0.0082) and continued to increase at T_{12} (P = 0.0019), in role-physical and social functioning scales at T_{12} (P = 0.0321, P = 0.0346) compared to Group B, in which we found no significant changes in any scales (Table 4).

DISCUSSION

The main result of this study is that in selected KTRs, a programme of 12 mo of supervised training performed one hour, three times per week in certified gyms does not affect the renal function, leading to significant improvement in aerobic fitness, muscle strength and HRQoL, with a significant decrease of BMI. Furthermore, the proposed organizational model led to a high exercise program adherence, *i.e.,* to a positive change in lifestyle.

The KTRs included in Group B who received only general information to promote regular physical activity at home, without a specific supervision, did not show any improvement in physical fitness outcomes, indicating a low adherence to non-supervised home-based physical activity. This demonstrated that without a direct or indirect supervision (*e.g.,* follow-up by calls or e-mails), patients tend to not carry out physical activity even if it is recommended by the physician.

van Adrichem *et al*[21] highlighted how perceived barriers of physical activity in KTRs such as physical limitations, lack of energy, and comorbidities cannot be omitted. Moreover, the lack of specific counselling by physicians about the benefits of physical activity is a critical issue. However, in the present study we recorded a dropout rate of 15% in Group A and 13% in Group B. Painter *et al*[6] reported a dropout rate of 33% at one year in their exercising group of patients who performed home-based training with regular phone follow-up. Greenwood *et al*[22] in their 12-wk study reported a dropout of 7 on 20 KTRs (35%) in both aerobic and resistance training supervised groups. Most of these patients reported difficulties attending classes following return to work after transplantation. Riess *et al*[4] reported a dropout of 2 out of 16 (13%) on their 12-wk study in the supervised exercise group and 1 out of 15 (7%) in the home based usual care

Table 4 Mean ± SD of 36-Item Short Form Health Survey questionnaire scales

	Group A (n = 44)			Group B (n = 41)		
	T_0	T_6	T_{12}	T_0	T_6	T_{12}
Physical function	84 ± 20	91 ± 11[a]	92 ± 12[c]	89 ± 10	86 ± 20	86 ± 23
Role physical	83 ± 25	88 ± 21	96 ± 15[c]	91 ± 19	91 ± 19	86 ± 24
Bodily pain	80 ± 24	80 ± 22	89 ± 20	86 ± 19	84 ± 22	84 ± 22
General health	63 ± 20	67 ± 21	68 ± 20	64 ± 21	67 ± 19	66 ± 17
Vitality	67 ± 16	70 ± 15	69 ± 19	67 ± 18	69 ± 14	68 ± 14
Social function	75 ± 19	80 ± 20	83 ± 17[c]	82 ± 19	78 ± 21	78 ± 21
Role emotional	85 ± 24	91 ± 20	90 ± 22	93 ± 16	96 ± 15	93 ± 17
Mental health	75 ± 16	75 ± 16	74 ± 19	74 ± 18	77 ± 16	74 ± 16

[a]$P < 0.05$ between T0 and T6; [c]$P < 0.05$ between T_0 and T_{12}.

group. O'Connor *et al*[23] in an un-supervised period of self-managed physical activity reported an attrition rate of 30% at the 12 mo time point that confirms a low exercise adherence without supervision.

Compliance with the treatment is a common barrier of health programmes based on exercise even if transplant recipients who have experienced a supervised exercise programme supported that it was beneficial to health and well-being[24]. Social, cognitive, personality, environmental, and socio-economic factors, unrelated to the recommended guidelines, seem to be of greater importance in considering behavioural adherence issues[25] in KTRs. To improve physical exercise programme compliance and longer-term outcomes, strategies to diversify and stimulate exercise training or change elements of training like introduce specific tracking devices designed for KTRs should be examined. Anyway, data from our study clearly show that recommendations alone are not sufficient to induce a change in lifestyle and physical fitness. On the other hand, the supervised training for long periods is costly and cannot be proposed for all the transplanted patients, so it is urgent to study new solutions, starting from the cooperation between transplantation, sports medicine, and exercise specialists.

Renal function data, expressed as creatinine, eGFR and proteinuria, were compatible with the framework of patients of a select population undergoing successful renal transplantation. The proposed training protocol had no negative effects on the renal function in the medium term and, more in detail, creatinine values tended to decrease in Group A and to increase in Group B at the same time, but the results were not significant after 12 mo. The tendency to decrease of creatinine and eGFR in Group A can be considered as positive effect of physical exercise and needs specific studies. Patients in Group A did not show any significant increase in muscle mass after 12 mo of resistance training such as to affect creatinine and eGFR, probably because of the low intensity of the resistance training.

To the best of our knowledge, 12 mo of observation period is one of the longest in the literature with reference to aerobic and resistance trainings; however, it is a relatively short-time period and further studies with larger populations are necessary to understand the long-term effects of exercise or sedentary lifestyle on the renal function of KTRs.

Regarding exercise capacity, in Group A we observed a 12% increase of $V'O_2$ peak at T_6. Similar results were obtained by van den Ham *et al*[26] in 33 KTRs after 12 wk of combination of endurance and strength training in which $V'O_2$ peak increase of 10% (from 21.6 ± 6.3 to 23.8 ± 6.1 mL/kg per minute). Riess *et al*[4] reported an increase of $V'O_2$ peak (from 20 ± 9 to 23 ± 10 mL/kg per minute) after 12 wk of supervised endurance training (three times/week) on cycle ergometer at 60%-80% of $V'O_2$ max involving 16 patients.

In a study of eight KTRs, Romano *et al*[27] utilised a supervised interval training technique for 10 wk, 40-min sessions for three times per week. They reported an increase of 13% of $V'O_2$ peak.

Another intervention was published by Kempeneers *et al*[28] who trained 16 KTRs for six months in preparation for the National Transplant Games. Their mean $V'O_2$ peak rose from 29.0 ± 7.8 to 37.5 ± 4.8 mL/kg per minute, with an increase of 27%.

In the other hand, Painter *et al*[6] prescribed an individualised home-based exercise training programme in 54 KTRs, consisting on 30 min, four times per week of training at an intensity corresponding to 60%-80% of maximal HR. Patients were contacted every two weeks by phone to assess progress and adherence to the programme and to adjust it as needed. After six months, $V'O_2$ peak increased from 24.0 ± 7.5 to 27.8 ± 11.0 mL/kg per minute (+ 16%) and to 30.1 ± 10.3 mL/kg per minute (+ 25%) after 12 mo.

We can conclude that the aerobic training in KTRs leads to a substantial increase in aerobic power[29]. In most cases, the type of training meets the minimal clinically significant difference of 3.5 mL/kg per minute (*i.e.*, 1 metabolic equivalent), which is associated with improved outcomes in CVD. However, Riess *et al*[4] after 12 wk of endurance training were unable to demonstrate any change in resting small or large arterial compliance, peak exercise systemic vascular resistance and Framingham Risk Assessment Score, indicating that exercise intensity and overall duration are probably the most critical factors affecting CVD risk

profile.

Our study did not reveal significant differences in blood pressure between the two groups, both showing normal blood pressure at rest, during and after maximal incremental exercise. This may be explained by the tightly controlled anti-hypertensive regimes in post-transplant care, as previously reported in other studies[23].

Reduced general muscular strength has been related to an increased risk of all-cause of cardiovascular mortality[30] and the handgrip test values are a recognised marker of health status[31]. In our study, the handgrip test values improved after six months of training and significantly improved after 12 mo, whereas in the Group B we found a trend in reduction in strength, even if it was not significant. Moreover, in the Group A the muscular strength of the lower limbs improved, and the power of the lower limbs increased after 12 mo. This increase in maximal strength and CMJ values may be associated with both neural adaptations and muscle trophism improvement[32]. This finding is consistent with prior studies[6].

In relation to anthropometric measures, the 12-mo supervised programme combining aerobic and resistance training was effective in reducing BMI[33] and fat mass in Group A, with a non-significant reduction in fat-free mass (-2%). However, in our study, the lipid profile remained the same; 39% of patients were taking a statin or ezetimibe as a regular drug (Table 2), which would make further improvement in the lipid profile unlikely. Moreover, the patients did not receive a diet programme.

Regarding quality of life, we found significant improvement in Group A in the self-perception of physical function, role-limitation to physical activity and social function. The KTRs in the usual care group (Group B) did not show any improvement in HRQoL scores. This finding confirms that supervised exercise training led to a better self-perception of quality of life[34].

The association of aerobic and resistance training was safe; no acute cardiovascular event, renal graft-related or serious adverse events due to endurance or strengthening exercises were recorded. The inclusion in the protocol of the CMJ test did not have any consequence to the musculoskeletal system, indicating that KTRs can safely perform supervised power exercises[35]. The accurate selection of the patients and the cardiovascular assessment at T_0 certainly contributed to these findings.

The present study has some limitations. First, it is a non-randomised study; we included the patients in the usual care group (Group B) on logistic and organisational grounds. Therefore, the two groups were different in baseline assessments of body mass.

Another limit is due to the workloads chosen for the aerobic and strength trainings. We adopted a steady state aerobic exercise protocol at intensity corresponding to the lactate aerobic threshold. Different training protocols, *i.e.,* interval training, or different duration of the sessions could be more effective. Furthermore, it is possible that with a higher percentage of 1RM and with a different progressive strengthening protocols the improvements would be higher, especially when training the upper limbs. The fact that the upper limb with the arteriovenous fistula was not trained, for safety precautions, also affected the final strength results. Furthermore, we checked both groups after 6 mo of training, so probably a more frequent adjustment in the prescription of the relative intensity of trainings would lead to better functional outcomes in Group A or would give further motivations in Group B.

The anthropometric assessment by the skinfolds technique has some limitations and probably it would be possible to detect significant changes with more precise methods (*i.e.,* dual energy X-ray absorptiometry). Another limit is that we administered the IPAQ only to Group B to reduce the bias between the two groups, but it was impossible to make any direct comparison with Group A.

Finally, patients included in our study were carefully selected and were thus not representative of the entire KTRs population. Furthermore, the prescribed exercise program was based on blood tests and other measurements that are not routinely performed in gyms and cannot be universally applied, so the generalizability of the results is limited.

Despite some limitations, this paper shows that developing a supervised exercise protocol for KTRs is a useful and safe non-pharmacologic contribution to usual after-transplant treatments, which can improve the physiological variables related to physical fitness and cardiovascular risks without consequences on renal function. Our study is an example of collaborative working between transplant centres, sports medicine and exercise facilities, aimed to apply the concepts of "exercise is medicine".

Further studies with longer follow-up and larger populations are necessary to understand the strategies that will improve adherence to training programmes, control costs and lead to steady and durable lifestyle changes in KTRs.

ARTICLE HIGHLIGHTS

Research background

Kidney transplant recipients (KTRs) are characterised by long term clinical complications and high risk of cardiovascular disease. After transplantation, physical activity is considered a key element in the prevention and management of chronic diseases, however the majority of KTRs maintain a sedentary lifestyle, often associated with an increase of body fat and weight gain. Whether exercise can positively affect outcomes in KTRs has only been addressed in few studies, with a small number of subjects and with different types, intensity and durations of interventions lasting almost always not more than six mo. Furthermore, few studies have investigated the effect of a combined aerobic and resistance training, and the effect of these protocols on kidney function is rather unknown. In this paper, we present some clinical and fitness outcomes of a 12-mo study conducted on KTRs, with the aim to evaluate the potential effects of supervised exercise combining aerobic and resistance training.

Research frontiers

Developing a supervised exercise protocol for KTRs is a useful and safe non-pharmacologic contribution to usual after-transplant treatments, which can improve the physiological variables related to physical fitness and cardiovascular risks without consequences on renal function. Further studies with longer follow-up and larger populations are necessary to understand the strategies that will improve adherence to training programmes, control costs and lead to steady and durable lifestyle changes in KTRs.

Innovations and breakthroughs

Selected KTRs can safely perform training protocols lasting 12 mo, with association of aerobic and resistance exercises.

Applications

The collaboration between transplant centres, sports medicine centres and exercise facilities is effective to prevent the low adherence to suggested and/or prescribed physical activity.

Terminology

Oxygen uptake ($V'O_2$) at the highest tolerated workload was referred to as $V'O_2$ peak (mLO_2/kg per minute).

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ORIGINAL ARTICLE

Clinical Practice Study

In vitro intracellular IFNγ, IL-17 and IL-10 producing T cells correlates with the occurrence of post-transplant opportunistic infection in liver and kidney recipients

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Abstract

AIM

To validate intracellular cytokine production functional assay as means of cell-mediated immunity monitoring of post-transplant patients with opportunistic infection (OI).

METHODS

Intracellular cytokine-producing $CD4^+$ and $CD8^+$ T-cell monitoring was carried out in 30 liver transplant (LTr) and 31 kidney transplant (KTr) recipients from 2010 to 2012. Patients were assessed in our Department of Immunology at the Clinical University 'Hospital Virgen de la Arrixaca-IMIB' in Murcia, Spain for one year following transplantation. FACS Canto Ⅱ flow cytometer was employed to quantify the intracellular production of IL-17, IFNγ and IL-10 cytokines on stimulated $CD4^+CD69^+$ and $CD8^+CD69^+$ T cells and BD FACS DIVA v.6 software was used to analysed the data. Statistical analysis was carried out using SPSS 22.0.

RESULTS

LTr with OI had significantly lower % of $CD8^+CD69^+IFN\gamma^+$ T cells at 60 (7.95 ± 0.77 *vs* 26.25 ± 2.09, $P < 0.001$), 90 (7.47 ± 1.05 *vs* 30.34 ± 3.52, $P < 0.001$) and 180 (15.31 ± 3.24 *vs* 24.59 ± 3.28, $P = 0.01$) d post-transplantation. Higher % of $CD4^+CD69^+IL\text{-}10^+$ as well as $CD4^+CD69^+IL\text{-}17^+$ T cells were yet reported at 30 (14.06 ± 1.65 *vs* 6.09 ± 0.53, $P = 0.0007$ and 4.23 ± 0.56 *vs* 0.81 ± 0.14, $P = 0.005$; respectively), 60 (11.46 ± 1.42 *vs* 4.54 ± 0.91, $P = 0.001$ and 4.21 ± 0.59 *vs* 1.43 ± 0.42, $P = 0.03$; respectively) and 90 d (16.85 ± 1.60 *vs* 4.07 ± 0.63, $P < 0.001$ and 3.97 ± 0.43 *vs* 0.96 ± 0.17, $P = 0.001$). Yet, KTr with OI had significantly lower percentage of $CD4^+CD69^+IFN\gamma^+$ at 30 (11.80 ± 1.59 *vs* 20.64 ± 3.26, $P = 0.035$), 60 (11.19 ± 1.35 *vs* 15.85 ± 1.58, $P = 0.02$), 90 (11.37 ± 1.42 *vs* 22.99 ± 4.12, $P = 0.028$) and 180 (13.63 ± 2.21 *vs* 21.93 ± 3.88, $P = 0.008$) d post-transplantation as opposed to $CD4^+CD69^+IL\text{-}10^+$ and $CD8^+CD69^+IL\text{-}10^+$ T cells which percentages were higher at 30 (25.21 ± 2.74 *vs* 8.54 ± 1.64, $P < 0.001$ and 22.37 ± 1.35 *vs* 17.18 ± 3.54, $P = 0.032$; respectively), 90 (16.85 ± 1.60 *vs* 4.07 ± 0.63, $P < 0.001$ and 23.06 ± 2.89 *vs* 10.19 ± 1.98, $P = 0.002$) and 180 (21.81 ± 1.72 *vs* 6.07 ± 0.98, $P < 0.001$ and 19.68 ± 2.27 *vs* 10.59 ± 3.17, $P = 0.016$) d post-transplantation. The auROC curve model determined the most accurate cut-off values to stratify LTr and KTr at high risk of OI and Cox Regression model confirmed these biomarkers as the most significant risk factors to opportunistic infection.

CONCLUSION

Post-transplant percentages of T-cell subsets differed significantly amongst infected- and non-infected-LTr and -KTr and yet this imbalance was found to contribute towards a worst clinical outcome.

Key words: Intracellular cytokine; Liver transplantation; Kidney transplantation; Opportunistic infection

Core tip: The aim of this research was to validate predictive biomarkers for the occurrence of post-transplant opportunistic infection in both liver and kidney recipients. The imbalance in the percentage of cytokine-producing cultured $CD4^+CD69^+$ and $CD8^+CD69^+$ T cells was shown to be the most significant recipient risk factor to develop opportunistic infection.

Boix F, Llorente S, Eguía J, Gonzalez-Martinez G, Alfaro R, Galián JA, Campillo JA, Moya-Quiles MR, Minguela A, Pons JA, Muro M. *In vitro* intracellular IFNγ, IL-17 and IL-10 producing T cells correlates with the occurrence of post-transplant opportunistic infection in liver and kidney recipients. *World J Transplant* 2018; 8(1): 23-37 Available from: URL: http://www.wjgnet.com/2220-3230/full/v8/i1/23.htm DOI: http://dx.doi.org/10.5500/wjt.v8.i1.23

INTRODUCTION

Despite the continuous improvement in the clinical management of solid organ transplant recipients (SOTr), opportunistic infection (OI) remains one of the leading causes of morbidity and mortality in this population[1]. Although current immunosuppressive regimens aim to prevent allograft acute rejection (AR)[2], clinicians still rely exclusively on therapeutic drug monitoring (TDM) of immunosuppression therapy (pharmacokinetics) to determine the immunological status of SOTr[3]. Indeed, the risk of an inadequate immunosuppression due to chronic exposure has been claimed to be one of the main reason of poor long-term outcomes[4,5]; hence there must be a balance to prevent not only AR but also reducing immunosuppression-related comorbidities, such as OI. Tailoring immunosuppressive regimens could potentially reduce the risk of life-threatening conditions, amongst other side effects, resulting in an improvement in the wellbeing of SOTr. Despite the aforementioned, TDM appears to be insufficient (intra- and inter-individual pharmacokinetic variability) in the provision of fulfilling information as to the real immunosuppressive status of SOTr[6].

In recent years new strategies, such as monitoring of cell-mediated immunity (CMI), have been seen to provide more accurate information with respect of the management of post-transplant SOTr. As such, CMI has been proposed as an alternative and reliable strategy in the search for predictive biomarkers of AR[7-10] and OI[11-14] amongst other clinical conditions.

The knowledge of T lymphocytes in host defense against infection has improved significantly over time. There is clear evidence that, upon pathogen derived-antigen contact, naïve T $CD4^+$ (T_H0) cells activate and differentiate into different functional subsets characterised by their cytokine secretion patterns (T_H1, T_H2, T_H9, T_H17,

Tregs)[15]. Furthermore, when stimulated by microbial products through pattern recognition receptors (PRRs), antigen presenting cells (APCs) acquire the capacity to activate naïve T cells and differentiate into effector T cells that mediate adaptive immune responses. APCs stimulated with pathogens such as *Bordetella pertussis*, *Klebsiella pneumoniae*, and *Mycobacterium tuberculosis* produce a significant amount of IL-23, resulting in the development of T_H17 cells, showing that this subset acts against both extracellular and intracellular infections[16,17]. Evidence has shown that T_H17 cells are also required for host defense against fungal infection[18]. The classically established T_H1/T_H2 paradigm yet describes the role of these two T lymphocyte subsets in host defense against infections. T_H1 cells are essential in the elimination of intracellular pathogens such as *Leishmania* and *Mycobacteria*[19], whereas T_H2 secreted IL-10 cytokine cells has emerged as a key immunoregulator during infection with viruses, bacteria, fungi, protozoa, and helminths[20].

We therefore hypothesised, that CMI could be used to tackle T cell differentiation as a therapeutic target, providing thorough understanding of the adaptive immune response against pathogens after SOT. Hence, the aim of this uni-centre study was to prospectively monitor T helper lymphocyte cytokine responses against overall OI in a cohort of liver and kidney transplant recipients. As such, CMI could aid clinicians in the provision of better prophylaxis therapies, potentially reducing the occurrence of post-transplant OI.

MATERIALS AND METHODS

Study design

From 2010 to 2012, 61 consecutive adult patients; of whom 30 patients diagnosed with end-stage liver disease underwent LT and 31 patients diagnosed with end-stage renal failure underwent KT, alongside 16 healthy control (HC) volunteers were recruited from the Immunology Service of the Clinical University Hospital 'Virgen de la Arrixaca', Murcia (Spain) for a prospective uni-centre study. Peripheral venous blood samples were obtained from individual participants for laboratory testing at baseline as well as at several different post-transplantation time points (7 d, 15 d, 1st month, 2nd month, 3rd month, 6th month and 1st year). Formal consent was obtained from both patients and healthy controls, with approval of the study protocol obtained by the institutional ethical committee. Pediatric, re-transplant and combined transplant patients were excluded. The inclusion criteria included primary liver and kidney transplantation, ABO compatibility and HIV negativity. The primary study outcome was the occurrence of overall OI, which took into consideration the following etiologies: Bacterial, fungal and viral post-transplant infection (including CMV disease, either viral syndrome or end-organ disease). The post-transplant follow-up period of 1 year was divided into three different intervals: early post-transplant period (up to the 1st month), intermediate (from the 1st to the 6th month) and long-term (from the 6th month to the 1st year). All post-transplant recipients were assessed on a regular basis by the consultant specialist in their respective outpatient transplant clinics, with a sample (urine or blood) taken for microbiological and biochemistry assessment. Based on laboratory findings, LTr and KTr were classified into two different study groups, with [INF; 60% of LTr (n = 18) and 61.3% of KTr (n = 19)] and without [NoINF; 40% of LTr (n = 12) and 38.7% of KTr (n = 12)] post-transplant OI.

Prophylaxis, immunosuppression and induction therapies

Cefuroxime (1500 mg/*iv* per 8 h) was administered to all methicillin-resistant *Staphylococcus* negative recipients, whereas Teicoplanin (200 mg/*iv* per 12 h) was given to patients positive for methicillin-resistant *Staphylococcus*. Oral Nystatin (5 cc/8 h) was also provided as *Candida sp* prophylaxis. Trimethoprim-sulfamethoxazole (160/800 mg/*iv* per 24 h) was given, over six months, as *Pneumocystis jiroveci pneumonia* (PJP) prophylaxis. Oral Itraconazole (200 mg/24 h) was also given over three months to prevent *Aspergillus sp.* infection. Oral Pyrimethamine (25 mg/24 h) + folic acid was given as prophylaxis against *Toxoplasma sp*, with treatment extended up to six months in cases where serology was positive. In patients CMV seropositive, Ganciclovir (5 mg/kg per 12 h) or Valganciclovir (900 mg/kg per 12 h) were given as induction prophylaxis treatment. CMV prophylaxis induction with iv-Ganciclovir or oral-Valganciclovir for 2 wk followed up by oral-Valganciclovir for 3 mo. In those cases of a CMV seronegative recipient and CMV seropositive donor, the induction treatment was extended for 4 wk and maintained up to 6 mo. Post-transplantation CMV infections were treated with iv-Ganciclovir for 2 or 3 wk in both types of transplant, and oral-Valganciclovir was maintained for 3 mo. Finally, BK viral infection was treated by the administration of oral leflunomide (100 mg/24 h) over five days.

Initial immunosuppressive therapy consisted of oral Tacrolimus (TRL) 1 mg (6 mg/24 h) or oral Mycophenolic acid (MMF) 500 mg (1 g/24 h for KTr or 1.5 g/24 h for LTr) with Prednisone 20 mg/d with progressing tapering. The average drug level achieved for TRL was 2.6-17.3 ng/mL. The average drug level achieved for MMF was 0.40-4.15 μg/mL. The initial dose was modified in case of adverse side effects, such as diarrhea or leucopenia. In case of AR, the rescue therapy provided was based on the administration of steroid boluses (500–1000 mg methylprednisolone/24 h) for 3 d. In case of chronic rejection (CR), the rescue therapy provided was based on the administration of oral TRL (FK506; 0.1 mg/kg per 24 h).

Induction therapy was based on the administration of either thymoglobulin (1-1.5 mg *iv*/kg; Genzyme

Table 1 List of type of opportunistic microorganisms that infected liver and kidney recipients during the post-transplant period *n* (%)

Type of opportunistic microorganism	Liver transplant recipients (*n* = 30)	Kidney transplant recipients (*n* = 31)
Presence of overall opportunistic infection (Yes/No)	18 (60)/12 (40)	19 (61.3)/12 (38.7)
Presence of bacterial infection (Yes/No)	12 (66.7)/6 (33.3)	17 (84.2)/3 (15.8)
Staphylococcus hominis	2 (16.7)/10 (83.3)	1 (5.8)/15 (94.2)
Staphylococcus epidermidis	5 (41.7)/7 (58.3)	5 (29.4)/11 (70.6)
Staphylococcus haemolyticus	2 (16.7)/10 (83.3)	2 (11.8)/14 (88.2)
Enterococcus faecalis	1 (8.3)/11 (91.7)	3 (17.6)/13 (82.4)
Enterococcus faecium	0	3 (17.6)/13 (82.4)
Clostridium difficile	2 (16.7)/10 (83.3)	3 (17.6)/13 (82.4)
Proteus mirabilis	1 (8.3)/11 (91.7)	4 (23.5)/12 (76.5)
Pseudomonas aeruginosa	2 (16.7)/10 (83.3)	5 (29.4)/11 (70.6)
Serratia marcescens	1 (8.3)/11 (91.7)	0
Escherichia coli	2 (16.7)/10 (83.3)	13 (76.5)/3 (23.5)
Treponema pallidum	1 (8.3)/11 (91.7)	0
Enterobacter aerogenes	2 (16.7)/10 (83.3)	1 (5.8)/15 (94.2)
Enterobacter cloacae	0	3 (17.6)/13 (82.4)
Streptococcus sp.	1 (8.3)/11 (91.7)	0
Citrobacter koseri	0	2 (11.8)/14 (88.2)
Morganella morganii	0	1 (5.8)/15 (94.2)
Klebsiella oxytoca	0	2 (11.8)/14 (88.2)
Klebsiella pneumoniae oxytoca	0	1 (5.8)/15 (94.2)
Hafnia alvei	0	1 (5.8)/15 (94.2)
Salmonella typhi	0	2 (11.8)/14 (88.2)
Presence of yeast infection (Yes/No)	4 (22.2)/14 (77.8)	3 (15.8)/16 (84.2)
Candida albicans	4 (100)/0	3 (100)/0
Presence of viral infection (Yes/No)	17 (94.4)/1 (5.6)	17 (89.5)/2 (10.5)
Cytomegalovirus	11 (64.7)/6 (35.3)	12 (70.6)/5 (29.4)
BK virus	3 (17.6)/14 (82.4)	6 (35.3)/11 (64.7)
Epstein-Barr virus	2 (11.8)/15 (88.2)	3 (17.6)/14 (82.4)
Varicella-Zoster virus	2 (11.8)/15 (88.2)	0
Presence of parasitic infection (Yes/No)	3 (16.7)/15 (83.3)	3 (15.8)/16 (84.2)
Toxoplasma gondii	3 (100)/0	2 (66.7)/1 (33.3)
Strongyloides stercoralis	0	1 (33.3)/2 (66.7)

Polyclonals S.A.S) or basiliximab (anti-CD25, 0.5-2 mg *iv*/kg; Simultec®, Novartis Farma), with 3 (10%) LTr and 23 (74.2%) KTr receiving basiliximab and 4 (12.9%) KTr receiving thymoglobulin.

Flow cytometry procedure for intracellular cytokine stain

The percentage of $CD4^+CD69^+IFN\gamma^+$, $CD8^+CD69^+IFN\gamma^+$, $CD4^+CD69^+IL\text{-}17^+$, $CD4^+CD69^+IL\text{-}10^+$ and $CD8^+CD69^+IL\text{-}10^+$ in individual samples was determined by flow cytometry following intracellular staining with anti-cytokine monoclonal antibodies. Upon venipuncture, whole peripheral blood was incubated with Ionomycin (Io) and Phorbol Myristate Acetate (PMA) for 4 h. Following activation, whole peripheral blood was stained with FITC-conjugated anti-human CD8, PE-conjugated anti-human CD69, and PerCP-conjugated anti-human CD3 for 30 min; then fixed and permeabilised using BD FACS™ Permeabilizing Solution (BD Biosciences), followed by intracellular staining with APC-conjugated anti-human IL-10, APC-conjugated anti-human IL-17A and APC-conjugated anti-human IFNγ. All monoclonal antibodies were supplied by Becton Dickinson (San Jose, CA, United States). The FACS CANTO Ⅱ cytometer (Becton Dickinson, San Jose, CA, USA) was used to acquire at least 20000 with data analysed on BD FACSDiva™ 6.0 Software. Figure 1 shows the gating strategy to quantify the intracellular cytokine production (IFNγ, IL-17 and IL-10) on peripheral $CD4^+$ and $CD8^+$ T lymphocytes.

Opportunistic infection diagnosis

The primary study outcome was the occurrence of overall OI during the 1st year post-transplantation. To the purpose of this study we took into consideration the occurrence of overall OI episode as the incident of any clinical event including all viral infection (Citomegalovirus, CMV and non-CMV infections, such as Herpes-Zoster Virus, HZV; Herpes Simplex Virus, HSV; Epstein-Bar Virus, EBV and BK virus), as well as bacterial, fungal and parasite infections as a whole. Table 1 summarises all opportunistic agents diagnosed in both LTr and KTr during the 1st year post-transplantation. Bacterial infection was diagnosed in those patients with a positive test in bloodstream and/or urine samples. Microbiological cultures were used to find bacterial microorganisms such as *Escherichia coli*, *Staphylococcus* sp., *Enterococcus* sp., *Pseudomonas* sp., *Serratia* sp., *Proteus* sp. with positivity considered in cases of > 10000 Colonial Forming Units (CFU)/mL. Urine tract infection due to yeast microorganisms was observed in all cases, due to *Candida albicans,* with diagnosis based on the presence of > 10000 CFU/mL following urine culture. In addition, the rapid test for detecting Clostridium's toxin was performed to diagnose

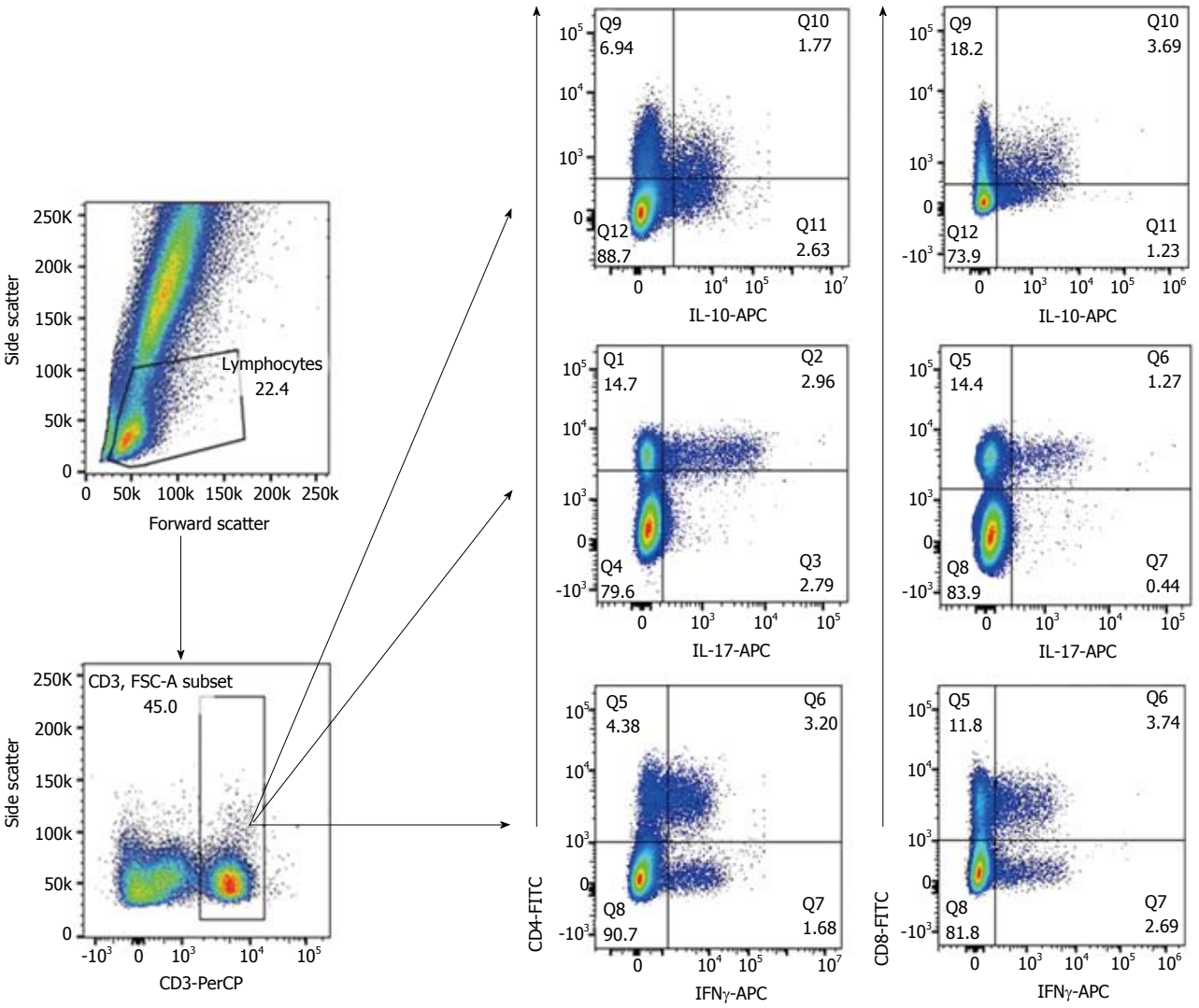

Figure 1 FACS analysis of cultured CD3$^+$ T lymphocyte. Whole blood peripheral CD4$^+$ and CD8$^+$ T lymphocytes from individual ESLF and ESRF patients as well as HC subjects were stain with anti-CD3, anti-CD8, anti-CD69, anti-IL-17, anti-IL-10 and anti-IFNγ monoclonal antibodies following manufacture's guidelines upon *in vitro* stimulation with Io and PMA. CD4$^+$ and CD8$^+$ lymphocytes were gated within CD3$^+$CD69$^+$ population following polyclonal activation for 4 h. CD4$^+$ T cells were consider to approximate CD3$^+$CD8$^-$ T cells. At least 50000 events were acquired. Io: Ionomycin; PMA: Phorbol myristate acetate.

infection by *Clostridium difficile*. Viral infection was determined using serological and molecular DNA-based methods. CMV infection was diagnosed by the presence of either IgM or IgG anti-CMV in symptomatic patients. CMV infection was assigned to either anti-CMV IgG antibody level ⩾ 0.6 UI/mL or anti-CMV IgM antibody ⩾ 30 UA/ml in symptomatic patients. Post-transplant active CMV and BK virus infections were confirmed using real-time polymerase chain reaction (qPCR) in plasma and/or urine samples. The presence of anti-EARLY IgG (⩾ 1/10) and/or anti-VCA IgM (> 0.400 DO) in symptomatic patients was considered evidence of EBV infection. Similarly, only the presence of anti-HSV type 1 and 2 IgM (⩾ 20 UI/mL) was considered evidence of active herpes virus infection.

Statistical analysis

Demographic data and results from our prospective follow-up study were collected and analysed in a unified database (SPSS 22.0, SPSS Inc., Chicago, IL, United States). Qualitative data are expressed as frequency and percentage. Quantitative data are shown as the mean ± SEM. Nonparametric Kolmogorov-Smirnov test was applied to identify whether the data followed a Gaussian distribution. Samples were adjusted to a nonparametric distribution. Nonparametric *U* Mann-Whitney test was applied to unpaired quantitative continuous variables, whereas nonparametric Wilcoxon test was applied to evaluate the relationship between paired quantitative continuous variables. Optimal biomarker cut-off points to discriminate between patients with and without OI were based on receiver operating characteristic (ROC) curves and calculated with the best Youden index (sensitivity + specificity-1)[21]. Discriminatory capacity was defined by the area under the curve (auROC) measure, with 0.7-0.8 deemed acceptable, 0.8-0.9

Table 2 Patient clinical and demographic characteristics

	Liver recipients (n = 30)			Kidney recipients (n = 31)		
	NoINF (n = 12)	INF (n = 18)	P	NoINF (n = 12)	INF (n = 19)	P
Donor age (yr)	60.75 ± 3.32	58.93 ± 4.71	0.905	51.21 ± 3.17[1]	55.67 ± 3.83[1]	0.017[1]
Recipient age (yr)	51.25 ± 2.87	53.79 ± 2.37	0.282	51.58 ± 3.25	51.42 ± 2.81	0.351
Recipient gender (M/F), n (%)	14 (87.5)/2 (12.5)	9 (64.3)/5 (35.7)	0.669	10 (41.7)/2 (28.6)	14 (58.3)/5 (71.4)	0.087
Total lymphocyte (%)	15.38 ± 3.63	11.39 ± 2.08	0.397	16.09 ± 2.85	11.66 ± 2.33	0.768
Total lymphocyte (cells/mm^3)	813.34 ± 163.70	750.02 ± 191.84	0.711	1121.67 ± 173.35	1073.16 ± 235.68	0.197
Total leukocyte (× 10^9/L)	6.29 ± 0.55	7.09 ± 1.10	0.652	8.36 ± 1.15[1]	10.98 ± 1.33[1]	0.006[1]
SGOT (U/L)	187.44 ± 97.28	117.16 ± 18.09	0.738	27.66 ± 4.61	19.17 ± 1.09	0.669
SGPT (U/L)	161.69 ± 69.20	135.56 ± 14.77	0.891	44.51 ± 9.81	27.06 ± 1.98	0.762
SALP (U/L)	177.47 ± 14.72	186.62 ± 14.40	0.847	106.84 ± 8.23[1]	85.36 ± 2.74[1]	0.008[1]
SGGT (U/L)	197.27 ± 23.65[1]	351.28 ± 42.44[1]	0.005[1]	109.97 ± 25.34	64.36 ± 11.74	0.074
Glomerular filtration (mL/min)	82.82 ± 7.36	81.28 ± 6.27	0.571	45.87 ± 2.90[1]	72.42 ± 2.52[1]	0.019[1]
Serum creatinine	0.93 ± 0.05[1]	1.08 ± 0.06[1]	0.027[1]	6.22 ± 0.60	5.83 ± 0.51	0.251
Induction therapy (thymoglobulin/basiliximab)	0(0)/0(0)	0(0)/1(3.3)		1(8.3)/11(91.7)	4(21.1)/12(63.2)	0.161
Post-transplant therapy (TRL/TRL + MMF), n (%)	7(58.3)/5(41.7)	10(55.6)/8(44.4)	0.880	0/0/12(100)	1(5.3)/2(10.5)/16(84.2)	0.350
Maintenance therapy (TRL/TRL + MMF), n (%)	7(58.3)/5(41.7)	13(72.2)/5(27.8)	0.461	0/0/12(100)	2(10.5)/2(10.5)/15(78.9)	0.235
TRL dose (mg/d)	7.92 ± 0.28[1]	6.67 ± 0.39[1]	< 0.001[1]	13.08 ± 1.51	12.35 ± 1.37	0.179
MMF dose (mg/d)	2062.50 ± 73.45[1]	1848.57 ± 79.00[1]	0.034[1]	1620 ± 164.70[1]	1917.89 ± 58.87[1]	< 0.001[1]
Cmin TRL (ng/mL)	10.46 ± 0.69	9.61 ± 0.47	0.445	10.54 ± 1.98[1]	6.53 ± 1.97[1]	0.012[1]
Cmin MMF (μg/mL)	2.91 ± 0.60[1]	0.97 ± 0.29[1]	0.016[1]	1.07 ± 0.18	3.80 ± 1.20	0.071

[1]Statistical significance. NoINF: Free-opportunistic infection study group; INF: Opportunistic infection study group; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; SALP: Serum alkaline phosphatase; SGGT: Serum Gamma-Glutamyl Transpeptidase; TRL: Tacrolimus; MMF: Mycophenolic acid.

excellent and > 0.9 outstanding[22]. The predictive value for the model was assessed with χ^2 test. Survival curves for the first episode of OI were plotted using the Kaplan–Meier method, and differences between groups compared with the log-rank test. Recipient and donor factors were entered into univariate Cox model and those factors found to be significant at $P < 0.25$ level were subsequently entered into multivariate model, using a backward stepping procedure, to find the best model. In addition in this model AR, induction therapy and average drug dose were added as controlled variables. Results were expressed as hazard ratios (HRs) with 95%CIs. All statistical tests were two-tailed, with a $P < 0.05$ representing statistical significance.

RESULTS

Patient clinical and demographic characteristics

Overall, 60% of LTr and 61% of KTr developed at least one post-transplant OI event during the 1st year post-transplantation. Generally, the infection pattern varied from bacterial, fungal and non-CMV infections following the first weeks post-transplantation to mainly CMV and non-CMV infections, such as HSV or HZV as previously described[15], seen towards the end of the follow-up period. Recipient's clinical and demographic data found to be significant between infected- and non-infected-LTr and KTr are shown in Table 2.

Percentage of T_H1, T_H2 and T_H17 lymphocytes in end-stage liver and renal failure patients

Prior to transplantation, circulating CD4$^+$ and CD8$^+$ T lymphocyte analysis found that, pre-transplant percentages of CD8$^+$CD69$^+$INFγ$^+$, CD4$^+$CD69$^+$IL-17$^+$ and CD4$^+$CD69$^+$IL10$^+$ lymphocytes (Figure 2A-C) in patients with ESLF and CD8$^+$CD69$^+$IL-10$^+$ lymphocytes (Figure 2E) in patients with ESRF were significantly greater compared to HC. In contrast, there were no significant differences in the percentage of CD4$^+$CD69$^+$INFγ$^+$ and CD4$^+$CD69$^+$IL-10$^+$ between ESRF patients and HC.

Monitoring of T_H1, T_H2 and T_H17 peripheral lymphocytes during follow-up period

Post-transplantation follow-up analysis of the different CD4$^+$ and CD8$^+$ T lymphocyte subsets showed that amongst LTr the percentage of CD8$^+$CD69$^+$IFNγ$^+$ decreased significantly within the early post-transplantation period, whereas the percentages of CD4$^+$CD69$^+$IL-10$^+$ and CD4$^+$CD69$^+$IL-17$^+$ experienced an up-regulation during the study period. Of particular interest was the IFNγ-producing-CD8$^+$ T lymphocytes, which observed a significant drop during the first weeks following transplant surgery compared to pre-transplant values, which then gradually recovered back to their basal levels (Figure 3A). On the other hand, T_H17 (Figure 3B) and T_H2 (Figure 3C) lymphocytes were significantly greater at the intermediate and long-term period compared to baseline levels. Likewise, the percentage of CD4$^+$ and CD8$^+$ T lymphocyte subsets amongst KTr also experienced changes during the post-transplantation follow-up period. Particularly, the percentage of CD4$^+$CD69$^+$INFγ$^+$ lymphocytes initially increased upon transplantation; however, levels dropped significantly during the post-transplantation intermediate-term (Figure 3D). On the other hand, percentages of CD4$^+$CD69$^+$IL-10$^+$ (Figure 3E) and CD8$^+$CD69$^+$IL-10$^+$

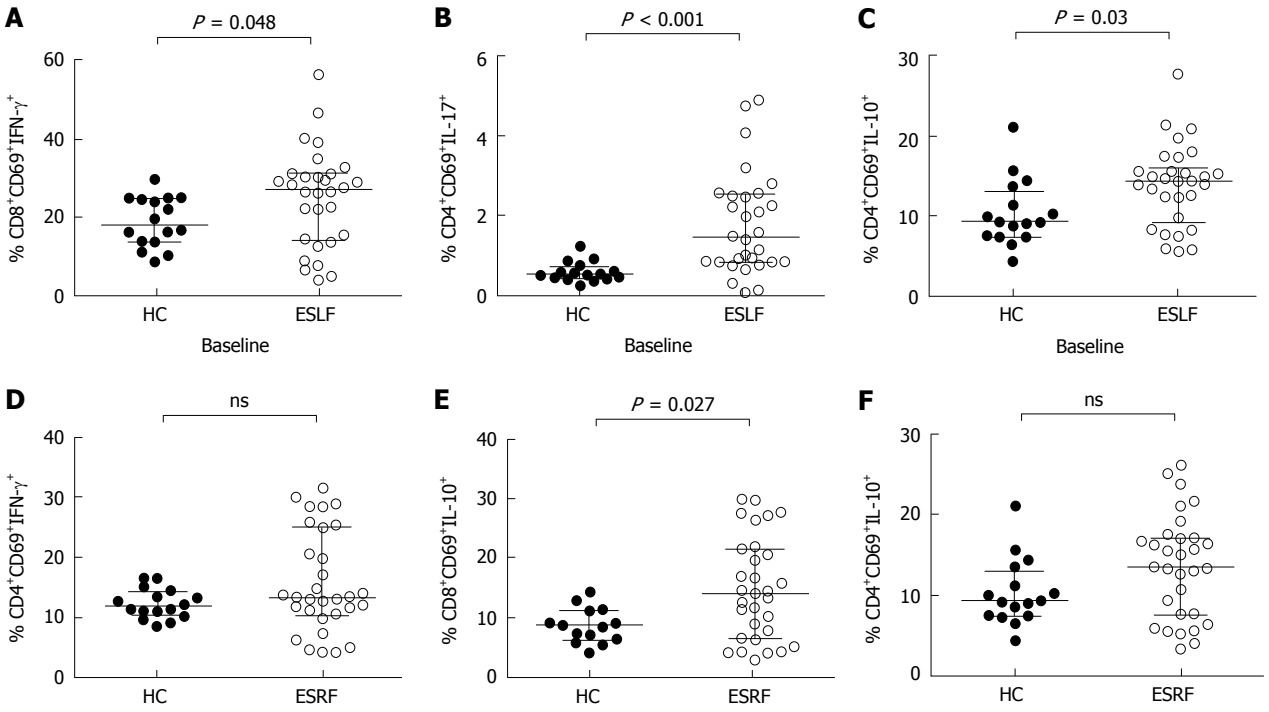

Figure 2 Quantitative analysis of cultured CD4$^+$ and CD8$^+$ T lymphocytes from individual end-stage liver failure, end-stage renal failure and healthy control subjects. A: % CD4$^+$CD69$^+$INFγ$^+$; B: CD4$^+$CD69$^+$IL-17$^+$; C: CD4$^+$CD69$^+$IL-10$^+$ cells in ESLF patients and HC individuals; D: % CD4$^+$CD69$^+$INF-γ$^+$; E: CD8$^+$CD69$^+$IL-10$^+$; F: CD4$^+$CD69$^+$IL-10$^+$ cells in ESRF patients and HC individuals. The horizontal lines reflect median values for each group and vertical lines reflect interquartile range. ESLF: End-stage liver failure; ESRF: End-stage renal failure; HC: Healthy control.

(Figure 3F) T lymphocyte subsets experienced a significant early up-regulation, which remained constant during the post-transplantation period compared to their basal levels. These data are summarized in supplementary Table 1.

Recipients with post-transplant opportunistic infection had significantly greater IL-17 and IL-10 and lower IFNγ intracellular production capacity on stimulated CD3$^+$CD69$^+$ T lymphocytes

The incidence of OI episodes was found to be higher within intermediate-term in both kinds of transplant recipients; with 54.8% and 64.1% of OI episodes occurring between the 1st and 6th month following LT and KT, respectively. Therefore, the post-transplantation T lymphocyte stratification analysis was focused within this period. In this regard, LTr who developed an OI episode displayed a lower percentage of CD8$^+$CD69$^+$IFNγ$^+$ compared to the OI-free study group at 60 (Figure 4A, $P < 0.001$), 90 (Figure 4A, $P < 0.001$) and 180 (Figure 4A, $P = 0.01$) d post-transplantation. On the other hand, LTr with OI had a significantly higher intracellular IL-10-cytokine production capacity by CD3$^+$CD4$^+$CD69$^+$ T lymphocytes at 30 (Figure 4E, $P = 0.0007$), 60 (Figure 4E, $P = 0.001$), 90 (Figure 4E, $P < 0.001$) and 180 d (Figure 4E, $P < 0.001$) post-transplantation in comparison with recipients who did not develop OI. In addition, the percentage of CD4$^+$CD69$^+$IL-17$^+$ in LTr with OI was significantly greater at 30 (Figure 4C, $P = 0.005$), 60 (Figure 4C, $P = 0.03$) and 90 d (Figure 4C, $P = 0.001$) post-transplantation.

The T lymphocyte kinetics amongst KTr showed a similar trend for both pro- and anti-inflammatory cytokine production capacities. In particular, KTr who developed an OI episode within the intermediate-term displayed a significantly less intracellular IFNγ production capacity by CD3$^+$CD4$^+$CD69$^+$ T lymphocytes compared to patients free of infection at 30 (Figure 4B, $P = 0.035$), 60 (Figure 4B, $P = 0.02$), 90 (Figure 4B, $P = 0.028$) and 180 d (Figure 4B, $P = 0.008$) post-transplantation. On the other hand, a higher IL-10-producing T lymphocytes capacity was seen in KTr who subsequently developed an OI episode. Post-transplant percentage of CD8$^+$CD69$^+$IL-10$^+$ in KTr who developed OI was significantly increased at 30 (Figure 4D, $P = 0.032$), 90 (Figure 4D, $P = 0.002$) and 180 d (Figure 4D, $P = 0.016$) post-transplantation. Similarly, a significantly increased percentage of CD4$^+$CD69$^+$IL-10$^+$ T lymphocytes at 30 (Figure 4F, $P < 0.001$), 60 (Figure 4F, $P = 0.002$), 90 (Figure 4F, $P = 0.001$) and 180 d (Figure 4F, $P = 0.01$) was observed in KTr who developed an OI episode within the intermediate post-transplantation term. These data are shown in supplementary Table 2.

Post-transplant cut-off values that accurately stratified liver and kidney transplant recipients at high risk of opportunistic infection

Following the stratification analysis, we wanted to find the potential capability of these T lymphocyte subsets as surrogate biomarkers capable of stratifying both LTr

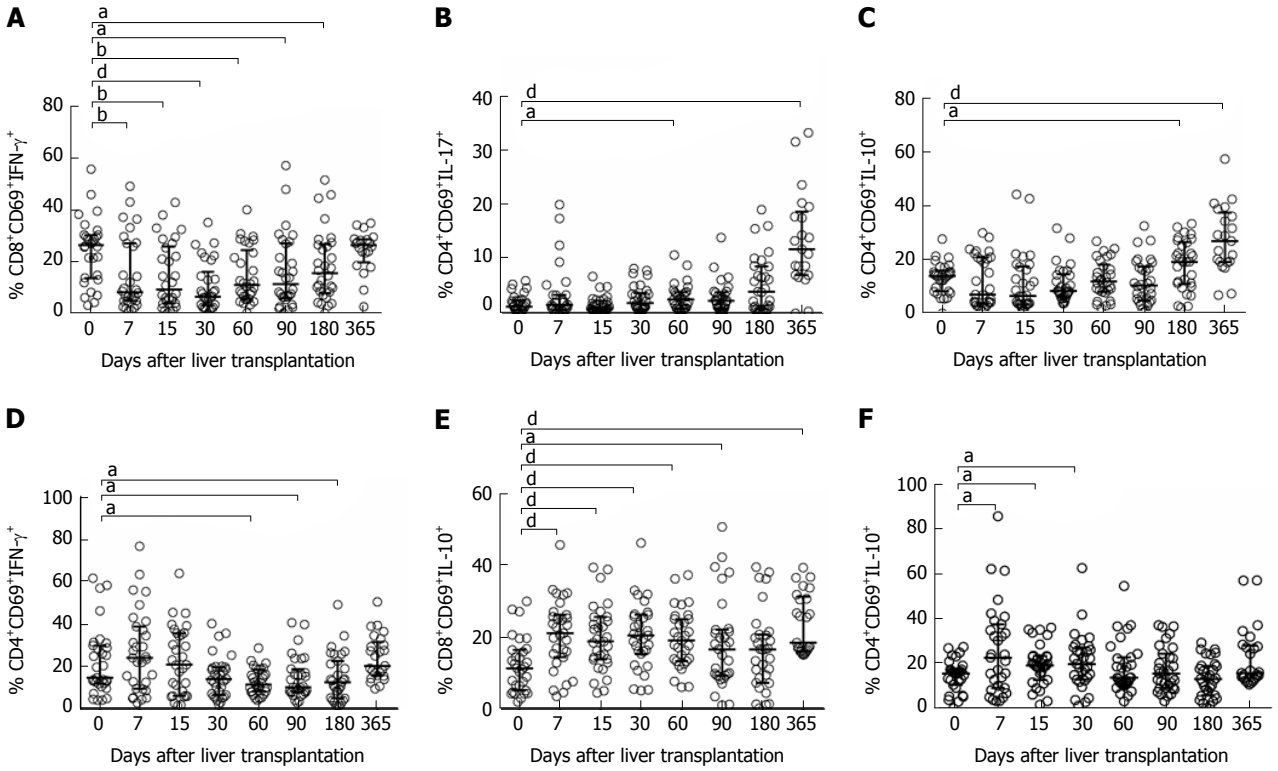

Figure 3 Cytokine-producing CD4$^+$ and CD8$^+$ T cells follow-up along first year after liver and kidney transplantation. A: % of cultured IFNγ-producing CD8$^+$ lymphocytes; B: % of cultured TH17 lymphocytes; C: % of cultured TH2 lymphocytes in LTr; D: % of cultured TH1 lymphocytes; E: % of cultured IL-10-producing CD8 T lymphocytes; F: % of cultured TH2 lymphocytes inKTr. The horizontal lines reflect median values for each group and vertical lines reflect interquartile range. $^aP < 0.05$, $^bP < 0.01$, $^dP < 0.001$. Significances express the difference against baseline level.

and KTr at high risk of overall post-transplant OI. The post-transplantation percentage of stimulated cytokine-producing CD3$^+$CD69$^+$ T lymphocytes was found to have an impact on OI incidence.

The auROC curve analysis showed that the disparity in T lymphocyte population was distinguishable amongst LTr and KTr at high risk of overall post-transplant OI. Particularly, patients with a percentage of CD8$^+$CD69$^+$IFNγ$^+$ ⩽ 14.95% (Figure 5A; AUC = 0.897, 95%CI: 0.834-0.960, $P < 0.001$) in LTr and a percentage of CD4$^+$CD69$^+$IFNγ$^+$ ⩽ 13.83% (Figure 5B; AUC = 0.750, 95%CI: 0.657-0.843, $P < 0.001$) in KTr were considered to be at a significantly high risk of post-transplant OI. In fact, 92.3% of LTr (n = 16) and 82% of KTr (n = 15) who developed OI displayed post-transplant levels of CD8$^+$CD69$^+$IFNγ$^+$ ⩽ 14.95% and CD4$^+$CD69$^+$IFNγ$^+$ ⩽ 13.83%, respectively at any time point between the 1st and 6th month post-transplantation. The Kaplan-Meier curve showed that time free of OI was significantly shorter for those LTr and KTr whose cut-off values were below the threshold (Figure 5A; $P < 0.001$, Long Rank test and Figure 5B; $P = 0.004$, Long Rank test).

With regards TH17 subset, a percentage of CD4$^+$CD69$^+$Il-17$^+$ T lymphocytes ⩾ 2.19% (Figure 5C; AUC = 0.840, 95%CI: 0.761-0.919, $P < 0.001$) was also capable to stratify LTr at high risk of post-transplant OI. 55.8% of LTr were classified at high risk of overall post-transplant OI with a percentage of TH17 above cut-off, of whom 15 out of 18 LTr (86.6%) developed OI and 3 recipients (13.4%) did not develop OI despite being stratified within the high risk group. A percentage of CD4$^+$CD69$^+$IL-17$^+$ ⩾ 2.19% in LTr at any time point between the 1st and 6th month post-transplantation resulted in a shorter time free of overall OI near of signification (Figure 5C; $P = 0.058$, Long Rang test).

Finally, a percentage of CD8$^+$CD69$^+$IL-10$^+$ T lymphocytes ⩾ 11.15% (Figure 5D; AUC = 0.734, 95%CI: 0.638-0.831, $P < 0.001$) in KTr and a percentage of CD4$^+$CD69$^+$IL-10$^+$ T lymphocytes ⩾ 9.35% (Figure 5E; AUC = 0.902, 95%CI: 0.834-0.969, $P < 0.001$) in LTr and ≥ 13.95% (Figure 5F; AUC = 0.856, 95%CI: 0.792-0.919, $P < 0.001$) in KTr accurately discriminated both cohort of patients at high risk of overall post-transplant OI. KTr with a percentage of CD8$^+$CD69$^+$IL-10$^+$ < 11.15% had significantly reduced overall OI episodes compared to those recipients with values ⩾ 11.15% (Figure 5D; $P = 0.002$, Long Rang test). 23 KTr (73.6%) were shown to be at high risk of OI, of whom 17 (75.3%) developed OI between the 1st and 6th month post-transplantation exhibiting a percentage above cut-off. Considering the intracellular IL-10 production capacity by the CD3$^+$CD4$^+$CD69$^+$ T lymphocyte subpopulation, a percentage ⩾ 9.35% in LTr (Figure 5E; $P = 0.003$, Long Rang test) and ⩾ 13.95% in KTr (Figure 5F; $P < 0.001$, Long Rang test) resulted in a worse outcome with significantly increased overall post-transplantation OI episodes. 50.5% (n = 15) of LTr as well as another 50% (n = 16) of KTr were

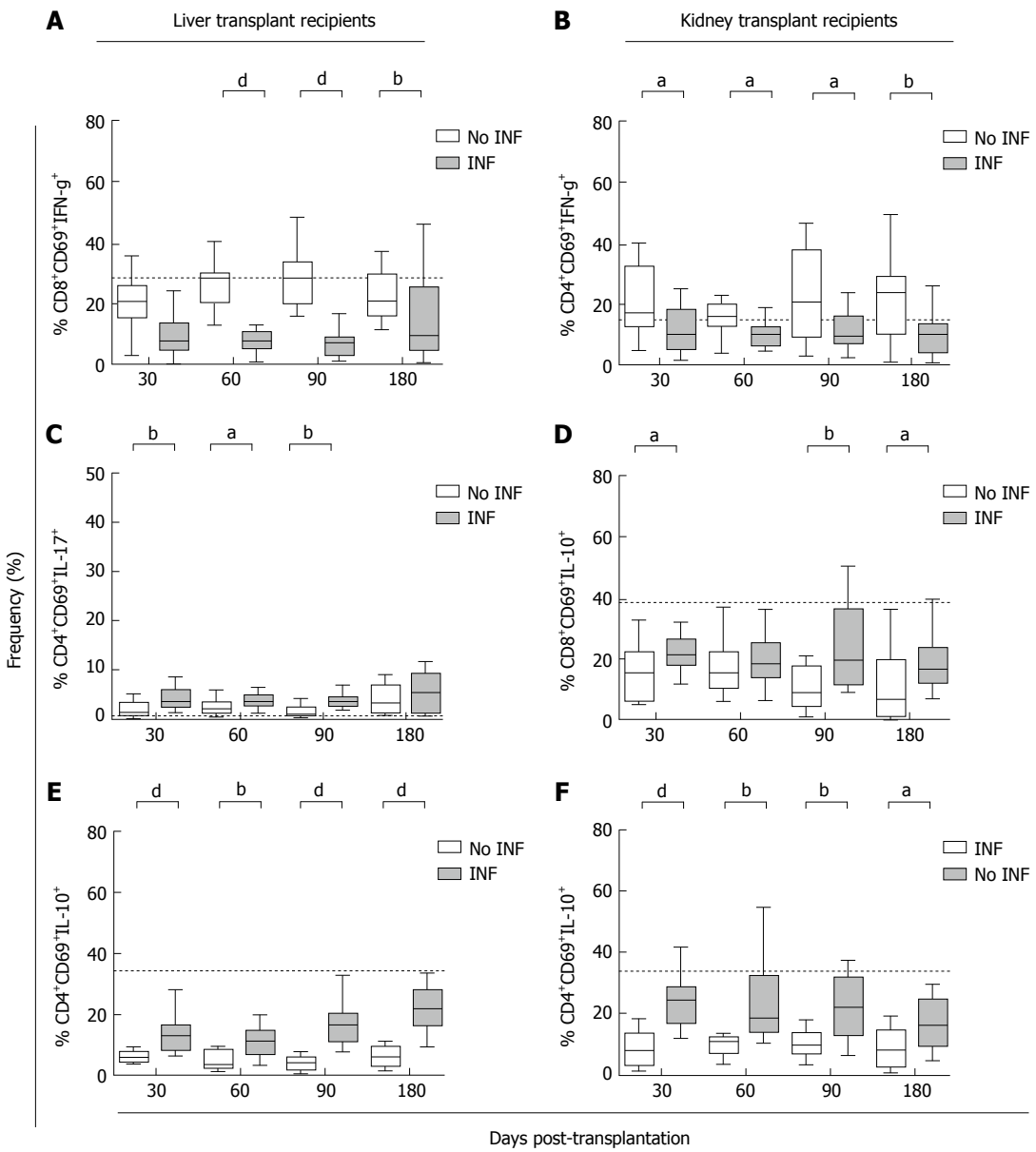

Figure 4 Stratification analysis of the percentage for the intracellular cytokine production capacity of CD4$^+$ and CD8$^+$ T lymphocytes between the 1st and 6th month post-transplantation. A: % of CD8$^+$CD69$^+$IFNγ$^+$ T lymphocytes in LTr with and without OI; B: % of CD4$^+$CD69$^+$IFNγ$^+$ T lymphocytes in KTr with and without OI; C: % of CD4$^+$CD69$^+$IL-17$^+$ T lymphocytes in LTr with and without OI; D: % of CD8$^+$CD69$^+$IL-10$^+$ T lymphocytes in KTr with and without OI; E: % of CD4$^+$CD69$^+$IL-10$^+$ T lymphocytes in LTr with and without OI; F: % of CD4$^+$CD69$^+$IL-10$^+$ T lymphocytes in KTr with and without OI. LTr: liver transplant recipients; KTr: kidney transplant recipients. ^{a}*P* < 0.05, ^{b}*P* < 0.01, ^{d}*P* < 0.001.

stratified at high risk of infection showing percentages of TH2 all above the threshold. Indeed, 96.2% of LTr and 87.1% of KTr of those at high risk developed at least one episode of OI between the 1st and the 6th month post-transplantation. Cut-off values, specificities and sensitivities for the surrogate biomarkers of post-transplant OI are shown in Table 3.

The imbalance between the TH1 and TH2 response as the most significant risk factor associated with post-transplant opportunistic infection in liver and kidney transplant

We further examined the relationship between different recipient/donor factors and the occurrence of post-transplant OI in LTr and KTr. Following auROC curve analysis; univariate and multivariate Cox regression models were carried out. Results of the univariate and multivariate analysis of recipients and donor factors are shown in Table 4. Several factors were shown to be associated with an increased risk of post-transplant OI in LTr as well as KTr. Amongst them, recipient gender and serum alkaline phosphatase (SALP) enzyme in LTr showed a trend (*P* = 0.067 and *P* = 0.078, respectively) to a worse post-transplant primary study point, whereas in KTr, recipient gender in conjunction with SALP, serum creatinine levels and dose of MMF, were significantly observed as independent risk factor of post-transplant OI episodes (*P* = 0.046, *P* = 0.016, *P* =

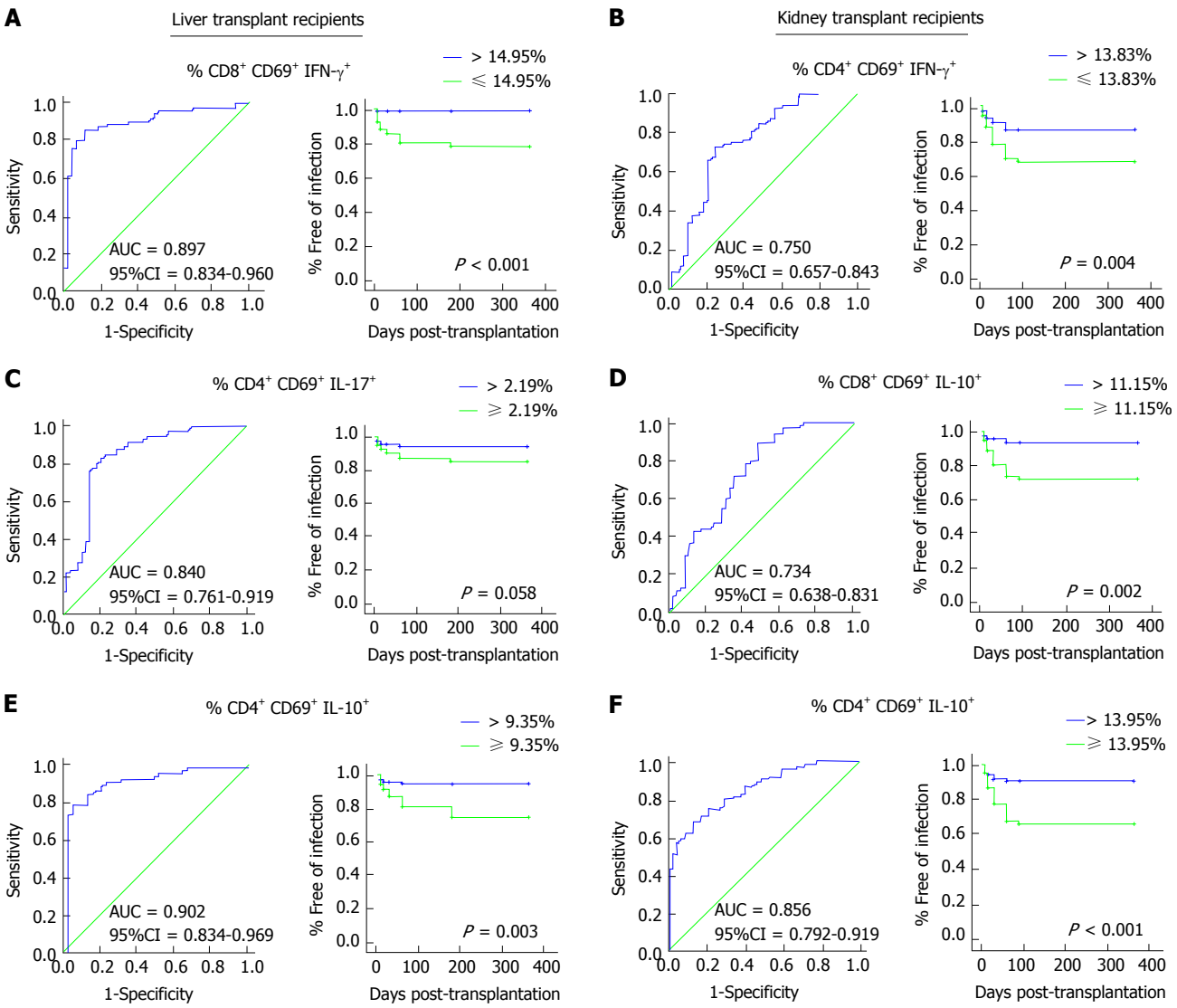

Figure 5 Post-transplantation receiver operating characteristic curve for the intracellular cytokine production capacity and the effect of the % of intracellular cytokine production capacity in stimulated T lymphocytes cut-off values for the discrimination of liver and kidney recipients likely to develop opportunistic infection between the 1st and 6th month post-transplantation (Kaplan–Meier analysis). A: Post-transplant % of $CD8^+CD69^+IFN\gamma^+$ in LTr; B: Post-transplant % of $CD4^+CD69^+IFN\gamma^+$ in KTr; C: Post-transplant % of $CD4^+CD69^+IL\text{-}17^+$ in LTr; D: Post-transplant % of $CD8^+CD69^+IL\text{-}10^+$ in KTr; E: Post-transplant % of $CD4^+CD69^+IL\text{-}10^+$ in LTr; F: post-transplant % of $CD4^+CD69^+IL\text{-}10^+$ in KTr. LTr: Liver transplant recipients; KTr: Kidney transplant recipients.

0.014 and P = 0.035, respectively). In addition to this, the percentage of total lymphocyte and serum Gamma-Glutamyl Transpeptidase (SGGT) (P = 0.022 and P = 0.035, respectively) was observed as the only clinical recipient factor having an impact in the occurrence of OI in LT. Amongst T lymphocyte subsets, LTr with a post-transplant percentage of $CD8^+CD69^+IFN\gamma^+$ below cut-off (P = 0.002) and a post-transplant percentage of $CD4^+CD69^+IL\text{-}10^+$ above cut-off (P = 0.006) resulted in a significantly worse outcome leading to an increase number of OI episodes, whereas the post-transplant percentage of $CD4^+CD69^+IL\text{-}17^+$ showed a trend (P = 0.07) towards a shorter time free of OI. Similarly, the post-transplant percentage of $CD4^+CD69^+IFN\gamma^+$ (P = 0.006), $CD4^+CD69^+IL\text{-}10^+$ (P = 0.001) and $CD8^+CD69^+IL\text{-}10^+$ (P = 0.007) in KTr also were shown as independent risk factor of OI.

In the multivariate analysis, with regards LT, the only donor factor resulting in a reduction of the risk of post-transplant OI was donor age (HR: 0.96, 95%CI: 0.93-0.99, P = 0.026). Percentage of total lymphocytes remained as a factor for a better outcome (HR: 0.94, 95%CI: 0.89-0.99, P = 0.036). On the other hand, recipient gender (HR: 4.56, 95%CI: 1.46-14.20, P = 0.009) was shown to have a negative impact in the occurrence of OI. Amongst the surrogate biomarkers for risk of infection, the imbalance between the T_H1 and T_H2 response was shown to be the most significant factor in poor post-transplant outcome (HR: 21.12, 95%CI: 2.80-159.31, P = 0.003 and HR: 2.84, 95%CI: 1.09-7.35, P = 0.032, respectively), resulting in an increased risk of overall post-transplant OI. Amongst KTr, in the multivariate analysis, TRL dose (HR: 0.92, 95%CI: 0.85-0.99, P = 0.037) and serum creatinine

Table 3 Post-transplant cut-off, area under curve, sensitivity and specificity values for the intracellular cytokine production capacity in liver transplant recipients and kidney transplant recipients as surrogate predictive biomarkers of post-transplant opportunistic infection

Biomarker	Cut-off	AUC (95%CI)	Sensitivity (95%CI)	Specificity (95%CI)
Liver transplant				
% $CD8^+CD69^+$ $IFN\gamma^+$ T Lymphocytes	14.95	0.897 (0.834-0.960)	85.71 (75.29-92.93)	88.37 (74.92-96.11)
% $CD4^+CD69^+IL\text{-}17^+$ T Lymphocytes	2.19	0.840 (0.761-0.919)	80.56 (69.53-88.94)	81.25 (67.37-91.05)
% $CD4^+CD69^+IL\text{-}10^+$ T Lymphocytes	9.35	0.902 (0.834-0.969)	78.12 (66.03-87.49)	94.87 (82.68-99.37)
Kidney transplant				
% $CD4^+CD69^+$ $IFN\gamma^+$ T Lymphocytes	13.83	0.750 (0.657-0.843)	72.37 (60.91-82.01)	75 (60.40-86.36)
% $CD4^+CD69^+IL\text{-}10^+$ T Lymphocytes	13.95	0.856 (0.792-0.919)	71.05 (59.51-80.89)	83.33 (69.78-2.52)
% $CD8^+CD69^+IL\text{-}10^+$ T Lymphocytes	11.15	0.734 (0.638-0.831)	89.33 (80.06-95.28)	52.17 (36.95-67.11)

AUC: Area under curve.

Table 4 Univariate and multivariate Cox regression model for overall opportunistic infection between 1st and 6th month post-transplantation

Predictive factors for OI	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P*-value	HR	95%CI	*P*-value
Liver transplant (n = 30)						
% $CD8^+CD69^+IFN\gamma^+$ T lymphocytes	22.56	3.01-169.56	0.002	21.12	2.80-159.31	0.003
% $CD4^+CD69^+IL\text{-}10^+$ T lymphocytes	3.77	1.46-9.75	0.006	2.84	1.09-7.35	0.032
% $CD4^+CD69^+IL\text{-}17^+$ T lymphocytes	2.43	0.93-6.35	0.07	1.37	0.45-4.16	0.584
Total leukocyte	1.08	0.98-1.18	0.111	0.95	0.78-1.17	0.666
Total lymphocytes (%)	0.94	0.90-0.99	0.022	0.94	0.89-0.99	0.023
Total lymphocyte (cells/mm^3)	1.24	1.18-1.30	0.150	1.00	0.99-1.04	0.261
Donor age	0.98	0.96-1.01	0.221	0.96	0.93-0.99	0.026
Recipient gender	2.40	0.94-6.09	0.067	4.56	1.46-14.20	0.009
TRL dose (mg/d)	0.96	0.87-1.10	0.815	0.82	0.59-1.13	0.230
MMF dose (mg/d)	0.87	0.49-1.52	0.619	0.99	0.99-1.01	0.454
Induction therapy	0.92	0.33-2.51	0.864	3.90	3.71-4.09	0.990
Post-transplant IS	1.89	0.78-4.59	0.157	1.98	0.64-6.15	0.238
Acute rejection	1.51	0.63-3.62	0.362	3.95	0.75-20.93	0.107
SALP (U/L)	1.02	0.92-1.17	0.078	1.00	0.99-1.05	0.706
SGGT (U/L)	1.19	1.13-1.25	0.035	1.01	0.91-1.03	0.333
Kindey transplant (n = 31)						
% $CD4^+CD69^+IFN\gamma^+$ T lymphocytes	2.36	1.28-4.33	0.006	3.29	1.71-6.35	< 0.001
% $CD4^+CD69^+IL\text{-}10^+$ T lymphocytes	3.49	1.68-7.30	0.001	4.76	2.05-11.08	0.003
% $CD8^+CD69^+IL\text{-}10^+$ T lymphocytes	4.17	1.49-11.68	0.007	2.52	0.74-8.57	0.139
Recipient gender	1.91	1.01-3.61	0.046	1.15	0.57-2.32	0.705
TRL dose (mg/d)	0.95	0.89-1.01	0.112	0.92	0.85-0.99	0.037
MMF dose (mg/d)	1.18	1.12-1.24	0.035	1.01	1.00-1.03	0.053
Induction therapy	1.32	0.91-1.91	0.143	1.50	0.96-2.35	0.076
Post-transplant IS	0.94	0.67-1.32	0.723	0.63	0.19-2.01	0.434
Acute rejection	1.34	0.64-2.81	0.439	1.44	0.59-3.48	0.417
Serum creatinine	1.02	1.00-1.03	0.014	1.02	1.00-1.04	0.013
SALP (U/L)	0.98	0.97-0.99	0.016	0.98	0.97-1.01	0.074
SGOT (U/L)	0.98	0.95-1.01	0.242	0.98	0.96-1.01	0.195

IS: Post-transplant immunosuppressive therapy; HR: Hazard ratio.

levels (HR: 1.02, 95%CI: 1.00-1.04, P = 0.013) were factors associated with post-transplant OI whereas MMF dose only showed a trend towards worse post-transplant outcome. (P = 0.053). The two main recipient factors associated with a worse impact to overall infection were the percentage of $CD4^+CD69^+IFN\gamma^+$ (HR: 3.29, 95%CI: 1.71-6.35, P < 0.001) and $CD4^+CD69^+IL\text{-}10^+$ (HR: 4.76, 95%CI: 2.05-11.08, P = 0.003). Post-transplant percentage of $CD8^+CD69^+IL\text{-}10^+$ T lymphocytes between months 1 and 6 was not associated with overall OI (P = 0.139). Further assessment of the presence of AR, as well as the administration of induction therapy in both LTr and KTr, showed an impact on the occurrence of post-transplant OI, in which the administration of induction therapy in KTr (HR: 1.5, 95%CI: 0.96-2.35, P = 0.076) was the only factor showing a trend towards a worse clinical outcome.

DISCUSSION

This prospective study describes the usefulness of *in vitro* stimulation of whole peripheral blood to quantify the intracellular cytokine production capacity from two independent cohorts of patients (LTr and KTr) with orthotopic liver and kidney transplantation as predictive biomarkers for overall post-transplant OI.

To our knowledge, this report is the first to analyze the impact of T_H1, T_H2 and T_H17 adaptive immune response in post-transplant OI outcome. The main objective was to analyze the occurrence of overall OI after OLT and LT, according to post-transplant percentages of $CD4^+CD69^+IFN\gamma^+$, $CD8^+CD69^+IFN\gamma^+$, $CD4^+CD69^+IL\text{-}17^+$, $CD4^+CD69^+IL\text{-}10^+$ and $CD8^+CD69^+IL\text{-}10^+$.

Our data show high occurrence of post-transplant OI due to an imbalance between T_H1/T_H2 adaptive immune response in LTr and KTr between the 1st and 6th month after transplantation. Besides, T_H17 adaptive immune response in LTr was also found to have an impact to post-transplant infection. Importantly, we have demonstrated that post-transplant percentages for both pro- and anti-inflammatory cytokine-producing T lymphocytes to be the most significant factors determining the overall OI susceptibility.

Pre-transplant levels of IFNγ, IL-17 and IL-10 producing T lymphocytes in patients with ELSF and ERSF were shown to be significantly different when compared to healthy individuals. Specifically, we found a significantly increased overall percentage of T_H1, T_H2 and T_H17 populations in LTr, however in KTr T_H1 and T_H2 cells were observed no significant; nevertheless, there was a trend towards higher percentage in ESRF patients compared to healthy individuals. This data are in concordance with previous evidence showing an increased level of T_H1 and T_H17 cells in patients with ESRF[23].

Overall, the IFNγ-dependent immune response was shown to be significantly reduced in LTr and KTr during the follow-up period compared to basal levels. This reduced IFNγ production capacity was more substantial in liver than kidney recipients but nevertheless, in both cases a significant reduction in IFNγ production capacity was found within the highest OI occurrence in post-transplant period (1st to 6th month). On the other hand, IL-17 as well as IL-10-dependant responses increased gradually from day 1 up until one year after transplantation in both types of transplants and this increase was found significant compared to pre-transplant levels. Erol *et al*[24] 2017 investigated the intracellular IFNγ and IL-17 levels in a cohort of 50 KTr during 6 mo after transplantation. No significant difference was observed between pre- and post-transplant levels at any time point. However, Loverre *et al*[25] 2011 investigated a cohort of 72 KTr in which intracellular IFNγ (T_H1), IL-4 (T_H2) and IL-17 (T_H17) production capacity was measured. Overall, they found a significant decrease in IFNγ expressing $CD4^+$ T lymphocytes at 24 mo in patients with delayed graft function (DGF) compared to pre-transplant, whereas T_H2 subset significantly increased after transplantation compared to baseline levels as measured by GATA3 protein expression in both patients with DGF as we all as acute tubular damage (ATD). Although our investigation did not compare post-transplant percentages of T lymphocytes with healthy individuals, our results shown concordance with the findings from P.J. van de Berg *et al*[26] 2012, in which they found a significantly decreased absolute number of $CD4^+$ and $CD8^+$ T cells in patients with stable graft function compared to healthy individuals at 6 mo, but more interestingly towards a differentiated and effector T-cell phenotype, specially observed in those CMV-seropositive recipients.

OI has been found to be more frequent in the first six months after orthotopic liver[27] and kidney[28] transplantation when the immunosuppressant reaches maximum levels. As shown in our results, stratification analysis showed that the percentage of $CD8^+CD69^+IFN\gamma^+$ in LTr and $CD4^+CD69^+IFN\gamma^+$ in KTr were significantly reduced in patients who developed OI. On the contrary, the IL-17 and IL-10-dependant immune response in both LTr and KTr was significantly augmented in patients who suffered OI during this period. Recently, a prospective study carried out in a cohort of 304 KTr found that recipients who subsequently developed OI had a significantly decreased count in total lymphocytes, $CD3^+$, $CD4^+$ and $CD8^+$ T-cells as well as NK-cells at month 1 post-transplantation[11]. Although, in our cohort of liver and kidney recipients the total count for both percentage and absolute number of peripheral lymphocytes was not statistically significant between both study groups, a trend was observed towards less count of total lymphocytes in patients with OI. The same study also used these T lymphocyte subpopulations as predictive biomarkers for OI, however they only took into consideration the quantitative side of the adaptive immune response against opportunistic pathogens. Thus, we believe that quantitative analysis, although rapid and affordable, could potentially miss beneficial information underlying the overall count of T $CD4^+$ and $CD8^+$. Therefore, qualitative assays which provide functional information should also be performed to monitor transplant recipients. Consequently, intra-cellular cytokine quantification was carried out in this research as an estimation of the functional adaptive immune response against opportunistic pathogens. Our group had reported the usefulness of quantifying the intracellular cytokine production as a surrogate predictive marker of adverse event, such as acute cellular rejection, in LTr and KTr[9]. Moreover, the intracellular-staining method based on flow cytometry was previously used to monitor patients infected by intracellular[29] and extracellular[30] pathogens as well as to define immune-status in non-infected individuals. In spite of the methodology requiring specific equipment for its implementation and well-trained scientists in cell culture and flow cytometry, the majority of Histocompatibility laboratories already use similar approaches for their phenotypical and functional assays leading us to believe that such methodology should not have a significant cost impact. Where implementation can prove impossible, as in the case of many small laboratories, there would be the option of using a referral laboratory service, therefore reducing any financial impact.

Our data has demonstrated the accuracy of this

cytokine-producing T cell functional assay by means of monitoring the susceptibility of post-transplant OI in LTr and KTr. As such, our auROC predictive model showed that LTr with post-transplant percentages of $CD8^+CD69^+INF\gamma^+ \leqslant 14.95\%$, $CD4^+CD69^+IL\text{-}17^+ \geqslant 2.19\%$ and $CD4^+CD69^+IL\text{-}10^+ \geqslant 9.35\%$ were stratified at high risk of OI between months 1 and 6. Similarly, KTr with post-transplant percentages of $CD4^+CD69^+INF\gamma^+ \leqslant 13.83\%$, $CD4^+CD69^+IL\text{-}10^+ \geqslant 13.95\%$ and $CD8^+CD69^+IL\text{-}10^+ \geqslant 11.15\%$ were also significantly found at high risk of OI throughout the same period of time. The deficiency of IFNγ production by stimulated $CD8^+$ T cells in LTr and $CD4^+$ T cells in KTr, in conjunction with an increased production of IL-10 cytokine by $CD3^+CD4^+CD69^+$ T cells in both types of transplant were significantly associated, confirmed by multivariate model, with a higher occurrence of overall OI and worse impact on patient wellbeing. Although post-transplant percentage of IL-17-producing $CD3^+CD4^+CD69^+$ T cells in LTr and IL-10-producing $CD3^+CD8^+CD69^+$ both had some impact on patient's outcome in univariate analysis, this effect was not seen in multivariate analysis. In line with the findings in this research, the negative effect of the imbalance of cytokine-producing T lymphocytes in the overall post-transplant OI seen at pre-transplant in our LTr and KTr had yet been seen (data not shown).

In addition, we have also reported several recipient and donor factors that still remain critical determinants for morbidity outcome in solid transplant patients. Donor age has been associated with an increased risk of OI infection in both LT and KT[31,32], amongst other donor factors, such as CMV serostatus and deceased donor source. Recipient gender[33] and immunosuppressive therapy, especially MMF[32,34], as well as induction therapy[35], have too been implicated in the susceptibility to some post-transplant OI. Our results confirm previous findings showing that donor age, recipient gender, immunosuppressive therapy with MMF and the administration of polyclonal antithymocyte globulin or basiliximab (anti-IL-2R or anti-CD25) should be taken into consideration as donor/recipient risk factors to post-transplant OI. On the other hand, we have also found serum levels of ALP and GGT in LTr and serum levels of ALP and creatinine in KTr to be associated with post-transplant OI; however, this data should be taken with caution as potential confounder may exist due to our small cohort of patients. Further investigation should be performed to elucidate these recipient factors as risks to post-transplant OI.

Analysis of our data has revealed several potential limitations. Likewise any uni-centre prospective study, the number of patients recruited to this purpose could have resulted as one limiting factor for the primary study outcome. Given that we have demonstrated significant associations in the basis of recipient/donor risk factors for post-transplant OI; these findings must be confirmed in larger (multi-center if possible) prospective study. Despite the limitations of the study.

In conclusion, our results add to the field of transplantation a validated, rapid and affordable CMI assay that provides basic functional information as to the monitoring of $CD4^+$ and $CD8^+$ T lymphocytes throughout the post-transplantation period. Based on these results, and those from recent studies, several post-transplant strategies could be proposed for the management of recipients. Particularly our study could be relevant in the setting of recipients showing an imbalance between the adaptive T_H1 and T_H2 immune response. Finally, our findings suggest that T_H17 adaptive immune response, along with several recipient characteristics and donor age should be not consider in isolation but as a whole based on recipient features.

ARTICLE HIGHLIGHTS

Research background

Nowadays liver and kidney transplant are well-established therapeutic options for patients with end stage liver and kidney diseases. However, the administration of immunosuppressant is not exempt of side effects that ultimately could lead to worse transplant outcome.

Research motivation

Monitoring of adaptive immune response by flow cytometry provides means of further understanding on how T lymphocytes vary throughout the post-transplant period.

Research objectives

In this study, the authors aim to validate the intracellular cytokine production functional assay as means of cell-mediated immunity monitoring of post-transplant patients with opportunistic infection.

Research methods

A longitudinal study was carried out in two cohorts of transplant recipients where patients were prospectively monitored for one year post-transplantation.

Research results

LTr with OI had significantly lower % of $CD8^+CD69^+IFN\gamma^+$ T cells at 60, 90 and 180 d post-transplantation. Higher % of $CD4^+CD69^+IL\text{-}10^+$ as well as $CD4^+CD69^+IL\text{-}17^+$ T cells were yet reported at 30, 60 and 90 d. KTr with OI had significantly lower % of CD4+CD69+IFNγ+ T cells at 30, 60, 90 and 180 d post-transplantation whereas IL-10-producing CD4+ and CD8+ T cells were significantly higher at 30, 90 and 180 d.

Research conclusions

The quantification of intracellular cytokine production by flow cytometry has been validated as a reliable functional assay that provides trustworthy information to a better management of transplanted patients. The occurrence of opportunistic infection was significantly correlated with an imbalance between T_H1, T_H2 and T_H17 cells in both liver and kidney transplant recipients.

Research perspectives

Post-transplant administration of immunosuppressant as well as prophylaxis therapies could be adapted according to the levels of T_H1, T_H2 T_H17 in an individual basis.

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MINIREVIEWS

Anastomotic techniques for rat lung transplantation

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Abstract

The first lung transplantation in the rat was achieved by Asimacopoulos *et al* using sutured anastomoses in 1971. Subsequent development of a cuffed technique to construct the anastomoses by Mizuta and colleagues in 1989 represented a breakthrough that resulted in simplification of the procedure and shorter warm ischemic times. Since then, a number of further variations on the technique of rat lung transplantation have been described. In spite of this, the procedure remains technically demanding and involves a long learning curve. This minireview describes the following new technical safeguards to further evolve the technique for cuffed anastomoses in rat lung transplantation: the use of anatomical landmarks to avoid twisting of the everted donor pulmonary vein and bronchus in the cuff, the use of the cuff tie as a landmark to avoid twisting of the anastomotic cuffs relative to the recipient vessels, distal ties on the recipient vessels to achieve a bloodless field and triangulation of the venotomy to avoid pulmonary vein tearing.

Key words: Lung transplantation; Rat; Surgery; Animal experiments; Technique

Core tip: This minireview describes the following new technical safeguards to further evolve the technique for cuffed anastomoses in rat lung transplantation: the use of anatomical landmarks to avoid twisting of the everted donor pulmonary vein and bronchus in the cuff, the use of the cuff tie as a landmark to avoid twisting of the anastomotic cuffs relative to the recipient vessels, distal ties on the recipient vessels to achieve a bloodless field and triangulation of the venotomy to avoid pulmonary vein tearing.

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INTRODUCTION

The first lung transplantation in the rat was achieved by Asimacopoulos *et al*[1] using sutured anastomoses in 1971. However, high complication rates and technical difficulties with the sutured anastomoses meant that this technique was not widely adopted. Subsequent development of a cuffed technique to construct the anastomoses by Mizuta *et al*[2] in 1989 represented a breakthrough that resulted in simplification of the procedure and shorter warm ischemic times. This technical breakthrough led to a surge in publications involving rat lung transplantation[3].

Since then, a number of further variations on the technique of rat lung transplantation have been described. In spite of this, the procedure remains technically demanding and involves a long learning curve[4]. Therefore many laboratories rely on dedicated microsurgeons who invest great deal of commitment and time in order to become facile with this model. However, the frequent turn-over of researchers in many laboratories calls for further technical improvements that shorten the learning curve and provide more reproducible outcomes by providing safeguards against complications. Here we describe in detail technical improvements and safeguards to further evolve the cuffed technique for rat lung transplantation.

ANASTOMOTIC CUFFS

Anastomotic cuffs should be made from PTFE, since this material was found to cause little foreign body reaction[5]. PTFE is also the most commonly used graft material for small caliber arteriovenous bypass grafting in human patients[6]. The anastomotic cuffs are made from PTFE angiocatheters that are clinically used for peripheral venous access (Exel Safelet Catheter, Exel International, Los Angeles, CA, United States). The angiocatheters are cut into sections of approximately 2 mm length, of which 1 mm forms the body of the cuff and a 1 mm elongation forms a wing (Figure 1)[7]. The wing is used to hold the cuff while the donor vessel is everted over the cuff. Additionally, we impress two grooves on the cuff with the back of a razor blade against the stylet of the angiocatheter to facilitate positioning of the circumferential ties[8]. Additionally, the surfaces of the cuff can be roughened with sandpaper to make the donor vessels less prone to slip while they are everted over the cuff[9,10].

Eighteen gauge and 16 gauge angiocatheters for the pulmonary artery (PA) and the pulmonary vein (PV) respectively for donor rats weighing approximately 300 g are used. Other investigators have used cuff sizes ranging from 24 gauge to 16 gauge for both PA and PV anastomoses[4,9-11]. We advocate against using small venous cuffs since a sufficiently large venous anastomosis is important to minimize complications from PV thrombosis. Smaller arterial cuffs are more acceptable since the pressure gradient across the arterial anastomosis is much higher than the pressure gradient across the venous anastomosis. For the bronchial anastomosis we also use 16 gauge cuffs. Others have used 14 gauge cuffs for the bronchus[9]. A large bronchial cuff size to optimize aeration of the donor lung is not unreasonable since the tough cartilaginous rings of the bronchus make this structure most resistant to tearing.

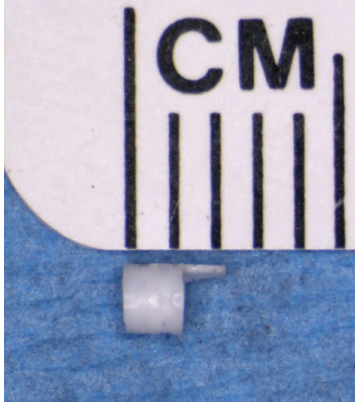

Figure 1 The anastomotic cuff.

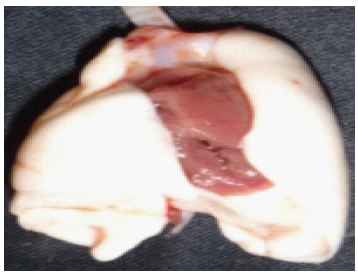

Figure 2 Donor pneumonectomy specimen.

MAGNIFICATION

Surgical magnification is necessary to perform rat lung transplantation. A 10 x dissection microscope (AmScope, Irvine, CA, United States) is ideal for hilar dissection and anastomoses. Others have described using 6-20 x magnification[4]. Ideally, frequent changing of the magnification strength should be avoided, since this negatively affects hand-eye coordination.

DONOR PROCEDURE

To prepare the donor lungs for anastomosis, it is necessary to isolate the donor PA, bronchus (B) and PV. This dissection starts with the heart-lung block in its anatomic position (Figure 2).

First, the PA is exposed by excising the thymus fat. The PA bifurcation is freed from the aortic arch by dividing the ligamentum arteriosum. The left PA is then divided at the bifurcation. Subsequently the left PA is circumferentially dissected free and traced towards the hilum of the lung.

Second, the bifurcation of the trachea is exposed by excising the surrounding mediastinal fat and lymphatic tissue. The left trachea is divided at its bifurcation and also circumferentially dissected free and traced towards

Figure 3 Eversion of the donor vein over the anastomotic cuff.

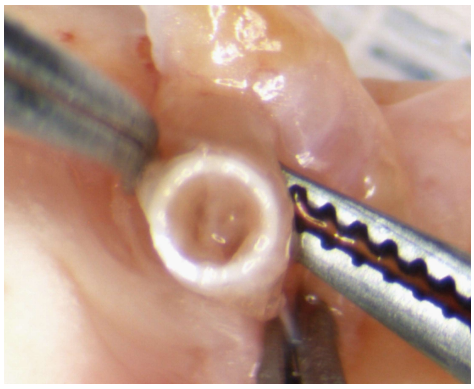

Figure 4 The pulmonary vein forms from the confluence of superior and inferior segmental veins. The ostia of these veins can be used as landmarks to avoid twisting the everted vein inside the anastomotic cuff.

the hilum of the lung.

Third, exposure of the PV, which lies dorsally, is improved by flipping the heart-lung block into prone position. The PV is freed from its attachments starting at its origin at the left atrium. Careful dissection of the vein is particularly important since the PV tears easily. Moreover, the pleura should be completely stripped from the vein in order to achieve the maximum PV length. Sufficient PV length is important to facilitate eversion of the PV over the cuff, since the elastic veins tend to retract. To free the proximal end of the vein, the left atrium is cut, leaving a rim of atrium on the ostium of the PV. The atrial rim greatly facilitates eversion of the PV over the venous cuff and does not interfere with perfusion of the anastomosis since it does not form part of the anastomotic lumen. The PV is then traced towards the hilum of the lung.

Fourth, the donor graft is freed from the remaining attachments to the heart. Each vessel is now carefully traced further towards the hilum by dissecting off the remaining parietal pleura in order to fully isolate the PA, B and PV. Isolation of the vessels serves to maximize the length of each vessel for eversion over the cuffs and prevents kinks after implantation of the graft.

ATTACHMENT OF THE ANASTOMOTIC CUFFS

Attachment of the cuffs should be performed on a tray covered with ice to control the temperature of the graft. The PV anastomosis is attached first, since the PV usually retracts to become the shortest vessel. This allows adjustment of the longer vessels to the same length as the cuffed PV. The cuff is held in place at the wing and the PV is pulled through the cuff. Next, the vein is everted over the cuff (Figure 3).

It is very important to avoid twisting the vein inside the cuff. In order to confirm that the vein is not twisted, the ostia of the superior and inferior lobar veins should be visualized inside the everted vein. If these ostia are found to be mal-aligned then the vessel should be rotated inside the cuff to orient them superiorly and inferiorly respectively (Figure 4).

When adequate orientation of the vein is confirmed, the everted vein is secured to the cuff with a circumferential tie. We use a 7-0 silk, but others have used 8-0 monofilaments[2]. The first loop secures the everted vessel to the middle of the cuff using a single knot. This loop need not enclose the entire circumference of the everted vessel as long as it holds the vessel in place. The ends of the tie are then looped around the everted vessel another time, this time close to the edge of the cuff. This loop is intended to form a circumferential seal around the everted vessel and is secured with two throws. The knots should be tied facing anterior relative to the donor lung. This allows the knot to serve as an anterior landmark to orient the cuff inside in the recipient vessel without twisting.

The bronchial cuff is attached second. Usually the bronchus is longer than the PV, so it needs to be cut to a length that is appropriate relative to the PV after it is pulled through the cuff. Twisting of the bronchus inside the cuff is avoided by orienting the membranous part of the bronchus posteriorly. The bronchus is everted over the cuff and secured to the cuff in the same fashion as the PV.

The PA cuff is attached last. The PA usually also needs to be cut to a length that is appropriate relative to the venous cuff. Analogous to the PV and bronchus, the PA is pulled through the cuff, everted and secured to the cuff in the same fashion as the PV. Special attention needs to be paid to avoid twisting the PA before everting it over the cuff, since there are no anatomical landmarks to help with its orientation. However, since the pressure inside the PA is higher than inside the PV or bronchus, perfusion of the arterial cuff is most resistant to twisting. Finally, the wings are cut off all three cuffs.

After all cuffs have been attached, the lung is re-wrapped in soaked gauze. It is important to make sure that fluid does not enter the bronchus through capillary action. This can be achieved by folding the gauze so that the hilum is left free.

RECIPIENT PROCEDURE

Exposure

First, the recipient left lung is brought into the wound by putting traction on the inferior pole of the lung with two cotton swabs. Once the inferior pole of the lung has been mobilized, the remainder of the inferior pulmonary ligament can be divided with scissors. Care must be taken not to damage the pulmonary vein, which is confluent with the inferior pulmonary ligament. The tidal volume is decreased before the recipient lung is clamped and retracted ventrally. This affords excellent exposure of the dorsal aspect of the pulmonary hilum. The pleura over the dorsal pulmonary hilum is divided. Bronchial arteries, which originate from the aorta and typically running over the membranous part of the bronchus, need to be cauterized or tied. This allows dissection of the PA, bronchus and PV. Care should be taken not to damage the vagus nerve, which is often brought over the root of the hilum by traction on the recipient lung. Once the posterior aspect of PA, bronchus and PV are dissected free, the ventral aspect of the hilum is exposed by retracting the recipient lung dorsally. The animal is also re-positioned on its back. This improves visualization of the hilum since the dissection microscope provides a top view only. Next, the pleura over the ventral aspect of the hilum is divided. Division of the hilar pleura invariably causes a pneumothorax of the accessory lobe, since the accessory lobe pleura is fused with the pleura covering the left PV. However, it is usually possible to avoid causing a right sided pneumothorax by starting the dissection of the PV hilar pleura distally and carefully preserving the pleura overlying the proximal PV, which is continuous with the right parietal pleura. Subsequent dissection completely isolates the PA, bronchus and PV. Particular care should be taken while dissecting the pulmonary vein, since it can tear easily. The goal of this dissection should be to provide maximum length of the recipient hilar vessels, since this makes it easier to create the anastomoses. In order to further increase the length of the donor vessels, the right accessory lobe vein can be divided[11] or interposition grafts can be fashioned from the donor descending aorta[7]. These additional steps are not necessary in rats weighing 300 g since sufficient length can be achieved by careful dissection.

CREATION OF THE ANASTOMOSES

The PV anastomosis is technically the most difficult because the recipient vein tears easily. We create the PV anastomosis first, since this affords the greatest degree of freedom to position the graft favorably so that the PV cuff can be pushed into the recipient vein without tension. It is important to avoid twisting the vein during creation of the anastomoses since the low blood pressure in the PV means that blood flow is compromised by relatively modest twisting or kinking. In order to minimize the potential for PV twisting or kinking, others have advocated constructing the bronchial anastomosis first to immobilize the graft before the PV anastomosis is constructed[9].

The PV typically forms from the confluence of an upper and a lower segmental vein. Rarely, the PV forms from the confluence of 3 or more veins. First, the upper segmental vein is divided between two ligatures. This increases the length of the vessel that is available to create the anastomosis. Moreover, leaving one strand of the proximal ligature long allows it to serve as a handle to triangulate the venotomy. Second, the distal PA is tied off with 7-0 silk to stop inflow of blood into the lung. It is important that this tie sits as distal as possible to maximize the length of PA available for anastomosis. Third, the remaining segmental PVs are tied off as distal as possible and the main PV is clamped as proximal as possible. This prevents antegrade or retrograde flow of blood into the part of the PV that will be used for the anastomosis. A bloodless represents an important safeguard for creating the anastomosis without tearing since it improves visualization. Fourth, the pulmonary vein is opened transversely with a microscissor. Again the venotomy should be as distal as possible to ensure sufficient length of the vessel is available to insert the cuff. The venotomy is then extended longitudinally along the vein to the ostium of the tied superior segmental vein. This maneuver opens a right-angled flap in the vein and allows triangulation of the venotomy between the long suture handle on the superior segmental vein, the intact back wall of the vein and a forceps holding the tip of the flap (Figure 5).

Triangulation is another important safeguard against tearing the fragile vein by opening a wide mouthed venotomy. Fifth, all blood is washed from the venotomy with heparinized saline. We use 50u, but others have used 500u[11]. The heparinized saline also displaces any air in the vein and thus acts as a safeguard against thrombi and against air emboli. Similarly, the donor PV cuff is filled with heparinized saline to displace any air. Sixth, the donor lung is brought up to the field and sandwiched between cooled wet gauze sponges. The graft is positioned such that the PV cuff is not under tension or twisted when it is inserted into the recipient vein. Finally, the cuff is then inserted into the venotomy and pushed into the recipient main PV. The correct orientation is preserved by using the knot on the anastomotic cuff as an anterior landmark. It is important to make sure that the cuff projects into the main PV to avoid any constriction of the graft venous outflow. The cuff is secured with a circumferential 6-0 silk tie.

The bronchial anastomosis is created second. In small rats it can be beneficial not to clamp the bronchus to avoid cluttering the field with clamps. In this case, the bronchial anastomosis needs to be completed quickly since the native contralateral lung is not ventilated while the left bronchus is open. First, the donor lung is re-

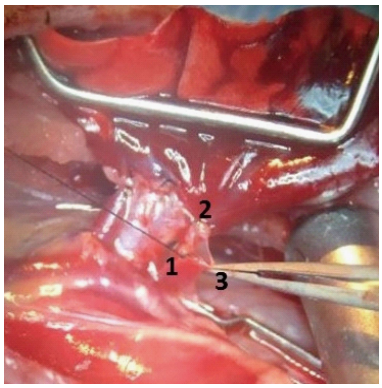

Figure 5 Triangulation of the venotomy by the superior segmental vein ligature (1), pulmonary vein back wall (2) and the tip of the flap (3) results in a wide-mouthed venotomy and serves as a safeguard that allows easy insertion of the donor cuff without tearing the recipient vessel.

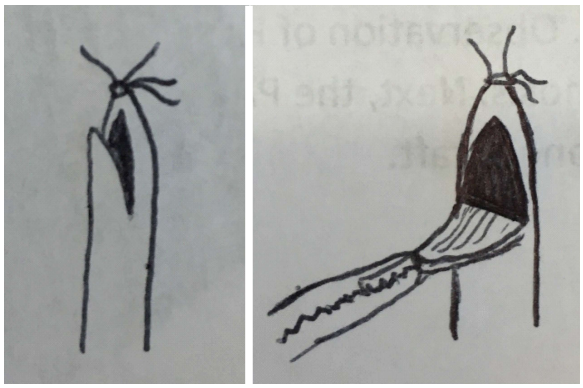

Figure 6 Oblique incision creates a V-shaped flap. Retraction on this flap with forceps results in a wide mouthed arteritomy that allows easy insertion of the donor cuff.

positioned so that there is no tension on the bronchial cuff after insertion into the bronchus. Avoiding tension prevents the cuff from slipping out of the recipient bronchus while the anastomosis is created. Second, the bronchus is incised distally between two cartilaginous rings. Intact cartilaginous ring make the bronchiotomy relatively resistant to tearing. Third, the cuff is inserted into the bronchus. The correct orientation is preserved by using the knot on the anastomotic cuff as an anterior landmark. Finally, the cuff is secured with a 6-0 silk tie. Following creation of the bronchial cuff, the tidal volume should be increased to baseline in order to accounting for the dead space of the non-perfused donor lung. Rarely, the bronchial anastomosis is constructed with sutures[4,12]. Studies with computer tomography have noted that rat bronchial anastomoses created by suturing have a trend to be wider than cuffed bronchial anastomoses, however this difference was not statistically significant[13]. We have not noted major problems with aeration of the graft if a sufficiently large cuff is used. Large cuffs can be used easily for the bronchial anastomosis since the bronchus is relative resistant to tearing.

The PA anastomosis is created third. First, the donor lung is again re-positioned to allow creation of the PA anastomosis without tension. Second, the artery is clamped proximally to control inflow into the segment that will be used to construct the anastomosis. Third, a V-shaped arteriotomy is made as close to the tie as possible to maximize the available length for anastomosis (Figure 6). This creates a wide mouthed arteriotomy. Fourth, the PA lumen is flushed with heparinized saline to remove any clots that may have formed in the occluded vessel and to displace any air. Similarly, air is displaced from the donor PA cuff by filling it with heparinized saline. The apex of the V-shaped flap provides a convenient handle for retraction to open a wide mouthed arteriotomy. Fifth, PA cuff is inserted into the PA. The correct orientation is preserved by using the knot on the anastomotic cuff as an anterior landmark. Finally, the cuff is secured with a circumferential 6-0 silk tie. Insertion of the PA cuff is easier than insertion of the venous cuff because the PA is elastic and far less prone to tears.

After all anastomoses have been completed, the old recipient lung is removed by cutting PA, bronchus and PV distal to the new anastomoses. This removes tension from the hilar vessels and allows the donor graft to sit in the orthotropic position. The donor lung is recruited by occluding the ventilator outflow in order for approximately 3 breaths. Recruitment of the graft before the clamps are released decreases the pulmonary vascular resistance of the donor lung and ensures homogenous reperfusion. The PV clamp is released first. This allows observation of retrograde blood flow through the cuff into the donor PV and segmental PV branches. Observation of PV backflow is an important safeguard for the quality of the venous anastomosis. Second, the PA clamp is released. This results in an immediate blush that indicates perfusion of the donor graft. Observation of a blush is an important safeguard for the quality of the arterial anastomosis.

CONCLUSION

Development of the cuff technique for lung transplantation by Mizuta popularized this model[2]. This technique was evolved by adding important safeguards to shorten the learning curve and result in more reproducible outcomes by providing safeguards against complications. This review describes highlight technical safeguards that were previously described and also describes the following new safeguards as improvements to the technique for lung transplantation: The use of anatomical landmarks to avoid twisting of the everted donor PV and bronchus in the cuff, the use of the cuff tie as a landmark to avoid twisting of the anastomotic cuffs with regards to the recipient vessels, distal ties on the recipient vessels to achieve a bloodless field and triangulation of the venotomy to avoid PV tearing.

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ORIGINAL ARTICLE

Observational Study

Cumulative positive fluid balance is a risk factor for acute kidney injury and requirement for renal replacement therapy after liver transplantation

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Abstract

AIM

To analyze whether fluid overload is an independent risk factor of adverse outcomes after liver transplantation (LT).

METHODS

One hundred and twenty-one patients submitted to LT were retrospectively evaluated. Data regarding perioperative and postoperative variables previously associated with adverse outcomes after LT were reviewed. Cumulative fluid balance (FB) in the first 12 h and 4 d after surgery were compared with major adverse outcomes after LT.

RESULTS

Most of the patients were submitted to a liberal approach of fluid administration with a mean cumulative FB

over 5 L and 10 L, respectively, in the first 12 h and 4 d after LT. Cumulative FB in 4 d was independently associated with occurrence of both AKI and requirement for renal replacement therapy (RRT) (OR = 2.3; 95%CI: 1.37-3.86, P = 0.02 and OR = 2.89; 95%CI: 1.52-5.49, P = 0.001 respectively). Other variables on multivariate analysis associated with AKI and RRT were, respectively, male sex and Acute Physiology and Chronic Health Disease Classification System (APACHE II) levels and sepsis or septic shock. Mortality was shown to be independently related to AST and APACHE II levels (OR = 2.35; 95%CI: 1.1-5.05, P = 0.02 and 2.63; 95%CI: 1.0-6.87, P = 0.04 respectively), probably reflecting the degree of graft dysfunction and severity of early postoperative course of LT. No effect of FB on mortality after LT was disclosed.

CONCLUSION

Cumulative positive FB over 4 d after LT is independently associated with the development of AKI and the requirement of RRT. Survival was not independently related to FB, but to surrogate markers of graft dysfunction and severity of postoperative course of LT.

Key words: Liver transplantation; Fluid balance; Acute kidney injury

Core tip: Whether fluid overload is an independent mediator of adverse outcomes on early postoperative liver transplantation (LT). The influence of fluid accumulation on morbidity and mortality after LT has not been well evaluated up to now. This study aims to analyze whether fluid management influences the early postoperative outcome after LT. Cumulative positive fluid balance (FB) over 4 d after LT influence the development of acute kidney injury and it is a risk factor for the requirement for renal replacement therapy. Survival is not independently related to FB but to surrogate markers of graft dysfunction.

Codes L, de Souza YG, D'Oliveira RAC, Bastos JLA, Bittencourt PL. Cumulative positive fluid balance is a risk factor for acute kidney injury and requirement for renal replacement therapy after liver transplantation. *World J Transplant* 2018; 8(2): 44-51 Available from: URL: http://www.wjgnet.com/2220-3230/full/v8/i2/44.htm DOI: http://dx.doi.org/10.5500/wjt.v8.i2.44

INTRODUCTION

It is well recognized that fluid overload in critically-ill patients may lead to anasarca, pulmonary edema, abdominal compartment syndrome (ACS) and also multiple organ dysfunction due to its deleterious effect in tissue perfusion[1-3]. In this regard, positive fluid balance (FB) has been shown to be associated with adverse outcomes in patients admitted to the intensive care unit (ICU) with sepsis and septic shock[4-6], acute respiratory distress syndrome (ARDS)[7,8], acute kidney injury (AKI)[9-15] and cancer[16]. Conversely, positive FB was also linked to increased morbidity and mortality after abdominal surgery[17-19], including esophagectomy[20], open aortic aneurysm repair[21] and rectal cancer surgery[22]. In most of these reports cumulative FB in the first 4 d were reliable indicators of worse outcomes in clinical[4] and surgical[21] ICU patients. On the contrary, restrictive fluid administration policies have led to a reduction in overall morbidity, including AKI, and increased survival in surgical[23-26] and medical[9,27,28] patients in the ICU. Few data is available in the literature concerning the impact of positive FB in the postoperative course of liver transplantation (LT)[29-32]. Some authors have described an increased frequency of postoperative pulmonary morbidity[29-32] and ileus[31] that could be prevented with restrictive administration of fluids[30,31]. No association between FB and AKI or survival after LT was disclosed in those aforementioned studies[29-32].

The aims of the present study were analyze whether cumulative positive FB is associated with the occurrence of AKI, requirement for renal replacement therapy (RRT) and 28-d mortality after LT.

MATERIALS AND METHODS

One-hundred twenty one patients submitted to LT at the Portuguese Hospital of Salvador, Bahia, Brazil who underwent surgery in a period of 5 years were retrospectively evaluated. All medical and surgical charts as well as electronic files were reviewed by a single observer in order to collect data regarding perioperative and postoperative variables, previously associated with adverse outcomes after LT, including demographics; etiology of liver disease; indication for LT; severity of liver disease assessed by MELD and Child-Pugh scores; perioperative parameters such as cold ischemia time, duration of surgery, need for inotropic support, FB and use of vasoactive drugs; Acute Physiology and Chronic Health Disease Classification System (APACHE II) score, peak lactate, AST and ALT levels; occurrence of postoperative complications, including early allograft dysfunction (EAD) and primary graft non-function (PGNF), biliary strictures or leaks, hepatic artery thrombosis or stenosis, AKI and requirement for RRT, acute rejection, sepsis and septic shock; cumulative FB in the first 12 h and 4 d; length of stay (LOS) in the ICU and in the hospital; mortality and causes of death in the first 28 d. The patients were evaluated in a single admission, when they entered the hospital to be transplanted

Child-Pugh, MELD and APACHE II scores were calculated as previously described[33-35]. Early allograft dysfunction was defined according to the definition of Olthoff *et al*[36] and PGNF was defining as EAD

Table 1 Baseline characteristics before liver transplantation (n = 121)

Male sex	106 (88%)
Age (yr)	50 ± 13
Etiology of chronic liver disease	
Hepatitis C	39 (32%)
Hepatitis C and alcoholic liver disease	12 (10%)
Alcoholic liver disease	36 (30%)
Cryptogenic and/or non-alcoholic steatohepatitis	10 (8%)
Hepatitis B	4 (3%)
Cholestatic liver disease	6 (5%)
Autoimmune hepatitis	4 (3%)
Others	10 (8%)
Indication for liver transplantation	
Decompensated cirrhosis	93 (77%)
Hepatocellular carcinoma	28 (23%)
Severity of liver disease at admission	
Child-Pugh score	9 ± 2
MELD score	18 ± 6

Data are expressed as mean ± SD. MELD: Model for end-stage liver disease.

Table 2 Intraoperative and postoperative features of the patients submitted to liver transplantation (n = 121)

Cold ischemia time (min)	520 ± 170
Duration of surgery (min)	333 ± 104
Use of blood products	77 (63%)
Number of packed red blood cell units	1.9 ± 3.1
Use of vasoactive drugs (norepinephrine)	38 (31%)
Peak of arterial lactate in the first 24 h (mmol/L)	2.3 ± 2.0
APACHE II score 24 h after admission	15 ± 4
Peak of AST levels (U/L)	3058 ± 4820
Peak of ALT levels (U/L)	1357 ± 1542
Postoperative complications	
Early allograft dysfunction	26 (22%)
Primary graft non-function	7 (6%)
Biliary strictures and/or leaks	5 (4%)
Arterial thrombosis or stenosis	5 (4%)
Acute rejection	32 (26%)
Sepsis or septic shock	38 (31%)
AKI	87 (72%)
AKI type 1	0
AKI type 2	44 (36%)
AKI type 3	43 (36%)
RRT	26 (22%)
Fluid balance (mL)	
Intraoperative	3829 ± 1904
Cumulative FB in the first 12 h	5473 ± 2417
Cumulative FB in the first 4 d	10956 ± 5117
Length of stay in ICU (d)	12 ± 11
Length of stay in the hospital (d)	19 ± 12
Mortality	11 (9%)

Data are expressed as mean ± SD. APACHE II: Acute Physiology and Chronic Health Disease Classification System; AKI: Acute kidney injury; RRT: Renal replacement therapy; FB: Fluid balance; ICU: Intensive care unit.

leading to death or retransplantation. The definition of AKI was based on The Kidney Disease Improving Global Outcomes (KDIGO) criteria published 2012[37]. Patients were evaluated by a nephrologist when dialysis was indicated. Fluid balance was defined as the difference between oral intake and/or intravenous fluid administration and urine output. Other potential causes for fluid losses including nasogastric aspirates, vomiting or diarrhea were not recorded. All patients received either normal saline or Ringer's lactate solution. Cumulative FB was calculated arbitrarily 12 h and 4 d after LT in order to evaluate the impact of fluid administration early in the postoperative period and thereafter after the initial phases of volume resuscitation. Cumulative FB in those chosen periods after admission to the ICU were also previously associated with adverse outcomes in other reports[4,26,30].

Cumulative FB in the first 12 h and 4 d after surgery were compared with three major adverse outcomes after LT, including the occurrence of AKI, requirement for RRT and 28-d mortality, as well as the other aforementioned variables previously known to influence morbidity and mortality after LT.

All patients granted informed consent at hospital admission. The study was approved by the Ethics Committee in Research of the Portuguese Hospital of Salvador, Bahia.

Statistical analysis

Descriptive analysis was performed. Continuous variables were expressed as mean ± SD and categorical variables as proportions. Univariate analysis of perioperative and postoperative parameters was tested using χ^2 test or the Fisher exact probability test when appropriate. Continuous variables were compared using the Mann-Whitney test[38]. Multivariate analysis using stepwise logistic regression was performed to evaluate the specific effect of each predictor[39]. Variables included in the multivariate model were those that achieved significance level of $P < 0.20$ in the univariate analysis. P value equal or less than 0.05 were considered significant. 95% confidence intervals were reported, when appropriate. The analysis of the residues was included in the steps of the logistic regression. All statistical analysis was performed using SPSS version 17.0 for Windows (SPSS Inc, Chicago, IL, United States).

RESULTS

Baseline clinical and laboratory data of those 121 patients included in the study are depicted in Table 1. Briefly most of the patients were males with a mean age of 50 ± 13 years and had decompensated cirrhosis (77%) due to hepatitis C and or alcoholic liver disease (72%) with mean Child-Pugh and MELD, respectively, of 9 ± 2 and 18 ± 6 (Table 1). The perioperative and postoperative information concerning the clinical course of those subjects are summarized in Table 2. Median cold ischemia time and duration of surgery were 520 ± 170 and 333 ± 104 min respectively. High peak AST and ALT levels were observed (Table 2) and the frequencies of EAD and PGNF encountered were 22% and 6%, respectively. Cumulative FB observed in the first 12 h and 4 d were, respectively, 5573 ±

Table 3 Comparison of baseline, intra-operative and postoperative features of patients submitted to liver transplantation according to the presence of acute kidney injury

	No AKI (n = 34)	AKI (n = 87)	P value
Age (yr)	51 ± 13	50 ± 12	0.643
Male sex	24 (71%)	82 (94%)	0.0001
Child-Pugh score at admission	8 ± 2	10 ± 2	0.840
MELD score at admission	17 ± 6	18 ± 6	0.868
APACHE II score 24 h after admission	14 ± 3	15 ± 4	0.142
Cold ischemia time (min)	537 ± 187	513 ± 164	0.267
Duration of surgery (min)	324 ± 131	336 ± 92	0.439
Use of blood products	59%	66%	0.490
Number of packed red blood cell units	1.0 ± 1.7	2.2 ± 3.4	0.010
Use of vasoactive drugs	6 (18%)	32 (37%)	0.032
Peak of arterial lactate in the first 24 h (mmol/L)	2.2 ± 1.4	2.4 ± 2.2	0.208
Peak AST levels (U/L)	1789 ± 1524	3535 ± 5511	0.022
Postoperative complications			
Early allograft dysfunction	3 (4%)	24 (28%)	0.019
Biliary strictures and/or leaks	1 (3%)	4 (5%)	0.567
Arterial thrombosis or stenosis	2 (6%)	3 (3%)	0.433
Acute rejection	10 (29%)	22 (25%)	0.402
Sepsis or septic shock	8 (24%)	30 (34%)	0.305
Cumulative FB in the first 12 h	4780 ± 1673	5743 ± 2610	0.050
Cumulative FB in the first 4 d	8690 ± 3463	11841 ± 5395	0.050
Length of stay in ICU (d)	8 ± 8	13 ± 11	0.087
Length of stay in the hospital (d)	15 ± 7	20 ± 12	0.001
Mortality	1 (3%)	10 (12%)	0.128

Data are expressed as mean ± SD. AKI: Acute kidney injury; MELD: Model for end-stage liver disease; APACHE II: Acute Physiology and Chronic Health Disease Classification System; FB: Fluid balance; ICU: Intensive care unit.

2417 and 10956 ± 5117 mL. AKI occurred in 87 (72%) patients, all with either type 2 (n = 44) or type 3 (n = 43) AKI. Twenty six patients required RRT 4 ± 2 d after surgery. The LOS in the ICU and in the hospital was, respectively, 12 ± 11 d and 19 ± 12 d. Eleven (9%) patients died due to PGNF (n = 7), septic shock (n = 2) and intraabdominal bleeding (n = 1) 10 9 d after surgery (Table 2).

The occurrence of AKI was associated with male sex (94% *vs* 71% of the patients without AKI, P = 0.0001), number of packed red blood cells transfused (2.2 ± 3.4 *vs* 1.0 ± 1.7 of subjects without AKI, P = 0.01), use of norepinephrine (37% *vs* 18% of patients without AKI, P = 0.032), peak AST levels (3535 ± 5511 *vs* 1789 ± 1524 of patients without AKI, P = 0.022), occurrence of EAD (28% *vs* 4% of patients without AKI) and cumulative FB in the first 12 h (5743 ± 2610 mL *vs* 4780 ± 1673 mL of patients without AKI, P = 0.05) and 4 d (11841 ± 5395 mL *vs* 8690 ± 3469 mL of patients without AKI, P = 0.05) (Table 3), but the difference remained significant in the multivariate analysis only for male sex and cumulative FB over 4 d.

In the univariate analysis, RRT was related to male sex (100% *vs* 84% in patients without RRT, P = 0.0001), APACHE II levels (18% ± 6% *vs* 14% ± 4% in patients without RRT, P = 0.03), use of blood products (81% *vs* 59% in patients without RRT, P = 0.03), use of norepinephrine (50% *vs* 26% in patients without RRT, P = 0.02), peak levels of arterial lactate in the first 24 h (3.3 ± 3.5 mmol/l *vs* 2.1 ± 1.3 mmol/L in patients without RRT, P = 0.0001), peak of AST level (6599 ± 9060 U/L *vs* 2144 ± 2157 U/L, in patients without RRT, p 0.0001), occurrence of EAD (50% *vs* 15% in patients without RRT, P = 0.0001), septic shock (58% *vs* 24% in patients without RRT, P = 0.0001), cumulative FB in the first 12 h (7146 ± 2538 mL *vs* 5014 ± 2181 mL in patients without RRT, P = 0.005) and cumulative FB over 4 d (14924 ± 7345 mL *vs* 9868 ± 3677 mL in patients without RRT, P = 0.0001) (Table 4). As expected, mortality (35% *vs* 2% in patients without RRT, P = 0.0001), LOS in the ICU (20 ± 14 *vs* 9 ± 9 in patients without RRT, P = 0.002) and in the hospital (24 ± 14 *vs* 17 ± 10 in patients without RRT, P = 0.007) were significantly increased in those patients requiring RRT (Table 4). However, only APACHE II levels, occurrence of sepsis or septic shock and cumulative FB in the first 4 d remained significant variables related to RRT in the multivariate analysis.

In respect to mortality in 28 d (Table 5), univariate analysis revealed an association with the number of packed red blood cell units transfused (3.6 ± 6 units *vs* 1.7 ± 2.6 units in survivors, P = 0.0001), peak of arterial lactate in the first 24 h (4.9 ± 4.2 mmol/L *vs* 2.1 ± 1.4 mmol/L in survivors, P = 0.0001), peak AST levels (11289 ± 13591 U/L *vs* 2372 ± 2280 U/L in survivors, P = 0.0001), EAD (72% *vs* 17% in survivors, P = 0.0001), acute rejection (0% *vs* 29% in survivors, P = 0.03), cumulative FB in 4 d (19073 ± 9416 mL *vs* 10144 ± 3656 mL in survivors, P = 0.00001), RRT (82% *vs* 15% in survivors, P = 0.001) (Table 5), but only APACHE II and AST levels remained significant in the multivariate analysis (Table 6).

Table 4 Comparison of baseline, intra-operative and postoperative features of patients submitted to liver transplantation according to requirement of renal replacement therapy

	No RRT (n = 95)	RRT (n = 26)	P value
Age (yr)	49 ± 12	53 ± 12	0.960
Male sex	80 (84%)	26 (100%)	0.0001
Child-Pugh score at admission	9 ± 2	10 ± 2	0.800
MELD score at admission	18 ± 6	19 ± 7	0.420
APACHE II 24 h after admission	14 ± 4	18 ± 6	0.030
Cold ischemia time (min)	506 ± 166	587 ± 175	0.470
Duration of surgery (min)	322 ± 103	372 ± 102	0.500
Use of blood products	59%	81%	0.030
Number of packed red blood cell units	1.6 ± 2.7	2.7 ± 4.2	0.080
Use of vasoactive drugs	25 (26%)	13 (50%)	0.020
Peak of arterial lactate in the first 24 h (mmol/L)	2.1 ± 1.3	3.3 ± 3.5	0.0001
Peak AST levels (U/L)	2144 ± 2157	6599 ± 9060	0.0001
Postoperative complications			
Early allograft dysfunction	14 (15%)	13 (50%)	0.0001
Biliary strictures and/or leaks	3 (3%)	2 (8%)	0.292
Arterial thrombosis or stenosis	5 (5%)	0 (0)	0.290
Acute rejection	27 (28%)	5 (19%)	0.249
Sepsis or septic shock	23 (24%)	15 (58%)	0.0001
Cumulative FB in the first 12 h	5014 ± 2181	7146 ± 2538	0.005
Cumulative FB in the first 4 d	9868 ± 3677	14924 ± 7345	0.0001
Length of stay in ICU (d)	9 ± 9	20 ± 14	0.002
Length of stay in the hospital (d)	17 ± 10	24 ± 14	0.007
Mortality	2 (2%)	9 (35%)	0.0001

Data are expressed as mean ± SD. RRT: Renal replacement therapy; MELD: Model for end-stage liver disease; APACHE II: Acute Physiology and Chronic Health Disease Classification System; FB: Fluid balance; ICU: Intensive care unit.

Table 5 Comparison of baseline, intra-operative and postoperative features of patients submitted to liver transplantation according to mortality in 28 d

	Survivors (n = 110)	Non survivors (n = 11)	P value
Age (yr)	50 ± 12	52 ± 13	0.780
Male sex	95 (86%)	11 (100%)	0.218
Child-Pugh score at admission	9 ± 2	10 ± 3	0.360
MELD score at admission	18 ± 6	19 ± 9	0.060
APACHE II 24 h after admission	14 ± 3	21 ± 6	0.060
Cold ischemia time (min)	512 ± 167	628 ± 177	0.080
Duration of surgery (min)	324 ± 98	417 ± 130	0.060
Use of blood products	64%	64%	0.620
Number of packed red blood cell units	1.7 ± 2.6	3.6 ± 6	0.000
Use of vasoactive drugs	32(29%)	6 (55%)	0.090
Peak of arterial lactate in the first 24 h (mmol/L)	2.1 ± 1.4	4.9 ± 4.2	0.0001
Peak AST levels (U/L)	2372 ± 2280	11289 ± 13591	0.0001
Postoperative complications			
Early allograft dysfunction	19 (17%)	8 (72%)	0.0001
Biliary strictures and/or leaks	5 (5%)	0	0.620
Arterial thrombosis or stenosis	5 (5%)	0	0.620
Acute rejection	32 (29%)	0	0.030
Sepsis or septic shock	34 (31%)	4 (36%)	0.740
Cumulative FB in the first 12 h	5205 ± 2233	8140 ± 2677	0.600
Cumulative FB in the first 4 d	10144 ± 3656	19073 ± 9416	0.00001
Length of stay in ICU (d)	12 ± 10	14 ± 11	0.360
Length of stay in the hospital (d)	18 ± 11	13 ± 10	0.070
AKI	77 (70%)	10 (90%)	0.140
RRT	17 (15%)	9 (82%)	0.0001

Data are expressed as mean ± SD. LT: Liver transplantation; MELD: Model for end-stage liver disease; APACHE II: Acute Physiology and Chronic Health Disease Classification System; FB: Fluid balance; ICU: Intensive care unit; AKI: Acute kidney injury; RRT: Renal replacement therapy.

DISCUSSION

Despite the development of several strategies to assess fluid responsiveness[40], fluid administration in the ICU remains largely empirical in daily practice. It is usually guided by bedside simple hemodynamic and laboratory parameters and urine output measurement. Early-goal directed therapy using large volume of

Table 6 Multivariate analysis of predictors of acute kidney injury, renal replacement therapy and mortality of patients submitted to liver transplantation

	Odds ratio	95%CI	*P* value
AKI			
Male sex	9.29	1.48-58.24	0.017
Cumulative FB in the first 4 d	2.3	1.37-3.86	0.020
RRT			
APACHE II 24 h after admission	2.5	1.36-4.62	0.003
Sepsis or septic shock	14.7	0.99-2.18	0.050
Cumulative FB in the first 4 d	2.89	1.52-5.49	0.001
Mortality			
AST levels (U/L)	2.35	1.1-5.05	0.020
APACHE II 24 h after admission	2.63	1.0-6.87	0.040

AKI: Acute kidney injury; RRT: Renal replacement therapy; APACHE II: Acute Physiology and Chronic Health Disease Classification System; FB: Fluid balance.

fluids to restore tissue perfusion in sepsis and septic shock has been shown to improve survival[41] and it is still considered today as the cornerstone of resuscitation in septic shock and sepsis-induced tissue hypoperfusion[42]. It may lead however, on the other hand, to post-resuscitation fluid overload with its detrimental effect in tissue perfusion leading to organ dysfunction and failure[1-3]. In surgical patients, fluid overload, usually assessed by cumulative FB, has been associated with impaired wound healing, ACS, postoperative pulmonary morbidity, as well as AKI with a detrimental influence not only on morbidity[1-3,10,11], but also on patient survival[13]. On the contrary, a restrictive approach on fluid administration has been shown to improve morbidity after major surgery, including LT[17,29-32], and mortality[17]. Concerning the influence of FB on the outcome of LT, other authors have shown detrimental effects of a cumulative positive FB concerning postoperative pulmonary complications and ileus[29-32]. Jiang *et al*[29], have demonstrated that a negative FB in the first 3 d after LT was linked to a decrease the frequency of early pulmonary complications. Lin *et al*[32] furthermore have described an increased incidence of postoperative pulmonary morbidity in patients submitted to LT who received a large amount of fluids and blood transfusions intraoperatively. Not surprisingly, protection from postoperative pulmonary morbidity was related to a negative FB in the first three days after LT. Later on, the same group of investigators demonstrated that employment of more than 100 mL/kg of blood transfusion intraoperatively and a FB equal or less than -14 mL/kg per day in the first three days after LT were inversely associated with postoperative pulmonary complications, when assessed by extubation time. Beneficial effects were also observed in frequency of postoperative ileus and ICU LOS. Reydellet *et al*[31] performed a before and after study comparing two resuscitation protocols after LT. The patients submitted to a liberal approach of fluid administration had significantly increased cumulative FB at 24 and 48 h when compared to their counterparts submitted to a more restricted fluid approach per protocol. Those patients submitted to a more restricted fluid approach had fewer days on mechanical ventilation and on postoperative ileus. None of the authors have investigated the influence of FB in the development of AKI after LT.

In the present study, most of the patients were submitted to a liberal approach of fluid administration with a mean cumulative FB over 5 and 10 L in the first 12 h and 4 d after LT. Several preoperative and postoperative variables were associated either with development of AKI and/or requirement for RRT, but only cumulative FB in 4 d were independently associated with occurrence of both AKI and requirement for RRT. Other variables on multivariate analysis associated with AKI and RRT were, respectively, male and APACHE II levels and sepsis or septic shock. Mortality was shown to be independently related to AST and APACHE II levels, probably reflecting the degree of graft dysfunction and severity of early postoperative course of LT. No effect of FB on mortality after LT was disclosed in the present study.

Although there is an increasing interest in the use of biomarkers to help identify AKI at an earlier stage, they were not used in the study. Patients with cirrhosis frequently have predisposing factors for the development of kidney diseases, such as advanced age, diabetes, and hypertension. In addition, specific liver diseases may be associated with kidney disease, such as HBV/HCV-associated glomerulonephritis or alcohol-related IgA nephropathy. In this study, the definition of AKI was based on The Kidney Disease Improving Global Outcomes criteria. This definition has been validated and it considers increases in serum creatinine from baseline known or presumed to have occurred within the prior 7 d. Early recognition of AKI in cirrhosis or in post-transplant is important in order to avoid factors that may contribute to further deterioration of renal function and to initiate appropriate management.

One of the major limitations of the present study is its retrospective design as well as the number of patients included in our cohort. We tried to control confounding variables through multivariate analysis. The authors also have to acknowledge that it is difficult to determine in such a study design whether cumulative FB may be a cause or consequence of disease severity or of AKI development, as postoperative resuscitation protocols were not standardized. However, our results do corroborate the detrimental effects of cumulative FB on the occurrence of AKI and requirement of RRT after LT, as demonstrated in several other clinical scenarios in the ICU[10-13,27,43].

In summary, cumulative positive fluid balance over 4 d after LT influence the development of AKI and is a risk factor for requirement of RRT. No effect on patient survival was independently related to FB, but to surrogate markers of graft dysfunction and severity of postoperative course of LT.

ARTICLE HIGHLIGHTS

Research background

Liver transplantation (LT) has become an option in treating a wide variety of liver diseases. Patients undergoing LT are at high risk of perioperative complications and death. Recently, there has been considerable interest in perioperative fluid therapy following major surgeries. Important question is whether fluid overload is an independent risk factor for adverse outcomes after LT. Previous reports indicate that restrictive strategy of fluids in surgical patients is beneficial. The influence of fluid accumulation on morbidity and mortality after LT has not been well evaluated up to now.

Research objectives

The aim of the study was to analyze whether cumulative positive fluid balance (FB) is associated with the occurrence of adverse outcomes after LT.

Research methods

Patients were retrospectively evaluated. In the present study, most of the patients were submitted to a liberal approach of fluid administration. Accumulated fluid balance (acFB), assessed within the first 12 hours and the 4 days following surgery, was compared with major adverse outcomes after LT.

Research results

Cumulative positive FB over 4 d after LT influences the development of acute kidney injury and it is a risk factor for the requirement for dialysis. No effect on patient survival was independently related to fluid balance.

Research conclusions

Our results show that fluid overload is a marker of severity of illness.

Research perspectives

We hope that these results may contribute to the management of liver grafted patients.

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REVIEW

Kidney exchange transplantation current status, an update and future perspectives

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Abstract

Kidney exchange transplantation is well established modality to increase living donor kidney transplantation. Reasons for joining kidney exchange programs are ABO blood group incompatibility, immunological incompatibility (positive cross match or donor specific antibody), human leukocyte antigen (HLA) incompatibility (poor HLA matching), chronological incompatibility and financial incompatibility. Kidney exchange transplantation has evolved from the traditional simultaneous anonymous 2-way kidney exchange to more complex ways such as 3-way exchange, 4-way exchange, *n*-way exchange,compatible pair, non-simultaneous kidney exchange,non-simultaneous extended altruistic donor, never ending altruistic donor, kidney exchange combined with desensitization, kidney exchange combined with ABO incompatible kidney transplantation, acceptable mismatch transplant, use of A2 donor to O patients, living donor-deceased donor list exchange, domino chain, non-anonymous kidney exchange, single center, multicenter, regional, National, International and Global kidney exchange. Here we discuss recent advances in kidney exchanges such as International kidney exchange transplantation in a global environment, three categories of advanced donation program, deceased donors as a source of chain initiating kidneys, donor renege myth or reality, pros and cons of anonymity in developed world and (non-) anonymity in developing world, pros and cons of donor travel *vs* kidney transport, algorithm for management of incompatible donor-recipient pairs and pros and cons of Global kidney exchange. The participating transplant teams and donor-recipient pairs should make the decision by consensus about kidney donor travel *vs*

kidney transport and anonymity *vs* non-anonymity in allocation as per local resources and logistics. Future of organ transplantation in resource-limited setting will be liver *vs* kidney exchange, a legitimate hope or utopia?

Key words: Kidney transplantation; Kidney exchange; ABO incompatible; Desensitization

Core tip: Reasons for joining kidney exchange transplantation are ABO blood group incompatibility, immunological incompatibility (positive cross match or donor specific antibody), human leukocyte antigen (HLA) incompatibility (poor HLA matching), chronological incompatibility and financial incompatibility. Here, we discuss recent advances in kidney exchange transplantation such as International kidney exchange transplantation in a global environment, three categories of advanced donation program, deceased donors as a source of chain initiating kidneys, donor renege myth or reality, pros and cons of anonymity in developed world and (non-) anonymity in developing world, pros and cons of donor travel *vs* kidney transport, need of algorithm for management of incompatible donor-recipient pairs and Global kidney exchange.

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INTRODUCTION

Chronic kidney disease is the global health problem with high prevalence rate of 11% to 13%[1,2]. Outcome of living donor kidney transplantation is two times better than deceased donor kidney transplantation. Kidney exchange transplantation is well established modality to increase living donor kidney transplantation and more useful in countries where deceased donor kidney transplantation is not well developed. Kidney exchange transplantation provides good quality of organs and increasingly used in developed[3-10] and de-veloping world[11-23]. Kidney exchange is more useful in countries with low deceased donation rates (China, South, Korea, Japan, India and Pakistan) due to cultural and regional factors. Reasons for joining kidney exchange programs are ABO blood group incompatibility, immunological incompatibility (positive cross match or donor specific antibody), human leukocyte antigen (HLA) incompatibility (poor HLA matching), chronological incompatibility and financial incompatibility. Kidney exchange transplantation has evolved from the traditional simultaneous anonymous 2-way kidney exchange to more complex ways. Table 1 shows types of kidney exchange. Table 2 shows key features of success in single center kidney exchange program in india. Table 3 shows key features of national kidney exchange program.

INTERNATIONAL KIDNEY EXCHANGE TRANSPLANTATION IN A GLOBAL ENVIRONMENT

Table 4 shows strength and weakness of international kidney Exchange. There is limited solution to O blood group patients with non-O donor and highly sensitized pairs in kidney exchange program due to blood group composition of the general and end stage kidney disease population[24]. International kidney exchange transplantation in a global environment of regulation imposed by World Health Organization and the Transplantation Society could increase transplantation for difficult to match donor-recipient pairs such as highly sensitized pairs and O blood group patients with non-O donor[25-28]. The heterogeneity in antigen antibody profile and blood group composition in different geographic area may be contributing factor for this increased transplant rate. International kidney exchange transplantation should be reviewed by the ethics committee according to international standards of Good Clinical Practice and as per local laws and regulations. It should be also abided by the Declaration of Helsinki and Declaration of Istanbul principles. National kidney exchanged may be first attempted to keep the logistics simple before participation in International kidney exchange transplantation. More studies are required about willingness of donor-recipient pairs, transplant professionals and society to participate in such kind on program in ethical and regulatory environment. There should be collaboration in the adjutant National kidney exchange registries in initial pilot project.

THREE CATEGORIES OF ADVANCED DONATION PROGRAM

Ethical concerns about advanced donation program include the management of uncertainty, the extent of donor and recipient consent, the scope of the obligation that the organization has to the kidney exchange recipient, and the potential to unfairly advantage the recipient[29-31].

Butt *et al*[32] reported "out-of-sequence donation" in which a donor donates in kidney exchange chain early because of time limits and their intended paired recipient receives a kidney transplant a short time later. The patient is already having identified matched kidney exchange donor but transplant could not be completed for whatever reason. The donating pair has to take calculated risk that other pairs will actually donate the kidney in short time. Flechner *et al*[33] reported "short-

Table 1 Types of kidney exchange

Simultaneous anonymous 2-way kidney exchange
3-way, 4-way, *n*-way exchange[13]
Compatible pair[14,21]
Non-simultaneous kidney exchange[16]
Non-simultaneous extended altruistic donor and domino[18]
Kidney exchange + desensitization therapy[15]
Kidney exchange + ABO incompatible transplant[18]
Acceptable mismatch transplant
Use of A2 donor to O patients[18]
Living donor-deceased donor list exchange[19]
National kidney exchange[20]
International kidney exchange[17]
Global kidney exchange[18]

term unmatched" donation in which recipient without a match at the time of his donation, was matched and transplanted few months later. The recipient then gets priority to be matched for a kidney.

Veale *et al*[34] reported first case of "voucher" donation in which a living donor donates a kidney to receive voucher for a intended named patient to be transplanted in the near future. Vouchers can be used for future kidney transplants to overcome "chronological incompatibility" between living donors and recipients in the modern era of living donor banking. However an exact time limit for matching cannot be guaranteed. The detailed written informed consent process of advance donation program should include the alternatives such as living donation, deceased donation, non-simultaneous extended altruistic donor chain and waiting until a transplant is indicated.

DECEASED DONORS AS A SOURCE OF CHAIN INITIATING KIDNEYS

Melcher *et al*[35] reported that deceased donor kidney can be used to start non-simultaneous extended altruistic donor chain. Standard criteria deceased donor kidney or deceased donor with kidney donor profile index below 35 should be used for optimum outcome.

DONOR RENEGE MYTH OR REALITY

It was standard practice to do surgery simultaneously when kidney exchange was started in 1986 in the traditional simplest form of 2-way exchange. The quality of kidney exchange matching and number of patients transplanted with kidney exchange improved further with increasingly complex strategies evolved utilizing non-simultaneous donor operations. Donor withdrawal is rare and has been minimized through careful and thorough medical evaluation including surgical, and psychiatric evaluations in addition to laboratory work, age-appropriate screening tests of potential donors, proper counselling, donor motivation, commitment, written informed consent; minimize time between consent and kidney donation and trust between transplant team and donor, and cryopreservation of donor blood preventing frequent laboratory visits for blood testing when new chains are constructed. The medical problems in donors such as pregnancy, trauma, prostate cancer, declined in glomerular filtration rate, donor or kidney declined by recipient surgeon can lead to donor withdrawal and broken chains. The logistics issues are less in short chain than longer chain decreasing the donor withdrawal. The optimum chain length is three and longer chain may not further increase quality of kidney exchange matching along with number of transplants. Decreasing the utilization of bridge donors and minimizing bridge donor wait time can also reduce donor renege. Cowan *et al*[36] reported a real-world renege rate of 1.5% and real-time swap failures as a subset of broken chains in 35% of cases in analysis of 1748 kidney exchange transplants from the National Kidney Registry from 2008 through May 2016. Gentry *et al*[37] estimated a bridge donor renege rate of 5% per month for non-simultaneous extended altruistic donor chains. The simulation was then run over 24 mo and resulted in 35% of chains broken by donor reneging, significantly higher than by recent study Cowan *et al*[36] of 1.7%. The data from India also reported donor renege rate of zero percent in single center study of 300 kidney exchange transplants. It shows that donor renege is rare and is not significant problem in modern kidney exchange practice.

PROS AND CONS OF ANONYMITY IN DEVELOPED WORLD AND (NON-) ANONYMITY IN DEVELOPING WORLD

There is disparity on standard practice of kidney exchange in developed and developing World in term of (non-) anonymity. There is variable practice on anonymity before and after surgery in different countries.

Conditional approach[38]: When the donor-recipient pairs give consent for meeting after surgery, they are allowed to meet each other after surgery in some countries such as the United States of America[39] and the United Kingdom[40]. In other countries, such as the Netherlands and Sweden[41], anonymity is absolute. Anonymity protects patients, donors and transplant hospital/ administration against the risks of revoking anonymity and prevents further commercialization of organs, and breach of patient donor privacy. An Ethical, Legal and Psychosocial Aspects of Organ Transplantation (ELPAT), a subsection of the European Society for Organ Transplantation reported that a conditional approach to anonymity should be possible after surgery[42]. Pronk *et al*[38] showed that most donor-recipient pairs who participated in anonymous donation process are in favour of a conditional approach to anonymity. Guidelines on how to revoke anonymity if both parties agree are needed and should include education about pros and cons of (non-) anonymity and a logistical plan on how, when, where, and by whom anonymity should be revoked.

Non-anonymous allocation[11,12]: Donor-recipient pa-

Table 2 Key features of success in single center kidney exchange program in India

Education, awareness, counselling of about risk and benefits of available transplant options[11-23]
Kidney exchange registry of incompatible pairs
Dedicated transplant team to overcome logistic problems
Uniform evaluation, care and follow-up
Complete work up of pairs before allocation avoids chain collapse
Standardization of HLA laboratory
Robust Immunological evaluation prevents unequal outcome in pairs
Non-anonymous allocation increases trust between pairs and transplant team
Exchange kidney of similar quality
Bonus for difficult to match and better HLA matched pairs
Use of short (⩽ 4-way exchange) *vs* long chain minimises logistic problems
Simultaneous surgeries avoid risk of donor reneging
Improve program using key features of other successful programs
Legal, ethical, fair, transparent, equitable and patient centric policy by Competent Authorities

HLA: Human leukocyte antigen.

Table 3 Key features of national kidney exchange program

Country[3-10]	Key features of kidney exchange program
Australia[3-4]	High transplant rate for highly sensitized, HLA-incompatible pairs due to accepting ABO-incompatible donor matching with ABO titers ⩽ 1:64, high-resolution HLA identification and virtual cross match
Canada[5]	Non-directed anonymous donors facilitate 62% of transplants
South Korea	Favourable due to less sensitized, more compatible pairs, more non-directed anonymous donors, non-O > O patients
United Kingdom[8]	Low transplant rate due to less use of altruistic donor, restriction on long chain, permit only ⩽ 3-way exchange, donor travel
Johns Hopkins University, United States	Kidney exchange + desensitization increases transplant rate for difficult to match and difficult to desensitize pairs
San Antonio, United States[10]	Use of compatible pairs and A2 donors increases transplant rate even in single canter program
National kidney registry, United States	Longer chain are used in matching
Donor *vs* kidney transport	Donors travel is preferred in Netherlands and Canada, kidney transport is preferred in United Kingdom and Australia
Alliance for paired donation, United States	Global kidney exchange

HLA: Human leukocyte antigen.

irs are allowed to meet each other before allocation of donor for surgery and even after surgery. They can share medical reports of exchange donors before surgery and kidney transplant and donor surgery outcome after surgery. Donor-recipient pairs do not choose their match but donor-recipient pairs may decline a match or can withdraw from participation in the kidney exchange program at any time, for any reason. Non-anonymous allocation has the potential of commercialization of organs in case of compatible donor-recipient pairs along with breach in privacy of donor-recipient pairs. Kute *et al*[11,12] reported that donor-recipient pairs are willing for non-anonymous allocation process in single center study of 300 kidney exchange transplants in India. They reported that non-anonymity is more helpful in manual allocation in absence of computer software allocation which also increases trust between patients, donors and transplant hospital/administration and legal team. More long term prospective studies are required to explore the donor and recipient perspective on anonymity in living kidney donation in different socio-economic regions and countries.

PROS AND CONS OF DONOR TRAVEL *VS* KIDNEY TRANSPORT[43-48]

The cold ischemia time is more detrimental in deceased donor kidney transplant than live donor kidney transplant. There is no statistically significant difference in live donor kidney transplant survival in shipped *vs* non-shipped kidney in data from various National registries (Scientific Registry of Transplant Recipients registry in the United States, National Kidney Registry in the United States, and Australian kidney paired donation program). This is feasible strategy to improve the quality of matching such as HLA matching in kidney exchange program. However, more studies are required to define long term safety of shipping donor kidneys and willingness of donor-recipient pairs to participate in donor travel *vs* kidney transport

In Canada with wide geographic distribution, donor travel is accepted and preferred over kidney transport whereas, in Australia kidney transport is accepted and preferred over donor travel.

Disadvantages of donor travel are variation in donor

Table 4 Strength and weakness of international kidney exchange

Strength	Weakness
Increase access to better and effective health care of end stage renal disease patients for transplantation	Inequalities between donor recipient pairs from participating countries result from differences in regulatory, legal and reimbursement policy. Increase inequality and inequity in participating countries particularly for low/middle income countries
Quality of medical care increase from existing and participating National programs	Logistics are complex in immunological evaluation of pairs, management of clinical data and simultaneous surgery
Increase pool size, optimization and diversity of pairs increase quality of matching, number of transplants and increase transplant rate for difficult to match pairs who remain unmatched within their own country	Emerging less well established programs are likely to benefit less than well-funded established program. Limiting development of national program to become self-sufficient in organ donation and transplantation
Mutual learning between different National programs. Promote collaboration, best practice and spread of kidney exchange in interested countries	Adequate financial support for effective and equitable follow-up must be available in low/middle income countries
	Risk for donor recipient pairs with less adequate health care system to manage medical complications and long term follow up care
Facilitate legal, ethical expansion of kidney exchange program with International organ donation and transplantation community	Risks reducing the effectiveness and equity of existing well established program due to practical, logistical and organisational considerations associated with trans-national kidney exchange program
Dialysis is replaced with kidney exchange which is best and cost effective living donor kidney transplantation	Reputational risk and loss of public trust interest confidence in organ donation and transplantation if international kidney exchange involve Nations without appropriate legal and ethical policy to support best practice

workup and donor surgery side of donor nephrectomy (right *vs* left), surgical method (open, laparoscopic, hand-assist or robotic), lack of family support/familiar surgical team, surgical skills and experience are different in different transplant centers as per surgical training and less patient trust and donor satisfaction.

Advantages of kidney transport are familiarity with the transplant team, presence of family and friends for logistical support. Disadvantage of kidney transport is the effect of prolong cold ischemia time on long term kidney allograft survival. However recent studies have shown that cold ischemia time of 16 h has minimal/no effect on long term kidney allograft survival. Cold ischemia time is short in kidney exchange programs where donor travel is used. The Global Positioning System tracking devices can be used to monitor the location of shipped kidneys. Donor-recipient pairs should discuss the best option with the transplant team as per available resources. The participating transplant teams should make the decision by consensus about kidney donor travel *vs* kidney transport as per local resources and logistics. Donor travel rather than kidney transport is likely to be logistically simpler to execute in the Indian situation.

EDUCATION, AWARENESS AND COUNSELLING OF INCOMPATIBLE DONOR-RECIPIENT PAIRS

Variations in practice for management of incompatible donor-recipient pairs will inevitably occur when clinicians take into account the needs of individual patients, available resources, and limitations unique to a clinical situation. There is need of clinical practice guideline document to be designed to provide information and assist decision-making in relation to kidney exchange *vs* desensitization. Each donor-recipient pairs should be given education, awareness, and counselling about risk, benefits and cost effectiveness of various renal replacement therapy options (ABO incompatible kidney transplantation *vs* kidney exchange, deceased donor kidney transplantation and dialysis) in an easy to understand format as early as possible in process of chronic kidney disease evaluation, treatment and transplant evaluation. This counselling can be performed by member of transplant team during dialysis sessions. Patients were encouraged for living donor kidney transplantation over deceased donor kidney transplantation. Patients with incompatible living donors should be encouraged for kidney exchange and ABO incompatible kidney transplantation depending on their phenotype. Infection is common cause of morbidity and mortality after kidney transplantation in developing world compared to developed world.

NEED OF ALGORITHM FOR MANAGEMENT OF INCOMPATIBLE DONOR-RECIPIENT PAIRS

The match/transplant rates for non-O group patients are higher with kidney exchange compared to O group patients. Such easy to match pairs (non-O group patients such as A donor and B recipient; B donor and A recipient and sensitised pairs) should be encouraged for kidney exchange over ABO incompatible kidney transplantation and desensitization protocol[11,12,49]. O group patients with ABO titer $\leqslant$ 128 or panel reactive antibody > 80% should undergo desensitization and ABO incompatible kidney transplantation with acceptable outcome[49]. O group patients with ABO titer > 128 should be first considered in kidney exchange than ABO incompatible kidney transplantation[49]. If no

Table 5 Advantages of global kidney exchange[50-53]

2-7 million people die World-wide from kidney failure due to poverty. Helping some of these poor patients would be good. GKE helps only those patients who have exhausted all the solutions in their home country and increases transplant opportunity for poor patients from low/middle income countries who are otherwise exposed to death[61-62]
GKE wants to support poor patients from low/middle income country legally, ethically, fairly and transparently following the rules established by the National Competent Authorities of each country
GKE does not induce donation but removes the financial barrier to donation for a willing donor recipient pairs where donor's motivation is altruistic and unpaid
Everybody wins in GKE: Low/middle income country's donor and recipient, low/middle income country's pre-and post-transplantation health care system, high income country's recipient, health care payers and high income country's Government and taxpayers
GKE can send high income country patient to high quality low/middle income country transplant centers, instead of reverse. This would be less expensive and build local infrastructure in low/middle income country and access to kidney transplantation to more low/middle income country patients
There can be oversight by organizations such as the World Health Organization and the Transplantation Society with strong International governance that is consistent with the highest ethical and legal standards

GKE: Global kidney exchange.

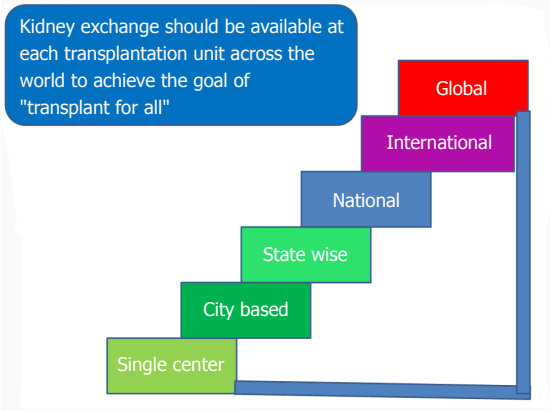

Figure 1 Stepwise progress in kidney exchange.

match is found with kidney exchange in a reasonable period of time they can be undergo ABO incompatible kidney transplantation with equally good results but with greater number of treatments and cost.

For sensitized donor-recipient pairs who have phenotypes that are either easy-to-match and/or difficult-to-desensitize are more likely to benefit from kidney exchange, whereas those who are either easy-to-desensitize and/or difficult-to-match should be considered for desensitization. For sensitized donor-recipient pairs with phenotypes that are both difficult-to-desensitize and difficult-to-match may benefit from a combination of kidney exchange and desensitization in which they are paired with a more immunologically suitable donor[49]. This will reduce waiting time for deceased donor kidney transplantation for patients with no living kidney donor. ABO incompatible kidney transplantation should continue to function in a complimentary way that enhances access to living donor kidney transplantation rather than competes with kidney exchange. ABO incompatible kidney transplantation should be performed after obtaining written informed consent of donor-recipient pairs. Patients with economic constrains; pre-transplant infections and baseline high ABO titer may be excluded from ABO incompatible kidney transplantation.

PROS AND CONS OF GLOBAL KIDNEY EXCHANGE

Table 5 Shows Advantages of Global Kidney Exchange (GKE). Figure 1 shows Stepwise Progress in Kidney Exchange. One third of donor-recipient pairs could not receive kidney transplantation due to immunological incompatibility (ABO incompatible or positive cross match/donor specific antibody). Financial incompatibility is much more common barrier to kidney transplantation than immunological incompatibility in developing countries in absence of universal access to health care for end-stage renal disease. Global kidney exchange increases access to living donor kidney transplantation for donor-recipient pairs from developing countries with financial incompatibility[50,51]. Global kidney exchange should be conducted in legal, transparent and an ethical way. Global kidney exchange will help rich donor-recipient pairs from developed countries with universal access to health care for end-stage renal disease and poor donor-recipient pairs from developing countries in absence of universal access to health care for end-stage renal disease. It should run in a way that enhances access to living donor kidney transplantation with kidney exchange along with national and regional KPD program. The collaboration of single center, regional, National, International and Global kidney exchange program should aim to provide cost effective kidney transplantation with better long term outcome for all patients with end-stage renal disease.

We believe that single center, regional, National kidney exchange program should be attempted before International and Global kidney exchange program to overcome transcultural and logistical issues with the later[52,53]. In addition, more studies are required for the definition of financial incompatibility and about willingness and feasibility of donor-recipient pairs from developing countries for International and Global kidney exchange program. Clearly, the heterogeneity in antigen-antibody profile of donor-recipient pairs from developing countries and developed countries increase

access to living donor kidney transplantation for difficult to match and highly sensitised donor-recipient pairs. The larger donor pool in International kidney exchange will increase HLA matching of donor-recipient pairs which is the best parameter to improve long- term kidney graft survival. Global kidney exchange appears to provide life-saving kidney transplantation to poor donor-recipient pairs from developing countries that otherwise could die due to economic constrain[50-53].

PAIRED EXCHANGE TO INCREASE LIVING DONOR LIVER TRANSPLANTATION

An exchange donor program for adult living donor liver transplantation appears to be a feasible modality for overcoming donor-recipient ABO incompatibility[54-56].

FUTURE OF ORGAN TRANSPLANTATION IN RESOURCE-LIMITED SETTING: LIVER *VS* KIDNEY EXCHANGE: LEGITIMATE HOPE OR UTOPIA?

Opportunity and necessity is the mother of invention. Suppose, there are two patients in developing countries with end stage kidney disease and end stage liver disease with no suitable living donors in family in area without deceased donor organ transplantation. The morbidity and mortality of end stage kidney disease and end stage liver disease is very high in developing countries in absence of national health care insurance, deceased donor organ transplantation program and economic constrains. The organ trafficking is regularly reported in media in underdeveloped World. There is no other outcome for these patients other than death if they did not undergo organ transplantation. The life of these patients can be saved by exchanging liver of patient with end stage kidney disease with kidney of patient with end stage liver disease with optimum patient care before organ harvesting. There is no better solution for such kind of patients other than exchange of organs (liver *vs* kidney). The patient who participate in such exchange should be medically, psychosocially suitable, fully informed of the risks and benefits as a donor, competent, willing to donate and free of coercion. Let us be clear: The intention of such kind of exchange is to save human life and without exchange of organs (liver *vs* kidney) such patients will never going to receive organ transplantation. No alternative existed for such patients and millions more like them. Such organ exchange even if inequitable would able to add years of life to patients who would have died without it.

The mortality rate is at least 10 times higher in living donor liver donation with mortality rate of 0.5% than living donor kidney donation with mortality rate of 0.03%[57-59]. The morbidity rate of 20% is also higher in living donor liver donation. There is regeneration of liver and not kidney in short period. The health care providers from developing and developed World including policy makers should come together to discuss challenges and solution to solve the disparity in access to organ transplantation in developing and developed World. This will be great service to mankind who are in real need. More discussion and studies are required for patient/donor selection, professional /public acceptance, legislation, logistics, exploitations, equity and ethical issues for such kind of organ exchanges in near future to solve the global problem of organ shortage especially in developing world on the International platform such as the World Health Organization and The Transplantation Society. This could be an alternative to xenotransplantation and may serve as Nobel service to Mankind.

CONCLUSION

Kidney exchange transplantation has increased living donor kidney transplantation for end stage renal disease patients with chronological incompatibility and financial incompatibility. The participating transplant teams and donor-recipient pairs should make the decision by consensus about kidney donor travel *vs* kidney transport and anonymity *vs* non-anonymity in allocation as per local resources and logistics. There is need of uniform algorithm for management of incompatible donor-recipient pairs.

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MINIREVIEWS

Utility of central venous pressure measurement in renal transplantation: Is it evidence based?

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Abstract

Adequate intravenous fluid therapy is essential in renal transplant recipients to ensure a good allograft perfusion. Central venous pressure (CVP) has been considered the cornerstone to guide the fluid therapy for decades; it was the only available simple tool worldwide. However, the revolutionary advances in assessing the dynamic preload variables together with the availability of new equipment to precisely measure the effect of intravenous fluids on the cardiac output had created a question mark on the future role of CVP. Despite the critical role of fluid therapy in the field of transplantation. There are only a few clinical studies that compared the CVP guided fluid therapy with the other modern techniques and their relation to the outcome in renal transplantation. Our work sheds some light on the available published data in renal transplantation, together with data from other disciplines evaluating the utility of central venous pressure measurement. Although lager well-designed studies are still required to consolidate the role of new techniques in the field of renal transplantation, we can confidently declare that the new techniques have the advantages of providing more accurate haemodynamic assessment, which results in a better patient outcome.

Key words: Fluid monitoring; Central venous pressure; Renal transplantation

Core tip: We suggest that central venous pressure (CVP) measurement should be abandoned in renal transplantation since it may be misleading. We recommend using intra-operative and post-operative cardiac output monitoring devices for guiding fluid therapy in renal transplant recipients. Although lager well-designed studies are still required to consolidate the role of new techniques in comparison to CVP monitoring in the field of renal transplantation. We Suggest that the new methods have the advantage of providing a more accurate haemodynamic assessment in renal transplant cases.

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INTRODUCTION

Central venous pressure (CVP) measurement have been in use for more than half a century to assess intravascular fluid status of renal transplant recipients and, thereby, be used as a guide for intravenous fluid therapy in renal transplantation. With the current advances in the diagnostic tools, the value of CVP is a point of debate. Several studies proved that CVP measurements are neither correlated to cardiac output nor have a precise correlation with intravascular volume status, therefore it's value in fluid management of renal transplant recipient is at the best speculative. On the other hand, the traditionalists continue to believe that CVP values are of sufficiently good enough as a benchmark in determining resuscitation goals for a given patient.

It is well recognised that optimum fluid resuscitation is essential to maximise the outcomes in critically ill patients. However, only a few studies have reliably endeavoured to assess the role of CVP in comparison to other modern techniques in the field of renal transplantation. We aim to answer this question in regards to clinical application of CVP and objectively review from the point of view of its benefits and inherent limitations.

HISTORICAL USE OF CVP

The clinical correlation between CVP and the intravascular fluid volume were established more than 50 years ago[1]. Theoretical basis of CVP is to measure the pressure in the superior vena cava (SVC) or right atrium pressure, which reflects the right ventricle preload[2]. Indeed, several textbooks have dogmatically stated that CVP provides a clinically relevant and reliable information in regards to circulatory and volume status of patients[3].

Marik *et al*[3] published a systematic review article that evaluated the relationship between CVP and the fluid status of the patients and concluded that CVP is an unreliable indicator of the fluid status and should not be used as a guide to fluid management. Furthermore, Marik *et al*[4] as per updated meta-analysis for evaluation of CVP reliability in clinical practice, reiterated abandoning the use of CVP as a guide in fluid management.

Cecconi *et al*[5] pointed that commonly used preload measurements such as CVP or end diastolic volume, when used in isolation, cannot be used reliably as a guide to fluid resuscitation. They rather recommend using more than one hemodynamic variable for patient evaluation and management. Nonetheless, the study validated the role of CVP in certain situations as severe congestive heart failure or hypovolemia, where the use of CVP is valuable in guiding fluid management[5].

CVP IN THE CURRENT PRACTICE

CVP measurement continues to be a pedestal in day to day clinical practice. A survey studying the resuscitation practices of Canadian physicians have shown that 89.2% of them would use CVP as a monitoring parameter in septic shock as shown in Figure 1[6]. Additionally, CVP-determined endpoints were considered the end-point of volume resuscitation in the early phases of septic shock by 78.7% of the Canadian clinicians as illustrated in Figure 2[6].

Bignami *et al*[7] addressed the current clinical practice in hemodynamic monitoring after cardiac surgery in Italy. They analysed data collected from 71 centres using a 33-item questionnaire from. For monitoring intravascular volume status, CVP was used most frequently (26.7%), followed by arterial BP (19.7%) and echocardiography (5.6%)[7]. Sondergaard *et al*[8] reported that CVP, though not a direct measure of preload, can be used to assess volume status, heart performance and systemic vascular resistance.

DRAWBACKS AND LIMITATIONS OF CVP IN RELATION TO RENAL TRANSPLANTATION

Recent medical advances in understanding haemodynamic of the vascular system together with the availability of new technology have changed the scope of diagnostic approaches. We strongly feel that CVP is not the right tool in assessing the fluid balance and guide fluid therapy in renal transplantation. CVP reading is affected by several physical and anatomical factors as illustrated in Table 1[9].

During kidney transplant operation, the recipient is exposed to many intraoperative factors which may alter the CVP reading, hence, can be misleading in decision

Table 1 Factors affecting the measured central venous pressure reading[9]

Central venous blood volume	Venous return/cardiac output
	Total blood volume
	Regional vascular tone
Compliance of central compartment	Vascular tone
	Right ventricular compliance:
	Myocardial disease
	Pericardial disease
	Tamponade
Tricuspid valve disease	Stenosis
	Regurgitation
Cardiac rhythm	Junctional rhythm
	Atrial fibrillation
	Atrio-ventricular dissociation
Reference level of transducer	Positioning of patient
Intrathoracic pressure	Respiration
	Intermittent positive pressure ventilation
	Positive end-expiratory pressure
	Tension pneumothorax

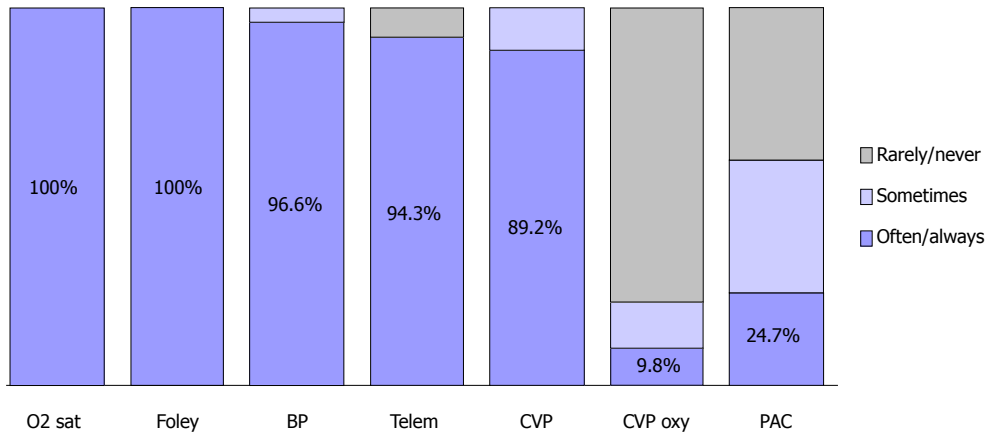

Figure 1 Monitoring parameters used by intensive care unit physicians[6]. BP: Intra-arterial blood pressure; CVP: Central venous pressure; CVP oxy: Continuous monitoring of central venous oxygen saturation; Foley: Foley catheter; O2 sat: Oxygen saturation; PAC: Pulmonary artery catheter; Telem: Telemetry.

making. These factors can be summarised in the following points: (1) During the operation, the position of the patient is not always in flat supine position. The surgeon may be tilting the table in a different direction, commonly head down while elevating the left or the right side to improve the access to the iliac vessels. The effect of posture changes on CVP reading was documented since a long time[10]; (2) transplant surgery always entails the use of abdominal retractors. These retractors must have a pressure effect on the viscera and subsequently affect the venous return. Moreover, the tension created by the retractors will resist movement of the diaphragm and will eventually affect the intrathoracic pressure. These mechanical factors again will give a false CVP reading[11]; (3) there is positive pressure ventilation (PPV) during the transplant operation will affect the CVP reading as mentioned in Table 1[9]. There is no convincing evidence demonstrating to how much the CVP is affected by PPV; (4) the target intra-operative CVP remains elusive. While aggressive hydration ensures good allograft perfusion. On the other hand, overhydration carries the risk of pulmonary congestion, pulmonary oedema, and prolonged intubation especially in patients with pre-existing cardiac conditions[12]; (5) CKD patients on dialysis fluctuate between the volume overload state and the dry state during the post-dialysis period, which makes it difficult to declare which CVP reading should be considered as a normal reading. Additionally, the effect of ageing, long-standing hypertension and the use of various medications affecting the peripheral vascular resistance (alpha blockers, beta blockers and calcium channel blockers) would be further confounding parameters[9]; and (6) we should not forget that placement of central venous catheters and other devices may result in central vein stenosis. Central vein stenosis can jeopardise the future of arteriovenous fistula and arteriovenous graft in the ipsilateral extremity when the renal graft fails, and the patient returns to dialysis[13-15].

Table 2 Advantages and limitations of some commercially available (minimally invasive) cardiac output monitoring[19,20]

Modality	Examples	Advantages	Limitations
Pulse wave analysis	LiDCO*rapid*™ and FloTrac/Vigileo™	Requires only arterial line; Beat-by-beat CO monitoring (this may help to evaluate response to IV fluids). - Validated by clinical studies in different medical and surgical conditions	Presence of arterial line with optimum waveform signal is a prerequisite; Accuracy may be reduced by sever arrhythmia; Needs frequent recalibration during periods of hemodynamic Instability
Lithium dilution	LiDCO*plus*™	Simple technique (can use peripheral arterial line); Continuous CO monitoring	Arterial line required; Accuracy affected by some neuromuscular blocking drugs; Lithium chloride is contraindicated in patients undergoing treatment with lithium salts
Electrical bioimpedance	BioZ®	Completely non-invasive	Numerous mathematical assumptions; Limited validity in patients with dysrhythmias
Partial CO2 rebreathing	NICO™	Easy to set up	Requires intubation and mechanical ventilation with minimal gas exchange abnormalities and fixed ventilator settings; Accuracy decreased with haemodynamic instability
Pulsed dye densitometry	DDG-330®	Non-invasive	Intermittent assessment; Accuracy may be affected by vasoconstriction, movement of the sensor and interstitial oedema

CO: Cardiac output; OR: Operating room.

POSSIBLE ALTERNATIVES FOR FLUID STATUS MONITORING

The introduction of commercially available equipment for assessing dynamic preload variables [*e.g.,* stroke volume variation (SVV)] considered a revolutionary advance in peri-operative fluid management. Srivastava *et al*[16] evaluated the use of intraoperative transesophageal Doppler (TED) to estimate the corrected flow time and variation in stroke volume values. TED was used to guide intraoperative fluid management in 110 living donor renal transplant recipients, and the outcome was compared with the historical records of 104 control recipients who received CVP guided fluid management over the previous year. They concluded that TED was associated with a similar rate of immediate graft function. Moreover, it was associated with a significantly less amount of intra-operative intravenous (IV) fluids, and reduced incidence of postoperative fluid overload[16].

Similarly, Kumar *et al*[17] studied the use of SVV (obtained from minimally invasive cardiac output monitor) to guide the perioperative fluid therapy in major abdominal surgery. The study documented a significantly lower amount of IV fluids used with the new technique, not only that but also there was a significantly shorter ICU stay, and a non-significant shorter hospital stay[17]. These non-invasive tools were used successfully as a part of enhanced recovery programs in kidney transplantation to improve patient outcomes and speed up patient's recovery after surgery[18].

Furthermore, several other non-invasive techniques are utilised for cardiac output assessment and IV fluid guidance like lithium dilution technology (*e.g.,* LiDCOplus™ machine) and arterial pulse wave analysis (*e.g.,* FloTrac/Vigileo™)[19,20]. However, each one of these novel, non-invasive techniques has its own limitations. Clinicians should be aware of the underlying principles and limitations of each technique to choose the best modality for each clinical scenario individually[19,20]. Advantages and limitations of some of the currently available non-invasive approaches are summarised in Table 2[19,20].

The reliability of these new techniques to guide fluid therapy in surgical cases has been investigated in several clinical trials. The conclusion of these trials is summarized in Table 3.

CONCLUSION

Although CVP measurement continues to be popular, yet it is not ideal for guiding and monitoring of fluid management in renal transplantation. It is noteworthy that there may be large variations in intravascular volume status and the patients have limited range of intravascular volume that can be called euvolemia (because of co-morbidities, vascular complications, drugs and the effects of disease on the autonomic nervous system). Therefore, the volume that is infused in a patient whose fluid balance status is doubtful is going to be imprecise if CVP is to be relied upon to appreciate their baseline value. Pulmonary oedema could be the first sign of fluid overload. Other variables such as the patient position, the use of abdominal retractors, and the positive pressure ventilation make any CVP reading meaningless. As clearly evident from the data presented in Tables 1-3, we suggest that CVP measurement be abandoned in renal transplantation since it may be misleading. Alternative to CVP, we recommend using intra-operative and post-operative cardiac output monitoring devices for guiding fluid therapy in renal transplant recipients. Understanding their limitations helps to provide more robust monitoring of fluid therapy. Giving that these novel tools are only

Table 3 Dynamic evaluation of fluid status in comparison to conventional approach

Author	Patients No.	Study group	Conclusion
Berkenstadt *et al*[21], 2001	15	Patients undergoing brain surgery	SVV could predict fluid responsiveness to even a small volume loading of 100 mL of 6% hydroxyethyl starch given for two minutes; There was no correlation between the changes in SV and the values of the CVP and heart rate before or after loading
Rex *et al*[22], 2004	14	Coronary artery bypass grafting (CABG) patients	The dynamic index SVV allowed real-time monitoring of left ventricular preload. Moreover, it allowed assessing the haemodynamic effect of a fluid challenge; Other preload variables (*i.e.*, PAOP, CVP, LVEDAI and ITBI) failed to predict fluid responsiveness
Preisman *et al*[23], 2005	18	Coronary artery bypass grafting (CABG) patients	Functional haemodynamic indices were superior to static indicators of cardiac preload in predicting fluid responsiveness; Use of CVP for the evaluation of intravascular volume status, have been found to lack any predictive value
Hofer *et al*[24], 2005	40	CABG patients	Stroke volume index was significantly correlated with SVV ($P < 0.001$) and PPV ($P < 0.001$) only; While CVP failed to have a significant correlation ($P = 0.235$)
Wiesenack *et al*[25], 2005	20	CABG patients	Stroke volume index correlated significantly with SVV and PPV derived from pulse contour analysis ($P < 0.05$) but not with CVP or pulmonary artery wedge pressure
Cannesson *et al*[26], 2006	18	CABG patients	Left ventricular stroke area measured by transoesophageal echocardiographic automated border detection is not only sensitive to changes in preload but also, can quantify the effects of volume expansion on cardiac output; The difference in CVP reading did not reach statistical significance in the study groups
Lee *et al*[27], 2007	20	Neurosurgical patients	Corrected flow time by oesophageal Doppler and PPV are better than CVP and LVEDAI in predicting fluid responsiveness
Cannesson *et al*[28], 2007	25	CABG patients	ΔPOP can predict response to volume expansion as well as quantify the effects of volume expansion on hemodynamic parameters during cardiac surgery; There was no statistically significant relation between CVP and increase in cardiac index after volume expansion
Belloni *et al*[29], 2008	19	CABG patients	Their results confirm the ability of SVV ($P = 0.0005$) and PPV ($P = 0.001$) to predict fluid responsiveness in ventilated patients during cardiac surgery No significant differences were found in mean LVEDA and CVP before and after fluid administration
Biais *et al*[30], 2008	35	Postoperative period of liver transplantation	SVV and PPV measurement by arterial waveform analysis can be used to predict the effects of volume expansion in mechanically ventilated patients after liver transplantation; The failure of CVP and PAOP to predict fluid responsiveness agrees with increasing evidence that static preload indicators are not suitable for functional haemodynamic monitoring
Hofer *et al*[31], 2008	40	CABG patients	Conventional static preload parameters failed to reflect the fluid status or to predict fluid responsiveness. CVP is therefore unsuitable for predicting ventricular response to fluid loading; SVV measured by the FloTrac™/Vigileo™ and the PiCCOplus™ systems exhibited similar performances regarding predicting fluid responsiveness
de Waal *et al*[32], 2009	18	CABG patients	SVV of > 8% can predict fluid responsiveness with 100% sensitivity and 78% specificity, while PPV ⩾ 10% can identify fluid-responders with 64% sensitivity and 100% specificity; CVP readings were not better in predicting fluid responsiveness than random chance
Cannesson *et al*[33], 2009	25	CABG patients	SVV of 10% helped in discrimination of responders to volume expansion with an 82% sensitivity and 88% specificity; SVV may be a potential alternative to DeltaPP which is an accurate predictor of fluid responsiveness in ventilated patients; SVV was significantly a better predictor of fluid responsiveness than CVP and PCWP in this study
Zimmermann *et al*[34], 2010	20	Elective major abdominal surgery	Both SVV and PVI are valid indicators of fluid responsiveness in ventilated patients during major abdominal surgery; CVP did not adequately reflect circulating blood volume and failed to predict fluid responsiveness in this study
Desgranges *et al*[35], 2011	28	CABG patients	PVI can predict fluid responsiveness during general anaesthesia whatever the site of measurement in the operating room (the finger, the ear, and the forehead); PCWP and CVP showed no significant difference between responders and non-responders
Shin *et al*[36], 2011	33	Elective living donor liver transplantation	Femoral SVV > 8% can predict responders to fluid loading with a specificity of 80% and a sensitivity of 89%; CVP and PAOP did not correlate with the changes in the cardiac index that occurred with a fluid challenge

Broch *et al*[37], 2011	81	CABG patients	SVV (P = 0.002) and PPV (P < 0.0001) were found to be reliable indicators for fluid responsiveness unlike CVP (P = 0.13) that failed to predict it; PVI ability to predict fluid responsiveness is limited in the presence of low perfusion indices
Cannesson *et al*[38], 2011	413	Multicentre study of different abdominal and cardiac surgeries	PPV [AUC 0.89 (0.86; 0.92)] is superior to CVP [AUC 0.57 (0.54; 0.59)] in prediction of fluid responsiveness (P < 0.001)
Yazigi *et al*[39], 2012	60	CABG patients older than 70 yr	PPV is a reliable predictor of fluid responsiveness while CVP and PAOP were not better than a random chance in predicting the response to fluid; PPV reliability was not affected by the decreased arterial compliance and increased arterial stiffness related to aging
Bogović *et al*[40], 2017	24	Major (abdominal or trauma) surgery	The study stressed on the inability of CVP to provide a valid evaluation of the preload; SVV and PPV monitored by LiDCO™ were better alternatives for preload assessment

AUC: Area under the receiver operator characteristic curve; CVP: Central venous pressure; DeltaPP: Respiratory variations in arterial pulse pressure; ITBI: Intrathoracic blood volume index; LVEDA: Left ventricular end-diastolic area; LVEDAI: Left ventricular end-diastolic area index; PAOP: Pulmonary artery occlusion pressure; PCWP: Pulmonary capillary wedge pressure; PPV: Pulse pressure variation; PVI: Pleth variability index; SV: Stroke volume; SVV: Stroke volume variation; ΔPOP: Respiratory variations in the pulse oximetry plethysmographic waveform amplitude.

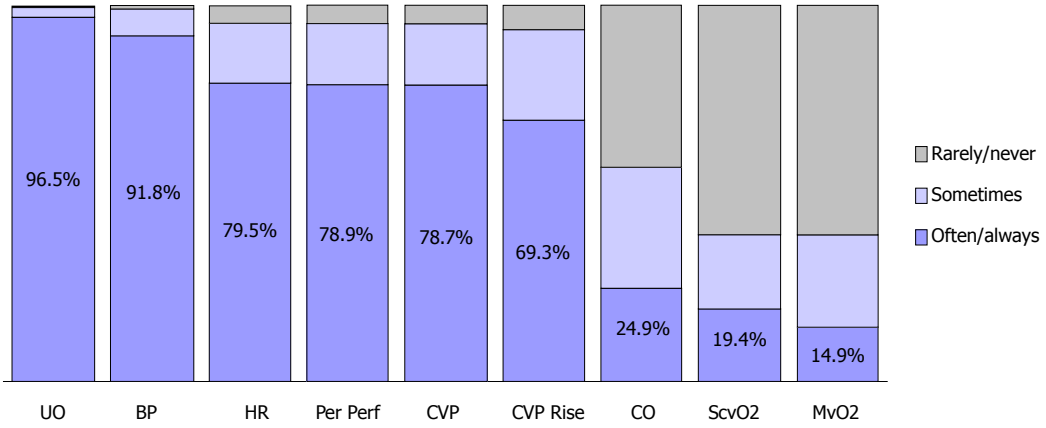

Figure 2 Volume resuscitation end-points[6]. BP: Blood pressure; CO: Cardiac output; CVP: Central venous pressure; CVP rise: Sustained rise in central venous pressure; HR: Heart rate; MvO2: Mixed venous oxygen saturation; Per Perf: Peripheral perfusion; ScvO2: Central venous oxygen saturation; UO: Urine output.

used in the ITU/HDU and operating theatre settings, management of these patients on the ward relies mainly on regular vital signs monitoring including daily body weight rather than being misled by erroneous CVP reading.

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MINIREVIEWS

Vaccination practices in End Stage Renal Failure and Renal Transplantation; Review of current guidelines and recommendations

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Abstract

Due to the increased burden of infectious complications following solid organ transplantation, vaccination against common pathogens is a hugely important area of discussion and application in clinical practice. Reduction in infectious complications will help to reduce morbidity and mortality post-transplantation. Immunisation history is invaluable in the work-up of potential recipients. Knowledge of the available vaccines and their use in transplant recipients, donors and healthcare providers is vital in the delivery of quality care to transplant recipients. This article will serve as an aide-memoire to transplant physicians and health care professionals involved in managing transplant recipients as it provides an overview of different types of vaccines, timing of vaccination, vaccines contraindicated post solid organ transplantation and travel vaccines.

Key words: Immunization; Travel vaccines; Infection; Immunosuppression; Inactivated vaccines; Vaccination post-transplant

Core tip: Patients in end-stage renal failure and those after renal transplantation have a higher risk of opportunistic infections with catastrophic complications and poor response to standard vaccines. Special individualized consideration is needed to immunize these

patients within the existing vaccination protocols.

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INTRODUCTION

End stage renal disease (ESRD) and long-term haemodialysis results in a state of immune compromise with increased risk of systemic infections. Similarly, Renal transplant (RT) recipients on maintenance immunosuppression, also have an increased life-time risk of opportunistic infections. Post-transplant infectious complications are one of the leading causes of morbidity and mortality in these patients. Although immunization against common pathogens can avoid potentially catastrophic complications, questions remain regarding safety, optimal timing and efficacy in these patients.

Transplant recipients are usually excluded from vaccine trials, leading to a scarcity of data regarding their safety and efficacy in these patients[1,2]. However, several guidelines have emerged based on individual case series and experience with other immunocompromised patients[3]. Nevertheless, there exists a clinical hiatus between published guidelines and routine clinical practice, due to safety concerns and fears of increased graft rejection after immunization[4].

IMMUNE STATUS AND IMMUNIZATION

Post-transplant immunosuppression has a cumulative effect on the immune system, including suppression of antigen presentation, T and B-cell proliferation and antibody production. Therefore, the host serological response to vaccination is suppressed and variable compared to the non-transplant individual[5]. Furthermore, transplant recipients have a state of hypogammaglobulinaemia, contributing to the low sero-conversion rates[6]. Therefore, patients with ESRD require a detailed and careful immunization history before enlisting for RT.

TYPE OF VACCINATION: LIVE, KILLED OR INACTIVATED

The place of live attenuated vaccines in transplant recipients remains an area of significant concern. Active viral replication following live vaccines has been demonstrated in immunocompromised hosts, leading to systemic infection. Viral replication can persist for several weeks after vaccination and such vaccines are recommended at least 6 wk prior to the planned transplant[7].

Killed and inactivated vaccines are safe in the transplant recipient. These can be administered in line with the immunization schedule for general population. Nevertheless, vaccination in general, is best avoided in the initial 6 mo after RT, where the immunosuppression is maximal. An exception is the influenza vaccine, which is safe as early as 4 wk after RT, to coincide with seasonal outbreaks[8,9].

TIMING OF VACCINATION

The optimum time for primary vaccination is the pre-transplant phase (Tables 1 and 2). Primary immunization should be carried out early after enlisting for RT due to the variable serological response rates[10]. This allows use of all types of vaccines including live vaccines, achieving adeqaute antibody titres and managing possible vaccine related reactions without compromising graft outcome. Live vaccination may interfere with the reading of Tuberculin skin test (TST) which is commonly done in most transplant cenetrs for all potential recipients. Therefore, the TST should be performed simultaneously with live vaccination or delayed by at least 28 d[11]. Similar difficulties with interpretation have also been reported with the newer interferon gamma release assay (IGRA)[12].

DOSING

Crespo *et al*[13] observed that following influenza vaccination, seroconversion rates were 33%, 42% and 82% in ESRD, post-RT and healthy controls respectively. A similar trend of poor sero-conversion is noted with other standard vaccinations among patients with ESRD and after RT. Furthermore, antibody titres tend to decline faster in these patients compared to healthy adults, requiring frequent monitoring of titres and booster vaccination in those who remain sero-negative or have suboptimal antibody levels.

VACCINATION OF HEALTH CARE PERSONNEL AND CARE GIVERS

Certain vaccines such as hepatitis-B are mandatory for all health care workers prior to assuming duties. Other vaccines (*e.g.*, Varicella, influenza) are recommended in most centers and have shown to minimize hospital-acquired infection. All killed or inactivated vaccines are safe in health care workers and close contacts of RT patients. However, live vaccines should be avoided as it can lead to viral shedding and active infection in the transplant recipient[14].

VACCINATION IN LIVING DONORS

In live donor RT, all donors need to be comprehensively

Table 1 Vaccination in end stage renal disease and pre-transplant

Vaccine	Live/inactivated	Comments
Hepatitis B	Inactivated	Higher concentration in 3-4 divided doses
		Check seroconversion after 6-12 wk
		Repeat dosing if HBsAb titre < 10 IU/L
Pneumococcal	Inactivated	(1) Adults (⩾ 19 yr), previously unvaccinated;
		PCV-13 followed 8 wk later by PPSV-23
		(2) Previously vaccinated;
		Single dose of PCV-13, one year after the last PPSV-23
HPV	Inactivated	All patients aged 9-26 yr
Influenza	Live (LAV)	Contra-indicated
	Inactivated (TIV)	Recommended annually
MMR	Live	Mandatory for all paediatric patients; 2 doses given 4 wk apart
		Single dose booster for all sero-negative adult patients
Rubella		For all seronegative female patients of child-bearing age
Varicella	Live attenuated	For all paediatric and adolescent patients, completed 6 wk before transplant
HZV	Live	Recommended for all elderly (> 60 yr) patients
		Optional for those 50-60 yr with a history of varicella or zoster
		No evidence of benefit in those < 50 yr
DTP	Inactivated	For all paediatric patients
Td/ Tdap	Inactivated	Td; Formerly (before 2005) recommended to all adult patients as a booster
		Tdap to all as a one-time dose followed by Td booster every 10 yr
BCG	Live	Routine neonatal vaccination done in Asia, Eastern Europe, Middle East, Africa and South America
		Elsewhere, recommended children < 5 yr deemed to be at high risk (see text)

HPV: Human papilloma virus; MMR: Mumps and rubella; DTP: Diptheria, tetanus and pertussis; BCG: Bacille Calmette-Guérin; LAV: Live attenuated vaccine; TIV: Trivalent inactivated vaccine.

checked for their immunization history. Potential donors should be up-to-date in their age appropriate immunization schedule. Live vaccinations should be avoided within 4 wk of a planned organ donation[7].

COMMON VACCINES IN THE TRANSPLANT PATIENT

Hepatitis B vaccine

Patients on long-term haemodialysis and after RT have a higher risk of hepatitis-B infection. It may manifest as an aggressive primary infection or reactivation of latent infection, requiring mandatory vaccination of all patients with ESRD, ideally before initiating dialysis. In case it had been missed, it is safe to be given while on dialysis or after RT. However, these patients have poor seroconversion rates (67%-86%), and require higher dosing, given as 20 or 40 (instead of the usual 10) micrograms of recombinant hepatitis-B in 3-4 doses at 0, 1, 2 and 6 mo[9,10,15].

Hepatitis-B surface antibody (HBsAb) titre should be checked 6-12 wk after completing the vaccination schedule and annually thereafter continuing beyond the transplantation. Those who fail to achieve desired titres (10 IU/L) are recommended a second course of vaccination. Those who fail to achieve the desired titres after two courses should be tested for active infection[3]. Booster dosing is also recommended for those with sub-optimal HBsAg titres at annual monitoring after RT.

Pneumococcal vaccine

Streptococcus pneumoniae infection can lead to severe and life-threatening pneumonia in ESRD and following RT. Furthermore, the incidence of invasive pneumococcal infection is also significantly higher in patients after RT compared to the general population. Therefore, routine vaccination is recommended in all patients with chronic kidney disease[15]. There are two common vaccine variants; the polysaccharide 23-valent (PPSV-23) and conjugated 13-valent (PCV-13), effective against different serotypes of the pathogen[16]. Both are inactivated vaccines and safe in the immunosuppressed host. Adult (⩾ 19 years) patients with chronic kidney disease who have not been previously vaccinated should receive a single dose each of PCV-13 followed 8 wk later by PPSV-23[15]. If previously vaccinated with PPSV-23, they should receive a single dose of PCV-13 after 1 year from the last dose of PPSV-23[17]. In immunocompromised hosts including those after RT, a second dose of PPSV-23 is recommended 5 years after the initial dose.

Human papilloma virus vaccine

Human papilloma virus (HPV) infection is one of the commonest prevalent infections among female transplant recipients. In the immunosuppressed host, specific strains of human papilloma virus may result in an increased risk of cervical, vulval or anal carcinoma[18]. The available trivalent and quadrivalent vaccines are both inactivated and safe in the immunocompromised host. It is recommended for all prospective male and female recipients aged 9-26 years, given prior to RT[4,15].

Influenza vaccine

Influenza infection can have devastating consequences in the immunosuppressed host. Early studies described prolonged viral shedding and risk of allograft rejection

Table 2 Common vaccinations contra-indicated post-transplant

Vaccine	Remarks
Influenza-Live attenuated	Inactivated is recommended annually
MMR	Recommended pre-transplant to all paediatric patients and sero-negative adult patients
Varicella	Recommended pre-transplant to all paediatric and adolescent recipients
HZV	Recommended pre-transplant to all those > 60 yr and those with a history of varicella or zoster infection (50-60 yr)
BCG	Trials under way for inactivated vaccine-currently not in routine clinical use post-RT
Oral polio vaccine	Inactivated injectable vaccine recommended when indicated
Typhoid	Travel vaccine, not routinely recommended
	Inactivated variant available for emergency travel

MMR: Mumps and rubella; BCG: Bacille Calmette-Guérin.

with influenza infection, leading to reservations regarding vaccination[19]. However, a direct causal effect of the vaccine on graft rejection has not been substantiated[20,21].

Two common vaccine variants exist; the live attenuated vaccine (LAV) and the trivalent inactivated vaccine (TIV). LAV and its intra-nasal variant are contraindicated after RT. The newer adjuvant vaccine is also contra-indicated as it has been shown to induce *de novo* anti-HLA donor specific antibodies, although with no proven clinical implications on the allograft[3].

Safety and efficacy of TIV is well documented and is recommended annually to all patients with ESRD and post-RT. It has been shown to be safe as early as one month after RT in line with seasonal influenza outbreaks. This current trend has led to a significant shift in practice pertaining to influenza vaccination after RT. A survey by Chon *et al*[22] covering 239 transplant centers across United States found that 95% of centers recommended influenza vaccine to their recipients compared to 84% in 1999.

Measles, mumps and rubella vaccine

Mumps and rubella (MMR) vaccine is a live attenuated vaccine and is contraindicated after RT. It is mandatory in all prospective paediatric recipients, recommended as a two-dose regimen approximately 4 wk apart after enlisting for RT[23]. In adults, serological testing is recommended and a single dose vaccination is undertaken for those who are seronegative.

Testing of rubella antibodies is recommended for all prospective female recipients of child-bearing age and vaccination performed if seronegative. Although adult rubella infection is self-limiting, immunization provides protection against congenital rubella syndrome in the event of post-RT pregnancy.

Varicella vaccine

Varicella can cause overwhelming disseminated disease in the immunosuppressed host. The varicella vaccine is live-attenuated and is contra-indicated after RT. It is recommended in all prospective paediatric and adolescent transplant recipients, completed at least 6 wk prior to transplantation[7,23]. If a deceased donor offer is received before completing 6 wk, RT can still proceed with a prophylactic regimen of acyclovir. In a study by Broyer *et al*[24], pre-transplant vaccination showed a dramatic reduction in post-RT varicella from 45% to 12%. Furthermore, the rate of late reactivation as zoster following vaccination (7%) was significantly lower than following primary infection (38%). In the event of a post-RT exposure in seronegative patients, prophylaxis is recommended with acyclovir, valacyclovir or intravenous immunoglobulins[25].

Herpes zoster virus vaccine

Herpes zoster reactivation (shingles) after transplant can lead to disseminated infection or troublesome herpetic neuralgia. Therefore, vaccination is recommended for all prospective elderly recipients (⩾ 60 years) at least 1 mo before RT. In those aged 50-60 years, vaccination is optional and can be considered in those who have a history of varicella or zoster infection[11]. There is no clear evidence for its benefit in recipients younger than 50 years.

Polio vaccine

The live oral polio vaccine is contra-indicated in transplant recipients and their contacts. Hence, paediatric transplant recipients and their household contacts are excluded from routine polio vaccination programs[3]. Instead, they are given the inactivated injectable vaccine in-line with the normal immunization schedule.

Diptheria, tetanus and pertussis vaccine

Diptheria, tetanus and pertussis (DTP) is an inactivated vaccine and is recommended to all prospective paediatric RT recipients. Until 2005, all prospective adult recipients were recommended a booster dose of tetanus-diphtheria (Td) only. However, a resurgence of pertussis related respiratory illness prompted the inclusion of pertussis vaccine to this schedule. The currently available tetanus toxoid-diphtheria-acellular pertussis (Tdap) vaccine is inactivated and safe in ESRD and after RT. Hence the current recommendation for both groups is a one-time dose of Tdap followed by Td boosters every 10 years[3,23].

Tuberculosis vaccine

The frequency of post-transplant active tuberculosis is

estimated to be 20-74 times higher than the general population, with a mortality rate reaching 30%[26]. Immunosuppressive medication may interfere with TST and IGRA used in diagnosis. Despite active disease, sputum smears may remain negative while the clinical manifestations are often atypical, leading to significant diagnostic delays. Furthermore, the disease may actively contribute to allograft dysfunction, resulting in the high morbidity and mortality[27].

The Bacille Calmette-Guérin (BCG) vaccine is a live vaccine and is contra-indicated after RT. Attempts at producing an effective inactivated vaccine have been largely unsuccessful. The only human trials to show efficacy of an inactivated vaccine was the Dar-Dar and DAR-901 trials conducted in Tanzania for patients with human immunodeficiency virus who were previously vaccinated with BCG at birth. The DAR-901 phase Ⅲ study showed the inactivated vaccine was well tolerated and did not cause post-vaccination tuberculosis[28].

Countries in Asia, Eastern Europe, Middle East, Africa and South America have universal neonatal BCG vaccination. In contrast, North America, United Kingdom, Australasia and Western Europe do not practice routine BCG vaccination due to low prevalence of TB, recommending it only to those neonates and children considered to be at a higher risk than the general population. This includes children < 5 years who live in an area of high prevalence, who have parents or grandparents born in a country of high prevalence, who live 3 or more months per year in a country with high prevalence or who have a close contact with diagnosed pulmonary TB[29].

Meningococcal vaccine

Meningococcal vaccine is usually recommended for patients undergoing splenectomy, those with complement deficiency or with HIV infection. In the transplant patients, it is widely recommended for those intending to travel to endemic regions. More recently, the meningococcal vaccine has been recommended for selected transplant candidates who are likely to receive eculizumab as immunosuppression[15]. Eculizumab is a complement inhibitor and has been linked to an increased incidence of meningococcal infection[30]. Accordingly, highly sensitized recipients who are likely to be given eculizumab post-transplant are recommended a two-dose regimen given 8 wk apart in the lead up to RT.

TRAVEL VACCINATION

Vaccinations of transplant recipients who intend to travel overseas to areas where certain infections are endemic, need special consideration. Preplanning allows serological testing before the intended travel to ensure protective serological status. In emergency travel circumstances, passive immunization with immunoglobulins can be considered[31].

Hepatitis-A vaccine

Transplant recipients have a poor seroconversion rate to hepatitis-A vaccination and show rapid decline in antibody titres[32]. For those travelling to endemic regions, the vaccine is recommended in two divided doses given six to twelve months apart. In addition to being a travel vaccine, hepatitis A vaccination is also recommended to RT recipients who are male homosexuals, recreational drug users, receive platelet regular concentrates and those who also have concomitant chronic liver disease[23].

Typhoid vaccine

The oral live attenuated vaccine is contraindicated after RT. If it is to be given, it must be done prior to transplant for those who reside in or travel to endemic areas. If emergency travel is needed, the inactivated injectable vaccine is recommended[33].

Polio vaccine

The live oral vaccine is contraindicated after transplant. Any transplant recipient travelling to endemic regions should receive a booster dose of the inactivated injectable vaccine[7].

Meningitis vaccine

The meningococcal vaccine is inactivated and is recommended to all travelers to endemic areas. This becomes especially important for transplant recipients who travel to regions such as Sub-Saharan Africa and Saudi Arabia, where it is a pre-requisite for travel[34].

Yellow fever vaccine

Yellow fever becomes endemic in peak seasons in Sub-Saharan Africa and certain regions of South America. The vaccine is a live attenuated and is contraindicated after RT. Hence, those who live or intend to travel to these regions need to be vaccinated before the transplant[35].

Rabies vaccine

Transplant recipients who are at constant risk of animal exposure such as veterinarians, should be considered for pre-transplant pre-exposure prophylaxis[15]. In all other transplant recipients, rabies vaccination becomes relevant only after possible rabid exposure. Such patients require comprehensive post-exposure prophylaxis. This comprises of injectable intramuscular vaccines in divided doses coupled with human rabies immunoglobulin[36].

Japanese encephalitis vaccine

Transplant recipients travelling to endemic East Asia and South-East Asia are recommended Japanese encephalitis vaccination. The newer killed inactivated vaccine is safe and recommended in two doses given 4

wk apart prior to intended travel[37].

CONCLUSION

Patients with ESRD and after RT are a distinct cohort that carry an increased risk of common infections, potentially catastrophic complications of such infections as well as reduced immunogenicity following immunization. In general, all immunization related details should be obtained prior to enlisting for RT. Any planned vaccines should be administered early in the pre-transplant phase at least 4 wk before the RT. While inactivated vaccines are considered safe beyond the first 6 mo after RT, live vaccines are contra-indicated throughout the post-transplant period. The reduced seroconversion rates and faster antibody clearance in these patients mandates regular screening for antibody titres and administration of booster doses when necessary.

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SYSTEMATIC REVIEWS

Perioperative glucose management and outcomes in liver transplant recipients: A qualitative systematic review

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Abstract

AIM

To investigate the relationship between post-liver transplantation (LT) glycemic control and LT outcomes.

METHODS

A qualitative systematic review on relevant prospective interventions designed to control glucose levels including insulin protocols. Studies investigating an association between glycemic control and post-LT outcomes such as mortality, graft rejection, and infection rate were reviewed. PubMed, EMBASE, and other databases were searched through October 2016.

RESULTS

Three thousands, six hundreds and ninety-two patients from 14 studies were included. Higher mortality rate was seen when blood glucose (BG) ⩾ 150 mg/dL (P = 0.05). BG ⩾ 150 mg/dL also led to higher rates of infection. Higher rates of graft rejection were seen at BG > 200 mg/dL (P < 0.001). Mean BG ⩾ 200 mg/dL was associated with more infections (P = 0.002).

Nurse-initiated protocols and early screening strategies have shown a reduction in negative post-LT outcomes.

CONCLUSION

Hyperglycemia in the perioperative period is associated with poor post-LT outcomes. Only a few prospective studies have designed interventions aimed at managing post-LT hyperglycemia, post-transplant diabetes mellitus (PTDM) and their impact on post-LT outcomes.

Key words: Diabetes; Liver transplant; Non-alcoholic steatohepatitis; Outcomes; Non-alcoholic fatty liver disease

Core tip: Despite the importance of post-liver transplantation (LT) glycemic control, there are no evidence-based guidelines on how to manage hyperglycemia in the post-LT period. The aim of this qualitative systematic review is to determine potential associations between glucose levels post-LT and outcomes such as mortality, graft rejection, infection rate, and other related post-LT outcomes. In addition, we analyzed methods for targeting glycemic control including specific therapeutic regimens or insulin protocols utilized in LT recipients.

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INTRODUCTION

Hyperglycemia after liver transplantation (LT) is a common phenomenon associated with increased risk of allograft rejection[1,2]. Poor glycemic control is also implicated in other post-LT complications including infection[3-5], acute kidney injury[6], new onset diabetes after transplantation (NODAT)[7,8], and malignancy, in addition to complications related to the metabolic syndrome including increased cardiovascular risk[9]. Despite the importance of post-LT glycemic control, there are no evidence-based guidelines on how to manage hyperglycemia in the post-LT period. Moreover, it is unclear what degree of glycemic control is associated with graft failure and complications such as infections. Similarly, predictors for poor glycemic control and NODAT in LT recipients have not been identified, apart from donor graft steatosis[9], post-LT immunosuppression[10-12], steroid use, and hepatitis C virus (HCV) infection[13]. These gaps in our existing knowledge necessitate a review of the literature on glycemic control and perioperative outcomes in LT recipients.

Given the increasing prevalence of non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome, many patients will arrive at transplant with some degree of insulin resistance. Post-LT hyperglycemic management will be essential to improving patient care and outcomes. The incidence of NODAT ranges from 20% to 44% among LT recipients, with rates varying depending on methodology used[8,9,11,14]. The aim of this qualitative systematic review is to analyze methods for targeting glycemic control including specific therapeutic regimens or insulin protocols utilized in LT recipients, and to determine associations between glycemic control and post-LT outcomes such as mortality, graft rejection, or infection rate. To achieve this goal, we reviewed prospective interventions targeting glucose control, as well as retrospective studies that examined the association between glucose control and relevant perioperative transplant outcomes.

MATERIALS AND METHODS

Overview

Our qualitative systematic review included *a priori* search criteria of journal articles and conference abstracts among adult (age ⩾ 18 years) human orthotopic or living donor LT recipients. Studies were limited to the English language and had to include at least one relevant outcome of interest such as patient survival, graft rejection, infection rate, acute kidney injury, and graft survival. Given the focus on perioperative glucose control, study outcomes were limited to glucose control during the first year post-LT.

Databases and search terms

A health sciences librarian with clinical input from our study team designed the *apriori* search strategy. PubMed (https://www.ncbi.nlm.nih.gov/pubmed), EMBASE (https://www.elsevier.com/solutions/embase-biomedical-research), SCOPUS (https://www.scopus.com), Clinical trials.gov (https://clinicaltrials.gov), and WHO ICTRP (http://www.who.int/ictrp/en) using the search terms outlined in Supplementary Table 1. Searches were performed on October 18, 2016 and updated in December 2017. All studies prior to this date were included.

Study selection

Using the various databases outlined above, our search yielded a total of 1624 results after removing duplicate results. Four reviewers (PP, SRL, RAL, and ASB) independently screened the titles and abstracts of 406 search results each for potential eligibility and a consensus was reached to include a total of 14 studies in the final analysis (Figure 1). Although the search strategy was designed to exclude patients receiving other transplants from this review, some of these studies included patients that received combined liver-

Table 1 Characteristics of retrospective studies

Ref.	Country	Year	*n*	Group 1	Group 2	Study outcome(s)	Comments
Ammori *et al*[5]	United States	2007	184	Strict glucose control (BG < 150 mg/dL)	Poor Glucose control (BG ⩾ 150 mg/dL)	Mortality Infection rate	
Chung *et al*[25]	South Korea	2014	211	BG decline during the Neohepatic Phase (Yes)	BG decline during the Neohepatic Phase (No)	Mortality, length of ICU stay, early allograft dysfunction, MELD Score recovery	Outcomes were assessed relative to the drop in hyperglycemia after the neohepatic phase
Gelley *et al*[21]	Hungary	2011	310	*De novo* diabetes	Control	HepC recurrence and association with NODAT	
Hartog *et al*[23]	United Kingdom	2014	430	DBD	DCD	NODAT	
Keegan *et al*[17]	United States	2010	161 (158 were available for analysis)	Pre-protocol	Protocol	Mortality Morbidity Graft function	
Linder *et al*[18]	United States	2016	114	PTDM	Non-PTDM	PTDM	BPAR, allograft failure, death, CMV infection are additional endpoints
Park *et al*[4]	United States/ Taiwan	2009	680	SSI (Yes)	SSI (No)	SSI	
Trail *et al*[20]	United States	1996	497	PTDM	Case-control	PTDM morbidity	PTDM leading to infections and graft rejection
Wallia *et al*[1]	United States	2010	144	BG > 200 mg/dL	BG < 200 mg/dL	Graft rejection, infection, and re-hospitalization	Graft survival and prolonged ventilation
Wallia *et al*[19]	United States	2011	73	Glucose management service	Non-Glucose Management Service	Graft rejection, infection, and re-hospitalization	Graft survival and prolonged ventilation
Yoo *et al*[6]	South Korea	2016	304	Normoglycemia (BG: 80-200 mg/dL)	Mild hyperglycemia (BG: 200-250 mg/dL)	AKI	Group 3: Moderate hyperglycemia (250-300 mg/dL) Group 4: Severe hyperglycemia (> 300 mg/dL)

DBD: Donated after brain death; PTDM: Post-transplant diabetes mellitus; Non-PTDM: Transplant diabetes mellitus free; DCD: Donated after circulatory death; NODAT: New onset diabetes after transplantation; AKI: Acute kidney injury.

kidney transplantation. These patients were included since the results were reported in a composite manner (*i.e.*, data for liver transplantation alone patients *vs* combined liver-kidney transplantation patients were not reported separately). Overall, the number of liver-kidney transplantation patients was relatively small, and the results were predominantly driven by LT recipients alone.

Quality assessment

Four reviewers independently assessed the risk of bias in each study. Selected studies were reviewed based on representativeness of study population, comparability of cohorts, adequate assessment of outcomes, sufficient length of follow-up, adequacy of follow-up, and source of study funding. The prospective randomized study was assessed using the Cochrane risk of bias tool and the Newcastle-Ottawa Scale (NOS) was used for the cohort/case-control studies[15,16].

RESULTS

This qualitative systematic review includes results from 14 full text articles. Of the 1624 records identified electronically, 780 were duplicates and 109 were eligible after abstract review. Of the 109, there were 22 articles that were reviewed and retrieved in full-text form. Of these, 8 were excluded and data from 14 full text articles (11 retrospective studies, 2 prospective studies and 1 cross-sectional study) were found to be eligible and included in this review (Figure 1).

Characteristics of included studies

The characteristics of the included studies are shown in Tables 1 and 2. A total of 3692 patients (3077 patients were retrospectively studied; 615 patients were prospectively studied) from 14 studies were included. The studies spanned 20 years from 1996 to 2016 with most occurring in the past decade and included transplants pe-

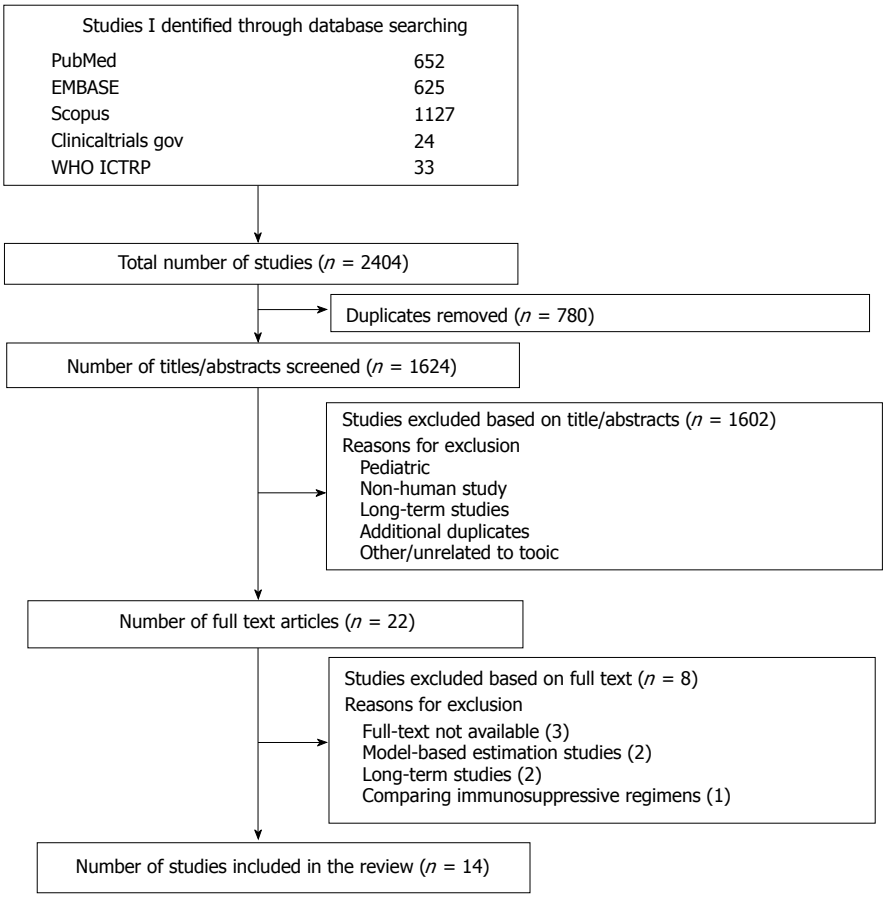

Figure 1 Consort diagram.

rformed in the United States, United Kingdom, Taiwan, Spain, South Korea, and Hungary.

Risk of bias in included trials

Supplementary Table 2 shows the risk of bias assessment of all the included trials. Of the 14 studies that were included, 11 were retrospective in nature and carry a potential to be inherently biased. NOS was used to assess risk of bias for the cohort/case-control studies and a modified version of the NOS was used for the single cross-sectional study. The single randomized prospective study, for the most part was deemed to have minimal bias utilizing the Cochrane risk of bias tool[15,16].

Study outcomes

Clinical outcomes for each trial are summarized in Table 3. Major outcomes of interest in relation to blood glucose (BG) level include mortality, graft rejection, infection rate, acute kidney injury (AKI), graft survival, post-transplant diabetes mellitus (PTDM), and NODAT.

Mortality

Three studies evaluated the relation between glycemic control and mortality in orthotopic liver transplantation (OLT) patients. Ammori *et al*[5] found a statistically significant association between glycemic control and mortality. The retrospective review compared patients with strict glycemic control (mean blood glucose < 150 mg/dL) *vs* those with poor control (mean blood glucose ⩾ 150 mg/dL). A total of 184 patients were analyzed (n = 60 for strict control, n = 124 for poorly controlled). The strict control group had a mean glucose of 135 mg/dL while the poorly controlled group had a mean glucose level of 184 mg/dL. Baseline donor and recipient characteristics for both groups were similar with the exception of recipient age (47 ± 2 years *vs* 53 ± 1 year; strict *vs* poor control, respectively). The Kaplan Meier survival analysis showed a significantly improved one-year survival rate in the strict glucose control group (91.2%) as compared to that in the poorly controlled group (78.1%). The one-year mortality rate was found to be 8.8% and 21.9% (P = 0.05) for patients in the strict controlled group and poorly glucose control group respectively.

Keegan *et al*[17] also evaluated the impact of perioperative glycemic control in OLT patients. This retrospective analysis studied the impact of the initiation of a nurse-initiated protocol for glycemic management (protocol group) *vs* glycemic management prior to the initiation of the protocol (pre-protocol group). Prior to the implementation of the protocol, a variety of insulin infusion protocols and ad hoc sliding scales were used at the discretion of the physician for glycemic control.

Table 2 Characteristics of prospective studies and the cross-sectional study

Ref.	Country	Year	*n*	Group 1	Group 2	Outcome	Comment
Alvarez-Sotomayor *et al*[24]	Spain	2016	344	Diabetes before transplantation	No diabetes before transplantation	PTDM	Cross-sectional study
Villanueva *et al*[22]	United States	2005	107	Rosiglitazone	-	PTDM	
Welsh *et al*[28]	United States	2016	164	Intensive glycemic control	Moderate glycemic control	Hypoglycemia	Insulin requirements

PTDM: Transplant diabetes mellitus.

Table 3 Summary of important findings of perioperative glucose control on liver transplant outcomes

Outcome of interest	Important findings	Data sources
Mortality	Mean BG ⩾ 150 mg/dL increases mortality	Ammori *et al*[5] (retrospective study)
	Nurse initiated insulin protocol did not impact mortality	Keegan *et al*[17] (retrospective study
	PTDM influenced glucose levels but did not change mortality	Linder *et al*[18] (retrospective study)
Graft rejection	Mean BG > 200 mg/dL increases risk of rejection	Wallia *et al*[1] (retrospective study)
	Although, mean BG were lower with the use of GMS, it did not lead to lower rate of rejection	Wallia *et al*[19] (retrospective study)
	Conflicting evidence exists relating to the development of PTDM and its relation to rejection	Linder *et al*[18] and Trail *et al*[20] (retrospective studies)
Infection rate	BG ⩾ 150 mg/dL is associated with higher infection rate	Ammori *et al*[5] (retrospective study)
	BG ⩾ 200 mg/dL increases risk of SSIs	Park *et al*[27] (retrospective study)
	Use of GMS led to lower rate of infection	Wallia *et al*[1] (retrospective study)
	Higher BG levels post-LT also led to increased incidence of HCV recurrence	Gelley *et al*[21] (retrospective study)
	No association between BG levels and post-LT CMV infection	Linder *et al*[18] (retrospective study)
	Development of PTDM did not lead to higher infection rate	Trail *et al*[20] (retrospective study)
Post-transplant diabetes mellitus/new onset diabetes mellitus	Rosiglitazone ± sulfonylurea is a potential option for the management of PTDM	Villanueva *et al*[22] (prospective study)
	Post-LT hyperglycemia is associated with the development of PTDM	Linder *et al*[18] (retrospective study)
	Insulin use was significantly higher in PTDM patients with inadequate BG	Alvarez-Sotomayor *et al*[24] (retrospective study)
Acute kidney injury and graft survival	High glucose variability is associated with post-LT acute kidney injury	Yoo *et al*[6] (retrospective study)
	No association between post-LT BG levels and graft survival	Wallia *et al*[1] and Trail *et al*[20] (retrospective studies)

BG: Blood glucose; PTDM: Post-transplant diabetes mellitus; GMS: Glucose management service; HCV: Hepatitis C virus; LT: Liver transplantation.

Under the protocol, a nurse would initiate a continuous intravenous (Ⅳ) insulin infusion within 48 h post-OLT if a patient's BG was greater than 130 mg/dL. The insulin infusion would be titrated as necessary (based on hourly readings) to reach a target BG goal of 80-130 mg/dL. The primary purpose of this quality improvement study was to identify the percentage of all measurements that were in the hypoglycemic (BG < 60 mg/dL) or severely hyperglycemia (BG > 250 mg/dL) range. These measurements were compared between pre-protocol and protocol groups. A total of 158 patients were available for analysis (n = 84 in the pre-protocol group; n = 77 in protocol group). Severe hyperglycemia was observed in 90 of the 581 measurements (15.5%) in the pre-protocol group and 15 of the 539 (2.8%) in the protocol group (OR for protocol group 0.16; CI: 0.09-0.28). Statistical significance, however, was not seen in one-year mortality between the protocol group and the pre-protocol group. Four out of 75 patients (5.3%) died in the protocol group, compared with 5 out of 83 patients (6.0%) in the pre-protocol group (OR for death in the protocol group, 0.89; 95%CI: 0.23-3.42; P = 0.86).

Linder *et al*[18] evaluated the insulin burden between liver transplant patients that developed PTDM *vs* patients that did not. BG levels between these two groups were reported as well as mortality rates. A total of 114 patients were retrospectively analyzed and while postoperative BG levels were similar in the ICU setting between the two groups, a statistically significant difference in floor (non-ICU) average BG levels (mg/dL) was seen between patients that developed PTDM and those that did not (184.7 ± 31.5 and 169.3 ± 31.4 respectively, P = 0.013). Statistically significant differences in one-month average BG levels were also seen-176.0 ± 31.1 for the PTDM group and 160.6 ± 28.0 for the non-PTDM group (P = 0.007). However, there was no significant difference in one-year mortality in the PTDM and non-PTDM groups.

Graft rejection

Four studies examined the association between glycemic

levels and graft rejection in liver transplant recipients. In a retrospective analysis conducted by Wallia *et al*[1] (n = 144), there was a statistically significant association between glucose level and graft rejection. Higher rates of rejection were seen in patients with a mean BG level > 200 mg/dL compared to those that had a mean BG level < 200 mg/dL (76.7% and 35.1% respectively; P < 0.001). A retrospective subgroup analysis by Wallia *et al*[19] (n = 73) studied the effect of a glucose management service (GMS) on blood glucose levels and its impact on clinical outcomes including graft rejection. The GMS consisted of a group of nurse practitioners supervised by an endocrinologist responsible for managing BG. The BG levels were managed by the primary transplant team in the non-GMS group. The mean inpatient BG level during the peri-transplant period was 189.0 ± 45.0 in the non-GMS group and 157.9 ± 32.3 in the GMS group (statistical significance data not provided). Although, patients in the non-GMS group had higher BG levels, hyperglycemia did not lead to higher rates of graft rejection (45% in the non-GMS group *vs* 29% in the GMS group, P = 0.156).

In the previously described retrospective analysis by Linder *et al*[18], biopsy-proven acute rejection (BPAR) was also studied as an outcome and there was a statistically higher incidence in PTDM *vs* non-PTDM patients (41.7% *vs* 24.2% respectively, P = 0.048). Similarly, a retrospective study by Trail *et al*[20] (n = 497), studied morbidity, including graft rejection, in DM patients after LT compared with matched control patients. Mean fasting blood glucose for patients with PTDM was 122.3 ± 5.0 mg/dL compared to 101.9 ± 3.9 mg/dL for the matched control patients (P < 0.01). Despite the statistically significant difference in glycemic levels between the PTDM group and matched control group, the number of rejection episodes was similar between the two groups, *i.e.*, rates of rejection were not significantly different between groups.

Infection

Six retrospective studies evaluated the association between glucose levels and infection. Park *et al*[4] studied the association between intraoperative hyperglycemia and surgical site infection (SSI) postoperatively in a retrospective study (n = 680). Of the 680 patients, 76 (11.2%) experienced SSI after LT. Severe hyperglycemia (defined as mean BG ⩾ 200 mg/dL) was seen in 37.8% of the 76 patients with SSIs compared to only 21.9% of the 604 non-SSI patients (P = 0.002) suggesting an association between the occurrence of SSIs and mean BG levels ⩾ 200 mg/dL. Similarly, In the study by Ammori *et al*[5], infectious complications when assessed 30 d post-LT were significantly associated with worse glucose control-among the strict glucose control group (mean BG < 150 mg/dL), there were 60 (30%) post-LT infections, compared to 124 (48%) infections in the poor glucose control group (mean BG ⩾ 150 mg/dL) (P = 0.02). The retrospective subgroup analysis by Wallia *et al*[19] found that the patients in the non-GMS group with higher BG levels exhibited higher rate of infection compared to the patients in the GMS group at one-year post-LT follow up (79% *vs* 51% respectively, P = 0.015). Gelley *at al*[21] found that higher early postoperative fasting plasma glucose led to higher incidence of HCV recurrence (diagnosed with histology criteria of the Knodell score), although no data was shown with regards to BG levels.

In contrast to the above studies, Linder *et al*[18] showed no association between glycemic level and post-LT CMV infection (patients with PTDM had higher BG levels compared to non-PTDM patients). Similarly, Trail *et al*[20] also showed no significant difference in infectious rates between patients with PTDM and those without PTDM. This study also evaluated the severity of infection as well as the type of infection and no differences were seen between the two groups.

Post-transplant diabetes mellitus and new-onset diabetes after transplantation

Villanueva and Baldwin evaluated the use of Rosiglitazone (ROSI) therapy for patients with PTDM. DM was diagnosed according to the American Diabetes Association (ADA) criteria (symptoms of hyperglycemia with post-prandial BG ⩾ 200 mg/dL, or fasting BG ⩾ 126 mg/dL on two separate occasions). The study followed 40 patients that developed PTDM that were initially stabilized by twice-daily NPH and regular insulin. These patients were subsequently started on ROSI 4 mg/d with the treatment goal to discontinue insulin while maintaining a target goal of HBA1c ⩽ 6.5%. Thirty of the patients that were initially treated with insulin were able to discontinue insulin within 3-4 mo. Three patients required chronic insulin therapy despite ROSI ± a sulfonylurea, and were considered insulin dependent. ROSI monotherapy was sufficient in 12 patients (30%), whereas 25 patients (62.5%) required ROSI + sulfonylurea to maintain insulin independence and normoglycemia. ROSI was continued at 4 mg/d in 25 patients while 15 patients required an increase to 8 mg/d. PTDM patients treated with ROSI maintained a mean HBA1C of 5.6% ± 0.8 (target BG levels were < 100 mg/dL for fasting glucose and < 140 mg/dL for post prandial glucose). A commonly seen side effect among patients treated with ROSI was edema (13%). These data suggest ROSI ± sulfonylurea may be a potential intervention that can reduce insulin burden in patients with PTDM[22].

Linder *et al*[18] also showed that patients who developed PTDM had significantly higher BG levels (1-mo average BG) suggesting post-LT hyperglycemia could play a role in the development of PTDM. Multivariate analysis for predictors of PTDM showed the use of Basiliximab was a negative independent predictor [AOR 0.182 (0.040-0.836), P = 0.03] and rejection was a positive independent predictor [AOR 3.237 (1.214-8.633), P = 0.019] for the development of

PTDM. Hartog *et al*[23] demonstrated that pulse high-dose steroids was an independent predictor of NODAT [OR 3.1 (1.7-5.6), P = 0.001]. In addition, this study also demonstrated donor graft type was associated with early occurrence of NODAT (within 15 d post-LT). Multivariate analysis showed donation after cardiac death (DCD) graft type was associated with significantly early occurrence of NODAT compared to donation after brain death (DBD) graft type [OR 6.5 (2.3-18.4), P = 0.001].

In addition to the previously mentioned outcomes, PTDM has also been associated with higher insulin use in post-LT patients. A cross-sectional study by Alvarez-Sotomayor *et al*[24] evaluated 344 patients of whom 141 patients experienced PTDM (157 total but 16 patients did not have HbA1c readings prior to enrollment). Patients with PTDM who had adequate glycemic control (defined as HbA1c < 7%), were significantly less dependent on insulin (39.4%) compared to patients with inadequate glycemic control (80.8%) (OR 6.6, 95%CI: 1.8-24.6, P < 0.001). Finally, Chung *et al*[25] found male sex, emergency surgery, surgical time (⩽ 9 h), and serum lactate (> 5 mmol/L) to be independent predictors for refractive hyperglycemia (RH), however, most post-LT outcomes were not significant in relation to RH.

Acute kidney injury and graft survival

Other outcomes of interest including AKI, graft survival, and complications related to hospitalization were not studied extensively. Three studies evaluated graft survival and no statistically significant association was seen between post-LT glycemic control and graft survival[1,19,20]. Similarly, no association was seen between BG levels and re-hospitalizations[1,19]. A study by Yoo *et al*[6] demonstrated no association between hyperglycemia and AKI in LT recipients; however, patients with greater glucose variability, as defined by the SD of blood glucose levels, more commonly presented with AKI (P = 0.019). Using SD as a surrogate marker for glucose variability, patients were divided into quartiles according to the SD of intraoperative and postoperative (initial 48 h of ICU admission) blood glucose levels. Patients with the lowest SD were assigned to the first quartile, ranging to those with the highest SD who were assigned to the fourth quartile. Glucose variability was significantly associated with AKI among patients in the third quartile (23.3% of patients with no AKI *vs* 30.3% with AKI, OR 2.47, CI: 1.22-5.00, P = 0.012) and fourth quartile (22.1% with no AKI and 31.1% with AKI, OR 2.16, CI: 1.05-4.42, P = 0.035).

DISCUSSION

This qualitative systematic review of 14 studies examined post-LT glucose control, interventions designed to target glucose control, and associations with post-LT outcomes including infection rate, PTDM, AKI, graft survival and mortality. Ultimately, this review concludes that perioperative hyperglycemia leads to unfavorable post-LT outcomes; however, the degree to which it plays a role may depend on the specific outcome in question. There is strong evidence to support an association between perioperative hyperglycemia and post-LT outcomes such as high infection rate and graft rejection[1,4,5,18-20,26]. A review by Park *et al*[27] that focused specifically on intraoperative hyperglycemia found a similar association between hyperglycemia and infection rate. In contrast, the strength of the evidence that exists to support an association between perioperative hyperglycemia and outcomes such as mortality and graft survival is not as well founded[1,5,17-20]. High glucose variability may also be a factor with the development of certain complications such as AKI[6]. In addition, donor graft type (DCD *vs* DBD) may also play a role in the early occurrence of NODAT (within 15 d post-LT)[23].

What was difficult to discern from these studies was the target BG level associated with poor post-LT outcomes. The studies in this review used different target BG levels to evaluate different outcomes, thus making it difficult to associate the degree of glycemic control with certain outcomes and also limiting comparisons that could be made between studies. The studies also varied in their definition of PTDM, the timing of glucose monitoring (immediate post-operative to days post-LT), and the medications used to manage hyperglycemia (ranging from insulin infusion to oral meds). The variability in the studies is what limits the comparisons that can be made and is the reason we can only perform a qualitative review of the literature. Additionally, most of the studies were retrospective observational studies and were not designed to study the specific association between hyperglycemia and post-LT outcomes. Finally, there were some studies that included a small number of combined liver-kidney transplant recipients and the results were reported in a composite manner, thereby making it difficult to detect LT-specific associations between glucose control and post-LT outcomes.

In this review, all of the relevant literature regarding glucose control and post-LT outcomes was compiled systematically using an apriori search strategy of the major medical literature databases. The data were compiled in a qualitative, descriptive manner due to the heterogeneity among research strategies and outcomes that exist in published literature

The conclusions from this review have robust implications for clinical practice. It is imperative to monitor glucose control pre- and post-LT. Along with hyperglycemia, it is also important to consider complications associated with strict glycemic control such as hypoglycemia and high insulin burden when deciding specific BG levels to target. Welsh *et al*[28] demonstrated the impact of hypoglycemia (defined as glucose ⩽ 70 mg/dL) pertaining to intensive and moderate glycemic control

in post-LT patients. There were a higher number of hypoglycemic patients in the intensive group and these hypoglycemic patients had significantly higher peak insulin drip rates, higher peak insulin glargine, and more importantly had significantly longer hospital stay. Therefore, it is crucial to target perioperative BG levels within a range that would limit complications associated with both hyper- and hypoglycemia. A reasonable target, based on our findings would be a range between 120 mg/dL to 150 mg/dL, given that BG ⩾ 150 mg/dL were associated with negative post-LT outcomes. In addition, interventions through nurse-initiated glucose management protocols to achieve specific target BG levels, early screening to identify patients at high-risk for PTDM, and use of oral agents for management of PTDM seem to be a promising approaches to minimize post-LT outcomes[17,22,24]. Although not discussed extensively in this review, optimizing immunosuppression regimens may also play an important role as noted by the potential association between basiliximab and pulse steroids with PTDM[18,23]. Song *et al*[29] conducted a retrospective study in China and demonstrated that lower exposure of tacrolimus (measured by mean tacrolimus concentration at 6 mo) was associated with less risk of developing NODAT and its related complications. This suggested that not only optimizing the regimen important but also the dosing of immunosuppressive drugs utilized in the regimen need to be optimized. Such recommendations would be strengthened by prospective randomized data and thus highlights the need for further study in this area.

The need for close monitoring of glucose levels post-LT will become even more important in the future. More patients with insulin resistance will come to transplant in the coming years. NAFLD is the fastest growing indication for transplant and will become the leading indication over the next decade[30,31]. The change in disease etiology may also be accompanied by donor grafts from older patients with DM and obesity that may be more susceptible to poor outcomes from hyperglycemic stressors[32]. As NAFLD increases prevalence, the transplant community will see more NAFLD among both living donors as well. A reliable assessment of hepatic steatosis is of paramount importance for living donor selection as significant steatosis can impact the postoperative outcomes of recipients and safety of the donor[33]. Because of these challenges, the focus could be on developing and establishing a standardized protocol for the monitoring of blood glucose levels. The frequency of test like hemoglobin A1c, glucose tolerance test, and use of tools such as continuous glucose monitoring should be further explored.

Prospective clinical studies need to further examine the impact of perioperative glycemic control in LT recipients with specific attention to the outcomes listed above. An ideal target range for BG levels needs to be determined and specifically investigated in terms of reducing negative outcomes associated with both hypo- and hyperglycemia, as well as adverse events related to post-LT complications due to impaired glucose control.

ARTICLE HIGHLIGHTS

Research background

There are no standard guidelines to properly manage hyperglycemia in the perioperative period of liver transplantation.

Research motivation

Understanding the importance of blood glucose level and proper strategies to manage post-liver transplantation hyperglycemia could help reduce adverse outcomes

Research objectives

The primary objective was to identify an ideal blood glucose level to achieve in the perioperative period for patients undergoing liver transplantation. In addition, exploring treatment regimens to achieve the target blood glucose can help identify better strategies for the management of these patients in the future.

Research methods

This is a qualitative systematic review that utilized key search terms to find studies on PubMed and other common databases. The search terms were in relation to liver transplantation and blood glucose level management in the perioperative period.

Research results

A total of 14 studies fit the criteria to properly study the objectives. The findings from this qualitative review suggests that blood glucose levels greater than or equal to 150 mg/dL in the perioperative period generally leads to negative post-liver transplantation outcomes. Specifically, there was an increased risk of infections, graft rejection, PTDM, and mortality. Graft survival was not impacted by hyperglycemia and there was an increased risk of acute kidney injury with high glucose variability in the perioperative period.

Research conclusions

The findings from the compiled studies in this review suggest a blood glucose level between 120 mg/dL and 150 mg/dL could potentially be an ideal target to manage hyperglycemia post-liver transplantation. In addition, early screening, use of oral agents, and utilizing resources such as a glucose management service could be potential strategies to limit adverse outcomes post-transplantation.

Research perspectives

Future studies can validate the findings from this review through a prospective study while implementing some of the strategies discussed in this review to minimize post-liver transplantation outcomes.

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REVIEW

Hepatitis C and renal transplantation in era of new antiviral agents

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Abstract

Data from World Health Organization estimates that the hepatitis C virus (HCV) prevalence is 3% and approximately 71 million persons are infected worldwide. HCV infection is particularly frequent among patients affected by renal diseases and among those in dialysis treatment. In addition to produce a higher rate of any cause of death, HCV in renal patients and in renal transplanted patients produce a deterioration of liver disease and is a recognized cause of transplant glomerulopathy, new onset diabetes mellitus and lymphoproliferative disorders. Treatment of HCV infection with interferon alpha and/or ribavirin had a poor efficacy. The treatment was toxic, expensive and with limited efficacy. In the post-transplant period was also cause of severe humoral rejection. In this review we have highlighted the new direct antiviral agents that have revolutionized the treatment of HCV both in the general population and in the renal patients. Patients on dialysis or with low glomerular filtration rate were particularly resistant to the old therapies, while the direct antiviral agents allowed achieving a sustained viral response in 90%-100% of patients with a short period of treatment. This fact to date allows HCV patients to enter the waiting list for transplantation easier than before. These new agents may be also used in renal transplant patients HCV-positive without relevant clinical risks and achieving a sustained viral response in almost all patients. New drug appears in the pipeline with increased profile of efficacy and safety. These drugs are now the object of several phases Ⅱ, Ⅲ clinical trials.

Key words: Hepatitis C virus; Renal transplantation; Hepatitis C virus and renal diseases; Interferon based therapies; Direct antiviral agents; Hepatitis C virus-positive donors

Core tip: The prevalence of hepatitis C virus (HCV) infection is high in patients with end-stage renal disease and HCV has clinical challenges in patients who undergo kidney transplantation. Historically, interferon-based treatment options have been limited by low rates of efficacy and significant side effects, including risk of precipitating rejection. Direct acting antiviral (DAA) drugs revolutionized the treatment of HCV. In this review we highlighted the most recent studies and clinical trial with DAA in renal patients including patients waiting for transplantation and already transplanted. In these studies all-oral DAA therapy appears to be safe and effective for such patients.

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HEPATITIS C VIRUS EPIDEMIOLOGY

The World Health Organization (WHO) estimates that the global prevalence of hepatitis C virus (HCV) infection averages 3%, and the incidence is 3-4 millions of new infections every year[1]. HCV prevalence is not similar worldwide and ranges from less than 0.1% in Northern Europe to 1%-5% in other countries, such as Eastern Europe and the Indian subcontinent[2], to 25% in Egypt[2]. HCV infection is considered to be an endemic disease in some country as Taiwan[3].

HCV prevalence is increasing annually and the October 2017 report from the WHO revealed that 71 million of people are infected worldwide. However, some population-based studies[4-6] have demonstrated that prevalence estimates based on blood donors, underestimate the true HCV prevalence in the general population.

HCV AND RENAL DISEASE

HCV prevalence increases in patients with kidney diseases. HCV may cause chronic kidney disease (CKD) *via* some forms of glomerulonephritis (GN), primarily membranoproliferative GN (MPGN), which may be caused by mixed cryoglobulinemia that represents HCV/anti-HCV immune complex associated with rheumatoid factor and complement[7]. Epidemiological studies in the United States (NHANES Ⅲ) and Taiwan have recently demonstrated the relationship between HCV infection and CKD[8,9].

HCV infection is a frequent consequence of CKD in stages 4-5. Blood transfusions and nosocomial transmission in dialysis units contribute to the much higher prevalence of HCV infection in CKD stage 5 than in the general population. Epidemiological studies documented that HCV infection is associated with a higher risk and shorter time to CKD despite the lower prevalence of many CKD risk factors (ERCHIVES Study)[10]. Another study[11] confirmed that HCV-positive patients exhibit 40% higher odds for renal insufficiency compared with HCV-negative patients after adjustment for age, race, gender, diabetes and hypertension. One retrospective study[12] did not confirm these findings, but the authors recognized the limitation of their study. A relevant longitudinal study including of 23820 adults aged 30-65 years old was performed in Taiwan. The study included 18541 anti-HCV serum-negative patients and 1095 anti-HCV serum-positive patients. The Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer (REVEAL)-HCV study is a large prospective community based cohort study in Taiwan, and long term diseases provide an excellent opportunity to investigate the natural history of chronic hepatitis C and long-term diseases associated with this chronic infection[13]. Lee *et al*[3] documented an association of HCV status and any cause of death. Lai *et al*[14] assessed the risk of developing end-stage renal disease (ESRD) in relation to HCV serostatus, HCV RNA level and HCV genotypes.

The Lai *et al*[14] study documented that chronic HCV infection is an independent risk factor for the development of ESRD. Participants with low and high HCV RNA levels exhibited a 2.6- and a 4.3-fold increased risk of developing ESRD, respectively, compared with participants who were not chronically HCV infected. Patients with HCV genotype 1 exhibit a higher risk of developing ESRD (Figure 1).

CLINICAL PROBLEMS OF HEPATITIS C IN RENAL TRANSPLANT PATIENTS

Survival of HCV-infected patients in ESRD is significantly lower in HCV-positive RNA-positive dialysis patients compared to HCV-positive RNA-positive kidney transplant recipients[15-17]. However, the persistence of HCV infection after renal transplantation is a true risk factor for graft and patient survival. The following complications primarily occur after renal transplantation in HCV-positive patients.

Liver disease

Immunosuppression facilitates HCV replication and accelerates liver disease to result in chronic hepatitis, fibrosing cholestatic hepatitis and rapidly progressive liver failure[18,19]. Therefore, preemptive treatment of HCV infection during dialysis is recommended.

Renal disease

HCV with associated cryoglobulinemia frequently causes MPGN even after renal transplantation[20,21]. Similarly, HCV may cause membranous nephropathy in renal transplant patients[20,22], and it may occur as a recurrent or de novo disease. A higher frequency of acute rejection

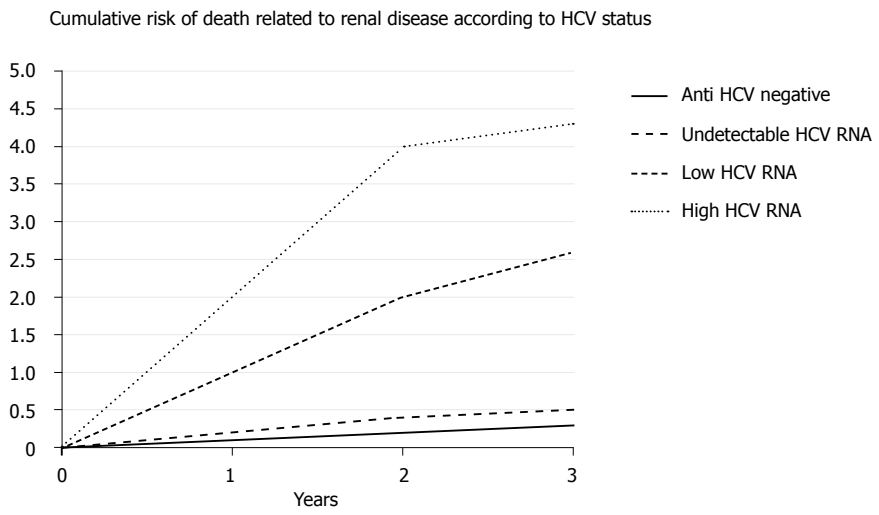

Figure 1 Hepatitis C virus infection is associated with an increased risk of renal disease, end-stage renal disease and renal-related mortality (REVEAL HCV Longitudinal Taiwanese study). HCV: Hepatitis C virus.

was found in HCV-positive patients, but this association is controversial[23,24]. Acute, often humoral, rejection is frequent in the patients receiving interferon (IFN) therapy[25]. Treatment of patients prior to transplantation is necessary, especially when IFN therapy is used. An increased risk of transplant glomerulopathy, the glomerular phenotype of chronic rejection, is associated with HCV infection[26,27]. An increased risk of new onset diabetes mellitus is associated with HCV infection[28,29]. An increase in post-transplant lympho-proliferative disorders was described in HCV patients transplanted with different organs[30].

These findings clearly document the need to manage HCV. The need for treatment during the dialysis period prior to transplantation is also clear. The standard therapy until recently consisted of IFN ± ribavirin administration, but the results were poor with this treatment. IFN was toxic, expensive and exhibited limited efficacy in the pre-transplant period. IFN treatment in the post-transplant period was also dangerous because it caused acute humoral rejections. HCV treatment may be divided in two periods: (1) IFN-based therapies; and (2) Direct acting antiviral (DAA) therapies.

IFN BASED THERAPIES

The first drug used for the treatment of HCV-positive patients with ESRD or transplantation was the recombinant alpha interferon (IFNα) eventually in combination with ribavirin, but the results in terms of sustained viral response (SVR) were poor.

Recombinant IFNα was first used as a monotherapy for chronic hepatitis C, but the drug only produced a modest SVR, several side effects were reported, and treatment was expensive and generated severe acute rejection when used after transplantation[25,31-35]. Fabrizi *et al*[36] performed a meta-analysis and concluded that the efficacy and safety of IFN-based therapies in renal transplant recipients were not satisfactory. The combination of IFNα with ribavirin increased the response rate, but induced the hemolysis as a new dose-dependent side effect[37]. This treatment was the standard of care until 1998. The introduction of pegylated IFNα increased the response rate by an additional 10%[38] and this treatment remained the standard of care until 2011.

The use of antiviral therapy was recommended for HCV patients in renal transplant candidates prior to transplantation because it was safer, effective and sustainable[1]. Several studies[39-44] confirmed these effects, including the Fabrizi *et al*[45] meta-analysis. One large randomized controlled trial recently demonstrated the greater efficacy and safety of combination antiviral therapy (pegylated IFN plus low-dose ribavirin, 200 mg daily) versus monotherapy (pegylated IFN alone) for HCV in a hemodialysis population[46]. The rates of sustained viral response were approximately 70%, and most dialysis patients tolerated the dual therapy well with appropriate patient monitoring.

DAA-BASED THERAPIES

Accumulating evidence and knowledge of the mechanism of action of HCV and the viral proteins involved in its replication during the 2000s allowed for the development of specific drugs for direct antiviral treatment (Figure 2). To date the DAAs may be divided in four classes according the mechanism of action (Table 1)

The first stage of this therapeutic revolution was the therapeutic introduction of protease inhibitors (PIs). The first generation of DAAs was represented by boceprevir and telaprevir, which inhibited NS3/4A protease activity. These drugs are inhibitors and substrates of the cytochrome (CYP) 3A4 isoenzyme in the liver and the intestinal P-glycoprotein (Pgp) transporter. However, these drugs may develop viral resistance. Therefore, these DAAs must be combined with pegylated IFN and

Table 1 The four classes of direct acting antivirals agents

The four classes of DAAs	Mechanism of action	Drugs (targeted genotypes in brackets)
NS3/4A PIs (PIs)	Block a viral enzyme (protease) that enables the HCV to survive and replicate in host cells	Glecaprevir (1-6) Paritaprevir (1, 4) Voxilaprevir (1-6) Grazoprevir (1, 3, 4)
Nucleoside and nucleotide NS5B polymerase inhibitors	Target the HCV to stop it from making copies of itself in the liver. So doing block the virus from multiplying	Sofosbuvir (1-4)
NS5A inhibitors	Block a virus protein, NS5A, that HCV needs to reproduce and for various stages of infection	Ombitasvir (1, 4) Pibrentasvir (1-6) Daclatasvir (3) Elbasvir (1, 4) Ledipasvir (1) Ombitasvir (1) Velpatasvir (1-6)
Non-nucleoside NS5B polymerase inhibitors	Stop HCV from reproducing by inserting themselves into the virus so that other pieces of the HCV cannot attach to it	Dasabuvir (1)

PIs: Protease inhibitors; HCV: Hepatitis C virus.

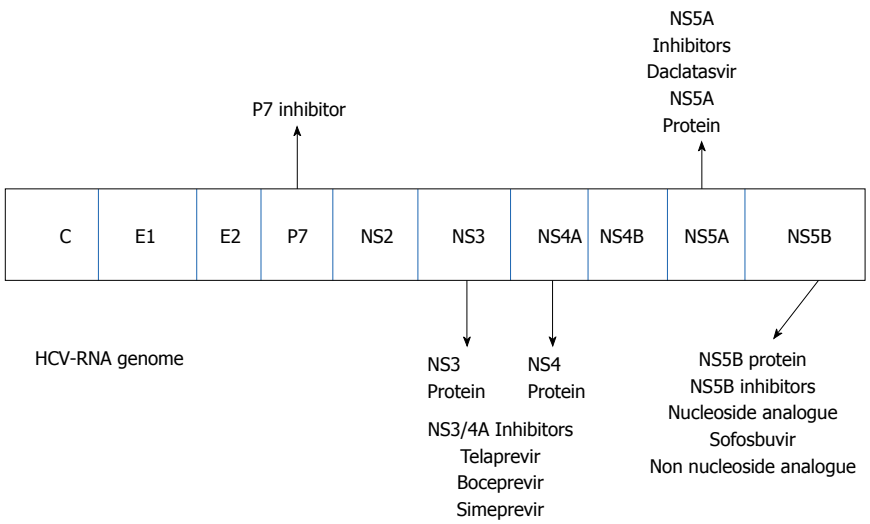

Figure 2 Development of new drugs for hepatitis C virus infection according the hepatitis C virus structure. HCV: Hepatitis C virus.

ribavirin. No dose adjustment is necessary for patients with hepatic or renal impairment[47-50].

New drugs target the 3 non-structural proteins of the NS3 serine protease. These serine PIs include simeprevir, paritaprevir and asunaprevir. Simeprevir is an inhibitor of gut cytochrome 3A4 and organic anion-transporting peptide 1B1/3 (OATP1B1/3), and treatment may produce indirect hyperbilirubinemia. Paritaprevir acts on the same cytochromes as simeprevir. These agents are better tolerated than boceprevir and telaprevir, but the antiviral activity is primarily limited to the HCV genotype Ⅰ. These drugs remain subject to viral resistance and are used in combination with other antiviral drugs. No dose adjustments are necessary in patients with renal impairment[51].

Another group of DAAs are inhibitors of NS5A, such as daclatasvir, ledispasvir and ombitasvir. These drugs inhibit the NS5A protein that controls phosphorilation/hyperphosphorilation and plays a vital role in HCV viral replication. These drugs also exhibit a low barrier of resistance and must be used in combination in combination with others antiviral[51]. No dose adjustments are necessary in patients with CKD.

The newest DAAs include the NS5B inhibitors. These agents are divided into two classes: Nucleoside and non-nucleoside inhibitors. Non-nucleoside inhibitors are less potent, produce viral resistance and are less frequently used[51]. The most important nucleoside NS5B inhibitor is sofosbuvir, which was recently approved for use in combination with other DAAs. Sofosbuvir targets HCV RNA synthesis at the catalytic site of the NS5B enzyme. Incorporation into the new RNA by the polymerase leads to premature chain termination. Numerous IFN-free regimens are in phase 2 and phase 3 clinical trials and

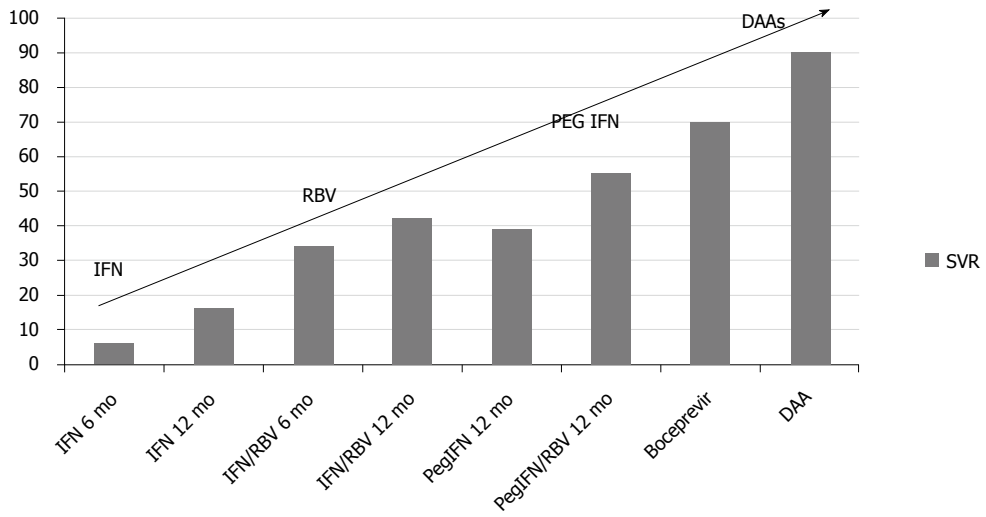

Figure 3 Sustained virological response with different therapies for hepatitis C virus genotype 1. HCV: Hepatitis C virus; SVR: Sustained virological response; IFN: Interferon; PEG IFN: Pegylated IFN; DAA: Direct acting antiviral; RBV: Ribavirin.

these combination regimens attained SVR in 90%-95% of patients[52-54]. Two publications summarize these drugs[55,56]. Figure 3 illustrates SVR improvement over time with different therapies for HCV genotype 1. These data refer to the general population.

EFFECTS OF DAAs IN PATIENTS WITH ESRD AND ON WAITING LISTS FOR RENAL TRANSPLANTATION

The prevalence of HCV infection in dialysis patients and patients on waiting lists for renal transplantation is high, between 6% and 40% and varies geographically[57,58]. In the Dialysis Outcomes Practice Patterns Study (DOPPS) the seroprevalence of HCV infection varies from 20% to 50% according the length on dialysis[59].

Patients with kidney disease are difficult to treat because they present with a high rate of co-morbid conditions, such as hypertension, diabetes mellitus and cardiovascular disease. Co-morbidities facilitate several adverse effects. Few data exist on the pharmacokinetics of DAAs in patients with reduced glomerular filtration rate (GFR). Drug-drug interactions between DAAs and drugs used for lipid-lowering and cardiovascular disease were reported[60]. Table 2 lists the currently available approved DAA-based regimens for the treatment of HCV in patients with renal failure based on HCV genotype[61].

The first-wave DAAs (*e.g.,* boceprevir and telaprevir) exhibited poor efficacy and few patients were treated with these agents. SVR was less than 70% and combination with IFN and ribavirin was mandatory because of viral resistance. Pockros *et al*[62] demonstrated that the combination of ombitasvir, paritaprevir and ritonavir produced SVR in 90% of patients with genotype 1 and stage 4/5 CKD. The regimen was well tolerated, and only the addition of ribavirin produced anemia (Study RUBY I NCT02207088). A more recent study[63] treated 104 patients with CKD and HCV genotypes 1, 2, 3, 4, 5 or 6 with the combination of the NS3/4A protease inhibitor glecaprevir and the NS5A inhibitor pibrentasvir for 3 mo. SVR was obtained in 98% of patients with few adverse events primarily consisting of pruritus, fatigue and nausea (NCT 02651194).

The C-SURFER study (NCT02092350) is a phase 3 study of the administration of NS3/4A protease inhibitor granzoprevir (100 mg) and the NS5A inhibitor elbasvir (50 mg) to 111 patients for 12 wk. The control group received placebo. SVR was obtained in 94.3% of patients, and only 4% of patients reported adverse events, which consisted of headache, nausea and fatigue[64,65]. The recent approval of the first pangenotypic NS5B inhibitor, sofosbuvir, revolutionized the treatment of HCV infection.

Sofosbuvir is a uridine nucleotide analog that inhibits hepatitis C RNA-dependent RNA polymerase and it is effective in all hepatitis C genotypes. Phase Ⅱ and phase Ⅲ studies reported that genotype Ⅰ patients who received sofosbuvir in combination with other DAAs achieved a sustained virological response rate greater than 90%. Different drug associations with sofosbuvir are suggested based on the HCV genotype[66]. Several studies demonstrated the efficacy and safety of these associations[67,68]. Some of these associations are principally useful in particular conditions. For example the association of sofosbuvir and velapatasvir revealed to be efficient in the case of HCV genotype 1, 2 and 3[69] and as rescue therapy in patients who developed viral resistance[53]. Many of these studies were performed in the context of the HCV-TARGET study.

HCV-TARGET is an observational longitudinal survey of patients affected by HCV different genotypes with different levels of renal function. The study is performed

Table 2 Available, approved direct acting antiviral-based regimens for treating hepatitis C virus in treatment-naive patients

Genotype 1a	Genotype 4
Ledipasvir + sofosbuvir	Ledipasvir + sofosbuvir
Paritaprevir + ritonavir + ombitasvir + dasabuvir	Paritaprevir + ritonavir + ombitasvir + dasabuvir + ribavirin
Sofosbuvir+ simeprevir ± ribavirin	Sofosbuvir + ribavirin + pegIFN
	Sofosbuvir + simeprevir + ribavirin
Genotype 1b	**Genotype 5**
Ledipasvir + sofosbuvir	Sofosbuvir + ribavirin
Paritaprevir + ritonavir + ombitasvir + dasabuvir	PegIFN + ribavirin
Sofosbuvir + simeprevir	
Genotype 2	**Genotype 6**
Sofosbuvir + ribavirin	Ledipasvir + sofosbuvir
	Sofosbuvir + ribavirin + pegIFN
Genotype 3	**Pangenotype**
Sofosbuvir + ribavirin	Glecaprevir + pibrentasvir
Sofosbuvir + ribavirin + pegIFN	Sofosbuvir + velatapasvir

pegIFN: Pegylated interferon.

at academic and community medical centers in North America and Europe. The study evaluates the efficacy and safety of antiviral regimens, including sofosbuvir, in 1893 patients (NCT01474811).

Sofosbuvir use was restricted to patients with an eGFR > 30 mL/min, and a few studies investigated the use of sofosbuvir in patients with ESRD[70-73]. Recently, the combination of sofosbuvir plus simeprevir was administered to 17 patients with ESRD. The SVR was 100% after 12 wk treatment. Few patients reported minor or mild adverse events[70].

Rostaing *et al*[74] recently reviewed the treatment of HCV infection in kidney transplant candidates with poor renal function or on dialysis. Saxena *et al*[75] reported the efficacy of sofosbuvir in association with ribavirin in 73 patients with an eGFR < 45 mL/min, and SVR was achieved in 83% of patients. However, these patients exhibited higher rates of anemia and deterioration of renal function regardless of the use of ribavirin. Because of this fact and because of pharmacokinetic studies, sofosbuvir should be administered with extreme caution to patients with reduced GFR. Indeed, the use of sofosbuvir in patients with renal impairment causes an increase in serum levels of sofosbuvir and an increase of the AUC of 171%. Desnoyer *et al*[76] performed a pharmacokinetic study in hemodialysis patients receiving two different doses of sofosbuvir and demonstrated that sofosbuvir did not accumulate in either regimen. Beinhardt *et al*[77] treated 25 patients (10 on dialysis and 15 had received renal or combined liver-renal transplantation with sofosbuvir in association with other DAAs. SVR was obtained in 96% of patients after 12 and 24 wk of treatment, but the treatment response was slower in hemodialysis patients[77]. Alternative treatments for patients with ESRD were reported recently from Japan, where the combined use of daclatasvir plus asuneprevir in genotype Ⅰ dialysis patients achieved a very high SVR rate[78-80].

EFFECTS OF DAAs ON KIDNEY TRANSPLANT PATIENTS WITH HCV INFECTION

We highlighted that the treatment of HCV renal transplant patients in the IFNα era was dangerous, poorly effective and frequently produced acute humoral rejection. Several recent studies demonstrated that the HCV infection eradication was feasible in renal transplant patients using DDAs, with few treatment-related side effects. However, these studies are recent, and the first guidelines for the use of DDAs in renal transplant patients were published at the end of 2017.

Colombo *et al*[81] performed a recent phase 2, open-label clinical trial to evaluate the safety and efficacy of the combination of ledipasvir and sofosbuvir in 5 European centers in 114 renal transplant patients infected with chronic genotype 1 or 4 HCV (NCT 02251717). The authors obtained SVR in 100% of patients after 12 wk of treatment. The eGFR remained stable, and adverse events were common (64%) and included headache, asthenia and fatigue. In one center the association of amiodarone and sofosbuvir probably caused a bradyarrhithmia and the patient interrupted the treatment[82]. The authors concluded that treatment with ledipasvir-sofosbuvir for 12 wk was well tolerated and achieved SVR in 12 wk with an acceptable safety profile. Sawinski *et al*[83] treated 20 renal transplant patients with HCV infection with a sofosbuvir-based therapy. SVR was obtained in all patients at 12 wk. Renal function remained stable, and no rejection occurred. However, 45% of patients required a dose reduction of the calcineurin inhibitor while receiving treatment. Saxena *et al*[84] reported the efficacy of DDAs therapy in 443 patients who received kidney (60) or liver transplant (347) or combined liver-kidney transplantation (36). The study was performed in the context of the vast HCV-TARGET study. Most patients had

HCV genotype Ⅰ. Patients were treated with sofosbuvir/ledipasvir ± ribavirin (85%), sofosbuvir plus daclatasvir ± ribavirin (9%) and ombitasvir/paritaprevir plus dasabuvir ± ribavirin (6%). SVR was achieved in 95.9% of patients after 12 wk of treatment. Six episodes of acute rejection occurred during HCV treatment. The authors concluded that different combinations of DAAs were effective and safe in kidney and/or liver transplant patients. Ribavirin did not influence SVR, and graft rejections were rare. Kamar *et al*[85] demonstrated the efficacy and safety of sofosbuvir-based antiviral therapy for HCV infection after renal transplantation in 25 patients. HCV RNA was not detectable in any patient 12 wk after completing DAA therapy. Treatment was well tolerated without graft rejections or reductions in renal function. Kamar did not observe any drug interaction with calcineurin inhibitors. These data differ from the findings of most studies. Hussein *et al*[86] reported the successful treatment of HCV genotype 4 in 3 renal transplant patients using the combination of sofosbuvir and ribavirin. Fernández *et al*[87] recently published data of the HepaC, which is a Spanish registry of 103 patients treated with DAAs after kidney transplantation. Most patients received a combination of sofosbuvir/ledipasvir or sofosbuvir/daclatasvir. The SVR at 12 wk was 98%. Three episodes of acute humoral rejection occurred, but there were no statistically significant differences in serum creatinine, eGFR or proteinuria before and after treatment. Most patients required immunosuppression dose adjustment, and 36% of patients, mostly cirrhotic, experienced renal dysfunction during antiviral treatment. The authors concluded that a close follow-up is required during treatment because of adjustments in immunosuppression therapy.

The phase 3, open-label, single-arm MAGELLAN-2 study evaluated a 12-wk course of the combination of the pangenotypic NS3/4A inhibitor glecaprevir and the pangenotypic NS5A inhibitor pibrentasvir in liver or renal transplant patients with chronic HCV genotype 1-6. Previous studies demonstrated that all these drugs exhibited a high barrier to resistance, sufficient potency against common NS3 and NS5A polymorphisms and synergistic antiviral activity. The study involved 80 liver transplant patients and 20 kidney transplant patients. The study demonstrated that the treatment with this combination for 12 weeks achieved a 99% SVR in patients with HCV genotypes 1-6. The treatment was well tolerated with few adverse events and confirmed the results obtained by Gane *et al*[63] in patients with ESRD. This new association represents an important alternative in treatment HCV patients after transplantation[88].

The American Association for the Study of Liver Diseases (AASLD) published the following HCV guidelines for kidney transplant patients in 2017[89] (Table 3): (1) The recommended drug association for the treatment of naïve and experienced kidney transplant patients with a genotype 1 or 4 infection: Daily fixed-dose combination of glecaprevir (300 mg)/pibrentasvir (120 mg) for 12 wk. An alternative is a daily fixed-dose combination of ledipasvir (90 mg) and sofosbuvir (400 mg) for 12 wk; and (2) The recommended association for the treatment of naïve and experienced kidney transplant patients with HCV genotypes 2, 3, 5 and 6: Daily fixed-dose combination of glecaprevir (300 mg)/pibrentasvir (120 mg) for 12 wk. An alternative is daily daclatasvir (60 mg) plus sofosbuvir (400 mg) and a low initial dose of ribavirin for 12 wk.

Pharmacokinetic warning for transplant patients

The most important factor to be considered in the treatment of HCV-infected renal transplant patients with DAAs is the possible interactions between DAAs and immunosuppressants. Kwo *et al*[90] recently reviewed this issue and found the following results: Sofosbuvir may be administered to transplant patients without any expected interaction with calcineurin inhibitors. A recent report[91] demonstrated no interaction with mycophenolate mofetil, prednisone or azathioprine. The combination of sofosbuvir and ledipasvir did not reveal any significant interaction with calcineurine inhibitors. No data on possible interactions with sirolimus or everolimus are available. The NS34A protease inhibitor simeprevir did not interact with tacrolimus (TAC), but recent pharmacokinetic studies demonstrated a 5.81-fold increase in the simeprevir AUC levels when administered with cyclosporine (CsA). Therefore, simeprevir should not be administered with CsA. A pharmacokinetic analysis was performed in patients receiving the combination of paritaprevir, ombitasvir and dasabuvir with TAC[92]. There was a 57-fold increase in the TAC AUC, and modeling suggested 0.5 mg of TAC every 7 d with strict monitoring of the TAC levels. A 5.8-fold increase in the CsA AUC was similarly observed, and CsA should be reduced to 1/5. No interaction data are available for paritaprevir, ombitasvir and dasabuvir with sirolimus and everolimus, and the co administration is not recommended. The NS5A inhibitor daclatasvir does not affect the CsA or TAC levels and no dose adjustment is required. The combination of elbasvir and grazoprevir produced a 15-fold increase in the grazoprevir AUC when administered with CsA, and this association is not recommended[89]. The combination of glecaprevir and pibrentasvir with CsA produced a 5-fold increase in the glecaprevir AUC when high doses of CsA were used. This same drug combination with TAC produced a 1.45-fold increase in the TAC AUC, and careful monitoring of the TAC levels is required[89]. Fernández-Ruiz *et al*[93] recently examined eGFR and 24-h proteinuria in 49 renal transplant patients who received sofosbuvir and ledipasvir for 12 mo after treatment. The TAC levels were higher at 12 mo compared to the end of treatment, and the eGFR was significantly decreased. The authors suggested adjusting immunosuppressants when DAAs are administered. Drug monitoring should also be performed after the end of the HCV treatment as well as monitoring of the renal

Table 3 Recommended regimens for kidney transplant patients

Recommended	Duration	Rating
Recommended regimens listed by evidence level and alphabetically for treatment-naive and experienced kidney transplant patients with genotype 1 or 4 infection, with or without compensated cirrhosis		
Daily fixed-dose combination of glecaprevir (300 mg)/pibrentasvir (120 mg)	12 wk	Ⅰ, A[1] Ⅱa, C[2]
Daily fixed dose combination of ledipasvir (90 mg)/sofosbuvir (400 mg)	12 wk	Ⅰ, A
Recommended and alternative regimens for treatment-naïve and experienced kidney transplant patients with genotype 2, 3, 4, 5 or 6 infection, with or without compensated cirrhosis		
Daily fixed-dose combination of glecaprevir (300 mg)/pibrentasvir (120 mg)	12 wk	Ⅰ, A[3] Ⅱa, C[4]
Alternative		
Daily daclatasvir (60 mg) plus sofosbuvir (400 mg) plus low initial dose of ribavirin (600 mg; increased as tolerated)	12 wk	Ⅱ, A

[1]Patients without cirrhosis; [2]Patients with compensated cirrhosis; [3]Genotypes 2, 3 and 6; [4]Genotype 5.

function.

TRANSPLANTING KIDNEYS FROM HCV POSITIVE DONORS INTO HCV POSITIVE RECIPIENTS

In the pre DAAs era, because of organ shortage, several transplant centers transplanted kidneys from HCV positive donors into HCV positive recipients. The issue was controversial. One of the largest reports using such strategy is the study of Morales *et al*[94]. In this study 162 HCV positive recipients received a kidney from HCV positive donors and were compared with 306 HCV positive recipients who received kidney from HCV negative donors. The 5 and 10 year patients survival was similar as well as the 5 and 10 years graft survival. The outcomes of the liver disease were also similar in both groups and the Cox regression analysis could not identify the donor's HCV serology as a significant risk factor. These data strongly suggest the use of kidneys from HCV positive donors in HCV positive recipients. Accordingly, the Kidney Disease Improving Global Outcomes (Kdigo)[1] recommended that transplantation of kidneys from HCV RNA positive donors should be directed to the HCV positive recipients. In United States, currently patients with untreated hepatitis C, who accept organ from HCV positive donors, may have a shorter time on transplant waiting list, while in other continents as Europe the positions differ according the different national programs. As afore mentioned direct-acting antiviral has revolutionized the treatment of hepatitis C infection also with implications for the use of HCV vermeil donors. Two recent papers reported the safety of transplanting kidneys from HCV positive donors to HCV positive recipients using DAAs[95,96]. The recommendation is to initiate early post-transplantation a pan-genotype therapy. A sustained SVR was near 100% and the DAA treatment after surgery was 125 d. Looking forward, the American Society of Transplantation (AST) held a consensus conference on the use of HCV viremic donors in solid organ transplantation[97].

The consensus conclusions established that: The term "HCV viremic donors" should be adopted; The provision of DAA to allow transplantation of HCV viremic donors into negative recipients is justified; The transplantation of organs from HCV viremic donors into HCV-negative recipients should be conducted only under monitored protocols and studies; There is a need for well-designed clinical trials of adequate power with conclusive findings to justify payer coverage of DAAs medications.

In this context the trial Exploring Renal Transplants Using Hepatitis C Infected Donors for HCV-negative Recipients (EXPANDER 1)[98] was started at the Johns Hopkins University. If the donor had genotype 1, the treatment included Grazoprevir and Elbasvir started immediately after transplantation and continued for 12 weeks. If the donor had genotype 1 with resistance variants, ribavirin was added. If the donor had genotype 2 or 3, sofosbuvir will be added. The data of this pilot study has been presented at the American Transplant Congress (ATC) 2017. Eight patients have been treated. After treatment no recipient had HCV-RNA detected and no graft failure was observed[99].

CONCLUSION

There has been a revolution in the treatment of chronic hepatitis C. Several oral regimens combining direct-acting antivirals (DAAs) from different families (NS5B nucleotide inhibitors, NS5B non-nucleoside inhibitors, NS5A replication complex inhibitors and NS3/4A PIs) have been developed. These regimens result in an increase in sustained virological response (SVR) rates to above 90% and reduce the duration of treatment to 12 wk or less. As of 2017 several regimens will be approved with additive potencies, without cross-resistance and with a good safety profile. Remaining issues will include increasing screening and access to care so that HCV may become the first chronic viral infection eradicated

Table 4 Main literature studies with direct acting antiviral therapy in patients with chronic hepatitis C and renal dysfunction

Ref.	Title	Journal	Year
[62]	Efficacy of direct-acting antiviral combination for patients with HCV genotype 1 infection and severe renal impairment or end-stage renal disease	*Gastroenterology*	2016
[63]	Glecaprevir and Pibrentasvir in patients with HCV and severe renal impairment	*N Engl J Med*	2017
[64]	Grazoprevir plus elbasvir in treatment-naive and treatment-experienced patients with HCV genotype 1 infection and stage 4-5 chronic kidney disease (the C-SURFER study): A combination phase 3 study	*Lancet*	2015
[65]	Elbasvir plus grazoprevir in patients with HCV infection and stage 4-5 chronic kidney disease: clinical, virological, and health-related quality-of-life outcomes from a phase 3, multicentre, randomized, double-blind, placebo-controlled trial	*Lancet Gastroenterol Hepatol*	2017
[70]	Use of sofosbuvir-based direct-acting antiviral therapy for HCV infection in patients with severe renal insufficiency	*Infect Dis*	2015
[71]	Safety, efficacy and tolerability of half-dose sofosbuvir plus simeprevir in treatment of hepatitis C in patients with end stage renal disease	*J Hepatol*	2015
[72]	Sofosbuvir and simeprevir in hepatitis C genotype 1-patients with end-stage renal disease on haemodialysis or GFR < 30 mL/min	*Liver Int*	2016
[74]	Use of direct-acting agents for HCV-positive kidney transplant candidates and kidney transplant recipients	*Transpl Int*	2016
[75]	Safety and efficacy of sofosbuvir-containing regimens in hepatitis C-infected patients with impaired renal function	*Liver Int*	2016

HCV: Hepatitis C virus.

Table 5 American Association for the Study of Liver Diseases Recommendation for treating hepatitis C virus in patients with renal impairment

Recommended	Rating	Genotype	Duration
Recommendations for patients with CKD stage 1, 2 or 3			
No dose adjustment is required when using			
(1) Daclatasvir (60 mg)			
(2) Daily fixed-dose combination of elbasvir (50 mg)/grazopevir (100 mg)			
(3) Daily fixed-dose combination of glecaprevir (300 mg)/pibrentasvir (120 mg)			
(4) Fixed-dose combination of ledipasvir (90 mg)/sofosbuvir (400 mg)	I , A		
(5) Fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)			
(6) Simeprevir (150 mg)			
(7) Fixed-dose combination of sofosbuvir (400 mg)/velpatasvir (100 mg)/voxilaprevir (100 mg)			
(8) Sofosbuvir (400 mg)			
Recommendations for patients with CKD stage 4 or 5 (eGFR < 30 mL/min or ESRD			
Daily fixed-dose combination of elbasvir (50 mg)/grazoprevir (100 mg)	I , B	1a, 1b, 4	12 wk
Daily fixed-dose combination of glecaprevir (300 mg)/pibrentasvir (120 mg)	I , B	1, 2, 3, 4, 5, 6	8 to 16 wk

CKD: Chronic kidney disease; ESRD: End-stage renal disease.

Table 6 European Association for the Study of the Liver Recommendations for treating hepatitis C virus in patients with reduced or absent renal function

Hemodialysis patients, particularly those who are suitable candidates for renal transplantation, should be considered for antiviral therapy (B1)
Hemodialysis patients should receive an IFN-free, if possible ribavirin-free regimen, for 12 wk in patients without cirrhosis, for 24 wk in patients with cirrhosis (B1)
Simeprevir, daclatasvir, and the combination of ritonavir-boosted paritaprevir, ombitasvir and dasabuvir are cleared by hepatic metabolism and can be used in patients with severe renal disease (A1)
Sofosbuvir should not be administered to patients with an eGFR < 30 mL/min per 1.73 m^2 or with end-stage renal disease until more data is available (B2)

worldwide.

The efficacy and safety of these new DAAs are primarily important in the field of renal diseases of patients affected by ESRD and of patients in dialysis waiting for a renal transplant and in patients already transplanted, but with HCV infection. The problem of HCV infection was particularly relevant in uremic patients in the pre-DAAs era and HCV was difficult to be eradicated.

The main studies in this field are cited in Table 4. Table 5 and Table 6 shows the recommendations for treating HCV in patients with renal impairment given from the American Association for the study of liver disease (AASLD)[90] and the European Association for the Study of the Liver (EASL)[60]. The access to transplantation to dialysis patients was allowed, but complications after transplantation were frequent and treatment was not possible after transplantation.

DAAs are able to eradicate HCV in dialysis patients with a short course therapy obtaining a SVR close to 100%. Additionally, DAA-treatment is successful even after transplantation. Particular attention must be devolved to the interference between DAAs and calcineurin inhibitors. Either an increase of CsA or TAC AUC or an increase of DAA AUC is possible and monitoring is essential even after long time after transplantation

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MINIREVIEWS

Review of stem cells as promising therapy for perianal disease in inflammatory bowel disease

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Abstract

Those patients with perianal Crohn's disease or ulcerative colitis experience a difficult to treat disease process with a delayed state and often inability to heal despite current therapies. The approaches currently used to treat these patients with corticosteroids, antibiotics, immunomodulators, anti-tumor necrosis factor-α drug, and surgical repair are limited in their healing ability. This review presents all current literature since emergence in the early 2000s of stem cell therapy for patients with perianal inflammatory bowel disease and analyzes the efficacy, outcomes and safety within these studies.

Key words: Crohn's disease; Stem cells; Mesenchymal; Perianal disease; Fistula; Inflammatory bowel disease

Core tip: Allogeneic and autologous mesenchymal stem cells (MSCs) are being researched for use in patients with refractory perianal Crohn's disease. Studies from 2003 until now demonstrate efficacy and safety of MSC therapy in this patient population. Up until now, there are no large multi-center, randomized double-blind, placebo-controlled studies examining this.

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INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory condition of the gastrointestinal tract that can disturb anywhere from the mouth to the anus. One of the most common manifestations of CD includes perianal disease, specifically including fistulas, abscesses, fissures, and stenosis. These complications frequently result in a significant burden for the patient due to abscess formation, perianal leakage, pain, and an overall decreased quality of life. Treatment options for perianal CD have traditionally included symptomatic management, antibiotics, and medications including immunomodulators and anti-tumor necrosis factor α agents, or surgery in cases with persistent refractory disease. However, surgical options are often limited and come with their own risks, which include incontinence and recurrence of disease. Recently, however, mesenchymal stem cells (MSCs) have been studied in perianal CD and results have been quite promising. This paper provides an up-to-date review on the use of MSC for perianal CD.

MSC therapy has been demonstrated to be a potentially effective treatment for perianal CD in a variety of ways. These stem cells are non-hematopoietic multipotent cells that can depress immune activation and encourage healing of inflamed tissue. MSCs have been found to hinder dendritic cell formation from monocytes, restrict naïve and memory CD4+ cells, stop T cell activation via inhibitory effects on mature dendritic cells, and encourage proliferation of regulatory T cells[1-6]. In addition, MSCs can travel to the site of inflammation and there contribute to local healing[7]. Over the last several years, multiple studies have evaluated autologous and allogeneic MSCs, to determine the safety and efficacy of treating perianal CD. The results are promising demonstrating significantly increased rates of healing for perianal disease refractory to conventional therapy alone. Here we will present studies involving autologous adipose, then autologous bone marrow studies, and then allogeneic adipose and bone marrow studies.

AUTOLOGOUS ADIPOSE STEM CELL STUDIES

The first report describing MSCs for perianal CD was a case report by García-Olmo *et al*[8] in 2003. Here, a rectovaginal fistula in CD was successfully healed seven days after the injection of adipose-derived MSCs. This same author then executed a phase Ⅰ clinical trial involving four individuals suffering from refractory complex Crohn's fistulas, again injecting the fistula tracts with autologous adipose-derived MSCs. Tissue repair was reported in three of four of patients at eight weeks, without adverse events during the one and two year follow up visits[9].

García-Olmo *et al*[10] then led a third study, a phase IIb trial, involving 49 patients with complex perianal cryptoglandular and CD fistulas comparing fibrin glue therapy to fibrin glue plus adipose-derived MSCs. Individuals in this latter group received a second dose of MSC if fistula healing did not appear after two months. In those with CD, fistula healing at twelve months occurred in five of seven (71%) in those given fibrin glue plus MSC as opposed to one of seven (14%) in those given fibrin glue alone[10]. Quality of life was also found to be better in the combined treatment group[10]. These early positive findings for MSCs treating perianal CD laid the groundwork for further work. In a dose-escalation phase Ⅰ trial led by Cho *et al*[11], ten individuals affected by perianal CD fistulas were given autologous adipose-derived MSCs. Following two months of treatment, fistula healing marked by epithelization was detected in three in ten (30%), with continued results at the eight month visit.

Lee *et al*[12] performed a follow-up phase Ⅱ study, including 33 treated subjects given injections of fibrin glue and adipose-derived MSCs with doses proportionate to fistula sizes, followed by repeat injections of increased doses if fistula closure did not complete by two months. Fistula healing was found in twenty-seven of thirty-three (82%) individuals by two months, with continued healing to twelve months in twenty-three of twenty-six (88%)[12]. The other six subjects of the original group developed an incomplete closure, five of which had a > 50% closure and decreased drainage[12].

Cho *et al*[13] did a further follow up study from their 2013 phase Ⅰ trial. Here adipose-derived MSC in fistulizing CD analyzed forty-one of forty-three patients for 12 mo and 24 mo weeks showing complete healing in 80.8% (21 of 26) patients in the complete healing pool and 75% (27 of 36) patients in the modified intention to treat pool[13]. The modified intention to treat pool included those patients who had efficacy data at one year in the phase Ⅱ study. Interestingly, regarding maintenance of complete closure, 27 patients achieved this at eight weeks, twenty-three of 26 (88.5%) at twelve months, twenty of 24 (83.3%) at twenty-four months[13]. Recurrence was seen in 11.5% at one year and 16.7% at two years. For the modified intention to treat group nine patients (25%) demonstrated an incomplete response at two years. Thus, the authors concluded that the use of MSC is safe and efficacious in perianal fistulizing disease.

For the Cho *et al*[13] study, one of the most unique aspects is the analysis of patients with MSC therapy and anti-TNF therapy. Of the twenty-four month group of twenty-seven patients showing complete healing, four patients receiving infliximab were documented. This was used due to enteric CD exacerbation, with 75% of these patients having complete closure prior to treatment with infliximab and having continued resolution of their fistula after infusion.

More recently, Dietz *et al*[14] led a phase Ⅰ clinical trial over a six month period assessing the safety and feasibility of autologous stem cell therapy for persistent,

Table 1 Summary of studies utilizing stem cell therapy in perianal Crohn's disease

Ref.	Study year	Stem cell therapy type	Type of study	Type of perianal disease	Method and amount of administration	Concurrent therapies	Outcome
[8]	2003	Autologous Adipose Stem Cell Studies	Case Report	Complex recurrent rectovaginal CD fistula	Local injection of 9 × 106 MSCs	Olsalazine (previously failed immunomodulators and biologics)	Healed 7 d after injection; no serious adverse events from MSC therapy were observed
[9]	2005	Autologous Adipose Stem Cell Studies	Phase Ⅰ Clinical Trial	Complex refractory CD fistulas, refractory to medical therapy and failing surgical therapy at least twice	Local injection of 3 × 106 MSCs	Immunosuppression without infliximab	Tissue repair in 75% (3 of 4) patients at 8 wk, no AE at 1 and 2 yr follow up; no serious adverse events from MSC therapy were observed
[10]	2009	Autologous Adipose Stem Cell Studies	Phase IIb Clinical Trial	Complex perianal cryptoglandular and CD fistulas, refractory to medical and surgical therapy (including at least one induction with anti-TNF)	Local injection of 2 × 106 MSCs plus fibrin glue vs fibrin glue alone; second local injection of 4 × 106 MSCs if no healing seen at 8 wk	Immunosuppression without infliximab, cyclosporine, or tacrolimus	71% (5 of 7) with fistula healing at 12 mo *vs* 14% healing in control group; higher quality of life in those with stem cell treatment; 1 serious adverse event from therapy (anal abscess)
[11]	2013	Autologous Adipose Stem Cell Studies	Dose-escalation Phase Ⅰ Clinical Trial	Perianal CD fistula, with CD confirmed by biopsy; 5 patients with previously unsuccessful surgical therapy	Local injection of 1 × 107, 2 × 107, 4 × 107 MSC, based on fistula size (total of 3-40 × 107 MSC)	Immunosuppression including infliximab	30% (3 of 10) patients with complete healing at two months and then continued eight month follow up; no serious adverse events from MSC therapy were observed
[12]	2013	Autologous Adipose Stem Cell Studies	Dose-proportional Phase Ⅱ Clinical Trial	Perianal CD fistula, less than 2cm in length	Local injection of 3 × 107 or 6 × 107 MSC, per 1 cm of fistula length; average 15.8 × 107 MSC, followed by second injection of 1.5 × previous (average 19 × 107 MSC) if incomplete closure at 8 wk	Immunosuppression including infliximab, but no infliximab within three months prior to MSC therapy	82% (27 of 33) patients with healing at 2 mo and continued healing of 88% these individuals (23 of 26) at 12 mo; of the 6/33 patients with incomplete closure, 5 had > 50% closure; no serious adverse events from MSC therapy were observed
[13]	2015	Autologous Adipose Stem Cell Studies	Phase Ⅱ Clinical Trial	Perianal CD fistulas	Local injection of 3 × 107 MSC, per 1 cm of fistula length; if second dose needed, 1.5 × previous dose administered	Immunosuppression including biologics	80.8% (21 of 26) patients with complete healing at 12 and 24 mo; recurrence in 11.5% at 12 mo and 16.7% at 24 mo; no serious adverse events from MSC therapy were observed
[14]	2017	Autologous Adipose Stem Cell Studies	Phase Ⅰ Clinical Trial	Refractory Perianal Fistulas in CD	Intra-operative placement of fistula plug, consisting of 20 × 106 MSC per plug attached to a bioabsorbable matrix	Biologic therapies (patients had failure to immunomodulators)	Healing in 83% (10 of 12) of patients at 6 mo; no serious adverse events from MSC therapy were observed
[15]	2011	Autologous Bone Marrow Stem Cell Studies	Phase Ⅱ Clinical Trial	Active complex perianal CD fistulas, refractory to medical and surgical therapies (including biologics)	Local injection of 1.5-3 × 107 MSC every 3 wk until improvement or until no longer available (2-5 injections total)	All patients took mesalamine and azathioprine, except for 2 taking prednisone with mesalamine and 2 on mesalamine monotherapy	Complete closure 67% (6 of 9) patients at 2 mo with continued closure at 12 mo; no serious adverse events from MSC therapy were observed

[16]	2017	Allogeneic Adipose Stem Cell Studies	Phase Ⅲ Randomized Clinical Trial	Refractory complex perianal CD fistulas; maximum of 2 internal and 3 external openings; draining for at least 6 wk	Local injection of 120 million C × 601 MSC or placebo; second injection of	Biologic therapies, immunomodulators, antibiotics	Closure at 24 wk in 50% (53 of 107) patients compared to placebo 34% (36 of 105) patients; shorter time to remission in treatment group *vs* placebo: 6.7 wk *vs* 14.6 wk; serious adverse events occurred in 6.8% of treatment subjects (7 of 103) and 6.9% of placebo subjects (7 of 102)-in both groups, the most common serious events were anal abscess/fistula and proctalgia
[17]	2015	Allogeneic Bone Marrow Stem Cell Studies	Phase IIa Randomized Clinical Trial	Refractory perianal CD fistulas to medical and surgical therapies, including all patients refractory to anti-TNF therapy	Local injections of 1 × 107 MSC for 5 patients; 3 × 107 MSC for 5 patients; 9 × 107 MSC for 5 patients; placebo for 6 patients	Stable doses of concurrent therapies, including mesalamine and steroids > 4 wk, immunomodulators > 8 wk, and anti-TNF > 8 wk	Healing in 47% (7 of 15) patients with MSC therapy vs 33% (2 of 6) with placebo at 12 wk; no serious adverse events from MSC therapy were observed

AE: Adverse events; MSC: Mesenchymal stem cell; CD: Crohn's disease; TNF: Tumor necrosis factor.

refractory perianal CD. This trial, dubbed Stem Cells on Matrix Plugs (STOMP), delivered concentrated, adipose-derived MSC attached to a bioabsorbable matrix to 12 patients. By three months, 9 of 12 patients (75%) achieved complete healing through clinical and radiographic determination; by six months, 10 of 12 of patients (83%) achieved this. There were no serious adverse events due to MSC therapy nor plug placement, and the study authors found these matrix plugs to be safe and effective for refractory perianal CD[14].

AUTOLOGOUS BONE MARROW STEM CELL STUDIES

There is much less data available regarding autologous bone marrow MSC treatment, compared to adipose-derived MSC treatment, in CD. A study led by Ciccocioppo utilized nine subjects with actively draining complex perianal fistulas who received intrafistular injections of bone marrow-derived MSC once monthly until healing was achieved or until they were no longer accessible. In all subjects, MSC expansion was successful. The fistulas were wholly closed in six of nine (67%) subjects at two months, with continued results at twelve months; in the other three cases incomplete closure was achieved[15].

ALLOGENEIC ADIPOSE STEM CELL STUDIES

A longer-term study evaluating allogeneic adipose-derived MSC for perianal CD was recently published with encouraging results. Led by Panes, this phase Ⅲ randomized clinical trial included 212 patients across 49 hospitals in Israel and Europe; 107 were given one injection of MSCs and 105 were given placebo with a saline injection. These participants had complex, medically refractory perianal fistulas draining for at least 6 wk, with a maximum of 2 internal and 3 external openings. The patients were kept on concurrent therapy during this study with biologics or immunomodulators or antibiotics. Twenty-four weeks after one local injection, those given MSC had significant clinical improvement delineated by closure of the external fistula tract and no fluid collections > 2 cm on magnetic resonance imaging (MRI). The authors found 53 of 107 subjects (50%) treated with MSC healed as opposed to 36 of 105 subjects (34%) given placebo (n = 36). Additionally, those given MSC experienced a much shorter time to remission of their disease: 6.7 wk as opposed to 14.6 wk. Explanations for why those in the placebo group experienced such high rates of fistula closure and remission include the fact that all patients received fistula curettage, internal orifice closure, and surgical drainage. While this study did not address the potential benefits of repeat injections of MSCs or dosage of injections based on size of fistula tract, it did provide large-scale, sustained positive results of MSCs for perianal CD. An expansion of this project has been developed in the United States, which is also a phase Ⅲ multicenter, randomized clinical trial evaluating allogeneic adipose-derived MSC for perianal CD[16].

ALLOGENEIC BONE MARROW STEM CELL STUDIES

Finally, Molendijk *et al*[17] studied allogeneic MSCs derived from bone marrow in a phase IIa randomized clinical trial in the Netherlands. There were twenty-one patients with refractory perianal fertilizing CD included; five were given a single shot of 1 × 107 MSCs, five were given 3 × 107 MSCs, five were given 9 × 107 MSCs, and six were given placebo. These injections were placed around the internal openings of fistula walls. Fistula healing was determined to be cessation of drainage and absence of fluid collections > 2 cm on MRI, and was observed in

seven of 15 (47%) of those administered MSCs and two of 6 (33%) of those given placebo. These encouraging results were found not only at the study's primary endpoint, week twelve, but also endured through week twenty-four. Amongst the range of dosages of MSCs given, the best effects were observed in those given 3 × 107. Notably, none of the treatment regimens were associated with an increase in adverse events (Table 1)[17].

CONCLUSION

Perianal CD is quite challenging for both patients and providers with delayed and difficult healing, despite current standard therapy including antibiotics, immunomodulators, anti-TNF treatment, and surgical repair. Need for novel treatment options to improve outcomes in these patients is obvious. Here, the promising results of recent and ongoing studies utilizing stem cell therapy-either allogeneic or autologous-for treatment of this patient population are presented. Given this data, the authors conclude that future randomized double-blind, placebo-controlled multi-center studies on the efficacy and safety of stem cell therapy for perianal disease in CD are warranted.

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ORIGINAL ARTICLE

Retrospective Study

Kidney transplantation in older recipients: Preemptive high KDPI kidney *vs* lower KDPI kidney after varying dialysis vintage

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Abstract

AIM

To evaluate the outcomes of transplanting marginal kidneys preemptively compared to better-quality kidneys after varying dialysis vintage in older recipients.

METHODS

Using OPTN/United Network for Organ Sharing database from 2001-2015, we identified deceased donor kidney (DDK) transplant recipients > 60 years of age who either underwent preemptive transplantation of kidneys with kidney donor profile index (KDPI) ⩾ 85% (marginal kidneys) or received kidneys with KDPI of 35%-84% (better quality kidneys that older wait-listed patients would likely receive if waited longer) after being on dialysis for either 1-4 or 4-8 years. Using a multivariate Cox model adjusting for donor, recipient and transplant related factors- overall and death-censored graft failure risks along with patient death risk of preemptive transplant recipients were compared to transplant recipients in the 1-4 and 4-8 year dialysis vintage groups.

RESUTLS

The median follow up for the whole group was 37 mo (interquartile range of 57 mo). A total of 6110 DDK transplant recipients above the age of 60 years identified during the study period were found to be eligible to be included in the analysis. Among these patients

350 received preemptive transplantation of kidneys with KDPI ⩾ 85. The remaining patients underwent transplantation of better quality kidneys with KDPI 35-84% after being on maintenance dialysis for either 1-4 years (n = 3300) or 4-8 years (n = 2460). Adjusted overall graft failure risk and death-censored graft failure risk in preemptive high KDPI kidney recipients were similar when compared to group that received lower KDPI kidney after being on maintenance dialysis for either 1-4 years (HR 1.01, 95%CI: 0.90-1.14, P = 0.84 and HR 0.96, 95%CI: 0.79-1.16, P = 0.66 respectively) or 4-8 years (HR 0.82, 95%CI: 0.63-1.07, P = 0.15 and HR 0.81, 95%CI: 0.52-1.25, P = 0.33 respectively). Adjusted patient death risk in preemptive high KDPI kidney recipients were similar when compared to groups that received lower KDPI kidney after being on maintenance dialysis for 1-4 years (HR 0.99, 95%CI: 0.87-1.12, P = 0.89) but lower compared to patients who were on dialysis for 4-8 years (HR 0.74, 95%CI: 0.56-0.98, P = 0.037).

CONCLUSION

In summary, our study supports accepting a "marginal" quality high KDPI kidney preemptively in older wait-listed patients thus avoiding dialysis exposure.

Key words: Preemptive kidney transplantation; Kidney donor profile index; Dialysis vintage; Kidney transplant outcomes; Older recipients; Waiting list

Core tip: Increasing waiting-time for deceased donor kidney (DDK) transplantation adversely impacts older patients disproportionately. Dialysis vintage and transplantation of "marginal kidneys" are associated with inferior post-transplant outcomes. Using OPTN/United Network for Organ Sharing database from 2001-2015, we compared the outcomes of preemptive transplantation of marginal [kidney donor profile index (KDPI) ⩾ 85%] DDKs compared to transplanting better quality DDKs (KDPI 35%-84%) after being on dialysis for 1-4 and 4-8 years in patient > 60 years old. Preemptive transplantation of marginal kidneys provided non-inferior graft and patient outcomes compared to transplanting better quality kidneys in older patients on maintenance dialysis. Early transplantation could also provide quality of life and cost benefits.

Chopra B, Sureshkumar KK. Kidney transplantation in older recipients: Preemptive high KDPI kidney *vs* lower KDPI kidney after varying dialysis vintage. *World J Transplant* 2018; 8(4): 102-109 Available from: URL: http://www.wjgnet.com/2220-3230/full/v8/i4/102.htm DOI: http://dx.doi.org/10.5500/wjt.v8.i4.102

INTRODUCTION

Number of patients waiting for kidney transplantation has been steadily growing in the United States with nearly 100000 currently on the waiting list. Organ shortage is the major limiting factor. With the intention to optimize utilization of deceased donor kidneys (DDKs), Organ Procurement and Transplant Network (OPTN) implemented the new kidney allocation system (KAS) in December 2014[1]. In the new KAS, each kidney is allocated a kidney donor profile index (KDPI) based on 10 donor variables. KDPI is derived from the prediction model termed kidney donor risk index (KDRI) which was originally proposed by Rao *et al*[2] in 2009. KDPI score ranges from 0%-100% with higher scores meaning lower quality kidneys. For instance, a KDPI score of 85% means that the kidney quality is worse than 85% of kidneys recovered for transplantation during the previous calendar year. The new KAS promotes allocation of better quality kidneys to recipients with better estimated post-transplant survival in a concept called longevity matching[3]. On the other hand, kidneys with higher KDPI are likely offered to older recipients.

Preemptive transplantation (transplantation before the need for maintenance dialysis) has been shown to be associated with better post-transplant outcomes[4,5]. Dialysis vintage is an independent predictor of adverse long-term outcomes following both deceased and living donor kidney transplantation[6-9]. Kidneys with KDPI ⩾ 85% are considered "marginal" and transplantation of such organs are associated with inferior outcomes when compared to transplanting kidneys with lower KDPI[10]. It is unclear whether preemptive transplantation of high KDPI kidneys and thus avoiding maintenance dialysis in older recipients would be beneficial compared to waiting for and transplanting lower KDPI kidneys after being on dialysis for varying lengths of time. We sought to answer this by utilizing the national transplant database.

MATERIALS AND METHODS

Study population

The study protocol was approved by the institutional review board and was conducted in accordance with the ethical standards laid down in the 2000 Declaration of Helsinki as well as 2008 Declaration of Istanbul. Using OPTN/United Network for Organ Sharing (UNOS) database, we identified patients older than 60 years who underwent first time DDK transplantation between January 2001 and December 2015, after receiving perioperative antibody induction and discharged on a calcineurin inhibitor (CNI) and Mycophenolate Mofetil (MMF) based maintenance immunosuppression. From this group, we further identified patients who underwent preemptive transplantation with kidneys with KDPI ⩾ 85% and those who underwent transplantation of kidneys with KDPI of 35%-84% after being on maintenance dialysis for either 1-4 years or 4-8 years. We chose KDPI of 35%-84% in the dialysis groups in order to approximate real life scenarios since older patients who wait longer will likely get offer for DDKs with mid-range quality with new KAS. KDPI was calculated retrospectively by OPTN/UNOS and is available in their

Table 1 Demographics

	Preemptive-high KDPI (n = 350)	1-4 yr dialysis vintage-lower KDPI (n = 3300)	Preemptive-high KDPI (n = 350)	4-8 yr dialysis vintage-lower KDPI (n = 2460)
KDPI	93 ± 4	62 ± 14	93 ± 4	62 ± 9
Dialysis duration (mo)	0	31 ± 10	0	67 ± 13
Age (donor)	61 ± 12	46 ± 13[b]	61 ± 12	46 ± 14[b]
Donor gender (M) %	46.8	56[d]	46.8	54.3[a]
DCD kidney (%)	8.6	14.4[d]	8.6	14.9[d]
ECD kidney (%)	89.4	25.6[b]	89.4	26[b]
HLA mismatch	4.5 ± 1.3	3.9 ± 1.7[b]	4.5 ± 1.3	4.3 ± 1.4[a]
Recipient age (years ± SD)	69 ± 5	67 ± 4[a]	69 ± 5	67 ± 4[b]
Recipient gender (M) %	52.4	63.5[b]	52.4	64[b]
African American Recipient (%)	14.7	20.9[a]	14.7	30.8[b]
Recipient diabetes (%)	30.4	51[b]	30.4	52.5[b]
Recipient BMI (%)	27 ± 4	28 ± 5[a]	27 ± 4	28 ± 5
Calculated PRA	4.6 ± 14	10 ± 25[b]	4.6 ± 14	13 ± 27[b]
Cold ischemia time (h)	19 ± 8	18 ± 9	19 ± 8	18 ± 9
Delayed graft function (%)	5.3	29[b]	5.3	37.5[b]
Depleting induction (%)	65.5	69.8	65.5	71.5[a]
Steroid maintenance (%)	64	69.6[a]	64	70.2[a]
Kidney pumped (%)	53.7	42.2[b]	53.7	44[d]
Transplant year	2009 ± 4	2008 ± 4[a]	2009 ± 4	2010 ± 3[b]

[a]$P \leqslant 0.05$, [b]$P \leqslant 0.001$, [d]$P \leqslant 0.005$, *vs* preemptive-high KDPI kidneys. BMI: Body mass index; DCD: Donation after cardiac death; ECD: Expanded criteria donor; HLA: Human leukocyte antigen; KDPI: Kidney donor profile index; PRA: Panel reactive antibody.

database. Patients were excluded from the analysis if they received previous transplant, underwent live donor kidney, or multi-organ transplantation. Patients were also excluded if they received no induction or were on maintenance regimen other than CNI/MMF.

Demographic variables for the three groups were collected. Overall and death-censored graft failure risks along with patient death risk associated with preemptive transplantation of high KDPI (⩾ 85%) kidneys were compared to these outcomes associated with transplantation of lower KDPI (35%-84%) kidneys among recipients who were on maintenance dialysis for 1-4 years and 4-8 years after correcting for pre-specified variables. The covariates used for correction in the multivariate model were: donor related including age, gender, expanded criteria donor kidney, donation after cardiac death kidney, cause of donor death; recipient related including age, African American race, diabetes mellitus, hepatitis B and C sero-positivity, ESRD cause, dialysis duration, panel reactive antibody (PRA) titer (peak PRA till 2009 and calculated PRA from 2009 onwards), human leukocyte antigen mismatch; transplant related including type of induction, cold ischemia time, pump perfusion of kidney, delayed graft function (defined as need for dialysis within the first week of transplantation), steroid maintenance, and transplant year.

Statistical analysis

Continuous variables were compared between groups using 2-tailed *t*-tests and categorical variables were compared using χ^2 test. Values were expressed as either mean ± standard deviation or as percentages. Missing values were addressed by imputing means of the variables. Cox model was used to compare adjusted graft and patient outcomes between the groups. Hazard ratios (HR) with 95% confidence intervals (CI) were calculated. A P value of < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software version 18 (IBM, Armonk, NY, United States).

RESULTS

Demographic characteristics

The median follow up for the whole group was 37 mo (interquartile range of 57 mo). A total of 6110 DDK transplant recipients above the age of 60 years identified during the study period were found to be eligible to be included in the analysis. Among these patients 350 received preemptive transplantation of kidneys with KDPI ⩾ 85. The remaining patients underwent transplantation of better quality kidneys with KDPI 35%-84% after being on maintenance dialysis for either 1-4 years (n = 3300) or 4-8 years (n = 2460).

The demographic features of the different groups are shown in Table 1. Preemptively transplanted kidneys had a KDPI of 93% ± 4% while the KDPI were 62% ± 14% and 62% ± 9% in patients who received the transplant after being on dialysis for 1-4 years and 4-8 years respectively. Mean dialysis duration was 31 ± 10 mo and 67 ± 13 mo respectively in patient groups with dialysis duration 1-4 years and 4-8 years. As shown there were significant differences between the preemptive transplant group and groups that received kidney transplant after being on maintenance dialysis. In the preemptive transplant group, donor age was higher with fewer male donors along with fewer

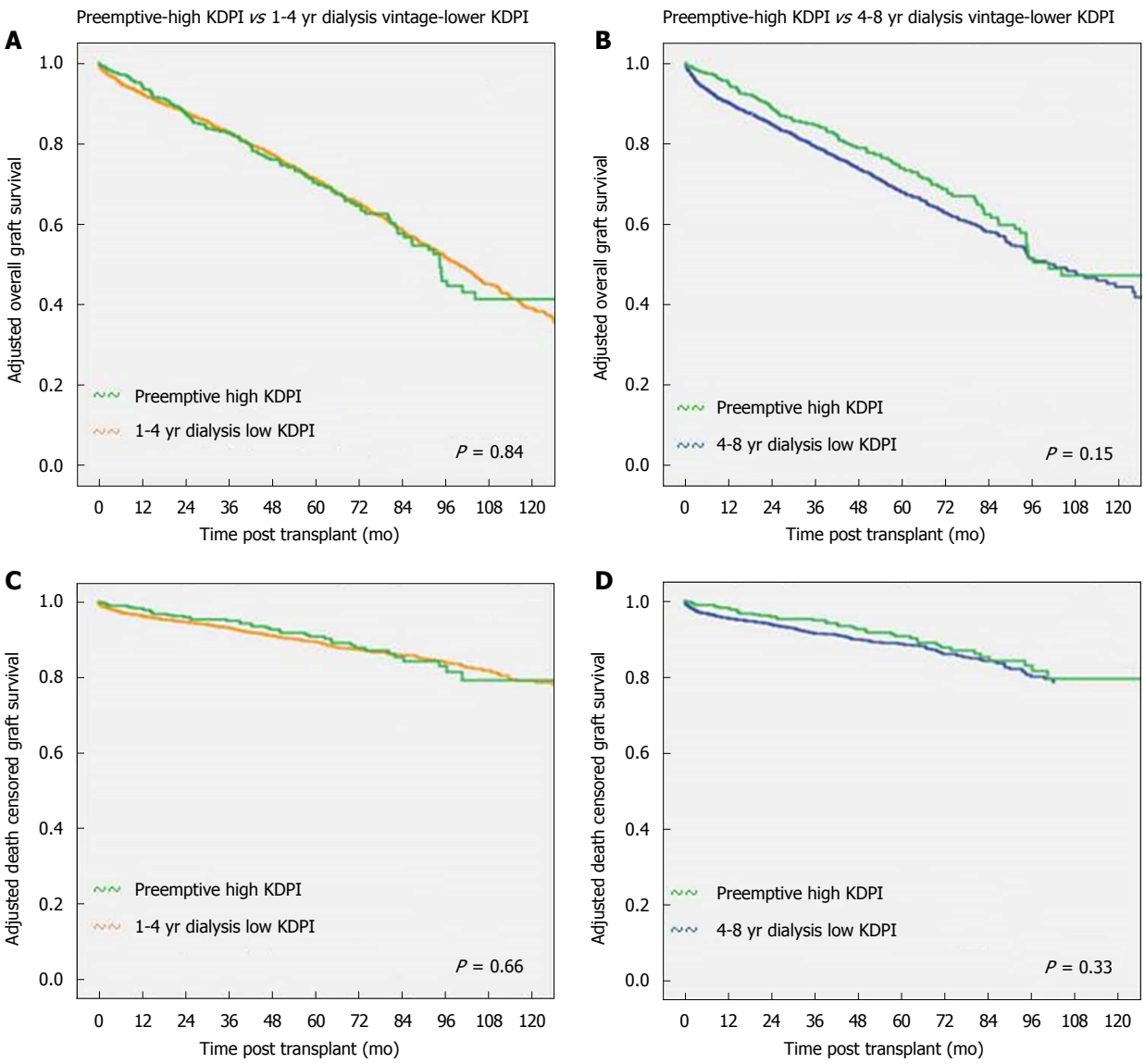

Figure 1 Adjusted graft survival. A: Overall graft survival for recipients of preemptive-high KDPI kidneys compared to 1-4 years dialysis vintage-lower KDPI kidneys; B: Overall graft survival for recipients of preemptive-high KDPI kidneys compared to 4-8 years dialysis vintage-lower KDPI kidneys; C: Death-censored graft survival for recipients of preemptive-high KDPI kidneys compared to 1-4 years dialysis vintage-lower KDPI kidneys; D: Death-censored graft survival for recipients of preemptive-high KDPI kidneys compared to 4-8 years dialysis vintage-lower KDPI kidneys. KDPI: Kidney donor profile index.

donation after cardiac death (DCD) and more expanded criteria donor (ECD) kidneys; recipients were older with fewer males, African Americans, and diabetics. Preemptive group also had higher proportion of kidneys pump perfused, lower PRA, higher HLA mismatches, lower DGF rates and lower steroid maintenance rates.

Graft and patient outcomes

Adjusted overall graft and death-censored graft survivals of preemptive high KDPI kidney recipients compared to recipients of lower KDPI kidneys with 1-4 years and 4-8 years dialysis vintage is shown in Figure 1. Adjusted overall graft failure risk and death-censored graft failure risk in preemptive high KDPI kidney recipients were similar when compared to group that received lower KDPI kidney after being on maintenance dialysis for either 1-4 years (HR 1.01, 95%CI: 0.90-1.14, P = 0.84 and HR 0.96, 95%CI: 0.79-1.16, P = 0.66 respectively) or 4-8 years (HR 0.82, 95%CI: 0.63-1.07, P = 0.15 and HR 0.81, 95%CI: 0.52-1.25, P = 0.33 respectively) as shown in Table 2.

Adjusted patient survival of preemptive high KDPI kidney recipients compared to recipients of lower KDPI kidneys with 1-4 years and 4-8 years dialysis vintage are shown in Figure 2. Adjusted patient death risk in preemptive high KDPI kidney recipients were similar when compared to groups that received lower KDPI kidney after being on maintenance dialysis for 1-4 years (HR 0.99, 95%CI: 0.87-1.12, P = 0.89) but lower compared to patients who were on dialysis for 4-8 years (HR 0.74, 95%CI: 0.56-0.98, P = 0.04) as shown in Table 2.

Table 2 Comparison of graft and patient outcomes between the groups

	Preemptive-high KDPI (n = 349) *vs* 1-4 yr dialysis vintage-lower KDPI (n = 3300)		Preemptive-high KDPI (n = 349) *vs* 4-8 yr dialysis vintage-lower KDPI (n = 2460)	
Adjusted overall graft failure risk	1.01 (0.90-1.14)	0.84	0.82 (0.63-1.07)	0.15
Adjusted death censored graft failure risk	0.96 (0.79-1.16)	0.66	0.81 (0.52-1.25)	0.33
Adjusted patient death risk	0.99 (0.87-1.12)	0.89	0.74 (0.56-0.98)	0.04

KDPI: Kidney donor profile index.

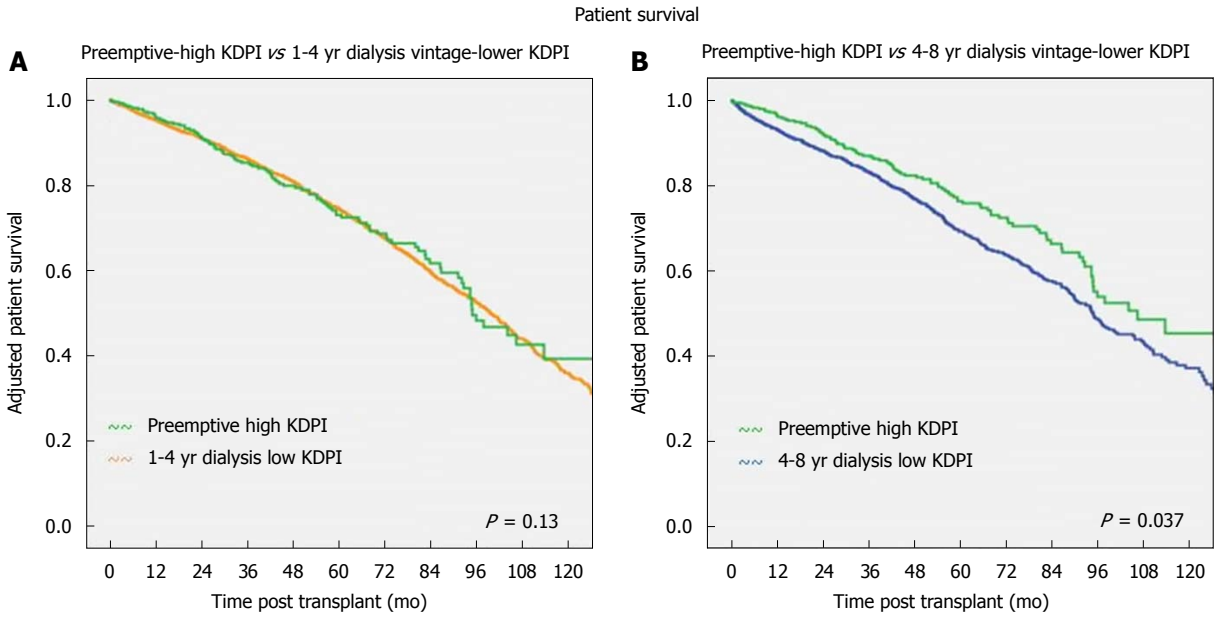

Figure 2 Adjusted patient survival. A: Patient survival for recipients of preemptive-high KDPI kidneys compared to 1-4 years dialysis vintage-lower KDPI kidneys; B: Patient survival for recipients of preemptive-high KDPI kidneys compared to 4-8 years dialysis vintage-lower KDPI kidneys. KDPI: Kidney donor profile index.

DISCUSSION

Our study showed that preemptive transplantation of high KDPI (⩾ 85%) kidneys in older first-time recipients conferred graft and patient outcomes that were not inferior when compared to transplanting better quality lower KDPI (35%-84%) kidneys in older recipients who were on maintenance dialysis for variable periods of time. In fact a patient survival benefit was emerging for preemptive high KDPI kidney recipients when compared to patient who got transplanted better quality kidney after a longer dialysis vintage. Our findings support favorable consideration of "marginal" kidneys for preemptive transplantation in older patients on the waiting list.

Living donor kidney transplantation in general offers the best patient and graft survival with the benefits extending to older recipients as well[11,12]. Living donor kidneys from 60-69 years old donors transplanted into older recipients' conferred superior patient survivals compared to standard criteria donor (SCD) and ECD DDKs while the graft survivals were superior compared to ECD but similar compared to SCD kidneys[13]. Patients without options for living donors are faced with an increasing time on the deceased donor wait list. The median time to transplant once listed has been steadily increasing, for instance from 5.5 years in 2003 to 7.6 years in 2007[11]. This is particularly disadvantageous to older wait listed patients, since longer they wait; the less likely they get transplanted since their health status can deteriorate thus running the risk of removal from the wait list or death[14]. Consideration of high KDPI kidneys can help to decrease the waiting time for such patients.

Transplantation of DDKs with high KDRI (from which KDPI is calculated) is associated with increased risk for allograft failure when compared to transplanting lower KDRI kidneys[2,10]. As mentioned, DDKs with KDPI ⩾ 85% are considered as "marginal" quality organs similar to the kidneys from ECD terminology used prior to the implementation of new KAS. Transplantation of ECD kidneys have been shown to be associated with higher risk for developing DGF, longer hospital length of stay and higher readmissions rates with higher cost of care along with increased risk for graft loss and mortality[15-18]. Because of these concerns, centers could understandably be reluctant to accept marginal kidneys for preemptive transplantation in their wait listed patients who have not started maintenance dialysis yet. However, it is hard to predict how long such patients

will have to wait to get offer for a more desirable kidney with a good chance that they could initiate dialysis while waiting. Our findings support the practice of careful consideration of marginal kidney offers compared to automatic decline of such kidney offers for preemptive transplantation in wait listed older recipients. This may also help to reduce the discard rate for these kidneys with KDPI ⩾ 85% which was at 60% at year 2 after the implementation of new KAS according to a recent UNOS report[19].

Despite a 70% increased risk for graft failure compared to non-ECD kidneys, transplantation of ECD kidneys which are considered "marginal" was found to confer survival benefit when compared to staying on waiting list[12,20-22]. Dialysis duration has been suggested as the strongest independent modifiable risk factor for renal transplant outcomes[8]. Increased comorbidity burden and immunological alterations that can develop in dialysis patients, along with adverse socioeconomic conditions associated with prolonged dialysis are some of the factors implicated towards inferior transplant outcomes observed in patients exposed to longer dialysis duration. Any adverse impact of transplanting high KDPI marginal kidneys in our preemptive group likely got mitigated by dialysis avoidance. On the other hand, any potential benefits of transplanting better quality lower KDPI kidneys in the dialysis groups are likely minimized by the impact of dialysis vintage on transplant outcomes. A previous analysis showed lower overall cumulative mortality associated with transplantation of high KDPI kidneys when compared to equivalent patients who forego high KDPI kidney transplantation with the hope of receiving lower KDPI kidney at a later time point while staying on dialysis[23]. Benefit was more pronounced in recipients > 50 years of age and at centers with wait time > 33 mo.

While our study demonstrated similar graft and patient outcomes for preemptive transplantation of high KDPI kidneys when compared to low KDPI kidney transplantation after varying dialysis vintage in older recipients, one also has to consider the quality of life advantage that can come with earlier transplantation. Previous studies have shown quality of life benefits in older patients who underwent kidney transplantation[24,25]. Earlier kidney transplantation could also translate into long-term cost savings. A recent economic analysis of contemporary kidney transplant practice found cost saving with living donor and low KDPI deceased donor transplants when compared to dialysis while transplantation using high KDPI DDK was cost effective[26].

Our study has limitations that merit discussion. Retrospective design only can prove associations but not causation. However, a prospective study addressing the same question will be difficult to conduct for logistical reasons. Residual confounding can still occur despite using a multivariate adjustment in our analysis. Doses or drug levels of maintenance immunosuppressive drugs and information about longitudinal changes in medication regimens which could impact transplant outcomes were not available. Even though our analysis showed favorable outcomes of preemptive transplantation of high KDPI kidneys in older recipients, this does not imply transplantability of each and every such kidney. The analysis was biased towards kidneys that actually got transplanted and kidneys may be rejected for reasons unrelated to KDPI.

In summary, our study supports accepting a "marginal" quality high KDPI kidney preemptively in older wait-listed patients thus avoiding dialysis exposure. Such preemptive transplantation results in graft and patient outcomes non-inferior to receiving a better quality kidney with lower KDPI after being on dialysis for a variable period. This practice could come with an added quality of life benefit associated with earlier transplantation and possibly cost benefit. In order to best serve such patients on the waiting list, clinicians should be open to offers of high KDPI kidneys and get the patients involved in this important and very personal decision making process.

ARTICLE HIGHLIGHTS

Research background

It is unclear whether preemptive transplantation of high kidney donor profile index (KDPI) (marginal quality) kidneys and thus avoiding maintenance dialysis in older recipients would be beneficial compared to waiting for and transplanting lower KDPI (better quality donor organ) kidneys after being on dialysis for varying lengths of time. We sought to answer this by utilizing the national transplant database.

Research motivation

The aim of this study was to evaluate the outcomes of transplanting marginal kidneys preemptively compared to better-quality kidneys after varying dialysis vintage in older recipients.

Research objectives

The objective of our study was to explore the benefits of transplanting marginal quality kidney preemptively compared to waiting for better quality kidney transplantation after exposure to varying times on dialysis.

Research methods

Using United Network for Organ Sharing database, we identified patients > 60 years who underwent first time deceased donor kidney (DDK) transplantation between January 2001 and December 2015, after receiving induction and discharged on calcineurine inhibitor/Mycophenolate Mofetil immunosuppression. We further identified patients who underwent preemptive DDK with KDPI ⩾ 85% and those who underwent DDK with KDPI of 35%-84% after being on maintenance dialysis for either 1-4 years or 4-8 years. Cox model was used to compare adjusted graft and patient outcomes between the groups. HR with 95%CI was calculated. A P value of < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software version 18.

Research results

Adjusted overall graft failure risk and death-censored graft failure risk in preemptive high KDPI kidney recipients were similar when compared to group that received lower KDPI kidney after being on maintenance dialysis for either 1-4 years or 4-8 years. Adjusted patient death risk in preemptive high KDPI kidney recipients were similar when compared to groups that received lower KDPI kidney after being on maintenance dialysis for 1-4 years but lower

compared to patients who were on dialysis for 4-8 years.

Research conclusions

Our study supports accepting a "marginal" quality high KDPI kidney pre-emptively in older wait-listed patients thus avoiding dialysis exposure. In order to best serve older patients on the waiting list, clinicians should be open to offers of high KDPI kidneys and get the patients involved in this important and very personal decision making process. A pre-emptive kidney transplant-even if it is a marginal organ, could come with an added quality of life benefit associated with earlier transplantation and possibly cost benefit. It is acceptable to use marginal quality kidneys in older transplant recipients, rather than having them wait on dialysis for better quality kidney. It has been widely accepted that marginal quality organs are acceptable for use in older transplant recipients. But there has been hesitance in accepting these kidneys for recipients who are not on dialysis yet. The purpose of this study was to evaluate the impact of avoiding dialysis vintage by preemptive transplantation of marginal kidneys in older recipients when compared to receiving better quality organ while remaining on dialysis. Avoiding dialysis with early transplantation should be favorably considered even with marginal quality kidneys. It will be logistically hard to design a prospective study trying to answer the same question; but that would be ideal. Future study should identify older patients who declined preemptive offer of marginal kidneys and went on to get better quality kidneys at a later point after being on dialysis. Control group should be older patients who accepted those marginal kidneys preemptively. Post-transplant outcomes between the 2 groups should be compared. It is acceptable to use a marginal quality kidney in an older recipient, thereby avoiding dialysis exposure. The current study supports the hypothesis of transplanting marginal quality kidney preemptively in older patients. The findings of this study enable transplant professionals to make a more informed choice when faced with the option of getting a marginal kidney offer for their older wait listed patients with chronic kidney disease who are not on dialysis yet.

Research perspectives

Avoiding dialysis exposure with early transplant even with a marginal kidney is potentially beneficial. Future studies should look at the outcomes of older patients who turned down a marginal kidney for preemptive transplantation and received better quality kidney after exposure to variable dialysis time compared to older patients who accepted the declined marginal kidneys preemptively and thus avoided dialysis exposure. Future study should identify older patients who declined preemptive offer of marginal kidneys and went on to get better quality kidneys at a later point after being on dialysis. Control group should be older patients who accepted those marginal kidneys preemptively. Post-transplant outcomes between the 2 groups should be compared.

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ORIGINAL ARTICLE

Observational Study

Renal transplants from older deceased donors: Is pre-implantation biopsy useful? A monocentric observational clinical study

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Abstract

AIM

To compare survival of kidney transplants from deceased extended criteria donors (ECD) according to: (1) donor graft histological score; and (2) allocation of high score grafts either to single (SKT) or dual (DKT) transplant.

METHODS
Renal biopsy was performed as part of either a newly adopted DKT protocol, or of surveillance protocol in the past. A total 185 ECD graft recipients were categorized according to pre-implantation graft biopsy into 3 groups: SKT with graft score 1 to 4 [$SKT_{(1-4)}$, n = 102]; SKT with donor graft score 5 to 8 [$SKT_{(> 4)}$, n = 30]; DKT with donor graft score 5 to 7 (DKT, n = 53). Graft and patient survival were analyzed by Kaplan-Meier curves and compared by log-rank test. Mean number of functioning graft years by transplant reference, and mean number of dialysis-free life years by donor reference in recipients were also calculated at 1, 3 and 6 years from transplantation.

RESULTS
There were no statistically significant differences in graft and patient survival between $SKT_{(1-4)}$ and $SKT_{(> 4)}$, and between $SKT_{(> 4)}$ and DKT. Recipient renal function (plasma creatinine and creatinine clearance) at 1 years did not differ in $SKT_{(1-4)}$ and $SKT_{(> 4)}$ (plasma creatinine 1.71 ± 0.69 and 1.69 ± 0.63 mg/dL; creatinine clearance 49.6 + 18.5 and 52.6 + 18.8 mL/min, respectively); DKT showed statistically lower plasma creatinine (1.46 ± 0.57, P < 0.04) but not different creatinine clearance (55.4 + 20.4). Due to older donor age in the DKT group, comparisons were repeated in transplants from donors older than 70 years, and equal graft and patient survival in SKT and DKT were confirmed. Total mean number of functioning graft years by transplant reference at 1, 3 and 6 post-transplant years were equal between the groups, but mean number of dialysis-free life years by donor reference were significantly higher in SKT (mean difference compared to DKT at 6 years: 292 [IQR 260-318] years/100 donors in $SKT_{(1-4)}$ and 292.5 [(IQR 247.8-331.6) in $SKT_{(> 4)}$].

CONCLUSION
In transplants from clinically suitable ECD donors, graft survival was similar irrespective of pre-implantation biopsy score and of allocation to SKT or DKT. These results suggest use of caution in the use of histology as the only decision criteria for ECD organ allocation.

Key words: Dual kidney transplant; Extended criteria donor; Graft survival; Pre-implantation biopsy score; Renal transplantation; Single kidney transplant

Core tip: Pre-implantation biopsy of grafts from elderly donors is under appraisal as a means to direct the acceptance/discard decision of organs for transplantation and the best allocation to single rather than dual transplant. Presented data shows that in recipients of grafts from older donors, rated suitable to donate according to clinical data and preserved renal function, graft and patient survival did not differ in the two categories of transplants with graft histological score in the lower (1-4) or higher (5-8) range of a scale in use. Additionally, allocation of higher score grafts to single or dual transplant did not result in different survival in time, but observed total number of dialysis free life years in recipients up to 6 years was lower for the dual kidney transplant (DKT) allocation. We suggest that older donors rated suitable to donation by clinical decision and preserved renal function may be allocated to single kidney transplant without biopsy; if biopsy is performed, higher scores than those in actual use should be considered for allocation to DKT.

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INTRODUCTION

Organ shortage is widely held the most urgent problem in the field of kidney transplantation[1]. In order to increase the donor pool and the chance of transplantation to patients on wait list most transplant programs are increasingly accepting suboptimal, so called "extended criteria", donors (ECD)[2,3]. Despite worse performance than transplants from young donors in terms of delayed graft function (DGF), primary non function (PNF), short and long term renal function and overall graft survival[2-4], transplants from ECD may offer a survival advantage in comparison with not-being transplanted and remaining on wait list, at least for specific patient categories[5-7]. In quantitative terms, several reports indicate that graft survival from adequately selected ECD may not be much lower as compared to grafts from "standard" donors[8-10]. In our series, a retrospective analysis of death-censored graft-survival of transplants from clinically suitable, *i.e.*, with preserved renal function and anatomy, donors older than 60 years was only 8.2% lower than that from younger than 60-year donors after 10 years (84.0% *vs* 92.2%). Thus, elderly donors may be a precious source of transplantable organs.

In some countries (among which Italy), dual (DKT) rather than single kidney transplantation (SKT) from ECD has gained popularity as a means of limiting elderly organ discard[11-15]; a simplistic rational is that quantity of functioning nephrons in one kidney from elderly donors may be insufficient to sustain adequate function in recipients, while double such a quantity may provide adequate compensation. Moreover organ senescence and age-related pathology might also benefit from doubling tissue mass. A critical issue is how to measure and quantitate these variables; common assumption is that histology, and its translation into quantitative scores, may allow a more objective evaluation of organ

Table 1 Histologic score in use for kidney allocation to single kidney transplant or dual kidney transplants of "high-risk" donors

Glomerular global sclerosis	0 = no glomeruli globally sclerosed 1 = less than 20% 2 = 20%-50% 3 = > 50%
Arteries/arterioles wall thickness[1]	0 = normal appearance 1 = less than lumen diameter 2 = equal/slightly higher than lumen diameter 3 = higher than lumen diameter/ severe lumen reduction
Tubular atrophy	0 = absent 1 = less than 20% tubuli affected 2 = 20%-50% 3 = > 50%
Interstitial fibrosis	0 = absent 1 = less than 20% parenchymal tissue substituted 2 = 20%-50% tissue 3 = > 50% tissue

[1]The most severe lesion determines the score. The final score is the sum of 4 individual scores: With final score up to 4 (included) organs are allocated to solitary kidney transplantation; from 5 to 7 organs are allocated to dual kidney transplantation; higher than 7 organs are discarded.

quality than clinical data (renal function, anatomy, comorbidities). Several reports have shown similar survival of high histological score organs (assumed to represent poor-quality grafts) used as DKT as compared to SKT with low histological score grafts (again assumed to represent better-quality organs)[11-13]; these results have been credited to support the validity of biopsy-based organ allocation. On the other hand, other reports have shown equal survival of grafts from elder donors (all allocated to SKT) independently of pre-implantation histological score, *i.e.*, low (1 to 3)[8,16,17] *vs* high [4 to 6(8) or 4 to 5[16,17]] score. Score 4 constitutes the limit for differential allocation of ECD grafts to SKT rather than DKT in the biopsy-based protocol in use in our transplant area. We and others[18] have reported that DKT recipients who lost one graft due to surgical complications were able to maintain adequate organ function, despite bad histological score of the surviving graft. Thus, it would appear that current biopsy protocol for allocation of ECD grafts to SKT or DKT may foster unbalanced allocation to DKT of grafts suitable for SKT, somehow reducing transplant benefits from available donors. In the present analysis, we have taken advantage of donor kidney pre-implantation biopsies performed in the past, *i.e.*, before adopting current biopsy-based DKT program, as a component of post-transplant surveillance protocol; we have reviewed all available biopsies from ECD and scored them according to current criteria within the DKT program. Several grafts, allocated to SKT, happened retrospectively to show > 4 histological score, a value which would actually indicate allocation to DKT. The aim of the study was to retrospectively compare the outcome of SKT from ECD categorized according to histological score, *i.e.*, up to 4, or higher than 4; in addition, outcome of SKT from grafts with low or high histological score was also compared to outcome of DKT from grafts with high histological score according to current protocol. Graft survival in time and measured renal function at one year in recipients were main outcomes; in addition, dialysis-free life years in recipients at 1, 3 and 6 years within each transplant category were also evaluated using the restricted mean survival time methodology[19-21].

MATERIALS AND METHODS

Donor categories and transplant types

All renal transplants from older than 60-year donors performed in our Centre from 1 Jan 2000 to 30 Oct 2017 were analyzed, provided that a pre-implantation biopsy was available. Up to 30 Nov 2010 only SKT were performed; irrespective of age and comorbidities, donor suitability was based on clinical data which included normal lower pre-donation plasma creatinine, eGFR (Cockroft-Gault formula) higher than 60 mL/min per 1.73 m^2, proteinuria absent or "trace", and anatomy permissive (echography and/or surgical inspection). Pre-implantation biopsy was not required, and was only performed for cause, *e.g.*, in case of pre-donation acute renal failure or more than trivial proteinuria, to ascertain any specific pathology, or as part of a post-transplant surveillance protocol, in which case histological data were analyzed only time after transplantation.

After 1 Dec 2010 our Centre joined to a biopsy-based DKT program designed and coordinated by our inter-regional regulatory agency, NITp[11], where it is publicly registered[22], and which is shared by all transplant Centers of the area. Within this program older than 60-year donors are allocated to SKT or DKT according to clinical and histological criteria: Donors older than 70 years, or aged 60-70 years with any of arterial hypertension treated with ⩾ 2 drugs, drug-treated type 2diabetes mellitus, death due to cerebrovascular event (with exclusion of trauma and aneurism rupture as cause of brain death), proteinuria higher than 0.5 g/L, eGFR (Cockcroft-Gault) less than 60 mL/min per 1.73 m^2 undergo pre-implantation biopsy, and are allocated to SKT if histological score is ⩽ 4, to DKT if mean score is 5-7, and discarded if mean score is > 7 (Table 1); these donors are collectively defined "high-risk" ECD. When only one of partner kidneys had a score > 4, it was at discretion of the transplant Centre to perform DKT or SKT with the lower score graft. Donors in the 60-70 year-range, without any of the above comorbidities, collectively defined "low-risk" ECD, are allocated to SKT without biopsy.

Application to the program is additive to that for standard donors and requires signature of a specific informed consent; in our Centre we also require recipient's age older than 62 years. Consent includes either DKT or SKT from the same donor categorized as "high-risk" ECD. All donors were brain-dead; transplants from

living, cardiac-death, ABO- or HLA-incompatible donors, as well as simultaneous kidney and any other organ transplants were not included. Both first and non-first transplants were included. A pre-transplant negative T and B-lymphocyte CDC was a pre-requisite for transplantation and forbidden donor antigens, according to actual or historical HLA antibodies in recipient, were carefully avoided by the allocation agency; allocation algorithm in use in our inter-regional area searches for best HLA match first, then for immunization status, listing time and age match in all transplant categories except in DKT protocol, where HLA match is not considered.

Informed consent was obtained from all the patients applying for renal transplantation in our Centre at the time of listing and at the time of transplantation, and additionally for applying to the DKT program. Consent for anonymous use of clinical data was included in the consent form. This study has been conducted according to principles of the declaration of Helsinki and complies with the declaration of Istanbul. As a standard of care, anonymous study no approval by ethic committee was needed.

Study design

We analyzed and compared 3 groups of transplants: Group 1, SKT from older than 60-year donors with pre-implantation graft biopsy score, either before or within the DKT protocol, $\leqslant$ 4 ($SKT_{(1-4)}$); group 2, SKT with graft pre-implantation biopsy score, either before or within the DKT protocol, $\geqslant$ 5 ($SKT_{(>4)}$); we included within these 2 SKT categories also 6 DKT recipients who had early removal of one graft for surgical complications with score in remaining graft $\geqslant$ 5 (5 patients) or < 5 (1 patient); group 3, DKT with graft pre-implantation biopsy score 4 to 7 according to the DKT protocol (DKT). As already said, only in the DKT protocol histological score was known before transplant and used for differential graft allocation, while in the pre-DKT period it was only a retrospective information.

For every donor-recipient pair, in each group, we collected and analyzed clinical data of interest, age, sex, HLA mismatches (loci A, B, DRB1), type and length of dialysis in recipients, plasma creatinine and eGFR in donor and plasma creatinine and creatinine clearance (24 h urine) at 3 mo and 1 years post-transplant in recipients, and biopsy-proven rejection of any type in the first 18 mo after transplantation in recipients. Outcomes of interest were death-censored graft survival (*i.e.*, freedom from dialysis or re-transplantation), overall graft survival (*i.e.*, graft loss or patient death with functioning graft, whichever came first, corresponding to patients alive with functioning graft), patient survival (*i.e.*, death with functioning graft) and renal function in recipients at 3 and 12 mo from transplantation; we also evaluated: Early graft losses (EGL, *i.e.*, no dialysis-freedom, or need of permanent dialysis, within 3 mo after transplantation), DGF (need of dialysis for any cause in the first week after transplantation), mean years of functioning graft at 1, 3 and 6 years from transplantation with reference to initial transplants and total dialysis-free life years at the same times with reference to donors.

Data base update was closed on 31 Jan 2018, allowing for at least 3 mo uncensored follow up in all patients; since only in few cases total follow-up was longer than 6 years in the DKT group, and longer of 10 years in both the SKT groups, follow up was censored at 6 years in DKT and 10 years in $SKT_{(1-4)}$ and $SKT_{(>4)}$.

Biopsies within the DKT program were either wedge or core biopsies, according to harvesting Centre practice, while our historical biopsies were all core needle. Score was evaluated on paraffin-embedded, hematoxylin-eosin stained slides; in the DKT program score was calculated by any of participating Centre pathologists and communicated to NITp; all our pre-DKT surveillance biopsies were viewed and scored by collaborative work of a pathologist (Camozzi MLP) and two nephropathologists (Colombo VG and Casati C). A minimum of at least 10 glomeruli were required for a biopsy to be representative.

Immunosuppression protocols

Immunosuppression protocols at our Centre did not change in all observation period (Jan 2000 to Oct 2017), and included in most patients rATG induction (3.5 mg/kg in 7 d, 7 mg/kg if $\geqslant$ 2nd transplant), cyclosporine-A starting pre-transplantation as a 10 mg/kg oral load, mycophenolate mofetil/mycophenolic acid starting on p.o. day 1 (1g or 720 mg bid) and corticosteroids (methyl-prednisolone 500 mg at reperfusion, rapidly tapered down to 8 mg/d on p.o. day 11 and 4 mg/d after 3 mo). In a minority of patients, tacrolimus, everolimus, belatacept or sirolimus were used (Table 2). Post-transplant heparin anticoagulation was started in 2011 only in DKT, after that a higher than usual graft vein thrombosis was observed in this type of transplant, as described also by others[23].

Statistical analysis

Descriptive statistics are given as numbers, percentages and mean ± SD or median (1st and 3rd interquartile range, IQR) according to data distribution; inter-category differences were checked by ANOVA, followed by Scheffé *post-hoc* test; Fisher's exact test was used for comparison of frequencies; Pearson's coefficient was used for correlation analysis between pairs of data. Survival analysis was estimated as event free cumulative survival using the Kaplan–Meier method and compared using the log-rank Mantel-Cox test.

We estimated the mean number of years the allo-grafts were functioning before loss for any cause (failure or death with functioning graft) by the restricted mean survival analysis[19-21]; it is computed as the total area under the survival curve at specific times (we repeated the procedure at 1, 3 and 6 post-transplant years), and indicates the mean time (years) the grafts remained functional at any defined time. Conceptually, this eva-luation indicates mean dialysis-free life years for every

Table 2 Baseline characteristics of donors and recipients in the 3 transplant categories

Transplant category[1]	SKT(1-4)	SKT(> 4)	DKT
n	102	30	53
Donors, M/F	54/48	16/14	29/24
Donor age, yr (mean, SD)	68.9 ± 5.7	66.9 ± 6.7	75.3 ± 5.0[b]
Score of transplanted graft, median (IQR)	3 (3-4)[d]	5 (5-6)[a]	5 (4-5)
Donor comorbidities			
Donor age > 70 yr, *n* (%)	47 (46)	8 (27)	47 (89)[b]
Arterial hypertension	40 (39)	21 (70)	34 (64)
Diabetes	9 (9)	6 (20)	7 (13)
Cerebrovascular cause of death	33 (32)	17 (57)	29 (55)
KDPI[1]	89.4 ± 8.0	89.9 ± 9.2	96.9 ± 3.4[b]
KDRI[1]	1.65 ± 0.27	1.7 ± 0.33	2.02 ± 0.31[b]
Recipients, M/F	68/34	20/10	37/16
Recipient age (mean ± SD, yr)	61.0 ± 7.2	60.2 ± 6.0	67.3 ± 4.6[b]
Years on dialysis, median (IQR)	3.5 (0.1-13.5)	3.4 (0.8-9.5)	2.1 (0.3-8.5)[b]
Dialysis mode, *n* (%)			
Hemodialysis	84 (82)	25 (83)	40 (75)
Peritoneal dialysis	16 (16)	5 (17)	12 (23)
Pre-emptive	2 (2)	0	1 (2)
Renal disease *n* (%)[1]			
GN/systemic	35 (34)	10 (34)	19 (36)
ADPKD	24 (23)	4 (13)	6 (11)
Vascular/hypertension	10 (10)	3 (10)	3 (6)
Diabetes	11 (11)	4 (13)	7 (13)
Other	14 (14)	6 (20)	12 (23)
Unknown	8 (8)	3 (10)	6 (11)
1st-2nd-3rd Tx	95-6-1	28-2-0	51-2-0
HLA-MM (median, IQR)	4 (3-5)	4 (3-5)	4 (4-5)
CITa (mean ± SD, h)	15.0 ± 3.6	15.9 ± 4.2	16.1 ± 3.1

[1]SKT(1-4), solitary kidney transplant, histologic score 1 to 4; SKT(> 4): Solitary kidney transplant, histological score 5 or higher; DKT: Dual kidney transplant, histological score 5 to 7; KDPI and KDRI: Kidney Donor Profile Index and Kidney Donor Risk Index; GN/systemic: Glomerulonephritis or systemic immunological disorder; CIT: Cold ischemia time. [b]$P < 0.01$ *vs* SKT (both categories); [d]$P < 0.001$ *vs* SKT(> 4) and DKT; [a]$P < 0.039$ *vs* DKT.

transplanted patient at any defined time. From this value we extrapolated total dialysis-free life years for every 100 donors at any time in each of the 3 groups of transplants; for this calculation each donor was made equal to 1.6 SKT, according to data of our regional agency on utilization of overall retrieved grafts[24], very close to the 1.67 figure for ECD of another transplant program[8], and to 1 DKT. SPSS Statistics software v.21 was used for all analyses. Two-tailed *P* values < 0.05 were considered significant.

RESULTS

In the DKT protocol (after Dec 2010) there were 196 older than 60-year donors, of which 131 qualified for biopsy showing score 4 or less in 66 (allocated to SKT) and score 5 or higher (up to 7) in 65, of which 59 were allocated to DKT and 6 to SKT, with score 5 in 5 and 6 in 1; we accepted these 6 grafts as SKT to avoid discard, since the corresponding partner grafts, with lower than 5 scores, had already been allocated to SKT or was anatomically unsuitable. Six of the 59 DKT, with early removal of one graft for surgical complications, have been included, according to score in remaining graft, in the SKT(> 4) (5 cases: score 6 in 4 cases and score 5 in 1) or SKT(1-4) (1 case, score 3) categories.

In the pre-DKT period, pre-implantation biopsy was available in 72 older than 60-year donors; in 18 cases available tissue was insufficient for adequate scoring, 35 grafts showed score 4 or less, and 19 score 5 or higher (range 5-8). Thus, our analysis concerns 102 SKT(1-4), 30 SKT(> 4), and 53 DKT. Summary data of baseline donor and recipient characteristics in the 3 transplant categories are given in Table 2 and main post-transplant events of interest in Table 3. Donor and recipient age was higher, and time on dialysis prior to transplant shorter, in the DKT category, while donor and recipient sex distribution was equal. Also KDPI and KDRI were higher in the DKT category, mostly as a consequence of older age (see below). Donors older than 70 years were 102, of which 47 were allocated to DKT and 55 to SKT. Histological score was lower by selection in SKT(1-4) than SKT(> 4) and DKT, and was also higher in SKT(> 4) than in DKT. Median and total follow-up was shorter in DKT, due to contribution to follow-up from the pre-DKT years only in the 2 SKT categories. All other donor and recipient characteristics, including donor comorbidities, recipient dialysis mode, renal disease, HLA mismatches, number of transplants, immunosuppression, graft cold ischemia time, were not different between categories. There were no major differences in events of interest along follow up between categories, apart higher incidence of DGF, *i.e.*, need of dialysis in the first week after transplantation, in SKT(> 4). Early graft losses were 9 (7.1%) in all 126

Table 3 Summary of main post-transplant characteristics and events in the 3 transplant categories

Transplant category[1]	SKT(1-4)	SKT(> 4)	DKT
n	102	30	53
Initial immunosuppression, *n* (%)			
rATG	95 (93)	25 (83)	53 (100)
Basilix imab	8 (8)	3 (10)	0
Cyclosporin	91 (89)	25 (83)	50 (94)
Tacrolimus	9 (9)	2 (7)	3 (6)
Mycophenolate	91 (89)	27 (90)	50 (94)
Everolimus	9 (9)	4 (13)	3 (6)
Sirolimus	1 (1)	1 (3)	0
Belatacept	2 (2)	1 (3)	0
Steroids	84 (82)	28 (93)	50 (94)
Tx duration[2], yr (median, IQR)	4.1 (1.6-7.4)	7.0 (2.6-9.9)	2.7 (1.4-4.8)[a]
Total follow-up, pt-years	467.8	180.5	161.7
DGF[3], %	42.1	56.6[c]	24.5
EGL[3], *n* (%)			
All	8 (7.8)	1 (3.3)	2 (3.8)
PNF[3]	4 (3.9)	1 (3.3)	0
Surgical	4 (3.9)	0	2 (3.8)
BPAR[3], *n* (%)	10 (9.8)	3 (10.0)	3 (5.6)
Graft failure[4], *n* (*n*/100 pt-yr)	10 (2.1)	6 (3.3)	3 (1.8)
Pt-death, *n* (*n*/100 pt-yr)	16 (3.4)	4 (2.0)	6 (3.5)

[1]SKT(1-4), solitary kidney transplant, histologic score 1 to 4; SKT(> 4): Solitary kidney transplant, histological score 5 or higher; DKT: Dual kidney transplant, histological score 5 to 7; [2]Right-censored at 6 (DKT) and 10 (SKT) years; [3]DGF: Need of dialysis in the first post-transplant week; EGL: Graft loss within 3 mo; PNF: Primary non-function from unknown cause; BPAR: Biopsy-proven acute rejection; [4]Censored for death with functioning graft; [a]$P < 0.03$ *vs* SKT (both categories); [c]$P < 0.05$ *vs* DKT.

original SKT, *i.e.*, excluding 6 original DKT included here in the SKT groups, of which 4 (3.2%) where associated to graft vascular thrombosis and 5 (4.0%) where "unexplained" PNF; in DKT there were 2 of 59 surgical (thrombosis and hemorrhage) early losses (3.4%), but overall vascular graft thrombosis occurred in 8 of 118 grafts (6.8%) ($P < 0.10$ *vs* SKT).

Donor histological score did not show any significant correlation with donor age ($r = 0.11$, $P > 0.10$), donor plasma creatinine ($r = 0.05$) and eGFR ($r = -0.01$), recipient creatinine clearance at 3 mo and 1 years after transplantation ($r = -0.05$ and 0.05, respectively; all $P > 0.25$), and donor KDPI and KDRI indices ($r = 0.05$ and 0.10, respectively, $P > 0.10$). Both KDPI and KDRI were strongly correlated with donor age ($r = 0.70$ and 0.78, respectively, $P < 0.0001$), and donor eGFR ($r = -0.31$ and -0.36, $P < 0.001$).

Survival analysis by transplant category

There were no statistically significant differences in graft, patient and overall survival in recipients of SKT(1-4) *vs* SKT(> 4) ($P = 0.41$, 0.78 and 0.31 for graft, overall and patients survival), and between DKT and both SKT(1-4) and SKT(> 4) (respectively $P = 0.40$ and 0.23 for graft, 0.71 and 0.85 for patient and graft, and 0.81 and 0.36 for patient survival) (Figure 1).

To account for differences in donor age, we repeated survival analysis in recipients of older than 70-year donors, *i.e.*, in the highest age risk range according to definitions in the DKT protocol in use: there were 47 older than 70 years donors with organs allocated to DKT and 55 to SKT (47 in SKT(1-4) and 8 in the SKT(> 4) categories); since survival data were equal for SKT(1-4) and SKT(> 4), we pooled together all SKT. Donor age was 76.4 + 4.0 in the DKT group, and 74.2 + 3.6 in the SKT group ($P < 0.004$). Recipient age was 67.3 + 4.8 in DKT and 63.2 + 6.2 in SKT ($P < 0.001$). Histological score was 5 (IQR 4-6) in DKT and 4 (IQR 3-4) in SKT ($P < 0.01$). For homogeneity, follow-up was closed at 6 years in both groups. Again, there were no statistically significant differences in graft ($P = 0.24$), patient ($P = 0.64$) and patient and graft survival ($P = 0.28$) (Figure 2).

Renal function in donors and recipients

Renal function in donors and recipients of each transplant category is shown in Table 4. Donor plasma creatinine and eGFR did not statistically differ between transplant categories.

At 3 and 12 post-transplant months, recipients alive with a non-failed graft showed similar levels of plasma creatinine and measured creatinine clearance in SKT(1-4) and SKT(> 4), while in DKT plasma creatinine was lower than in SKT at both times, with statistical significant difference at 3 mo *vs* both SKT(1-4) and SKT(> 4) and only versus SKT(1-4) at 12 mo. Differences in creatinine clearance did not reach statistical significance (Table 4).

Restricted mean number of functioning graft years by transplant and projection of dialysis-free life years by donors

Table 5 shows that the mean number of functioning graft years by transplant reference at 1, 3 and 6 years from transplantation was equal for all 3 transplant categories; for clarity, calculations were referred to 100 transplants.

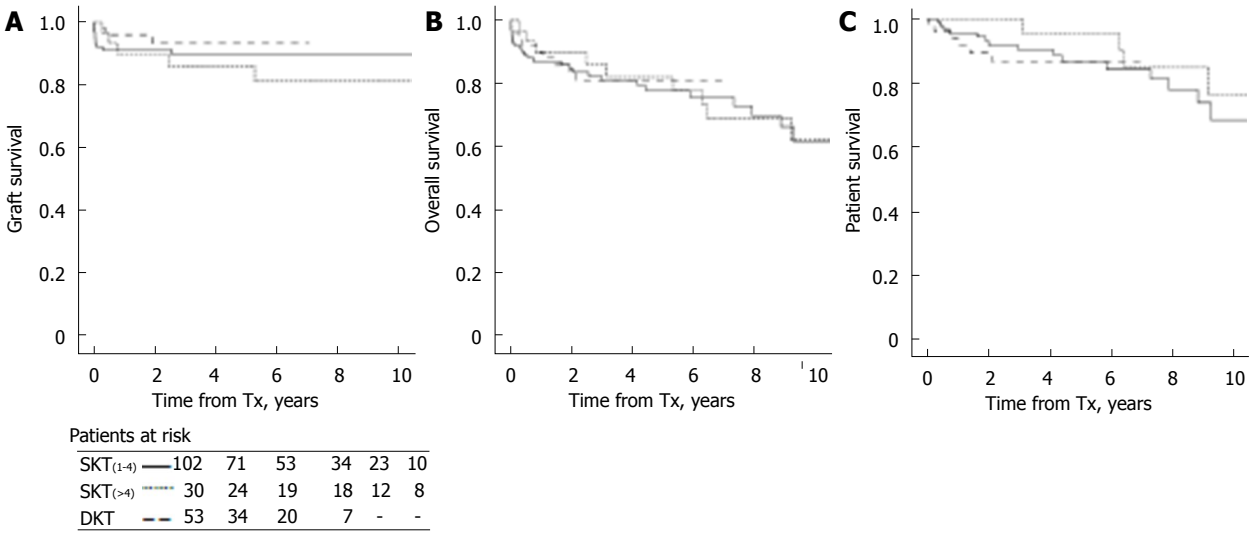

Figure 1 Kaplan Meier plots of graft (death-censored) (A), overall (including death as cause of graft loss) (B) and patient survival (C) according to transplant category. SKT(1-4): SKT with score 1 to 4 grafts; SKT(>4): SKT with score 5 or higher grafts; DKT: Dual kidney transplants with score 4 to 7 grafts. Follow up was censored at 6 years for DKT and 10 years for SKT. There were no statistically significant differences in survival for any of the 3 outcomes. SKT: Single kidney transplant; DKT: Dual kidney transplants.

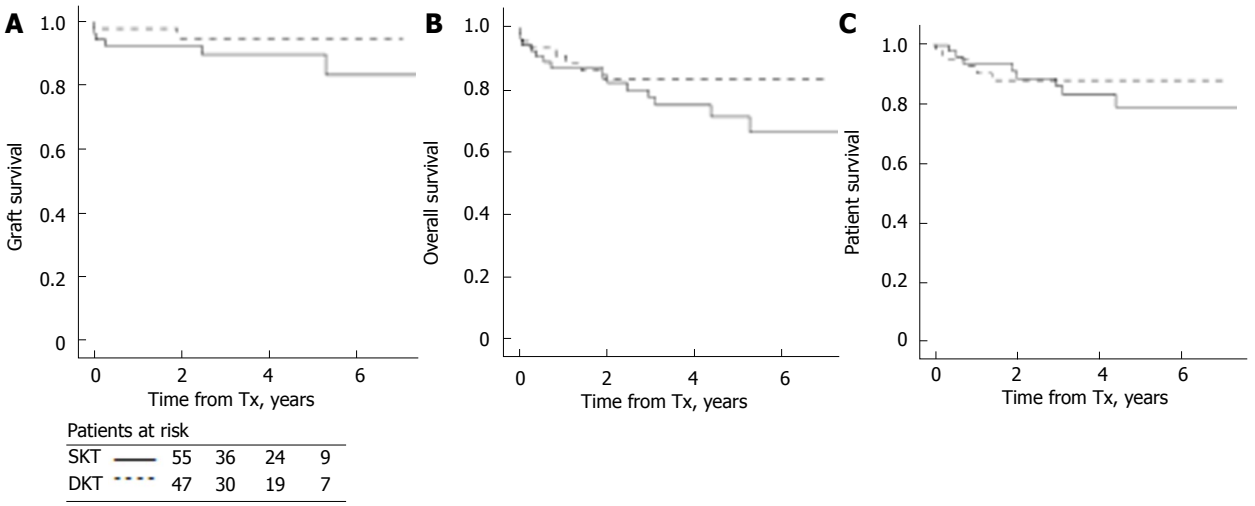

Figure 2 Kaplan Meier plots of graft (death-censored) (A), overall (including death as cause of graft loss) (B) and patient survival (C) in recipients of older than 70-year donors, according to transplant category. SKT: Solitary kidney transplant with any graft score; DKT: Dual kidney transplants with score 4 to 7 grafts. Follow up was censored at 6 years for both SKT and DKT. There were no statistically significant differences in survival for any of the 3 outcomes.

Extrapolation of total dialysis free life years by donor reference at the same time showed significant differences by allocation (*i.e.*, SKT or DKT), with statistically higher figures for both SKT categories at any time. In this extrapolation, we have conservatively chosen an utilization factor of 1.6, rather than 2, SKT for each donor according to published statistics[8,24]. Thus, our data are a minimal realistic estimation of benefits of SKT *vs* DKT, accounting for observed differences in overall survival.

DISCUSSION

Key findings

Our data shows that graft and overall survival in recipients of renal transplants from elderly donors, allocated to SKT, is not statistically different according to histological score of transplanted grafts, *i.e.*, score 4 or lower as compared to score 5 or higher (up to 8); additionally, they also show that survival of grafts of score 5 or higher does not differ by organ allocation to SKT rather than DKT, at least within the available follow-up of 6 years. Also measured GFR at one year from transplantation in not-failed grafts (a generic predictor of survival expectation) does not differ between SKT with differential score grafts, and is marginally better in DKT than in SKT. Thus, our data would indicate that, for organs rated suitable for transplantation clinically, histological information has uncertain usefulness to predict outcome; additionally, current score scale for allocation to DKT appears to hold little discrimination power between grafts which could or could not perform adequately as SKT.

While the biopsy protocol for allocation of elderly donors to SKT or DKT according to score actually in use in our transplant area, which operates on a 19 million

Table 4 Mean ± SD of plasma creatinine and GFR (Cockcroft-Gault formula in donors or 24 h-creatinine clearance in recipients) in donors (D) and recipients (R; at 3 and 12 mo after transplantation) in each transplant category

Transplant category[1]	SKT(1-4)	SKT(> 4)	DKT
D-Pcr, mg/dL	0.88 ± 0.31	0.92 ± 0.28	0.81 ± 0.23
D-eGFR	83.9 ± 27.7	87.1 ± 26.0	79.1 ± 21.8
n	102	30	53
R-Pcr 3 mo	1.92 ± 0.98	2.12 ± 1.12	1.56 ± 0.75[a]
R-CCr 3 mo	45.0 ± 19.3	43.4 ± 21.8	49.6 ± 19.6
n	94	28	49
R-Pcr 12 mo	1.71 ± 0.69	1.69 ± 0.63	1.46 ± 0.57[c]
R-CCr 12 mo	49.6 ± 18.5	52.6 ± 18.8	55.4 ± 20.4
n	83	25	45

[1]SKT(1-4): Solitary kidney transplant, histologic score 1 to 4; SKT(> 4): Solitary kidney transplant, histological score 5 or higher; DKT: Dual kidney transplant, histological score 5 to 7; [a]$P < 0.02$ *vs* SKT(1-4) and SKT(> 4); [c]$P < 0.04$ *vs* SKT(1-4). Pcr: Plasma creatinine; D: Donors; R: Recipients.

population area, dictates allocation of organs with score higher than 4 to DKT, we were able to find out from SKT performed in the past recipients who happened to receive higher than score 4 grafts, as disclosed by surveillance biopsies which were a posteriori scored using criteria of the protocol in use. To these recipients, we added 5 DKT recipients who retained a single high-score graft due to early loss of the corresponding partner graft for vascular complications and 6 other high score grafts in the DKT era whose paired graft had been allocated to SKT in other Centers or was unsuitable for transplantation. The SKT(1-4) recipients were part of both the recent DKT protocol and past transplant activity with available surveillance biopsy. SKT(1-4) and SKT(> 4) groups were well matched concerning donor and recipient characteristics, and differed only in donor graft score by intended, afterward selection; thus, observational data in these 2 groups offer unbiased, clinically relevant, information. DKT category instead showed older age in donors and accordingly in recipients. To overcome this bias, we repeated outcome survival analysis considering only transplants from donors in the most extreme age range, *i.e.*, older than 70 years and up to 88 in DKT and 85 in SKT. In this analysis, due to observed equal graft and patient survival between SKT with different score ranges, we only compared DKT to SKT allocation. Again, there were no statistically significant differences in graft and patient survival between DKT and SKT recipients. Unfortunately, also in this sub-analysis the two populations were not homogeneous, since mean donor and recipient ages were 2 and 4 years older, respectively, and histological score higher in DKT; we think that these small differences have little impact on interpretation of results, even though we recognize that we cannot evade the general assumption that equal outcome with worst graft histology may sustain the validity of DKT allocation by score.

Comparison with literature data

"High risk donors" as defined in our regional DKT protocol (older than 70 years, or 60-69-year-old with comorbidities) are 10 years ahead of canonical ECD definition (older than 60-year or 50-59-year-old with comorbidities)[3,25]. The overwhelming majority of our ECD (84%) were "high risk" according to the above definition. Despite this donor connotation, our medium (in DKT) and long-term data (in SKT(1-4) and SKT(> 4)) shows not inferior graft and patient survival in recipients of these donor grafts than that commonly described for ECD in general[9,10], and confirms the potential wealth of older donor organs. Survival figures did not change, too, by restricting survival analysis to donors older than 70 years, indicating that also very old donors may be safe, if renal function is permissive. Others have described similar survival in recipients of grafts from donors older than 75 years as compared to grafts from younger ECD[10], or in recipients of grafts from ECD donors which differed by decades in the range from 60 to 80 years[12].

Our data that histology appears a poor predictor of transplant outcome confirms other published reports: Hofer *et al*[8] showed similar medium term (8 years) survival of grafts with score 0-3 as compared to score 4-6, with worst survival only for grafts with extremely high score (*i.e.*, 7-12). These latter were only 8 out of 106 ECD (7.5%), and 4 out of 305 SCD (1.3%); it is uncertain if so severe histology entailed any degree of impaired function, which might have indicated for cause biopsy. Carta *et al*[17] report equal short term (3 years) graft and patient survival in SKT recipients of score 4-5 as compared to score 0-3 grafts. Foss *et al*[26] allocated to SKT by clinical criteria 54 grafts from older than 75-year donors and retrospectively could not find any relationship between 5 years graft survival and pre-implantation score (ranging 0 to 8), with equal 1-year plasma creatinine levels in recipients of score 0-4 as compared to score 5-8 grafts.

No single component of histological score has been shown to be consistently associated to post-transplant outcome[8,17,27]; definition of a score limit for graft allocation or for acceptance/discard has so far entailed some empiricism. The original DKT protocol in NITp area contemplated a score above 3 for organ allocation to DKT[28,29], and has been changed to score 4 as a result of favorable outcome of SKT with score 4 grafts[14,30]. Our and others'[8,17,26] data suggests that even higher score grafts, from clinically suitable donors, may perform well as SKT. So, further appraisal from clinical series comparing outcome of grafts with equal histology but differentially allocated to SKT or DKT appears at least desirable. Ideally, such a comparison of outcome should be implemented with the new concept of population-average dialysis-free life years by donors, which may somehow temper the interpretation of the more direct and usual concept of time survival by recipients (see below).

Clinical correlates of histological score

As reported[8,16,17,26] also in our hands histological score,

Table 5 Restricted number (95%CI) of functioning graft years at 1, 3 and 6 years post-transplantation, and projected number of total dialysis-free life years in recipients for every 100 transplants or 100 donors in each transplant category. Differences indicated in bold indicate a statistically significant difference ($P < 0.05$)

		1 yr	3 yr	6 yr
RNFGY (× 100 Tx)	$SKT_{(1-4)}$	93.3 (86.9-99.7)	261.0 (253.3-268.7)	499.6 (490.5-508.7)
	$SKT_{(>4)}$	93.8 (85.2-102.4)	279.5 (266.9-292.1)	499.9 (482.5-517.2)
	DKT	97.7 (91.4-104.1)	275.0 (264.1-285.8)	507.3 (496.0-524.3)
TDFLY (× 100 donors)	$SKT_{(1-4)}$	149.3 (139.1-159.6)	417.6 (405.4-429.9)	799.3 (784.7-813.9)
	$SKT_{(>4)}$	150.1 (136.2-163.9)	447.2 (427.1-467.4)	799.8 (772.1-827.6)
	DKT	97.7 (91.4-104.1)	275.0 (264.1-285.8)	507.3 (496.0-524.3)
Vs DKT, difference				
	$SKT_{(1-4)}$	**51.6 (35.0-68.2)**	**142.7 (119.5-165.8)**	**292.0 (260.4-317.9)**
	$SKT_{(>4)}$	**52.3 (32.2-72.5)**	**172.3 (141.2-203.3)**	**292.5 (247.8-331.6)**

RNFGY: Restricted number (95%CI) of functioning graft years; TDFLY: Total dialysis-free life years; DKT: Dual kidney transplant.

despite being credited as a senescence index, had no relationship with donor age, nor did it correlate with renal function in donors and recipients. It was shown to correlate mostly with hypertension and vascular disease in donors[8,16], a finding consistent with the marginally lower incidence (just below statistical significance) of hypertension in our $SKT_{(1-4)}$ donors in comparison with $SKT_{(>4)}$ and DKT. It was even not correlated with donor KDPI and KDRI, as shown by equal values of these indices in either SKT category. Higher KDPI/KDRI in DKT were almost the exclusive effect of older donor age, as indicated by the very strong correlation of these indices with donor age, much stronger than that with donor eGFR. Thus, our data adds evidence that current tools to predict organ quality, *i.e.*, histology, KDPI and even pump perfusion[31] have little reliability in predicting individual graft outcome and are no better than clinical evaluation.

Lack of correlation between histological score and graft outcome we have shown has to be commented within the frame of donors with well-preserved renal function; while there is no doubt that donor grafts with severe pathology are poor candidates for transplantation, it is disputable that such grafts associate with well-preserved renal function. In healthy kidney live donors it was shown that while number of glomeruli falls with age, single nephron GFR does not change up to 70 years, so that total GFR proportionally falls[32]; thus preserved GFR may select donors with a lesser degree of age-related nephron loss. Our results indicate that reliance only in histology for organ allocation may not always be well founded, and that even though function does not predict histology it remains a reliable predictor of graft outcome. In ECD with well-preserved renal function, as the majority of ECD in the present series, biopsy should better be avoided. Causes of discordance between histology and outcome have already been commented, and may reside in any of recognized biases of histology, including its randomness, differences in technique and process, and pathologist expertise among others[8,26,27].

We underscore that donors (of any category) who present with impaired renal function, either long standing, acute or uncertain, are a different context. Biopsy in these donors is of definite help in defining specific underlying pathologies (*i.e.*, acute *vs* chronic, reversible *vs* irreversible lesions); while grafts with acute, reversible pathologies (more commonly acute tubular necrosis) perform well as SKT[33], grafts with chronic lesions require integration of both clinical and histological information to guide mainly in the decision between DKT *vs* discard. We think that donors with pre-existing marginal renal function and anatomy should be the main candidates to histological evaluation, with the aim to ascertain that at least 50% of renal mass is viable. We acknowledge that such an achievement may not be easy; within the frame of current score scale, we suggest that a level of at least up to 2 for any individual score should be allowed, summing up to a total of 8 as acceptable score for DKT.

Benefits of SKT vs DKT allocation

DKT was proposed as a means to reduce discard rate of grafts from marginal donors (defined on the basis of vascular disease and/or older age)[34]; organs from these donors have been often perceived to offer inadequate function if used as SKT. Indeed, survival in time of these organs allocated to SKT is lower in comparison with grafts from younger, or standard, donors[9,10]. In one study early graft loss from any cause was 10.1% (4.2% from unexplained PNF) in recipients of ECD grafts against 4.1 (all causes) and 1.5 (PNF), respectively, in standard donor grafts[35]; these and other's[36] figures in ECD transplants are not far from ours in all SKT (7.1% early loss for any cause, with 4.0% PNF). As for survival in time, the population-average relative risk of graft failure (including patient death) at 10 years from transplantation was 1.7 times higher in recipients of an ECD graft as compared to a standard donor graft[8,10]. Translated into quantitative numbers, after 10-year follow-up the mean time to graft failure was only 8 mo shorter for recipients of an ECD graft as compared to standard donor graft[10]. Thus, despite inherent detriments as compared to younger donor grafts, absolute benefits of ECD organs at a population level are not trivial, and foster in many European transplant communities a call to a wider use rather than to discard of these organs[10,20]. In this perspective DKT, even assuming that it effectively reduces early and long-time losses, may not allow an equally efficient use of available organs as

SKT. It is claimed that, due to bad histology, these organs could not perform adequately if allocated to SKT. We have shown instead that SKT of grafts with bad histological score is associated with similar graft and patient survival in recipients as compared to DKT.

We have tried to quantitate benefits from ECD transplants according to allocation to SKT or DKT; from the observed survival curves, we calculated mean number of functioning graft years at specific time points in recipients by transplant reference and mean dialysis-free life years by donor reference. Dialysis-free life years may be viewed as a good indicator of transplant benefits, as far as it includes both quality of life related to transplantation and social cost savings. Dialysis-free life years were greater in SKT than DKT at any time of our analysis, and the difference increased rather than lessen in time. This data would favor SKT over DKT from the same donors; moreover, since our follow up was not long, longer-reaching series are needed to confirm maintenance in time of these benefits. Better renal function at 1 years justifies a longer survival expectation in time for DKT; on the other hand, it has to be appreciated that in the long-term immunological mechanisms are a prevalent cause of graft loss[37], and may become the main determinant of graft survival. Thus, any long-term scenario remains simple speculation unless longer term observational data is available.

Study strengths and limitations

Despite a rather small number of cases, this study allows an unbiased comparison of clinical outcome of renal transplants categorized by graft histology and allocation. Donor and recipient characteristics, immunosuppression and clinical management were homogeneous between groups, except for donors' and recipients' older age in the DKT group. In addition to canonical survival analysis by Kaplan Meier methodology, this study has evaluated novel outcome data in use in clinical transplantation based on the restricted mean survival time methodology, allowing to infer on quantitative dialysis-free life years made possible by differential allocation.

Main limit of the study is the rather short follow up of our DKT population, which advocates for a longer time analysis. Older age in donor and recipients of DKT may also constitute a bias in comparison to SKT categories, however reanalysis of results in older than 70-year donors, with very small mean donor and recipients age difference, confirmed the results in the whole series.

In conclusion, our data shows that grafts older than 60 years of age from deceased donors, allocated to SKT on the basis of clinical suitability, perform equally well in recipients irrespective of categorization according to histological score, up to 4 or greater than 4, and that high-score grafts perform equally well in recipients irrespective of allocation to SKT or DKT. With respect to observed survival figures at 1, 3 and 6 years, overall dialysis-free life years per any donor number were greater for SKT than DKT allocation of equally scored grafts. For clinically suitable organs, histology appears unable to predict and improve the population-average graft survival. Thus, indications for DKT allocation of ECD grafts should perhaps be revised, with DKT being limited to use mainly for organs clinically unsuitable for SKT due to inadequate function and/or imaging/anatomy. In this context, new criteria have to be sought to guide decision not on allocation, but rather on acceptance *vs* discard.

ARTICLE HIGHLIGHTS

Research background

In renal transplantation a hot topic is the best use of older donor grafts: these organs are associated with an higher risk of early and late graft failure, yet this donor category has become the most prevalent one in western countries. Pre-implantation biopsy of grafts from elderly donors is commonly used to guide in the acceptance/discard of organs, and/or in their allocation to single or dual kidney transplant.

Research motivation

There is no universal agreement in the literature on usefulness of biopsy to predict post-transplant graft outcome; additionally, a main concern with dual kidney allocation is a reduction of transplants made possible by available donors.

Research objectives

The main objectives of our study were to retrospectively compare outcome data of transplants with older donor grafts categorized according to pre-implantation histology into a low-score or high-score category; additionally, high-score grafts were compared by allocation to either dual kidney or single kidney transplant category.

Research methods

All renal-only transplants in our Center from 1 Jan 2000 to 30 Oct 2017 from donors older than 60 years and with available pre-implantation graft biopsy were retrospectively evaluated. Before Dec 2010 grafts were allocated only to single kidney transplant, irrespective of histology; after that date we adopted a biopsy-based protocol (DKT protocol), which dictated allocation to single kidney transplant of grafts with low histological score (1 to 4), and to dual kidney transplant of grafts with high histological score (4 to 7).

Research results

A total of 185 patients with pre-implantation biopsy were available, 102 with low histological score (4 or less), 83 with high histological score (5 to 8), of which 30 were allocated to single kidney transplant (score 5 to 8) and 53 to dual kidney transplant (score 5 to 7). Donors allocated to single kidney transplant did not differ between the low score and high score categories as concerns age, sex distribution, renal function, comorbidities, KDPI and KDRI indices, while they were older and with higher KDPI/KDRI indices in the dual kidney transplant category. Up to 10 years after transplant, we did not observe any differences in graft, patient and overall survival between recipients of a single kidney transplant with either low or high histological score, or between recipients of high histological score grafts allocated either to single or dual kidney transplant. These results were confirmed in a sub-analysis based only on the oldest donors (older than 70 years). We also calculated the total number of dialysis free life years in recipients of either a single or dual kidney transplant by available donors, showing a significantly higher value for recipients of a single kidney transplant up to the available follow-up of 6 years.

Research conclusions

Our study shows that the histological score in use in our transplant area does not predict post-transplant outcome in recipients of a single kidney transplant; additionally, allocation of grafts with similar histological score to single or dual kidney transplant is associated with equal survival up to the available follow-up of 6 years. We propose that renal biopsy is not indicated in older donors with preserved renal function and anatomy, and that organ allocation to single kidney transplant allows the best use of these donors. We propose that pre-implantation biopsy be limited to donors of any age with abnormal renal function, to ascertain

type and reversibility of underlying pathology; dual kidney transplant allocation should be considered for bad function grafts with chronic histological pathology, provided that at least 50% viable tissue be reasonably ascertained.

Research perspectives

Main lesson of our study is that histological score scale in current clinical use does not allow to discriminate between organs which could or could not function adequately as single kidney transplant. This implies the risk of underutilization of available donors. A prospective randomization of equal score grafts to single or dual kidney transplant, and a longer follow-up are strongly desirable to ascertain any advantages or inconveniences of dual *vs* single kidney allocation.

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REVIEW

Thrombotic microangiopathy after renal transplantation: Current insights in *de novo* and recurrent disease

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Abstract

Thrombotic microangiopathy (TMA) is one of the most devastating sequalae of kidney transplantation. A number of published articles have covered either *de novo* or recurrent TMA in an isolated manner. We have, hereby, in this article endeavored to address both types of TMA in a comparative mode. We appreciate that *de novo* TMA is more common and its prognosis is poorer than recurrent TMA; the latter has a genetic background, with mutations that impact disease behavior and, consequently, allograft and patient survival. Post-transplant TMA can occur as a recurrence of the disease involving the native kidney or as *de novo* disease with no evidence of previous involvement before transplant. While atypical hemolytic uremic syndrome is a rare disease that results from complement dysregulation with alternative pathway overactivity, *de novo* TMA is a heterogenous set of various etiologies and constitutes the vast majority of post-transplant TMA cases. Management of both diseases varies from simple maneuvers, *e.g.*, plasmapheresis, drug withdrawal or dose modification, to lifelong complement blockade, which is rather costly. Careful donor selection and proper recipient preparation, including complete genetic screening, would be a pragmatic approach. Novel therapies, *e.g.*, purified

products of the deficient genes, though promising in theory, are not yet of proven value.

Key words: Kidney transplantation; *De novo* thrombotic microangiopathy; Thrombotic microangiopathy; Recurrent thrombotic microangiopathy; Atypical hemolytic uremic syndrome

Core tip: Many articles in the literature have covered either *de novo* or recurrent thrombotic microangiopathy (TMA) in an isolated manner; we tried here in this article to gather the criteria of both types in one review for comparison. Contrary to what was believed in the past, *de novo* TMA is more common and its prognosis is poorer. On the other hand, recurrent TMA relies on a wide base of genetic backgrounds, with mutation errors differing in their impact on disease behavior and consequently on allograft and patient survival. This base for instance is rapidly expanding, and ultimately warrants a parallel robust work up regimen.

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INTRODUCTION

Thrombotic microangiopathy (TMA) is a debilitating complication of kidney transplantation that is associated with poor patient and graft outcomes. The incidence of post-transplant TMA has been reported to be 5.6 cases per 1000 renal transplant recipients per year with a 50% mortality rate three years after diagnosis[1]. TMA after transplantation can be classified into either: (1) *De novo* TMA, *i.e.*, developed for the first time without any evidence of the disease before transplant; and (2) Recurrent TMA, *i.e.*, native kidneys failed as a result of TMA and it came back in renal transplantation. Since renal biopsy of native kidney is not performed in many patients with end stage renal disease (ESRD), missed diagnosis of TMA prior to kidney transplantation is likely. With the advent of the drug eculizumab, an anti C5 monoclonal antibody, that is highly effective in prevention as well as treatment of atypical hemolytic uremic syndrome (aHUS), it would be crucial to know the etiology of ESRD in order to differentiate *de novo* from recurrence. Such distinction will invariably have clear clinical and therapeutic implications. In this review, we shall try to discuss the main differences between the two categories in the pathophysiology, clinical course and available approaches of prevention and treatment.

DE NOVO TMA

In the presence of acquired or genetic dysregulation of the alternative complement pathway (AP), a number of precipitating factors have been identified in the context of renal transplantation that trigger the development of *de novo* TMA. These factors include the following: (1) Antibody mediated rejection (AMR); (2) Immunosuppressive-associated TMA: Calcineurin inhibitors (CNI) or mTOR inhibitors (mTORi), single or combined; (3) Other medications: *e.g.*, anti-vascular endothelial growth factor inhibitors (anti-VGFI); (4) Viral infection: *e.g.*, HCV, CMV, BK and parvovirus; (5) Genetic abnormalities in the complement cascade; (6) Phenotypical shift of C3 glomerulopathy (with ESRD), to an aHUS post transplantation; and (7) Missed diagnosis of TMA in the native kidney as a cause of ESRD (*i.e.*, recurrent TMA)[2].

Which is more prevalent, de novo or recurrent TMA?

Reynolds *et al*[1], in a United States Renal Data System (USRDS)-based study, declared that the number of recurrent TMA cases was only 12 compared to 112 patients with *de novo* TMA, though the risk of post-transplant TMA recurrence was 36.5 times higher in kidney transplant recipients with ESRD due to hemolytic uremic syndrome (HUS) as compared to other etiologies (29.2% *vs* 0.8%)[1]. Langer *et al*[3] reported the incidence of *de novo* TMA to be 1.5%. However, the incidence of *de novo* TMA is mentioned to be as high as 3%-14%[4,5]. It is clear that *de novo* TMA is more prevalent after kidney transplantation and presumably underestimated. Graft loss rate of 40% is reported in *de novo* TMA within a couple of years of diagnosis[5,6].

Etiopathogenesis of de novo TMA

AMR and medications are the two main causes of *de novo* TMA. In addition, the role of complement abnormalities is becoming more apparent with one study reporting an underlying complement mutational abnormality in one third of patients with *de novo* TMA[7].

Calcineurin-induced TMA: The link between CNI (CyA and tacrolimus) administration and the evolution of *de novo* TMA is not a new concept. Three underlying mechanisms could explain the role of CNI in TMA development: (1) Loss of the normal balance between the vasodilator peptides (*e.g.*, prostaglandin (PG) E2 and prostacyclin (PG12)) and the vasoconstrictor peptides (*e.g.*, thromboxane A2 and endothelin), results in arteriolar vasoconstriction[8,9], renal ischemia and establishment of endothelial injury[10]; (2) CNI-induced platelet activation, pro-coagulant and anti-fibrinolytic activity have been shown to be involved in TMA evolution, particularly so, with an injured endothelium due to AMR, ischemia-reperfusion injury or any other etiology[10-12]; and (3) Microparticle production from endothelial cells, a known effect of CyA that can result in activation of the AP, a well-known mechanism that is implicated in

TMA evolution[13]. However, three trap points have been speculated to oppose the role of CNI: (1) Patients utilizing CNI to maintain immunosuppression represent more than 95% of kidney transplant recipients (KTR), and only a small percentage can develop TMA, which suggests the presence of another underlying predisposing factor (s)[14]; (2) CNI withdrawal in *de novo* TMA does not always guarantee a favorable graft outcome[6]; (3) A USRDS-based study demonstrates a significantly higher incidence of TMA in the group of KTR that was not under CNI maintenance therapy (11.9/1000/year), as compared to those on CNI maintenance (5.0/1000/year)[1].

mTOR inhibitor-associated TMA: mTORi can inhibit cell cycle progression and proliferation. Both sirolimus and everolimus have been reported to be implicated in the pathogenesis of *de novo* TMA. The following explanations have been given: (1) mTORi has antiangiogenic properties, and can decrease renal expression of vascular endothelial growth factor (VEGF) with death of the endothelial progenitor cells. These effects are proven to be implicated in TMA pathogenesis[15,16]; (2) The VEGF inhibition has been recently proven to be associated with reduced renal levels of complement factor H (CFH)[17]. Patients with underlying CFH genetic mutations are more susceptible to develop *de novo* TMA, particularly with mTORi exposure[7]; (3) Repair of endothelial injury could be hampered by mTORi use[18-20]; and (4) Furthermore, the procoagulant and the antifibrinolytic activity of mTORi might play additional roles in *de novo* TMA development[21,22].

The exact role of mTORi in the evolution of *de novo* TMA is not fully understood[3,18,23]. Some authors have suggested that the impact of these medications may exceed that of CNI in the development of *de novo* TMA[1,24]. However, interpretation of these data may be limited by the fact that mTORi itself, *e.g.*, sirolimus, may be used as a rescue medication in the case of diagnosis of CNI-induced TMA[1,24]. The risk of development of TMA with combined CNI and mTORi protocols is higher than using mTORi alone, an effect that has been documented in several studies. While Fortin *et al*[18] reported that the highest risk of *de novo* TMA was in the group using CNI and mTORi, Nava *et al*[20] studied 396 KTR, 36 (7.3%) developed TMA and 17 of them were drug-related. Not only were the drug levels of CNI and mTORi higher in the TMA group, but the sum of both drug levels in the TMA group was also higher[18-20]. An explanation for this additive risk is that the repair of the endothelial injury induced by CNI is hampered by mTORi[18-20]. Therefore, immunosuppression protocols using drug combinations should be planned cautiously, when high doses of these agents are usually used in the early post-transplant period[7].

AMR-associated *de novo* TMA: The role of AMR in the development of post-transplant TMA is commonly reported and well-recognized[1]. Endothelial cells are a well-known target of allo-immune response. The peritubular capillary (PTC) C4d staining (a well-recognized surrogate marker of AMR) has been reported to be present in 16.2% of biopsied recipients with TMA[1,25]. Moreover, Satoskar *et al*[6] reported an incidence of 55% of *de novo* TMA patients who express diffuse PTC C4d positivity. The observed prevalent administration of CyA in this study argued that it may have an augmenting effect on TMA prevalence. However, the observed difference between TMA in patients with C4d positive biopsy (13.6%) and that in C4d negative biopsies (3.6%) favors a postulated role of humoral rejection in the evolution of post-transplant TMA[2]. Both studies, for instance, demonstrated that clustering of both AMR and TMA would predict much worse graft outcome[6,26].

Other causes: Several less common etiologies have been reported to be involved in TMA pathogenesis and include: Viral infection, *e.g.*, CMV infection[27,28], BK virus[29], parvovirus[30,31], chronic hepatitis C virus (with or without anti-cardiolipin seropositivity)[32,33], and antiviral medications, *e.g.*, ribavirin and interferon[34] and disseminated histoplasmosis[35,36]. Ischemia-reperfusion injury can augment complement-associated injury through complement activation[37]. An acquired disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) deficiency- another rare risk factor- has been shown in one case to represent post-transplant TMA[38,39]. Unfortunately, the role of rare risk factors is rather difficult to evaluate in controlled studies. Living donation, on the other hand, has not been shown to guarantee any protection against graft dysfunction[5]. Interestingly, a C3 glomerulopathy disease in a native kidney can undergo phenotypical shift and present after kidney transplantation as *de novo* TMA[40].

Complement gene mutations: Chua *et al*[41] reported that renal complement activation is the common denominator in such a heterogeneous condition. They observed C4d deposits in more than 88% and C4d with localized C5b-9 in about 60% of 42 biopsy samples from patients with histologically confirmed diagnosis of TMA from a heterogenous group of patients[41]. Moreover, Le Quintrec *et al*[7] reported the presence of genetic mutations in CFH, Complement Factor I (CFI) or both in 29% of their studied *de novo* TMA patients, 25% showed low Complement Factor B (CFB) and/or low C3, suggesting an AP complement activation. No mutations have been found in healthy controls (100) or in TMA-free KTR controls[7].

Relation to TMA evolution: The AP depends on two main regulators: CFH and CFI. CFH has the ability to inhibit the C3 cleaving enzyme C3bBb. Moreover, it can serve as co-factor for FI, and the latter has the ability to inactivate C3b. Consequently, inactivation of these proteins either due to genetic mutations or development of neutralizing antibodies, can trigger an uncontrolled AP activity, leading to endothelial injury, the pathogenetic

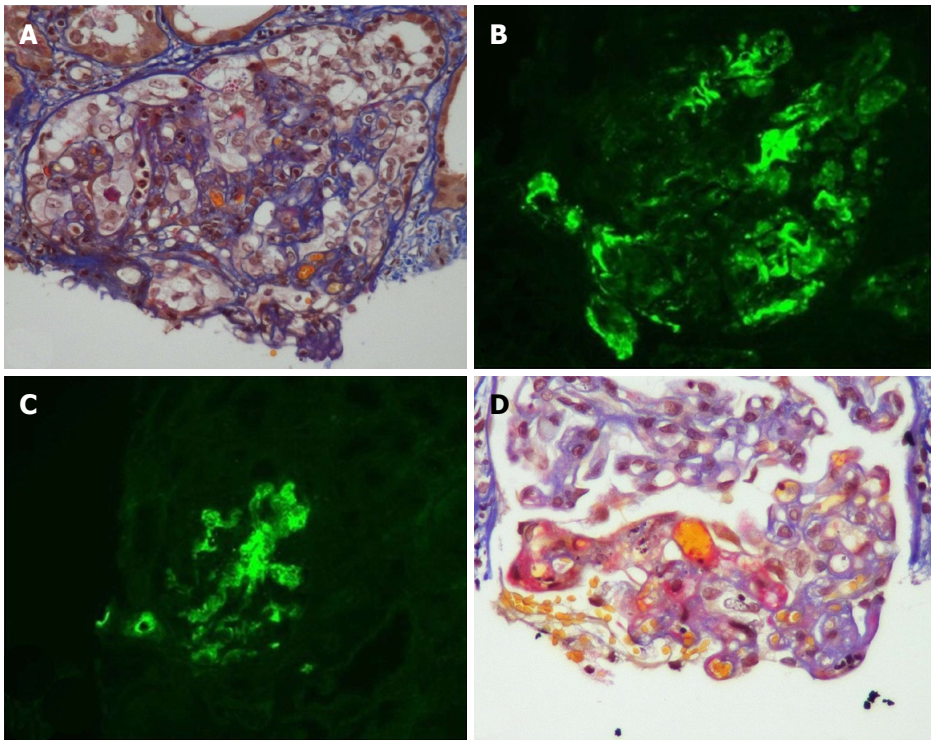

Figure 1 Acute and chronic thrombotic microangiopathy and calcineurin inhibitors-associated arteriolopathy with severe acute ischemic tubular lesions. A: Advanced interstitial inflammatory fibrosis (Masson trichrome stain); B: Immunofluorescence, diffuse and segmental C3; C: C1q deposits within glomerular capillary walls; D: Diffuse acute and chronic arteriolar and glomerular thrombotic microangiopathy lesions on light microscopy (LM). (Adapted from: Yassine *et al*[45]).

basis of TMA. Interpreting the results of the above study may suggest an overlap between aHUS and TMA. However, multiple mutational gene varieties related to complement and the coagulation-fibrinolysis cascades have been recently recognized in TMA patients[42].

Clinical manifestations

Timing: TMA could develop at any time in the post transplantation course[5,43], however this syndrome is mostly encountered in the first 3-6 mo post transplantation. This is probably when the CNI immunosuppressive trough levels are relatively higher[1].

Salient features: TMA manifestations are quite variable and can vary from a limited form confined to the kidney to a full blown systemic variant[4,6,44]. The systemic form of TMA consists of the classic triad of thrombocytopenia, microangiopathic hemolytic anemia (MAHA) and acute kidney injury (AKI). Features of MAHA include raised lactic acid dehydrogenase (LDH), drop in hemoglobin (HB) and decreased haptoglobin with schistocytes on peripheral blood smear. Localized (limited) TMA is usually presented later in TMA course, as compared to the systemic form, which can be explained by the urgency of the systemic type, necessitating the diagnostic allograft biopsy[4]. When a renal transplant recipient has significant renal dysfunction and the biopsy does not show any acute rejection, one must suspect two possibilities: (1) TMA or (2) Renal artery stenosis. The histopathologic changes are usually non-specific but vary in the acute status to the chronic angiopathic changes. In the active stage, there is evidence of endothelial cell injury with platelet aggregation (thrombosis), fibrinoid necrosis and glomerular ischemia. In the chronic stage, the basement membranes undergo duplication and multilayering with increased matrix layers and vessel wall cells, which ultimately ends in the unique onion skin formation (Figure 1)[2,45].

Once the diagnosis of TMA has been established, a prompt revision of the etiology of the native kidney ESRD should be instituted. In aHUS patients who do not show systemic manifestations, the diagnosis could be obscure. In the absence of renal biopsy, many cases can be misdiagnosed as hypertensive nephrosclerosis[2]. Consequently, a prompt testing for genetic mutations should be accomplished to unmask an underlying complement dysregulation and avoid missing the diagnosis of a recurrent aHUS. This approach has key therapeutic implications, since *de novo* TMA has limited therapeutic options, in contrast to recurrent aHUS after transplantation, which has a better chance of C-5 blockade through the monoclonal antibody eculizumab, an effective therapeutic agent not only for treatment, but also for prevention of recurrence[2,46].

Prognosis of *de novo* TMA: The prognosis of post-transplant *de novo* TMA is quite poor for the patient and as well as the allograft. About one half of the patients loses their graft within the first two years after diagnosis[4,6]. This is supported by the USRDS-based

Table 1 Morphological features in microangiopathy

Active lesions	Chronic lesions
Glomeruli: Thrombi - Endothelial swelling or denudation - Fragmented RBCs - Subendothelial flocculent material. EM: Mesangiolysis - Microaneurysms Arterioles: Thrombi - Endothelial swelling or denudation-Intramural fibrin-Fragmented red blood cells-Intimal swelling-Myocyte necrosis Arteries: Thrombi - Myxoid intimal swelling -Intramural fibrin- Fragmented red blood cells	Glomeruli: LM: Double contours of peripheral capillary walls, with variable mesangial interposition - EM: New subendothelial basement membrane - Widening of the subendothelial zone Arterioles: Hyaline deposits Arteries: Fibrous intimal thickening with concentric lamination (onion skin)

Adapted from: Goodship *et al*[58]. EM: Electron microscopy; LM: Light microscopy.

report presented by Reynolds *et al*[1] that reported a patient mortality rate of 50% after three years of diagnosis. Many studies support these results[4-6,18]. To compare systemic versus localized TMA, Schwimmer *et al*[4] reported that 54% of systemic TMA develops dialysis-requiring AKI and 38% lost their grafts. On the other hand, none of the patients with localized TMA developed TMA-related early graft loss or required dialysis. Unfortunately, this variation in both types of behavior has not reflected on graft survival, as both types of TMA face poor long-term graft survival[2,4].

RECURRENT TMA AFTER RENAL TRANSPLANTATION

Etiology of recurrent TMA

aHUS; thrombotic thrombocytopenic purpura (TTP); and autoimmune diseases: *e.g.*, scleroderma and systemic lupus erythematosus, with or without anti-phospholipid antibody syndrome[2].

aHUS: Recurrence of TMA in the allograft depends on the underlying type involving the native kidney. Overactivation of the AP is known to be the underlying etiology of aHUS. By far, aHUS is the most common diagnosis in TMA associated with recurrence. Risk of recurrence is greatly dependent on the underlying associated abnormality[47]. For example, mutational abnormality involving CFH and CFI, regulatory complement components produced by the liver, results in aberrant CFH and CFI. After transplant, CFH and CFI have a robust impact in the evolution of aHUS recurrence. The reported rate of aHUS recurrence approached 70%-90%[47,48]. Membrane co-factor protein (MCP), a transmembrane complement regulatory component that is produced by kidney endothelial cells even in post-transplant period, keeps aHUS recurrence lower unless other mutational gene defects have been associated[47-49]. Additional MCP mutations (> 22%), as reported by Bresin *et al*[50], led to graft loss due to recurrence of aHUS in one third of patients. The global rate of recurrence in aHUS patients is reported to be as high as 60%. Untreated patients, however, ultimately develop graft loss at a rate of 90%, with 80% of them occurring in the first year[50].

TTP: TTP is the second recognized etiology in TMA. Genetic or acquired lack of ADAMTS13 has been recognized. For a long period, differentiation between TTP and HUS relied primarily on the presence of neurologic manifestation in TTP and renal dysfunction in HUS to settle the diagnosis. Serological evaluation of ADAMTS13 activity is now feasible. However, complete distinction between the two clinical entities is not always possible because of overlap in manifestations. Recently, Zafrani *et al*[51] documented the presence of AKI in more than half of TTP patients (with low ADAMTS13 activity) and 50% progression of CKD and even ESRD. It is reasonable to expect TTP recurrence as long as the underlying defect is present after transplantation[52]. The same explanation can be applied to the autoimmune diseases, *e.g.*, lupus nephritis, wherein patients can develop TMA in 5%-10% with documented recurrence after kidney transplantation[53-57].

Pathology: aHUS is a variety of TMA that represents the tissue response to an ongoing endothelial injury. Thrombotic features, *e.g.*, fibrin/platelet plugging and intraluminal fibrin are not always seen in renal allograft biopsy. Non-thrombotic features can appear as denuded and swollen endothelium, mesangiolysis, glomerular basement membrane double contour, as well as accumulation of electrolucent material in the subendothelium. Arterial and arteriolar intraluminal fibrin, myxoid intimal thickening as well as concentric myointimal proliferation (onion skin appearance) have also been described[58] (Table 1).

PATHOPHYSIOLOGY OF TMA RECURRENCE

The AP is constitutively active and is, therefore, fine-tuned. The regulatory components exist either in the serum (fluid phase) or attached onto cell membranes. CFH is the main inhibitor of the AP. CFH has the ability to work in fluid phase as well as on cell surfaces. Furthermore, CFH can act as a co-factor to CFI[59,60]. Regulatory components on cell surfaces, or "membrane regulators" include the following: (1) Membrane cofactor protein (MCP/CD46); (2) Complement receptor 1 (CR1/CD35); (3) Decay accelerating factor (DAF/CD55); and (4) Protectin (CD59), which prohibits MAC formation[61,62].

Any disturbance involving any of this protective

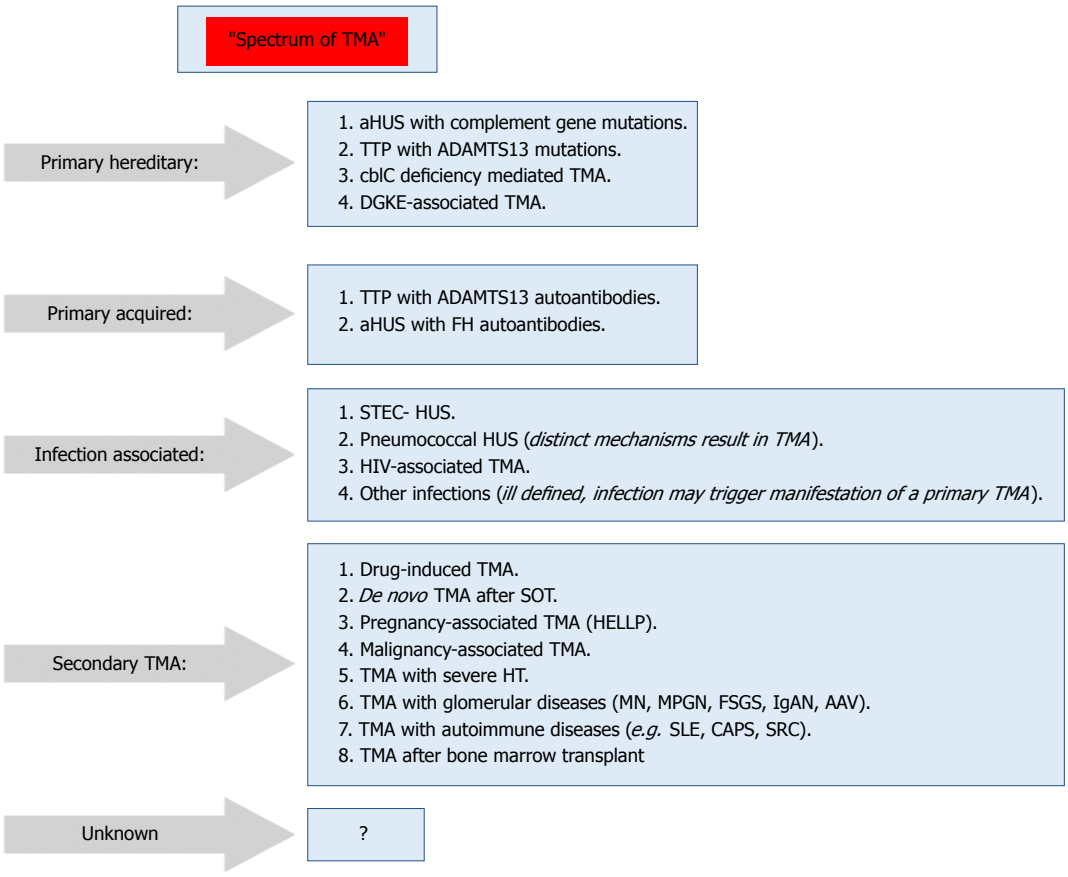

Figure 2 Spectrum of thrombotic microangiopathy[64]. AAV: ANCA-associated vasculitis; ADAMTS13: A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; aHUS: Atypical hemolytic uremic syndrome; C3G: C3 glomerulopathy; CAPS: Catastrophic antiphospholipid syndrome; cblC: Cobalamin C type; *DGKE*: Gene encoding diacylglycerol kinase ε; FH: Factor H; HELLP: Syndrome of hemolysis, elevated liver enzymes, and low platelets; HUS: Hemolytic uremic syndrome; IgAN: IgA nephropathy; MN: Membranous nephropathy; MPGN: Membranoproliferative GN; SRC: Scleroderma renal crisis; STEC: Shiga toxin–producing *Escherichia coli*; TMA: Thrombotic microangiopathy; TTP: Thrombotic thrombocytopenic purpura.

shield will ultimately lead to complement activation with subsequent endothelial cell derangement[63]. It is increasingly recognized that complement dysregulation is the fundamental etiology involved in TMA evolution. Both genetic aberrations as well as autoantibodies can be involved in this process. Usually, there is (are) an inciting environmental trigger factor(s).

Current classification of TMA includes the following

Primary hereditary TMA: Includes mutations in ADAMTS13, MMACHC (cb1c deficiency), or in genes encoding complement components.

Primary acquired TMA: Autoantibodies to ADAMTS13 or to CFH, which occurs with homozygous CFHR3/1 deletion.

Infection-associated TMA: Shiga toxin-producing Escherichia coli-HUS (STEC-HUS) and pneumococcal HUS have distinct mechanisms that result in TMA; in other infections, the processes are ill-defined and sometimes can trigger manifestations of the primary TMA.

Secondary TMA: Presents in a variety of conditions, and in many conditions the culprit mechanisms are usually multifactorial or unknown. The shown classification (Figure 2) is not unequivocal, *i.e.*, in some secondary forms of TMA, *e.g.*, pregnancy-associated TMA or *de novo* TMA after transplantation, a significant percentage of cases may be associated with genetic predisposition (Figure 2)[64].

The most common complement mutation in aHUS is CFH, with 40% of cases inherited and 25% sporadic[65,66]. Furthermore, not only CFH has its impact on TMA evolution, but the CFH-related genes (CFHR1-5) have additional roles. Through deletion, hybrid protein formation and duplication[67] of these genes, the endothelial cell surface becomes denuded from its protective shield, and consequently aHUS may supervene[65,68].

The risk of aHUS recurrence could be four times higher with CFH mutations or with the carriers of CFH/CFHR1 hybrid genes[24]. On the other hand, the impact of CFI mutations is controversial. While early reports about CFI mutations documented a high rate of recurrence and graft loss[69-71], Bienaime *et al*[72] denied any risk of recurrence associated with CFI mutations. Le Quintrec *et al*[24] were in agreement with them. As MCP can normally be expressed by the endothelial cell surface of

Table 2 Risk of atypical hemolytic uremic syndrome recurrence according to the implicated genetic abnormality

Gene mutation	Location	Functional impact	Mutation frequency in aHUS (%)	Recurrence after transplantation (%)
CFH	Plasma	Loss	20-30	75-90
CFI	Plasma	Loss	2-12	45-80
CFB	Plasma	Gain	1-2	100
C3	Plasma	Gain	5-10	40-70
MCP	Membrane	Loss	10-15	15-20
THBD	Membrane	Loss	5	One case
Homozygous	Circulating	Undetermined	14-23	NA
CFHR1 del (3%-8%)			(> 90% with anti-CHF AB)	

Adapted from Salvadori *et al*[74]. NA: Not available; *CFH*: Complement factor H; *CFI*: Complement factor I; CFB: Complement factor B; C3: Complement component 3; *MCP*: Membrane cofactor protein; *THBD*: Thrombomodulin.

the allograft, aHUS recurrence is seldom influenced by MCP gene mutations. No more than three cases of MCP-associated recurrence have been reported[73,74], where recurrence was attributed either to combined gene mutations[49] or microchimerism related to the recipient's endothelium[74] (Table 2).

There is a paucity of data on the role of thrombomodulin (THBD) gene mutations in aHUS. Like MCP, THBD is membrane-anchored, so the possibility of recurrence is rarely seen. Only a few cases have been reported[75,76]. Gain of function mutation (C3 and CFB) is vulnerable for recurrence. Recurrent aHUS with subsequent graft loss have been reported in up to four cases of CFB carriers[77,78]. On the other hand, data related to C3-asociated recurrence are conflicting. While Le Quintrec *et al*[24] documented recurrence in four of five allografts, Noris *et al*[79] reported only two cases out of seven transplants with *C3* mutations. Zuber *et al*[80] postulated that normal C3 supplied by the graft tissues might have a protective effect.

Role of diacylglycerol kinase-ε (*DGKE*) mutations: Until recently, the vast majority of aHUS patients were thought to be associated with AP dysregulation. On the contrary, most patients with *DGKE* mutations exhibit no evidence of complement overactivity. Homozygous mutations in the gene encoding for DGKε and DGKε-associated nephropathy have been recently uncovered. Complete loss of function is associated with acute renal failure, thrombocytopenia and hemolytic anemia. Consequently, it has been postulated that the DGKε protein may play a fundamental role in regulating thrombosis in renal tissues, a robust fact that urged expert renal clinicians to include DGKE mutations in the pathophysiology of aHUS[81,82] (see treatment below).

Environmental triggers: The process of aHUS recurrence can be triggered by anti-HLA antibodies[6], viral infection, ischemia-reperfusion injury and immunosuppressive medications[83], either isolated or in clusters, which can initiate the cascade of complement activation in susceptible patients.

Clinical assessment of aHUS: Any HUS that is not due to STEC-HUS has been called aHUS[75]. The recent progress in understanding the pathophysiology and the underlying genetic factors led to the current classification of aHUS[84]. Consequently, the term "primary HUS" has been addressed by some clinicians when there is underlying abnormality in the AP. However, patients with underlying complement abnormality need a trigger factor, *e.g.*, infection, including pneumococcal infection (T-antigen associated TMA), surgery, medications, pregnancy, so that aHUS can clinically manifest[85,86].

Acute vs chronic lesion?

Timing of an aHUS episode is not easily predictable. Many patients are at persistent risk of recurrence. In medical genetics, penetrance of any disease-causing mutation means the percentage of subjects with genetic mutations who can express clinical symptoms[87]. Penetrance in aHUS is age-related, by age 70, penetrance reaches 64%[88], which supports the presence of disease modifiers by the aging process. The fact that certain patients (3%-5%) may express more than one genetic variant supports the postulation that mutation burden determines the magnitude of disease penetrance. The late presentation of aHUS reflects the impact of the environmental triggers. However, dissociation between the pathological entities and the clinical presentation have been reported. For example, TMA can be diagnosed in tissue biopsy without simultaneous decline in platelet count. Moreover, the current use of eculizumab has its impact on the natural history of aHUS[89]. Complement inhibition can improve glomerular perfusion enough to maintain kidney function. Once this biological agent is withdrawn, the renal endothelium may interact with the complement system through an unknown mechanism. More studies are obviously warranted to declare these alterations[58].

Extrarenal manifestation: Twenty percent of aHUS patients can express extrarenal manifestations in the form of digital gangrene, cerebral artery thrombosis, myocardial infarction, in addition to ocular, GIT, pulmonary and neurologic involvement[42,90-98]. Drusen formation is not common in aHUS[99].

Laboratory investigations and differential diagnosis: Once the diagnosis of aHUS is suspected,

Table 3 Complement studies for atypical hemolytic uremic syndrome (aHUS)

Complement test	aHUS
Complement protein levels	C3, C4, FB[1], C5[1]
Complement regulatory protein levels	FH, FI, Properdin[1], CD46[2]
Complement split products	C3c[1], C3d[1], Bb[1], sC5b-9[1]
Complement functional assays	CH50, AH50, hemolytic assays, FH assays[1]
Autoantibodies	Anti-FH
Genetic screening	CFH, CFI, C3, CD46, CFB
	Genomic rearrangements across the FH-FHR locus (*e.g.*, by MLPA)
	Sequencing of coding regions and assessment of CNV
	Non-complement genetic screening includes THBD and DGKE

[1]Currently available only at specific laboratories; they are research and not clinically validated assays; [2]CD46 is also known as MCP. Adapted from: Goodship *et al*[58]. AH50: Alternative pathway hemolytic assay; C3: Complement component 3; C4: Complement component 4; C5: Complement component 5; *CFB*: Complement factor B gene; *CFH*: Complement factor H gene; *CFHR*: Complement factor H related genes; CFI: Complement factor I gene; CH50: Classical pathway hemolytic assay; CNV: Copy number variation; *DGKE* gene: Diacylglycerol kinase epsilon gene; FB: Complement factor B; FH: Complement factor H; FI: Complement factor I; MLPA: Multiplex ligation-dependent probe amplification; sC5b-9: Soluble C5b-9; THBD: Thrombomodulin; aHUS: Atypical hemolytic uremic syndrome.

exclusion of ADAMTS13 activity is urgently mandated to exclude TTP diagnosis. In children, TTP is less common; therefore, eculizumab therapy should be instituted early without waiting for the results of ADAMTS13 activity. In addition, 5% of STEC-HUS patients have no prodromal diarrhea and 30% of complement-mediated aHUS patients can present with a diarrheal prodrome[100].

Complement assessment in aHUS: Before commencing plasma therapy, serum complement component should be thoroughly evaluated. C3 is low in 30% of aHUS patients and, therefore cannot be used as a screening criteria for aHUS[97,101]. CD46 surface expression should be assessed by flow cytometry. Functional parameters as well as activation markers should be also determined. Whether these biological markers can be used to guide therapy requires further investigation[102] (Table 3).

Panel of genetic testing: The diagnostic list of genes of aHUS should include at least CFH, CFI, C3, CFB, THBD, CFHR1, CFHR5 and DGKE[48,65,75,103-105]. Genotyping workup should also include CFH-H3 and MCP ggaac haplotypes[106]. Recent advances in genetic surveys addressed the use of copy number variation (CNV), hybrid genes, and the complex genomic rearrangements of CFH/CFHRs genomic region[68,107-111]. The full-detailed genetic mapping, however, allows proper diagnosis and therapeutic plans, and helps in genetic counseling, particularly in living related-donation[112]. The role of living-related kidney donor transplantation in aHUS is that the culprit agent(s), either acquired or genetic, should be well-recognized, and the donor should be free of this factor(s) at the same time. Consequently, the presence of CFH or MCP mutations in the donor is not-per se- a contraindication for donation[58].

Rationale for genetic screening: The current progress in understanding the underlying genetic background of aHUS and its molecular basis makes it paramount to provide a full detailed genetic map before transplant, and the following explanations have been given: (1) Determination of the actual cause of the disease that allows for correct genetic counseling; (2) Drawing the plan of disease management; (3) Evaluating the expected response for therapy; and (4) Defining the prognostic course as well as patient and allograft survival. These studies, however, did not hamper the progress in clinical diagnosis and therapy institution before irreversible sequalae have been established[113]. A schematic presentation for the "genetic drivers" of aHUS is supplied in Figure 3[58].

Interpretation of the genetic variants: Genetic mutations can be interpreted as: (1) Benign; (2) Likely benign; (3) Variant of uncertain significance; (4) Likely pathogenic; or (5) Pathogenic, according to the international guidelines[114].

The pathogenic mutations in aHUS have the ability to hamper the capacity to protect the endothelial lining and the platelet from the devastating effect of complement or its activation[78,115-121]. It is well-documented now that pathogenic variant combinations as well as clustering of risk factors facilitate the evolution of aHUS[49,88,122-125]. Genetic designation also has its impact on therapeutic plans, response to therapy as well as the chance for aHUS recurrence[79,126] (Table 4).

Acquired drivers of aHUS: The FH autoantibodies are the best reported example. It is typically characterized by homozygosity for delCFHR3-CFHR1. Test results need to be confirmed after two weeks if the initial results were positive. According to the consensus guidelines in pediatrics, CFH autoantibodies assessment should be confirmed, if positive, on a regular basis[84]. About a quarter of patients with anti-CFH-associated HUS are vulnerable for relapse.

Diagnosis of aHUS recurrence: A full detailed clinical history is usually warranted. A proven tissue diagnosis

Table 4 Genotype-phenotype correlations in atypical hemolytic uremic syndrome (data refer to the period before introduction of eculizumab)

Gene	Risk of death or ESRD at onset or first yr	Risk of recurrence	Risk of death or ESRD after 3-5 yr	Risk of recurrence in allograft
CFH or *CFH-CFHR1/3* hybrid genes	50%-70%	50%	75%	75%-90%
CFI	50%	10%-30%	50%-60%	45%-80%
MCP single	0%-6%	70%-90%	6%-38%	< 20%
MCP combined[1]	30%-40%	50%	50%	50%-60%
C3	60%	50%	75%	40%-70%
CFB	50%	100%	75%	100%
THBD	50%	30%	54%	?
Anti-FH	30%-40%	40%-60%	35%-60%	Depends on antibody titers

[1]Combined with *CFH* or *CFI* or *C3* mutations. Adapted from: Goodship *et al*[58]. *CFB*: Complement factor B gene; *CFH*: Complement factor H gene; *CFHR*: Complement factor H-related genes; *CFI*: Complement factor I gene; FH: Factor H protein; *THBD*: Thrombomodulin gene.

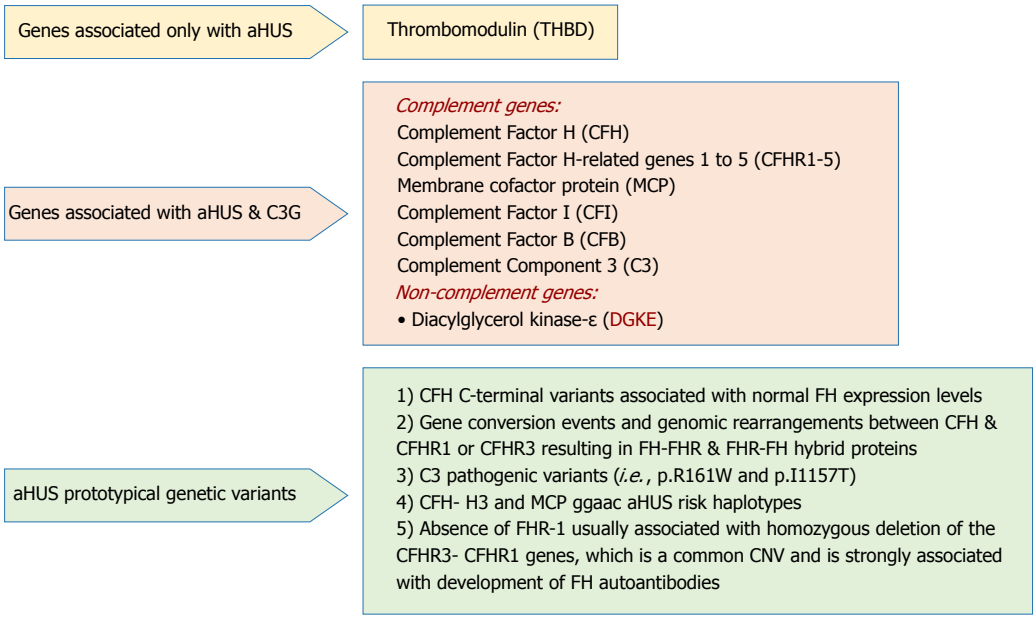

Figure 3 Genetic drivers in atypical hemolytic uremic syndrome (Adapted from: Goodship *et al*[58]). aHUS: Atypical hemolytic uremic syndrome; C3G: C3 glomerulopathy; CNV: Copy number variation; SCR: Short consensus repeat.

with light microscopy (LM), immunofluorescence (IF) and electron microscopy (EM) studies supporting the diagnosis of aHUS in the native kidney should be available. However, once diagnosis of aHUS is suspected, a full battery of biochemical, genetic as well as pathological investigations of the AP should be accomplished[127], including the following: (1) Estimation of the anti-CFH AB; (2) MCP screening on the peripheral blood WBCs; (3) Examination of the recombination in CFHR region; and (4) Screening of the genetic mutations related to CFH, CFI, CFB, C3, and MCP.

The impact of various genetic mutations on allograft survival is not universally quantifiable. Not all of the genetic mutations share the same magnitude of risk on allograft survival. Despite the fact that genetic screening is difficult and complex and the spectrum of gene mutation is a continuously expanding field[102], performing such studies is fundamental to determining the possible outcome of the kidney transplant in the set of aHUS recurrence[128].

THERAPY OF POST-TRANSPLANT TMA

Treatment of de novo TMA

In view of the extreme heterogenicity of the mechanisms related to variable etiologies of TMA, therapeutic maneuvers should be individualized for each patient. Institution of therapeutic options is highly dependent on diagnosis as well as the patient's response. The following approaches have been suggested: (1) Immunosuppressive medication management: the role of immunosuppressive medications (*e.g.*, CNI or mTORi) has been reported in the literature, with a documented better response after switching from one CNI member to another or to an mTORi)[5,129-134]. However, this was not agreed by Satoskar *et al*[6], who denied any difference in outcomes between temporary discontinuation, dose modulation, withdrawal or continuation of CyA in man-

agement of *de novo* TMA. Whatever the situation would be, the withdrawal of the offending agent should be the first line in treating *de novo* TMA, a fundamental step that ultimately results in correction of the hematological profile[2]; (2) Plasmapheresis (PE) and intravenous immunoglobulins (IVIG): The following rationales have been addressed in favor of PE/IVIG therapy: Depending on its efficacy in treating patients with TTP[135,136], and previous choice as a first line therapy for aHUS (replaced now by eculizumab), PE with IVIG has been extrapolated to be used early in treating *de novo* TMA patients. In 2003, Karthikeyan *et al*[43] reported a graft salvage rate with PE approaching 80%. Two benefits have been postulated for this type of therapy: Removal of the platelet aggregation factors, *e.g.*, thromboxane A2 and the simultaneous replenishment of the deficient factors, *e.g.*, PGI2-stimulating factor[43]. With the possibility of the presence of underlying complement dysregulation in patients undergoing kidney transplantation due to systemic TMA[7], in the same manner, it is reasonable to speculate that PE can be beneficial for two reasons: Removal of the abnormal mutant complement proteins and supplying normally functioning complement components[7]. In AMR-associated TMA, an improved outcome has been reported, which was attributed to removal of the anti-HLA antibodies[6,137]. A 100% response has been reported to be associated with PE/IVIG therapy in five solid organ transplantation with systemic TMA with no evidence of relapse after withdrawal of the culprit agent (*e.g.*, tacrolimus) in a recent study[2]; (3) Belatacept: A promising alternate option that allows withdrawal of the offending drug incriminated in TMA evolution. Belatacept is an immunosuppressive co-stimulatory blocker against CD80 and CD86 surface ligands and CD28 on T cells. The first case report in 2009 documented TMA resolution after belatacept therapy used for immunosuppression in post-transplantation TMA due to CNI-induced endothelial toxicity[138]. Two case series have followed, thereafter documenting fair graft outcome due to resolution of the CNI-induced TMA[139,140]. Of note, belatacept has nothing to do with the underlying endothelial derangement, its role is only to replace/displace the culprit drug[2]; and (4) Complement inhibition: Eculizumab, an anti-C5 agent, blocks the lytic C5b-9 membrane attack complex generation. This recombinant monoclonal antibody addressed a breakthrough in the management of aHUS, as it was proven to be effective in treatment as well as in prevention of recurrent aHUS after renal transplantation[141]. A large percentage of patients with diagnosed TMA express complement activation, including those patients with unrecognized complement genes[2]. For example, Chua *et al*[41] reported C4d renal deposition in all histologically documented cases with post-transplantation TMA. These data delineate that complement overactivation can be considered as one of the final common pathways incriminated in TMA evolution[2]. Consequently, anti-complement therapy has been suggested to have a fundamental role in the management of *de novo* post-transplantation TMA. Efficacy of eculizumab has been documented in several case reports and case series in management of resistant cases of medication-associated TMA, including cases with unrecognized genetic defects[142-147]. This efficacy has been also documented in patients with refractory AMR with TMA[147-156].

On the other hand, Cornell *et al*[157] reported no difference in death-censored graft survival or biopsy finding at one year when they compared the outcome of eculizumab-treated patients with positive cross matching with controls, even though the incidence of acute AMR was less in the eculizumab group. So, in view of these conflicting results as well as considering the high cost of the drug, the use of this vital biological agent should be confined to a specified subset of *de novo* TMA patients, presumably: (1) AMR-associated TMA; (2) Patients who became PE-dependent; and (3) Refractory hemolysis persists despite maximum doses of PE therapy. However, more efforts are still warranted to declare the best way to utilize this unique agent and which subset of TMA patients are the best candidates for this costly drug. An urgent need for new biomarkers is also warranted for early detection of complement overactivity[2] (see kidney transplantation without eculizumab prophylaxis below).

Treatment of recurrent TMA

Recommendations for recurrent TMA: First of all, it is worthy to remember that most of the recommendations about recurrence and therapeutic advices relied primarily on case reports (level 4 evidence) as well as experts' opinions (level 5 evidence) rather than on randomized controlled trials (level 1b evidence). (1) The minimal list of genetic screening should include: CFH, CFI, CFHR, CFB, MCP and C3[158]; (2) All patients with primary or suspected aHUS, should be surveyed for all complement components and its related proteins; (3) Patients with isolated MCP associated mutations (not combined with other mutations) may be safe for kidney donation; (4) Patients with documented aHUS and with lack of definite genetic mutations can proceed in renal transplantation under the umbrella of intensive plasma exchange therapy[159]; and (5) Polygenic pattern for aHUS patients should be handled with extreme caution in case of living donation[80].

Prevention of aHUS: The following strategies are suggested to decrease/prevent aHUS: (1) Complement activity incited by an injury to endothelium, *e.g.*, ischemia-reperfusion injury, viral infection and immunosuppressive medications[127], should be avoided; (2) Certain relations have been reported between CNI use and aHUS recurrence[160], which is not confirmed by other authors[15,112], even the usual substitute in such a case (an mTOR) is not innocent and can induce recurrence[15,112]; (3) We cannot depend solely on PE therapy in management of aHUS recurrence for several reasons: PE failed to prevent aHUS recurrence in many cases[161]; PE cannot guarantee prevention of aHUS recurrence after cessation of therapy; Many cases under PE therapy were

Table 5 Eculizumab dosing in atypical hemolytic uremic syndrome based on dosing goal, one additional monitoring may be required during intercurrent events (*e.g.*, infection, surgery, vaccination) to detect unblocked complement activity

Minimal dose
Desire to continue dosing with the minimal dose required to achieve a pre-identified level of complement blockade 1
Dose reduction or interval extension
Goal CH50 < 10% (recommended)
Goal AH50 < 10% (recommended)
Goal eculizumab trough > 100 μg/mL
Discontinuation
Desire to discontinue complement blockade: No consensus exists regarding tapering of dose

Adapted from: Goodship *et al*[58]. AH50: Alternative pathway hemolytic activity; CH50: Total complement activity.

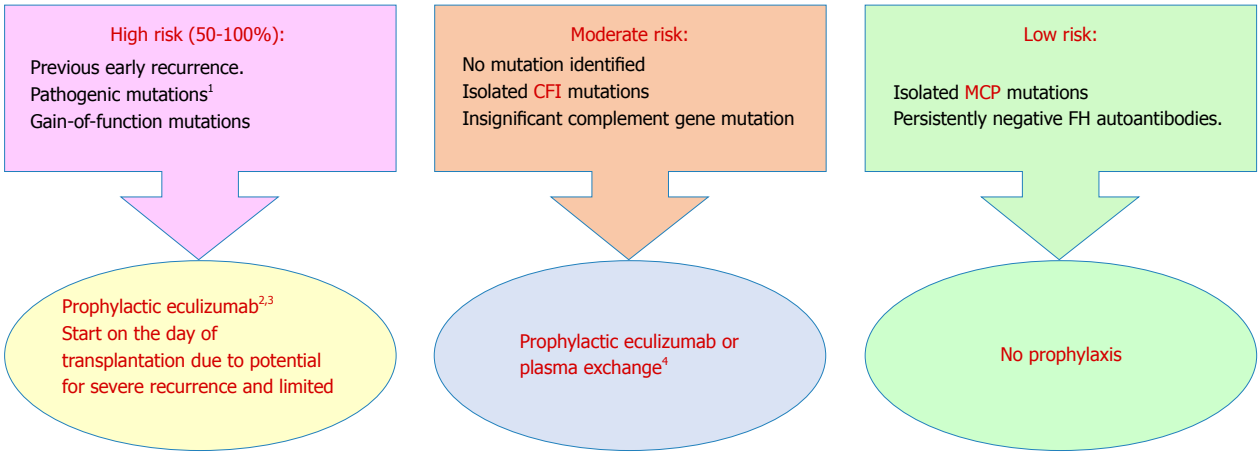

Figure 4 Prophylaxis against atypical hemolytic uremic syndrome recurrence in allograft based on a risk-assessment strategy[96] (Adapted from: Goodship *et al*[58]). [1]Requires complete screening of all genes implicated in atypical hemolytic uremic syndrome; [2]Prophylactic regimens are based on local center protocols; no trial data exist to support superiority of one protocol over another; [3]Liver transplantation can be considered for renal transplant recipients with liver-derived complement protein abnormalities, uncontrolled disease activity despite eculizumab therapy or financial considerations regarding cost of long-term eculizumab therapy; [4]Decision to perform or not to perform prophylactic plasma exchange or complement inhibition is left to the discretion of the clinician. aHUS: Atypical hemolytic uremic syndrome; *CFI*: Complement factor I gene; FH: Complement factor H protein; *MCP*: Membrane cofactor protein gene.

proved to develop "subclinical" aHUS recurrence, which means that PE therapy cannot influence complement activity; Prophylactic use of rituximab proved to be efficacious as anti-CFH-antibodies[162], the beneficial effect of rituximab can be enhanced by adding PE therapy[163,164]; and (4) The anti-C5 monoclonal antibiotic eculizumab has been reported to be used successfully to prevent aHUS recurrence in patients with CFH, CFH/CFHR1 hybrid genes as well as with C3 gene mutations[165-168] (see below).

Prophylactic complement blockade: Gene abnormalities have been reported to be associated with aHUS recurrence in 80% of patients[112]. In light of robust evidence of increased complement activity during aHUS episodes[169,170] after exposure to a trigger, *e.g.*, surgery or infection, clinical indication of complement blockade is suggested[171]. However, this explanation lacks enough evidence (Figure 4[58]).

Therapeutic protocols for aHUS recurrence: Once the diagnosis of primary aHUS has been established, complement blockade therapy should be instituted. The available data points to two strategies: (1) Minimal dosage to establish complement blockade; and (2) Dose withdrawal scheme[142]. Both options, however, lack enough evidence and require precise monitoring of complement blockade (Table 5).

FH autoantibody-driven aHUS: Anti-cellular therapy is recommended, with close monitoring of the antibody titer (Figure 5). How to monitor complement blockade? Detailed description is shown in Table 6.

Duration of therapy: There is not enough data supporting life-long therapy for aHUS. Cessation of therapy appears to be plausible in certain situations (Figure 6). Enough time, however, should be permitted to optimize renal recovery and satisfy TMA resolution. Early biomarkers of disease relapse due to complement activation or endothelial derangement as well as their inciting triggers should be thoroughly investigated in the future.

Unanswered questions: There is paucity of information about this biological agent, *e.g.*, what is the most optimal dose? What are the ideal dose-intervals? For how long should this kind of costly therapy be continued?[175] What impact does this agent have on the spectrum of renal transplantation[113]?

Table 6 Monitoring eculizumab therapy

Description	
CH50 (total complement activity)	Measures the combined activity of all of the complement pathways Tests the functional capability of serum complement components to lyse 50% of sheep erythrocytes in a reaction mixture Low in congenital complement deficiency (C1-8) or during complement blockade Normal range is assay dependent Recommended goal during therapeutic complement blockade: < 10% of normal
AH50 (alternative pathway hemolytic activity)	Measures combined activity of alternative and terminal complement pathways Tests the functional capability of alternate or terminal pathway complement components to lyse 50% of rabbit erythrocytes in a Mg^{2+}-EGTA buffer Will be low in congenital C3, FI, FB, properdin, FH, and FD deficiencies or during terminal complement blockade Normal range is assay dependent Recommended goal during complement blockade: < 10% of normal
Eculizumab trough	May be a free or bound level ELISA: Using C5 coated plates, patient sera, and an anti-human IgG detection system Not affected by complement deficiencies Recommended trough level during complement blockade: 50-100 μg/mL
Alternative assays	The following assays are under investigation (or awaiting to be replicated in different laboratories)[83] as a means to monitor therapeutic complement blockade Free C5 *In vitro* human microvascular endothelial cell test sC5b -9 (also referred to as sMAC and TCC) may remain detectable in aHUS patients in remission and therefore is not recommended as a monitoring tool

Adapted from: Goodship *et al*[58]. aHUS: Atypical hemolytic uremic syndrome; C3: Complement component 3; C5: Complement component 5; EGTA: Ethyleneglycol tetraacetic acid; ELISA: Enzyme-linked immunosorbent assay; FB: Complement factor B; FD: Complement factor D; FH: Complement factor H; FI: Complement factor I; sC5b-9: Soluble C5b-9; sMAC: Soluble membrane attack complex; TCC: Terminal complement complex.

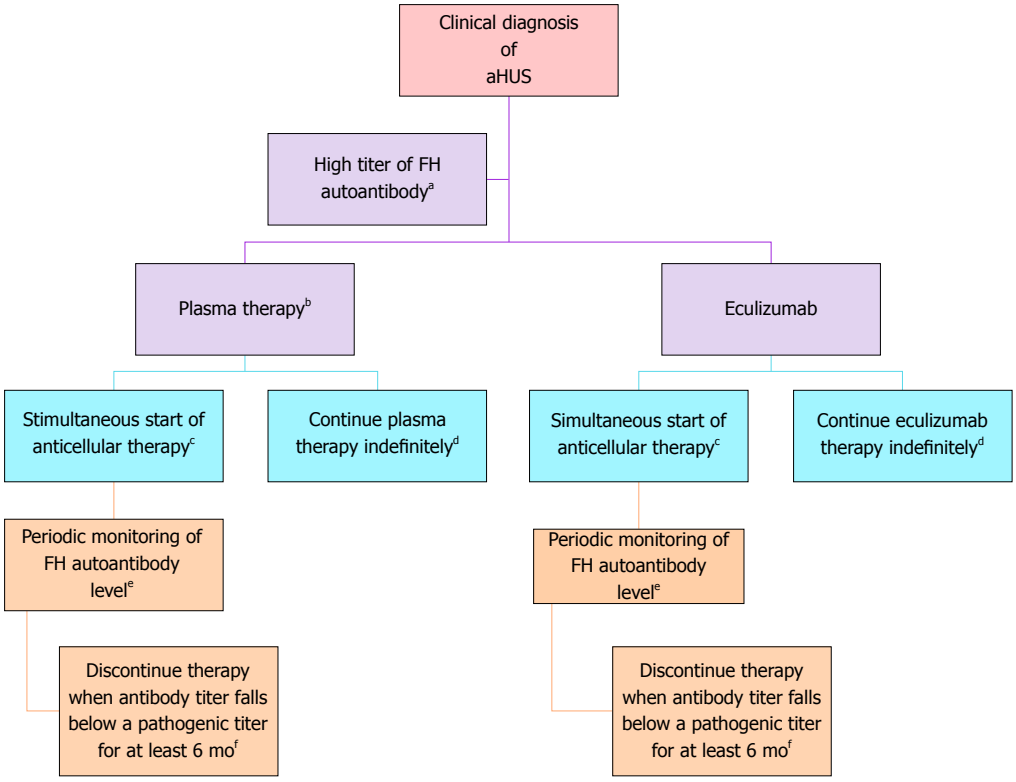

Figure 5 Treatment of complement factor H autoantibody-mediated atypical hemolytic uremic syndrome. There are no prospective controlled studies in patients with atypical hemolytic uremic syndrome (aHUS) due to anti–factor H protein (FH) antibodies, and thus the proposed management is based on a pediatric consensus[84] (Adapted from: Goodship *et al*[58]). [a]Abnormal titer depends on the testing laboratory; [b]The decision to use plasma therapy versus eculizumab will be based on patient age and local resource availability; [c]Cyclophosphamide, rituximab, or mycophenolate mofetil; [d]The decision to continue anticomplement therapy indefinitely is not informed by data; [e]The interval may be monthly or quarterly and is based on local resources; [f]This recommendation is based on limited retrospective case reviews[172-174].

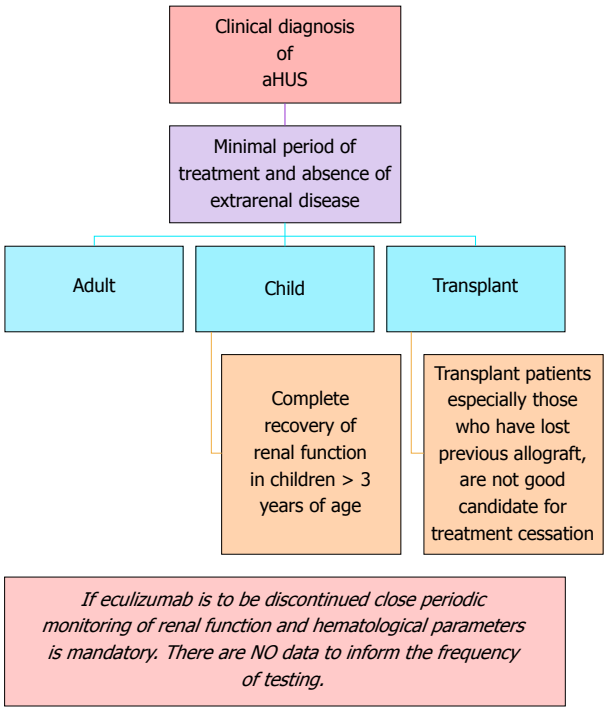

Figure 6 Recommendations for cessation of treatment with complement inhibitors. There are no prospective controlled studies in patients with atypical hemolytic uremic syndrome (aHUS) to define criteria for discontinuation of eculizumab therapy. This flow diagram is based on expert opinion[176-178]. Discontinuation can be considered on a case-by-case basis in patients after at least 6-12 mo of treatment and at least 3 mo of normalization (or stabilization in the case of residual CKD) of kidney function. Earlier cessation (at 3 mo) may be considered in patients (especially children) with pathogenic variants in MCP if there has been rapid remission and recovery of renal function. Patients on dialysis, eculizumab should be maintained for at least 4 to 6 mo before discontinuation. In this setting, assessment of fibrotic changes in kidney biopsy may be helpful. In transplant patients, especially patients who have lost previous allografts, discontinuation is not recommended. Adapted from: Goodship *et al*[58]. aHUS: Atypical hemolytic uremic syndrome.

Cessation of therapy: The following scheme is suggested for withdrawal of complement blockade therapy (Figure 6).

Kidney transplantation without eculizumab prophylaxis: A case series presented by Verhave *et al*[179] described successful kidney transplantation without recurrence in four high risk aHUS patients. They received living donor kidney with therapeutic protocol consisted of: Basiliximab for induction, tacrolimus in low dose, and prednisone and mycophenolate mofetil as immunosuppressive in addition to a statin. Additional precautions include lowering the blood pressure and minimizing the cold ischemic time. No recurrence or rejection has been observed after 16-21 mo. This case series heralds the possibility of successful kidney transplantation in recurrent aHUS without the need for prophylactic eculizumab through minimizing cold ischemic time, decreasing the risk of rejection and, thereby, providing endothelial protection[179].

Treatment of DGKE mutation associated TMA: The role of complement blockade here is questionable. Many cases experienced disease remission with no specific therapy. Azukaitis[82] and colleagues reported the feasibility of kidney transplantation in five patients with no recurrence after transplantation.

RENAL TRANSPLANTATION

Timing

Renal transplantation should be postponed six months after institution of dialysis, as limited kidney recovery can occur several months after commencing eculizumab therapy[170,180]. Disappearance of the extrarenal manifestations as well as resolution of TMA hematological parameters are the prerequisite for kidney transplantation. The magnitude of risk of recurrence can be utilized to guide the necessity of anti-complement blockade (Table 2).

Risk of kidney donation

Two risks have been reported to be associated with living-related kidney donation: (1) Recurrent disease in the recipient; and (2) *De novo* disease in the donor, if he/she is a genetic mutation carrier[169]. Any potential donor proved to exhibit alternative pathway dysregulation should be excluded. On the other hand, any potential living-related donor devoid of complement gene abnormalities can be permitted[113]. "Liver transplantation" may be reserved for patients with liver-derived complement protein aberrations, particularly in patients poorly responding to complement blockade[181].

Future therapy

The following future therapeutic agents have been addressed: (1) Purified products of the deficient genes; and (2) C3 convertase inhibitors[182].

Research targets

The following agents are under investigation: (1) The anti-C3b blocker, compstatin analog Cp40[183]; and (2) The anti-C3 convertase monoclonal antibodies[184].

CONCLUSION

The impact of TMA, either *de novo* or recurrent, on allograft longevity is underestimated. The spectrum of the culprit genes implicated in the evolution of TMA is currently expanding. Despite the landmark breakthrough of immense efficacy of complement blockade therapy, the outlook of this devastating syndrome remains poor if the diagnosis is delayed. In contrast, the recurrent TMA is much more optimistic if there is timely intervention by complement blockade before permanent damage sets in. More efforts targeting genetic mutation management as well as the advent of early predictors of TMA recurrence are warranted for better disease control and, thereby, better patient and allograft outcome.

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MINIREVIEWS

Early urological complications after kidney transplantation: An overview

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Abstract

Urological complications, especially urine leaks, remain the most common type of surgical complication in the early post-transplant period. Despite major advances in the field of transplantation, a small minority of kidney transplants are still being lost due to urological problems. Many of these complications can be traced back to the time of retrieval and implantation. Serial ultrasound examination of the transplanted graft in the early post-operative period is of key importance for early detection. The prognosis is generally excellent if recognized and managed in a timely fashion. The purpose of this narrative review is to discuss the different presentations, compare various ureterovesical anastomosis techniques and provide a basic overview for the management of post-transplant urological complications.

Key words: Anastomotic leak; Urinoma/s; Postoperative complications; Ureterostomy; Nephrostomy

Core tip: Urological complications, especially urine leaks, remain the most common type of surgical complication following kidney transplantation. Preservation of the peri-ureteric tissue during kidney retrieval, Lich-Gregoir ureteroneocystostomy technique and routine prophylactic ureteral stenting has been shown to decrease the incidence of these complications. Routine post-operative allograft ultrasound is important for their early detection.

The majority of recipients can be effectively managed percutaneously, avoiding the morbidity associated with open surgery. The prognosis is generally excellent if recognized and treated successfully in a timely manner.

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INTRODUCTION

Kidney transplantation remains the best renal replacement modality for most patients with end-stage kidney disease[1]. Yet, as with everything else in the medical field, it is not devoid of risk. The patients who manage to get a kidney transplant in a timely fashion face a constant struggle for successful long-lasting survival. The vast majority of graft failure is attributed to alloimmune-mediated injury, recurrent glomerulonephritis, infections, cardiovascular mortality and malignancy[2,3]. Nonetheless, a number of renal allografts are lost due to urological complications, especially in the early post-transplant period. The purpose of this review is to discuss different presentations and provide an evidence-based management plan for patients who present with such complications.

OUTLINE OF SURGICAL AND UROLOGICAL COMPLICATIONS

Complications in the immediate post-transplant period can be broadly subdivided into vascular, urological, fluid collections and wound healing problems. Vascular complications encompass hemorrhage, thrombosis, aneurysm, dissection and stenosis, while urological complications mainly involve leaks and/or obstruction of the collecting system[4,5]. In essence, hematomas form due to poor tissue handling, insecure knot tying and inadequate hemostasis. The lymphoceles result from severed lymph channels, which should be tied or clipped rather than diathermied, leading to extravasation of lymph. Urine leaks can result in the formation of urinomas. These collections can compress vascular structures or urine outflow, causing transplant dysfunction. In addition, urine leaks are associated with increased risk of surgical site infection, which can lead to peri-nephric abscesses[6,7]. Wound healing complications are generally more common when mammalian target of rapamycin (mTOR)-based immunosuppression is used[8].

Ultrasonography is the first-line imaging modality for graft evaluation in the immediate post-transplant period, especially when suspecting vascular problems, fluid collections and/or obstruction[9,10]. Apart from being non-invasive, it can provide some additional information on the graft function by measuring the intra-renal resistivity indices[11]. Differentiating between different types of collections on ultrasound can be difficult. A urinoma usually appears as a well-defined, rapidly enlarging non-echoic fluid collection without septations, whereas a hematoma usually has a complex and echogenic appearance with numerous septations[9,12]. Computed tomography may assist in the diagnosis by further elucidating the ultrasound findings such as the extent or exact relationship of the fluid collection to the transplanted kidney[10]. ^{99m}TC-MAG-3 radionuclide isotope scan is useful to confirm the presence of a urine leak outside the anatomical space of the urinary tract, as the radionuclide tracer accumulates in the excreted urine as opposed to other types of fluid collections[13]. A cystogram can provide additional information to establish the exact site of urine leak, especially if it is at the ureterovesical junction (Figure 1). Antegrade pyelography performed during nephrostomy tube insertion remains the investigation of choice to identify the exact site and extent of urine leak. Ultrasound and/or computed tomography-guided needle aspiration followed by biochemical and bacteriological analysis is essential in diagnosing the exact etiology of fluid collections[4]. A fluid creatinine well above the serum level indicates a urine leak as opposed to a lymphocele which has levels similar to that of serum. Gram stain and cultures are important because any fluid collection can potentially become infected[6].

RISK FACTORS AND PRESENTATION OF URINE LEAKS

The incidence of urological complications following kidney transplantation as portrayed in early studies (*i.e.*, including patients between 1970-1990s) ranged between 4.2% to 14.1%[14-18], while in later studies (*i.e.*, including patients between 1990-2000), it ranged between 3.7% to 6.0%[19-21]. The incidence of urine leaks described in studies that included patients between the 1990s and 2000 ranged between 1.5% to 6.0%[19-23]. This variability is probably a reflection of the different transplantation era, diagnostic tools and surgical proficiency. Indeed, the incidence of urological complications has been shown to diminish considerably with increasing center experience[24]. These complications are associated with significant patient morbidity, including graft loss and mortality[17,25].

Urine leaks generally present in the immediate or early post-transplant period (3 mo)[26]. Clinical presentation can include pain and swelling in the transplant area, rising creatinine, oliguria and/or signs of systemic infection[27]. In the immediate post-transplant period, urine leaks can manifest *via* the drains or through the wound, leading to delayed healing and increased risk of infection[7,28]. In addition, leaking urine can translocate into the retroperitoneal space, pelvis and occasionally in the pre-sacral and scrotal area[29]. The leaking of infected urine could lead to peri-nephric infections and abscess

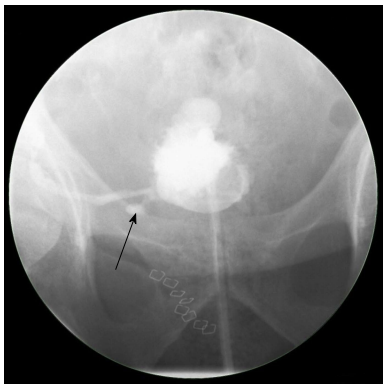

Figure 1 A cystogram showing urinary leak (arrow) at the anastomosis between the newly implanted graft ureter and urinary bladder.

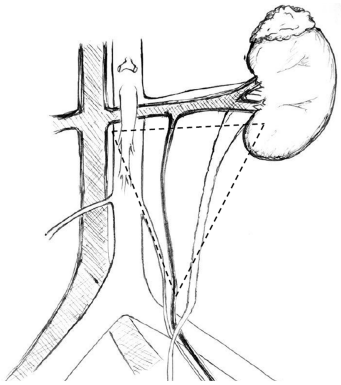

Figure 2 The golden triangle. Bordered by the lower pole of the kidney on the left, the junction between the renal vein and the inferior vena cava on the right and gonadal vein.

formation. This is important considering that urinary tract infections occur in about 23% of patients receiving a kidney transplant[30].

Most urological complications can be traced back to technical errors during retrieval, bench dissection or implantation[28]. The vast majority of leaks occur at the distal portion of the ureter, most commonly at the site of the ureteroneocystostomy[26]. Distal ureteral ischemia and necrosis secondary to compromised blood supply is thought to be the main culprit for early ureteral complications in most patients in the absence of technical difficulties during the transplant operation[31]. In contrast to the native ureters, which derive their blood supply *via* both renal arteries and pelvic collaterals, the transplanted ureter depends solely on the blood supplied by the branches of the renal artery that traverse in peri-ureteric tissues. This area, also known as the "golden triangle" (Figure 2), contains important arterial branches, such as the lower polar artery, which supplies the distal ureter. Indeed, the importance of preserving the peri-ureteral connective tissue in order to prevent disastrous urinary complications is well documented in the literature[14,32-35]. Male donors, male recipients, African American recipients, Taguchi technique, graft arterial reconstruction, multiple renal arteries and recipient diabetes were established as independent risk factors for urinary complications[36-39]. We believe that gentle handling of the ureter and peri-ureteric tissue, and keeping the length of the ureter as short as possible without tension is of key importance. A ureter that appears ischemic after reperfusion should be resected proximally until an adequately perfused area is reached. In this situation, achieving a tension-free urinary anastomosis may require special techniques, such as ipsilateral uretero-ureterostomy (joining the transplant ureter to the native ureter of that side), pyelovesicostomy, psoas hitch, Boari flap or fashioning of an ileal ureter, in that order of priority. In general, the risk of urinary complications following laparoscopic donor nephrectomy has decreased substantially over time, now comparable to open nephrectomy[40].

The ureterovesical anastomosis associated with the lowest rate of complications continues to be a subject of debate. The Leadbetter-Politano technique (Figure 3) was primarily used in the early days of kidney transplantation[41]. This has been largely superseded by the less technically demanding Lich-Gregoir technique (Figure 4)[42]. The Taguchi technique (Figure 5) has been associated with unacceptably higher incidence of complications compared to the Lich-Gregoir technique[43,44]. In a recent meta-analysis, which included two randomized controlled studies and 24 observational studies, the Lich-Gregoir technique was found to significantly reduce the incidence of ureteral leaks when compared to the Leadbetter-Politano and Taguchi techniques[45]. The incidence of ureteral stricture and reflux, however, did not differ significantly. The use of a shorter ureter and the avoidance of a separate cystostomy are two hypothetical advantages over the Leadbetter-Politano technique[46]. A modification of the Lich-Gregoir technique, using a short muscular tunnel over the distal ureter, has been shown to reduce complications in two separate retrospective studies[46,47]. In one Chinese study, primary termino-terminal ipsilateral ureteroureterostomy, was associated with significantly less urinary fistulas when compared to the established Lich-Gregoir technique[23].

Currently, many centers have adopted the routine use of ureteric stent during kidney transplantation. A meta-analysis, which included seven randomized controlled studies, confirmed that routine prophylactic stenting is generally well tolerated and significantly reduces major urological complications[48]. In a recently published Cochrane database systematic review, it was established that 13 transplant recipients need to be treated (with using JJ stent) in order to prevent one major urological complication[48]. Despite some opposition due to the higher incidence of urinary tract infections, current evidence recommends the routine use of prophylactic stenting.

MANAGEMENT OF URINARY LEAKS

In general, one can select between two main approaches

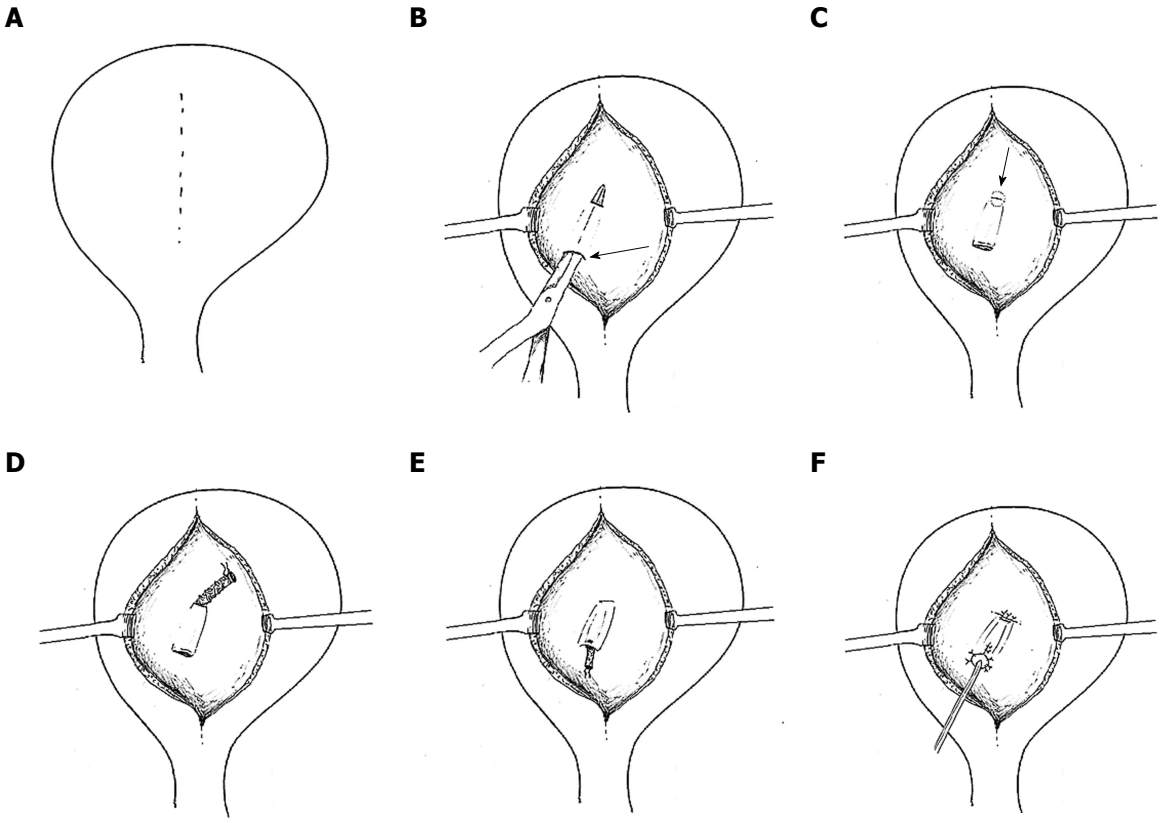

Figure 3 Leadbetter-Politano technique. A: A longitudinal bladder incision is performed to gain access to the interior of the bladder; B: A second cystotomy is done to introduce the neo-ureter in the bladder. Subsequently, an Overholt is inserted from the second cystotomy and tunnelled close to the bladder wall for about 3 cm; C: A new hiatus is created at the end of the tunnel; D: The neo-ureter is pulled through the mucosal tunnel and the new mucosal hiatus using a free suture as a guide rail; E: Closure of the second cystotomy and then sub-mucosal transposition of distal neo-ureter; F: Fixation of the neo-ureter orifice and closure of the bladder mucosa.

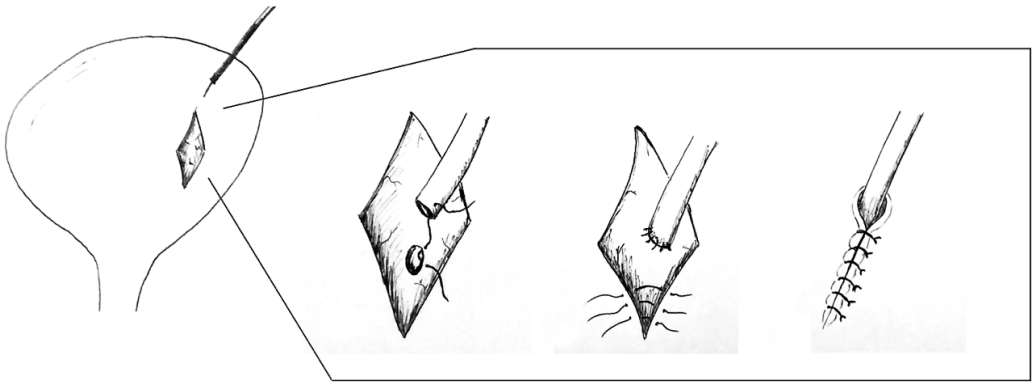

Figure 4 Lich-Gregoir technique. A: Bladder wall incision through the detrusor muscle is performed, leaving a very thin layer of muscle and uroepithelium unbreached; B: The distal part is completely incised to create a neo-ureter-bladder anastomosis; C: Suturing of the neo-ureter is performed *via* the same access used to introduce it into the bladder; D: The ureter is positioned in the groove and in direct contact to the uroepithelium, followed by closure of the muscle over the ureter while carefully avoiding constriction of the neo-ureter.

(conservative *vs* reconstructive surgery) depending on the site, cause and extent of the leak. One has to keep in mind that these treatment strategies are not based on robust scientific evidence and tend to vary between centers based on anecdotal experiences. The current best available evidence is merely based on retrospective studies.

A conservative approach typically involves insertion of a percutaneous nephrostomy followed by antegrade stenting of the collecting system (unless already performed during the transplant operation), together with a Foley catheter replacement. Retrograde stenting of a transplant ureter is technically demanding and often impossible, even by the most skilled urologists, because of the atypical position of the ureteric orifice. Antegrade stenting, although generally easier, can still pose technical challenge in the absence of pelvi-caliceal dilatation. Interventional radiologists and transplant surgeons

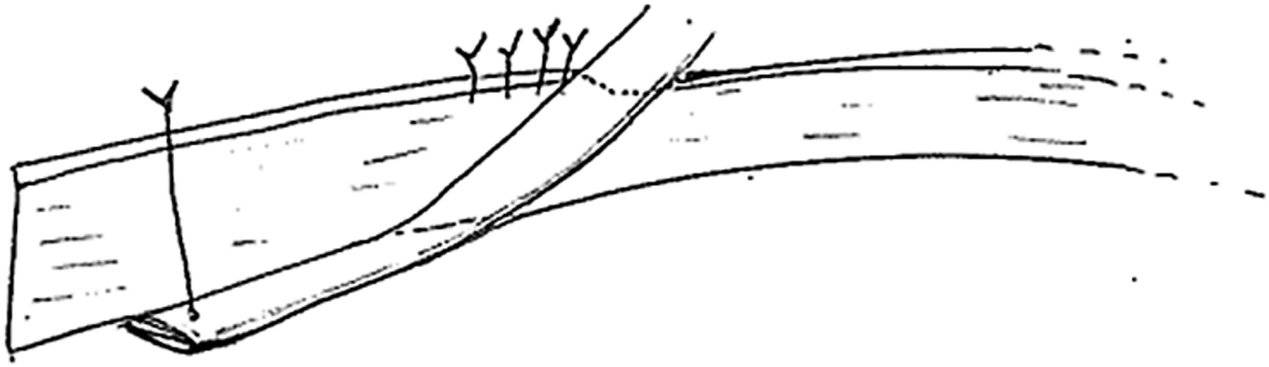

Figure 5 Taguchi technique. A suture is positioned at the distal end of the neo-ureter and subsequently introduced in the bladder *via* a cystotomy. The neo-ureter is later fixed to the bladder wall by bringing the suture out through the bladder wall and closed.

can work together to manage difficult cases[49]. This procedure diverts the urinary flow away from the leaking site and, thereby, fully decompresses the collecting system in order to allow for healing to take place. The Foley catheter is usually removed once the leak has resolved. Many centers report stent deployment for a period of 6-12 wk[14,33,35,46]. The presence of recurrent urinary tract infection may hasten the time for stent removal.

Surgical exploration is required if the urine leak fails to resolve following maximal decompression, especially when dealing with major urine extravasations or necrotic ureters. During the surgical procedure, the necrotic ureter should be resected proximally until healthy tissue is reached, followed by re-implantation. If the remaining viable ureter is short, an ipsilateral uretero-ureterostomy, pyelovesicostomy, psoas hitch, Boari flap or fashioning of an ileal ureter are alternative techniques that could be employed for tension-free ureteric anastomosis[50]. A psoas hitch (Figure 6) involves extensive dissection and mobilization of the urinary bladder to allow mobilization towards the transplant ureter, usually up to 5 cm. Subsequently, the bladder is anchored to the ipsilateral psoas muscle. Alternatively, a Boari flap (Figure 7) can be fashioned to attain an additional 10 cm. If required, this can be used in conjunction with the psoas hitch technique to bridge larger gaps between the short transplant ureter and the bladder. Contracted or atrophic urinary bladders in anuric patients seriously limit these options. In this circumstance, an ipsilateral uretero-ureterostomy can be an alternative option if the cause of native kidney failure was not reflux disease. A pyelovesicostomy or an ileal ureter can be fashioned, the latter being preferred for larger gaps, in situations where no donor or recipient ureter can be salvaged[51]. Both these techniques are devoid of an anti-reflux mechanism. In all cases, serial ultrasound examinations together with close monitoring of the transplant excretory function is of chief importance to anticipate any secondary ureteral strictures.

Traditionally, urine leaks have been corrected by open reconstruction. Over the last two decades, advances in interventional radiology have allowed several patients to be effectively managed percutaneously, avoiding the morbidity associated with open surgery[49,52]. This conservative approach has been shown to be successful in a number of retrospective studies, with a success rate varying between 30% and 87%[19,21,53-55]. This considerable inter-center variability is probably related to different baseline characteristics. We believe that the outcome largely depends on the etiology, site and extent of the urine leak. In general, small leaks at the ureter implantation site tend to do well with conservative management, while extensive leaks, especially if related to ureter necrosis, do better with open surgery. When in doubt, we treat conservatively in the first instance and then proceed to surgical reconstruction only if the patient fails to respond. The type of surgery is frequently dictated by the intra-operative findings and the overall state of the patient. Surgical reconstruction is usually successful in the majority of cases[19,21,23,55]. Nonetheless, some patients required more than one surgical procedure for complete resolution[23].

LIMITATION

This narrative review is intended to provide a general overview of the early urological complications after kidney transplantation. Although we performed an extensive literature search, this review lacks the scientific rigor of article selection found in a systematic review, and is therefore susceptible to selection bias. In addition, the selected articles have not been subjected to quality evaluation.

CONCLUSION

Urological complications, especially urine leaks, remain the most common type of surgical complication following kidney transplantation. The preservation of peri-ureteric tissue during kidney retrieval, employing the Lich-Gregoir ureteroneocystostomy technique and routine prophylactic ureteral stenting, have been associated with lower incidence of such complications. Serial ultrasound examination of the transplanted graft in the early post-operative period is of key importance for early detection of these potential complications. The first line

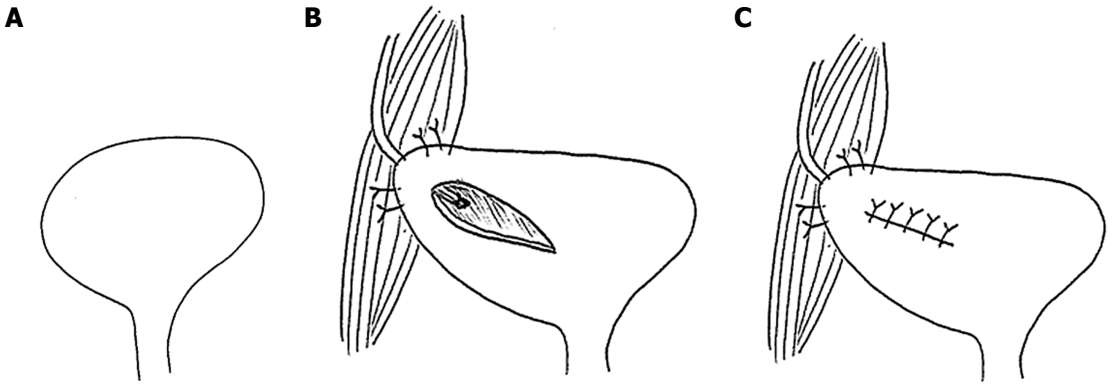

Figure 6 Psoas hitch. A: A psoas hitch procedure is used to bridge the gap between the urinary bladder and a short ureter; B: Mobilization of the urinary bladder is achieved by dissecting the attachments of the urinary bladder, which is subsequently hitched to the Psoas muscle; C: Ureter implantation is performed *via* a transverse incision, which is later closed.

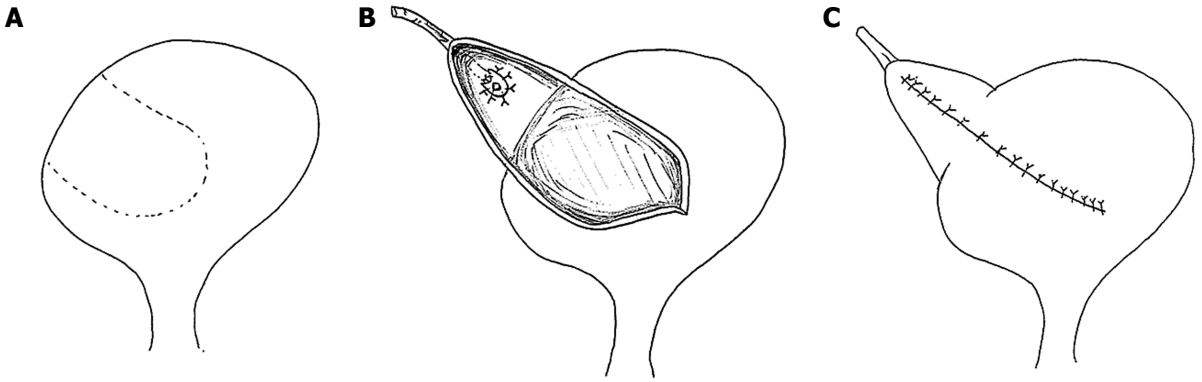

Figure 7 Boari flap. A: A Boari flap is used when a Psoas hitch is not enough to bridge the gap between the bladder and a short ureter to allow for a tension-free anastomosis. A U-shaped flap composed of all tissue layers is created. The base should be proportional to the length of the flap to avoid ischemia; B: The ureter is implanted to the apex of the flap *via* end-to-end anastomosis or a sub-mucosal tunnel; C: The bladder incision together with the flap are subsequently closed.

management of urine leaks is usually percutaneous urinary decompression. Failing this approach, surgical intervention is usually required, especially if dealing with major leaks or necrotic ureters. Although urological complications are associated with significant morbidity and occasionally mortality, the prognosis is generally excellent if recognized and treated successfully in a timely manner.

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MINIREVIEWS

Introduction of everolimus in kidney transplant recipients at a late posttransplant stage

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Abstract

This minireview focuses on the current knowledge about the introduction of everolimus (EVL), a mammalian target of rapamycin inhibitor, with calcineurin inhibitor (CNI) elimination or minimization in kidney transplant recipients at a late posttransplant stage. Within, we have summarized two major clinical trials, ASCERTAIN and APOLLO, and seven other retrospective or nonrandomized studies. In the open-label multicenter ASCERTAIN study, the estimated glomerular filtration rate (eGFR) at 24 mo after conversion was not significantly different between three groups-EVL with CNI elimination, CNI minimization and continued CNI unchanged-at a mean of 5.4 years after transplantation. However, recipients with baseline creatinine clearance higher than 50 mL/min had a greater increase in measured GFR after CNI elimination. In the open-label multicenter APOLLO study, adjusted eGFR within the on-treatment population was significantly higher in the EVL continuation group than in the CNI continuation group at 12 mo after conversion at a mean of 7 years posttransplantation. Other studies on recipients without adverse events and already having satisfactory renal function showed favorable graft function by EVL late-induction with CNI elimination or reduction. These studies showed that chronic allograft nephropathy, CNI nephrotoxicity, CNI arteriolopathy, cancer and viral infection (especially cytomegalovirus infection) may be good indications for late conversion to EVL.

Key words: Kidney transplantation; Everolimus; mTOR inhibitor; Late conversion; Calcineurine inhibitor

Core tip: Current immunosuppressive protocols consisting of calcineurin inhibitors (CNIs) and mycophenolate mofetil have improved short-term graft survival. However, improvements in long-term graft survival are restricted by nephrotoxicity associated with CNI. Everolimus is an exceedingly useful immunosuppressant for kidney transplant recipients when administered in combination with low-dose CNIs or with elimination of CNIs. Here, we summarize the current knowledge about the introduction of everolimus with CNI elimination or minimization in

kidney transplant recipients at late posttransplant stage.

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INTRODUCTION

Excellent short-to medium-term graft survival has been achieved in kidney transplantation owing to the low acute rejection rate of calcineurin inhibitor (CNI), cyclosporine (CsA) and tacrolimus (Tac)-based immunosuppressive therapies[1]. Therefore, the next step is to determine how to improve long-term graft and patient survival rates. CNIs are known to induce nephrotoxicity, malignancies and cardiovascular diseases and to promote interstitial fibrosis/tubular atrophy[2-5], strongly influencing long-term graft and patient survival. Thus, efforts to reduce CNI exposure have become extremely valuable.

Everolimus (EVL) is an inhibitor of the mammalian target of rapamycin (mTOR), an evolutionarily conserved serine/threonine kinase playing an important role in the regulation of many cellular functions, which include metabolism, growth, proliferation, survival and memory[6]. EVL binds to the cytosolic FK-binding protein (FKBP)-12. The resulting complex then binds with high affinity to the FKBP12-rapamycin binding domain of mTOR, which inhibits mTOR activity, resulting in the inhibition of B cell and T cell proliferation, angiogenesis and cell metabolism[7,8]. EVL exhibits little nephrotoxicity and pleiotropic effects, such as antiproliferative[9], anti-neoplastic[10], antiviral[11] and antiatherosclerotic[12] properties. Therefore, it can be speculated that EVL is an exceedingly useful immunosuppressant for kidney transplant recipients in combination with low-dose or elimination of CNIs.

In the *de novo* use of EVL with low-dose CsA study (A2309) - a 24-mo randomized controlled study that compared EVL plus low-dose CsA against mycophenolate mofetil (MMF) plus standard-dose CsA in 833 kidney transplant recipients - the two treatment groups showed comparable graft function[13]. Meta-analysis of the CNI-sparing regimen in kidney transplantation showed an increase in graft failure rate associated with the combined use of mTOR inhibitors (mTORi) and mycophenolate, although improved graft function was noted among those surviving with functioning grafts[14].

In the early conversion of CNI to EVL study (ZEUS[15]), kidney transplant recipients were randomized at 4.5 mo for either conversion to EVL or continuance of CsA, and a higher estimated glomerular filtration rate (eGFR) was observed in the EVL group at year 3. However, the biopsy-proven acute rejection (BPAR) rate was 13.0% in the recipients who converted to EVL and 4.8% in the recipients who continued CsA (P = 0.015), although a statistically significant difference was not associated with long-term graft loss. In addition, the discontinuation rate of the EVL group was high (28.4%).

In a recent open-label, 24-mo study (the ELEVATE trial[16]), 715 kidney transplant recipients were randomized for either conversion to EVL or continuance of CNI at 10-14 wk after kidney transplantation. As a result, eGFR was comparable between the two groups, but the BPAR and discontinuation rates were higher in the EVL group (9.7% *vs* 4.8%, P = 0.014). Subsequently, some studies have been undertaken to explore the benefits of delayed introduction of EVL following initial CNI therapy in kidney transplantation (Tables 1 and 2). Possible pros and cons of late conversion to EVL with CNI elimination or minimization are shown in Table 3.

The aim of this minireview was to summarize the current knowledge on the introduction of EVL in kidney transplant recipients at a late posttransplant stage.

GRAFT FUNCTION

Only two major clinical trials are available for the introduction of EVL in kidney transplant recipients at a late posttransplant stage, namely the ASCERTAIN[17] and APOLLO[18] trials (Table 1). In the open-label multicenter ASCERTAIN study, kidney transplant recipients receiving CNI were randomized to EVL with CNI elimination (n = 127), CNI minimization (n = 144) and continuation of CNI unchanged (controls, n = 123) at a mean of 5.4 years after transplantation. The eGFR at 24 mo was not significantly different among the three groups. However, recipients with baseline creatinine clearance higher than 50 mL/min had a greater increase in measured GFR after CNI elimination.

In the open-label multicenter APOLLO study, kidney transplant recipients were randomized to EVL with CNI elimination (n = 46) or for remaining on standard CNI-based immunosuppression (controls; n = 47) at a mean of 7 years after transplantation. Within the on-treatment population, adjusted eGFR was significantly higher in the EVL continuation group than in the CNI continuation group at 12 mo after conversion. In addition, the 5-year follow-up results showed that eGFR in the EVL continuation group was significantly higher, by 11 mL/min·1.73 m^2 (P = 0.031), in recipients who remained on their randomized study regimen until 60 mo[19].

Other studies[20-26] have shown that favorable graft function was sustained by EVL late-induction with CNI elimination or reduction (Table 2). Our previous study[24] demonstrated that eGFR was significantly improved in stable kidney transplant recipients already having favorable renal function, after remaining on EVL treatment for 12 mo after conversion. As a histological assessment, Chow *et al*[22] demonstrated that EVL rescue therapy and CNI inhibitor minimization strategy slowed down the disease progression by reducing the tubular atrophy and interstitial fibrosis score in renal transplant recipients with biopsy-confirmed chronic

Table 1 Summary of late everolimus conversion clinical trials

Ref.	No. of subjects/ follow-up	EVL treatment	Groups	Outcomes
ASCERTAIN[17] (2011)	394/2 yr	Conversion to EVL with CNI elimination or minimization at mean of 5.6 yr	Gp 1: CNI elimination (EVL C0, 8-12 ng/mL), n = 127 Gp 2: CNI minimization (EVL C0, 3-8 ng/mL and CNI reduced to 80%-90% below baseline), n = 144 Gp 3: control (CsA C2, > 400 ng/mL; Tac C0, > 4 ng/mL), n = 123	Graft survival: 96.9%, 94.6%, 95.1% (P = NS) Patient survival: 97.6%, 97.1%, 100% (P = NS) Comparable eGFR in 3 groups; recipients with baseline CrCl > 50 mL/min had greater increase in measured GFR after CNI elimination Adverse events resulted in discontinuation: 28.3%, 16.7%, 4.1% (Gp 1 *vs* GP 3, P < 0.001; Gp 2 *vs* Gp 3, P = 0.020)
APOLLO[18] (2015)	93/1 yr	Conversion from CNI to EVL at mean of 7 yr	Gp 1: CNI elimination (EVL C0, 6-10 ng/mL), n = 46 Gp 2: control (CsA C0, 80-150 ng/mL; Tac C0, 5-10 ng/mL), n = 47	Graft survival: 100%, 100% Patient survival: 97.8%, 97.9% (P = NS) Adjusted eGFR was significantly higher in Gp 1 within on-treatment population Adverse events resulted in discontinuation: 32.6%, 10.6% (P < 0.01)

C0: Zero hour blood level; CNI: Calcineurin inhibitor; CrCl: Creatinine clearance; CsA: Cyclosporine; eGFR: Estimated glomerular filtration rate; EVL: Everolimus; Gp: Group; No.: Number; NS: Not significant; Tac: Tacrolimus.

Table 2 Summary of retrospective or nonrandomized studies for late everolimus conversion

Ref.	No. of subjects/ follow-up	EVL treatment	Outcomes
Morales *et al*[20] (2007)/ retrospective	8/1-16 mo	Conversion to EVL with CNI elimination or reduction at mean of 5 yr	CrCl increased by 42% in recipients with CAN (grade 1 or 2) and CNI nephrotoxicity (P = 0.017)
Sanchez-Fructuoso *et al*[21] (2012)/ retrospective	220/1 yr	Conversion from CNI to EVL at mean of 69.4 mo	CrCl increased in recipients with baseline CrCl ⩾ 40 mL/min and baseline proteinuria < 550 mg/d (P = 0.005) Median proteinuria increased from 304 mg/d to 458 mg/d (P < 0.001) EVL discontinuation rate was 24%
Chow *et al*[22] (2015)/ open-label, single arm	17/1 yr	Conversion to EVL with CNI minimization in recipients with CAN at mean of 4.2 yr	Mean slope of eGFR was - 4.31 mL/min/1.73 m^2 per yr before conversion, as compared with 1.29 mL/min/1.73 m^2 per yr at 12 mo after conversion (P = 0.036) Renal biopsy showed significant decrease of tubular atrophy (15.7% *vs* 7.1%, P = 0.005) and interstitial fibrosis (14.8% *vs* 7.2%, P = 0.013)
Miura *et al*[23] (2015)/ retrospective	13/1 yr	Conversion to EVL with Tac reduction in recipients with CNIA at mean of 43 mo	aah scores improved in 5 recipients (38%); No improvement was observed in recipients with aah3; No deterioration was observed. eGFR improved from 44.3 mL/min/1.73 m^2 to 49.8 mL/min/1.73 m^2 (P < 0.01).
Uchida *et al*[24] (2016)/ retrospective (our report)	26/1 yr	Conversion from antimetabolites (MMF or MZ) to EVL with CNI minimization at mean of 39.5 mo	eGFR significantly increased from 50.7 mL/min/1.73 m^2 to 53.6 mL/min/1.73 m^2 in the EVL continuation group EVL discontinuation rate was 42.3%
Nojima *et al*[25] (2017)/ retrospective	56/1 yr	Conversion to EVL with CNI reduction in recipients with CNI nephrotoxicity or IF/TA at mean of 7.4 yr	eGFR increased by 7% (P < 0.005) EVL discontinuation rate was 11%
Nanmoku *et al*[26] (2017)/ nonrandomized	86/ 1 yr	Conversion to EVL with Tac minimization, MMF reduction and steroid withdrawal in cases of complications such as diabetes, viral infection *etc*	Conventional group (n = 50); EVL group (n = 36) Biopsy-proven acute rejection rate exhibited no significant difference between these groups (12% *vs* 17%, P = 0.55) Serum creatinine significantly improved in the EVL group (P = 0.031) EVL discontinuation rate was 13.8%

CAN: Chronic allograft nephropathy; CNI: Calcineurin inhibitor; CNIA: Calcineurin inhibitor arteriolopathy; CrCl: Creatinine clearance; eGFR: Estimated glomerular filtration rate; EVL: Everolimus; IF/TA: Interstitial fibrosis/tubular atrophy; MMF: Mycophenolate mofetil; MZ: Mizoribine; No.: Number; Tac: Tacrolimus.

allograft nephropathy. Miura *et al*[23] reported that Tac reduction with EVL addition histologically improved CNI arteriolopathy in 5 out of 9 selected recipients, whose alternate quantitative scoring for hyaline arteriolar

Table 3 Pros and cons of late conversion to everolimus with calcineurin inhibitor elimination or minimization in kidney transplant recipients

Advantage	Disadvantage
Due to EVL introduction	Due to EVL introduction
Antitumoral effect (especially on nonmelanoma skin carcinoma)	Adverse events (gastrointestinal disorders, hyperlipidemia, interstitial pneumonitis, edema, mouth ulcers, proteinuria, impaired wound healing, hematotoxicity and so on)
Antiviral effect (especially on CMV and BKV infection)	
Antiproliferative effect	
Antiatherosclerotic effect	
Due to CNI elimination or minimization	Due to CNI elimination or minimization
Favorable graft function	Risk of *de novo* DSA

BKV: BK virus; CMV: Cytomegalovirus; CNI: Calcineurin inhibitor; DSA: Donor-specific HLA antibodies; EVL: Everolimus.

thickening (aah scores) was under 3.

REJECTION

There was no significant difference in the number of BPAR episodes between the intervention group and the control group in both the ASCERTAIN and APOLLO studies. It was reported that EVL-based immunosuppression in early conversion from CNI was associated with an increased risk of developing donor-specific HLA antibodies (DSA) and antibody-mediated rejection[27]. In contrast, late conversion to CNI-free therapy with mTORi did not appear to affect the risk of *de novo* DSA[28], but there is concern about the development of DSA and antibody-mediated rejection because CNI level variability is a strong risk factor for *de novo* DSA development and death-censored graft loss[29].

ADVERSE EVENTS

Generally, mTORi administration has been associated with several adverse events, such as gastrointestinal disorders, hyperlipidemia, interstitial pneumonitis, edema, mouth ulcers, proteinuria, impaired wound healing, hematotoxicity and so on[7]. It was reported that adverse events of mTORi accounted for 20%-40% of the drop-out rate in a clinical phase Ⅲ trial[30]. In the late conversion to EVL studies, the discontinuation of EVL treatment due to adverse events occurred at about the same rate (approximately 30%). In our report[24], the discontinuation rate of EVL treatment was relatively high, at 42.3%.

The common adverse events leading to discontinuation have been aphthous stomatitis, pneumonitis, progressive renal deterioration and proteinuria. Proteinuria is a well-known prognostic factor for graft and patient survival rates in kidney transplantation[31]. Sanchez-Fructuoso *et al*[21] reported that risk factors for the development of proteinuria ⩾ 900 mg/d at 1 year after late conversion were creatinine clearance of < 60 mL/min, serum triglycerides of ⩾ 150 mg/d, no treatment with steroid, baseline proteinuria of ⩾ 550 mg/d and conversion at ⩾ 3 years after transplantation. An interaction was observed between baseline proteinuria and time to conversion, and the authors concluded that the success of EVL conversion with CNI elimination depended on not making so late conversions and not converting recipients with high baseline proteinuria. On the other hand, Nojima *et al*[25] demonstrated that late immunosuppression conversion, at > 3 years after kidney transplantation, using EVL in addition to a reduction in CNI dose safely and significantly improved graft function.

MALIGNANCIES

Kidney transplant recipients late-converted to sirolimus-based, CNI-free immunotherapy had a lower risk of malignancies at 2 years postconversion, with a high degree of heterogeneity attributed in the CONVERT trial[32]. The reduction was driven by a significant reduction in nonmelanoma skin carcinoma rate ($P < 0.001$), while the rate of all other malignancies was numerically lower, although without statistical significance ($P = 0.058$). It has been reported that switching from CNIs to sirolimus had an antitumoral effect among kidney transplant recipients with previous nonmelanoma skin carcinoma[33]. In the cases of late EVL conversion, however, the ASCERTAIN study[17] showed that the incidence rates of malignancies were 7.1%, 7.6% and 5.7%, respectively in the CNI elimination, CNI minimization and control groups at 2 years after EVL conversion.

CAUSE OF LATE CONVERSION TO EVL

Chronic allograft nephropathy, CNI nephrotoxicity and CNI arteriolopathy may be good indications for late conversion to EVL[20-23,25]. Furthermore, cancer is one of the main indications for late conversion to EVL[20,21]. As mentioned in the above section on "malignancies", there is no evidence to date for the superiority of EVL in suppressing malignancies at late conversion. However, Lim *et al*[34] published that *de novo* use of EVL with reduced exposure to CNIs may enable a reduction in malignancy burden after transplantation.

Viral infection is also an indication for late conversion to EVL. It is well known that kidney transplant recipients receiving mTORi have a lower risk of developing cytomegalovirus (CMV) infection[35]. Furthermore, cases with ganciclovir-resistant cytomegalovirus infection have been reported to be cured after switching to mTORi[36].

Kidney transplant recipients who have BK virus infection may benefit from conversion to mTORi[35]. Polanco *et al*[37] reported a recent prospective study of 15 recipients with BK virus-associated nephropathy. As a result, MMF elimination and conversion from Tac to EVL occurred in 9 recipients (60%), and 6 (67%) of the 9 recipients had improvement and 3 maintained stable renal function. In addition, BK viremia cleared in 5 (56%) of the recipients and decreased more than 95% in the remaining 4. With respect to Epstein-Barr virus infection, there is lack of evidence on whether the use of mTORi reduces the risk of infection in solid organ transplant recipients[35].

ABO-INCOMPATIBLE KIDNEY TRANSPLANTATION

Only two short-term pilot studies have been published about the introduction of EVL in ABO-incompatible kidney transplant recipients at a late posttransplant stage[38,39]. In our study, 16 stable ABO-incompatible kidney transplant recipients were switched from MMF to EVL with CNI minimization. Our results showed that conversion to EVL with CNI minimization for 3 mo did not induce acute rejection and C4d deposition in all recipients, and the mean eGFR value significantly increased at 3 mo after conversion compared to baseline[38]. In another study, 7 stable ABO-incompatible kidney transplant recipients were converted from mycophenolate acid to EVL at a late posttransplant phase because of active BK virus replication, and then compared with a reference group of 14 ABO-incompatible patients receiving standard Tac and mycophenolate acid[39]. Conversion from mycophenolate acid to EVL decreased the BK viral load in 5 patients. Thus, this study demonstrated that ABO-incompatible kidney transplant recipients with an active BK virus infection may benefit from conversion to EVL[39].

CONCLUSION

In this minireview, we summarized reports published on the introduction of EVL in kidney transplant recipients at a late posttransplant stage. Selected recipients, who can continue EVL treatment without adverse events and who already have satisfactory renal function, may profit by late conversion to EVL with CNI elimination or minimization. In addition, chronic allograft nephropathy, CNI nephrotoxicity, CNI arteriolopathy, cancer and viral infection (especially cytomegalovirus infection) may be good indications for late conversion to EVL.

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ORIGINAL ARTICLE

Basic Study

Interaction of immunosuppressants with HCV antivirals daclatasvir and asunaprevir: combined effects with mycophenolic acid

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Abstract

AIM

To investigate the specific effects of immunosuppressants on the antiviral action of daclatasvir and asunaprevir.

METHODS

The antiviral activity of daclatasvir (DCV) and asunaprevir (ASV) combined with immunosuppressants was tested using two *in vitro* models for hepatitis C virus (HCV) infection.

RESULTS

Tacrolimus, rapamycin and cyclosporine did not negatively affect the antiviral action of DCV or ASV. Mycophenolic acid (MPA) showed additive antiviral effects combined with these direct acting antivirals (DAAs). MPA induces interferon-stimulated genes (ISGs) and is a potent GTP synthesis inhibitor. DCV or ASV did not induce ISGs expression nor affected ISG induction by MPA. Rather, the combined antiviral effect of MPA with DCV and ASV was partly mediated *via* inhibition of GTP synthesis.

CONCLUSION

Immunosuppressants do not negatively affect the antiviral activity of DAAs. MPA has additive effect on the antiviral action of DCV and ASV. This combined benefit needs to

be confirmed in prospective clinical trials.

Key words: Immunosuppressant; Hepatitis C; Daclatasvir; Asunaprevir; Liver; Transplantation

Core tip: Since 2013, several new generation direct acting antivirals (DAAs) have been approved for the treatment of hepatitis C virus (HCV), including daclatasvir (DCV) and asunaprevir (ASV). Although a few reports investigated the effectivity of DAAs after liver transplantation, the effects of specific immunosuppressants on the antiviral efficacy remain largely unknown. We investigated the effect of the immunosuppressants on the antiviral action of DCV and ASV in two *in vitro* models for HCV. We observed that none of the immunosuppressants negatively affected the antiviral activity of these DAAs, and that mycophenolic acid has an additive effect on their antiviral action.

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INTRODUCTION

Liver disease caused by chronic hepatitis C virus (HCV) infection is still the major indication for liver transplantation worldwide. Factors that contribute to the recurrence of HCV after transplantation include viral factors (*e.g.*, HCV RNA levels at the time of transplantation and HCV genotype), host factors (immune response and HCV cryoglobulinemia), and the use of immunosuppressive medication[1].

Glucocorticosteroids like prednisolone are commonly used as immunosuppressant, both as an induction agent to prevent acute rejection and as maintenance immunosuppressive therapy. Some clinical observations suggest that steroid boluses used to treat acute rejection are associated with an increase in HCV viral load and with severity of HCV recurrence. However, no direct effect of prednisolone on HCV replication could be demonstrated *in vitro*. We have previously shown that prednisolone does not affect the action of direct-acting antivirals against hepatitis C, but that it acts on the antiviral function of plasmacytoid dendritic cells by inhibiting the production of interferon-alpha[2,3].

Calcineurin inhibitors (CNIs) are the most widely prescribed immunosuppressants after liver transplantation. Cyclosporine A (CSA) and tacrolimus (TAC) form complexes with immunophilins, resulting in the inhibition of the activity of calcineurin[4]. CSA can inhibit HCV replication *in vitro* by blocking the activity of cyclophilins that interact with viral protein NS5B[5,6]. The antiviral action of CSA is independent of calcineurin signaling[7]. CSA also has a broad antiviral activity against Influenza A and B viruses[8]. TAC has no effect on HCV replication[9,10].

Mycophenolic acid (MPA), the active form of mycophenolate mofetil (MMF) is a non-competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH). This protein, in particular the isoform IMPDH2, is crucial for the *de novo* synthesis of guanosine nucleotides. Next to its immunosuppressive properties, MPA has potent and broad anti-viral activity: replication of rotavirus, influenza, and hepatitis E virus[11-13], as well as of the Flaviviridae Yellow Fever, West Nile virus, Zika virus and HCV is inhibited by MPA[5,14,15]. The antiviral action of MPA against HCV is partially dependent on the inhibition of IMPDH, but also on the increased expression of antiviral interferon stimulated genes (ISGs) caused by MPA[16].

Until recently, the standard therapy for recurrent HCV infection after transplantation was the combination of pegylated interferon alpha and ribavirin. However, the sustained virological response (SVR) rates were limited between 17% to 45%[17]. The development of direct acting antivirals (DAAs) has led to profound changes in the treatment of HCV. Since 2013, several new generation DAAs have been approved for the treatment of HCV. These include the pan-genotypic NS5A inhibitor daclatasvir (DCV) and the NS3/4A protease inhibitor asunaprevir (ASV)[18,19]. Daclatasvir was approved by the EMA in 2014 and by the FDA in 2015 for treatment of HCV infected individuals. Both drugs were approved by the Japanese Ministry of Health for the treatment of HCV in July 2014. The combination of DCV and ASV was the first combination of DAAs approved for use in Korea in 2015, and in 2017 the combination of DCV and ASV was approved for the treatment of HCV genotype 1 in China[20,21]. The prevalence of HCV infection in Japan, Korea and China is 1.3%, 1.5% and 0.8% respectively, affecting the lives of millions of people[22]. In 2017, a Japanese multicenter study was published about the use of ASV and DSV for recurrence of HCV after liver transplantation, where an SVR12 rate of 80.3% was achieved[23]. According to the authors this SVR rate was unsatisfactory, and indeed in other patient studies in the pre-transplant setting higher SVR rates were reported[21,24,25]. A meta-analysis of 41 studies showed a pooled SVR rate of 89.9% for HCV genotype 1[26]. Although some drug-drug interactions were reported on the pharmacokinetics of DAAs and immunosuppressants[27-32], the potential interference of immunosuppressants with the antiviral activity of DAAs post-transplantation is largely unknown. The aim of our study is to investigate the antiviral action of DCV and ASV in the presence of several different classes of immunosuppressants, using *in vitro* model systems for HCV replication.

MATERIALS AND METHODS

Reagents and cell culture media

Daclatasvir (DCV) and asunaprevir (ASV) were kindly

provided by Bristol-Meyers Squibb (New York, NY, United States). MPA and guanosine were obtained from Sigma (Sigma-Aldrich Chemie, Zwijndrecht, the Netherlands). TAC and CSA were from Abcam (Cambridge, MA, United States). RAPA was obtained from Merck (Amsterdam, the Netherlands). Beetle luciferin potassium salt was from Promega (Promega Benelux BV, Leiden, the Netherlands). All cell lines were cultured in DMEM (Lonza Benelux, Breda, the Netherlands), with 10% fetal calf serum (Sigma-Aldrich Chemie), 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 U/mL streptomycin. Huh7-ETluc cells were cultured in the presence of 500 μg/mL G418 (Life Technologies Europe BV, Bleiswijk, the Netherlands).

HCV quantification

The human hepatoma cell line Huh7-ETluc, stably transduced with the HCV bi-cistronic replicon (I389/NS3-3V/LucUbiNeo-ET) containing the nonstructural coding sequences of HCV and the luciferase gene, was used as a model for HCV replication[27]. Huh7-ETluc cells were seeded in white walled, clear bottom 96-well plates (Cellstar, Greiner Bio-one, Alphen a/d Rijn, the Netherlands) at a density of 50000-100000 cells per well. After 16 h the compounds were added in triplicate wells. Cells incubated with vehicle (DMSO) were used as a control. DCV (0.001, 0.01 and 0.1 nmol/L) and ASV (0.1, 1 and 10 nmol/L) were combined with rapamycin (10, 100 and 1000 nmol/L), tacrolimus (0.1, 0.5 and 5.0 μg/mL), cyclosporine A (0.1, 0.5 and 5.0 μg/mL) or MPA (0.1, 0.5 and 5.0 μg/mL). Guanosine (50 μmol/mL) was added to cultures with 0.1 nmol/L DCV and 10 nmol/L ASV in the presence or absence of 5.0 μg/mL MPA to investigate the involvement of the IMPDH pathway on the antiviral action of these compounds. After 24 h luciferase activity was measured. 10 mmol/L Beetle luciferin was added to the cultures and after 30 min luminescence was measured using a Lumistar Optima luminometer. The HCV luciferase activity was calculated as a percentage of the control wells. Huh7 cells stably transduced with a lentiviral vector continuously expressing firefly luciferase (Huh7-PGK-luc) were used as a control to assess non-specific effects of the compounds on luciferase activity and cell growth.

Huh7 cells harboring the full-length JFH-1 derived viral genome were used as an infectious HCV model[28]. 24h after infection the cells were treated with DCV (0.01 and 0.1 nmol/L) and ASV (1 and 10 nmol/L), in combination with 0.5 μg/mL CSA, 5 μg/mL MPA or 5 μg/mL MPA with 50 μmol/mL guanosine. After 48h the cells were lysed, RNA was isolated (Macherey-Nagel Nucleospin RNA kit, Bioké, Leiden, the Netherlands) and quantified using a Nanodrop ND-1000 (Wilmington, DE, United States). cDNA was synthesized using the Primescript RT Master Mix from Takara (Westburg, Leusden, the Netherlands). The levels of HCV-IRES, with GAPDH as a reference gene, were quantified by Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) method using SYBR green (SYBR Select Master Mix, Life Technologies). The relative expression of HCV-IRES (normalized for GAPDH) was calculated as a percentage of the HCV expression in cells that were treated with vehicle only.

Expression of interferon stimulated genes

Naïve Huh7 cells were cultured in the presence of 5 μg/mL MPA in combination with 0.1 nmol/L DCV or 10 nmol/L ASV. DMSO was used as a vehicle control. After 48 h RNA was isolated and quantified and cDNA was synthesized. The levels of Interferon regulatory factor 1 (IRF1), Interferon regulatory factor 9 (IRF9), and Interferon-induced transmembrane protein 3 (IFITM3), with GAPDH as a reference gene, were quantified with RT-qPCR using SYBR green.

RT-qPCR analysis

RT-qPCR was performed using the StepOnePlus Real-Time PCR System from Applied Biosystems (Fisher Scientific, Landsmeer, the Netherlands). All reactions were performed in duplicate, 40 cycles of 15′ at 95 ℃, 15′ at 58 ℃ and 1 min at 72 ℃, followed by a meltcurve. Primer sequences: IRF1 forward 5-TGCCTCCTGGGAAGATG-3, reverse 5-CCTGGGATTGGTGTTATG-3, IRF9 forward 5-CAAGTGGAGAGTGGGCAGTT-3, reverse 5-ATGGCATCCTCTTCCTCCTT-3, IFITM3 forward 5-CTGGGCTTCATAGCATTCGCCT-3, reverse 5-AGATGTTCAGGCACTTGGCGG-3, IRES forward 5-GTCTAGCCATGGCGTTAGTATGAG-3, reverse 5-ACCCTATCGGCAGACCACAAG-3, GAPDH forward 5-AGAAGGCTGGGGCTCATTTG-3, reverse 5-AGGGGCCATCCACAGTCTTC-3.

Statistical analysis

All luciferase assays were performed in triplicate and repeated in at least three independent experiments. RT-qPCR analyses were performed in duplicate and repeated in at least two independent experiments. Statistical analysis was performed using GraphPad Prism version 5.01 (Graphpad Software, Inc., La Jolla, California, United States). All data are presented as a mean ± SE. We used a non-parametric Mann-Whitney test (two-tailed, 95%CI) to evaluate the significance of our date. A *P*-value < 0.05 was considered statistically significant.

RESULTS

Antiviral action of daclatasvir and asunaprevir

Huh7-ETluc cells were cultured in the presence of different doses of daclatasvir (DCV) and asunaprevir (ASV) and after 24h treatment, HCV replication was measured as luciferase counts. Both DCV and ASV caused a 75% inhibition of HCV replication compared to control levels (Figure 1A and B, $P < 0.001$). The inhibition of luciferase in Huh7-ETluc cells cannot be attributed to effects of ASV or DCV on cell growth or luciferase activity: when Huh7-PGK-luc cells that stably express luciferase were cultured with ASV or DCV, no inhibition of

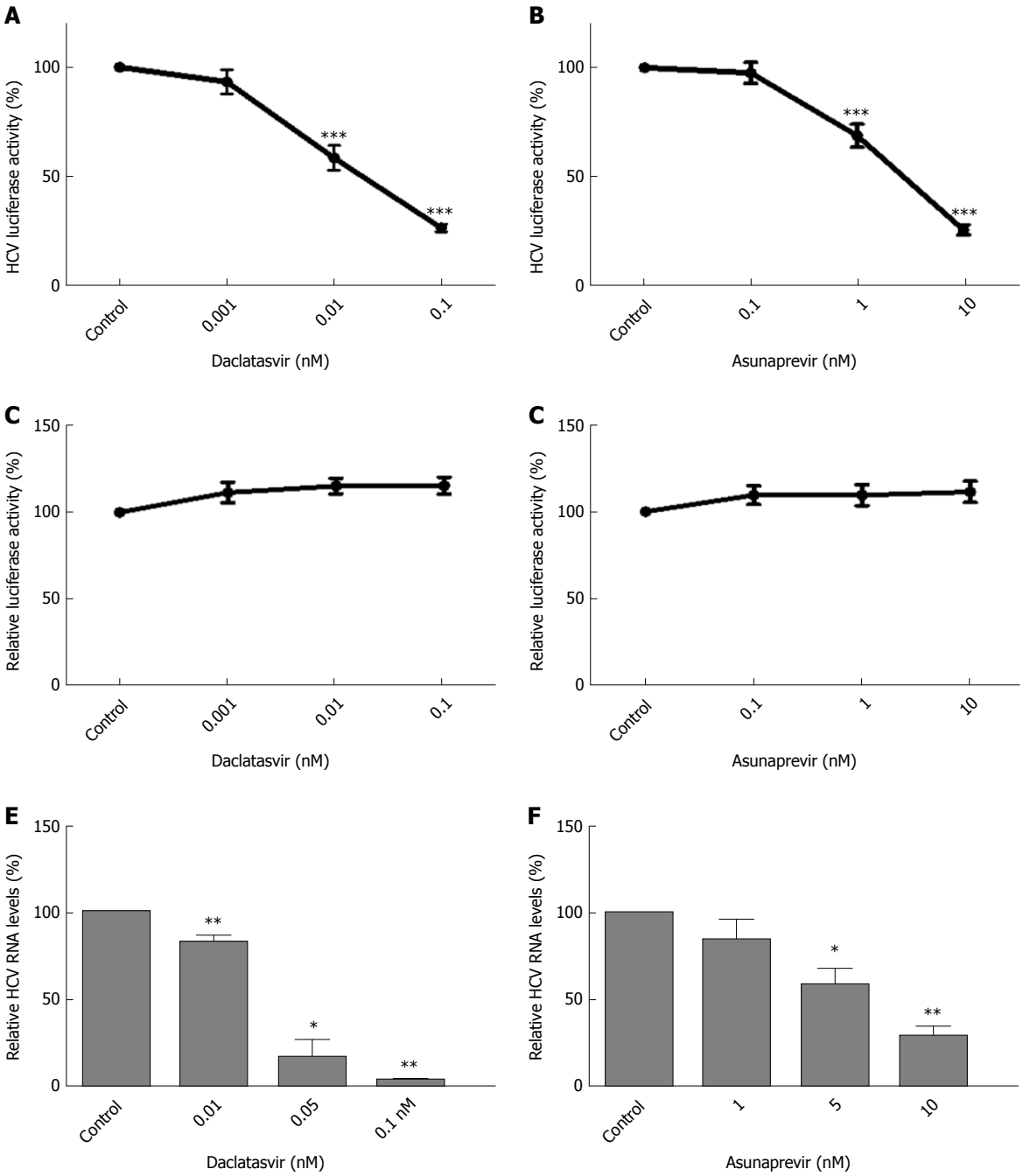

Figure 1 Hepatitis C virus replication is effectively inhibited by daclatasvir and asunaprevir. Huh7-ETluc cells were cultured with increasing concentrations of DCV (A) or ASV (B). The luciferase activity in these cells is a direct measure of HCV replication. HCV replication was significantly inhibited by 0.01 and 0.1 nmol/L DCV and 1 and 10 nmol/L ASV (mean of 13 independent experiments performed in triplicate, $P < 0.001$ Mann-Whitney test); The luciferase signal in Huh7-PGK-luc cells, stably expressing luciferase, was not affected by any concentration of DCV (C) or ASV (D), indicating that the observed effect in HUh7-ETluc is not due to non-specific inhibition of luciferase (mean of 7 experiments performed in triplicate); HCV replication in the infectious JFH model was effectively inhibited by DCV (E) at all tested concentrations (mean of 4-6 independent experiments measured in duplicate, $P = 0.004$ for 0.01 nmol/L DCV, $P = 0.11$ for 0.05 nmol/L DCV and $P = 0.007$ for 0.1 nmol/L DCV), as well as by 5 nmol/L and 10 nmol/L ASV (F) (mean of 4-6 independent experiments measured in duplicate, $P = 0.01$ for 5 nmol/L ASV and $P = 0.007$ for 10 nmol/L ASV). HCV: Hepatitis C virus; DCV: Daclatasvir; ASV: Asunaprevir; MPA: Mycophenolic acid; DAAs: Direct acting antivirals.

the luciferase signal could be observed, confirming that the decrease in luciferase signal in Huh7-ETluc cells by DCV and ASV is caused by inhibition of HCV replication (Figure 1C and D). Also in the JFH-derived infectious HCV model, DCV and ASV effectively inhibited HCV replication, with almost complete inhibition by 0.1 nM DCV (Figure 1E, $P = 0.004$ for 0.01 nM DCV, $P = 0.011$ for 0.05 nmol/L DVC, $P = 0.007$ for 0.1 nmol/L DCV), and a 78% reduction compared to control levels by 10nM ASV (Figure 1E and F, $P = 0.01$ for 5 nmol/L ASV and $P = 0.007$ for 10 nmol/L ASV).

Rapamycin has no effect on the antiviral action of DCV and ASV

Huh7-ETluc cells were cultured in the presence of different doses of DCV and ASV, in combination with

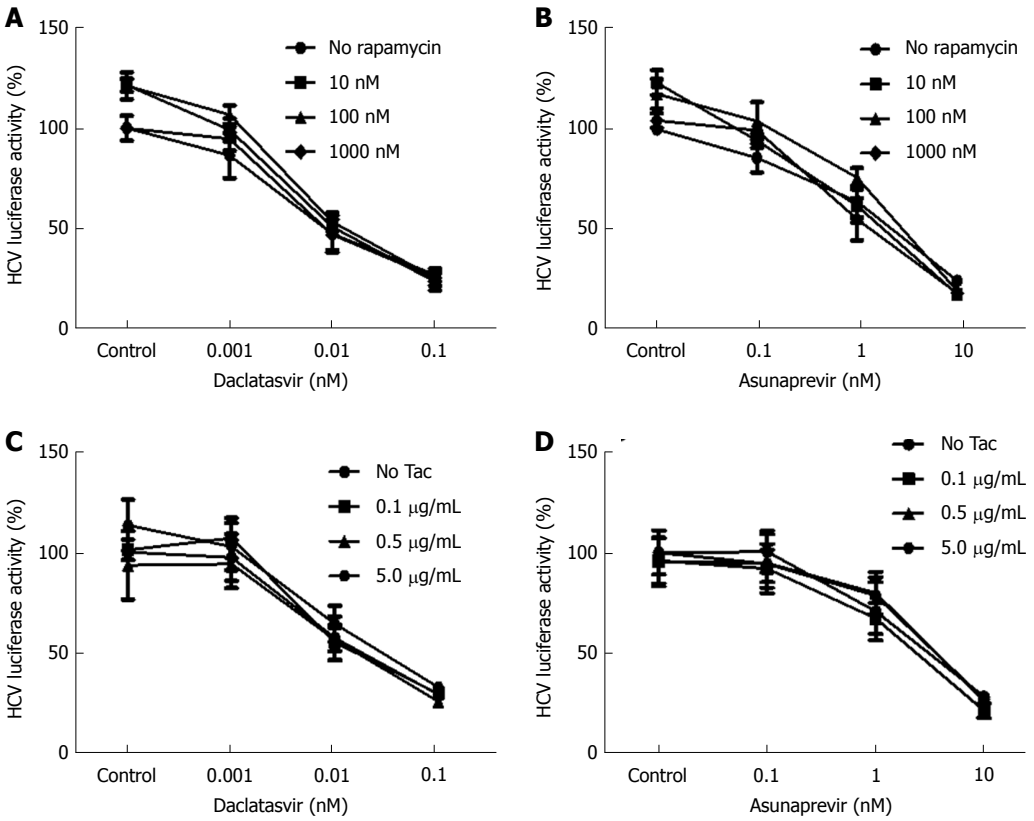

Figure 2 Dose-dependent inhibition of hepatitis C virus replication by daclatasvir and asunaprevir is not affected by rapamycin and tacrolimus. A, B: Huh7-ETluc cells were cultured with increasing concentrations of DCV (A) or ASV (B), in combination with different concentrations of RAPA; C, D: Huh7-ETluc cells were cultured with increasing concentrations of DCV (C) or ASV (D), in combination with different concentrations of TAC. After 24 h incubation luciferase was measured. HCV replication was effectively inhibited by ASV and DCV but not by rapamycin. RAPA and TAC had no effect on the antiviral action of ASV and DCV. Results are mean ± SE of 3 independent experiments performed in triplicate. HCV: Hepatitis C virus; DCV: Daclatasvir; ASV: Asunaprevir; RAPA: Rapamycin; TAC: Tacrolimus.

10, 100 or 1000 nmol/L rapamycin (RAPA). After 24 h of culture HCV replication was measured as luciferase counts. RAPA itself had no effect on viral replication, and the antiviral action of both DCV and ASV was not affected by the addition of RAPA (Figure 2A and B).

Effect of calcineurin inhibitors on the antiviral activity of DAAs

We investigated the effects of the calcineurin inhibitors tacrolimus (TAC) and cyclosporine A (CSA) on the antiviral activity of DCV and ASV. As shown in Figure 2C and 2D, the antiviral action of DCV and ASV was not affected by TAC. As shown in Figure 3A and 3B, contrary to TAC, 5 μg/mL CSA significantly inhibited HCV replication by maximal 76% of control levels (P = 0.03 with DCV, P = 0.04 with ASV).

When combined, the antiviral activity of ASV and DCV was not negatively affected by the addition of CSA. The observed antiviral action of CSA, ASV or DCV in Huh7-ETluc cells cannot be attributed to effects on cell growth or nonspecific effects on luciferase activity. When Huh7-PGK-luc cells were cultured in the presence of ASV or DCV combined with CSA, there was no effect on the luciferase signal (Figure 3C and 3D). In the infectious HCV model, comparable results were found. We observed that HCV replication was inhibited by both ASV and DCV. The addition of 0.5 μg/mL CSA completely inhibited HCV replication at the RNA level and did not negatively affect the inhibition of HCV replication by DCV and ASV (Figure 3E and 3F).

Daclatasvir and asunaprevir show a combined antiviral effect with MPA

MPA is an immunosuppressant that also affects HCV replication in *in vitro* cell culture systems. In Huh7-ETluc cells, the addition of MPA resulted in a 70%-76% inhibition of HCV replication compared to control levels. MPA provided additive antiviral effects when combined with ASV or DCV, resulting in an extra inhibition of HCV replication. At the highest doses of DCV and ASV, 1 and 5 μg/mL MPA significantly further decreased HCV replication by an extra 12%-16% (DCV) or 12% (ASV) (Figures 4A and B, P = 0.02 for 1 μg/mL and P = 0.08 for 5 μg/mL MPA with 0.1 nmol/L DCV; P = 0.01 for 1 μg/mL and 5 μg/mL MPA with 10 nmol/L ASV). To investigate if the combined effect of MPA and DAAs on the replication of HCV was not due to non-specific inhibition of luciferase or effects on cell viability, Huh7-PGK cells were cultured with ASV or DCV combined with MPA. The expression of luciferase was not significantly affected by treatment with ASV, DCV or MPA (Figures 4C and D).

In the Huh7 infectious model, 5 μg/mL MPA inhibited

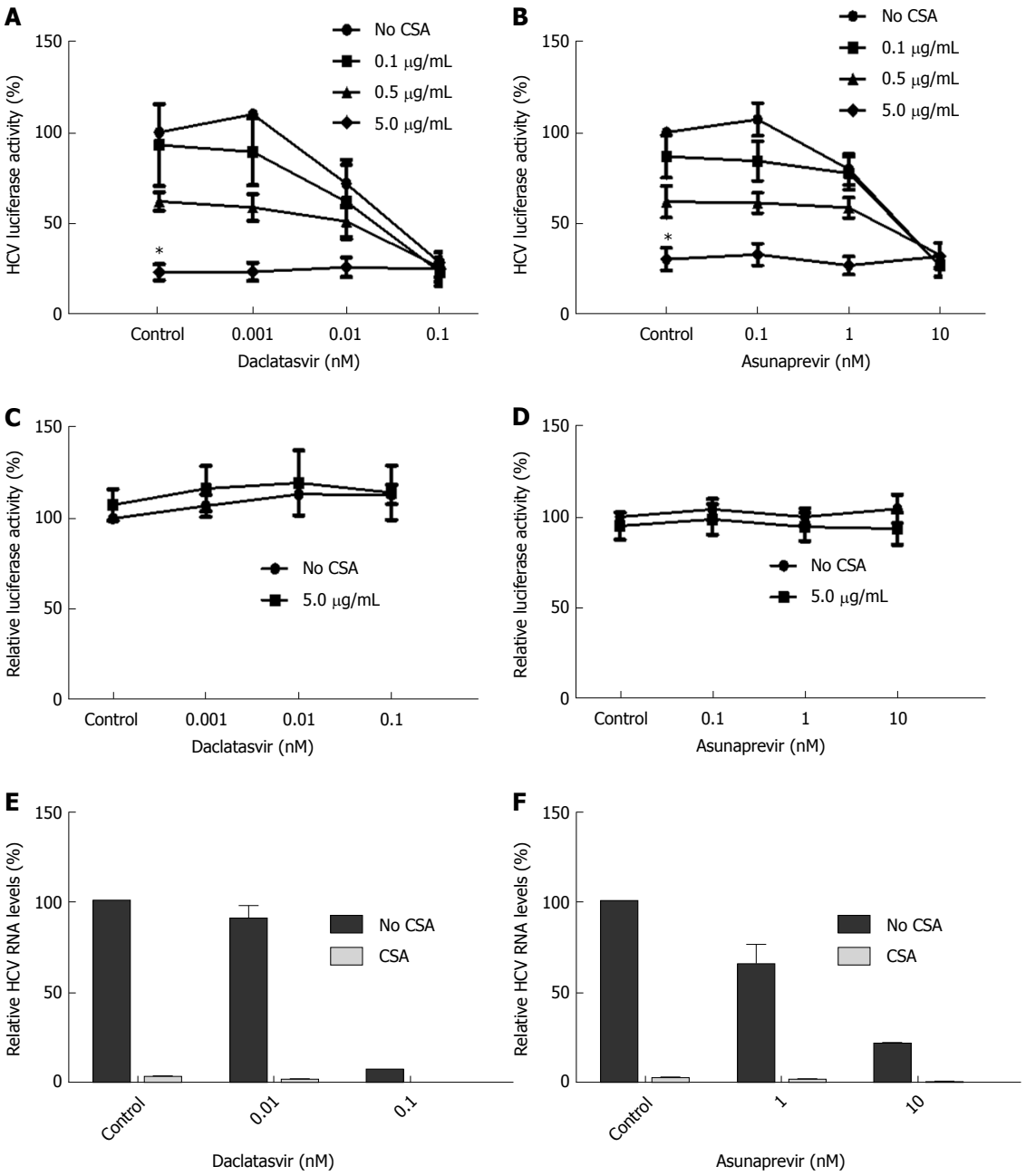

Figure 3 Calcineurin inhibitor cyclosporine A does not affect the antiviral activity of direct acting antivirals. A, B: Huh7-ETluc cells were cultured with increasing concentrations of DCV (A) or ASV (B), in combination with different concentrations of CSA. After 24 h luciferase was measured. HCV replication was inhibited by CSA: 5 μg/mL CSA significantly inhibited HCV replication compared to control (Mann-Whitney test, $P = 0.03$ for DCV, $P = 0.04$ for ASV). The antiviral action of DCV of ASV was not negatively affected by CSA and vice versa; C, D: The luciferase signal in Huh7-PGK-luc cells, stably expressing luciferase, was not affected by any concentration of DCV (C) or ASV (D) with or without CSA, indicating that the observed effect in HUh7-ETluc is not due to non-specific inhibition of luciferase. Results are mean ± SE of 4 independent experiments performed in triplicate; E, F: In the JFH infectious HCV cell culture model, HCV RNA levels were inhibited to by > 99% of control levels by both DCV (E) and ASV (F). The addition of 0.5 μg/mL CSA completely inhibited HCV replication (E, F). Shown are the results of two independent experiments, measured in duplicate by RT-qPCR. CSA: Cyclosporine A; HCV: Hepatitis C virus; DCV: Daclatasvir; ASV: Asunaprevir.

HCV replication by 68% of control levels. MPA further inhibited the inhibition of HCV replication by DCV. The highest dose of DCV (0.1 nmol/L) inhibited HCV replication by 96.5% of control levels with an extra reduction by 99.4% of control by MPA (Figure 4E). ASV was less effective in the Huh7 infectious model: when cells were cultured with 10 nmol/L ASV, HCV replication was inhibited by 54% of control levels, and the addition of MPA did not lead to an extra inhibition of HCV replication (Figure 4F).

From our previous research, it is known that the antiviral effect of MPA is partially exerted via upregulation of antiviral ISGs[16]. DCV and ASV show a combined antiviral effect with MPA, so we investigated whether the expression of antiviral ISGs was enhanced by the addition of DCV or ASV. Naïve Huh7 cells were cultured for 48 h in the presence of MPA with or without DCV or ASV. After 48 h, total RNA was isolated and the expression of Interferon

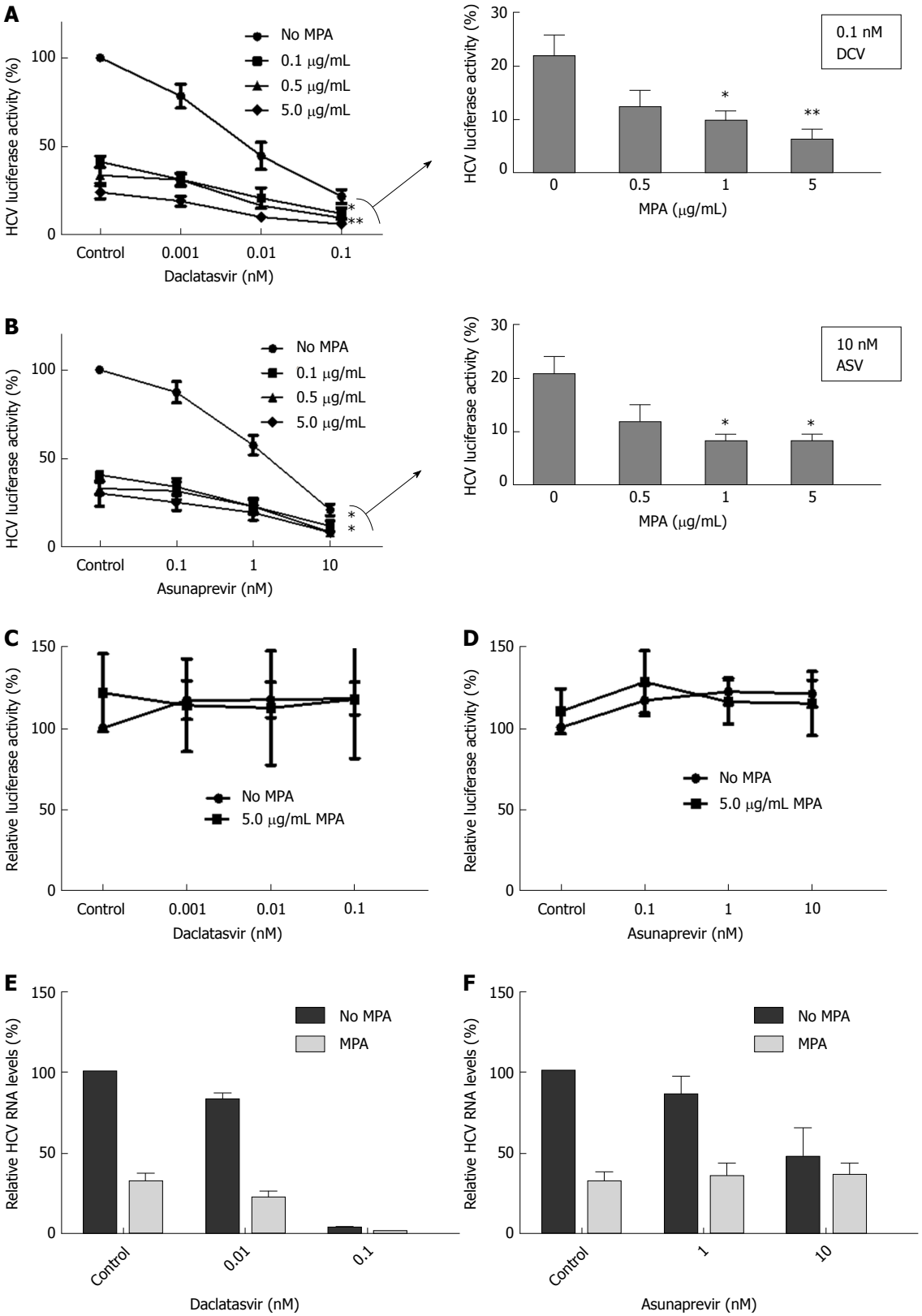

Figure 4 Daclatasvir and asunaprevir show a combined antiviral effect with mycophenolic acid. A, B: Huh7-ETluc cells were cultured with increasing concentrations of DCV (A) or ASV (B), in combination with different concentrations of MPA. After 24 h incubation luciferase was measured. HCV replication was effectively inhibited by ASV and DCV and by increasing concentrations of MPA. As shown in the bar graphs, when cells were treated with 0.1 nmol/L DCV or 10 nmol/L ASV, the addition of 1 and 5 μg/mL MPA further significantly inhibited HCV replication (P = 0.02 for 1 μg/mL and P = 0.08 for 5 μg/mL MPA and 0.1 nmol/L DCV; P = 0.01 for 1 μg/mL, 5 μg/mL MPA and 10 nmol/L ASV, Mann-Whitney test). Results are means ± SEM of 4 or 5 independent experiments performed in triplicate; C, D: The luciferase signal in Huh7-PGK-luc cells, stably expressing luciferase, was not affected by any concentration of DCV (C) or ASV (D) with or without MPA, indicating that the observed effect in HUh7-ETluc is not due to non-specific inhibition of luciferase. Results are mean ± SE of 3 independent experiments performed in triplicate; E: In the Huh7 infectious model, 5 μg/mL MPA reduced HCV replication by 68% of control levels. The inhibition of HCV replication by DCV was further reduced by MPA. The highest dose of DCV (0.1 nmol/L) inhibited HCV replication by 96.5% of control levels with an extra reduction by 99.4% of control levels by MPA; F: When HCV infected Huh7 cells were treated with 10 nmol/L ASV, HCV replication was reduced by 54% of control levels, with no additional effect of MPA. Results are mean of 6 experiments, performed in duplicate. HCV: Hepatitis C virus; DCV: Daclatasvir; ASV: Asunaprevir; MPA: Mycophenolic acid.

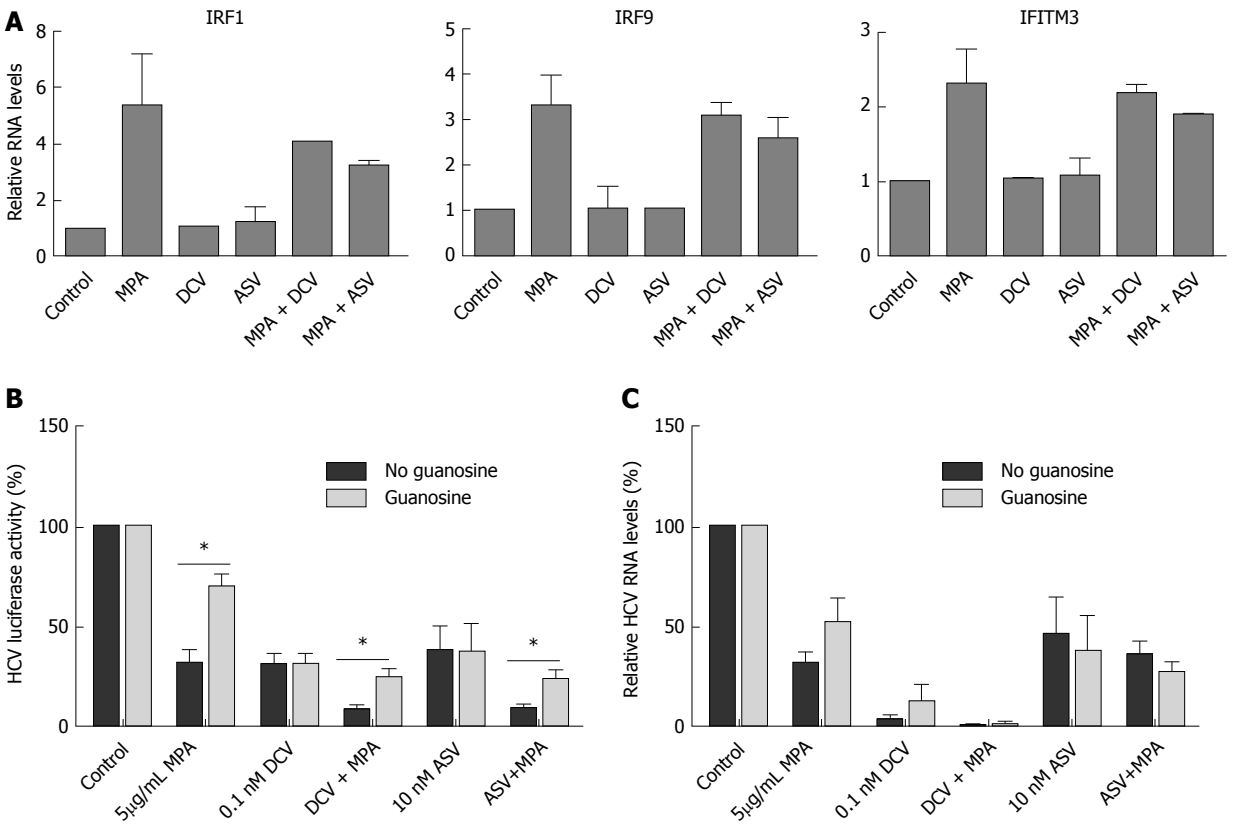

Figure 5 The combined antiviral action of DAAs with MPA is not caused by increased expression by ISGs and is partly reversed by guanosine. A: The expression of IRF1, IRF9, and IFITM3 was upregulated after 48h culture with 5 mg/ml MPA. 0.1 nM DCV and 10 nM ASV had no effect on the expression of these genes and did not affect the MPA induced expression. The results are means ± SEM of 2 independent experiments, performed in duplicate; B: The effect of guanosine (GU) supplementation on the combined antiviral action of DCV and ASV with MPA was investigated in Huh7ET-luc cells: MPA inhibited HCV replication by 69% of control, and this was significantly reversed by the addition of 50 μmol/ml guanosine by 30% of control (Mann-Whitney test, P = 0.03). Guanosine did not affect the antiviral action of DSV or ASV, and significantly reversed the combined antiviral effect of DSV or ASV with MPA (Mann-Whitney test P = 0.03 for DSV + MPA and P = 0.03 for ASV + MPA) Results are mean of 4 independent experiments performed in triplicate; C: In the infectious JFH model, HCV replication was effectively inhibited by 5mg/ml MPA, 0.1 nM DCV and 10nM ASV (by 68%, 96.5% and 54% of control respectively).The addition of 50 μmol/ml guanosine partly reversed the antiviral action of MPA by 49% of control, and had no effect on the antiviral action of DSV or ASV, either in the absence or presence of MPA. Results are mean ± SEM of 4-6 independent experiments, performed in duplicate.

regulatory factor 1 (IRF1), Interferon regulatory factor 9 (IRF9), and Interferon-induced transmembrane protein 3 (IFITM3) was measured by RT-qPCR. GAPDH was used as a reference gene. The expression of IRF1, IRF9, and IFITM3 was upregulated by 5 μg/mL MPA, but ASV and DCV did not affect the expression of these ISGs, either in the absence or presence of MPA (Figure 5A).

Part of the antiviral effect of MPA on HCV is exerted *via* inhibition of IMPDH, and subsequent inhibition of guanosine nucleotide biosynthesis. Supplementation with exogenous guanosine can partly reverse the antiviral action of MPA[16]. Therefore, we investigated the role of guanosine supplementation on the antiviral action of DCV or ASV in combination with MPA. As shown in Figure 5B, the addition of 50 μmol/ml guanosine indeed partially reversed the antiviral action of MPA from 69% inhibition to 30% inhibition compared to control levels in Huh7-ETluc cells (P = 0.03) but did not affect the action of DCV or ASV. The combined antiviral effect of MPA and DCV or ASV could significantly be reversed by the addition of guanosine (Figure 5B, P = 0.03 for DSV + MPA and P = 0.03 for ASV + MPA)

We also investigated the effect of guanosine supplementation on the antiviral action of MPA, DCV and ASV in the JFH derived infectious model. After infection, the cells were cultured with DCV or ASV in combination with MPA with or without guanosine. After 48 h, HCV RNA levels were determined by RT-qPCR. MPA inhibited HCV replication by 68% of control levels. This could be partly (but not significantly) reversed to 49% inhibition compared to control levels by the addition of guanosine. DCV (0.1 nmol/L) inhibited HCV replication by 96.5% of control levels, with no significant effect of guanosine. The addition of MPA further reduced HCV replication to more than 99% of control levels, however with no effect of guanosine supplementation. 10 nmol/L ASV reduced HCV replication by 54% of control levels, with no additional effect of MPA. The addition of guanosine also had no effect on the inhibition of HCV replication by ASV, either in the presence or absence of MPA (Figure 5C).

DISCUSSION

The potential interference of immunosuppressants with

the antiviral activity of DAAs post-transplantation is largely unknown. In 2017, Ikegami *et al*[23] showed in their study that the SVR rate of 80.3% that was achieved in patients who were treated with DCV and ASV after transplantation was not satisfactory. We aimed to investigate the interaction between immunosuppressants and DCV and ASV, both newer generation DAAs for the treatment of HCV. In our two *in vitro* HCV culture models, the mTOR inhibitor rapamycin and the calcineurin inhibitor tacrolimus did not negatively affect the antiviral action of DCV and ASV.

The calcineurin inhibitor CSA inhibited HCV replication, as described previously[6,10]. The addition of CSA did not negatively affect the antiviral action of DCV and ASV. The CSA concentrations we used in our study (between 100 and 5000 ng/mL) are in a clinically relevant range. Cyclosporine A target levels in patients range between 700-1300 ng/mL measured in blood[33], and peak levels vary between 800-2285 ng/mL[34]. In liver tissue, CSA levels can be 2.7 times higher as compared to plasma levels[35].

MPA, like CSA, inhibited HCV replication *in vitro*. The concentrations of MPA we used (0.1-5 μg/mL) are clinically achievable. In patients receiving MMF or MPA, serum peak levels range from 0.6 to11.5 μg/mL and trough levels average around 3 μg/mL[36]. Animal studies have shown that MPA accumulates in the liver[37]. When DCV and ASV were combined with MPA in our experiments, there was a difference in effect on the antiviral action compared to the experiments with CSA. When MPA was combined with the highest concentrations of DCV and ASV, an extra inhibition of HCV replication was observed, that could not be achieved with DCV or ASV alone. The combined antiviral effect was also observed in an infectious HCV model, but only with MPA and DCV. MPA exerts its antiviral action on HCV *via* two pathways: through the induction of antiviral ISGs and *via* inhibition of IMPDH, leading to depletion of the GTP pool in the cell. We did not observe upregulation of antiviral ISGs in cells that were cultured with DCV or ASV, and the upregulation of ISGs by MPA was not affected by the addition of these DAAs. In Huh7-ETluc cells, supplementation of the GTP pool by guanosine partly reversed the antiviral effect of MPA, and also the combined antiviral action of DCV or ASV with MPA. However, in the infectious model, only the antiviral activity of MPA was (partly) reversed by guanosine, and not the combined antiviral action of MPA and DCV. These results indicate that the inhibition of GTP synthesis by MPA is (partly) involved in the combined antiviral action of MPA with DSV and ASV. The difference in responsiveness to DCV or ASV we observe between Huh7-ETluc cells and the JFH infectious model might be explained by the fact that DCV is a pan-genotypic HCV inhibitor, while ASV is more specific for genotype 1b and is less active against genotypes 2 and 3[38,39]. The genotype of HCV in the JFH infectious model is 2a and the HCV construct in the Huh7-ETluc cells is derived from genotype 1b.

Although the *in vitro* antiviral action of MPA has been well documented, the clinical effects of MPA on HCV replication remain controversial. Some patient studies showed a significant reduction of HCV viral load by MMF treatment[40,41], while others reported no effects on HCV infection[42-44]. Ikegami *et al*[23] show in their study that 46.9% of patients who achieved SVR were treated with MMF, whereas 38.4% of the no-SVR group received MMF. However, this putative positive effect of MMF on DAA-induced SVR was not significant[23].

Our *in vitro* study shows that none of the immunosuppressants we tested negatively interfered with the antiviral action of DSV and ASV. The combination of MPA with DSV and ASV resulted in a higher reduction of HCV replication than that could be achieved by treatment with these compounds alone. Although the antiviral action of MPA is evident in cell culture systems, the antiviral effect in patients might be masked by the suppressive effects of MPA on the immune response. Our results can, however, complement the still emerging clinical findings on the effectivity of DAAs in the presence of immunosuppressants. Based on this *in vitro* study, there is no rationale or evidence to withhold or adjust DCV or ASV in combination with immunosuppressants in the post-transplantation management of HCV.

ARTICLE HIGHLIGHTS

Research background

Liver disease caused by chronic Hepatitis C virus (HCV) infection is a leading indication for liver transplantation. Factors that contribute to the recurrence of HCV after transplantation include viral factors (*e.g.*, HCV RNA levels at the time of transplantation and HCV genotype), host factors (immune response and HCV cryoglobulinemia), and the use of immunosuppressive medication. Current treatment of HCV is based on direct acting antivirals (DAAs), including daclatasvir (DCV) and asunaprevir (ASV). Recently a study reported reduced sustained virological response rates with DCV/ASV therapy after transplantation, indicating potential interference with immunosuppressants.

Research motivation

Although some drug-drug interactions were reported on the pharmacokinetics of DAAs and immunosuppressants, the potential interference of immunosuppressants with the antiviral activity of DAAs post-transplantation is largely unknown.

Research objectives

The aim of our study is to investigate the antiviral action of DCV and ASV in the presence of several different classes of immunosuppressants.

Research methods

The antiviral activity of DCV and ASV combined with immunosuppressants was tested using two *in vitro* cell culture models for HCV infection. The cells were cultured with different concentrations of DCV or ASV in combination with immunosuppressants from several different classes. The effects on HCV replication were quantified by luciferase assay or quantitative RT-PCR. Effects on the expression of antiviral interferon-stimulated genes were also assessed by quantitative RT-PCR.

Research results

Tacrolimus, rapamycin and cyclosporine did not negatively affect the antiviral action of DCV or ASV. Mycophenolic acid (MPA) showed additive antiviral effects combined with these DAAs. MPA induces interferon-stimulated genes (ISGs) and is a potent GTP synthesis inhibitor. DCV or ASV did not induce expression of ISGs nor affected ISG induction by MPA. Rather, the combined

antiviral effect of MPA with DCV and ASV was partly mediated via inhibition of GTP synthesis.

Research conclusions

Our *in vitro* study shows that none of the immunosuppressants we tested negatively interfered with the antiviral action of DSV and ASV. The combination of MPA with DSV and ASV resulted in a higher reduction of HCV replication than that could be achieved by treatment with these compounds alone. Although the antiviral action of MPA is evident in cell culture systems, the antiviral effect in patients might be masked by the suppressive effects of MPA on the immune response. Our results can, however, complement the still emerging clinical findings on the effectivity of DAAs in the presence of immunosuppressants.

Research perspectives

Based on this *in vitro* study, there is no rationale or evidence to withhold or adjust DCV or ASV in combination with immunosuppressants in the post-transplantation management of HCV.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Trends of characteristics and outcomes of donors and recipients of deceased donor liver transplantation in the United States: 1990 to 2013

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Author contributions: Ayloo S contributed to this paper with conception, design, analysis, and manuscript drafting; Pentakota SR contributed to data analysis; Molinari M contributed to data analysis and manuscript drafting.

Institutional review board statement: The study did not require approval by the ethics review board of our institution because it was conducted and reported per STROBE statement recommendations which was acknowledged in the methods section of the manuscript. This data is available to everybody and is provided by OPTN/UNOS as SRTR files.

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Abstract

AIM

To compare trends in donor/recipient characteristics and outcomes using four period cohorts of liver transplant recipients from 1990 to 2009.

METHODS

Seventy thousand three hundred and seventy-seven adult first-time recipients of whole-organ deceased-donor liver grafts from 1990 to 2009 were followed up until September 2013. Four periods based on transplantation dates were considered to account for developments in transplantation. Descriptive statistics were used to describe donor/recipient characteristics and transplant outcomes. Statistical comparisons between periods were performed using χ^2/Fischer's exact test (categorical variables) and *t*-tests/Mann-Whitney *U* test (continuous variables). Univariate descriptive statistics/survival data were generated using Kaplan-Meier curves. Cox Proportional Hazards models were used for regression analyses of patient and graft survival.

RESULTS

Mean age (years), body mass index (kg/m^2), and the proportion of males were, respectively, 39.1 (± 17.4), 25.9 (± 5.7) and 60.3 for donors, and 51.3 (± 10.5), 27.7 (± 5.6), and 64.4 for recipients. Donor and transplantation rates differed between racial/ethnic groups. Median (Q1-Q3) cold and warm ischemia, waitlist, and hospital stay times were 8 (6.0-10.0) h and 45 (35-59) min, 93 (21-278) d, and 12 (8-20) d. Total functional assistance was required by 8% of recipients at wait-listing and 13.4% at transplantation. Overall survival at 1, 3, 5, 10, 15, and 20 years was 87.3%, 79.4%, 73.6%, 59.8%, 46.7%, and 35.9%, respectively. The 2005-2009 cohort had better patient and graft survival than the 1990-1994 cohort overall [HR 0.67 (0.62-0.72) and 0.66 (0.62-0.71)] and at five years [HR 0.73 (0.66-0.80) and 0.71 (0.65-0.77)].

CONCLUSION

Despite changes in donor quality, recipient characteristics, and declining functional status among transplant recipients, overall patient survival is superior and post-transplant outcomes continue to improve.

Key words: UNOS database; OPTN database; Liver transplant surveillance; Liver transplant outcomes; Liver transplant survival

Core tip: The objective of this study was to compare trends in liver transplant donor/recipient characteristics and outcomes using four period cohorts of adult, first-time whole-organ deceased donor recipients from 1990-2009 using historical data from the OPTN/UNOS database. The landscape of donors and recipients undergoing liver transplantation (LT) in the United States has changed. Donor age, body mass index, and the contribution of racial minorities have increased. Transplant recipients are older, more deconditioned and obese, and with changing causes of cirrhosis. Despite this, the long-term patient survival has improved over time. This paper provides an overview of the landscape of LT in the United States.

Ayloo S, Pentakota SR, Molinari M. Trends of characteristics and outcomes of donors and recipients of deceased donor liver transplantation in the United States: 1990 to 2013. *World J Transplant* 2018; 8(5): 167-177 Available from: URL: http://www.wjgnet.com/2220-3230/full/v8/i5/167.htm DOI: http://dx.doi.org/10.5500/wjt.v8.i5.167

INTRODUCTION

Liver transplantation (LT) is a life-saving surgical option for many people with end-stage liver disease. According to annual data from the OPTN, 5710 deceased donor and 211 living donor LT were performed in 139 centers across the United States in 2013[1]. Although several short-term studies have analyzed the OPTN/UNOS database, few have evaluated LT over an extended period[2-7], leading to uncertainty regarding the long-term course of LT.

Numerous advances have occurred in LT management over the last several decades, including advancements in surgical techniques, anesthesia, and perioperative care in intensive care units, evolution of immunosuppressive medications and regimens[8,9], changes in organ allocation policies, institution of the Model for End-stage Liver Disease score (MELD)[10-12] to prioritize transplant candidates, improvements in tissue and organ preservation[13,14], and refinements in histocompatibility matching[15]. Therefore, we hypothesize that overall patient survival during this time has improved. However, transplant programs have extended their acceptance of grafts from donors who are older, higher risk, and have increased comorbidities to alleviate the paucity of available organs. The objective of this study was to compare donor and recipient characteristics and outcomes among four cohorts of LT recipients from 1990 to 2009.

MATERIALS AND METHODS

Historical data from the OPTN/UNOS database were obtained for all LT performed in the United States from 1989 to 2013. The primary objective was to evaluate post-transplant patient survival (1, 3, 5, 10, and 20 years), and the secondary objective was to evaluate transplant outcomes, including cold ischemia time (CIT) and warm ischemia time (WIT), hospital length of stay (LOS), waitlist time (WL), MELD, re-transplantation, rejection of graft, graft failure, reasons for graft failure, and post-transplant causes of death.

Data were provided by OPTN/UNOS as Standard Transplant and Research files. The study did not require approval by the ethics review board of our institution because it was conducted and reported per STROBE statement recommendations[16-18]. Analyses were limited to first-time, adult, whole-organ LT from a deceased donor from January 1st, 1990 to December 31st, 2009. Patients with missing data on liver type, donor type, previous LT, with multiple records, or who underwent multi-organ transplantation or re-transplantation were excluded from the study. Study subjects were grouped arbitrarily into four cohorts representing five-year intervals (1990-1994; 1995-1999; 2000-2004; 2005-2009) by transplant date. Study follow-up extended from transplant date until re-transplant, death, or September 06, 2013 (the last follow-up date recorded in the UNOS database), whichever occurred first. Data were updated with the date of death listed in the Social Security Death Master File for patients marked as "alive" or "lost to follow-up."

Demographic and clinical variables analyzed for both donors and recipients included: age, gender, highest education level, race/ethnicity, and body mass index (BMI). The World Health Organization classification

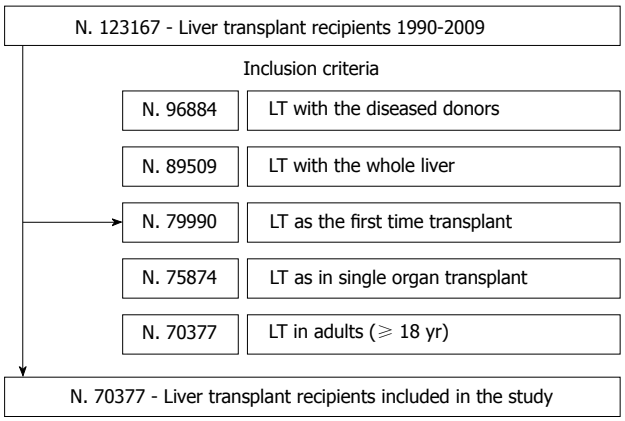

Figure 1 Records meeting inclusion criteria. LT: Liver transplantation.

was used to categorize the weight status of donors and recipients as follows: underweight (BMI < 18.5 kg/m^2), normal weight (BMI = 18.5-24.9 kg/m^2), overweight (BMI = 25.0-29.9 kg/m^2), class Ⅰ obesity (BMI = 30.0-34.9 kg/m^2), class Ⅱ obesity (BMI = 35.0-39.9 kg/m^2), and class Ⅲ obesity (BMI ≥ 40.0 kg/m^2). The donor cause of death was also analyzed.

Several recipient-specific variables were included in the analyses. These variables were related to transplant (CIT, WIT, LOS, and WL), recipient comorbidities including hypertension (HTN: no, yes, unknown), chronic obstructive pulmonary disease (COPD: no, yes, unknown), diabetes [no, type 1 (insulin-dependent diabetes mellitus), type 2 (non-insulin-dependent diabetes mellitus or other types of diabetes), unknown, angina (no, yes, unknown), dialysis in the week prior to LT, recipient functional status (no, some, or total assistance for activities of daily living), and recipient medical condition (admitted to ICU, hospitalized, not hospitalized). Individuals with coronary artery disease since 2004 were included in the angina group, whereas no such categorization was available prior to 2004.

Functional status was classified into three simple, clinically-useful categories. Patients requiring "total assistance" carried out 50% or less of daily activity functions and needed frequent medical care, or were severely disabled or moribund. Patients required "some assistance" if they were able to carry out 60%-80% of their daily functional activities and care for themselves, with some disease-related symptoms affecting daily activities. Patients requiring "no assistance" could perform 90%-100% of daily activities without substantial disease-related limitations.

Statistical analysis

Descriptive statistics were used to describe donor/recipient characteristics and transplant outcomes for the overall and the four period cohorts. Categorical variables were described using counts and proportions. Continuous variables were described using means and standard deviation, or with medians and interquartile ranges when skewed. Statistical comparisons of donor/recipient characteristics and transplant outcomes between period 1 (1990-1994) and period 4 (2004-2009) were performed using χ^2 and Fischer's Exact test as appropriate (categorical variables), *t*-tests (normally distributed continuous variables), and Mann-Whitney *U* test for skewed continuous variables. Univariate descriptive statistics and survival data on patient survival, both overall and by the four period cohorts, were generated using Kaplan-Meier curves. Cox Proportional Hazards models were used for regression analyses of patient and graft survival data, which was analyzed for overall and five year survival. Unadjusted and adjusted Cox Proportional Hazards regression models were run for patient and graft survival with "period" as the main exposure variable. In addition to period, the adjusted models included donor characteristics (age, gender, race/ethnicity, BMI, and cause of death) and recipient characteristics (age, gender, race/ethnicity, BMI, cause of liver failure, wait-list time, angina, diabetes, HTN, COPD, CIT, and functional and medical status). Given the numerous statistical tests performed, the level of statistical significance for interpretation of statistical results was assumed to be 1% (a two-sided alpha of < 0.01) instead of the traditional cut-off value of 5%. All analyses were performed using SAS version 9.4 (SAS, Cary, NC) and SPSS version 24 (IBM Corp., Armonk, NY).

RESULTS

A total of 70,377 LT met the inclusion criteria (Figure 1). Transplants were mostly performed in OPTN/UNOS Region five (14.7%) and three (14.5%). The mean age of donors was 39.1 ± 17.4 years, 60.4% were men, and the majority (73.3%) were white. The mean (± SD) BMI was 25.9 kg/m^2 (± 5.7), and 40.3% of donors had a normal body weight. The leading primary causes of donor deaths were cardiovascular adverse events (42.3%) and head traumas (39.9%) (Table 1).

In the subset analyses, mean donor age and BMI were significantly higher in period four than in period one. Donors with normal BMI dropped from 47.4% to 36.47% in Periods two to four, while the overweight donor group steadily increased from 14.5% to 33.3%. The percent of livers retrieved from obese donors more than tripled in period four compared with period one.

The mean age of recipients was 51.3 ± 10.5 years, and 64.4% were men (Table 2). The majority (76%) of recipients were white. The mean (± SD) BMI was 27.7 kg/m^2 (± 5.6), and 31% of recipients were normal body weight. Overall, 30.2% of recipients were either high school graduates or received a general education diploma. The leading primary causes for liver failure were hepatitis C (25%) followed by alcoholic cirrhosis (14%) (Table 3). The median (Q1-Q3) MELD at listing and transplant were 16 (12-24) and 18 (14-28), respectively. The median (Q1-Q3) wait-list time including days inactive on the list was 93 (21-278). The median (Q1-Q3) CIT in hours, WIT in minutes, and LOS during index transplant surgery were 8.0 (6.0-10.0), 45.0 (35-59), and 12.0

Table 1 Donor characteristics *n* (%)

Donor characteristics	Total	5 yr periods				*P*-value[1]
		Period 1	Period 2	Period 3	Period 4	
		1990-1994	1995-1999	2000-2004	2005-2009	
Age, mean (SD)	39.1 (17.4)	32.3 (15.2)	37.0 (17.4)	40.6 (17.6)	42.4 (17.2)	< 0.001
Gender						< 0.001
Female	27884 (39.6)	3831 (35.4)	6397 (40.1)	8077 (41.0)	9579 (40.1)	
Male	42492 (60.4)	6998 (64.6)	9573 (59.9)	11625 (59.0)	14296 (59.9)	
BMI, mean (SD)	25.9 (5.7)	23.8 (4.6)	24.8 (5.2)	26.0 (5.6)	27.0 (6.0)	< 0.001
BMI						< 0.001
Underweight	3310 (4.7)	502 (4.6)	1077 (6.7)	910 (4.6)	821 (3.4)	
Normal	29093 (41.3)	3655 (33.8)	7698 (48.2)	8730 (44.3)	9010 (37.7)	
Overweight	20441 (29.1)	1582 (14.6)	4461 (27.9)	6345 (32.2)	8053 (33.7)	
Obese - Class Ⅰ	7921 (11.3)	384 (3.6)	1369 (8.6)	2459 (12.5)	3709 (15.5)	
Obese - Class Ⅱ	2722 (3.9)	84 (0.8)	367 (2.3)	801 (4.1)	1470 (6.2)	
Obese - Class Ⅲ	1496 (2.1)	47 (0.4)	196 (1.2)	446 (2.3)	807 (3.4)	
Unknown	5394 (7.7)	4575 (42.3)	802 (5.0)	12 (0.1)	5 (0.0)	
Ethnicity						< 0.001
White	51594 (73.3)	8747 (80.8)	12334 (77.2)	14444 (73.3)	16069 (67.3)	
Black	9195 (13.1)	1044 (9.6)	1741 (10.9)	2481 (12.6)	3929 (16.5)	
Hispanic	7460 (10.6)	812 (7.5)	1396 (8.7)	2144 (10.9)	3108 (13.0)	
Asian	1327 (1.9)	135 (1.3)	255 (1.6)	395 (2.0)	542 (2.3)	
Other	662 (0.9)	47 (0.4)	164 (1.0)	224 (1.1)	227 (1.0)	
Unknown	139 (0.2)	44 (0.4)	80 (0.5)	15 (0.1)		
Causes of death						< 0.001
Anoxia	7848 (11.2)	483 (4.5)	1256 (7.9)	2028 (10.3)	4081 (17.1)	
Cerebrovascular/stroke	29788 (42.3)	3778 (34.9)	6645 (41.6)	8929 (45.3)	10436 (43.7)	
Head trauma	28087 (39.9)	3576 (33.0)	7592 (47.5)	8171 (41.5)	8748 (36.6)	

[1]Contrast between period 1 and 4. CNS: Central nervous system; SD: Standard deviation; BMI: Body mass index.

(8-20) days, respectively (Table 3).

In the subset analyses, mean recipient age and BMI were significantly higher in the later period. Significant decrease in transplanting normal weight recipients was observed with a rise in transplanting obese liver failure patients. Significant differences were noted in the recipient utilization of livers among different ethnicities and trends over different periods. Furthermore, recipients in the later period had higher education then period one. In terms of recipient functional status, the most common adult daily living functional status was the "no assistance" group at both wait-listing and transplantation. Similarly, 68.3% of recipients were not hospitalized for their medical condition at the time of their transplantation (Table 4). In terms of recipient comorbidities, diabetes was the most common medical comorbidity, followed by HTN. Approximately 4.3% of recipients were receiving dialysis before their transplantation (Table 5).

Analysis by different periods showed the WL for LT decreased from a median (Q1-Q3) of 151 (45-332) days in period two (1995-2000) to 68 (15-235) days in period four (2005-2009). Similarly, significant factors that affect transplant outcomes of median CIT and WIT decreased in later periods *vs* early periods of transplantation.

Rejection was treated in 9.5% of patients within 12 months post-transplantation. Primary graft failure (9.3%) and recurrence of hepatitis (9.1%) were the leading identifiable causes of graft failure (Table 6), with 8.2% of LT patients undergoing re-transplantation.

Percent cumulative patient survival at 1, 3, 5, 10, 15 and 20 years is 87.3, 79.4, 73.6, 59.8, 46.7 and 35.9, respectively (Figure 2). Of the identifiable causes, infection and malignancy were the leading causes of death in recipients, accounting for 13% and 12% of deaths, respectively (Table 7).

When adjusted for donor age, gender, BMI, ethnicity, causes of death and recipient age, gender, BMI, causes of liver failure, ethnicity/race, functional status, medical condition, CIT, WL, comorbidities of diabetes, COPD, HTN, angina and dialysis, the adjusted hazard ratio of patient and graft survival in period four in comparison to period one was 0.67 (0.62-0.72) and 0.66 (0.62-0.71), respectively. When the analysis was limited to five years of follow-up, the adjusted hazard ratios of patient and graft survival were 0.73 (0.66-0.80) and 0.71 (0.65-0.77), respectively (Figure 3 and Table 8).

DISCUSSION

This study describes the landscape of LT in the United States over a period of 20 years. It is important to understand the impact of changes that have occurred in the United States over this period of time on LT outcomes. Therefore, we analyzed UNOS data on LT performed from 1990 to 2009, followed up to September 2013. Cox proportional hazards regression analysis highlights an interesting fact; over the 20-year period, the graft loss has decreased by 34% and patient survival has improved by 33% after adjusting for donor and recipient age, gender, BMI, ethnicity, CIT, donor cause of death, recipient cause of liver failure, WL, comorbidities of diabetes, chronic obstructive pulmonary disease,

Table 2 Recipient characteristics *n* (%)

Recipient characteristics	Total	5 yr periods				*P*-value[1]
		Period 1	Period 2	Period 3	Period 4	
		1990-1994	1995-1999	2000-2004	2005-2009	
Age, mean (SD)	51.3 (10.5)	48.2 (11.4)	49.8 (10.5)	51.5 (9.7)	53.5 (9.9)	< 0.001
Gender						< 0.001
Female	25073 (35.6)	4724 (43.6)	6272 (39.3)	6544 (33.2)	7533 (31.6)	
Male	45304 (64.4)	6105 (56.4)	9698 (60.7)	13159 (66.8)	16342 (68.5)	
BMI, mean (SD)	27.75 (5.6)	26.26 (5.3)	27.47 (5.6)	28.03 (5.6)	28.35 (5.6)	< 0.001
BMI						< 0.001
Underweight	1458 (2.1)	371 (3.4)	319 (2.0)	364 (1.9)	404 (1.7)	
Normal	22533 (32.0)	4580 (42.3)	5395 (33.8)	5868 (29.8)	6690 (28.0)	
Overweight	24550 (34.9)	3436 (31.7)	5494 (34.4)	7077 (35.9)	8543 (35.8)	
Obese - Class I	13417 (19.1)	1488 (13.7)	2771 (17.4)	3991 (20.3)	5167 (21.6)	
Obese - Class II	5583 (7.9)	527 (4.9)	1166 (7.3)	1623 (8.2)	2267 (9.5)	
Obese - Class III	2084 (3.0)	203 (1.9)	452 (2.8)	629 (3.2)	800 (3.4)	
Unknown	752 (1.1)	224 (2.1)	373 (2.3)	151 (0.8)	4 (0.0)	
Ethnicity						< 0.001
White	53474 (76.0)	8839 (81.6)	12501 (78.3)	14844 (75.3)	17290 (72.4)	
Black	5448 (7.7)	631 (5.8)	1097 (6.9)	1565 (7.9)	2155 (9.0)	
Hispanic	7907 (11.2)	901 (8.3)	1655 (10.4)	2294 (11.6)	3057 (12.8)	
Asian	2785 (4.0)	317 (2.9)	555 (3.5)	785 (4.0)	1128 (4.7)	
Other	719 (1.0)	99 (0.9)	160 (1.0)	215 (1.1)	245 (1.0)	
Unknown	44 (0.1)	42 (0.4)	2 (0.0)			
Highest education level						0.2
Unknown	26282 (37.3)	9872 (91.2)	5735 (35.9)	5897 (29.9)	4778 (20.0)	
Less than high school	2335 (3.3)	55 (0.5)	513 (3.2)	704 (3.6)	1063 (4.5)	
High school (9-12) or GED	21249 (30.2)	438 (4.0)	4846 (30.3)	6835 (34.7)	9130 (38.2)	
College less than graduate	17559 (25.0)	384 (3.6)	4183 (26.2)	5359 (27.2)	7633 (32.0)	
Graduate	2952 (4.2)	80 (0.7)	693 (4.3)	908 (4.6)	1271 (5.3)	
Causes of liver failure						< 0.001
Alcoholic cirrhosis	9857 (14.0)	2165 (20.0)	2366 (14.8)	2497 (12.7)	2829 (11.9)	
Alcoholic cirrhosis with hepatitis C	4467 (6.4)	302 (2.8)	1373 (8.6)	1244 (6.3)	1548 (6.5)	
Cirrhosis: Autoimmune	2486 (3.5)	568 (5.3)	696 (4.4)	629 (3.2)	593 (2.5)	
Cirrhosis: Cryptogenic (Idiopathic)	5918 (8.4)	1397 (12.9)	1565 (9.8)	1515 (7.7)	1441 (6.0)	
Cirrhosis: Fatty liver (NASH)	1442 (2.1)		9 (0.1)	173 (0.9)	1260 (5.3)	
Cirrhosis: Hepatitis type B (HBSAG+)	2367 (3.4)	509 (4.7)	675 (4.2)	694 (3.5)	489 (2.1)	
Cirrhosis: Hepatitis type C	17611 (25.0)	1849 (17.1)	4024 (25.2)	6058 (30.8)	5680 (23.8)	
Other	13851 (19.7)	2344 (21.7)	3195 (20.0)	4196 (21.3)	4116 (17.2)	
PLM: Hepatoma (HCC) and cirrhosis	4960 (7.1)	143 (1.3)	272 (1.7)	984 (5.0)	3561 (14.9)	
PLM: Hepatoma - HCC	1954 (2.8)	173 (1.6)	141 (0.9)	423 (2.2)	1217 (5.1)	
Primary biliary cirrhosis (PBC)	3762 (5.4)	1122 (10.4)	1105 (6.9)	814 (4.1)	721 (3.0)	
PSC: Ulcerative colitis	1702 (2.4)	257 (2.4)	549 (3.4)	476 (2.4)	420 (1.8)	

[1]Contrast between period 1 and 4. GED: General education development; NASH: Non-alcoholic steatohepatitis; HCC: Hepatocellular carcinoma; PBC: Primary biliary cholangitis; PLM: Primary liver malignancy; PSC: Primary sclerosing cholangitis; (HBSAG+): Hepatitis B surface antigen-positive; SD: Standard deviation.

Table 3 Recipient perioperative data *n* (%)

Recipient characteristics	Total	5 yr periods				*P*-value[1]
		Period 1	Period 2	Period 3	Period 4	
		1990-1994	1995-1999	2000-2004	2005-2009	
MELD (median) (Q1-Q3)						
Listing	16 (12-23)	NA	NA	16 (12-23)	16 (12-23)	
Transplant	18 (13-26)	NA	NA	18 (13-25)	19 (14-27)	
CIT (median hours) (Q1-Q3)	8 (6.0-10.0)	10.3 (8.0-13.2)	8.5 (6.5-10.9)	7.3 (5.7-9.5)	7 (5.1-8.7)	< 0.001
WIT(median Minutes) (Q1-Q3)	45 (35.0-59.0)	58 (45.0-75.0)	48 (38.0-60.0)	40 (31.0-50.0)	40 (31.0-49.0)	< 0.001
Waiting list/inactive (median days) (Q1-Q3)	93 (21-278)	53 (14-31)	151 (45-332)	124 (27-386)	68 (15-235)	< 0.001
Hospital stay (median days) (Q1-Q3)	12 (08-20)	20 (14-31)	13 (09-21)	10 (07-17)	10 (07-16)	< 0.001

[1]Contrast between period 1 and 4. CIT: Cold Ischemia Time; WIT: Warm Ischemia Time; MELD: Model for End Stage Liver Disease; (Q1-Q3): 25th Quartile - 75th Quartile.

hypertension, angina, on dialysis, functional status and medical condition.

In terms of race/ethnicity, white patients were the most common transplant donors and recipients, however our study showed that the contribution from this group has been decreasing while that of other racial/ethnic

Table 4 Functional status and medical condition *n* (%)

Recipient characteristics	Total	5 yr periods				*P*-value[1]
		Period 1	Period 2	Period 3	Period 4	
		1990-1994	1995-1999	2000-2004	2005-2009	
Functional status - listing						< 0.001
Unknown	16999 (24.2)	7131 (65.9)	3916 (24.5)	4279 (21.7)	1673 (7.0)	
ADL with no assistance	30882 (43.9)	1527 (14.1)	8019 (50.2)	11006 (55.9)	10330 (43.3)	
ADL with some assistance	16803 (23.9)	2096 (19.4)	3772 (23.6)	4101 (20.8)	6834 (28.6)	
ADL with total assistance	5693 (8.1)	75 (0.7)	263 (1.7)	317 (1.6)	5038 (21.1)	
Functional status - transplant						< 0.001
Unknown	22251 (31.6)	7686 (71)	6695 (41.9)	6722 (34.1)	1148 (4.8)	
ADL with no assistance	23277 (33.1)	1338 (12.4)	5959 (37.3)	8363 (42.5)	7617 (31.9)	
ADL with some assistance	15434 (21.9)	1686 (15.6)	2876 (18.0)	3986 (20.2)	6886 (28.8)	
ADL with total assistance	9415 (13.4)	119 (1.1)	440 (2.8)	632 (3.2)	8224 (34.5)	
Medical condition - listing						< 0.001
Unknown	14394 (20.5)	83 (0.8)	76 (0.5)	5 (0.0)	14230 (59.6)	
ICU	4549 (6.5)	1354 (12.5)	1208 (7.6)	1339 (6.8)	648 (2.7)	
Hospitalized not in ICU	5949 (8.5)	1447 (13.4)	1615 (10.1)	1819 (9.2)	1068 (4.5)	
Not Hospitalized	45485 (64.6)	7945 (73.4)	13071 (81.9)	16540 (84.0)	7929 (33.2)	
Medical condition - transplant						< 0.001
Unknown	28 (0.0)	2 (0.0)	26 (0.2)			
ICU	10220 (14.5)	1883 (17.4)	2824 (17.7)	2946 (15.0)	2567 (10.8)	
Hospitalized not in ICU	12076 (17.2)	2219 (20.5)	3467 (21.7)	2613 (13.3)	3777 (15.8)	
Not Hospitalized	48053 (68.3)	6725 (62.1)	9653 (60.4)	14144 (71.8)	17531 (73.4)	

[1]Contrast between period 1 and 4. ICU: Intensive care unit; ADL: Adult daily living; Unknown: Data not available.

Table 5 Medical comorbidities *n* (%)

Recipient characteristics	Total	5 yr periods				*P*-value[1]
		Period 1	Period 2	Period 3	Period 4	
		1990-1994	1995-1999	2000-2004	2005-2009	
Diabetes						< 0.001
Unknown	11392 (16.2)	9331 (86.2)	848 (5.3)	714 (3.6)	499 (2.1)	
No DM	47401 (67.4)	1310 (12.1)	12792 (80.1)	15326 (77.8)	17973 (75.3)	
Type 1 DM	702 (1.0)			63 (0.3)	639 (2.7)	
Type 2 DM	10882 (15.5)	188 (1.7)	2330 (14.6)	3600 (18.3)	4764 (20.0)	
COPD						0.4
Unknown	26589 (37.8)	9359 (86.4)	1387 (8.7)	1159 (5.9)	14684 (61.5)	
No	43172 (61.3)	1449 (13.4)	14412 (90.2)	18280 (92.8)	9031 (37.8)	
Yes	616 (0.9)	21 (0.2)	171 (1.1)	264 (1.3)	160 (0.7)	
Hypertension						< 0.001
Unknown	26387 (37.5)	9412 (86.9)	1115 (7.0)	1159 (5.9)	14701 (61.6)	
No	37629 (53.5)	1288 (11.9)	13356 (83.6)	15664 (79.5)	7321 (30.7)	
Yes	6361 (9.0)	129 (1.2)	1499 (9.4)	2880 (14.6)	1853 (7.8)	
Angina						0.5
Unknown	28259 (40.2)	9365 (86.5)	1019 (6.4)	2103 (10.7)	15772 (66.1)	
No angina	40926 (58.2)	1416 (13.1)	14567 (91.2)	17081 (86.7)	7862 (32.9)	
Angina	1192 (1.7)	48 (0.4)	384 (2.4)	519 (2.6)	241 (1.0)	
Dialysis						< 0.001
Unknown	9682 (13.8)	8532 (78.8)	629 (3.9)	471 (2.4)	50 (0.2)	
No	57690 (82.0)	2234 (20.6)	14789 (92.6)	18282 (92.8)	22385 (93.8)	
Yes	3005 (4.3)	63 (0.6)	552 (3.5)	950 (4.8)	1440 (6.0)	

[1]Contrast between period 1 and 4. DM: Diabetes mellitus; COPD: Chronic obstructive pulmonary disease; Unknown: Data not available.

groups is growing. Hispanic (10.6%) and Asian (1.9%) individuals were the lowest contributors to the liver organ donation pool but were recipients more often (11.2% and 3.9%, respectively). Black donors and recipients showed a different distribution, constituting 13.1% of donors but only 7.7% of recipients. The discrepancy may be at least partly attributable to the higher mortality of blacks candidates while on the LT waitlist relative to that of Hispanic and Asian candidates[1].

Hepatitis C was the foremost identified cause of liver failure in our study, with a 25.0% incidence over the 20 year time period. This underscores the importance of efforts to intensively treat hepatitis C in order to prevent both end-stage liver disease and graft failure after transplantation. Recurrence of hepatitis was the leading cause of graft failure (9.1%) in our study. However, it is important to note that our results mostly reflect patients treated in the era of low-efficacy treatment options for

Table 6 Graft status *n* (%)

Recipient characteristics	Total	5 yr periods				*P*-value[1]
		Period 1	Period 2	Period 3	Period 4	
		1990-1994	1995-1999	2000-2004	2005-2009	
Graft status						< 0.0001
Not Failed	35460 (50.4)	2879 (26.6)	6221 (39)	10361 (52.6)	15999 (67)	
Failed	34917 (49.6)	7950 (73.4)	9749 (61.1)	9342 (47.4)	7876 (33)	
Treated for rejection ≤ 12 mo						< 0.0001
Unknown	37869 (53.8)	10081 (93.1)	12610 (79)	8066 (40.9)	7112 (29.8)	
No	25835 (36.7)	145 (1.3)	2000 (12.5)	9392 (47.7)	14298 (59.9)	
Yes	6673 (9.5)	603 (5.6)	1360 (8.5)	2245 (11.4)	2465 (10.3)	
Causes of graft failure						
Biliary						< 0.001
Unknown	23875 (68.4)	6299 (79.2)	6558 (62.3)	5989 (64.1)	5029 (63.9)	
No	10098 (28.9)	1514 (19)	2981 (30.6)	3112 (33.3)	2491 (31.6)	
Yes	944 (2.7)	137 (1.7)	210 (2.1)	241 (2.6)	356 (4.5)	
Hep *de novo*						0.0006
Unknown	23854 (68.32)	6329 (79.61)	6551 (67.2)	5971 (63.92)	5003 (63.52)	
No	10968 (31.41)	1591 (20.01)	3168 (32.5)	3356 (35.92)	2853 (36.22)	
Yes	95 (0.27)	30 (0.38)	30 (0.31)	15 (0.16)	20 (0.25)	
Hep recurrence						0.9
Unknown	23670 (67.79)	6230 (78.36)	6523 (66.91)	5929 (63.47)	4988 (63.33)	
No	8086 (23.16)	1232 (15.5)	2387 (24.48)	2403 (25.72)	2064 (26.21)	
Yes	3161 (9.05)	488 (6.14)	839 (8.61)	1010 (10.81)	824 (10.46)	
Infection						<0.001
Unknown	23794 (68.14)	6213 (78.15)	6564 (67.33)	5986 (64.08)	5031 (63.88)	
No	9429 (27)	1333 (16.77)	2690 (27.59)	2897 (31.01)	2509 (31.86)	
Yes	1694 (4.85)	404 (5.08)	495 (5.08)	459 (4.91)	336 (4.27)	
Primary graft failure						0.0013
Unknown	23289 (66.7)	5921 (74.48)	6475 (66.42)	5901 (63.17)	4992 (63.38)	
No	8392 (24.03)	1369 (17.22)	2432 (24.95)	2521 (26.99)	2070 (26.28)	
Yes	3236 (9.27)	660 (8.3)	842 (8.64)	920 (9.85)	814 (10.34)	
Recurrent disease						0.3
Unknown	23686 (67.84)	6177 (77.7)	6536 (67.04)	5965 (63.85)	5008 (63.59)	
No	9548 (27.34)	1464 (18.42)	2862 (29.36)	2890 (30.94)	2332 (29.61)	
Yes	1683 (4.82)	309 (3.89)	351 (3.6)	487 (5.21)	536 (6.81)	
Acute rejection						0.6
Unknown	23854 (68.32)	6318 (79.47)	6546 (67.15)	5969 (63.89)	5021 (63.75)	
No	10374 (29.71)	1530 (19.25)	2999 (30.76)	3180 (34.04)	2665 (33.84)	
Yes	689 (1.97)	102 (1.28)	204 (2.09)	193 (2.07)	190 (2.41)	
Chronic rejection						<0.001
Unknown	26635 (76.28)	6507 (81.85)	7441 (76.33)	6927 (74.15)	5760 (73.13)	
No	7018 (20.1)	1124 (14.14)	1949 (19.99)	2128 (22.78)	1817 (23.07)	
Yes	1264 (3.62)	319 (4.01)	359 (3.68)	287 (3.07)	299 (3.8)	
Vascular thrombosis						0.3
Unknown	23750 (68.02)	6231 (78.38)	6535 (67.03)	5970 (63.9)	5014 (63.66)	
No	9635 (27.59)	1473 (18.53)	2780 (28.52)	2964 (31.73)	2418 (30.7)	
Yes	1532 (4.39)	246 (3.09)	434 (4.45)	408 (4.37)	444 (5.64)	

[1]Contrast between period 1 and 4. Unknown: Data not available; Hep: Hepatitis.

hepatitis C. With the advent of direct-acting antiviral agents[19], we suspect that these trends will change in the future[20,21].

Consistent with the worldwide obesity epidemic, cirrhosis due to non-alcoholic steatohepatitis (NASH) has risen as an indication for LT from 1.2% in 2001 to 9.7% in 2009. Currently, NASH is the third-most common cause for LT in the United States, and it has been projected to become the leading cause by 2025[22]. Our results showed a similar trend, with NASH cirrhosis increasing substantially from 0.06% in 1995-1999 to 5.3% in 2005-2009, coinciding with the increasing obesity rates in the United States and improved understanding of NASH. When we evaluated the causes of end-stage liver disease from 2009 to 2013, the latest available data in the dataset, NASH cirrhosis constituted 8.2%. In this period, NASH remained the third leading cause of liver failure following hepatitis C (22.0%), cirrhosis with HCC (18.9%), and alcoholic cirrhosis (12.3%). NASH-associated liver failure had been the least prevalent identifiable etiology of liver failure in the early 1990s (Table 3), highlighting its significant growth[23].

While the two leading causes of liver failure (hepatitis C and alcoholic cirrhosis) decreased in the second decade of our study, the rates of primary liver malignancy, both alone and in combination with cirrhosis, rose substantially from 1990-1999 to 2000-2009. This increase likely reflects the 2002 UNOS allocation policy assigning

Table 7 Recipient status *n* (%)

Recipient characteristics	Total	5 yr periods				*P*-value[1]
		Period 1	Period 2	Period 3	Period 4	
		1990-1994	1995-1999	2000-2004	2005-2009	
Re-transplantation						< 0.001
No	64588 (91.8)	9586 (88.5)	14350 (89.9)	18180 (92.3)	22472 (94.1)	
Yes	5789 (8.2)	1243 (11.5)	1620 (10.1)	1523 (7.7)	1403 (5.9)	
Causes of death						< 0.001
Cardiovascular/cardio	2893 (9.9)	718 (10.7)	783 (9.6)	735 (9.4)	657 (10.2)	
Cerebrovascular	647 (2.2)	177 (2.6)	191 (2.4)	146 (1.9)	133 (2.1)	
Graft Failure	3363 (11.6)	677 (10.1)	895 (11)	948 (12.1)	843 (13)	
Hemorrhage	825 (2.8)	237 (3.5)	213 (2.6)	222 (2.8)	153 (2.4)	
Infection	3794 (13)	1032 (15.4)	1011 (12.4)	893 (11.4)	858 (13.3)	
Malignancy	3477 (12)	704 (10.5)	847 (10.4)	931 (11.9)	995 (15.4)	
Multiorgan failure	2192 (7.5)	349 (5.2)	536 (6.6)	669 (8.6)	638 (9.9)	
Other	3378 (11.6)	629 (9.4)	919 (11.3)	1004 (12.9)	826 (12.8)	
Pulmonary	965 (3.3)	187 (2.8)	260 (3.2)	269 (3.4)	249 (3.9)	
Renal failure	708 (2.4)	208 (3.1)	237 (2.9)	167 (2.1)	96 (1.5)	
Unknown	6861 (23.6)	1788 (26.7)	2232 (27.5)	1825 (23.4)	1016 (15.7)	

[1]Contrast between period 1 and 4. Unknown: Data not available.

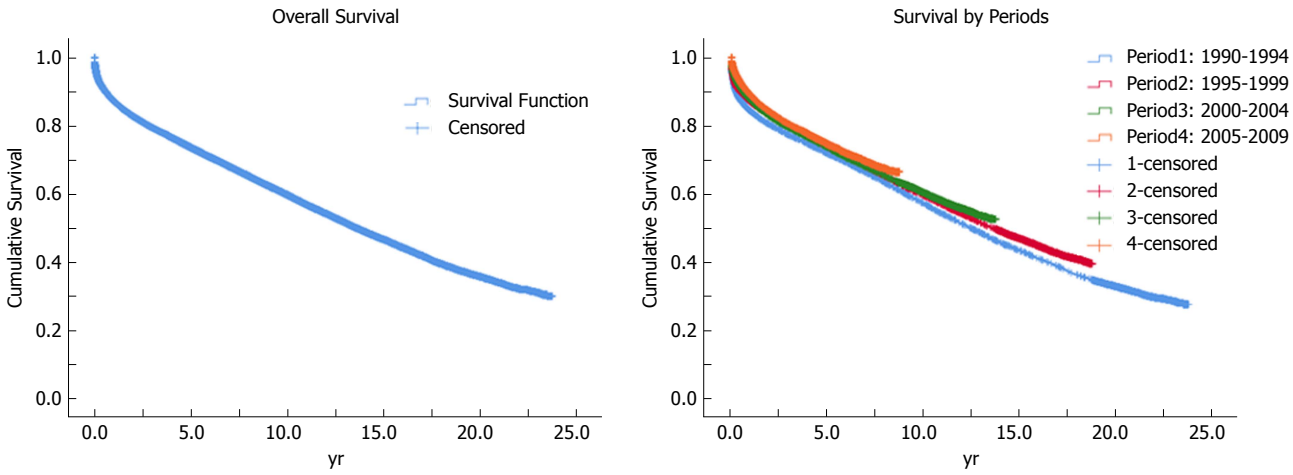

Figure 2 Kaplan-Meier patient survival curves for entire follow-up and for 5 years.

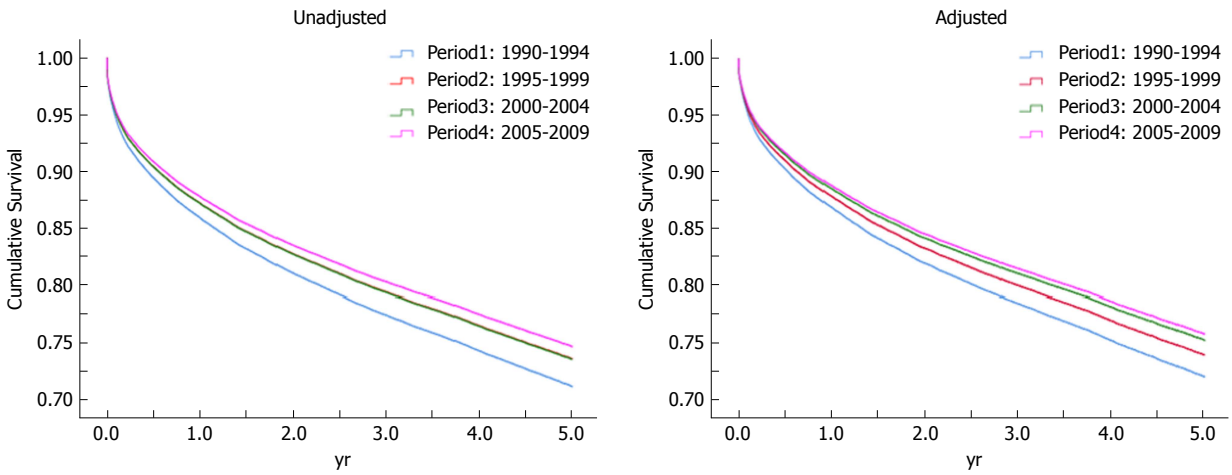

Figure 3 Cox Proportional Hazard patient unadjusted and adjusted patient survival by periods.

exceptional (additional) MELD score points for HCC.

OPTN annual data from 2013 reported that of the 15,027 patients placed on the wait-list, 1,767 (11.8%) died while on the wait-list and 1,223 (8.1%) were too

Table 8 Cox proportional hazards regression of survival after liver transplantation

	Unadjusted HR (95%CI)	Adjusted HR (95%CI)[1]
Over all patient survival		
Period 2 (1995-1999 *vs* 1990-1994)	0.90 (0.87-0.93)	0.90 (0.84-0.94)
Period 3 (2000-2004 *vs* 1990-1994)	0.87 (0.84-0.90)	0.76 (0.72-0.81)
Period 4 (2005-2009 *vs* 1990-1994)	0.83 (0.80-0.86)	0.67 (0.62-0.72)
5 yr patient survival		
Period 2 (1995-1999 *vs* 1990-1994)	0.90 (0.86-0.95)	0.90 (0.82-0.98)
Period 3 (2000-2004 *vs* 1990-1994)	0.90 (0.86-0.95)	0.80 (0.73-0.88)
Period 4 (2005-2009 *vs* 1990-1994)	0.86 (0.82-0.90)	0.73 (0.66-0.80)
Over all graft survival		
Period 2 (1995-1999 *vs* 1990-1994)	0.90 (0.87-0.93)	0.88 (0.83-0.93)
Period 3 (2000-2004 *vs* 1990-1994)	0.84 (0.82-0.87)	0.74 (0.70-0.79)
Period 4 (2005-2009 *vs* 1990-1994)	0.80 (0.76-0.81)	0.66 (0.62-0.71)
5 yr graft survival		
Period 2 (1995-1999 *vs* 1990-1994)	0.91 (0.87-0.95)	0.89 (0.82-0.96)
Period 3 (2000-2004 *vs* 1990-1994)	0.87 (0.84-0.91)	0.77 (0.71-0.84)
Period 4 (2005-2009 *vs* 1990-1994)	0.81 (0.76-0.84)	0.71 (0.65-0.77)

[1]Adjusted for donor age, gender, BMI, ethnicity, cause of death and recipient's age, gender, BMI, ethnicity, CIT, cause of liver failure, waitlist time, diabetes, COPD, HTN, dialysis, angina, functional status, medical condition. HR: Hazard ratio; CI: Confidence interval; CIT: Cold ischemia time; BMI: Body mass index; COPD: Chronic obstructive pulmonary disease; HTN: Hypertension.

sick to undergo transplantation[1]. With a median WL of 93 d, it is not surprising that we observed a decrease in functional status between the time liver transplant candidates were placed on the list and the time of transplantation. The percentage of transplant candidates requiring no assistance in daily functioning decreased by approximately 10% from the time of listing to the time of transplantation, whereas the percentage of candidates requiring total assistance increased. A similar study by Orman *et al*[24], using data from the OPTN/UNOS database from 2005 to 2015, likewise reported that the proportion of patients with Karnofsky performance status A (able to carry out normal activity or work) decreased, whereas the proportion with a status of B and C (unable to work plus able (B) or not able (C) to carry out personal care) increased. In patients with cirrhosis, worsening of performance status was associated with increased risk of mortality. Several other studies have previously reported functional status as a predictor of WL and post-transplant mortality[25-27].

Despite recipients' deteriorating functional status at the time of transplantation, the median LOS for LT in our study was 12 d, which is relatively short considering the complexity of, and complications associated with, the procedure. We also noted a decrease in LOS by about 10 d from the earliest to the latest period. This may reflect improvements in perioperative care, growth in follow-up management experience, ease in outpatient management of immunosuppressive medications, and the recent trend of encouraging earlier hospital discharge.

About 9.5% of transplants experienced rejection within one year of transplantation. Primary graft failure and hepatitis recurrence were the leading causes of graft failure. About 8.2% of patients in this dataset underwent re-transplantation. The percentage of re-transplantations improved over the different time periods, from 11.5% to 5.9%, which probably reflects multifactorial improvement in every aspect of transplantation. The leading causes of mortality in transplant recipients were infection and malignancy, suggesting that aggressive screening for post-transplant malignancies and prompt treatment of infections may be important ways to improve future survival. Since the leading cause of graft failure is the recurrence of hepatitis, we anticipate that implementation of new anti-viral therapeutic regimens before and after transplantation may improve graft survival rates. Reducing obesity is another strategy to potentially improve survival. Not only is obesity a modifiable risk factor for cardiovascular adverse events, which accounted for 9.9% of deaths in our study, but it is also a major contributor to NASH, which is becoming an increasingly common indication of LT. In addition to lifestyle changes and medically-supervised weight loss, the role of metabolic surgery needs to be explored very early in the course of liver failure[28,29].

Although this study was restricted to adults undergoing first-time single whole-organ deceased donor LT, with multi-organ and re-transplanted recipients excluded to improve homogeneity and adjusted for broad changes, there is an intrinsic drawback of using data from a 20 year period. Many advances in LT occurred over this extended period, which likely affected the findings. Dividing the time period arbitrarily into four epochs provided insight into the potential impact of these advances. In order to maintain the homogeneity, we have excluded donation after cardiac death, split liver and living donor recipients, who were directly related to advancements in the field of transplantation at the study period. It is also significant to note that there are a high number of recipients in the 'unknown' category, especially in the function condition category, which makes it difficult to draw a confident conclusion. This study also did not address the impact of introducing new immunosuppressive medications on graft and patient

survival.

In conclusion, this paper provides an overview of the landscape of LT in the United States from 1990 to 2009 in adults receiving first-time, deceased donor whole-organ LT. The landscape of donors and recipients undergoing transplantations in the United States has changed. Donor age and BMI, and the contribution of racial minorities, have increased. Recipient characteristics have also changed; we are transplanting recipients who are older, more deconditioned, more obese, and with changing causes of cirrhosis. Despite this, the long-term patient survival has improved over time. There is a potential for further improvement by understanding the leading causes of patient death and graft failure in the post-transplant period.

ARTICLE HIGHLIGHTS

Research background

The long-term impacts of clinical advancements and policy interventions over the past two decades on liver transplant outcomes have been poorly studied.

Research motivation

The motivation for such a study is the vast amount of large data that are mandatorily reported from 1989 by all transplant institutions in the United States, from which key observations could be made for future policy changes in transplantation.

Research objectives

The objective of this study was to compare trends in donor/recipient characteristics and outcomes over time. Subjects included 70,377 adult first-time recipients of whole-organ deceased donor liver grafts between 1990 and 2009 who were followed up until September 2013.

Research methods

Descriptive statistics were used to describe donor/recipient characteristics and transplant outcomes. Statistical comparisons between periods were performed using χ^2/Fischer's Exact test (categorical variables) and *t*-tests/Mann-Whitney *U* test (continuous variables). Univariate descriptive statistics/survival data were generated using Kaplan-Meier curves. Cox Proportional Hazards models were used for regression analyses of patient and graft survival.

Research results

Mean age (years), body mass index (BMI) (kg/m^2), and proportion males were, respectively, 39.1 (± 17.4), 25.9 (± 5.7) and 60.3 for donors, and 51.3 (± 10.5), 27.7 (± 5.6), and 64.4 for recipients. Donor and transplantation rates differed between racial/ethnic groups. Overall survival at 1, 3, 5, 10, 15, and 20 years was 87.3%, 79.4%, 73.6%, 59.8%, 46.7%, and 35.9%, respectively. The 2005-2009 cohort had better patient and graft survival than the 1990-1994 cohort overall [HR 0.67 (0.62-0.72) and 0.66 (0.62-0.71)] and at five years [HR 0.73 (0.66-0.80) and 0.71 (0.65-0.77)].

Research conclusions

The key findings were that despite changes in donor quality, recipient characteristics, and declining functional status among transplant recipients, overall patient survival is superior and post-transplant outcomes continue to improve. The long duration that this study encompassed involving the entire United States transplant institutions data has not been previously evaluated.

Research perspectives

This is the first study to show that over time, despite transplanting high-risk recipients and utilizing high-risk deceased donors, transplant outcomes are getting better with the accumulation of experience. Future studies involving more specified liver transplant groups (such as transplant for hepatitis *vs* non-alcoholic steatohepatitis *vs* Laennec cirrhosis) would give insight into long-term outcomes within the category of end-stage liver disease.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Treatment with plasmapheresis, immunoglobulins and rituximab for chronic-active antibody-mediated rejection in kidney transplantation: Clinical, immunological and pathological results

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Abstract

AIM

To evaluate the role of a therapeutic regimen with plasma exchange, intravenous immunoglobulins and rituximab in chronic-active antibody-mediated rejection (cAMR) settings.

METHODS

We compared 21 kidney transplant recipients (KTRs) with a diagnosis of cAMR in a retrospective case-control analysis: nine KTRs treated with plasmapheresis, intravenous immunoglobulins and rituximab (PE-IVIG-RTX group) *vs* 12 patients (control group) not treated with antibody-targeted therapies. We examined kidney survival and functional outcomes 24 mo after diagnosis. Histological features and donor-specific antibody (DSA) characteristics (MFI and C1q-fixing ability) were also investigated.

RESULTS

No difference in graft survival between the two groups was noted: three out of nine patients in the PE-IVIG-RTX group (33.3%) and 4/12 in the control group (33.3%) experienced loss of allograft function at a median time after diagnosis of 14 mo (min 12-max 18) and 15 mo (min 7-max 22), respectively. Kidney functional tests and proteinuria 24 mo after cAMR diagnosis were also similar in both groups. Only microvascular inflammation (glomerulitis + peritubular capillaritis score) was significantly reduced after PE-IVIG-RTX in seven out of eight patients (87.5%) in the PE-IVIG-RTX group (median score 3 in pre-treatment biopsy *vs* 1.5 in post-treatment biopsy; $P = 0.047$), without any impact on kidney survival and/or DSA characteristics. No functional or histological parameter at diagnosis was predictive of clinical outcome.

CONCLUSION

Our data showed no difference in the two year post-treatment outcome of kidney grafts treated with PE-IVIG-RTX for cAMR diagnosis, however there were notable improvements in microvascular inflammation in post-therapy protocol biopsies. Further studies, especially involving innovative therapeutic approaches, are required to improve the management and long-term results of this severe condition.

Key words: Chronic-active antibody-mediated rejection; Kidney transplantation; Donor-specific antibody; Rituximab

Core tip: Chronic-active antibody-mediated rejection (cAMR) is one of the major causes of poor long-term outcome in kidney transplantation, with no effective treatments currently available. We retrospectively compared 21 kidney transplant recipients with a diagnosis of cAMR, nine treated with plasmapheresis, intravenous immunoglobulins and rituximab *vs* 12 patients not treated with antibody-targeted therapies. Our data showed improvement in microvascular inflammation in post-therapy protocol biopsies without differences in functional outcomes at 24 mo, suggesting the lack of a prompt and marked effect of this therapeutic protocol. Further studies are required to improve the management and long-term results of this severe condition.

Mella A, Gallo E, Messina M, Caorsi C, Amoroso A, Gontero P, Verri A, Maletta F, Barreca A, Fop F, Biancone L. Treatment with plasmapheresis, immunoglobulins and rituximab for chronic-active antibody-mediated rejection in kidney transplantation: Clinical, immunological and pathological results. *World J Transplant* 2018; 8(5): 178-187 Available from: URL: http://www.wjgnet.com/2220-3230/full/v8/i5/178.htm DOI: http://dx.doi.org/10.5500/wjt.v8.i5.178

INTRODUCTION

Chronic-active antibody-mediated rejection (cAMR) due to de novo or pre-formed donor specific antibody (DSA) is currently considered the main cause of long-term allograft losses[1,2].

From the first pilot test with intravenous immunoglobulins (IVIG) and rituximab (RTX) reported by Billing *et al*[3], based on the aim of reducing or eliminating DSA, some authors antagonized their detrimental effects on the graft and proposed different therapeutic regimens for cAMR treatment. All of these protocols were derived from previous experience using acute antibody-mediated rejection and desensitization protocols, and mainly consisted of steroids, plasma exchange (PE), IVIG and RTX in various modalities[4-7]. More recently, bortezomib and eculizumab were also proposed[8-10].

Specifically, an antibody-directed treatment combining high-dose IVIG and RTX showed beneficial effects [reduction in allograft losses and/or stabilization of glomerular filtration rate (GFR)] in some patients with cAMR[3-5,11], but these positive results have now been partially questioned[12-15].

The role of functional and histological parameters (*i.e.*, GFR proteinuria at diagnosis, microvascular inflammation) in predicting response to antibody-targeted therapy has also been evaluated[6,16].

In spite of the aforementioned studies, the question of when these protocols should be adopted (in all patients or in only specific histopathological and functional settings) is still open.

In our Transplantation Center, we adopted a thera-

peutic protocol from 2011 that includes PE, IVIG and RTX in patients with a diagnosis of cAMR. In this paper, we compare, in a retrospective case-control analysis, nine patients treated with a combination of PE, IVIG and RTX (PE-IVIG-RTX group) for cAMR with a historical cohort of 12 kidney transplant recipients (KTRs) (control group). These control patients displayed similar histological and clinical profiles to the experimental patients, however they were not treated with antibody-targeted therapies. The primary outcome of our analysis was the difference in graft survival at 12 and 24 mo following diagnosis. Renal functional tests (including proteinuria), changes in histological features and/or DSAs-MFI, and C1q-binding ability were considered as secondary endpoints.

MATERIALS AND METHODS

Twenty-one adult KTRs with a diagnosis of cAMR according to the BANFF 2015 criteria (see Histology section) were included in this retrospective study. These 21 patients included nine with a consecutive diagnosis of cAMR from January 1, 2011 to December 31, 2014 who were treated with PE, IVIG and RTX (PE-IVIG-RTX group), and 12 KTRs with the same consecutive diagnosis performed in the period between January 2009 and December 2012 (control group). In that early period, antibody-targeted therapies were not currently adopted, or patients did not give their consent to these therapies.

At the time of diagnosis, patients were treated with a CNI-based immunosuppression (28.6% Cyclosporine A, 71.4% Tacrolimus, equally distributed into two groups), with Mycophenolate Mofetil/Mycophenolic Acid (77.8% in the PE-IVIG-RTX group and 66.7% in the control group) or an mTOR inhibitor drug (11.1% in the PE-IVIG-RTX group and 37.3% in the control group). Azathioprine was used only in one patient in the PE-IVIG-RTX group, and 77.8% of patients in the PE-IVIG-RTX group *vs* 66.7% in the control group were treated with steroids, respectively.

After cAMR diagnosis, maintenance therapy was reinforced in both groups by either introducing MMF and/or steroids, (with contemporary suspension of the mTOR inhibitor drug, if used) or switching from Cyclosporine A to Tacrolimus.

The PE-IVIG-RTX schedule was defined as follows: (1) Four or five PE (one plasma volume removal and 5% Albumin or plasma infusion) sessions in the first two weeks, (2) subsequent high-dose 2 g/kg IVIG (in one or two days), and (3) intravenous RTX (375 mg/m^2, one dose) after IVIG. Three patients in both groups also received steroid boluses after diagnosis (4 mg/kg methylprednisolone, tapered in five to seven days with a total steroid dose of about 1.5 g). One patient in the PE-IVIG-RTX group received a second RTX dose (375 mg/m^2) because of a concomitant diagnosis of membranous nephropathy.

Renal function was measured by serum creatinine (sCr) and GFR (estimated using the Cockroft-Gault formula). Patients were also tested repeatedly pre-transplantation for anti-HLA antibodies using the panel reactive lymphocytotoxicity assay, and maximum values from this assay were considered for our analysis.

We obtained an informed consent about potential complications and adverse events from all treated patients.

All biopsies were performed for cause, *i.e.,* in case of a significant and/or unexplained increase of serum creatinine > 25% from baseline, proteinuria, or both. Biopsies were reviewed according to the Banff 2015 classification[17], and only patients with a diagnosis of cAMR meeting all the requested criteria were included in this study. These criteria are as follows: (1) Histologic evidence of chronic tissue injury (transplant glomerulopathy - expressed by a cg score > 0, and/or severe peritubular capillary basement membrane multilayering, and/or arterial intimal fibrosis of new onset; (2) evidence of antibody-endothelium interaction [C4d > 0 in paraffin sections of peritubular capillaries and/or microvascular inflammation (MVI) – expressed by a g + ptc score ⩾ 2, considering that in the presence of acute TCMR, borderline infiltrate, or infection, g must be ⩾ 1]; and (3) serologic evidence of DSAs. We also evaluated a chronicity score (ci + ct), as reported by other authors[18].

In the PE-IVIG-RTX group, we also performed a protocol kidney biopsy at a median time of ten months after therapy (as discussed below in the Results section) in order to assess histopathological improvement when present.

Sera were evaluated twice, at both the time of biopsy and after 12 mo. As discussed in our previous paper[19], we tested all sera with a Luminex platform and commercially-available SAB kits (LABScreen One Lambda, Canoga Park, CA, United States) in order to identify HLA Classes Ⅰ and Ⅱ IgG DSA. Sera were also studied with the C1qScreen (One Lambda) to assess DSA complement-fixing ability. The cut-off was set at the normalized MFI value of 1000 for both tests.

Statistical analysis

Statistical analysis was performed with SPSS (IBM SPSS Statistics, vers. 22.0.0). Continuous variables are presented, according to their distribution, as mean ± SD or as median (min-max). Inter-group differences were analysed with *t*-test or Mann-Whitney test, respectively. We expressed categorical variables as fractions, and Pearson's χ^2 or, for small samples, Fisher's exact test was adopted to compare groups. The odds ratios (OR) with 95%CI were used as a measure of relative risk. Survival analysis was performed with the Kaplan-Meier method, comparing groups with Log Rank test. Significance level (α) was set at $P < 0.05$ for all tests.

RESULTS

Baseline characteristics

The PE-IVIG-RTX and control groups are comparable

Table 1 Clinical and demographical data of PE-IVIG-RTX and control group *n* (%)

	PE-IVIG-RTX group (*n* = 9)	Control group (*n* = 12)	*P*-value
Recipient age at diagnosis, yr	47 (24-65)	52 (26-67)	0.234
Gender (M/F ratio)	5/4	8/4	0.604
Donor age, yr	58 (37-80)	49 (18-82)	0.203
Living donor transplantation	2/9 (22.2)	0/12 (0)	0.086
Previous transplants	1/9 (11.1)	3/12 (25)	0.422
Maximum PRA	0% (0-89)	27.5% (0-95)	0.061
Mismatches HLA A-B-DR, n	2 (1-4)	3 (1-4)	0.639
Previous episodes of acute rejection			
(acute AMR – ACR)	1/9 (11.1)-1/9 (11.1)	1/12 (8.3)-1/12 (8.3)	0.586
Immunosuppression: Induction[1]	9/9 (100)	10/12 (83.3)	0.198
Clinical data at diagnosis			
Time between transplantation and diagnosis of cAMR, mo	51 (21-108)	79 (20-258)	0.201
Serum creatinine, mg/dL	1.9 (1.2-3)	1.9 (0.9-3.7)	0.477
GFR[2], mL/min	55,4 (23.9-65.4)	42.35 (18.9-88.1)	0.887
Proteinuria, g/d	1.6 (1-4)	1.55 (0.3-7.3)	0.886

[1]All patients in both groups were treated with basiliximab except the two patients in control group who received only steroid induction. [2]GFR estimated by Cockroft-Gault formula. Data are expressed as median (min-max). GFR: Glomerular filtration rate; PRA: Panel reactive lymphocytotoxicity assay; AMR: Antibody-mediated rejection; ACR: Acute cellular rejection.

Table 2 Donor-specific HLA antibody specificity and C1q-fixing assessment in PE-IVIG-RTX and control groups at diagnosis *n* (%)

	PE-IVIG-RTX group (*n* = 9)	Control group (*n* = 12)	*P*-value
Class I	2/9 (22.2)	6/12 (50)	
Class II	5/9 (55.6)	2/12 (16.7)	0.166
Class I + II	2/9 (22.2)	4/12 (33.3)	
MFI at diagnosis[1]	9800 (2700 – 24400)	4500 (900-24700)	0.327
C1q-fixing DSA[1]	4/9 (44.4)	4/10[2] (40)	0.845

[1]Considering immunodominant antibody; [2]Two patients were not tested for serum unavailability. DSA: Donor-specific antibodies.

(*P* = NS) for the time between transplantation and cAMR diagnosis, age at diagnosis, donor age, immunosuppressive therapy (induction and maintenance), number of mismatches and previous episodes of acute rejection (acute AMR and acute cellular rejection). In addition, the evaluation of renal functional tests (sCr, GFR) and proteinuria showed no difference between the two groups at diagnosis (Table 1).

DSA findings

Two out of nine patients (22.2%) in the PE-IVIG-RTX group and 6/12 (50%) in the control group expressed antibodies towards class I HLA. In 5/9 (55.6%) and 2/12 (16.7%), respectively, only anti class II HLA antibodies were found. Two out of nine patients (22.2%) in the PE-IVIG-RTX group and 4/12 (33.3%) in the control group showed both anti-class I and anti-class II HLA DSA (*P* = 0.166 for the analysis of distribution) (Table 2).

Considering the immunodominant antibody (DSA with the higher MFI), the median MFI was similar between the two groups (9800 in the PE-IVIG-RTX group *vs* 4500 in the control group, *P* = 0.327). Additionally, C1q-fixing ability showed no difference in the two populations: 4/9 patients (44.4%) in the PE-IVIG-RTX group and 4/10 (40%) in the control group expressed a C1q-fixing DSA ability (two patients were not tested for serum unavailability).

Considering the whole population, the median MFI was higher in patients with C1q-fixing DSA (median 15000, min 4700 - max 24700) in comparison with patients with non-C1q-fixing DSA (median 3000, min 900 - max 13400; *P* = 0.010).

Histology at diagnosis

Assessing cAMR histological scores according to the BANFF 2015 criteria[17] at diagnosis, the two populations were comparable for all of the considered variables: chronic glomerulopathy (cg), glomerulitis (g), peritubular capillaritis (ptc), microvascular inflammation (MVI) score (g + ptc), interstitial inflammation (ci), C4d positivity, and C4d score. Only tubular atrophy (ct) was statistically different between the PE-IVIG-RTX and control groups (median score 0, min 0 - max 1 *vs* 1, min 0 - max 1, respectively; *P* = 0.04). This was in spite of the chronicity composite score (ci + ct), which was quite similar in both groups (1, min 0 - 3 in the PE-IVIG-RTX group *vs* 2, min 0 - max 3 in the control group; *P* = 0.831) (Table 3).

Graft survival

No difference in graft survival was noted 12 and 24 mo after cAMR diagnosis. At the end of the follow-up, five out of the nine patients in the PE-IVIG-RTX group (55.6%) and 7/12 (58.3%) in the control group had a functioning graft (Figure 1A). Three out of nine patients in the PE-IVIG-RTX group (33.3%) and 4/12 in the control group lost their allograft, at a median time after diagnosis of

Table 3 Analysis of Banff scores at diagnosis

	PE-IVIG-RTX group (n = 9)	Control group (n = 12)	P-value
Chronic glomerulopathy (cg)	2 (1-3)	1.5 (0-3)	0.792
Glomerulitis (g)	2 (1-3)	2 (0-3)	0.23
Peritubular capillaritis (ptc)	1 (0-2)	0.5 (0-3)	0.122
Microvascular inflammation (g + ptc)	3 (2-5)	2.5 (2-3)	0.219
Interstitial inflammation (ci)	1 (0-3)	1 (0-2)	0.624
Tubular atrophy (ct)	0 (0-1)	1 (0-1)	0.04
Chronicity score (ci + ct)	1 (0-3)	2 (0-3)	0.497
Arteriolar hyaline thickening (ah)	2 (0-3)	2 (0-3)	0.075
C4d+, n (%)	7/9 (77.8)	7/12 (58.3)	0.35
C4d score	2 (0-3)	1 (0-3)	0.831

Data are expressed as median (min-max).

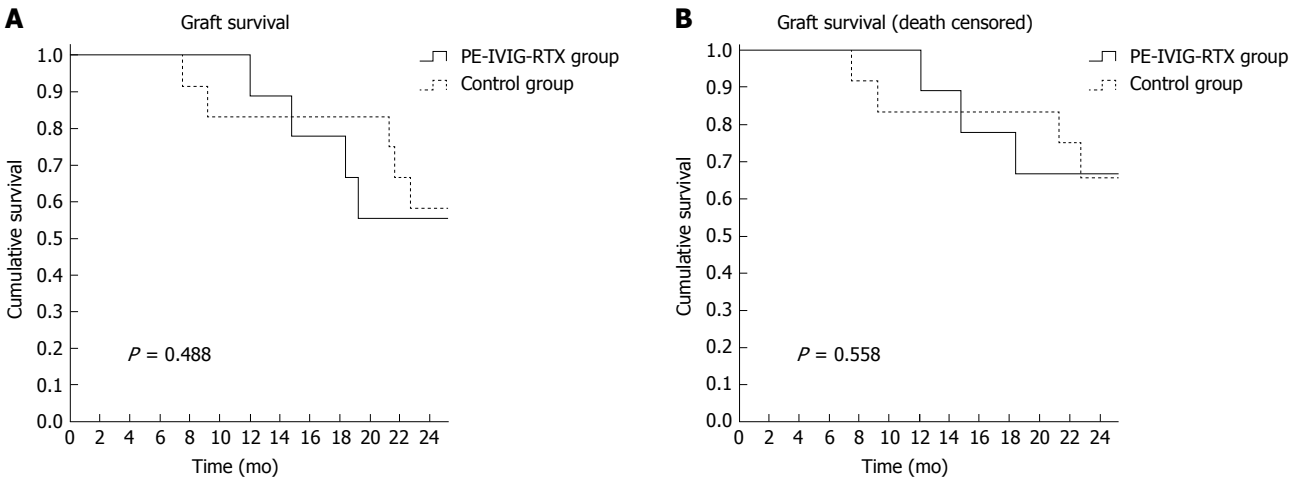

Figure 1 Survival Kaplan-Meier curves following diagnosis in PE-IVIG-RTX and control groups. A: Graft survival; B: Graft survival (death-censored).

14 mo (min 12 - max 18) and 15 mo (min 7 - max 22), respectively. One patient in both the PE-IVIG-RTX group and control group died with a functioning graft, and the adjusted death-censored graft survival remained similar between the PE-IVIG-RTX and control groups (Figure 1B, P = 0.558). Considering kidney functional tests (Figure 2A and B) and proteinuria (Figure 2C) in patients with a functioning graft, no difference was observed between the two groups at 12 and 24 mo (Figures 1 and 2).

Changes in pre- and post-treatment histology and DSA characteristics in the PE-IVIG-RTX group

Eight out of nine patients in the PE-IVIG-RTX group were subjected to a protocol biopsy at a median time of 10 mo (min 4 - max 20). We observed (Table 4) a significant reduction in MVI score in 7/8 (87.5%) of patients (median score 3 in pre-treatment biopsy *vs* 1.5 in post-treatment biopsy, P = 0.047); a trend in the reduction of C4d positivity was also noted (7/9 - 77.8% in pre-treatment biopsy *vs* 3/8 - 37.5% in post-treatment biopsy, P = 0.083), without differences in pre- and post-treatment cg and chronicity score (Tables 4 and 5).

Considering DSAs (Table 5), two out of nine patients (Pt. 4 and 6) had a negative post-treatment Luminex test. Despite the response in these two patients, considering the entire cohort, median MFI (9800 pre-treatment *vs* 8200 post-treatment; p = NS) and the percentage of C1q-fixing ability (4/9 - 44.4% pre-treatment *vs* 3/9 - 33.3% post-treatment) were unchanged after treatment.

Risk factors for allograft lost

To investigate whether some factors could be considered risk-prone for kidney failure, we analyzed both histological and clinical parameters at diagnosis.

Considering histopatological features (Table 6), no significant difference in cg and microvascular inflammation scores (g, ptc, g + ptc) or C4d positivity was observed between patients with functioning and non-functioning grafts at 24 mo in the PE-IVIG-RTX group, despite the fact that patients with non-functioning grafts showed a trend towards a more pronounced chronicity score at diagnosis (median 0.5 in patients with functioning grafts *vs* 2 in patients with non-functioning grafts; P = 0.29). Patients with a functioning graft in the control group showed a significantly higher g score (median 2 *vs* 1; P = 0.043) and lower ptc score (median 0 *vs* 1; P = 0.037), however the MVI score was quite similar in the two subgroups (median 2.5 in both subgroups; P = 0.727).

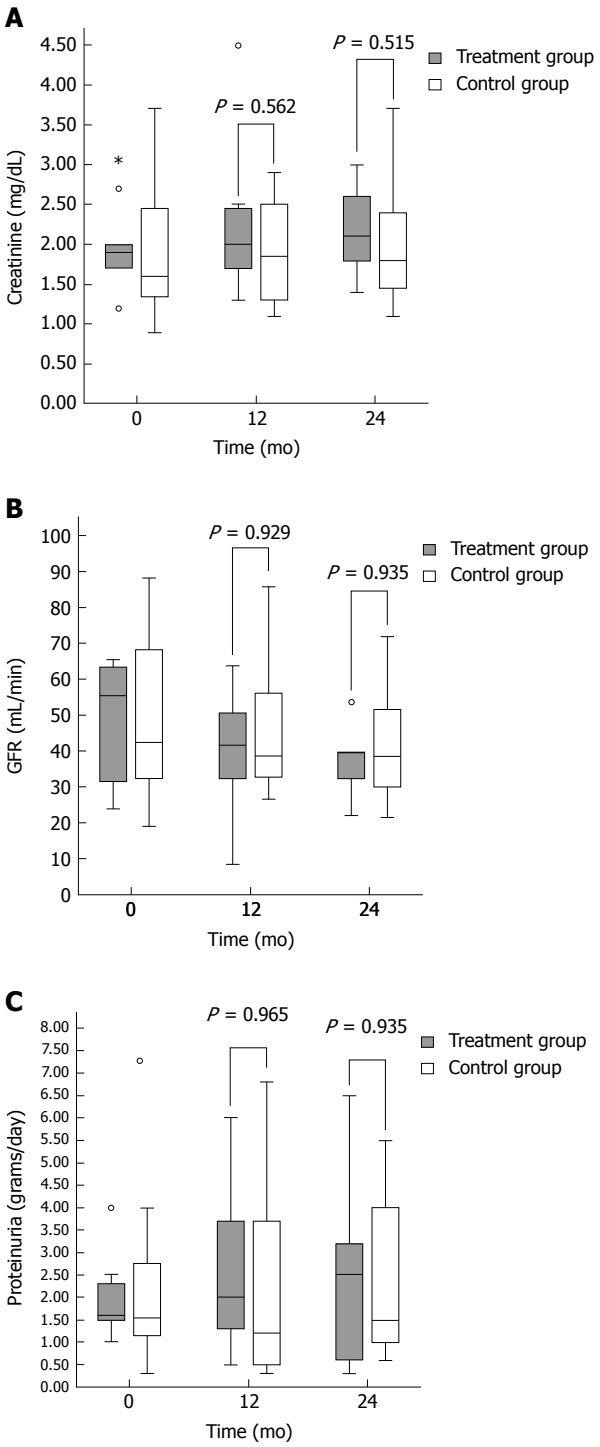

Figure 2 Serum creatinine, glomerular filtration rate and proteinuria at diagnosis. A: Serum creatinine at diagnosis (12 and 24 mo); B: Glomerular filtration rate at diagnosis (12 and 24 mo); C: Proteinuria at diagnosis (12 and 24 mo).

Kidney functional tests showed different patterns in the two groups (Table 7 and Figure 3). Data were examined at biopsy time. Proteinuria values were similar in all subgroups. sCr and GFR were comparable in patients with functioning and non-functioning grafts in the PE-IVIG-RTX group (Figure 3A and Table 7). On the contrary, functional data were significantly lower in patients with non-functioning *vs* functioning grafts at 24 mo in only the control group (median sCr 2.9 *vs* 1.4 mg/dL; P = 0.04 - median GFR 30.5 *vs* 52 mL/min; P = 0.04) (Figure 3B and Table 7).

The donor age was similar between failed and unfailed grafts in both groups (Table 7). Despite patients with functioning and non-functioning grafts in the PE-IVIG-RTX group, DSA characteristics were comparable for MFI and C1q-fixing ability. In the control group, patients with non-functioning grafts showed a trend towards a higher MFI and C1q-fixing ability when compared with patients who had functioning grafts (median MFI 13200 *vs* 4500; P = 0.533 - C1q-fixing DSA in 2/3 *vs* 2/7; P = 0.333) (Table 7).

Safety

In the 24 mo follow-up after cAMR diagnosis, two patients died: one in the control group due to pulmonary cancer, and one in the PE-IVIG-RTX group due to a cardiovascular complication that occurred 19 mo after diagnosis and cAMR treatment. Four patients in the PE-IVIG-RTX group experienced five clinically-relevant bacterial infections (all recovered after appropriate treatments). No such infections were recorded in the control group (P = 0.03; Odds ratio for bacterial infection in the PE-IVIG-RTX group = 4, 1.7-9.3) (Table 8).

DISCUSSION

In this study, we performed retrospective case-control analysis to study the mid-term clinical outcomes (24 mo) in 21 KTRs with a diagnosis of cAMR. We compared nine patients treated with PE, IVIG and RTX with a historical cohort of 12 patients who featured similar clinical and histological characteristics yet did not receive these antibody-targeted therapies.

Our data showed no clinical improvement after therapy with PE-IVIG-RTX, either in graft survival or in renal functional tests. In addition, proteinuria values were not influenced by the treatment.

On the contrary, upon evaluating histological features in protocol biopsies after PE-IVIG-RTX, microvascular inflammation (estimated by g + ptc score) was found to improve after PE-IVIG-RTX treatment. These data are quite similar to what was observed in the RITUX-ERAH trial in patients with acute AMR who were treated with PE, IVIG and steroids, either in association or not in association with RTX[18]. In Muller's paper[15], patients treated for cAMR with only Rituximab improved in g + ptc score after one year. Despite different histological settings (acute AMR in tge RITUX-ERAH trial *vs* cAMR in our study and in Muller *et al*[15]) and different follow-ups (12 mo in the RITUX-ERAH trial and in Muller *et al*[15] *vs* 24 mo in our study), the evidence for an improvement in renal histology was not supported by an amelioration in kidney survival at a mid-term follow-up.

As for DSA, a lowering effect was not obtained in all patients (the median value was unchanged after treatment). These data may suggest that, in the context of chronic antibody production, the B cell target for

Table 4 Analysis of Banff score changes in PE-IVIG-RTX group

	Pre PE-IVIG-RTX (*n* = 9)	Post PE-IVIG-RTX (*n* = 8)	*P*-value
Chronic glomerulopathy (cg)	2 (1-3)	2 (1-3)	0.705
Glomerulitis (g)	2 (1-3)	0.5 (0-2)	0.054
Peritubular capillaritis (ptc)	1 (0-2)	0.5 (0-2)	0.160
Microvascular inflammation (g + ptc)	3 (2-5)	1.5 (0-4)	0.047
Interstitial inflammation (ci)	1 (0-3)	1 (1-3)	0.480
Tubular atrophy (ct)	0 (0-1)	1 (0-2)	0.059
Chronicity score (ci + ct)	1 (0-3)	2 (1-5)	0.084
C4d+, *n* (%)	7/9 (77.8)	3/8 (37.5)	0.083
C4d score	2 (0-3)	0 (0-3)	0.102

Data are expressed as median (min-max).

Table 5 Analysis of MFI and C1q-fixing ability changes in PE-IVIG-RTX group

	Immunodominant DSA specificity	Pre PE-IVIG-RTX (*n* = 9)		Post PE-IVIG-RTX (*n* = 8)	
		MFI	C1q-fixing	MFI	C1q-fixing
Patient 1	DPw3	13400	No	8200	Yes
Patient 2	DQ9	3000	No	10300	No
Patient 3	A24	9800	Yes	21200	No
Patient 4	DR4	2700	No	0	No
Patient 5	B35	10300	No	2500	No
Patient 6	DQ5	7000	Yes	0	No
Patient 7	DR53	15000	Yes	24000	Yes
Patient 8	DQ7	24400	Yes	9000	Yes
Patient 9	DR51	7400	No	3400	No
Median (min-max)		9800 (2700-24400)[1]	4/9[2]	8200 (0-24000)[1]	3/9[2]

[1]*P* = 0.767 for difference in pre- and post-PE-IVIG-RTX MFI; [2]*P* = 1 for difference in pre- and post-PE-IVIG-RTX C1q-fixing ability.

Table 6 Analysis of Banff scores at diagnosis in functioning and non-functioning grafts at 24 mo

	PE-IVIG-RTX group (*n* = 9)		*P*-value	Control group (*n* = 12)		*P*-value
	Functioning graft (*n* = 6)	Non-functioning graft (*n* = 3)		Functioning graft (*n* = 8)	Non-functioning graft (*n* = 4)	
Chronic glomerulopathy (cg)	2.5 (1-3)	1 (1-3)	0.57	2.5 (1-3)	1 (0-2)	0.226
Glomerulitis (g)	2 (1-3)	1 (1-3)	0.472	2 (2-3)	1 (0-2)	0.043
Peritubular capillaritis (ptc)	1 (0-2)	1 (0-2)	0.829	0 (0-1)	1 (1-3)	0.037
Microvascular inflammation (g + ptc)	2.5 (2-5)	3 (2-3)	0.269	2.5 (2-3)	2.5 (2-3)	0.727
Interstitial inflammation (ci)	0.5 (0-2)	2 (1-2)	0.131	1 (0-1)	1 (1-2)	0.852
Tubular atrophy (ct)	0 (0-1)	0 (0-0)	0.667	1 (0-1)	1 (1-1)	0.255
Chronicity score (ci + ct)	0.5 (0-2)	2 (1-3)	0.29	1.5 (0-3)	2 (1-3)	0.807
C4d+, *n* (%)	5/7 (71.4)	2/3 (66.7)	0.583	3/8 (37.5)	4/4 (100)	0.071

Data are expressed as median (min-max).

PE-IVIG-RTX may elude the RTX effect and is likely represented by CD20-negative cells, as previously reported by other authors[12,20]. In two patients, we observed no DSA detection after treatment, although this was in association with highly different functional data (stabilization of GFR in one patient, graft failure in the other one).

No significant difference was noted in pre- and post-treatment C1q-fixing ability, or in DSA fixing complement ability at diagnosis. In addition, the clinical outcomes were similar at 24 mo. Our analysis is underpowered for the evaluation of DSA C1q-fixing ability as a marker of severe cAMR, which was positively reported in a larger cohort study[21]; however, we have recently observed in 35 KTRs with a transplant glomerulopathy diagnosis and de novo DSA (dnDSA) that a higher percentage of patients with dnDSA-associated transplant glomerulopathy was C1q-negative, and that the presence of C1q-fixing dnDSA did not significantly correlate with graft outcome[19].

We are aware that the lack of difference in the immunodominant DSA-MFIs before and after treatment may be due to technical limitations related to the "prozone" effect[22]. However, it is remarkable that the MFI titer in three patients increased after treatment and that in 6/9 it remained higher than 3000, a threshold

Table 7 Analysis of kidney functional tests, proteinuria, MFI and DSAs-C1q fixing ability at diagnosis in functioning and non-functioning grafts at 24 mo

	PE-IVIG-RTX group (n = 9)		*P*-value	Control group (n = 12)		*P*-value
	Functioning graft (n = 6)	Non-functioning graft (n = 3)		Functioning graft (n = 8)	Non-functioning graft (n = 4)	
Creatinine, mg/dL	1.75 (1.2-2.7)	2 (1.9-3)	0.167	1.4 (0.9-2.3)	2.9 (2.4-3.7)	0.04
GFR, mL/min	47.9 (31-65.4)	55.4 (23.9-63.8)	0.905	52 (34.5-88.1)	30.5 (18.9-33.6)	0.04
Proteinuria, g/d	1.55 (1.3-2.5)	1.8 (1-4)	0.905	1.7 (0.8-7.3)	1.1 (0.3-2.6)	0.154
Donor age, yr	61 (37-63)	44 (43-80)	0.796	50.5 (18-82)	48 (25-55)	0.799
MFI	11600 (2700-24400)	7400 (7000-10300)	0.714	4500 (900-19300)	13200 (1700-24700)	0.533
C1q-fixing DSA, n (%)	3/6 (50)	1/3 (33.3)	0.595	2/7(28.6)	2/3(66.7)	0.333

DSA: Donor-specific antibodies.

Table 8 Adverse events after cAMR diagnosis in the 24 mo follow-up (number of total events)

	PE-IVIG-RTX group (n = 9)	Control group (n = 12)
Infections		
Pyelonephritis and urinary tract infections	1	0
Gastrointestinal (diarrhea, ileitis)	2	0
Respiratory infection (bronchiolitis)	1	0
Acute cholecystitis	1	0
Cancers	0	2
Death	1	1

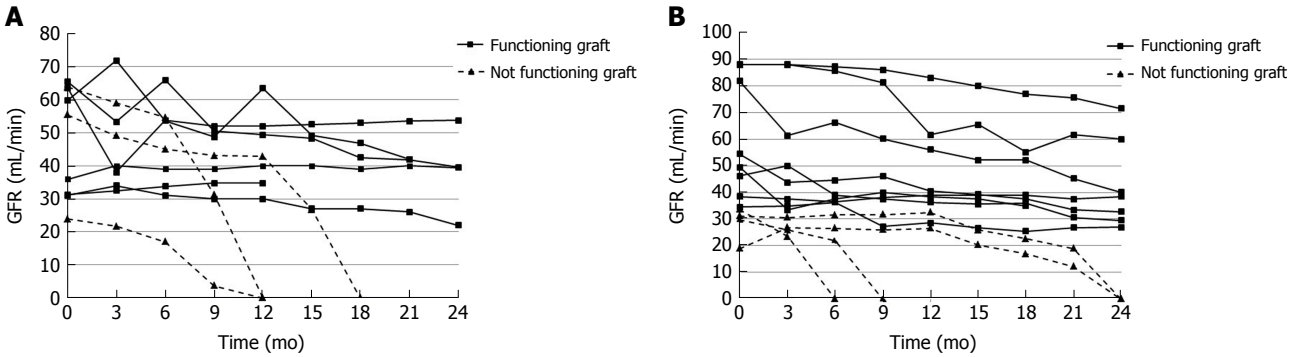

Figure 3 Glomerular filtration rate in functioning and non-functioning grafts at 24 mo and follow-up. A: PE-IVIG-RTX group; B: Control group.

value considered by several centers.

We also evaluated functional, histological and immunological parameters at diagnosis to detect potential risk factors for allograft loss. In the control group, we found a trend towards a higher DSA-MFI titer, C1q-fixing DSA positivity, a higher sCr, and a lower GFR. On the contrary, the histological findings at diagnosis showed no significant difference between failed and unfailed grafts at 24 mo in both groups.

Based of our analysis, we are unable to define any characteristics at diagnosis that influence prognosis. The goal of any study on this topic should be to identify a certain population who would benefit from therapy (in this case Rituximab associated with PE and IVIG). Unfortunately, no study has fulfilled this scope to the best of our knowledge[15,16]. The search for characteristics that label the population that would benefit from these therapies is even more important when we consider the significant risk associated with these therapies. In our study, we noted a significant increase in the bacterial infection rate in the PE-IVIG-RTX group (OR: 4, 1.7-9.3).

Upon comparing our results to the literature data, Bachelet *et al*[13] also reported no improvements in graft survival or renal functional tests in 21 patients with cAMR-associated severe transplant glomerulopathy who received IVIG and two doses of RTX. Similar outcomes (no differences in eGFR decline, increase of proteinuria, Banff scores at one year, or MFI of the immunodominant DSA) were also shown in a very recent randomized clinical trial evaluating efficacy and safety of IVIG combined with RTX in 25 patients with cAMR[14].

All these data are in contrast with previous evidence from Billing's paper, showing a GFR improvement or stabilization at 12 mo in four out of six pediatric patients who were IVIG and RTX treated[3]. A subsequent analysis of 20 pediatric patients, published by the same author,

reported a lower median GFR loss in the 24 mo follow-up after IVIG and RTX, compared with GFR loss in the 6 mo prior to treatment[23]. When also excluding differences between pediatric and adult KTRs, and the absence of a control group in the two studies by Billing *et al*[23], it is clear that a minor GFR-worsening might not result from a therapeutic effect, but instead represent the natural history of the disease and its early diagnosis.

In a retrospective analysis, Redfield *et al*[2] examined 123 patients with severe cAMR; Kaplan–Meier survival showed an association of steroids/IVIG (together or in combination with rituximab and/or Thymoglobulin) with better graft survival. However, the association between the addition of rituximab or Thymoglobulin to steroids/IVIG with better graft survival did not reach statistical significance.

We acknowledge the limitations of our study, which include the low numerosity, the retrospective design, and the absence of protocol biopsies in the control group. Nonetheless, a low number of treated subjects, the absence of a control group, and retrospective analysis can be found in most studies that involve treatment of this clinical condition[2,4,5,13]. Moreover, we also recognize that three patients in both groups were also treated with steroid boluses in low doses. This observation may be considered as a bias in interpretation due to a possible "positive" effect in the control group, however this may also be seen as a negligible aspect since two out of two of these patients lost their graft.

We recognize that protocol biopsies could have enlightened the question as to whether early lesions could be a marker for a better response to treatment. The absence of protocol biopsies in the control group precludes an adequate histological comparison between the populations. We are therefore able to compare the histopathological findings inside the treatment group, but we are unable to evaluate the progression of the chronic lesions in the control group. However, protocol biopsies are not a current practice for some centers, and cAMR is often diagnosed only after appearance of clinical abnormalities that trigger biopsy indication.

Regarding microvascular inflammation lesions, which are considered to be crucial for disease progression[6,24], we found a reduction in g + ptc score after treatment with PE-IVIG-RTX. One could speculate that if the amelioration of these lesions have a significant clinical impact, it could potentially be noted in a longer follow-up.

In conclusion, no guidelines about the therapeutic management of cAMR is currently available. Our data, along with the results of other groups[12-15], suggest the lack of a prompt and marked effect of a therapeutic protocol with PE, IVIG and RTX, despite good histological improvement (reduction in microvascular inflammation) in the majority of treated patients. It is possible that this treatment could have greater efficacy with a longer follow-up, or in a subset of patients not yet identified, as suggested by other authors[15,16]. Further prospective studies, especially involving innovative therapeutic approaches, are required to improve both the management and long-term results of this severe condition.

ARTICLE HIGHLIGHTS

Research background

Chronic-active antibody-mediated rejection (cAMR) due to de novo or pre-formed donor specific antibody (DSA) is now considered the most important cause of allograft losses. Treatment is focused on reducing or eliminating DSA, antagonizing their detrimental effects on the graft with different approaches, without available guidelines.

Research motivation

An antibody-directed treatment combining high-dose immunoglobulin and rituximab showed beneficial effects (reduction in allograft losses and/or stabilization of glomerular filtration rate) in some patients with cAMR, but these results have now been partially questioned. The role of functional and histological parameters (*i.e.*, GFR proteinuria at diagnosis, microvascular inflammation) in predicting response to antibody-targeted therapy is also a matter of debate.

Research objectives

To evaluate the role of a therapeutic regimen with plasma exchange, intravenous immunoglobulins and rituximab in cAMR settings. To identify in which cases these protocols should be adopted (in all patients or only in specific histopathological and functional settings).

Research methods

Retrospective case-control analysis in 21 kidney transplant recipients with a diagnosis of cAMR, 9 treated with plasmapheresis, intravenous immunoglobulins and rituximab and 12 patients not treated with antibody-targeted therapies. Primary outcomes were kidney survival and functional outcomes 12 and 24 mo after diagnosis. Histological features (according to BANFF 2015 criteria) and donor specific antibodies characteristics (MFI and C1q-fixing ability) were also evaluated.

Research results

No difference in graft survival was noted 12 and 24 mo after cAMR diagnosis. Three out of nine patients in the PE-IVIG-RTX group (33.3%) and 4/12 in the control group (33.3%) lost their allograft, at a median time after diagnosis of 14 mo (min 12 - max 18) and 15 mo (min 7 - max 22), respectively. Kidney functional tests (serum creatinine and eGFR) and proteinuria 24 mo after cAMR diagnosis were strictly similar in both groups. Microvascular inflammation (glomerulitis + peritubular capillaritis score) was significantly reduced after PE-IVIG-RTX in seven out of eight patients (87.5%) in the PE-IVIG-RTX group (median score 3 in pre-treatment biopsy *vs* 1.5 in post-treatment biopsy; P = 0.047), without any impact on kidney survival. Two out of nine patients had a negative post-treatment Luminex test. However, considering the entire cohort, the median MFI of immunodominant DSA (9800 pre-treatment *vs* 8200 post-treatment; P = NS) and the percentage of C1q-fixing ability (4/9 - 44.4% - pre-treatment *vs* 3/9 - 33.3% - post-treatment) were unchanged after treatment with PE-IVIG-RTX. No functional or histological parameter at diagnosis was predictive of clinical outcome.

Research conclusions

No clinical improvement after therapy with PE-IVIG-RTX, either in graft survival or in renal functional tests (serum creatinine, eGFR, proteinuria) was observed. In addition, the reduction in the MVI score was not supported by an amelioration in kidney outcomes. Considering our results, we are unable to define any functional or histological characteristics at diagnosis that could influence prognosis.

Research perspectives

Future prospective studies that involve innovative therapeutic approaches, longer follow-ups and protocol biopsies are required to: (1) Improve the management and long-term results of this severe condition; and (2) identify a

certain population who would benefit from therapy.

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ORIGINAL ARTICLE

Randomized Clinical Trial

Clinical features and determinants of VO_{2peak} in *de novo* heart transplant recipients

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Abstract

AIM

To study exercise capacity and determinants of early peak oxygen consumption (VO_{2peak}) in a cohort of *de novo* heart transplant (HTx) recipients.

METHODS

To determine possible central (chronotropic responses, cardiopulmonary and hemodynamic function) and peripheral factors (muscular exercise capacity and body composition) predictive of VO_{2peak}, a number of different measurements and tests were performed, as follows: Cardiopulmonary exercise testing (CPET) was performed mean 11 wk after surgery in 81 HTx recipients > 18 years and was measured with breath by breath gas exchange on a treadmill or bicycle ergometer. Metabolic/respiratory measures include VO_{2peak} and VE/VCO_2 slope. Additional measures included muscle strength testing, bioelectrical impedance analysis, echocardiography, blood sampling and health-related quality of life. Based on the VO_{2peak} (mL/kg per minute) median value, the study population was divided into two groups defined as a low-capacity group and a high-capacity group. Potential predictors were analyzed using multiple regression analysis with VO_{2peak} (L/min) as the dependent variable.

RESULTS

The mean ± standard deviation (SD) age of the total study population was 49 ± 13 years, and 73% were men. This *de novo* HTx cohort demonstrated a median VO_{2peak} level of 19.4 mL/kg per min at 11 ± 1.8 wk post-HTx. As compared with the high-capacity group, the low-capacity group exercised for a shorter time, had lower maximal ventilation, O_2 pulse, peak heart rate and heart rate reserve, while the VE/VCO_2 slope was higher. The low-capacity group had less muscle strength and muscular exercise capacity in comparison with the high-capacity group. In order of importance, O_2 pulse, heart rate reserve, muscular exercise capacity, body mass index, gender and age accounted for 84% of the variance in VO_{2peak} (L/min). There were no minor or major serious adverse events during the CPET.

CONCLUSION

Although there is great individual variance among *de novo* HTx recipients, early VO_{2peak} measures appear to be influenced by both central and peripheral factors.

Key words: Cardiopulmonary exercise testing; Early VO_{2peak}; *De novo* heart transplant; Health related quality of life; Muscle strength

Core tip: This *de novo* heart transplant (HTx) cohort demonstrated a median peak oxygen consumption (VO_{2peak}) level of 19.4 mL/kg per min at 11 ± 1.8 wk post-HTx, which is comparable to what is shown in maintenance HTx recipients. VO_{2peak} in this study was determined by both central and peripheral factors. The strongest predictors were O_2 pulse, heart rate reserve and muscular exercise capacity. Maximal exercise testing provides valuable information for clinical use and future prognosis and can be safely performed as early as 11 wk post-HTx.

Rolid K, Andreassen AK, Yardley M, Bjørkelund E, Karason K, Wigh JP, Dall CH, Gustafsson F, Gullestad L, Nytrøen K. Clinical features and determinants of VO_{2peak} in *de novo* heart transplant recipients. *World J Transplant* 2018; 8(5): 188-197 Available from: URL: http://www.wjgnet.com/2220-3230/full/v8/i5/188.htm DOI: http://dx.doi.org/10.5500/wjt.v8.i5.188

INTRODUCTION

Cardiac rehabilitation, including exercise training to improve exercise capacity and health-related quality of life (HRQoL) is recommended after heart transplant (HTx)[1], but there are no clear and specific guidelines for how, how often or at what intensity exercise training should be performed.

Exercise capacity is often severely reduced shortly after HTx with peak oxygen consumption (VO_{2peak}) levels reported to be between 9.2 and 19.7 mL/kg per min[2-12]. However, early measurement of VO_{2peak} is not routine in most centers. VO_{2peak} is the gold standard to objectively assess functional limitation and give an assessment of the integrative physiology involving cardiovascular, pulmonary, muscular, cellular and oxidative systems[13,14]. It has also been reported that VO_{2peak} is a strong predictor for survival in HTx recipients[15,16]. In studies of maintenance HTx patients, VO_{2peak} seems to be determined by both central (chronotropic incompetence, reduced stroke volume and cardiac output, impaired systolic and diastolic function, pulmonary dysfunction) and peripheral factors (diminished skeletal muscular capacity)[1,17-19]. Other factors, like donor characteristics, diagnosis and deconditioning before transplantation may also be associated with reduced exercise capacity after HTx[18]. However, we have recently reported that the most important variables predicting VO_{2peak} in maintenance HTx patients are mostly of peripheral origin[20,21]. In *de novo* HTx patients, only two studies exist (n = 43[6] and n = 24[12]), which report limiting factors for VO_{2peak}. These studies indicate that both central and peripheral

factors could be involved in the early phase, but the knowledge is scarce and thus, a better understanding of factors that are associated with peak exercise shortly after HTx could guide clinicians and physiotherapist for more individualized therapy and specific exercise recommendations.

We hypothesized that both central and peripheral factors are associated with reduced exercise capacity in *de novo* HTx recipients. In the present study, we performed cardiopulmonary exercise testing (CPET) in a cohort of *de novo* HTx patients with the aim to determine clinical, hemodynamic and peripheral factors that contribute to the reduced exercise capacity.

MATERIALS AND METHODS

Patients and settings

This study was conducted in three centers in Scandinavia (Oslo, Gothenburg and Copenhagen). Altogether, 155 *de novo* HTx patients were assessed for eligibility. Of these, 72 were excluded for various reasons: did not meet inclusion criteria (cognitive issues, physical disabilities, medical complications, language barriers, contagion, no physical therapist available) (n = 43); were not motivated (n = 15); logistic reasons (n = 14). In addition, two were excluded after they had given their consent, one due to medical complications and one withdrawal. A total of 81 patients underwent CPET. The study was approved by the South-East Regional Committee for medical and health research ethics in Norway and the Committee for medical and health research ethics in Sweden and Denmark. The study was conducted in accordance with the recommendations in the Helsinki Declaration.

The current study is based on the baseline data from an ongoing randomized controlled trial (RCT): The High-intensity Interval Training in de novo heart Transplant recipients in Scandinavia (HITTS) study. The design and rationale of this study is described elsewhere[22]. In short, the RCT compares the effect of a 9-mo long two-armed intervention: High-intensity interval training versus moderate intensity continuous training.

Inclusion criteria

The inclusion criteria were: Clinically stable HTx recipients approximately 8-12 wk after HTx; Age > 18 years; Both sexes; Receiving immunosuppressive therapy according to local protocols; Patient willing and able to give written informed consent for study participation, and motivated to participate in the study for nine months.

Measurements

The primary endpoint, VO_{2peak}, was measured on a treadmill or a bicycle ergometer applying an individualized protocol with an incremental workload until exhaustion[23]. The Norwegian populations were tested on a treadmill, except for four subjects, who could not comply and were tested on a bicycle ergometer. All patients in Sweden and Denmark were tested on a bicycle, which is the customary form for exercise testing in these countries. The variables from the CPET have been described previously[22]. Common heart rate (HR) variables and abbreviations used in this study were: Peak heart rate (HR_{peak}); Percentage of age-predicted maximum HR (% HR_{max}) = [(HR_{peak}/220 - age) × 100]; Chronotropic response index (CRI) = (HR_{peak} -HR_{rest})/(220 - age/HR_{rest}); Heart rate reserve ($HR_{reserve}$) = HR_{peak} - HR_{rest}; $HR_{recovery}$ (difference between HR_{peak} and HR after 30 s, 1, 2, 3 and 4 min).

Secondary endpoints

Potential variables influencing VO_{2peak}, such as lung function, maximum muscle strength and muscular exercise capacity, bioelectrical impedance analysis, echocardiography, blood samples and HRQoL were measured.

Lung function

Different lung function variables were measured in relation to the CPET, both at rest and during exercise. Spirometry was performed at rest before CPET: Peak expiratory flow (PEF), forced expiratory volume at 1 min (FEV1), forced vital capacity (FVC) during exercise, maximum ventilation (Vmax) and ventilatory efficiency (VE/VCO_2)[14] were calculated.

Muscle strength and muscular exercise capacity

Muscle strength and muscular exercise capacity in the quadriceps and hamstring muscle groups were measured isokinetically. Five repetitions at an angular velocity of 60°/s were performed when measuring muscle maximal strength. For the muscular exercise capacity, 30 isokinetic contractions at 240°/s were performed. In the analyses, we used the bilateral sum of m. quadriceps and m. hamstrings[20,22].

Bioelectrical impedance analysis

Bioelectrical impedance is a simple and fairly valid method to measure body composition[24]. In this study, the Tanita (Tanita, Arlington Heights, IL, United States) system was used to measure body fat, body water, muscle mass, bone mass, visceral fat, metabolic age and basal metabolic rate.

Echocardiography

Standard Doppler-echocardiography was performed by experienced technicians and assessed by cardiologists to determine myocardial size and function.

Biochemistry

All patients underwent blood sampling in the morning in a fasting state. Two EDTA tubes were collected, inverted ten times and immediately placed on ice. Samples were centrifuged within 20 min. Plasma was transferred into four vials and frozen at -80 ℃. One serum tube was collected and placed in room temperature for 60 to 120 min for coagulation before centrifugation. The sample was then transferred into two vials and frozen at -80 ℃.

Plasma concentrations of N-terminal pro brain natriuretic peptide (NT-proBNP) was determined using an electrochemiluminescence immunoassay on a Modular platform (Roche Diagnostica, Basel, Switzerland), high sensitive C-reactive protein (hs-CRP) levels using a particle-enhanced, high-sensitive immunoturbidimetric assay (hsCRP, Tina-Quant CRP Gen.3), and high-sensitive troponin T (hs-TnT) was measured by electrochemiluminescence immunoassay (hsTnT, Elecsys Troponin T high sensitive, Roche Diagnostics).

HRQoL and symptoms of anxiety and depression

HRQoL was measured with the generic questionnaire short form-36, version 2 (SF-36v2)[25]. The results were transformed into norm-based scores on a standardized scale with a mean of 50 and a standard deviation (SD) of 10[25]. Subscales were aggregated into two sum-scores; physical component summary (PCS) and mental component summary (MCS). Symptoms of anxiety and depression were measured with the Hospital Anxiety and Depression Scale (HADS)[26]. The values were dichotomized using a cut-off score ⩾ 8, which was considered to represent symptoms of depression or anxiety.

Statistical analysis

All data were analyzed using IBM SPSS, version 23 and version 25.0 (IBM corporation, United States). Continuous data are expressed as mean ± SD or median first quartile (Q1), third quartile (Q3), and categorical data are presented as percentages. Patients were divided by the median VO_{2peak} (mL/kg per min) value into a low-capacity group (⩽ 19.4) and a high-capacity group (> 19.4). Between-group comparisons were performed using two independent samples t or Mann Whitney *U* test. χ^2 or *F* were used for categorical data, where appropriate. Bivariate relationships were explored and univariate regression analyses were performed with potential predictors (Tables 1 and 2). To identify the degree of association with VO_{2peak}, all relevant variables with $P < 0.05$ and other potential variables from the univariate analyses of linear regression were selected for further multiple regression analyses. VO_{2peak} (L/min), adjusted for age, sex and BMI, was used as the dependent variable. The final model was built using a series of multiple regression analyses with the enter method (Table 3). Assumptions were checked for normality and linearity.

RESULTS

Clinical characteristics

The mean ± SD age of the total study population was 49 ± 13 years, and 73% were men. Patients were on average 11.1 ± 1.8 wk after HTx. The mean VO_{2peak} was 20.4 mL/kg per min, which is 56% of expected compared to the reference values described in the 9th edition of the American College of Sports Medicine's (ACSM) guidelines for exercise testing and exercise prescription[27]. Further demographic and clinical characteristics are presented group-wise in Tables 1 and 2.

Compared to the high-capacity group, the low-capacity group was characterized by a higher body mass index (BMI) and a higher fat content, they were more often ex-smokers, had lower PCS score, had less muscle strength and muscular exercise capacity, had lower FEV1, FVC and ejection fraction (EF) as measured by echocardiography. The low-capacity group more often used beta blockers and less mycophenolate, had higher NT-proBNP, hs-TnT, triglycerides and lower hemoglobin (Hgb). Duration of heart failure before HTx, primary diagnosis, donor age, ischemic time, rejection scores, MCS score and HADS depression score were similar between the two groups (Table 1).

Exercise variables

Exercise variables are shown in Table 2. As compared with the high-capacity group, the low-capacity group exercised for a shorter time, had lower maximal ventilation, O_2 pulse, HR_{peak} and $HR_{reserve}$, while VE/VCO_2 slope was higher (Table 2). The respiratory exchange ratio (RER), rated perceived exertion (RPE) and blood pressure responses were similar between the groups (Table 2).

Predictors of VO_{2peak}

Univariate predictors of VO_{2peak} are shown in Tables 1 and 2. There were strong correlations ($P < 0.001$) between VO_{2peak} and $HR_{reserve}$, O_2 pulse and muscular exercise capacity (Figures 1-3). In multiple regression analyses, O_2 pulse, $HR_{reserve}$, muscular exercise capacity, BMI, gender and age accounted for 84% of the variance in VO_{2peak} (L/min). Only O_2 pulse, $HR_{reserve}$ and muscular exercise capacity were important determinants in the final model ($P < 0.001$, $P < 0.001$ and $P < 0.015$, respectively). Other potential predictors were also analyzed in the multiple regression analyses, but these did not reach statistical significance. VO_{2peak} (L/min) was chosen as the dependent variable in order to be able to adjust for and see the impact of age, gender and BMI directly, as the VO_{2peak} (mL/kg per min) variable is already weight-based.

Safety

All measurements performed in this study, including the CPET and muscle strength testing, were completed without any minor or serious adverse events.

DISCUSSION

The main findings in this study were that *de novo* HTx patients display reduced exercise capacity compared with a general population: The reference population in ACSM[27] and Astrand[28], and that maximal exercise capacity was determined by both central (O_2 pulse and $HR_{reserve}$) and peripheral factors (muscular exercise capacity) (Table 3 and Figures 1-3). Furthermore, CPET can be safely performed as early as an average of 11 wk after HTx and is a valuable basis for individual tailoring of the further rehabilitation program.

Table 1 Clinical characteristics and health-related quality of life of the study population

[6]*N* = 55-81	Total	Low-capacity group (*n* = 41) VO_{2peak} ≤ 19.4 mL/kg per min	High-capacity group (*n* = 40) VO_{2peak} > 19.4 mL/kg per min	t (*P*-value)	Univariate regression Standardized coefficient Beta [95%CI], *P* VO_{2peak} (L/min)	[7]R^2
Clinical characteristics						
Sex (% men)	73%	66	80	0.152[1]	-0.45 [-0.61, -0.23], < 0.001	0.2
Age (yr)	49 ± 13	51 ± 11	46 ± 15	0.08	-0.19 [-0.01, -0.001], 0.093	0.04
Body mass index	25.3 ± 3.7	26.3 ± 3.4	24.2 ± 3.8	0.01	0.28 [0.007, 0.056], 0.013	0.08
Body fat (%)	25.1 ± 8.7	29.0 ± 8.3	21.0 ± 7.1	<0.001	-0.34 [-0.03, -0.006], 0.003	0.11
Donor age (yr)	34 (24, 49)	37 (27, 48)	33 (23, 52)	0.825[2]	0.09 [-0.004, 0.009], 0.447	0.01
Ischemic time (min)	210 (95, 237)	215 (99, 249)	185 (87, 227)	0.072[2]	-0.01[-0.001, 0.001], 0.938	8.2^{-5}
Weeks after HTx	11 ± 1.8	11.3 ± 2	10.9 ± 1.5	0.307	-0.001 [-0.05, 0.05], 0.990	2.0^{-5}
Duration of HF prior to HTx (yr)	4 (1.5, 10)	4 (1.5, 10.5)	4 (1.0, 9.3)	0.718[2]	-0.05 [-0.02, 0.01], 0.681	0.002
Time on HTx waiting list (d)	75 (24, 193)	96 (29, 227)	47 (12, 131)	0.06[2]	-0.14 [-0.001, 1.5-4], 0.202	0.02
Rejections grade 1-2 (% yes)	45	48	43	0.653[1]	0.09 [-0.11, 0.27], 0.408	0.01
VO_{2peak} preHTx (mL/kg per min)	11.6 ± 3.3	11.1 ± 3	12.1 ± 3.5	0.248	0.03 [-0.032, 0.039], 0.826	0.001
LVAD (% yes)	15	22	8	0.067[1]	-0.14 [-0.43, 0.097], 0.211	0.02
Preoperative IABP/ECMO (% yes)	16	15	18	0.725[1]	0.05 [-0.20, 0.32], 0.637	0.003
Postoperative IABP/ECMO (% yes)	10	15	5	0.264[3]	-0.26 [-0.68, -0.066], 0.018	0.07
Etiology HF (%)				0.138[3]		
Cardiomyopathy	65	56	75			
Ischemic heart disease	25	34	15			
Other	10	10	10			
Smoking (%) no/yes/ex-smoker	49/0/51	34/0/66	65/0/35	0.005[1]	-0.19 [-0.34, 0.03], 0.100	0.03
24 h ambulatory blood pressure						
Overall systolic BP	133 ± 12	133 ± 13	132 ± 10	0.672		
Overall diastolic BP	81 ± 7	80 ± 8	82 ± 7	0.493		
Medication (%)						
Ciclosporin	70	63	78	0.165[1]		
Tacrolimus	28	32	23	0.352[1]		
Everolimus	34	43	25	0.098[1]		
Mycophenolate	90	81	100	0.005[3]	0.29 [0.10, 0.71], 0.009	0.08
Prednisolone	100	100	100			
Beta-blocker	28	40	15	0.012[1]	-0.19 [-0.39, -0.03], 0.086	0.04
Calcium blocker	25	25	25	1.000[1]		
ACE inhibitors	3	3	3	1.000[3]		
AII-blocker	9	13	5	0.263[3]		
Diuretics	79	80	78	0.785[1]		
Statins	99	98	100	1.000[3]		
Blood samples						
TG (mmol/L)	1.7 (1.3, 2.5)	2.1 (1.5, 2.8)	1.5 (1.1, 2.2)	0.013[2]	-0.24 [-0,19, -0.002], 0.045	0.06
LDL (mmol/L)	2.9 ± 1.0	3.0 ± 1.2	2.9 ± 0.7	0.416	0.12 [-0.05, 0.15], 0.308	0.01
HDL (mmol/L)	1.5 ± 0.5	1.5 ± 0.5	1.6 ± 0.5	0.432	0.04 [-0.16, 0.22], 0.755	0.001
Cholesterol (mmol/L)	5.1 ± 1.3	5.3 ± 1.5	5.0 ± 1.0	0.329	0.03 [-0.07, 0.09], 0.830	0.001
Hemoglobin (g/dL)	11.8 ± 1.7	11.3 ± 1.9	12.2 ± 1.4	0.017	0.38 [0.042, 0.15], 0.001	0.14
hs-CRP (mg/L)	2.3 (1.0, 6.1)	2.7 (1.3, 6.7)	1.6 (0.6, 3.9)	0.052[2]	-0.17 [-0.015, 0.002], 0.125	0.03
NT-proBNP (ng/L)	968.3 (625.8, 1680.8)	1348.9 (765.4,2006.4)	790.7 (522.2, 1351.0)	0.005[2]	-0.36[-2.7E-4, -6.5^{-5}], 0.002	0.13
hs-TnT (ng/L)	32.5 (20.0, 61.8)	42.0 (27.8, 66.7)	24.0 (18.0, 50.8)	0.009[2]	-0.18 [-0.005, 0.001], 0.128	0.03
HbA1c (%)	5.6 ± 0.8	5.8 ± 0.9	5.4 ± 0.7	0.038	-0.15 [-0.19, 0.04], 0.213	0.02
Glucose (mmol/L)	5.9 ± 1.8	6.3 ± 2.1	5.5 ± 1.4	0.046	-0.19 [-0.1, 0.01], 0.109	0.04
Leukocytes (× 10^{-9}/L)	5.4 ± 2.3	6.0 ± 2.7	4.7 ± 1.6	0.017	-0.06 [-0.05, 0.03], 0.580	0.004
Creatinine (μmol/L)	117.4 ± 31.4	118.0 ± 31.9	116.9 ± 31.3	0.868	-0.05 [-0.004, 0.002], 0.669	0.002
Carbamide (mmol/L)	9.8 ± 3.4	9.9 ± 4.0	9.7 ± 2.7	0.865	-0.003 [-0.03, 0.03], 0.977	1.00E-05
eGFR (mL/min per 1.73 m^2)	55 ± 16	54.1 ± 17.0	56.1 ± 15.0	0.586	0.23 [3.9E-5,0.01], 0.049	0.05
Muscle strength and muscular exercise capacity						
Muscle strength (Nm)	279 ± 129	231 ± 128	326 ± 113	0.001	0.66 [0.002, 0.003], < 0.001	0.43
Muscular Exercise capacity (J)	3229 ± 1660	2423 ± 1351	4015 ± 1567	< 0.001	0.64 [0.0001, 0.0002], < 0.001	0.41
Spirometry						
FEV1 (%)	81 ± 16	74 ± 14	88 ± 16	< 0.001	0.39 [0.004, 0.02], 0.001	0.16
PEF (%)	85 ± 22	79 ± 23	91 ± 20	0.018	0.37 [0.003, 0.01], 0.001	0.14
FVC (%)	86 ± 17	81 ± 16	90 ± 16	0.026	0.17 [-0.002, 0.01], 0.152	0.03
Echocardiography						
EF (%)	57.9 ± 5.6	56.2 ± 5.4	59.4 ± 5.4	0.011	0.26 [0.003, 0.04], 0.025	0.07
LVEDD (cm)	4.9 ± 0.5	4.9 ± 0.5	4.9 ± 0.4	0.996	0.42 [0.19, 0.59], < 0.001	0.18
FS (%)	36.7 ± 5.9	35.9 ± 6.8	37.5 ± 4.9	0.242	0.23 [-4.7E-5, 0.03], 0.051	0.05
CO (L/min)	6.1 ± 1.2	6.0 ± 1.2	6.2 ± 1.2	0.467	0.39 [0.06, 0.21], 0.001	0.15

Health-related quality of life						
PCS	43 ± 8	41 ± 7	45 ± 8	0.029	0.35 [0.008, 0.03], 0.001	0.13
MCS	54 ± 11	53 ± 10	55 ± 11	0.416	0.17 [-0.002, 0.02], 0.127	0.03
Symptoms of anxiety and depression						
HADS-A ⩾ 8 (%)[4]	15	17	13	0.562[1]	-0.26 [-0.56, -0.05], 0.02	0.07
HADS-D ⩾ 8 (%)[5]	5	5	5	1.000[3]	-0.16 [-0.73, 0.13], 0.165	0.03

Groups are divided according to the median VO_{2peak} (mL/kg per min). Variables are presented as percentages, mean ± SD or as median (Q1, Q3) where appropriate. [1]χ^2; [2]Mann Whitney U-test; [3]F; [4]HADS-A score ⩾ 8 indicates symptoms of anxiety; [5]HADS-D score ⩾ 8 indicates symptoms of depression; [6]The actual N varies from 55 to 81 for different variables; [7]Unadjusted R^2. ACE: Angiotensin-converting enzyme; ATII: Angiotensin Ⅱ; BP: Blood pressure; CO: Cardiac output; ECMO: Extracorporeal membrane oxygenation; EF: Ejection fraction; FEV_1: Forced expiratory volume at 1 min; FVC: Forced vital capacity; FS: Fractional shortening; HADS: Hospital anxiety and depression scale; HbA1c: Hemoglobin A1c; HDL: High density lipoprotein; hs-CR: High-sensitive C-reactive protein; hs-TnT: High-sensitive troponin T; HTx: Heart transplantation; IABP: Intra-aortic balloon pump; LVAD: Left ventricle assist device; LVEDD: Left ventricular end diastolic diameter; MCS: Mental component summary; Nm: Newton meter; NT-pro BNP: N-terminal pro brain natriuretic peptide; PEF: Peak expiratory flow; PCS: Physical component summary; Q1: First quartile; Q3: Third quartile; SD: Standard deviation; TG: Triglyceride.

Table 2 Cardiopulmonary responses to exercise of the study population

[2]*N* = 63-81	Total	Low-capacity group VO_{2peak} ⩽ 19.4 mL/kg per min (*n* = 41)	High-capacity group VO_{2peak} > 19.4 mL/kg per min (*n* = 40)	t (*P*-value)	Univariate regression Standardized coefficient Beta [95%CI], *P* VO_{2peak} L/min	[3]R^2
VO_{2peak} (mL/kg per min)	20.4 ± 4.9	16.4 ± 2	24.3 ± 3.6	< 0.001	0.75 [0.05, 0.08], < 0.001	0.56
VO_{2peak} (L/min)	1.6 ± 0.4	1.3 ± 0.3	1.8 ± 0.4	< 0.001		
%expected VO_{2peak}	55.8 ± 12.4	46.5 ± 7.4	65.3 ± 8.6	< 0.001	0.60 [0.01, 0.03], < 0.001	0.36
RER	1.2 ± 0.1	1.2 ± 0.14	1.2 ± 0.10	0.898		
HRrest (echocardiography)	87 ± 10	87 ± 11	86 ± 9	0.85	-0.07 [-0.013, 0.007], 0.551	0.01
Peak systolic BP (mmHg)	188 ± 30	188 ± 31	189 ± 30	0.865	0.19 [-0.001, 0.006], 0.108	0.04
Peak diastolic BP (mmHg)	82 ± 17	82 ± 18	82 ± 16	0.917	0.09 [-0.004, 0.008], 0.467	0.01
VE/VCO_{2slope}	34.8 ± 7.7	37.3 ± 7.2	32.6 ± 7.6	0.008	-0.42 [-0.035, -0.01], < 0.001	0.18
Vmax (L)	71.4 ± 22.8	60.5 ± 17.5	81.7 ± 22.7	< 0.001	0.76[0.01, 0.02], < 0.001	0.58
O_2 pulse (mL/beat)	12.4 ± 3.3	11.0 ± 3	13.7 ± 3	< 0.001	0.80 [0.08, 0.12], < 0.001	0.65
AT (L/min)	1.08 ± 0.3	0.95 ± 0.2	1.2 ± 0.3	0.001	0.73 [0.74, 1.2], < 0.001	0.53
METS	6.5 ± 1.6	5.4 ± 0.8	7.8 ± 1.3	< 0.001	0.77 [0.16, 0.24], < 0.001	0.59
HRpeak (beats/min)	128 ± 19	121 ± 19	134 ± 17	0.001	0.31 [0.002, 0.01], 0.005	0.1
%HRmax	75 ± 12	72 ± 12	78 ± 11	0.021	0.20 [-0.001, 0.02], 0.071	0.04
HRreserve (beats/min)	43 ± 16	35 ± 13	50 ± 15	< 0.001	0.47 [0.01, 0.02], < 0.001	0.22
CRI	0.51 ± 0.2	0.45 ± 0.18	0.57 ± 0.2	0.004	0.31 [0.20, 1.12], 0.005	0.1
RPE (Borg scale)	18.6 ± 0.8	18.5 ± 1	18.6 ± 0.5	0.638		
Test duration (min)	9.5 ± 2.8	7.8 ± 1.5	11.1 ± 2.7	< 0.001		
HRrecovery						
Beats /min at 2 min	-1.0 (-3.0, 1.0)	-1.0 (-3.0, 1.0)	-2.0 (-3.3, 1.3)	0.697[1]		

Groups are divided according to the median VO_{2peak} (mL/kg per min). Variables are presented as mean ± SD or as median (Q1, Q3) where appropriate. [1]Mann Whitney *U*-test; [2]The actual N varies from 63 to 81 for different variables; [3]Unadjusted R^2. BP: Blood pressure; CI, confidence interval; CRI, chronotropic response index; HR, heart rate; METS, metabolic equivalents; Vmax, maximum ventilation; Q1, first quartile; Q3, third quartile; RER, Respiratory Exchange Ratio; RPE, rated perceived exertion; SD, standard deviation.

Table 3 Multiple regression analysis

N = 66	Model 1 Standardized coefficient Beta [95% CI]	*P*-value	Model 2 Standardized coefficient Beta [95% CI]	*P*-value
O_2 pulse (mL/beat)	0.707 [0.075, 0.104]	< 0.001	0.675 [0.069, 0.102]	< 0.001
HRreserve (beats/min)	0.382 [0.007, 0.013]	< 0.001	0.397 [0.008, 0.013]	< 0.001
Muscular exercise capacity (Joule)	0.162 [1.1E-5, 7.1E-5]	0.008	0.155 [8.0^{-5}, 7.1^{-5}]	0.015
BMI (kg/m^2)			0.067 [-0.004, 0.020]	0.211
Sex			-0.029 [-0.142, 0.086]	0.630
Age (yr)			0.019 [-0.003, 0.004]	0.719
Adjusted R^2	0.85		0.84	

Dependent variable VO_{2peak} L/min. Final model for *n* = 66. BMI: Body mass index; CI: Confidence interval; HR: Heart rate.

In addition to the main predictors mentioned above, self-reported physical function was also positively associated with VO_{2peak} in this cohort, which is in accordance with an earlier paper from our research team[15]. Similar findings are reported from the general population in the Norwegian HUNT study, in which physical activity level

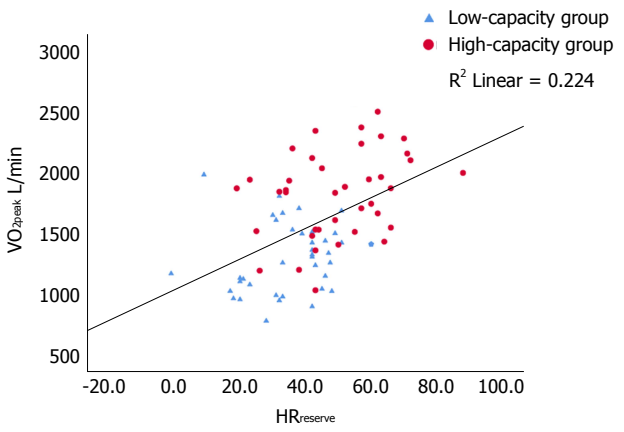

Figure 1 Scatterplot of the correlation between peak oxygen consumption (L/min) and heart rate reserve with inserted regression line. R^2 = 0.224. Pearsons *r* 0.473, *P* < 0.001. VO_{2peak}: Peak oxygen consumption; $HR_{reserve}$: Heart rate reserve.

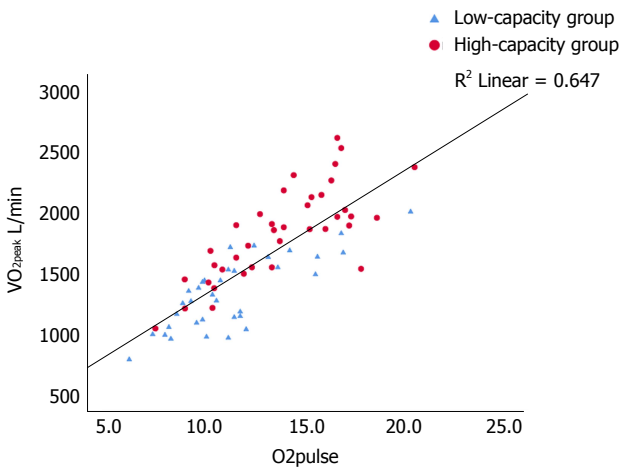

Figure 2 Scatterplot of the correlation between peak oxygen consumption (L/min) and O2 pulse with inserted regression line. R^2 = 0.647. Pearsons *r* 0.804, *P* < 0.001. VO_{2peak}: Peak oxygen consumption.

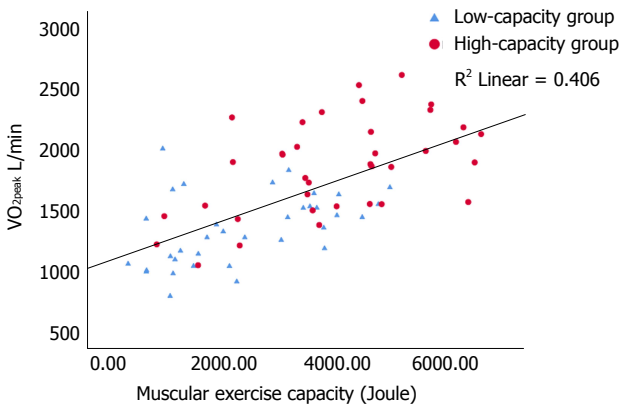

Figure 3 Scatterplot of the correlation between peak oxygen consumption (L/min) and muscular exercise capacity (Joule) with inserted regression line. R^2 = 0.406. Pearsons *r* 0.637, *P* < 0.001. VO_{2peak}: Peak oxygen consumption.

was associated with VO_{2peak}[29]. Although both groups in our current study had a lower score on the physical function subscale compared to the norm values described in Ware *et al*[25], the high-capacity group had a clinical meaningful and significantly higher score than the low-capacity group on physical function. The high-capacity group also had higher score on the PCS. On the other hand, there were no differences between the two groups regarding the psychosocial subscales or MCS in SF-36v2.

As previously mentioned, only two previous studies exist that describe determinants for VO_{2peak} in *de novo* HTx recipients[6,12]. Kitagaki *et al*[6] found that knee extensor muscle strength and cholinesterase were important predictors for VO_{2peak} 55 d after surgery. Salyer *et al*[12] found that age was the only predictor of $VO_{2\ peak}$ 68 d after HTx, but they did not include muscular exercise capacity or chronotropic variables in their regression analyses. A small study (*n* = 15) by Oliveira Carvalho *et al*[30] described that $HR_{reserve}$, as the only important variable, was associated with VO_{2peak} six months after HTx, while in maintenance HTx recipients, $HR_{reserve}$ was no longer strongly associated with VO_{2peak}. In $HR_{recovery}$ after exercise, there was an important difference between early and late HTx recipients, suggesting a partial reinnervation in maintenance HTx recipients[30]. However, peripheral factors such as muscular exercise capacity were not measured in Oliveira Carvalho's study[30]. Borelli *et al*[31] followed HTx recipients for two years and found that both central and peripheral factors contributed to the reduced VO_{2peak} both early (5.3 mo) and late (2 years) after HTx, but that the improvements in VO_{2peak} seen over two years were mostly related to peripheral factors.

In the present study, both $HR_{reserve}$ and O_2 pulse were independent predictors of VO_{2peak}. The chronotropic responses, CRI, $\%HR_{max}$ and HR_{peak} were, as expected, lower than normal both in the low-capacity and the high-capacity group. However, the high-capacity group had better chronotropic responses than the low-capacity group (CRI, *P* = 0.004; $\%HR_{max}$, *P* = 0.021, HR_{peak}, *P* = 0.001; $HR_{reserve}$, *P* < 0.001). $HR_{recovery}$ was markedly delayed in both groups, with no difference between the groups. Previous studies in maintenance HTx recipients have reported conflicting results whether chronotropic incompetence is associated with a reduced VO_{2peak} or not. Schwaiblmair *et al*[32] and Kemp *et al*[33] found a higher VO_{2peak} in patients with a greater $HR_{reserve}$, compared to patients with a lower $HR_{reserve}$. In contrast, Squires *et al*[34] found no difference in VO_{2peak} between patients with high versus low $HR_{reserve}$ (46 ± 15 *vs* 33 ± 15). In a previous study by our research group, where maintenance HTx recipients demonstrated a close to normal chronotropic response, $HR_{reserve}$ was not a strong determinant of VO_{2peak}[20]. However, in this current study of *de novo* HTx recipients, it is (Figure 1). The findings described above suggest that as the initially impaired chronotropic responses improve over time, they become less predictive of VO_{2peak}.

O_2 pulse derived from CPET is considered a surrogate for stroke volume[14,35,36]. In the current study, there was a strong correlation between $VO_{2\ peak}$ and O_2 pulse (Figure 2). In line with this, the high-capacity group also had a

higher O_2 pulse ($P < 0.001$), increased left ventricular EF, as well as lower NT-proBNP and hs-TnT levels, reflecting a better preserved myocardial function compared with the low-capacity group.

De novo HTx recipients have reduced muscle mass mostly due to inactivity prior to HTx[18]. The high-capacity group had higher muscular exercise capacity ($P < 0.001$) and muscular strength ($P = 0.001$) than the low-capacity group (Figure 3), and this finding supports the previously described association between muscle function and VO_{2peak}[20]. Comparing the muscle strength values from our previous study on maintenance recipients[20] with the values in this current study, they are not surprisingly much lower in the *de novo* recipients. As muscular exercise capacity is the only peripheral predictor for VO_{2peak} in the current study, peripheral factors might be less dominant than central factors in the early phase after HTx. However, from a clinical point of view, resistance training in the early rehabilitation after HTx is of high importance in order to prevent and restore loss of muscle mass and bone density and is likely to contribute to an improved VO_{2peak} level[37].

In the existing literature, VO_{2peak} in *de novo* HTx patients is reported to range from 9.2 mL/kg per min up to 19.7 mL/kg per min (1-3 mo after HTx)[2-12]. One small study of nine patients with left ventricle assist device (LVAD) prior to HTx had a mean VO_{2peak} of 24.6 mL/kg per min 12 wk after HTx, which is higher than what has been reported in other studies and may be explained by the LVAD effect and the patients' relatively high VO_{2peak} before HTx[38]. Except for this study, our cohort's mean VO_{2peak} level of 20.4 mL/kg per min (measured 11 wk post HTx) is higher than what is previously reported in *de novo* HTx recipients. Compared to an earlier exercise study in maintenance HTx recipients from our center with a median VO_{2peak} value of 27.3 mL/kg per min[20], this *de novo* HTx cohort is below this value, but compared to other international studies in maintenance HTx recipients, our current *de novo* HTx recipients are close to these reported values[18]. This may be partially related to the early and individualized exercise program conducted at our centers, where the patients are attended to daily by a physical therapist from the multidisciplinary HTx team.

Results from a CPET test can be important in many aspects in the early phase after HTx. First of all, a maximal exercise test is of great value to the individual patient in terms of contributing to increased confidence in their new heart and the body's tolerance to high-intensity exercise. Secondly, an early CPET is useful for deciding and tailoring the individual exercise programs and for the further rehabilitation, both for monitoring patients' status and prognosis and measuring effect of exercise. In addition to the many gas exchange variables, the CPET also provides other valuable and useful measurements, such as lung function and chronotropic responses. Finally, as we know that measures of physical capacity are strong predictors for long-term survival in HTx recipients[15,16], we suggest that such measures should be routinely included both in the early phase after HTx and at yearly controls thereafter. We underscore that the safety aspect is very important when performing a CPET and it should always be supervised by competent and experienced health personnel.

Limitations

Selection bias is a common risk in all voluntary studies, and although our aim was to include every newly transplanted HTx recipient, the recipients had to be medically stable and able to perform a maximal CPET and other physical tests. Thus, as the median VO_{2peak} value in this *de novo* cohort is comparable to maintenance HTx recipients' VO_{2peak} values, this may be due to a possible selection bias.

This is a cross-sectional study, based on the baseline data from an ongoing RCT, and no causal relationships should be drawn from such a study design. We present only associations between VO_{2peak} and different possible determinants. A rather small sample size ($n = 81$) may also imply type 2 errors, but all the performed statistics were carefully checked for underlying assumptions.

In this *de novo* HTx cohort, the age-predicted mean $VO_{2\ peak}$ value was 56% of age-expected values, which is comparable to previously reported values in maintenance HTx[18]. Predictors for VO_{2peak} in *de novo* HTx recipients seem to be of both central (O_2 pulse and $HR_{reserve}$) and peripheral (muscular exercise capacity) origin. A CPET and determination of muscular exercise capacity provide important information for patient motivation, rehabilitation and prognosis and thus, measurements for physical function should be considered as routine examinations early after HTx.

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ARTICLE HIGHLIGHTS

Research background

Peak oxygen consumption (VO_{2peak}) is reduced after heart transplant (HTx). Both peripheral and central factors are determinants of the reduced VO_{2peak} in maintenance HTx recipients, but there are still few studies among *de novo* HTx patients. A higher VO_{2peak} is associated with better prognosis after HTx, and knowledge about predictors for VO_{2peak} in *de novo* HTx is important for the rehabilitation process. A cardiopulmonary exercise test (CPET) is the gold standard for measuring VO_{2peak} and should be performed as a routine test early after HTx.

Research motivation

More knowledge about predictors for VO_{2peak} in *de novo* HTx patients may contribute to a better understanding of the reduced exercise capacity early after

HTx. Individualized exercise prescriptions are very important after HTx, and a CPET early after HTx will guide both clinicians and physiotherapists in this vulnerable phase of the rehabilitation process.

Research objectives

The aim of this study was to investigate determinants of early VO_{2peak} and exercise capacity in a cohort of *de novo* HTx recipients.

Research methods

This study used baseline data from an ongoing randomized controlled trial investigating high-intensity interval training compared to moderate continuous exercise training among *de novo* HTx recipients, the HITTS study. A cross sectional analysis was performed on the baseline data from the 81 patients included in the study, and all baseline tests were performed an average of 11 wk after surgery. The primary endpoint was VO_{2peak} measured by CPET. Secondary endpoints were lung function, maximum muscle strength and muscular exercise capacity, bioelectrical impedance analysis, echocardiography, blood samples and health-related quality of life.

Research results

The main findings in this study were that *de novo* HTx patients display reduced exercise capacity compared to a general population, but comparable with maintenance HTx recipients. This *de novo* HTx cohort demonstrated a median VO_{2peak} level of 19.4 mL/kg per min at 11 ± 1.8 wk post-HTx. Maximal exercise capacity was determined by both central (O_2 pulse and $HR_{reserve}$) and peripheral factors (muscular exercise capacity). The CPET tests were performed without any serious adverse events mean 11 wk after HTx. This is a cross-sectional study, and no causal relationships should be drawn from such a study design. We present only associations between VO_{2peak} and different possible determinants.

Research conclusions

In this *de novo* HTx cohort, the age-predicted mean $VO_{2\ peak}$ value was 56% of age-expected values, which is comparable to previously reported values in maintenance HTx. Predictors for VO_{2peak} in *de novo* HTx recipients seem to be of both central and peripheral origin.

Research perspectives

A CPET and determination of muscular exercise capacity provide important information for patient motivation, rehabilitation and prognosis and thus, measurements for physical function should be considered as routine examinations early after HTx.

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EDITORIAL

Surgeon's perspective on short bowel syndrome: Where are we?

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Abstract

Short bowel syndrome (SBS) is due to a massive loss of small bowel: the reduction of gut function is below the minimum necessary to maintain health (in adults) and growth (in children) so intravenous supplementation is required. Parenteral nutrition represents the milestone of treatment and surgical attempts should be limited only when the residual bowel is sufficient to increase absorption, reducing diarrhea and slowing the transit time of nutrients, water and electrolytes. The surgical techniques lengthen the bowel (tapering it) or reverse a segment of it: developed in children, nowadays are popular also among adults. The issue is mainly represented by the residual length of the small bowel where ileum has shown increased adaptive function than jejunum, but colon should be considered because of its importance in the digestive process. These concepts have been translated also in intestinal transplantation, where a colonic graft is nowadays widely used and the terminal ileum is the selected segment for a living-related donation. The whole replacement by a bowel or multivisceral transplant is still affected by poor long term outcome and must be reserved to a select population of SBS patients, affected by intestinal failure associated with irreversible complications of parenteral nutrition.

Key words: Parenteral nutrition; Bowel rehabilitation; Surgical rescue; Intestinal transplantation; Short bowel syndrome

Core tip: Short bowel syndrome represents a surgical dilemma: parenteral nutrition is considered the gold standard of care and any surgical attempt must be limited by the universal principle "first do not harm." The surgical rehabilitation should be pursued when there are enough residual intestines to obtain a better bowel function: lengthening the intestine or reversing a loop of it with different techniques should have the only aim of slowing the transit while increasing the absorptive surface. When intestinal failure is associated to life-threating parenteral nutrition complications, bowel transplantation should be considered as an option.

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INTRODUCTION

Short bowel syndrome (SBS) results from a reduced length of the small intestine. A "normal small bowel length," measured from the duodeno-jejunal flexure to ileocolic valve, is estimated at 250 cm ± 40 cm at birth, and the growth is maximal during the first year of life[1]. In adults, the small bowel length varies from 275 cm to 850 cm, with a mean of 350 cm ± 60 cm, depending on the method used, radiologic, surgical, or per autopsy[2]. The massive loss of small bowel represents the most frequent mechanism of intestinal failure, defined by the European Society for Clinical Nutrition and Metabolism as "the reduction of gut function below the minimum necessary for the absorption of macronutrients and/or water and electrolytes, such that intravenous supplementation is required to maintain health and/or growth"[3]. Among children "the minimum necessary for the absorption" is a residual small bowel length of more than 25% of the expected for gestational age[3], in adults SBS usually appears when the small bowel length is less than 200 cm (67% of the normal length)[4]. Malabsorption and diarrhea represent the classical symptoms, associated to deficit of growth in the pediatric population. Wilmore *et al*[5] first demonstrated long-term survival with parenteral nutrition (PN) in a child affected by SBS. Nowadays home PN represents the standard of care in patients affected by massive loss of small bowel with excellent long term results[6-12]. PN does not replace physiologically the bowel function because uses the intravenous route to supplement nutrients and it could be affected by several life-threating complications. Under this perspective, a surgical rehabilitation in case of SBS should be represented by: (1) the possibility to slow the transit and obtain an adequate absorptive surface of the remnant intestine through lengthening procedures and (2) whole replacement of the massive intestinal loss with a bowel transplant. It is worthwhile to analyze briefly the main reported studies on the issue in international literature, in order to develop an updated perspective under the surgical point of view.

OVERVIEW OF THE LITERATURE

SBS is mainly, but not only, a matter of length. In children, the massive resection of the small bowel could lead to a "very short bowel syndrome" (≤ 40 cm)[13,14], "ultra-short bowel syndrome" (between < 30 and < 10 cm)[15-17] or "no gut syndrome" (only the duodenum is left)[18-20]. Adults with less than 200 cm but more than 75 cm of small bowel[21] have a potentially functional intestine especially if the colon (and specifically the ileocolic valve) is preserved in continuity. Among SBS patients, the role of the colon in the process of digestion has been demonstrated since the '90s[22-25]. The presence of remaining colon is associated with a lower dependency on PN[26,27] and there is agreement that the remaining small bowel after massive intestinal loss is supported by the colon (if in continuity) for completion of the digestion process. On the other hand, jejunum and ileum have different roles in digestion and ileum has probably a greater adaptive potential than jejunum[28]. A remnant ileum (especially in continuity with the colon) could probably guarantee a faster weaning from PN. Clinical experience shows that patients with a jejuno-colonic anastomosis (SBS type II), even better with a jejuno-ileo-colonic anastomosis (SBS type III), have an improved absorption with time after a period of intestinal rehabilitation, whereas patients with end-terminal jejunostomy without colon (SBS type I) do not show that. When the colon is missing, among adults 115 cm of small bowel with an end enterostomy are considered the limit before SBS.

SURGEON'S PERSPECTIVE

In SBS the remaining small bowel may dilate. This is important for surgeons in order to lengthen the intestine, tapering it. It has been shown that the extent of dilation is associated with the bowel length, and both are related to enteral autonomy[29]. Two surgical procedures are popular in order to lengthen the bowel: Bianchi and Serial Transverse Enteroplasty Procedure (STEP). The Bianchi procedure, summarized by Bianchi in 1997[30], is also known as longitudinal lengthening and tailoring (LILT). The small bowel mesentery is separated as two leaves with a GI anastomosis stapler to create a tunnel, and then the two resulting small bowel segments of smaller diameter are connected with an end-to-end anastomosis in an iso-peristaltic fashion. In the STEP, first described by Kim in 2003[31], the dilated small bowel is narrowed by serial transverse applications of the GI stapler from opposite directions, creating a new lengthened small intestine (zig-zag channel). This procedure does not require an intestinal anastomosis and the mesenteric vascular supply is untouched. Since its first description, STEP has become a widespread procedure, sometimes repeated on the same patient (re-STEP) to obtain a longer intestinal segment. Bianchi and STEP procedures have been performed at first in children and more recently also in adults[32-35]. Most of the studies are on STEP: while enteral autonomy (median time: 21 mo) is eventually possible in some patients[36], improved enteral tolerance can be achieved in a majority[37,38]. STEP can be performed on shorter intestinal segments or intricate segments such as the duodenum, which is technically not feasible for Bianchi procedure, and it seems to have a lower mortality but an overall progression to transplantation[39]. The spiral intestinal lengthening and tailoring procedure is a new

surgical technique based on a spiral shape incision of the dilated intestine (at 45°-60° to its longitudinal axis), and re-tubularization in a longer but narrower fashion. It does not alter the orientation of the muscle fibers like STEP, offering minimal mesenteric handling compared to Bianchi procedure. It has been reported in a 3-year-old girl[40] where, 6 mo after the procedure, PN was weaned off. Another manuscript described the technique in a 10-month-old child[41] showing at 1-year follow-up a growth on the 15-25th centile on 82% oral calories and 18% PN, passing 2-3 daily stools. Three children with "no gut" syndrome and dilated duodenum underwent a novel surgical procedure of "duodenal lengthening" combined with a technical modification of STEP[18]: duodenal tapering was performed with sequential transverse applications of an endoscopic stapler on the anterior and posterior wall of the duodenum, avoiding bilio-pancreatic injury. Two patients weaned PN off at 12 mo post-surgery and the last one's PN caloric requirements decreased by 60%. The surgical rescue of "no gut" syndrome has been reported in adults as well. Bueno *et al*[20] demonstrated the feasibility of lengthening a dilated duodenum in a patient where his mega-duodenal stump was tapered by STEP, restoring his digestive continuity through an end-to-side duodeno-colonic anastomosis. After 24 mo of follow-up, the time on daily PN was shortened from 24 to 9 h and the volume and calorie requirements were reduced by half.

Since lengthening procedures slow the bowel transit time, a "reversed anti-peristaltic segmental bowel loop" has been proposed with the same aim: this procedure can be indicated in patients with an adequate remnant bowel length. Median oral autonomy was described up to 100% ± 38% with a lower amount of parenteral calories, as well as PN dependence[42]. In another report[43] 56% of patients improved their enteral autonomy.

The different graft types used in intestinal transplantation are the isolated small bowel, combined liver-intestine, multivisceral and modified multivisceral ones[44]: liver-containing grafts have shown the longest survivals. Apart from cadaveric donation, living-related intestinal transplantation has been pursued especially in a pediatric setting[45]: terminal ileum represents the used graft, because of technical feasibility and its greater adaptive potential than jejunum[28]. Short term results of intestinal transplantation have recently improved in terms of survival and digestive autonomy, due to advances in surgery and immunosuppression. Immunosuppressive therapy has evolved significantly over the past 20 years: the tacrolimus-based therapy as maintenance, preceded by induction with anti-thymocyte globulin or an interleukin-2 blocker, is the main used protocol worldwide. A "secondary" agent like steroids, azathioprine, mycophenolate mofetil or an mTOR inhibitor is recommended after an episode of rejection. Innovative cross match strategies and optimizing organ allocation could improve the long-term outcome, but the main causes of death and graft loss remain sepsis and rejection. Challenges for long-term results are chronic rejection and immunosuppressant-related complications[46,47]. According to Intestinal Transplant Registry reports[44], 1611 children were transplanted worldwide between 1985 and 2013, with an overall patient survival rate of 51%. In the 2014-2016 Scientific Registry of Transplant Recipients[48], the 6 American centers that in 2016 performed 10 or more intestinal transplants in adults reported a 1-year graft survival from 61% to 83% and a 3-year graft survival from 29% to 73%. In an earlier report from 2008 to 2010, the 1-year graft survival in adults was 71%, illustrating the relatively modest gains achieved[47]. Intestinal transplantation should be suggested to a very select subset of SBS patients with severe and irreversible complications of PN and no hope of intestinal rehabilitation. In conclusion, among SBS patients the surgical rehabilitation (Table 1) of the remnant bowel must be performed to slow the intestinal transit time increasing at the same time the absorptive surface: only in cases of irreversible intestinal failure with PN life-threating complications, intestinal transplantation could represent a therapeutic option even if still encumbered by suboptimal long term results.

Table 1 Surgical rehabilitation of short bowel syndrome

SBS surgical rehabilitation
Lengthening procedures
Bianchi
STEP
SILT
Duodenal lengthening
Reversed anti-peristaltic segmental bowel loop
Intestinal transplantation

SBS: Short bowel syndrome; STEP: Serial transverse enteroplasty procedure; SILT: Spiral intestinal lengthening and tailoring.

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REVIEW

Complement-mediated renal diseases after kidney transplantation - current diagnostic and therapeutic options in *de novo* and recurrent diseases

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Author contributions: Abbas F designed the study, data collection, writing the manuscript; El Kossi M, Kim JJ, Shaheen IS and Sharma A reviewed and edited the manuscript; Halawa A contributed to conceptualization, designing the study, supervising the data collection and reviewing and editing the manuscript.

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Abstract

For decades, kidney diseases related to inappropriate complement activity, such as atypical hemolytic uremic syndrome and C3 glomerulopathy (a subtype of membranoproliferative glomerulonephritis), have mostly been complicated by worsened prognoses and rapid progression to end-stage renal failure. Alternative complement pathway dysregulation, whether congenital or acquired, is well-recognized as the main driver of the disease process in these patients. The list of triggers include: surgery, infection, immunologic factors, pregnancy and medications. The advent of complement activation blockade, however, revolutionized the clinical course and outcome of these diseases, rendering transplantation a viable option for patients who were previously considered as non-transplantable cases.

Several less-costly therapeutic lines and likely better efficacy and safety profiles are currently underway. In view of the challenging nature of diagnosing these diseases and the long-term cost implications, a multidisciplinary approach including the nephrologist, renal pathologist and the genetic laboratory is required to help improve overall care of these patients and draw the optimum therapeutic plan.

Key words: Complement-related diseases; Kidney transplantation; *De novo*; Recurrent diseases

Core tip: The recent progress in our understanding of the pathophysiology of complement-mediated diseases is gaining considerable popularity. Complement dysregulation due to inherited or acquired factors is currently the culprit mechanism. Several constitutional abnormalities usually trigger the process of recurrence, with a subsequent high rate of graft loss. The development of the terminal complement inhibitor "eculizumab" is a breakthrough in controlling abnormal complement activation. While diagnosing complement abnormalities is one challenge, treatment cost with this new agent is another major hurdle in any health care system. New lines of promising therapies are currently in the pipeline.

Abbas F, El Kossi M, Kim JJ, Shaheen IS, Sharma A, Halawa A. Complement-mediated renal diseases after kidney transplantation - current diagnostic and therapeutic options in *de novo* and recurrent diseases. *World J Transplant* 2018; 8(6): 203-219 Available from: URL: http://www.wjgnet.com/2220-3230/full/v8/i6/203.htm DOI: http://dx.doi.org/10.5500/wjt.v8.i6.203

INTRODUCTION

The complement components can be seen in biopsies of almost all types of glomerulonephritis, which can be broadly divided into two main groups: (1) "complement over-activation" includes IgA nephropathy (IgAN) and immune complex membranoproliferative glomerulonephritis (MPGN); and (2) "complement dysregulation" that encompasses atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy (C3G)[1]. While complement activation is triggered by immune complex formation in the former group, genetic mutations are the driver of complement over-activation in the latter one. This explains why the disease process in the former class is potentially modifiable by immunosuppression in the post-transplantation period, which is not the case in the latter class. Our understanding of the biogenetic causes of C3G and aHUS/thrombotic microangiopathy (TMA) has been expanding. The mechanisms of these diseases not only affect their clinical history, but also affect the recurrence rate[2]. The role of complement in C3G evolution is now well-recognized[3]. Recent progress in understanding the pathophysiology of MPGN led to newer classifications of MPGN into immune complex-mediated and complement-mediated subtypes. The hallmark of complement-mediated MPGN is the deposition of C3 and other complement products in glomerular tissues[4]. This is caused by dysregulation and loss of control of the AP complement pathway[5]. The AP is tightly regulated under physiological conditions. It can be disrupted through either inherited (mutations/polymorphisms) or acquired (autoantibodies) interferences to the regulating components. Histological staining using immunofluorescence (IF) is currently the best determinant technique, and C3G is defined by dominant C3 with dispersed, reduced or absent immunoglobulin (Ig). Based on electron microscopy (EM) examination, C3G subdivides into complement three glomerulonephritis (C3GN) and dense deposit disease (DDD). In C3GN, discrete deposits can be seen in the mesangium and capillary walls (subendothelial and subepithelial regions). On the contrary, DDD deposits are large in size, extremely dense (osmiophilic) and intramembranous, which leads to a characteristic thickening of the glomerular basement membrane (GBM)[5]. The term aHUS is applied to a heterogenous group of diseases (Figure 1) that share TMA manifestations with an associated decline in renal function (classically, no IF staining of C3 or any other complement components). In aHUS, complement abnormalities (either genetic mutations or acquired autoantibodies) are well-recognized mechanisms with a clearly associated complement-mediated TMA[1]. In this article, we will discuss various types of complement-mediated renal diseases after kidney transplantation and their current therapeutic options.

Methodology

In view of the lack of prospective controlled trials concerned with complement-mediated diseases post-kidney transplant, we tried to shed the light in this review on the most recent expert opinions, with regard to the best tools of management for these devastating diseases.

CLINICAL PRESENTATION

Salient features of C3G

DDD and C3GN share some salient features that include proteinuria, hematuria and increased serum creatinine concentration[6,7]. Recurrence of C3G is typically encountered one to two years after transplant[7]. C3G comprises a spectrum of diseases that result from aberrant control of complement activation, deposition and dysregulation, leading to C3 glomerular deposition with characteristic electron-dense deposits (EDD) in EM (Table 1).

Table 1 Morphological features of C3 glomerulopathy

	Morphological features of C3G
Light microscopy	Active lesions Mesangial expansion with or without hypercellularity Endocapillary hypercellularity including monocytes and/or neutrophils Capillary wall thickening with double contours (combination of capillary wall thickening + mesangial increase is referred to as a membranoproliferative pattern) Fibrinoid necrosis Cellular/fibrocellular crescents Chronic lesions Segmental or global glomerulosclerosis Fibrous crescents
IF microscopy	Typically dominant C3 staining
Electron microscopy	DDD: Dense osmiophilic mesangial and intramembranous electron dense deposits. C3GN: Amorphous mesangial with or without capillary wall deposits including subendothelial, intramembranous and subepithelial EDD Subepithelial "humps" may be seen in both DDD and C3GN

Adapted from Goodship *et al*[12]. C3G: C3 glomerulopathy; DDD: Dense deposit disease; C3GN: C3 glomerulonephritis; EDD: Electron dense deposits, fibrinoid necrosis.

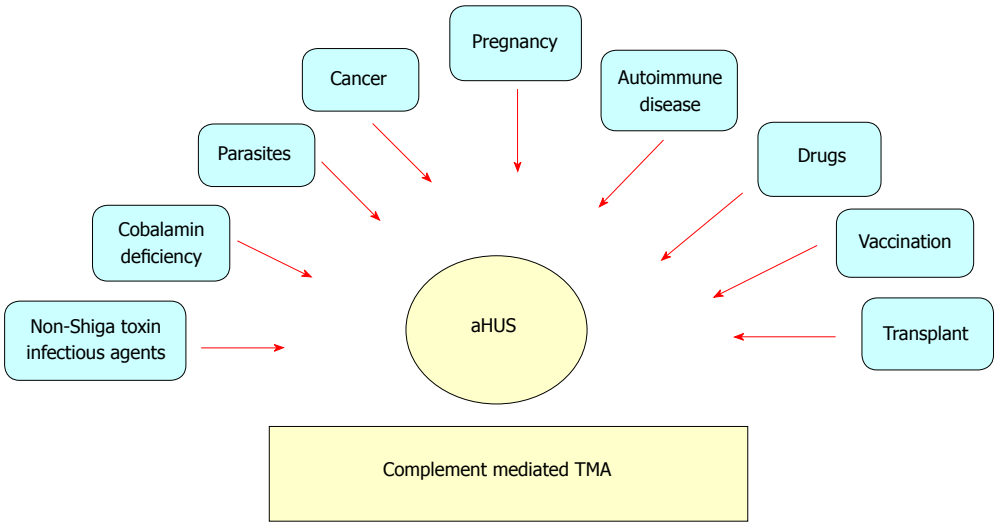

Figure 1 Heterogeneity of atypical hemolytic uremic syndrome. Adapted from Salvadori *et al*[1]. TMA: Thrombotic microangiopathy; aHUS: Atypical hemolytic uremic syndrome.

Pathology

Renal biopsy is crucial for C3G diagnosis. LM is not helpful, due to its extremely diverse appearance. IF is the mainstay for diagnosis. A unique criterion in IF studies is the presence of dominant C3 staining, which is twice as intense as any other immunoreactant (IgG, IgM, IgA, and C1q)[8]. Ninety percent of DDD patients, but fewer C3GN patients, can be diagnosed through applying this criterion[8]. Repeated biopsy may be required to confirm the diagnosis. As C3G may present in acute infection, C3 can be observed with post-infectious GN. Humps are no longer pathognomonic criteria of post-infectious GN, however they can also be encountered in C3G. However, the presence of double contours in the GBM raises the possibility of C3G diagnosis. To differentiate DDD from C3GN, EM studies should be accomplished, as it has pivotal clinical implications. Moreover, staining for IgG as well as light chains on pronase-digested paraffin should be applied for all cases of C3GN on standard IF, particularly in adults (Figure 2 and Table 1)[9,10].

Salient features of TMA

TMA is mostly presented 3-6 mo post-transplant, but it can occur at any time after renal transplantation[13]. Presentation of TMA is not universal, ranging from the renal-limited form up to a complete systemic picture with its classic triad of thrombocytopenia, microangiopathic hemolytic anemia (MAHA) and decline in renal function[14]. MAHA is defined as increased LDH, decline in HB and haptoglobin, and appearance of schistocytes in peripheral blood smears. On the other hand, localized (renal-limited) TMA usually presents later in the post-transplant course. In the acute stage, evidence of endothelial injury with platelet aggregation (thrombosis), fibrinoid necrosis, as well as

Table 2 Morphological features in microangiopathy

Active lesions	Chronic lesions
Glomeruli: Thrombi - Endothelial swelling or denudation - Fragmented RBCs - Subendothelial flocculent material. EM: Mesangiolysis - Microaneurysms	Glomeruli: LM: Double contours of peripheral capillary walls, with variable mesangial interposition - EM: New subendothelial basement membrane - Widening of the subendothelial zone
Arterioles: Thrombi - Endothelial swelling or denudation - Intramural fibrin - Fragmented red blood cells - Intimal swelling - Myocyte necrosis	Arterioles: Hyaline deposits
Arteries: Thrombi - Myxoid intimal swelling - Intramural fibrin - Fragmented red blood cells	Arteries: Fibrous intimal thickening with concentric lamination (onion skin)

Adapted from Goodship *et al*[12]. EM: Electron microscopy; LM: Light microscopy.

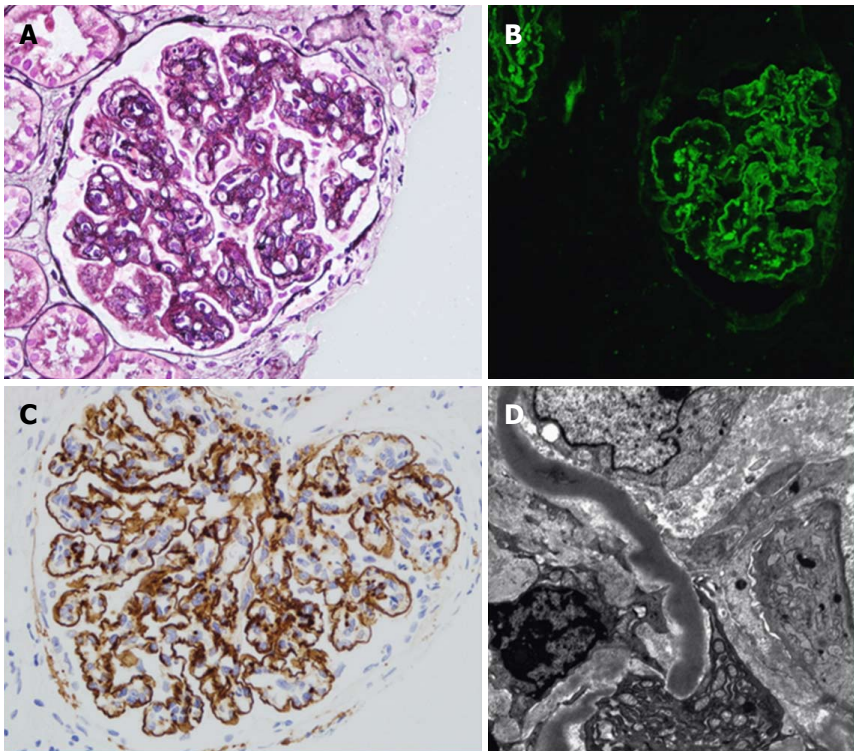

Figure 2 Renal histology in individuals with dense deposit disease. A: Light microscopy with silver stain showing a membranoproliferative glomerulonephritis pattern with double contours of the glomerular basement membrane; B: Immunofluorescence; C: Immunohistochemistry with immunoperoxidase showing strong capillary wall staining of C3 and some granular mesangial C3; D: Characteristic sausage-like, intramembranous, osmiophilic deposits on electron microscopy. Adapted from Barbour *et al*[11].

glomerular ischemia can be seen. On the other hand, chronic lesions show duplication and multilayering of the GBM, with clustering of the matrix layers and vessel wall cells leading to the characteristic onion skin shape appearance (Table 2)[15]. As TMA is not always present with full-blown systemic pictures, genetic studies to unmask the underlying complement defect are ultimately mandated, particularly if no other clear cause has been associated (*e.g.*, AMR-associated TMA). AMR can give a TMA-like picture, as it is an antibody interaction with the endothelium. This is also a fundamental maneuver to differentiate *de novo* from recurrent disease (positive genetic testing), with consequent clinical therapeutic implications[16].

Extrarenal manifestations of aHUS and C3G

Twenty percent of aHUS patients express extrarenal manifestations. Their relation to complement activation and TMA evolution is unclear. Drusen is rarely seen in TMA[17]. Drusen formation, which represents an accumulation of lipids and complement-rich proteins between Bruch's membrane and the retinal pigmentary epithelium, is commonly reported in age-related macular degeneration but present at a much earlier age with C3G[18]. In C3G, retinal drusen and acquired partial lipodystrophy have been commonly reported. The latter is most commonly encountered with C3 nephritic factors. Factor D, an essential agent for C3 convertase formation, is highly concentrated in adipocytes that undergo C3 nephritic factor-induced complement-dependent lysis[19] (Table 3).

Pathogenesis and classification of C3G

The new classification of MPGN encompasses two

Table 3 Extrarenal manifestations reported in atypical hemolytic uremic syndrome, dense deposit disease, and C3 glomerulonephritis

aHUS	DDD/C3GN
Digital gangrene, skin	Retinal drusen
Cerebral artery thrombosis/stenosis	Acquired partial lipodystrophy
Extracerebral artery stenosis	
Cardiac involvement/myocardial infarction	
Ocular involvement	
Neurologic involvement	
Pancreatic, gastrointestinal involvement	
Pulmonary involvement	
Intestinal involvement	

Adapted from Goodship *et al*[12]. aHUS: Atypical hemolytic uremic syndrome; C3GN: C3 glomerulonephritis; DDD: Dense deposit disease.

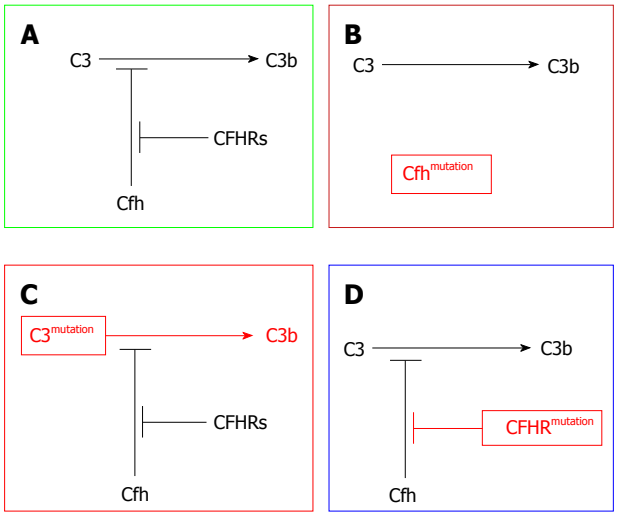

Figure 3 Disease mechanisms in C3 glomerulopathy, based on genetic defects identified in family studies. A: Physiological regulation of C3 activation to C3b *via* the alternative pathway is mediated by complement factor H (CFH) (Cfh). Competitive inhibition of CFH by CFHR proteins is termed CFH deregulation; B: Homozygous deficiency or dysfunction of CFH results in excessive C3 activation; C: Hyper-functional C3 produces excessive C3 activation despite normal CFH activity; D: Abnormal CFHR proteins enhance CFH deregulation, leading to excessive C3 activation. Adapted from Barbour *et al*[11].

subtypes: the immune complex-mediated GN (ICGN) and complement-mediated GN (CGN), recently named (C3G). The former is characterized by both Ig as well as complement component deposition in kidney tissues as recognized by IF studies. The latter is characterized by dominant complement deposition with smaller amounts of Ig deposition. Further subdivision of C3G into C3GN and DDD can be attained through EM studies[20]. Both subtypes are triggered through dysregulation of any part of the AP. For example, patients may develop the C3 convertase-stabilizing factor called C3NeF, which leads to uncontrolled complement activation. Loss-of-function mutations in complement regulatory proteins (CFH or CFI)[20-23] or gain-of-function mutations in C3 leads to CFH resistance, which has been postulated as an underlying mechanism (Figure 3).

Pathophysiology and recurrence of C3G

Pathophysiology of AP activation in DDD and C3GN is nearly the same. In both disorders, disturbance of the fluid phase is triggered as a result of aberrant gene mutations or the presence of autoantibodies. However, the presence of C3 nephritic factor (C3NeF) is by far the most commonly acquired complement defect. C3NeF has the ability to block CFH-mediated decay by stabilizing C3 convertase[5,24]. By binding to C3 convertase, C3NeF has the ability to trigger it approximately ten times[25,26]. C3 convertase can also block the action of CFH, CR1, as well as decay-accelerating factor (DAF).

C3NeF is prevalent in 50%-80% C3G patients[27]. Other autoantibodies have also been found (*e.g.*, autoantibodies against factor B[28], CFH[29,30] and C3 convertase)[28]. In C3G, CFH mutations have been frequently reported. Different forms of mutations can be presented as defective or completely absent protein H. These mutations can be seen in homozygous or heterozygous forms[31,32]. C3NeF can also be encountered, which denotes the clustering of different risk factor varieties. More recently, genetic mutations involving the CFHR gene have been reported in the C3G cohort of patients[33]. CFHR group genetic mutations[34], deletions[35], duplications[36], as well as hybrid genes[37] have also been observed in C3G patients, either in an isolated manner or in a familial cohort. Malik and his associates[38] reported that members of one family can develop C3G as an result of aberrant copies of CFHR3 and CFHR1 loci. The presence of familial C3G underscores the genetic basis of several C3G varieties and their relation to AP dysregulation.

To summarize, complement dysregulation is the specific etiology of C3G, which could be genetic or acquired. While genetic causes encompasse complement gene mutations, acquired causes include the C3NeFs, which have the ability to impede normal complement regulation[1]. Moreover, genetic varieties constitute the pathophysiologic basis of C3G and aHUS evolution (Table 4). Recently, a robust correlation between CFH-related proteins and a variety of complement-mediated diseases have been documented. Functional parameters (*e.g.*, complement regulators and CFH competitors) have recently attained significant popularity[39].

TMA or C3G?

Both TMA and C3G have a common underlying

Table 4 Overview of mutations in complement factor H-related protein genes

Genetic defect	Phenotypical expression
Duplication in *CFHR5* gene	C3 glomerulopathy (CFHR5 nephropathy)
Duplication in *CFHR1* gene	C3 glomerulopathy
Hybrid *CFHR3/CFHR1*	C3 glomerulopathy
Hybrid *CFHR2/CFHR5*	C3 glomerulopathy
Hybrid *CFH/CFHR1*	aHUS
Hybrid *CFH/CFHR3*	aHUS

Adapted from Salvadori *et al*[1]. aHUS: Atypical hemolytic uremic syndrome; CFH: Complement factor H.

causation: AP dysregulation. However, the question that arises is "which factors influence the evolution of one disease rather than the other?"[40]. The prevalence of the fluid phase complement activation dysregulation in animal models suggests that C3G is the responsible factor. On the other hand, complement activation involving capillary walls can result in TMA evolution[41]. Furthermore, absolute CFH deficiency is in favor of an activation of the fluid phase complement with subsequent C3G evolution, while the lack of an aberrant CFH binding region is in favor of TMA evolution[41]. It has also been postulated that CFH and CFH/CFHR mutations induce aHUS to inhibit CFH-binding to many cell surfaces, while C3G-associated mutations in CFHRs cannot inhibit CFH binding to endothelial cell surfaces[42]. The prevalence of familial C3G mutations serves as a robust indicator of the genetic base of C3G recurrence[1].

Risk of DDD recurrence

Despite the well-known DDD variants of C3, its pathogenesis has only recently been recognized. The five-year graft survival rate was only 50% in one retrospective study of 75 children[6]. In adults, a majority of the recipients developed recurrence in post-transplant periods, with 25% of them losing their allografts[43]. In another broader cohort that included eighty adults and children with C3G, Medjeral-Thomas *et al*[44], reported histological recurrence in all six DDD recipients. Graft loss had resulted in 50% of his cases. For recipients who developed DDD recurrence, the ten-year graft survival rate has been reported to be up to 57.5% in an UNOS review[45]. Risk factors for DDD recurrent disease and graft loss are not well-recognized. However, the histological recurrence rate was reported to be more than 70%[46,47]. Recurrence may present spontaneously in post-transplant periods, though it may take several years to manifest[47]. This discrepancy raises some questions, such as the impact of the longevity of follow-ups, the need for tissue diagnosis, and the real rate of DDD recurrence.

Risk of C3GN recurrence

There is no documented relation between mode of presentation, C3 serum levels, or C3NeF levels and C3GN recurrence[48]. The only trustworthy risk factor correlated with C3 recurrence is the presence of heavy proteinuria, with two thirds of C3 patients showing vulnerability to recurrence and a high incidence of graft loss[5,7,27]. All the available data about recurrence are based on case series, with the largest by Zand *et al*[7] that failed to reveal robust evidence of recurrence risk. This observation is partially explained by the heterogeneity of complement defects implicated in C3GN evolution. Early reports postulated HLA-B8 DR3 and living related donation as possible risk factors for recurrence[49]. However, the more recent reports suggested the following: (1) history of graft loss owing to recurrence[50]; (2) aggressive histopathological alterations in native kidney biopsy; and (3) hybrid CFHR3 1 gene-related C3GN. Wong *et al*[51] have recently reported a high rate of C3G recurrence (five patients received a total of eight kidney transplants). Four (50%) renal allografts had disease recurrence, of which three had biopsy-proven recurrence, with time to recurrence ranging from as early as 2 wk following living-related donor transplantation, to 93 and 101 mo for the two remaining allografts, respectively[51].

Diagnosis of C3G recurrence

The declining appearance of proteinuria, hematuria or eGFR is a strong indicator of C3G recurrence. Final diagnosis is usually made through LM, IF, and EM studies of kidney biopsy. After histopathological examination, a thorough evaluation of any genetic mutation in the AP should be accomplished, especially if these studies were not previously fulfilled with the native kidney disease.

Diagnosis of C3G/TMA recurrence

A robust work-up of analytic studies including genetic, biochemical and pathological evaluation should be instituted, including the following: (1) complement components and complement regulatory protein levels; (2) peripheral WBC MCP levels; (3) screening for antibodies to CFH and C3NeFs; and (4) mutation screening of CFH, CFI, CFB, C3, and MCP. Furthermore, recombination in the CFHR region should be tested[52].

Prognosis of DDD/C3GN

In both DDD and C3GN, recurrent disease is usually associated with allograft loss[6,44,53]. The one-year allograft survival was reported to be 94%, with 69% at five years, and 28% at ten years. Three predictive criteria for progression to ESRD were recognized: (1) crescentic GN; (2) severe arteriolar sclerosis by LM; and (3) decline of renal function at the time of first biopsy[44].

Prognosis of TMA

Compared to recurrent TMA, the prognosis of *de novo* TMA is quite poor. Fifty percent of patients may lose their graft within a couple of years after diagnosis[54,55]. Many reports were in favor of this attitude[54-56]. Before

Table 5 Recommended therapy approach for C3 glomerulopathy based on small prospective trial, case reports, and expert opinion

All patients	Moderate disease	Severe disease
Lipid control Optimal BP control (< 90% in children and ≤ 120/80 mm Hg in adults) Optimal nutrition for both normal growth in children and healthy weight in adults	Urine protein > 500 mg/24 h despite supportive therapy, or Moderate inflammation on renal biopsy or Recent increase in serum creatinine suggesting risk for progressive disease Recommendation Prednisone Mycophenolate mofetil	Urine protein > 2000 mg/24 h despite immunosuppression and supportive therapy or Severe inflammation represented by marked endo- or extracapillary proliferation with/without crescent formation despite immunosuppression and supportive therapy or Increased S. Cr suggesting risk for progressive disease at onset despite immunosuppression and supportive therapy Recommendations Methylprednisolone pulse-dosing as well as other anti-cellular immune suppressants have had limited success in rapidly progressive disease Data are insufficient to recommend eculizumab as a first-line agent for the treatment of rapidly progressive disease

Adapted from Goodship *et al*[12].

the era of eculizumab (EZ), Schwimmer *et al*[54] reported that 54% of systemic TMA can develop dialysis requiring AKI, and about 38% lost their allograft. However, no one patient with localized TMA has complicated with TMA-related allograft loss or a need for dialysis. Nevertheless, both systemic and localized forms may experience unfavorable long-term graft survival[54,57].

THERAPY OF COMPLEMENT DYSREGULATION-RELATED DISEASES

Treatment of de novo C3G

The therapeutic approach for *de novo* C3G therapy is similar to that of recurrent disease. Very minimal information is available regarding *de novo* C3G[58].

Treatment of recurrent C3G

In light of the paucity of data from controlled studies, some experts have suggested an approach that depends on disease severity (*i.e.,* mild, moderate and severe) based on the degree of proteinuria and the magnitude of allograft dysfunction (Table 5): (1) conservative measures, as with other glomerulotides, including RAS blockade and lipid-lowering agents; (2) glucocorticoids, MMF, rituximab and PE have been used with variable success[59,60]. In selected patients, MMF has been reported to be effective in C3GN controls in a retrospective study[12,61]; and (3) EZ was firstly reported by Bomback *et al*[62], in treating six patients with C3G (three with DDD and three with C3GN) in an open-labelled trial. EZ dose is guided by previous experience in aHUS and used for one year. Improved kidney function was observed in two patients; one patient showed partially improved proteinuria, while another patient showed better histological and laboratory findings[62]. Notably, elevated serum membrane attack complex (MAC) levels were associated with clinical improvement[63]. Duration of therapy is not yet defined. The beneficial effects of EZ in DDD recurrence[46] and C3GN recurrence[64] have been shown in case reports[65]. However, histopathological evidence of disease progression has been observed in subsequent biopsies. This highlights the fact that there is no standard accepted biomarker for disease monitoring, which can be used to assess the patient's response to treatment and predict better renal function.

In 2018, Garg *et al*[66] described the spectrum of C3 pathophysiology and its clinical implications. The observed variability of the degrees of upstream (site of C3 convertase) and downstream (site of C5 convertase) complement dysregulation may result in variable phenotypic differences[67,68]. Consequently, the nature of this spectrum will be reflected clinically on disease progress in two ways: firstly, the variability in response to EZ therapy (Figure 4)[66]. In C3G, if the dominant process focused on activation of C5 convertase (resulting in increased soluble C5b-9 levels), EZ will be of therapeutic benefit. On the other hand, patients with the dominant process focused on dysregulation at the level of C3 convertase (increased C3 split product levels), the impact of EZ therapy will be less impressive, and the process of uncontrolled complement dysregulation will persist with consequent ongoing renal injury. Secondly, future application of "soluble C5b-9" as well as "C3 degradation product" measurements will be feasible in monitoring EZ therapy (and other newly introduced C3 convertase inhibitors agents) and, thereby, will help in predicting its response[66]: (1) compstatin is a C3 inhibitory peptide that can block C3 and its convertase interaction, so that all of the three complement pathways are activated; (2) CP40 is a compstatin analog with a selective C3 inhibitor property. CP40 can prevent *in vitro* complement-mediated hemolysis induced by C3GN patient sera. Moreover, it can abort dysregulated AP activation induced by autoantibodies and genetic

A

Underlying defect	Lab response		Tissue response (histopathology)	Reference
	SCr	PCR		
None	○	●	●	Bomback *et al*, 2012[62]
C3Nef	●	●	Not performed	McCaughan *et al*, 2012[46]
C3Nef	●	●	●	Sa'nchez-Moreno *et al*, 2014[85]
None	●	●	●	Le Quintrec *et al*, 2015[80]

B

Underlying defect	Lab response		Tissue response (histopathology)	Reference
	SCr	PCR		
C3Nef	○	○	●	Bomback *et al*, 2012[62]
C3Nef, CD46 mutation	●	Non-proteinuric throughout	○	Bomback *et al*, 2012[62]
C3Nef, CFH mutations	○	● > ●	● (Increased fibrosis and continuously active C3GN)	Gurkan *et al*, 2013[64]
CFH and CFI mutations	●	●	● Improved tubulointerstitial injury, recovered ischemic injury; presistent 2-3 C3 deposition	Garg N *et al*, 2018[66]

● = Improved ○ = No change ● = Worsened ● > ● = Improved, then worsened

Figure 4 Response of complement 3 glomerulopathy subtypes to eculizumab therapy based on laboratory parameters and tissue (histopathological) response. A: Dense deposit disease response to eculizumab therapy[66]; B: Complement 3 glomerulonephritis response to eculizumab therapy[66]. CFH: Complement factor H; CFI: Complement factor I; C3Nef: C3 nephritic factor.

mutations[63]. Since C3d is the major complement fragment deposited in C3GN and DDD, CP40 represents a promising therapeutic agent. CP40 has been evaluated in paroxysmal nocturnal hemoglobinuria and hemodialysis-induced inflammation[69,70]. If CP40 is able to offer a disease-specific targeted therapy, this agent may represent a breakthrough in C3G control; (3) other novel therapeutics: antibody-based agents targeting complement function by blocking particular components of C3 convertase to hamper its formation and/or function (*e.g.*, anti-C3b monoclonal antibodies reported by Paixao-Cavalcante *et al*[71], anti-FB antibodies as described by Subias[72], and anti-properdin antibodies as professed by Pauly *et al*[73] targeting complement blockade are all under thorough evaluation[74]). Soluble complement receptor1 (CR1): a robust regulator of complement activity *in vitro,* soluble CR1 can prevent dysregulation of the AP C3 convertase. The safety and efficacy of the soluble CR1 in normalizing complement activity in pediatric patients with ESRD have been reported. With its ability to breakdown active C3b, soluble CR1 infusion can induce clinical improvement in C3GN as well as in the serum levels of MAC in patients with DDD recurrence[37].

Methods of achieving C3GN control are summarized in Table 5[34,75-86]. Until enough data from randomized control trials become available, the guidelines related to complement blockade therapy of C3GN should be based on those applied in aHUS (Table 6)[12].

Renal transplantation for C3G

Minimal data is available concerning renal transplantation for C3G. The available recommendations (Table 7) are currently based on expert opinion. Recurrence post-transplant is common, with about half of the patients with C3G at risk of losing their grafts[12].

TREATMENT OF POST-TRANSPLANT TMA

For cases of TMA secondary to medication, switching of the culprit drug to another agent (mTOR or CNI) is associated with a better response[88-90]. The first line of therapy of *de novo* TMA should encompass withdrawal of the offending drug, an essential step that is usually associated with correction of the hematological profile[57].

Plasmapheresis (PE) and intravenous immunoglobulins (IVIG) (particularly with AMR-associated TMA):

Table 6 Monitoring eculizumab therapy

CH50 (total complement activity)	AH50 (alternative pathway hemolytic activity)	Eculizumab trough	Alternative assays
Measures the combined activity of all of the complement pathways	Measures combined activity of alternative and terminal complement pathways	May be a free or bound level	The following assays are under investigation
Tests the functional capability of serum complement components to lyse 50 % of sheep erythrocytes in a reaction mixture	Tests functional capability of alternate or terminal pathway complement components to lyse 50% of rabbit erythrocytes in a Mg^{2+}-EGTA buffer	ELISA: using C5-coated plates, patient sera, and an anti-human IgG detection system	Free C5
Low in congenital complement deficiency (C1-8) or during complement blockade	Will be low in congenital C3, FI, FB, properdin, FH, and FD deficiencies or during terminal complement blockade	Not affected by complement deficiencies	*In vitro* human microvascular endothelial cell test
Normal range: Assay dependent	Normal range is assay-dependent.	Recommended trough level during complement blockade: 50-100 μg/mL	SC5b-9 (also referred to as sMAC and TCC) remain detectable in aHUS remission, so not recommended as a monitoring tool
Recommended goal during therapeutic complement blockade: < 10% of normal	Recommended goal during complement blockade: < 10% of normal		

Adapted from Goodship *et al*[12]. aHUS: Atypical hemolytic uremic syndrome; C3: Complement component 3; C5: Complement component 5; EGTA: Ethyleneglycol tetraacetic acid; ELISA: Enzyme-linked immunosorbent assay; FB: Complement factor B; FD: Complement factor D; FH: Complement factor H; FI: Complement factor I; sC5b-9: Soluble C5b-9; sMAC: Soluble membrane attack complex; TCC: Terminal complement complex.

Table 7 Transplant considerations in C3 glomerulopathy[1]

Timing	Donor selection	Risk reduction
Avoid transplantation during acute period of renal loss	No specific recommendation can be made on donor choice. When considering living donors, high risk of recurrence should be weighed against presumed risk of waiting on cadaveric donor list	C3G histological recurrence is as high as 90%[7,87]
Avoid transplantation during acute inflammation		Limited data suggest: rapid progression to ESRD in native kidneys increases recurrence risk[87]
No data supporting whether specific complement abnormalities (*e.g.*, high titer C3Nef, low C3 or high soluble C5b-9) predict increased risk for relapse		There are no known strategies to reduce recurrence risk of C3G Clinical recurrence should drive decision to treat[7] In absence of clinical trials, use of anti-complement therapy is based solely on a small open-label trial and positive case reports[62] (the impact of publication bias is unknown) C3G associated with monoclonal gammopathy has a high rate of recurrence[7]

[1]Based on limited retrospective cohort data. Adapted from Goodship *et al*[12]. C3: Complement component 3; C3G: C3 glomerulopathy; C3Nef: C3 nephritic factor; ESRD: End-stage renal disease.

fresh-frozen plasma (FFP) is advised as a reposition fluid, which must be type-specific, ordered in advance and thawed before use, despite the high risk of reactions; however, it replaces all plasma constituents and is appropriate for patients with TMA[91]. Before the era of EZ, the following supportive explanations have been provided: (1) proven efficacy in TTP[92]; (2) a graft salvage rate of more than 80%, as reported by Karthikeyan *et al*[13]. He addressed two possible benefits for this type of therapy: clearance of the platelet aggregation factors (*e.g.*, thromboxane A2) and replenishment of the deficient agents (*e.g.*, PGI2-stimulating factor)[13]; (3) with frequent possibility of the presence of underlying complement dysregulation, commencing PE therapy will also be beneficial in two ways: clearance of the aberrant complement components, and replacement with normally functioning complement proteins[93]; (4) clearance of the anti-HLA antibodies in AMR-associated aHUS improved patient outcome[55,94]; (5) PE/IVIG therapy was successfully associated with a 100% response rate in five solid organ transplants complicated by a systemic form of TMA. There was no evidence of relapse after cessation of the culprit drug (*e.g.*, tacrolimus) in a recent report[57].

Belatacept, a fusion protein composed of the Fc fragment of human IgG1 linked to the extracellular domain of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), selectively inhibits T cell activation through a co-stimulatory blockade[95].

EZ, an anti-C5 agent that blocks lytic C5b-9 MAC generation, not only revolutionized aHUS therapy but was also effective in preventing its recurrence[96]. The role of complement activation in TMA evolution has been recognized in a majority of *de novo* TMA patients. Chua *et al*[97], for example, reported deposition of C4d in all biopsies of post-transplant TMA. Efficacy of this

Table 8 Eculizumab dosing in atypical hemolytic uremic syndrome based on dosing goal

Minimal dose	Discontinuation
Desire to continue dosing with the minimal dose required to achieve a pre-identified level of complement blockade[1]	Desire to discontinue complement blockade
Dose reduction or interval extension Goal CH50 < 10% (recommended) Goal AH50 < 10% (recommended) Goal eculizumab trough >100 μg/mL	No consensus exists regarding tapering of dose

[1]Additional monitoring may be required during intercurrent events (*e.g.*, infection, surgery, vaccination) to detect unblocked complement activity. Adapted from Goodship *et al*[12]. AH50: Alternative pathway hemolytic activity; CH50: Total complement activity.

agent has also been documented in the management of resistant cases of medication-associated *de novo* TMA, including those with unidentified genetic mutations[98-103]. Moreover, efficacy of EZ has been also shown in some cases of resistant AMR-associated TMA[103-111]. However, Loupy *et al*[112] reported a similar graft survival (95.8% *vs* 89.7% at two years post-transplant, respectively) and estimated GFR (52.6 mL/min *vs* 46.7 mL/min) in comparing PE-treated recipients with the EZ-treated group. Considering the high cost of this drug, utilization of this agent is better confined to PE-dependent patients, AMR-associated TMA and to cases with refractory hemolysis.

Treatment of recurrent TMA

Minimal work-up of genetic studies should include: CFH, CFI, CFHR, CFB, MCP and C3[113]. All cases with suspected TMA should be screened for all complement components and its related proteins. Cases with isolated membrane cofactor protein (MCP) mutations (not combined with other gene defects) may be safe for kidney donation. Cases with documented TMA and with a lack of definitive genetic defects may proceed with kidney transplantation under the umbrella of intensive PE therapy[114]. Polygenic patterns of TMA should be dealt with cautiously in case of living donation[115].

Prevention of aHUS

Avoid trigger factors that stimulate complement activity (*e.g.*, ischemia-reperfusion injury, viral infection and culprit medications)[52]. Immunosuppressive regimens devoid of medications related to TMA evolution[116] are advised. PE therapy alone is not sufficient for TMA cure and prevention, with the following explanations postulated: (1) PE alone frequently failed to prevent TMA recurrence[117]; (2) TMA regression cannot be preserved after cessation of therapy; and (3) recipients treated with PE showed an evidence of "subclinical" disease[118], which declares that PE has no influence on complement activity. Prophylactic use of rituximab proved to be beneficial as an anti-CFH-antibody[119], and this effect can be augmented with the addition of PE therapy[120,121]. The anti-C5 monoclonal antibody EZ has been reported to be successful in preventing TMA recurrence in recipients with CFH, CFH/CFHR1 hybrid gene mutations as well as in C3 gene mutations[122-125].

Prophylactic complement blockade

Eighty percent of kidney transplantation recipients with TMA proved to be associated with genetic mutations[126]. Based on the fact that a TMA episode is suspected with trigger factor (*e.g.*, surgery), a robust suggestion is to protect the patient with complement blockade, if not already instituted[127]. Unfortunately, this suggestion lacks appropriate evidence[12].

Therapeutic protocols for aHUS recurrence

Given a clear role of complement blockade in the management of TMA, two regimens have been suggested: (1) minimal dosage to achieve complement blockade; and (2) a dose withdrawal scheme (Table 8)[84]. EZ monitoring, however, is mandated for better response (Table 6)[128-131].

HOW TO MONITOR COMPLEMENT BLOCKADE - TABLE 6 DESCRIBES EZ THERAPY MONITORING

Duration of therapy

There is not enough data supporting life-long therapy. However, sustaining EZ seems to be reasonable in certain situations. Figure 5 represents a small guide, meanwhile early biomarkers of disease recurrence and complement activation became available.

Unanswered questions

The lacunae in satisfactory data still present as proper dosage, dose intervals, and duration of therapy[132], as well as the impact of this type of therapy on transplant spectrum[133].

Cessation of therapy

Figure 5 represents a guiding scheme suggested for EZ withdrawal[12].

Is EZ therapy the end of the road?

In 2013, Verhave *et al*[118] reported the feasibility of successful kidney transplantation without EZ therapy in four patients with high-risk aHUS. Patients received living donor kidneys with a therapeutic regimen consisting of: Basiliximab for induction, tacrolimus in low dosage, prednisone, and MMF for maintenance

Table 9 Risk of atypical hemolytic uremic syndrome recurrence according to the implicated genetic abnormalities

Gene mutation	Location	Functional Impact	Mutation frequency in aHUS (%)	Recurrence after transplantation (%)
CFH	Plasma	Loss	20-30	75-90
CFI	Plasma	Loss	2-12	45-80
CFB	Plasma	Gain	1-2	100
C3	Plasma	Gain	5-10	40-70
MCP	Membrane	Loss	10-15	15-20
THBD	Membrane	Loss	5	One case
Homozygous *CFHR1* del (3%-8%)	Circulating	Undetermined	14-23 (> 90% with anti-CHF AB)	NA

Adapted from Salvadori *et al*[1]. aHUS: Atypical hemolytic uremic syndrome; NA: Not available; CFH: Complement factor H; CFI: Complement factor I; CFB: Complement factor B; C3: Complement 3; MCP: Membrane cofactor protein; THBD: Thrombomodulin.

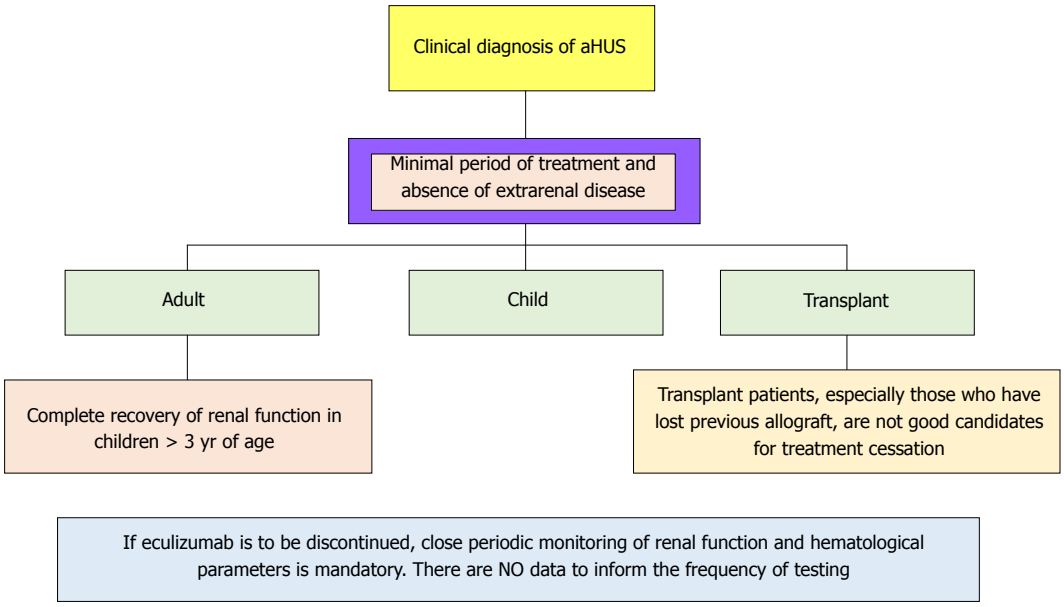

Figure 5 Recommendations for cessation of treatment with complement inhibitors. There are no prospective controlled studies in patients with atypical hemolytic uremic syndrome (aHUS) to define criteria for discontinuation of eculizumab therapy. This flow diagram is based on expert opinion[134-137]. Discontinuation can be considered on a case-by-case basis in patients after at least 6-12 mo of treatment and at least 3 mo of normalization (or stabilization in the case of residual chronic kidney disease) of kidney function. Earlier cessation (at 3 mo) may be considered in patients (especially children) with pathogenic variants in membrane cofactor protein if there has been rapid remission and recovery of renal function. Patients on dialysis or eculizumab should be maintained for at least 4 to 6 mo before discontinuation. In this setting, assessment of fibrotic changes in kidney biopsy may be helpful. In transplant patients, especially patients who have lost previous allografts, discontinuation is not recommended. Adapted from Goodship *et al*[12].

immunosuppression. A statin has also been added. Further precautions include: lowering BP as much as tolerable and minimizing the cold ischemic time. For the next 16-21 mo, no recurrence or rejection events have been reported[118]. The following conclusion has been addressed: successful kidney transplantation in recurrent aHUS patients can be achieved with an EZ-free regimen through: (1) decreasing cold ischemic time; (2) minimizing the risk of rejection; and (3) preserving endothelial integrity[118].

Renal transplantation in TMA

Timing of transplant: six months after commencing, dialysis should elapse before proceeding in transplant, as renal recovery can be observed several months after initiation of EZ therapy[137,138]. Two prerequisites should be fulfilled before commencing renal transplantation: (1) resolution of the extrarenal manifestations of TMA; and (2) recovery of TMA hematological parameters. The magnitude of recurrence risk may be used to evaluate the recipient's need for complement blockade (Table 9)[1].

CONCLUSION

The role of complement cascade in the evolution of kidney diseases either in the native kidney or post-transplant is well recognized. The prognosis of aHUS and, in some cases, C3G is greatly improved after commencing complement blockade. These agents are not only curative, but also successful in preventing post-transplant disease recurrence. Owing to the inherited nature of most of these diseases, the maintenance of this therapy is recommended despite cost burden. Consequently, the need for regimens allowing safe withdrawal of these agents is urgently required. However, newer therapies (*e.g.*, new monoclonal

antibodies, recombinant proteins, and small interfering RNA (siRNA) agents) hold promise for the near future[139,140].

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SYSTEMATIC REVIEW

Impact of machine perfusion of the liver on post-transplant biliary complications: A systematic review

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Abstract

AIM

To review the clinical impact of machine perfusion (MP) of the liver on biliary complications post-transplantation, particularly ischaemic-type biliary lesions (ITBL).

METHODS

This systematic review was performed in accordance with the Preferred Reporting Systematic Reviews and Meta-Analysis (PRISMA) protocol. The following databases were searched: PubMed, MEDLINE and Scopus. The keyword "liver transplantation" was used in combination with the free term "machine perfusion". Clinical studies reporting results of transplantation of donor human livers following *ex situ* or *in situ* MP were analysed. Details relating to donor characteristics, recipients, technique of MP performed and post-operative biliary complications (ITBL, bile leak and anastomotic strictures) were critically analysed.

RESULTS

Fifteen articles were considered to fit the criteria for this review. *Ex situ* normothermic MP was used in 6

studies, *ex situ* hypothermic MP in 5 studies and the other 4 studies investigated *in situ* normothermic regional perfusion (NRP) and controlled oxygenated rewarming. MP techniques which have *per se* the potential to alleviate ischaemia-reperfusion injury: Such as hypothermic MP and NRP, have also reported lower rates of ITBL. Other biliary complications, such as biliary leak and anastomotic biliary strictures, are reported with similar incidences with all MP techniques. There is currently less clinical evidence available to support normothermic MP as a mitigator of biliary complications following liver transplantation. On the other hand, restoration of organ to full metabolism during normothermic MP allows assessment of hepatobiliary function before transplantation, although universally accepted criteria have yet to be validated.

CONCLUSION

MP of the liver has the potential to have a positive impact on post-transplant biliary complications, specifically ITBL, and expand extended criteria donor livers utilisation.

Key words: Liver transplantation; *Ex situ* machine perfusion of the liver; Donation after circulatory death; Non-anastomotic intra-hepatic stricture; Ischemic-type biliary lesions; Extended criteria donors

Core tip: Post-transplant biliary complications are one of the main culprits responsible for the high patient morbidity following extended criteria donor liver transplantation. In its most severe form, ischaemic-type biliary lesions, can lead to graft failure and re-transplantation. Machine perfusion (MP) of the liver is a promising approach in reconditioning high-risk organs. Clinical studies have, so far, focussed on the impact of MP on hepatocellular function recovery and assessment. In this review we present the clinical evidence of the effect of MP on post-transplant biliary complications and discuss how, in the future, this approach can reduce these complications further.

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INTRODUCTION

Post-transplant biliary complications: The current scenario

Post-transplant biliary complications often require laborious and costly interventions, placing a heavy burden on health resources and adversely affecting patient outcomes[1,2]. The incidence of these complications is increasing as a result of the growing utilisation of extended criteria donor (ECD) organs, mainly from donation after circulatory death (DCD). Biliary complications such as biliary leak and anastomotic strictures are primarily related to surgical technicalities and are usually successfully managed with endoscopic procedures[3]. The most severe form of post-transplant biliary complication is non-anastomotic intrahepatic strictures (NAS). NAS is characterised by the occurrence of diffuse intra-hepatic strictures in the biliary tree and it was initially associated with hepatic artery thrombosis[4]. The ischaemic donor biliary tree was found to develop necrosis with fibrotic strictures, dilatations and potentially biliary casts[4]. Thereafter it was demonstrated that similar lesions occurred in the presence of a patent hepatic artery without evidence of recurrence of biliary disease. This entity was subsequently classified as ischaemic-type biliary lesion (ITBL)[5].

The reported incidence of ITBL is approximately 10%-30% for controlled DCD and 1%-3% for donation after brain death (DBD) organs[6-10]. Patients generally present with elevated liver function tests suggesting cholestasis (bilirubin, alkaline phosphatase and gamma-glutamyltransferase) within a few months of transplantation and may be asymptomatic initially. Initial work-up includes exclusion of hepatic artery thrombosis and anastomotic biliary strictures. Imaging investigations consist of non-invasive magnetic resonance cholangiopancreatography (MRCP) and computed tomographic cholangiography, or direct cholangiographic methods, such as endoscopic retrograde cholangiopancreatography and percutaneous transhepatic cholangiography. Due to the high reliability of current non-invasive imaging techniques in diagnosing biliary strictures, invasive procedures are currently reserved for scenarios where an intervention is planned, such as stricture dilatation, stenting or stone extraction[11,12]. With ITBL, imaging confirms the presence of fibrotic strictures, in most cases located around the bifurcation of the common bile duct leading to dilatation of the intra-hepatic biliary system[1,8]. Figure 1 illustrates these typical imaging features of ITBL following liver transplantation. The obstructive strictures cause cholestasis with formation of sludge and casts that predispose to cholangitis, frequently requiring surgical or endoscopic intervention. Despite these measures, approximately 50% of patients with ITBL require re-transplantation or die[13].

Although the pathogenesis of ITBL is still not fully understood a growing body of evidence suggest that it is partially associated with ischaemia-reperfusion injury (IRI)[14,15]. Noack *et al*[16] in a well-designed *in-vitro* study using rat-derived bile duct cells showed that they were more resistant to anoxia than hepatocytes, however during reoxygenation they produced higher amounts of reactive oxygen species (ROS). This was associated with increased rates of bile duct cell death when compared to hepatocytes[16]. It has been shown that mitochondrial ischaemic induced injury leads to ROS production during reperfusion which in turn causes

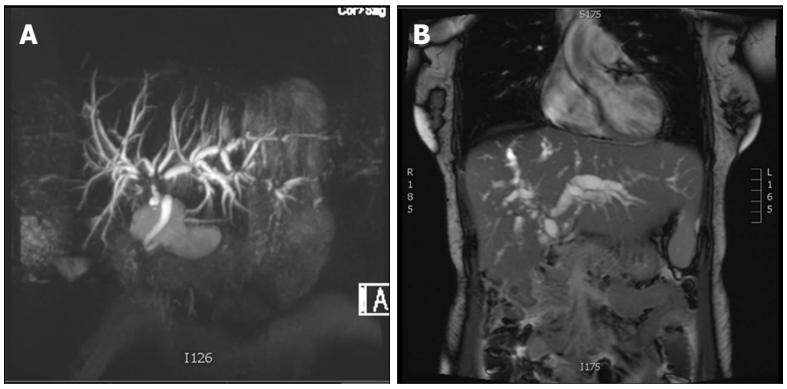

Figure 1 Magnetic resonance cholangiopancreatography images of ischemic-type biliary lesions following liver transplantation. The images show two recipients of livers from donation after circulatory death donors that developed ischemic-type biliary lesions within 60 d following transplantation. Hepatic artery thrombosis and anastomotic biliary strictures were ruled out. A: A typical lesion is seen affecting the bifurcation of the common hepatic bile duct with moderate dilatation of the intrahepatic biliary tree; B: The image shows strictures at the bifurcation of the common hepatic bile duct, diffuse intra-hepatic strictures and a severe dilatation of the intrahepatic biliary tree.

oxidative injury and activation of the inflammatory cascade[17,18]. Conversely, clinical series have reported severe injury to the biliary epithelium just after cold static storage[19,20]. Garcia-Valdecasas *et al*[15] using a porcine transplantation model suggested a direct relationship between prolonged ischaemic times and cell injury. Indeed, other clinical series have confirmed the association of longer cold ischaemic time (CIT) and higher rates of ITBL[21-24]. A similar relationship has been observed with warm ischemic time in DCD liver transplantation[15,25]. A large clinical series of donor bile duct biopsies before liver transplantation showed similar injury to the biliary epithelium after static cold storage (SCS), and that it was exacerbated after reperfusion; however, this did not correlate with the development of ITBL[26]. Nevertheless, the authors reported a strong association between ITBL and damage to the peribiliary vascular plexus and peribiliary glands. As progenitor biliary cells are known to reside in the peribiliary glands, the former finding suggests an association between ITBL and an attenuated regenerative capacity of the biliary epithelium[26,27]. Ischaemic injury is likely to play a major role in ITBL pathogenesis, although other factors have also been shown to be implicated. Immunological mediated injury to the biliary epithelium has been associated with ITBL[28]. It may be the result of direct immunological damage to the biliary epithelium *via* a rejection reaction[29]; or, indirect, secondary to the development of arteriopathy[29,30]. This cross reactivity is described in scenarios of cytomegalovirus infection[30], ABO incompatibility[31] and transplantation for primary sclerosing cholangitis[1]. Bile salt toxicity has also been investigated as a potential cause for ITBL by having a direct detergent effect on phospholipid cellular membranes of the biliary epithelium[28]. Flushing of the biliary tree during organ procurement is necessary in order to remove all bile salts that could damage cholangiocytes[5,28]. Furthermore, an imbalance in the post-transplant bile composition, with a higher bile salt/phospholipid ratio, due to inefficient ATP-dependent biliary transporters has been suggested as a predictive factor for ITBL[32]. While detail of the pathogenesis of ITBL is beyond the scope of this review, information on the implicated mechanisms can be found in a number of published reviews[9,28].

Machine perfusion of donor livers

The utilisation of DCD livers is increasing. In 2017, in the United Kingdom, they constituted 28% of the livers transplanted[33]. Furthermore, the rising prevalence of donor obesity (body mass index greater than 30 kg/m^2) and an ageing population continue to compound the risks to those livers[33]. These high-risk ECD organs are associated not only with a higher risk of graft dysfunction post-transplantation but also increased rates of ITBL[34]. Despite these disadvantages, their utilisation is required to tackle the ever-growing discrepancy between organ donor supply and demand. Machine perfusion (MP) of the liver is being developed as a means of assessment and reconditioning of ECD donors, potentially allowing for safer transplantation of these high-risk livers[34,35]. Different techniques of MP have been developed; it can be performed *in situ* during organ procurement or *ex situ* after the procedure. With regards to livers, the only technique of *in situ* MP described so far is normothermic regional perfusion (NRP)[8]. *Ex situ* MP protocols vary in terms of oxygenation (active or pre-charged oxygenation), perfusate temperature (hypothermic, subnormothermic, gradual rewarming and normothermic), timing of perfusion (preservation or end-ischemic) and via of organ perfusion (portal vein alone or dual portal vein and hepatic artery perfusion)[34,36].

Hypothermic machine perfusion (HMP) has been performed around 10 ℃ in most studies[37,38]. At this temperature liver metabolism is reduced; and, passive oxygen delivery by diffusion in an oxygen carrier-free perfusate is enough to support the organ[39]. The first published clinical series employed pre-charged oxygen delivery to the organs[37], technique that was later followed by active oxygenation of the perfusate[40].

Hypothermic oxygenated MP can be performed *via* portal vein alone (HOPE) or *via* portal vein and hepatic artery (dual hypothermic oxygenated perfusion - D-HOPE)[41-43]. Both techniques have shown the capacity of improve mitochondrial oxidative function prior to rewarming, resulting in increased adenosine triphosphate (ATP) synthesis and a reduction in ROS production, oxidative tissue injury and activation of the inflammatory cascade[42,43].

Normothermic machine perfusion (NMP) maintains the organ at physiological temperatures (37 ℃) and therefore restores full metabolic activity. This enables the possibility of functional or viability assessment prior to transplantation, a major advantage of NMP when compared to other perfusion techniques[44,45]. It also opens up a window of opportunity for *ex situ* therapeutic interventions[34]. Furthermore, previous studies have reported on the safety of extended normothermic perfusion of organs, which may facilitate transportation and logistical management of busy transplant units[46]. However, potential drawbacks of NMP are that it requires obligatorily the inclusion of an oxygen carrier in the perfusate, and NMP inevitably induces reperfusion injury to some extent.

Subnormothermic machine perfusion (SMP) has been performed at around 20 ℃ in most studies. It encompasses purely SMP and the controlled oxygenated rewarming (COR) from 10 ℃ to 20 ℃[47,48]. The increase in temperature from HMP to SMP is suggested to be enough to increase liver metabolism to an extent that it would allow assessment of organ function without inducing the detrimental changes associated with organ reperfusion at normothermic temperatures[48]. Evidence for the clinical benefits is available for COR perfusions, it was associated with lower markers of hepatocellular injury after transplantation and enhanced graft function through the avoidance of subtle changes in organ temperature[47].

For DCD livers, there are encouraging reports of *in situ* oxygenated NRP. It has been successfully applied to controlled DCD donors (withdrawal of life support in patients with irreversible clinical conditions) and uncontrolled DCD (witnessed cardiac arrest without response to resuscitative measures)[8,49,50]. NRP limits ischaemia and prevents depletion of energy stores prior to SCS and this is suggested to be essential for uncontrolled DCD donors and beneficial for controlled DCD[8].

More recently, combinations of MP techniques have been shown to merge the advantages of individual protocols, enhancing the rescue of liver function what may potentially improve graft function after transplantation[51,52]. Despite differences between techniques, MP has the potential to limit ischaemic injury to the organ, thus offering a safer preservation environment and an opportunity for organ reconditioning which could mitigate IRI.

As discussed herein, the current evidence shows that cholangiocytes are more vulnerable to IRI than hepatocytes and that the pathogenesis for biliary injury goes beyond IRI. Therefore, investigation of the impact of MP on biliary function specifically, and not only on hepatocellular function, is fundamental. The aim of this review was to investigate the current clinical evidence available regarding the effect of MP on post-transplant biliary complications, focusing on ITBL.

MATERIALS AND METHODS

This systematic review was performed in accordance with the Preferred Reporting Systematic Reviews and Meta-Analysis (PRISMA) protocol[53].

The following databases were searched for the development of this review: PubMed, MEDLINE and Scopus. The keyword "liver transplantation" was used in combination with the free term "machine perfusion". The literature review was performed until June 20, 2018 and there were no limits on the date for inclusion of publications. The literature search strategy used for one database is presented in the Supplementary Table S1.

The screening and selection of articles were independently performed by two authors (Yuri L Boteon and Amanda PCS Boteon). There was no disagreement in study selection between authors. Manuscript titles that were not related to the main scope of the review were excluded. Full abstracts were then read and excluded if found not to be relevant to the review. Finally, full papers were assessed for eligibility and included in this review. The flow diagram for the literature selection process is shown in Figure 2.

Inclusion criteria were: (1) clinical studies reporting results of transplantation of donor human livers following *ex situ* or *in situ* MP; and (2) articles written in English and published. Exclusion criteria were: (1) absence of transplantation following MP; (2) exclusively animal models; (3) single case report; (4) review articles; and (5) articles not written in English.

Details relating to donor characteristics [type, age, donor risk index (DRI), warm ischaemic time (WIT), CIT], recipients [age, model for end-stage liver disease (MELD)], perfusion (type of perfusion, oxygenation, timings) and post-operative biliary complications (ITBL, leak and anastomotic strictures) were retrieved from each manuscript and critically analysed. Studies were assessed in terms of study design, methods and outcomes. No review protocol was registered before this review was started. No simplifications or assumptions were made, and any identified risk of bias is discussed throughout the review.

RESULTS

Fifteen articles were considered to fit the criteria for this review. A diagrammatic summary of the screening process is provided in Figure 2.

MP and ischemic type biliary lesions (ITBL)

Eight out of fifteen clinical studies utilised an end-

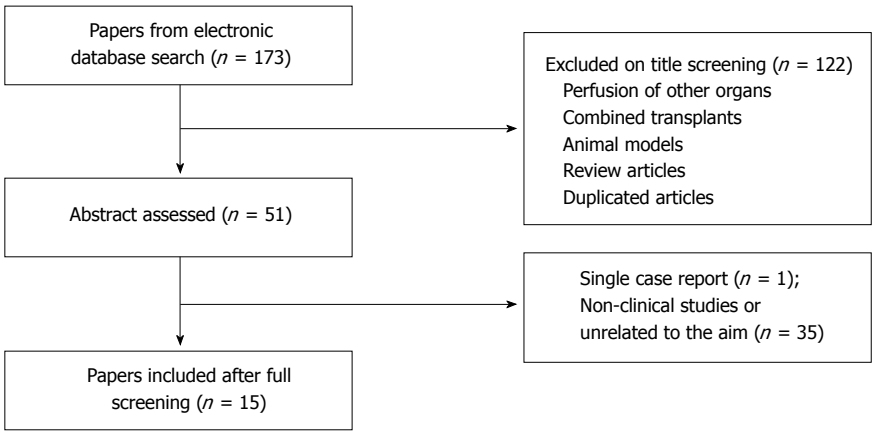

Figure 2 Study flow diagram for systematic review of the literature on the impact of machine perfusion of the liver and post-transplant biliary complications. Following literature search duplicate articles were excluded and the titles screened. The selected abstracts were then read and non-clinical studies or reports unrelated to the aim of the review were excluded.

ischemic model of MP (MP commenced after a variable period of SCS), 4 studies utilised preservation MP (MP from organ procurement up to transplantation) and 3 employed NRP. NMP was used in 6 studies, HMP in 5 studies and the other 4 studies investigated NRP and COR. HMP with active perfusate oxygenation (HOPE and D-HOPE) studies were seen to be currently focused on DCD organs and HMP with pre-charged oxygenation on DBD organs. NMP studies used both donor types, however preservation studies explored a higher proportion of DBD compared to DCD organs. The contrary was seen for end-ischemic NMP.

Donor and recipients characteristics, of the cases included in individual studies, are presented in Table 1. It also reports the rates of ITBL. Table 2 describes the incidence of bile leak and anastomotic biliary stricture within the different studies. Studies characteristics were described therein, as it was their design.

NMP and post-transplant biliary complications

The largest clinical trial involving NMP as a preservation strategy was recently published by Nasralla *et al*[46]. Following procurement, transplantable livers were randomised and allocated to the intervention group that had NMP up to the point of transplantation or a control group that had conventional SCS. From the 121 livers perfused, 87 were from DBD donors and 34 from DCD donors. Results did not shown differences in bile duct complications between groups, with one patient in each arm developing ITBL within the first year, both requiring re-transplantation. On MRCP, the rates of NAS were similar between groups for DBD (NMP 7.4% *vs* SCS 5.4%; $P = 0.678$) and DCD (NMP 11.1% *vs* SCS 26.3%; $P = 0.180$). The incidence of anastomotic strictures was also similar for DBD or DCD organs (NMP 40.7% *vs* SCS 41.8%; $P = 0.909$; and, NMP 48.1% *vs* SCS 57.9%; $P = 0.515$, respectively)[46].

Other clinical studies investigating NMP using a preservation approach[54-56] involved smaller patient numbers, the majority of which were from DBD donors, and did not specifically report the incidence of ITBL (Table 1). Ravikumar *et al*[55] published the first phase 1 clinical trial demonstrating the safety and feasibility of NMP in a preservation approach, as an alternative to SCS. In all, 20 donor livers (16 DBD and 4 DCD) were transplanted following NMP. The 30-day graft survival was similar to static cold stored livers and the median peak aspartate aminotransferase within the first 7 post-operative days was lower. In terms of biliary complications, the authors reported the occurrence of 4 cases of anastomotic biliary strictures in the NMP group[55].

The two studies of NMP after a period of SCS (end-ischaemic model) involved organs that were deemed too high risk for transplantation[57,58]. These studies predominantly used DCD livers and applied predefined viability criteria prior to transplantation. Mergental *et al*[57] did not observe any biliary complications at 7 mo of follow up post-transplantation. Watson *et al*[58] reported the occurrence of 4 cases of ITBL in 16 DCD liver transplants, of which 3 needed re-transplantation. The authors of the latter study concluded that that NMP per se does not prevent ITBL but may provide biomarkers to identify livers that are high risk, such as maximum bile pH > 7.5 and bile glucose ≤ 3 mmol/L or ≥ 10 mmol less than perfusate glucose[58].

HMP and post-transplant biliary complications

The first clinical study using HMP prior to transplantation was performed by Guarrera *et al*[37] Twenty DBD livers were perfused after a period of SCS in a non-actively oxygenated model of HMP. ITBL rate was reported as 5%, half of the incidence of the control matched cohort that was subjected to SCS. Additionally, there was one case of bile leak and 1 report of anastomotic biliary stricture[37]. The same approach was repeated later in a study of DBD livers declined by the United Network for Organ Sharing region for transplantation[59]. The authors found a significant decrease in the rate of biliary stricture in comparison with SCS (10% *vs* 33%, $P = 0.031$). One report of bile leak was noted in the HMP group and 3 in

Table 1 Comparison between donor, recipient, perfusion characteristics and the reported rates of ischemic-type biliary lesions

Ref.	Yr	Perfusion type	Timing MP	*n*	Donor age	Donor risk index	Recipient age	Recipient MELD	DBD (*n*)	DCD (*n*)	DBD ITBL (%)	DCD ITBL (%)	CIT (min)	Func. WIT (min)	Re-Tx (*n*)
Ex situ normothermic machine perfusion															
Nasralla *et al*[46]	2018	NMP	Preserv	121	56 (16-84)	1.7[3]	55	13 (6-35)	87	34	7.4	11.1	126	21	3
Selzner *et al*[54]	2016	NMP	Preserv	10	48 (17-75)	1.9	57	21 (8-40)	8	2	0	0	103	NA	0
Bral *et al*[56]	2017	NMP	Preserv	9	56 (14-71)	1.6 (0.9-2.7)	53 (28-67)	13 (9-32)	6	3	0	0	167 (95-293)	22	0
Ravikumar *et al*[55]	2016	NMP	Preserv	20	58 (21-85)	NA	NA	12 (7-27)	16	4	0	0	NA	21	0
Watson *et al*[58]	2018	NMP	End-Isc	22	57	2.3	NA	NA	6	16	0	25	386	12	3
Mergental *et al*[57]	2016	NMP	End-Isc	5	49 (29-54)	2.3	56 (47-66)	8 (8-13)	1	4	0	0	422	28	0
Ex situ hypothermic non-oxygenated machine perfusion															
Guarrera *et al*[59]	2015	HMP	End-Isc	31	57 (± 18)[1]	1.9 (± 0.5)[1]	57 (± 8.0)[1]	19 (± 5.9)[1]	31	0	9.7	NA	558	NA	0
Guarrera *et al*[37]	2010	HMP	End-Isc	20	39 (± 2.5)[1]	NA	55 (± 6.2)[1]	17 (± 7.4)[1]	20	0	5	NA	306	26	0
Ex situ hypothermic oxygenated machine perfusion															
van Rijn *et al*[43]	2017	DHOPE	End-Isc	10	53 (47-57)	1.9 (1.5-2.2)	57 (54-62)	16 (15-22)	0	10	NA	10	331	15	0
Dutkowski *et al*[38]	2015	HOPE	End-Isc	25	54 (36-63)	NA	60 (57-64)	13 (9-15)	0	25	NA	0	188 (141-264)	31 (26-36)	0
Dutkowski *et al*[40]	2014	HOPE	End-Isc	8	54 (NA)	2.2 (NA)	60 (NA)	12 (NA)	0	8	NA	0	141 (NA)	31 (22-41)	0
In situ normothermic regional perfusion															
De Carlis *et al*[60][2]	2017	NRP	NRP	7	48[1]	NA	54[1]	10.6[1]	0	7	NA	0	414[1]	33	0
Oniscu *et al*[49]	2014	NRP	NRP	11	46 (16-74)	NA	68 (43-74)	NA	0	11	NA	0	389 (169-450)	26 (13-48)	0
Minambres *et al*[50]	2017	NRP	NRP	11	58 (50-67)	NA	55 (± 13)[1]	NA	0	11	NA	0	266 (± 82.7)[1]	12 (11-16)	0
Controlled oxygenated rewarming															
Hoyer *et al*[47]	2016	COR	End-Isc	6	58 (51-71)	1.9 (1.5-2.5)	52 (43-65)	18 (11-23)	6	0	0	NA	508 (369-870)	NA	0

[1]Data presented as median or median (± SD), if available. Otherwise, all data presented as median (Interquartile range); [2]Combined hypothermic oxygenated machine perfusion after normothermic regional perfusion. Six uncontrolled DCD were included in this study; [3]Eurotransplant DRI. MP: Machine perfusion; MELD: Model for end stage liver disease; DBD: Donation after brain death; DCD: Donation after circulatory death; ITBL: Ischemic-type biliary lesions; CIT: Cold ischemic time; Func; WIT: Functional warm ischemic time; Re-Tx: Re-transplantation; NA: Not applicable or not available; Preserv: Preservation; End-Isc: End ischemic; NMP: Normothermic machine perfusion; HMP: Hypothermic machine perfusion; DHOPE: Dual vessel hypothermic oxygenated machine perfusion; HOPE: Hypothermic oxygenated machine perfusion; NRP: Normothermic regional perfusion; COR: Controlled oxygenated rewarming.

SCS respectively (Table 2).

Following these initial studies, the Zurich group developed the concept of HOPE, with active oxygenation of the perfusate, and applied this MP strategy to DCD donors[38,40]. Their first clinical trial was published in 2015, reporting the results of transplantation of 25 DCD livers[38]. The authors reported no cases of ITBL at one year follow-up of patients who received perfused DCD livers, whereas control livers subjected to SCS developed a significantly higher rate of ITBL (0/25 *vs* 11/50, $P = 0.013$). The same benefit of HOPE was not seen for extra-hepatic biliary complications, as the reported rates of leaks and anastomotic strictures were similar (HOPE 5/25 *vs* Control 12/50)[38].

The Groningen group published the first clinical series using D-HOPE in 2017[43]. Ten DCD livers were transplanted following two hours of D-HOPE, one patient in the perfusion group developed ITBL compared to 7 out of 20 in the control group. The case in the D-HOPE group was described as NAS in segments II and III of the liver and was managed with endoscopic stenting. Three control livers which developed ITBL required re-transplantation. The rate of anastomotic biliary strictures was comparable between groups (D-HOPE 2 *vs* Control 3, $P = 1.000$) as was the reported rate of biliary cast formation (D-HOPE 3 *vs* Control 3, $P = 0.372$)[43].

Normothermic reginal perfusion and post-transplant biliary complications

The first series reporting the results for transplantation of livers following NRP was published in 2014 by Oniscu *et al*[49] The authors reported the results of transplantation of

Table 2 Prevalence of bile leak and anastomotic biliary strictures between clinical studies using different techniques of machine perfusion of donor livers

Ref.	Yr	Study design	Perfusion type	Timing machine perfusion	*n*	DBD (*n*)	DCD (*n*)	Bile leak (*n*)	Anastomotic stricture (*n*)
Ex situ normothermic machine perfusion									
Nasralla *et al*[46]	2018	RCT	NMP	Preservation	121	87	34	0	0
Selzner *et al*[54]	2016	PS	NMP	Preservation	10	8	2	0	0
Bral *et al*[56]	2017	PS	NMP	Preservation	9	6	3	0	0
Ravikumar *et al*[55]	2016	PS	NMP	Preservation	20	16	4	0	4 (DBD)
Watson *et al*[58]	2018	DS	NMP	End-Ischaemic	22	6	16	0	0
Mergental *et al*[57]	2016	DS	NMP	End-Ischaemic	5	1	4	0	0
Ex situ hypothermic non-oxygenated machine perfusion									
Guarrera *et al*[59]	2015	PS	HMP	End-Ischaemic	31	31	0	1	0
Guarrera *et al*[37]	2010	NCS	HMP	End-Ischaemic	20	20	0	1	1
Ex situ hypothermic oxygenated machine perfusion									
van Rijn *et al*[43]	2017	PS	DHOPE	End-Ischaemic	10	0	10	0	2
Dutkowski *et al*[38]	2015	PS	HOPE	End-Ischaemic	25	0	25	5 (in total)	
Dutkowski *et al*[40]	2014	PS	HOPE	End-Ischaemic	8	0	8	1	1
In situ normothermic regional perfusion									
De Carlis *et al*[60][1]	2017	DS	NRP	NRP	7	0	7*	0	1
Oniscu *et al*[49]	2014	DS	NRP	NRP	11	0	11	1	1
Minambres *et al*[50]	2017	DS	NRP	NRP	11	0	11	NA	NA
Controlled Oxygenated Rewarming									
Hoyer *et al*[47]	2016	PS	COR	End-Ischaemic	6	6	0	NA	NA

[1]Combined hypothermic oxygenated machine perfusion after normothermic regional perfusion. Six uncontrolled DCD were included in this study. RCT: Randomised controlled trial; PS: Single-arm non-randomised pilot study; DS: Descriptive study; NCS: Non-randomised cohort studies; DBD: Donation after brain death; DCD: Donation after circulatory death; NA: Not applicable or not available; NMP: Normothermic machine perfusion; HMP: Hypothermic machine perfusion; DHOPE: Dual vessel hypothermic oxygenated machine perfusion; HOPE: Hypothermic oxygenated machine perfusion; NRP: Normothermic regional perfusion; COR: Controlled oxygenated rewarming.

11 controlled DCD livers, with a minimum follow-up of 3 mo, with no clinical or radiological evidence of ITBL. One patient developed an anastomotic stricture, treated endoscopically by cholangio-pancreatography (exact intervention performed is not described), and one patient had a bile leak[49]. Minambres *et al*[50] 2017, studying controlled DCD transplantation after NRP, reported no cases of ITBL after 1-year follow-up. De Carlis *et al*[60] 2017 performed NRP on 1 controlled DCD liver and 6 uncontrolled DCD. On arrival at the transplant centre, the livers were subjected to D-HOPE until transplantation. No cases of ITBL were observed and one patient had an anastomotic biliary stricture 45 d after transplantation, which was successfully treated with endoscopic stenting[60]. In terms of SMP, Hoyer *et al*[47] reported transplantation of 6 DBD livers following COR perfusion. No biliary complications were reported within a follow-up period of six months.

DISCUSSION

Post-transplant biliary complications are associated with high rates of morbidity and re-transplantation and are a major obstacle to the wider clinical utilisation of ECD livers. There is a growing body of evidence suggesting that MP can offer safer organ preservation when compared to SCS, and also offer an opportunity for organ assessment and/or reconditioning prior to transplantation[38,43,46,49,58]. In this review we have assessed the available literature investigating the impact of MP on post-transplant biliary complications, with special reference to ITBL. MP techniques which have per se the potential to alleviate IRI, such as HMP and NRP, have also reported lower rates of ITBL. Other biliary complications, such as biliary leak and anastomotic biliary strictures, are reported with similar incidences with all MP techniques.

Liver IRI is thought to be a major driver of biliary injury and, therefore, it is associated with complications following transplantation. More specifically, during ischemia, without oxygen as a terminal acceptor of electrons in the electron transport chain, succinate accumulates and acts as a store for electrons. Succinate oxidation during the early stage of reperfusion, blocks mitochondrial complex II of the electron transport chain resulting in a reverse flow of electrons towards mitochondrial complex I leading to accentuated leakage of electrons, and generation of ROS[61]. Various experimental findings using the HOPE technique have shown that oxygen at hypothermic temperatures is able to promote mitochondrial metabolism of succinate prior to reperfusion[36,42,62]. By re-establishing adequate mitochondrial oxidative function, HOPE is able to recover ATP stores, since during hypothermia mitochondria have lower energy requirements due to a minimum activation of the organ metabolism. Therefore, mechanistically, HOPE can in theory prevent the reverse flow of electrons during reperfusion, ROS generation and activation of the inflammatory cascade[36]. These factors may mitigate IRI, which would be beneficial not only

for hepatocellular function but also for the prevention of further biliary injury.

Extensive research focussing on the effect of oxygenated HMP on post-transplant biliary complications has been performed by the Groningen group. In a recent publication exploring the effects of D-HOPE on bile duct biopsies from a previous published series of cases, they showed less injury to deep and periluminal peribiliary glands after reperfusion during transplantation in the perfused group in comparison with SCS control livers[43,63]. Peribiliary glands have been described as stores for biliary progenitor cells, therefore injury to them would potentially decrease the regenerative capacity of the biliary system[64,65]. The authors acknowledge that definitive evidence to support this would require a clinical randomized trial that has since been initiated at their centre[63].

There is currently less clinical evidence available to support NMP as a mitigator of biliary complications following liver transplantation[58]. Preservation NMP shortens the ischaemic injury and offers a more physiological environment for the organ before transplantation. Nevertheless, as previously discussed, the injury to biliary cells would not be restricted to an ischaemic mechanism but may also be worsened during reperfusion. This observation could imply that NMP is of limited benefit in terms of biliary complications, since biliary injury may worsen during organ reperfusion on the machine and is not prevented or mitigated beforehand. NMP restores the full metabolism of the organ, resulting inevitably in the production and circulation of ROS and potential activation of the inflammatory response leading to tissue injury[66]. On the other hand, restoration of organ to full metabolism allows assessment of hepatobiliary function before transplantation, although universally accepted criteria have yet to be validated[35]. Watson *et al*[58] suggested bile pH and glucose content as markers of bile duct injury and associated those with the development of ITBL, however the authors recognise that NMP was not able to prevent biliary damage.

Promisingly, *in situ* NRP has shown excellent biliary outcomes after transplantation of DCD livers[49,50,60]. NRP may potentially prevent ischaemic injury and deterioration of ATP stores during organ procurement. Additionally, NRP allows assessment of the liver metabolism even before SCS[8]. Despite these points, there is no mechanistic evidence available to demonstrate any alleviation in IRI after reperfusion. It is also difficult to rule out the possibility that this beneficial effect was as a result of a potential selection bias when recruiting organs for transplantation during the procedure.

The present body of work has several limitations. First and foremost, donor livers and recipient characteristics as well as MP technique protocols exhibit a high degree of variability between studies. So far, there has been no standardisation in terms of methodology and reporting of results. Furthermore, some studies neglect to report important data variables, such as DRI, recipient age, recipient MELD and CIT. All these features are presented in Table 1 to allow an unbiased assessment of the retrieved information by the readers. Additionally, few clinical studies from each MP technique are available and most of them are originated from small pilot studies, which limit definitive interpretation of the data. MRCP was performed in some of the studies at different post-operative periods, but the significance of findings without clinical correlation is not clear. In addition, they have focussed mainly on evaluation of hepatocellular function rather than biliary function and injury. Despite the subject of this review being a relevant topic with important clinical implications, the direct effects of MP on biliary tree integrity are still relatively under-researched. More clinical randomized trials will be reported in the field in the next few years.

Higher rates of ITBL following transplantation of ECD livers, mainly DCD, place a major restraint on the wider use of these marginal livers. Each technique of MP offers different advantages and they all have the potential to tackle this problem. A feasibility study has shown that a combination of HOPE and NMP increased the rescue of metabolic parameters of high-risk ECD organs[52]. This approach may derive benefits from the individual methods, thus optimising gains also in terms of biliary function. Pharmacological interventions during NMP may potentially alleviate IRI, positively affecting biliary cells[67], and may have a direct effect on post-transplant biliary complications. Supplementation of the perfusate with substances that may induce proliferation and maturation of progenitor cells from peribiliary glands may be a feasible option to be considered[9]. We hypothesize that therapies promoting increase in secretion of phospholipids and cholesterol in the bile would equilibrate the phospholipids/bile salts balance mitigating further injury to the biliary tree. Although promising, these are options that still need to be explored in future studies. A diagrammatic summary of the current and future impact of MP on ITBL is presented in Figure 3.

The high incidence of post-transplant biliary complications, specifically ITBL, is a major constraint to wider utilisation of ECD livers. MP is currently considered a promising tool to increase ECD utilisation. However, the focus of most of the studies up to date has been the effect of MP on hepatocellular function. In this review we explored the clinical evidence currently available for the impact of MP on post-transplant biliary complications. From those studies that have looked at the effects of MP on biliary integrity, oxygenated HMP and NRP studies have been shown to exhibit better postoperative biliary outcomes in comparison with NMP and non-oxygenated HMP. However, larger clinical studies and randomised clinical trials powered for the occurrence of biliary complications as a primary endpoint are needed to confirm this data.

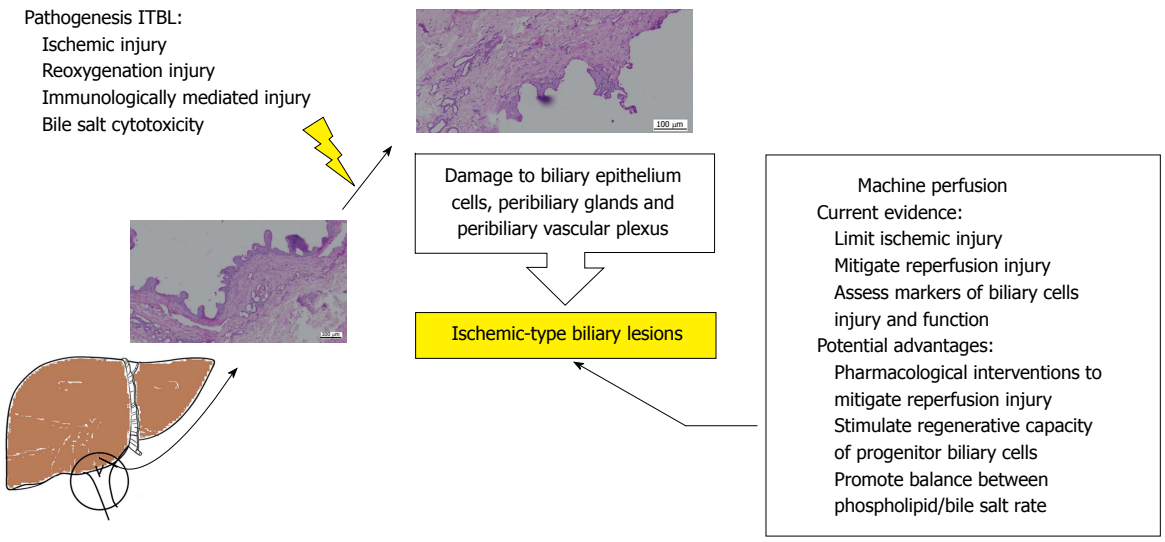

Figure 3 Diagrammatic summary of the current evidence for the impact of machine perfusion of the liver on post-transplant ischemic-type biliary lesions and future perspectives. The current evidence suggests that ischaemic-type biliary lesions (ITBL) have a multifactorial pathogenesis. These diverse factors lead to injury to the biliary epithelium, peribiliary glands and peribiliary vascular plexus. Currently, there is evidence for the potential benefits of machine perfusion on post-transplant ITBL. The figure summarises those and possible future interventions that could enhance increase these benefits further.

ARTICLE HIGHLIGHTS

Research background

The ever-growing discrepancy between donor organ availability and patients on the transplant waiting list has led to increased acceptance of extended criteria donors (ECD). However, ECD liver transplantation, mainly donation after circulatory death, is associated with poor patient and graft outcome. A major factor is the increased risk of biliary complications, in particular ischaemic type biliary lesions (ITBL). Machine perfusion (MP) of the liveris a promising tool to recondition ECD organs prior to transplantation. Therefore investigation of the impact of MP on post-transplant biliary complications is a highly relevant topic.

Research motivation

Understanding the current evidence available for the effect of MP on post-transplant biliary complications, in particular ITBL, may guide further studies in this field.

Research objectives

Revise the current clinical evidence available regarding the effect of MP on post-transplant biliary complications, focusing on ITBL.

Research methods

A systematic review was carried out with literature searches in PubMed, MEDLINE and Scopus databases. The keyword "liver transplantation" was used in combination with the free term "machine perfusion". Only clinical studies reporting results of transplantation of donor human livers following ex situ or in situ MP were included.

Research results

MP techniques which have demonstrated the potential to mitigate ischaemia reperfusion injury, such as *ex situ* oxygenated hypothermic MP and *in situ* normothermic regional perfusion, have also reported lower rates of ITBL. Other biliary complications, such as biliary leak and anastomotic biliary strictures, are reported with similar incidences with all MP techniques. Clinical studies have focused on evaluation of hepatocellular function rather than biliary function and injury so far. The direct effects of MP on biliary tree integrity are still relatively under-researched and further studies are needed.

Research conclusions

Post-transplant biliary complications are a major obstacle to the wider utilisation of ECD livers. MP has the potential to have a positive impact on this issue, specifically ITBL, and expand ECD livers utilisation. Mechanistically, mitigation of ischaemia-reperfusion injury appears to be the key mechanism involved.

Research perspectives

Supplementation of the perfusion fluid during *ex situ* MP with drugs can stimulate protective/regenerative mechanisms of the biliary tree. Pharmacological strategies may potentially modulate progenitor cells proliferation and equilibrate the phospholipid/bile salts balance in the bile.

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CASE REPORT

Treatment of transplant renal artery pseudoaneurysm using expandable hydrogel coils: A case report and review of literature

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Abstract

Transplant renal artery (TRA) pseudoaneurysm can result in bleeding, infection, graft dysfunction and graft loss. We report the management of a renal transplant recipient who presented five months after renal transplantation with deterioration of renal function, who was found to have TRA pseudoaneurysm and TRA stenosis. Both were treated radiologically by using expandable hydrogel coils (EHC) in combination with stenting. Improvement in clinical, biochemical and radiological parameters were observed after the intervention. To our knowledge, this is the first report in the transplant literature on the use of EHC for the treatment of a TRA pseudoaneurysm.

Key words: Pseudoaneurysm; Transplant renal artery; Expandable hydrogel coils; Outcomes

Core tip: Transplant renal artery (TRA) pseudoaneurysm is an uncommon complication after renal transplantation, which can cause transplant dysfunction, bleeding, infection

and graft loss. Expandable hydrogel coils should be considered in the treatment of TRA pseudoaneurysm as they have been effective in our patient.

Marie Y, Kumar A, Hinchliffe S, Curran S, Brown P, Turner D, Shrestha B. Treatment of transplant renal artery pseudoaneurysm using expandable hydrogel coils: A case report and review of literature. *World J Transplant* 2018; 8(6): 232-236 Available from: URL: http://www.wjgnet.com/2220-3230/full/v8/i6/232.htm DOI: http://dx.doi.org/10.5500/wjt.v8.i6.232

INTRODUCTION

Transplant renal artery (TRA) pseudoaneurysm can result in bleeding, infection, graft dysfunction, graft loss, lower limb ischaemia, limb loss and mortality[1]. The treatment of TRA pseudoaneurysm remains challenging. The expandable hydrogel coil (EHC) embolization system is a relatively new type of device that has been described to successfully treat intracranial and peripheral pseudoaneurysms. These are helical platinum coils coated with exapandable hydrogel polymer. The hydrogel coating undergoes full expansion within 20 min attending a size between 4-5 times the size of the coils on coming in contact with blood. The stasis of the blood causes organization of thrombus, which fills the aneurysm causing complete its occlusion[1]. To our knowledge, there is no published data in the transplant literature on the application of EHC in the treatment of TRA pseudoaneurysm. We describe the successful management of a case of TRA pseudoaneurysm using EHC and review the pertinent literature.

CASE REPORT

A 38-year-old male patient received a renal transplant (RT) of a kidney from a donation after circulatory death donor. The right donor kidney had a single real artery on an aortic patch and the short renal vein which was elongated by using a segment of inferior vena cava. The kidney was implanted in the right iliac fossa by anastomosing the renal artery to the external iliac artery in an end-to-side fashion using continuous 5/0 prolene sutures (Ethicon Inc., United Kingdom) and renal vein to the external iliac vein in the similar fashion. An extravesical ureteroneocystostomy was performed as describe by Lich-Gregoir. The vascular anastomosis time was 45 min while the total cold ischaemic time was 15 h and 38 min. The patient received basiliximab (Sandoz, United Kingdom) and methyl prednisolone as induction therapy and tacrolimus, mycophenolate mofetil and prednisolone as maintenance immunosuppression.

The transplant had delayed graft function and required haemodialysis during the first week until renal function started to improve. The initial ultrasound scan of the transplant kidney showed a well perfused graft with no evidence of hydronephrosis or any collection and the resistive indices (RI) were within normal limits. The renal function was stable with a serum creatinine of 136 μmol/L and an estimated glomerular filtration rate (eGFR) of 51 mL/min per 1.73 m^2 at 3 mo post-transplantation.

At five months post-transplantation, on routine outpatient review, deterioration in kidney renal function with a rise in serum creatinine to 633 μmol/L (eGFR 13 mL/min per 1.73 m^2) was observed. A duplex ultrasound scan showed a well-perfused kidney with no evidence of hydronephrosis. An ultrasound-guided biopsy of the kidney, which was treated with three pulses of intravenous methyl prednisolone, showed features of acute cellular rejection. However, there was no improvement in renal function. A repeat duplex ultrasound scan showed damped flow signals on the intra-renal blood vessels with reduced RI ranging between 0.4 and 0.45. There were associated high velocities at the transplant artery origin which were suspicious of TRA stenosis. A computerized tomography (CT) scan was done which showed a 20 mm × 25 mm pseudoaneurysm arising from the aortic patch and the TRA origin lying adjacent to the pseudoaneurysm was tightly narrowed (Figures 1 and 2). After discussion in the departmental multidisciplinary team meeting and with patient's informed consent, he underwent radiological intervention as described below.

Under ultrasound guidance, the right common femoral artery was punctured, and a 5Fr sheath was inserted. 7500 unit of heparin was administered intravenously. The dimensions of the TRA were confirmed and were found to be like those of CT scan findings. The TRA was catheterized using a size 4Fr Berenstein® catheter and Terumo® wire (Terumo Medical Corporation, United States), subsequently exchanged for a 0.014 Thruway wire (Boston Scientific Inc., Ireland) and was left in situ as a "safety wire". The left common femoral artery was punctured and a 6Fr destination sheath was placed over the aortic bifurcation. Through the ipsilateral 6Fr sheath, a 10 mm percutaneous transluminal angioplasty (PTA) balloon was placed opposite the aneurysm neck. From the 6Fr sheath, a 4Fr Cobra (Cook Medical, United States) and 2/7 Progreat® microcatheter (Terumo Medical Corporation, United States) were used to gain access to the aneurysm sac. Within the right external iliac artery, the PTA balloon was inflated to reduce the risk of coil prolapse and migration and the aneurysm was embolized using two Azur® 20 mm Framing coils (Terumo Medical Corporation, United States), and packed with Azur® Hydrogel Coils. The pseudoaneurysm was filled with coils and hydrogel leading to its complete occlusion. Subsequently, the stenosed transplant RA was stented using a 6 mm × 20 mm Hippocampus stent (Medtronic, United Kingdom), which restored the patency of the stenosed renal artery and normal blood flow (Figure 3).

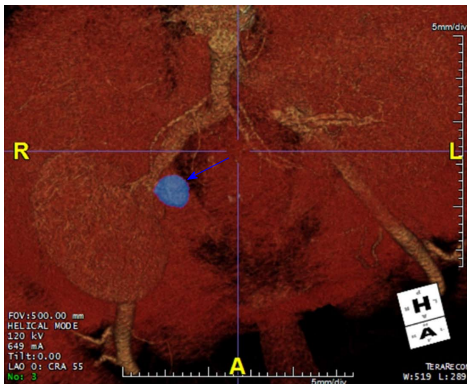

Figure 1 Computerized tomography angiogram showing a 20 mm x 25 mm pseudoaneurysm arising from the aortic patch (blue arrow).

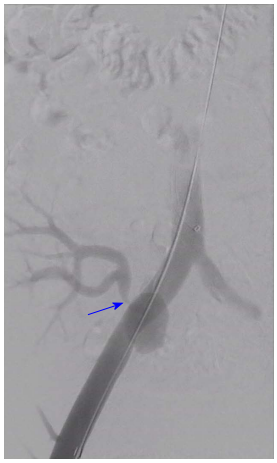

Figure 2 Angiogram showing transplant renal artery stenosis (blue arrow) due to compression caused by the pseudoaneurysm. Guide wire is present within the right common and external iliac artery.

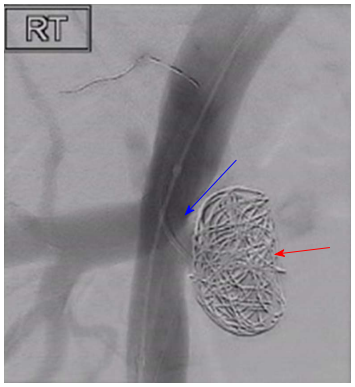

Figure 3 Successful coiling of the pseudoaneurysm (red arrow) and stenting of the transplant renal artery stenosis (blue arrow).

Following embolization of the pseudoaneurysm and stenting of the TRA, improvement in renal function occurred leading to a fall in the serum creatinine level to 159 μmol/L (eGFR 47 mL/min per 1.73 m^2). A follow-up CT angiogram one month after the intervention showed a patent TRA with successful coiling of the pseudoaneurysm and satisfactory position of the stent with no evidence of TRA stenosis. The patient continues to be followed up in the routine RT clinic and has a serum creatinine of 150 μmol/L (eGFR 49 mL/min per 1.73 m^2).

DISCUSSION

Renal Transplantation remains the treatment of choice in end stage renal disease patients. Vascular complications after RT include TRA stenosis, TRA thrombosis, transplant renal vein thrombosis, arteriovenous fistula and TRA pseudoaneurysm[2]. TRA pseudoaneurysm is an uncommon complication and can be classified anatomically as intrarenal or extrarenal based on the involvement of either TRA or iliac artery, respectively. Aetiologically, TRA pseudoaneurysms can be of infective or non- infective origin. Infective pseudoaneurysms are more common and can be of fungal (mycotic) and non-fungal (non-mycotic) origin. Amongst the infective pseudoaneurysms, Candida albicans and Aspergillus species have been reported to be the predominant microorganisms, while Pseudomonas species were the leading cause of non-mycotic infective pseudoaneurysms[2-8]. Non-infective TRA pseudoaneurysms can result from injury to the arterial wall, faulty suture techniques[9-11] or following a biopsy[12,13].

TRA pseudoaneurysms can be asymptomatic[14] or can present with RT dysfunction, fever, pain (mainly at the site of the transplant) or a combination of these presentations. Graft loss is a recognized complication of TRA pseudoaneurysms and sometimes bleeding from the ruptured pseudoaneurysm can lead to hemorrhagic shock or death of the patient[8,15]. Lumbar plexopathy has been reported in a previous literature because of pressure effect of the pseudoaneurysm[16], while malignant hypertension is a rare presentation[17].

The choice of the modality of treatment of TRA pseudoaneurysms depends on several factors including the aetiology, haemodynamic stability of the patient, presentation, anatomy, graft function and the radiological features of the pseudoaneurysm[18-22]. Aneurysms larger than 25 mm, progressive enlargement, deterioration of renal function or presentation with symptoms are the main indications for repair[18]. Treatment modalities include minimally invasive techniques using mainly exclusion stents to the external iliac artery, but this may sacrifice the graft[14]. Ultrasound-guided percutaneous thrombin injection in combination with a covered stent has been reported as a successful way of treating TRA pseudoaneurysm with preservation of renal function[18,19].

Traditional aneurysm coiling in general can be associated with complications such as migration, non-target embolisation, inadequate filling, compaction and the technical difficulty in placing the coils leading to added risk to organs, patients and increase in cost[23].

Expandable hydrogel technology coils have been described to treat intracranial and peripheral pseudo-aneurysms successfully for number of years. The main

advantage of using EHC is related to their superior mechanical occlusion properties resulting in fewer coils deployed and a lower recurrence rate. They are also compatible with imaging modalities[24-26].

In our case, successful radiological and clinical outcomes were achieved with return of serum creatinine to baseline within 48 h of intervention without any complication related to the RT or lower limb. We have employed EHC system to treat TRA pseudoaneurysm, achieved excellent volumetric filling and targeted embolisation and subsequently deployed a stent leading to restoration of transplant renal function to its normality. It offers a new non- invasive technique to treat TRA pseudoaneurysms with preservation of renal grafts; therefore, it should be considered as a first line treatment modality in this clinical situation.

ARTICLE HIGHLIGHTS

Case characteristics

A 38-year-old male, who had received a deceased donor renal transplant presented with deterioration of renal function five months post-transplantation.

Clinical diagnosis

On examination, there were clinical features pointing to definitive diagnosis.

Differential diagnosis

Differential diagnosis included obstructive uropathy, acute rejection, infections, drug nephrotoxicity and transplant renal artery stenosis.

Laboratory diagnosis

The serum creatinine was significantly elevated.

Imaging diagnosis

The Duplex ultrasound scan showed reduced resistive index with high velocity flow in the renal artery suggestive of transplant renal artery stenosis. A computerized tomography angiogram showed a 20 mm × 25 mm pseudoaneurysm at the anastomosis site and stenosis of the transplant renal artery adjacent to the pseudoaneurysm.

Treatment

Endovascular embolisation of the pseudoaneurysm using expandable hydrogel coils (EHC) followed by deployment of stent lead to resolution of the pseudoaneurysm and transplant renal artery stenosis and restoration of renal function to normality.

Related reports

Follow-up of the patient was satisfactory with no adverse events related to the procedure.

Experiences and lessons

This is the first reported case of treatment of a transplant renal artery pseudoaneurysm with wide neck with the use of EHC leading to successful outcomes.

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REVIEW

Solid pancreas transplant: Pushing forward

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Abstract

Pancreas transplant has evolved significantly in recent years. It has now become a viable treatment option on type 1 diabetic patients with poorly controlled diabetes on conventional treatment, insulin intolerance, hypoglycaemia unawareness, brittle diabetes and/ or end-stage kidney disease. The purpose of this review is to provide an overview of pancreas transplant historical origins and current barriers to broader utilization of pancreata for transplant, with a focus on areas for future improvement to better pancreas transplant care. Donor pancreata remain underutilized; pancreatic allograft discard rates remain close to 30% in the United States. Donations after cardiac death (DCD) pancreata are seldom procured. Study groups from Europe and the United Kingdom showed that procurement professionalization and standardization of technique, as well as development of independent regional procurement teams might increase organ procurement efficiency, decrease discards and increase pancreatic allograft utilization. Pancreas transplant programs should consider exploring pancreas procurement opportunities on DCD and obese donors. Selected type 2 diabetics should be considered for pancreas transplant. Longer follow-up studies need to be performed in order to ascertain the long-term cardiovascular and quality of life benefits following pancreas transplant; the outcomes of which might eventually spearhead advocacy towards broader application of pancreas transplant among diabetics.

Key words: Pancreas transplant; Whole pancreas transplant; Donations after cardiac death pancreas transplant; Obese pancreas donors; Pancreas transplant for type 2 diabetes

Core tip: Pancreas transplant has become a viable treatment option on type 1 diabetics. The purpose of this review is to describe current barriers to broader pancreatic allograft utilization, and focus on areas for future improvement. Donor pancreata, especially Donations after cardiac death (DCD), remain underutilized. Procurement professionalization might decrease discards and increase pancreatic allograft utilization.

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Pancreas procurements should be extended to DCDs and suitable obese donors. C-peptide positive non-obese brittle diabetics may be suitable transplant candidates. Longer studies on pancreas transplant cardiovascular benefits are needed; this might eventually drive pancreas transplant advocacy among diabetics.

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BACKGROUND

In 1894, Williams[1] reported the implantation of minced sheep's pancreas to a 15-year-old diabetic boy for the treatment of his ketoacidosis. In 1922, Banting *et al*[2] reported the use of pancreatic extract to treat diabetes mellitus (DM) in human, seemingly heralding the end of this scourge for all time. The discovery of insulin detracted from pancreatic transplant until 1966, at which time Kelly and Lillehei performed the first simultaneous human kidney-pancreas allotransplant from a deceased donor into a 28-year-old woman at the University of Minnesota, 3 years after the first reported kidney allotransplant[3]. The first living donor pancreas transplant was performed at the University of Minnesota, in 1979[4].

Other early efforts included islet cell transplant. Ballinger and Lacy demonstrated islet of Langerhans' isolation and subsequent *in vivo* post-transplant function in rats in 1972[5]. Najarian and Sutherland performed the first clinical islet transplant in 1974[6]. Further subsequent efforts culminated in the introduction of the Edmonton Protocol for islet cell transplant by Shapiro *et al*[7] in 2000.

According to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR), as of end of 2014, over 48000 pancreas transplants were reported internationally, with approximately 29000 transplants performed in the United States alone[8]. Nonetheless, pancreas transplant rates have declined in the United States by 33% from 2004 (approximately 1500) to 2014 (approximately 1000)[9]. Similar trends were identified in the Organ Donation and Transplant (ODT) report in the United Kingdom[10]: during 2015-2016, the total number of pancreas and kidney/pancreas transplants decreased by 37.9% and 3.5% respectively.

Paradoxically, this pancreas transplant decline has occurred despite of reported improvements in graft and patient survival outcomes. According to the Organ Procurement and Transplant Network (OPTN)/ Scientific Registry of Transplant Recipients (SRTR) 2014 Annual Data Report, graft and patient survival improved[8]. These positive outcomes were attributed to improvements in recipient and organ selection, introduction of T-cell depleting agents for immunosuppression induction, and combined use of tacrolimus and mycophenolate mofetil for maintenance immunosuppression[11].

In an era of an increasingly aggressive approach in other solid organ transplant categories, the transplant community seems to have remained conservative with pancreas allograft utilisation, at least within the United States territory[9]. This is presumed to be multifactorial[12].

Aim of this review is to outline the current pancreas transplant status, address barriers in pancreas donation and transplant, and describe ways to optimise pancreatic allograft utilisation and transplant of previously considered as unconventional pancreas transplant candidates.

Indications and types of pancreas transplant

Pancreas transplant has become an accepted treatment modality for both uremic and non-uremic patients with type 1 diabetes mellitus (T1DM). Pancreas transplant restores glucose homeostasis, relieving the patient from the need of ongoing glucose monitoring, insulin injections and the risk of life-threatening diabetic hypoglycemia or ketoacidosis. Nonetheless, considering the transplant-related morbidity and mortality plus the lifetime need for immunosuppression, not all T1DM patients should be considered for pancreas transplant.

Pancreas transplant has also become a viable option on T1DM patients with poorly controlled diabetes despite conventional treatment, insulin intolerance,

hypoglycaemia unawareness, brittle diabetes or end-stage kidney disease. There are currently 7 types of pancreas transplant: (1) simultaneous pancreas and kidney transplant (SPK). As per UNOS guidelines, SPK is indicated for T1DM patients or those with detectable C-peptide levels [as a surrogate indicator of type 2 diabetes mellitus (T2DM)], who are insulin dependent, have a body mass index (BMI) < 30 kg/m^2, and end stage renal disease, who are currently on dialysis or expected to require dialysis within 6 mo[13]; (2) pancreas transplant alone (PTA), indicated primarily for T1DM with hypoglycaemia unawareness, non-compliance with insulin treatment and/or impaired quality of life and adequate glomerular filtration rate to render the need of kidney transplant unlikely[14,15]; (3) pancreas-after-kidney transplant (PAK), indicated for patients who would qualify for a PTA and already have a viable renal allograft[16,17]; (4) simultaneous deceased donor pancreas and live donor kidney transplant, indicated for patients who would qualify for SPK. This approach is expected to result in reduced waiting times, lower delayed graft function (DGF) rates and better outcomes[18]; (5) total pancreatectomy and islet cell autotransplant (TPIAT). According to the PancreasFest consensus, TPIAT is indicated in selected patients with intractable pain related to chronic pancreatitis despite other appropriate treatment modalities, and no psychosocial or medical contraindications[19]. In the United States, TPIAT is subject only to regulation of human cells and tissues (the tissue rules). The centers performing it should be registered with the Federal Drug Administration (FDA) and follow the Current Good Tissue Practices, without being required to submit FDA drug application[20]; (6) laparoscopic donor distal pancreatectomy for living donor solid pancreas or isl*et al*lotransplant and pancreas-kidney transplant[21,22]; and (7) isl*et al*lotransplant. The implantation of deceased donor islets of Langerhans is a promising treatment for T1DM with labile diabetes, recurrent hypoglycaemia and hypoglycaemia unawareness[19]. In the United States, islet cell allotransplant is currently investigational and subject to both the FDA published guidelines on the tissue rules and the biologic and drug provisions.

SPK is by far the commonest pancreas transplant type. According to the SRTR data (United States), in 2014, 77% of pancreas transplants were SPKs, while PAK and PTA accounted for 13.6% and 9% of the transplants performed, respectively[8].

Outcomes

According to IPTR, in 2007, PTA, SPK and PAK 1-year unadjusted patient survival was 95%-97%; the 5-year survival was 91%, 87% and 83%, respectively. PTA recipients were by definition non-uremic. These findings raised the question whether T1DM patients benefit from a pancreas transplant over a kidney transplant alone. Gruessner *et al*[23] assessed mortality of pancreas transplant recipients over those on the waiting list (WL). Transplant recipients had elevated hazard ratios in the immediate post-transplant period up to 3 mo post-transplant[23]. However, 4 years' follow-up showed SPK patient survival benefit compared to WL (90% *vs* 59%). PAK and PTA survival benefits were indeterminate in 4 years, possibly because WL mortality in these cohorts was lower due to their non-uremic status and younger age (PTA)[23].

On their mortality assessment, Gruessner *et al*[23] reported that, kidney allograft failure after SPK/PAK increases patient death risk by eleven-fold. The pertinent question remains whether these patients benefit from a functioning pancreas allograft. Most studies provided conflicting reports, partly due to insufficient follow-up and dependence on registry data[24-30]. Morath *et al*[31,32] (Heidelberg University, Germany) performed a very long term follow-up analysis based on the International Collaborative Transplant Study and observed that SPK graft and patient survival allograft outcomes were equivalent to living donor kidney transplant (LDKT) outcomes at 10 years; and, most importantly, that very long term survival (18-20 years) was superior among the SPK over the kidney transplant alone (on both LDKT and deceased donor kidney transplant recipients). The authors also noted decreased long-term cardiovascular events among the SPK patients[31,32]. These findings should trigger extension of follow-up analysis across more pancreas transplant centers.

It remains unclear if re-establishment of long-standing euglycemia can halt or reverse end-organ diabetic complications. Fioretto *et al*[33] estimated that a period of 10 years of euglycemia is a necessary interval to reverse diabetic nephropathy features.

DONOR PANCREATA

Current status

Across the United States, transplant surgeons often appear reluctant to consider pancreas allografts from donors considered as marginal for pancreas donation. As marginal are characterized older (> 50 years of age), obese, and donation after cardiac

death (DCD) donors. According to OPTN/UNOS, between 2003 to 2014, there has been a decrease in donors aged over 50, with 83% of donors aged less than 35 years[8]; among the organs recovered, there were more recorded pancreatic discards from donors 50 years or older[8]. During the same period, obese pancreas donors decreased from 56.3% to 34.6%[8]. These findings may indicate diminished intent to use pancreata from marginal donors[8].

Expanding the pancreas donor pool

According to the OPTN/ SRTR 2016 Annual Data Report, since implementation of the new pancreas allocation system in October 2014, there has been an increase in the number of pancreas transplants for the first time over a decade[34]. At the same period, total active listings have also decreased, reaching a historic low[34]. Despite the above, the average WL times have remained largely unchanged, with 34.2% of patients waiting between 1 and 3 years[34]. Even though WL mortality has improved marginally over the recent years, there is still remarkable geographical variation across the United States, ranging from 0 to 15%[34]. At the same time, pancreas transplant programs have become more liberal with their candidates' selection, as indicated by an increased proportion of T2DM patients (9.9% in 2016), of recipients aged over 50 years, and of candidates with higher BMI[34]. Unless the pancreas donor pool is expanded, this more aggressive approach is expected to attract increasing numbers of transplant candidates and stretch the WL times further. In order to restrain WL times, decrease WL mortality and eliminate regional disparities in pancreas transplant access, it is necessary to expand the pancreas donor pool and increase pancreas transplant rates.

Utilization of pancreatic allografts from obese donors: Steatosis is a primary concern in evaluating pancreas allograft quality[35]. The effect of steatosis on the pancreas allograft is presumably twofold: first, macrovesicular pancreatic steatosis may result in microvascular occlusion and thrombosis; second, adiponecrosis can potentially trigger inflammation and post-reperfusion graft pancreatitis[35,36]. Donor obesity, the latter defined as donors with BMI of 30 kg/m^2 or greater, is a surrogate indicator of pancreatic steatosis; as such, obesity has been associated with poor pancreas transplant outcomes. For this reason, transplant centers commonly decline pancreatic allografts from obese donors. An OPTN database analysis of 9916 SPKs performed during period 2000-2013 compared the effect of donor BMI on graft outcome. The donors were categorized into 4 BMI groups: 20-25, 25-30, 30-35, and > 35 kg/m^2. BMI 20-25 kg/m^2 donor outcomes were compared to the rest of the groups. Only BMI > 35 kg/m^2 was associated with inferior kidney and pancreas allograft survival. BMI 30-35 kg/m^2 did not affect 3 mo, 1-, 5-, and 10-year kidney and pancreas graft survival. The authors concluded that pancreata from donors with BMI 30-35 kg/m^2 might be used safely for transplant[37]. Certainly, this retrospective analysis is skewed due to potential discards upon visual of organs with significant interacinar fat infiltration or evidence of acute or chronic inflammation.

DCD pancreas utilization: DCD allografts have been used successfully in liver and kidney transplant. The concept of DCD pancreas transplant is not new; it has become an increasingly common practice in several European countries and the United Kingdom[38,39]. In the latter, DCD pancreas transplant accounts for up to 19.5% of transplanted pancreatic allografts[38]. However, in the United States, DCD pancreatic donation has remained out of favor, accounting for as low as 1.5 % of transplanted pancreata over period 1996 to 2014[40].

Various studies have compared DCD *vs* DBD pancreas transplant outcomes (Table 1). The University of Wisconsin has been pioneering DCD pancreas utilization in the United States, reporting no difference in graft survival, function, complication or rejection rates between DBD and DCD pancreata; even though it did report longer renal DGF in the DCD cohort[41-43]. Similarly, an OPTN/UNOS registry analysis by Salvalaggio *et al*[44] reported comparable outcomes, even though DCD SPK recipients had longer hospital stay and, not unexpectedly, more protracted renal allograft DGF. The Oxford group performed a United Kingdom registry analysis which reported equivalent patient and graft survivals among 134 and 875 pancreas transplants performed between 2006 and 2010[45]. A systematic review and meta-analysis published by Shahrestani *et al*[46] in 2017 reported no difference in 10-year survival among the DCD and DBD cohorts. Kopp *et al*[39] (Leiden University Medical Center, Netherlands) recently published a single-center cohort study, which indicated comparable outcomes among DCD and DBD pancreas transplants. The DCD donors were younger. The authors concluded that donor age was the most significant allograft survival prognosticator; therefore, younger DCD grafts might be a better option than DBD grafts from older donors[39].

Table 1 Studies comparing pancreas transplant outcomes between donations after cardiac death *vs* donation after brain death pancreas allograft recipients

First author/ yr	Country	Type of study	No. transplants	Mean donor age (yr)	Donor BMI [Median, IQR]	Warm ischemia time (min)	Cold ischemia time (hours)	Follow-up (yr)	Comments/conclusions
D'Alessandro *et al*[41], 2004	United States	Cohort	31 DCD; 455 DBD	Unclear	ns	15.3 (SD ns)	15.9 (SD ns)	5	No difference in 5-yr graft survival in SPKs
Fernandez *et al*[43], 2005	United States	Cohort	37 DCD; 539 DBD	31	ns	17.5 (SD = 9.9)	15.8 (SD = 3.4)	5	Indistinguishable patient and graft 5-yr survival in SPKs. Elevated DGF rate on DCD kidneys, with no significant long-term impact.
Salvalaggio *et al*[44], 2006	United States	Cohort; OPTN/UNOS Registry	57 DCD; 3948 DBD	DCD= 30.1; DBD = 29	ns	ns	15.7	5	For SPK recipients, the wait for DCD organs was shorter. DCD SPK recipients had longer hospital stay. Renal DGF was higher with DCD organs. Higher thrombosis rates (12.8% *vs* 6.1%)
Bellingham *et al*[42], 2011	United States	Cohort	72 DCD; 903 DBD	DCD= 30	ns	20.8 (SD = 9.4)	ns	10	No difference in surgical complications, rejection or hemoglobin A1c levels.
Muthusamy *et al*[45], 2012	United Kingdom	Cohort	134 DCD; 875 DBD	DBD = 32; DCD= 28	23	12	12.5	1	Similar patient and graft survival, with improved DCD pancreas graft survival if performed as an SPK. Early graft loss in the DCD cohort was mainly due to thrombosis (8% *vs* 4%)

Shahrestani *et al*[46], 2017	Australia	Systematic review and meta-analysis	762 DCD; 23609 DBD (included 10 cohort studies and 8 case reports)	DBD = 37 ns	21-25 ns	ns	ns	0.3-15	No significant difference in 10-yr graft or patient survival. Higher graft thrombosis risk with DCDs [95%CI: 1.04-2.67; *P* = 0.006]. Thrombosis risk not higher when DCD donors were given ante-mortem heparin (*P* = 0.62)
Kopp *et al*[39], 2018	The Netherlands	Cohort	21 DCD; 83 DBD	[a]	[a]	31 (median)	11 (median)	5	Without the DCD factor, PDRI from DCD donors was lower. Donor age was the only donor-related risk factor associated with graft survival. Post-op bleeding and renal DGF were more common with DCDs. Graft survivals were comparable. DCD pancreata had lower thrombosis incidence. DCD donors yield similar outcomes for low PDRI. Most DCD donors were younger. DCD grafts may be a better option rather than older DBD donors.

[a]Range not significantly different between DCD *vs* DBD donors. BMI: Body mass index; SD: Standard deviation; ns: Not stated in the study; DCD: Donation after cardiac death; DBD: Donation after brain death; SPK: Simultaneous kidney-pancreas transplant; DGF: Delayed graft function; PDRI: Pancreas donor risk index.

Graft thrombosis has been the DCD pancreas transplant Achilles heel. DCD pancreatic allografts appear to be more vulnerable to ischemia-reperfusion injury due to sustained peri-procurement ischemic insult, which may predispose them to higher risk of graft thrombotic events, even though its impact on overall graft survival has not been demonstrated yet[39]. OPTN/UNOS registry analysis published in 2006 did demonstrate higher thrombosis risk in the DCD cohort (12.8 *vs* 6.1%)[43]. Shahrestani *et al*[46] meta-analysis has estimated that the odds of graft thrombosis were 1.67 times higher in DCD organs; however, that thrombosis risk was not significant if the donors had been given ante-mortem heparin[45]. Interestingly, Kopp *et al*[39] reported lower DCD graft thrombotic risk.

Professionalization and standardization of the pancreas procurement process:

According to SRTR, 27.7% of pancreata were discarded after recovery[47], often due to pancreatic trauma occurring at the time of procurement. Ausania *et al*[48] performed a retrospective ODT Registry analysis, and demonstrated that pancreatic allografts are indeed more vulnerable to procurement damage. More than 50% of recovered allografts had at least one reported injury, most commonly a short portal vein[48]. Arterial and parenchymal damage were associated with higher graft loss risk[48]. DCD status was not related to graft damage; increased BMI, aberrant hepatic artery anatomy, concurrent liver donation, and non-pancreas transplant procurement team increased the risk of pancreatic injury[48]. The Dutch Transplant Foundation (DTF) developed a digital scoring system for abdominal organs donated and accepted in the Netherlands. According to DTF, pancreatic injury was reported in 25% of the recovered organs, of which only 2% led to organ discard[49]. The authors identified higher donor BMI and DCD status as risk factors associated with organ discard due to procurement-related injury (Table 2)[49].

The same research group (Leiden University Medical Center, Netherlands) also reported that organ recovery from surgeons accredited on standardized abdominal organ procurement methods, who also performed pancreas transplants in high-volume centers, was associated with more frequent recovery of the pancreas from DCD donors, less discards due to organ damage, and higher overall pancreatic allograft utilization[50]. They developed a course named "Multi Organ Donor Procurement Surgery", which has since been assimilated by the European Society for Organ Transplant[50]. Aim of this course is to standardize abdominal organ procurement surgery training, including a step-by-step e-learning module and hands-on training, with documented completion of a set number of procurements under supervision and examination before certification[50]. A same approach has been recently introduced and endorsed by the ODT in the United Kingdom.

The Netherlands is divided in 5 fully independent regional organ procurement teams, which procure all abdominal organs at their respective regions. Each of these teams consists of at least one certified surgeon, an assistant, two procurement scrub nurses and anesthesia team, and carries all necessary instruments to the donor hospital[49]. Similarly, procurements in the UK are performed by regional independent organ procurement teams, each manned by at least one certified procurement surgeon, procurement scrub nurses/perfusionists, carrying their own surgical equipment to the site of donation. This procurement model results in standardization of the procurement technique and eliminates the donor hospital-related hazards (such as lack of appropriate equipment or non-acquaintance of the local scrub team to the demands of a multi-organ, especially a DCD, procurement). It further mitigates the inter-surgeon variation on the procurement technique and therefore procurement quality, degree of organ damage, and derivation of organ description to the receiving transplant surgeons. It also results in better team coordination and time management and, therefore, more efficient execution of the procurement surgery, both of which are critical factors for a successful rapid DCD organ procurement[49,51]. Finally, this procurement model may lead to more experienced surgeons, and therefore, higher procurement quality and potentially less discards[49-51].

The outcomes of the Dutch (DTF) and United Kingdom (ODT) procurement models indicate that pancreatic allograft utilization may be optimized and pancreatic discards minimized with standardization of the procurement technique and development of independent organ procurement teams, which should be organ procurement organization rather than transplant center-based. In the United States, standardization of the procurement technique and formal credentialing of procurement surgeons may be achieved *via* institutional initiatives and through the American Society of Transplant Surgeons; based on the European and United Kingdom experience, this may result in higher procurement quality, less discard rates, and increased procurement and utilization of DCD pancreatic allografts for the purpose of whole organ or islet transplant (Table 2)[49-51].

Pancreas transplant centralization: A study published in 2017 by Kopp *et al*[52] on the outcomes of 1276 pancreas transplants in the Eurotransplant region, demonstrated that patient and graft survival after pancreas transplant are superior in higher volume centers; the outcomes remain superior even after using organs with the higher Pancreas Donor Risk Index (PDRI). An OPTN/ UNOS study published in the same year, indicated better pancreas survival rates at high-volume centers across all PDRI categories (Table 2)[53]. PDRI is a predictive model described by Axelrod *et al*[54] in 2010, that may be used at the time of organ offering, in order to better assess which allografts would be associated with good survival. Identified risk factors were increased donor age, DCD and black race[54]. In the United Kingdom, PDRI has been validated as a tool to predict survival in SPK transplant, but not in PTA or PAK transplant[55].

Table 2 Studies on the effect of pancreas procurement professionalization and center volume on pancreas transplant outcomes

First author, yr	Study aim	Region, country	Study period	No. cases	Results/comments
Boer *et al*[49], 2017	Analysis of abdominal organ procurement quality and clinical impact.	Eurotransplant, The Netherlands	2012-2013	591 procurements	13% surgical injuries on procured pancreata, leading to 3% pancreas discards. Higher BMI, DCD donation in liver procurement were risk factors for discard due to injury. High procurement volume centers were associated with less pancreatic injury.
Lam *et al*[50], 2017	Analysis on the effect of the abdominal recovery team professionalization on the pancreatic procurement injury and acceptance for transplant.	Eurotransplant, The Netherlands	2002-2015	264 procurements	31.8% pancreatic surgical injuries. 85.6% of procured pancreata were eventually transplanted. Surgeons certified in abdominal organ procurements recovered more grafts from older donors, DCDs, and had less surgical injuries. Predictors to proceed with pancreas transplant were: certified procurement surgeons; surgeons from a pancreas transplant center; DBD donation; and lower donor BMI. Procurement certification results in less surgical damage and more pancreata transplanted.
Kopp *et al*[52], 2017	Analysis of the effect of the transplant center volume on pancreas transplant outcomes.	Eurotransplant, The Netherlands	2008-2013	1276 pancreas transplants	Centers were classified into: low (< 5 transplants/yr); medium (5-13/yr); high volume (≥ 13/yr). Patient and graft survival were superior in higher volume centers. High center volumes were protective for graft failure, even though they transplanted organs with higher PDRI.
Alhamad *et al*[53], 2017	Analysis of the effect of the transplant center volume on the pancreas allograft failure risk.	UNOS, United States	2000-2013	11568 SPKs and 4308 solitary pancreas transplants	Centers were categorized into low, medium, and high tertiles. Low volume centers were associated with higher pancreatic failure risk. High volume centers had better graft survival rates irrespective of PDRI.

BMI: Body mass index; DCD: Donation after cardiac death; DBD: Donation after brain death; PDRI: Pancreas donor risk index; SPKs: Simultaneous kidney-pancreas transplants.

Living donor segmental pancreas transplant: SPK candidates are often advised to pursue LDKT, followed by PAK[56]. Inevitably, this exposes the recipient to two operations. The SPK option from a living kidney-pancreas donor has also been advocated[56-59]. This offers a pre-emptive kidney transplant, thus abolishing dialysis-related morbidity and mortality; allows the recipient to forego a second transplant operation (PAK); decreases the historically high early rejection risk-since the recipient

will be exposed to a single donor rather than two.

Living donor pancreatectomy was the first extrarenal organ to be successfully transplanted[59]. The first living donor pancreas transplant was performed at the University of Minnesota, in 1979[4]. According to Kirchner *et al*[59] between 1994-2013, 46 living donor segmental pancreas transplants have been performed, with 0% mortality. 15% of donors developed post-donation DM requiring oral hypoglycemics, and 11% developed insulin-dependent DM. A risk stratification model for post-donation DM using 3 pre-donation risk factors (oral glucose tolerance, basal insulin and fasting glucose) and 1 post-donation risk factor (ΔBMI > 15) predicted 100% of donors who developed post-donation DM[59]. In conclusion, living donor segmental pancreas transplant is a viable option, after appropriate donor selection.

PANCREAS TRANSPLANT CANDIDATES

Conventionally, pancreas transplant is intended to restore function of the endocrine portion of the pancreas, in effect restoring normoglycemia in diabetic patients devoid of insulin producing capacity, *i.e.*, T1DM patients, especially those with labile or brittle diabetes, poor response or low compliance to insulin therapy, hypoglycemia unawareness, and/or renal failure. According to the SRTR, in 2014, 9.2% of these transplants were performed on T2DM patients, increased from 7% in 2010[60]. In 2016, T2DM pancreas recipients increased further to 9.9%[34].

On this latter part of this review we will endeavor to explore the potential of pancreas transplant application to previously considered "unconventional" pancreas transplant candidates, such as T2DM ("C-peptide positive") patients, overweight and mildly obese T1DM patients, and patients with chronic pancreatitis.

The C-peptide positive recipient

Pancreas transplant on T2DM contradicts traditional wisdom. T2DM has been attributed to insulin resistance rather than low or nil insulin production; in the presence of insulin resistance, pancreas transplant will arguably confer little or no benefit upon the recipient. There is also the potential to harm: pancreas transplant carries a high complication risk in a population with a multitude of inherent comorbidities; and, it places the transplant recipient under obligatory lifetime immunosuppression. Lastly, pancreas transplant on a T2DM may result in the waste of a precious commodity and the opportunity cost of its use on a T1DM patient.

Multiple studies have attempted to explore the effect of C-peptide presence on SPK outcomes (Table 3)[61,62,64-66]. Stratta *et al*[62], performed a single center retrospective analysis of 162 SPK patients, including 30 (18.5%) of C-peptide positive (C-peptide levels ≥ 2.0 ng/mL) *vs* 132 C-peptide negative patients. In a mean follow-up period of 6.5 years, there were no differences between the two groups in terms of patient, pancreas and kidney graft survival, acute rejection, HbA1c, serum creatinine levels or estimated glomerular filtration rate. However, C-peptide positive patients had higher post-transplant C-peptide levels and T2DM phenotype (overweight or obese, hyperlipidemia, family history of diabetes, progressive insulin resistance)[63]. The authors concluded that positive C-peptide "should not be used exclusively to determine candidacy for SPK transplant"[62].

Light *et al*[64] performed a retrospective analysis of 173 SPK recipients, of whom 66.5% had negligible C-peptide ("C-peptide negative", < 0.8 ng/mL). The elevated C-peptide group ("C-peptide positive", ≥ 0.8 ng/mL) tended to have T2DM phenotype and C-peptide levels > 5 ng/mL. In long-term follow-up (up to 20 years), "C-peptide negative" patients had significantly improved survival (P = 0.019); "C-peptide positive" recipients showed a trend to better survival (P = 0.069). Similar to Stratta *et al*[62], this study indicates that "C-peptide positive" (T2DM phenotype) patients can have favorable outcomes post SPK transplant[64]. A more recent by Shin *et al*[65] compared 5-year outcomes among 151 T1DM and 42 T2DM pancreas transplant recipients. There was no difference in hemoglobin A1c levels, fasting insulin levels, homeostasis model assessment of insulin resistance or the insulinogenic index between the groups. Notably, insulin resistance decreased between both groups, even though T2DM recipients kept significantly higher C-peptide levels[65].

The overweight and obese T1DM recipient

C-peptide positive or not, overweight (BMI 25-30 kg/m^2) and obese (BMI > 30 kg/m^2) pancreas transplant candidates are becoming increasingly common[34]; possibly reflecting the global obesity epidemic[66]. T1DM patients may be overweight or obese and still benefit from pancreas transplant. That being said, such patients are not immune to the general obesity-linked surgical risk[68,71]. On a large scale SRTR analysis of 21000 pancreas transplant recipients, Bedat *et al*[72] showed that overweight and

Table 3 Studies on Simultaneous pancreas and kidney transplant outcomes of C-peptide positive *vs* C-peptide negative recipients

First author, yr	Country	No. patients	Study period	C-peptide positive (%)	BMI (kg/m²) Mean (SD)	Follow-up (yr)	Outcomes	Conclusion
Chakkera *et al*[61], 2010	United States	80	2003-2008	[a]15	T1DM 24.8 (4.2); T2DM 27 (3)	1	No difference in graft (kidney and pancreas) or patient survival.	SPK should be considered in selected patients with T2DM and ESRD. C-peptide measurements for ESRD patients can be misleading.
Light *et al*[64], 2013	United States	173	1989-2008	[c]33.5	T2DM 26.1 (ns)[d]; T1DM 22.5 (ns)[d] (P < 0.0001)	20	T2DM were older at diabetes diagnosis, older at transplant, and heavier pre- and post-transplant, and had better graft survival. T1DM had better patient survival	There was a difference in patient but not graft survival in 20 yr follow-up.
Stratta *et al*[62], 2015	United States	162	2001-2013	[b]18.5	T2DM 26.1 (3.3); T1DM 24.4 (3.2)	5.6 (median)	No difference in patient and graft survival or surgical complications, rejections, serum creatinine, HbA1c, eGFR, C-peptide and weight gain were higher in the C-peptide positive group.	C-peptide "positive" patients appear to have a T2DM phenotype. Outcomes were similar between the two groups, suggesting that C-peptide should not be used exclusively when assessing for SPK transplant candidacy.
Shin *et al*[65], 2017	Republic of Korea	217	2004-2015	[e]ns	T2DM 38 (9); T1DM 18 (7)	5	Similar post-operative HbA1c (< 6%), fasting insulin, HOMA of insulin resistance, and insulinogenic index. Higher post-transplant C-peptide in T2DM recipients.	No significant difference in insulin resistance or β-cell function in 5 yr.

[a]T2DM definition: C-peptide presence, negative glutamic acid decarboxylase antibody, no diabetic ketoacidosis, use of oral hypoglycemics;
[b]C-peptide "positive" (T2DM) = C-peptide ≥ 2.0 ng/mL; C-peptide "negative" = C-peptide < 2.0 ng/mL;
[c]Patients with undetectable C-peptide (< 0.8 ng/mL) were considered T1DM; patients with detectable C-peptide (> 0.8 ng/mL) were considered T2DM;
[d]SD not stated;
[e]Patients were classified as T1DM and T2DM, based upon the American Diabetes Association and the World Health Organization definitions of T2DM. As such, there were 151 T1DM [C-peptide 0.92 (SD = 0.58) ng/mL] and 42 T2DM [C-peptide 3.49 (SD = 3.95) ng/mL] patients. T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; ESRD: End-stage renal disease; eGFR: Estimated glomerular filtration rate; HbA1c: Glycosylated hemoglobin A1; SPK: Simultaneous kidney-pancreas transplant; ns: Not stated; HOMA: Homeostasis model assessment.

obesity are independent predictors of increased early mortality and graft loss, and obesity is associated with inferior long-term graft survival. In an earlier series, Sampaio *et al*[73] reached similar conclusions.

Is there a role for bariatric surgery?

Bariatric and metabolic surgery is an established method of treatment of T2DM and metabolic syndrome[74-76]. It is yet to be clarified whether a metabolic procedure, may it

be sleeve gastrectomy or a more complex restrictive and malabsorptive procedure such as Roux-en-Y gastric bypass, would provide survival benefit on a patient with negligible insulin production.

T2DM patients with BMI ≥ 32 kg/m^2, currently non-eligible for pancreas transplant in most United States centers, should be considered for metabolic surgery[74-76] ; if their post-bariatric surgery BMI drops to ≤ 30 kg/m^2 but they remain insulin-dependent, suffer from brittle diabetes, insulin intolerance and/or hypoglycemia unawareness, they may be channeled towards pancreas transplant.

T1DM patients with BMI > 28 kg/m^2, who are currently considered poor pancreas transplant candidates, may be reconsidered for transplant after adequate weight loss. Excess weight loss prior to pancreas transplant may improve pancreatic graft survival[72]; plus, it will probably temper the obesity-related cardiovascular morbidity and mortality[77]; even though its benefit on T1DM population post-pancreas transplant is yet to be described.

The chronic pancreatitis patient: Islet autotransplant after total pancreatectomy

Total pancreatectomy without pancreatic endocrine function replacement will result in brittle diabetes and life-threatening hypoglycemia due to vanished pancreatic α- and β-cell function. According to 2014 PancreasFest consensus and 2015 National Institute of Diabetes and Digestive and Kidney Diseases, TPIAT is a potential treatment option for selected patients with impaired quality of life due to severe painful chronic pancreatitis, where conservative measures have failed[19,20]. TPIAT should not be performed in patients with active alcoholism or illicit substance use, T1DM, pancreatogenic diabetes, portal vein thrombosis, portal hypertension, significant liver disease, severe cardiopulmonary disease, pancreatic cancer, untreated or uncontrolled psychiatric disorder or history of poor compliance[78]. A retrospective review of 75 children undergoing TPIAT showed sustained pain relief and improved quality of life, whereas beta-cell function was dependent on islet yield[78]. Fan *et al*[79] from Johns Hopkins University recently published a smaller series of 32 patients who underwent laparoscopic TPIAT, resulting in sustained pain relief, earlier recovery and variable insulin dependence. There is vast potential for future research in this emerging field.

DISCUSSION

Pancreas transplant is a potentially curative option for T1DM, re-establishing euglycemia and, therefore, independence from the need of external insulin administration and glucose monitoring. The Heidelberg group analysis of > 20 year outcomes based on International Collaborative Transplant Study data, demonstrated that pancreas transplant benefits become obvious after 10 years, at which time it confers survival benefit superior to LDKT among uremic T1DM patients[31,32]. The group also reported diminished death rates from cardiovascular events beyond 10 years[31,32]. Despite these obvious benefits, the transplant community maintains a rather conservative approach. Donor pancreata remain underutilized[8]; the United States pancreatic discard rates are close to 30%[47]. DCD pancreata are seldom procured[40]; steatotic pancreatic allografts are commonly discarded; and obese donors are commonly considered poor pancreatic donation candidates[35,36]. European study groups showed that procurement professionalization is associated with increased pancreatic allograft utilization, and that high-volume pancreas transplant centers are associated with superior outcomes (Table 2)[49-53]. United Kingdom and OPTN registry analyses demonstrated that DCD and DBD SPKs could have indistinguishable outcomes (Table 1)[41,43-46]. OPTN registry analysis indicated that heavier donor (BMI 30-35 kg/m^2) pancreata might provide comparable outcomes[37]. On the recipient end, pancreas transplant has been shown to be beneficial to selected C-peptide positive patients (Table 3)[64-66].

This study has several limitations. It is a narrative review; as such, it has strong vulnerability to article selection bias; and databases have not been searched in a systemic way. There is limited number of studies exploring the various topics discussed, with series of publications often reported by the same institutions. Another inherent limitation is that most studies included were prospective or retrospective OPTN/UNOS, United Kingdom or DTF cohort reports or case series, which were founded on skewed datasets, since surgeons had already balanced donor-recipient risk at the time of organ/recipient selection and transplant.

CONCLUSION

Pancreas donors remain underutilized. DCD and obese donors should be considered for pancreas donation; the pancreas procurement process should be audited, standardized and optimized. Selected T2DM patients should be considered for pancreas transplant.

More very long-term follow-up studies should be performed in order to delineate the long-term cardiovascular and quality-of-life benefits of pancreas transplant; the results of which might eventually ascertain the pancreas transplant role in the armamentarium of definitive diabetes treatment.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Graft *vs* host disease impacts overall survival post allogeneic hematopoietic stem cell transplantation for acute lymphoblastic leukemia/lymphoma

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Author contributions: Damlaj M designed the study and analyzed data; Damlaj M, Ghazi S and Snnallah M collected data; all authors provided patients, wrote and reviewed the manuscript, and approved final version of the manuscript.

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Abstract

AIM

To examine the outcome and prognostic factors for high risk patients with acute lymphoblastic leukemia/lymphoma (ALL/LBL) who underwent allogeneic hematopoietic stem cell transplantation (HCT) at our center during the period of 2010-2017

METHODS

After due institutional review board approval, patients with high risk ALL/LBL post HCT were identified and included. All records were retrospectively collected. Time to event analysis was calculated from the date of HCT until event of interest or last follow up with Kaplan-Meir means. Cox regression model was used for multivariable analysis calculation.

RESULTS

A total of 69 patients were enrolled and examined with a median age of 21 (14-61). After a median follow up of 15 mo (2-87.3), the 2-year cumulative incidence of relapse, cumulative incidence of non-relapse mortality, progression free survival and overall survival (OS) were 34.1%, 10.9%, 54.9% and 62.8%, respectively. In a multivariable analysis for OS; acute graft *vs* host disease (GVHD) and chronic GVHD were significant with corresponding hazard ratio 4.9 (1.99-12; P = 0.0007) and 0.29 (0.1-0.67; P = 0.0044), respectively.

CONCLUSION

Allogeneic-HCT for high risk ALL/LBL resulted in promising remissions particularly for patients with cGVHD.

Key words: Acute lymphoblastic leukemia; Allogeneic hematopoietic stem cell

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transplant; Lymphoblastic lymphoma; Complete remission

Core tip: Allogeneic hematopoietic stem cell transplantation (HCT) is a potentially curative therapy for acute lymphoblastic leukemia/lymphoma (ALL/LBL) patients. We examined the outcome and prognostic factors of HCT for high risk ALL/LBL at our center. After due institutional review board approval, 69 patients were enrolled. After a median follow up of 15 mo (2-87.3), the 2-year overall survival (OS) was 62.8%. In a multivariable analysis; acute graft *vs* host disease (GVHD) and chronic GVHD predicted OS. In conclusion, allogeneic-HCT for ALL/LBL results in promising remissions in high risk disease and early referral for HCT to be considered for young and fit patients.

Damlaj M, Snnallah M, Alhejazi A, Ghazi S, Alahmari B, Alaskar A, Al-Zahrani M. Graft *vs* host disease impacts overall survival post allogeneic hematopoietic stem cell transplantation for acute lymphoblastic leukemia/lymphoma. *World J Transplantation* 2018; 8(7): 252-261
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INTRODUCTION

Acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LBL) constitute around 5% of all adult lymphoid malignancies and is typically diagnosed in the second to third decade of life. Complete morphologic remission, evident by presence of less than 5% clonal blasts in the bone marrow, following induction therapy can be achieved in the majority of patients. Incidence of relapse (IR) remains high; therefore, optimization of post remission therapy is vital. Furthermore, outcome of patients post relapse is dismal[1].

The role of allogeneic hematopoietic stem cell transplantation (HCT) in adult ALL/LBL in first complete remission (CR1) is debated. This is in part due to conflicting evidence with regards to the utility of this therapy due to on-going developments in the field. Typically accepted indications for allogeneic HCT in CR1 include elevated white blood count (WBC) > 30 × 10^9/L in B-cell disease and > 100 × 10^9/L in T-cell disease, age > 35 years, CD20 expression in B-cell disease, high risk cytogenetics including Philadelphia chromosome (Ph +ve), among others[2,3].

A number of prospective studies have examined the role of allogeneic HCT in CR1 spanning an enrolment period of almost two decades (1986-2005). The French Leucemie Aigue Lymphobalstique del'Adulte (LALA) group reported outcomes on over 400 patients from two studies (LAL-87 and LALA-94) and found that allogeneic HCT in CR1 resulted in improved survival in high risk patients[4,5]. Similar conclusions were drawn from the Groupe Ouest-Est des Leucémies Aiguës et Maladies du Sang (GOELAL02) clinical trial[6]. Conversely, the Eastern Cooperative Oncology Group/Medical Research Council (ECOG/MRC) and the Haemato-Oncology Foundation for adults in the Netherlands (HOVON) clinical trials demonstrated that this survival advantage is restricted to patients with standard risk disease[7,8]. Collectively, these results created some controversy within the transplant community on the optimal indication for all-HCT in CR1. The American Society of Blood and Marrow Transplantation recently published recommendations for the indications of various diseases for HCT, and they endorsed transplant for ALL in high risk disease in CR1 or CR2; however, these recommendations were not consistent with their European counterparts[9,10].

Out our center, we reserve allogeneic HCT for patients exhibiting conventional high risk features or evidence of minimal residual disease (MRD) at end of induction. We also perform allogeneic HCT for patients in second or subsequent CR (≥ CR2) due to its curative potential, albeit lower, in these patients and lack of better therapeutic strategies in this setting. Our aim from this analysis is to examine the prognostic factors and outcome in these high risk patients.

MATERIALS AND METHODS

Patient cohort

The project was approved by the institutional review board (IRB) prior to commencing. We identified all patients ≥ 14 years of age at our institution that underwent HCT for ALL during the time period of 2010-2017. All clinical records with regards to patient, disease, therapy and outcome were collected retrospectively from electronic medical records at our institution. The inclusion criteria were; patients who received allogeneic HCT for ALL using different conditioning intensity from matched related donor (MRD), matched unrelated donor (MUD) or haploidentical donors. The intensity of the conditioning regimen was based on the criteria suggested by the Centre of International Blood and Marrow Transplant Research (CIBMTR)[11]. Choice of regimen was based on the Hematopoietic Stem Cell Co-morbidity index (HCT-CI); patients scoring < 3 were considered for a myeloablative (MAC) regimen while the remaining patients received reduced intensity conditioning (RIC) regimen. Patients preferentially received a total body irradiation (TBI) regimen if they were candidates for a MAC regimen. We excluded patients who received a cord blood or bone marrow graft, second transplant and any patient that underwent in vivo or in vitro T-cell depletion. All records were retrospectively collected. Cytogenetics with hypodiploid karyotype, translocations at (4;11), (11q23), (9;22) and (1;19) were classified as high risk while all others were deemed standard risk.

Treatment protocol and indications for allogeneic HCT

The majority of patients received hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone with high dose methotrexate and cytarabine (HyperCVAD) given in alternating cycles (A and B) with cycle A consisting of 300 mg/m^2 of intravenous (IV) cyclophosphamide every 12 h on days 1-3 for a total of 6 doses with appropriate mesna dose for bladder protection; vincristine 1.4 mg/m^2 (maximum dose 2 mg) IV for two days (day 1 and 11); doxorubicin 50 mg/m^2 IV on day 4 followed by dexamethasone 40 mg IV on days 1-4 then 11-14. Cycle B contained of high dose methotrexate 1 g/m^2 given over 24 h on day 1 with appropriate hydration with sodium bicarbonate, leucovorin and therapeutic drug monitoring; cytarabine 3000 mg/m^2 IV over 2 h given every 12 h on days 2-3 for a total of 4 doses and methylprednisolone 50 mg IV every 12 h on days 1-3. Patients with CD20 expression were given the monoclonal antibody rituximab on days 1 and 8 at a dose of 375 mg/m^2. Ph positive ALL patients were given tyrosine kinase inhibitor (TKI) dasatinib 140 mg daily days 1-14 of each cycle of therapy and reinitiated post HCT once immunosuppression is tapered. Central nervous system prophylaxis consisted of intrathecal (IT) methotrexate 12 mg and hydrocortisone 50 mg given on day 2 of cycles A and B, and cytarabine 50 mg on day 8 of cycle A only. Patients were given at least 6 doses of IT chemotherapy prior to HCT. Patients were given 4 cycles of therapy (until 2B) prior to proceeding to HCT.

Supportive care consisted of granulocyte colony stimulating factor (GCSF) 300 mcg given starting day 5 until neutrophil recovery; ciprofloxacin 500 mg orally or IV equivalent twice daily; acyclovir 200 mg orally or IV equivalent twice daily; fluconazole 200 mg orally or IV equivalent twice daily and prednisolone 1% eye drops in each eye four times daily 1 d prior to and continued for 3 d post completion of cytarabine.

Bone marrow aspirate and trephine biopsy was done on day 28 post cycle 1A induction to assess for remission status with morphologic remission defined as < 5% blasts in the bone marrow with complete count recovery. The following high risk features were considered as indications for allogeneic HCT in first remission; presenting WBC > 30 × 10^9/L or 100 × 10^9/L in B- *vs* T-cell ALL, respectively; high risk cytogenetics as indicated above or evidence of persistent MRD post induction with HyperCVAD. Patients with relapsed disease and successfully achieved CR2 following salvage chemotherapy proceeded to HCT.

Preparative regimens and graft vs host disease prophylaxis

The MAC preparative regimen for matched related or unrelated donors (MRD or MUD) consisted of cyclophosphamide 60 mg/kg IV for a total of two days then a total of 1200 cGy of TBI divided twice daily for three days. Mesna was given for bladder protection. The MAC preparative regimen for haploidentical HCT consisted of fludarabine 25 mg/m^2 IV for 3 d and TBI 1200 cGy fractionated twice daily for 4 d as previously described[12]. For RIC regimens and MRD or MUD donors, patients received fludarabine 30 mg/m^2 IV on a daily basis for a total of 5 d with melphalan 70 mg/m^2 IV for 2 d. For those with RIC haploidentical HCT, the preparative regimen consisted of fludarabine 30 mg/m^2 IV daily for 5 d, cyclophosphamide 14.5 mg/kg IV daily for 2 d and TBI 200 cGy in a single fraction[13].

Prophylaxis for graft *vs* host disease (GVHD) contained methotrexate and cyclosporine for MRD and MUD HCT. Methotrexate was administered at 15 mg/m^2

on day +1 then at 10 mg/m^2 on days +3, +6 and +11. GVHD prophylaxis for haploidentical HCT consisted of tacrolimus 0.1 mg/kg per day orally twice daily (or IV equivalent) starting on day +6 adjusted to trough level of 10-15 ng/mL, mycophenolate mofetil (MMF) 15 mg/kg/dose three times daily starting on day +6 until +36 and cyclophosphamide 50 mg/kg IV daily on days +3 and +5 with appropriate mesna dose for bladder protection.

Definitions and transplant related outcomes

We defined overall survival (OS) as the time from transplant until the time of death of any cause or last patient encounter while progression free survival (PFS) was defined as the time from transplant until death due to any cause or relapsed disease. Cumulative incidence of relapse (CIR) was defined as the time from transplant until evidence of disease relapse or last patient encounter. While cumulative incidence of non-relapse mortality (NRM) was defined as the time from transplant until death due to any cause without evidence of relapse. Absolute neutrophil count (ANC) of 0.5 × 10^9/L or for 3 d constituted neutrophil engraftment while platelet count greater than 20 × 10^9/L for 7 d without transfusion support constituted platelet engraftment.

Statistical analysis

All baseline variables relating to patient, disease or treatment characteristics were reported in a descriptive fashion. Pearson's χ^2 and Wilcoxon/Kruskal-Wallis tests were used to analyze categorical or continuous variables, respectively. The Kaplan-Meir method with log ranks was used to estimate the probability of OS and PFS. Grey's model was used to estimate the incidence of events with competing nature, *i.e.*, CIR and cumulative incidence of NRM (CI-NRM). Cox regression model was used for univariate and multivariate analysis with outcome expressed as a hazard ratio (HR) with 95% confidence interval (CI) and P value. Variables with a $P \leq 0.05$ were inserted into the multivariate model. Analysis was performed using JMP and EZR[14].

RESULTS

Patient and transplant variables

During the study period, 69 patients were identified per our inclusion criteria and were further analyzed. The median (range) age was 21 (14-61) years with 41 (59%) being male. B-cell ALL was the most common pathology representing 50 (72%) of cases with the remaining being T-cell subtype. Ph-ALL was detected in 16/50 (32%) of B-cell ALL. LBL was seen in 17 (25%) of cases. 35 (51%) of patients had high risk cytogenetics. A total of 42 (61%) of patients received HCT in CR1 while the remaining patients were in second or subsequent CR. Indications for HCT in these patients were; 27 (64%) for high risk cytogenetics including Ph-ALL; 11 (26%) for high presenting WBC at diagnosis and 4 (10) for persistent MRD post induction. Matched sibling donor (MSD) was the most common donor type in 58 (84%) of cases and the majority of patients received MAC regimen (90%) containing TBI (87%). The baseline characteristics of the cohort are shown in Table 1.

Engraftment and GVHD

The median total of CD34 cells infused was 6 × 10^6/kg of recipient weight (range; 8.9-2) and all collected cells were infused through a Hickman catheter or a peripherally inserted central catheter (PICC). Infusion was over one day for all patients. GCSF was used in 33 (47.8%) of patients at the discretion of the treating physician. Median time to ANC engraftment, defined as ANC ≥ 0.5 × 10^6/L sustained over three days was 17 d (range; 9-28). There was no significant difference between time to ANC engraftment between patients receiving GCSF and those who did not. On the other hand, the median time to platelet engraftment was 12 (range; 0-29).

Acute GVHD (aGVHD) developed in a total of 20 patients (29%), with grades II, III or IV with 8 (40%), 8 (40%) and 4 (20%), respectively. All of them required systemic corticosteroid therapy, 5/20 (25%) required second line immune-suppressants while 2/20 (10%) required third line immune-suppressants. A high incidence of mortality was noted within these patients with 8/20 (40%) dying due to organ toxicity or infectious etiology. On the other hand, chronic GVHD (cGVHD) developed in a total of 30 patients (43.5%) with mild, moderate or severe forms in 8 (26.7%), 15 (50%) and 7 (23.3%), respectively. A total of 9 patients had overlap GVHD syndrome.

Post-transplant outcomes

Overall cohort: The median follow up was 15 mo (2-87.3), following which the 2 year CIR, CI-NRM, PFS and OS were 34.1%, 10.9%, 54.9% and 62.8%, respectively as shown in Figure 1). Stratified by remission status at the time of HCT, patients in CR1 had an

Table 1 Baseline characteristics of the cohort *n* (%)

Characteristic	Entire cohort (*n* = 69)
Patient age in years, median (range)	21 (14-61)
Recipient gender, male	41 (59%)
Cell subtype	
B-cell	50 (72)
T-cell	19 (28)
Philadelphia chromosome (B-cell)	16/50 (32)
Disease subtype	
Lymphoblastic leukemia	52 (75)
Lymphoblastic lymphoma	17 (25)
Cytogenetic status	
Standard	30 (43)
High risk	35 (51)
Missing	4 (6)
ECOG, median (range)	0 (0-2)
HCT-CI, median (range)	0 (0-5)
Gender mismatch	28 (41)
Female donor/male recipient	11 (16)
Donor type	
MSD	58 (84)
MORD	2 (3)
MUD	3 (4)
Haploidentical	6 (9)
Status at HCT	
CR1	42 (61)
≥ CR2	27 (39)
ABO matching	
Match	50 (73)
Major/bidirectional	10 (14)
Minor	9 (13)
TBI containing regimen	60 (87)
Conditioning intensity	
MAC	62 (90)
RIC/NMA	7 (10)

ECOG: Eastern Cooperative Oncology Group; HCT-CI: Hematopoietic stem cell transplant comorbidity index; MSD: Matched sibling donor; MORD: Matched other related donor; MUD: Matched unrelated donor; CR: Complete remission; TBI: Total body irradiation; MAC: Myeloablative conditioning; RIC/NMA: Reduced intensity conditioning/non-myeloablative.

improved survival compared to those in CR2 or CR3 with 2-year OS of 69.5% *vs* 46.5% *vs* 25% with a trend towards significance (P = 0.083) as shown in Figure 2A. On the other hand, when stratified by presence of cGVHD post HCT, patients with evidence of cGVHD had a significantly improved outcome with a 2-year OS of 70% *vs* 47.6% (p = 0.033) as shown in Figure 2B.

Predictors of outcome: In multivariable analysis for PFS or OS as the outcome of interest, the following variables were included; age at HCT, cell subtype, ALL *vs* LBL, Ph-chromosome status, female donor to male recipient, donor gender mismatch, MSD *vs* other donor source, TBI containing regimen, MAC regimen *vs* other, CR1 *vs* other, acute or cGVHD. For PFS, aGVHD and cGVHD were significant for PFS with corresponding HR of 3.14 (1.36-7.1; P = 0.008) and HR 0.38 (0.15-0.89; P = 0.026), respectively. Whereas for OS aGVHD and cGVHD were significant at the multivariable analysis with HR 4.9 (1.99-12; P = 0.0007) and 0.29 (0.1-0.67; P = 0.0044), respectively. These results are shown in Table 2.

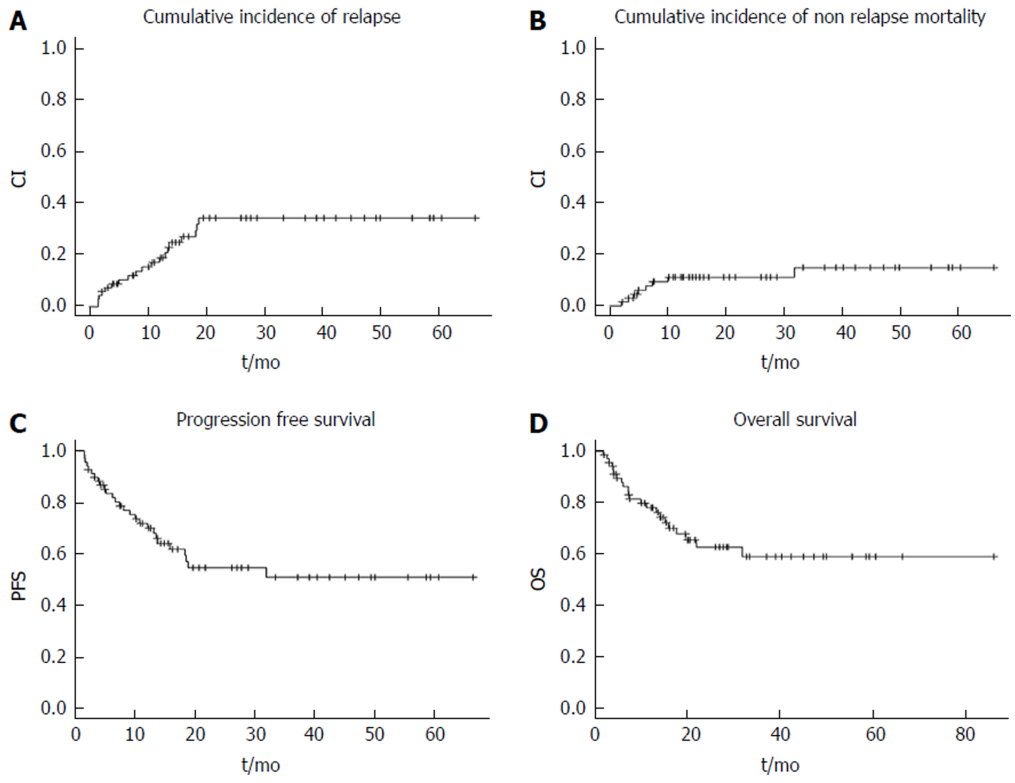

Figure 1 Outcome of post hematopoietic stem cell transplant for high risk acute lymphoblastic leukemia/lymphoma. A: Cumulative incidence of relapse; B: Cumulative incidence of non-relapse mortality; C: Progression free survival; D: Overall survival. PFS: Progression free survival; OS: Overall survival; CI: Cumulative incidence.

DISCUSSION

The optimal post remission therapy in ALL/LBL continues to be debated amongst experts given the ongoing developments in the field. On the one hand, allogeneic HCT offers good disease control relative to chemotherapy alone but the potential toxicity depending on prior therapy and hematopoietic stem cell transplant comorbidity index (HCT-CI) can be a hindering factor for some patients[15]. On the other hand, more refined methods of risk stratification specifically with the use of MRD and the utilization of a pediatric inspired regimens in eligible patients have significantly reduced relapse rates[16]. Importantly, optimal therapy should be delivered upfront as outcome of these patients post relapse are inferior. Oriol *et al*[17] reported on outcome of ALL patients with relapsed disease treated on one of four risk adapted trials by the PETHEMA study group. Only 10% of patients were alive at 5 years but more favorable outcomes were seen in younger patients and those relapsing late beyond 2 years.

A large comparative study examined 422 Ph negative ALL patients who underwent HCT in CR1 from the Center of International Blood and Marrow Transplantation Research (CIBMTR) to an age matched concurrent cohort of 108 patients treated with the Dana-Farber Consortium (DFC) Pediatric protocol found that while the relapse rate was similar among both approaches, patients fared significantly better with the DFC mainly due to a transplant related mortality (TRM) of 37%[18]. With regards to chemotherapy regimen comparison, the MD Anderson Cancer Center performed a comparative analysis between HyperCVAD, a common regimen for ALL used at their institution and the Augmented Berlin-Frankfurt-Munster (ABFM)[19]. Both regimens were associated with comparable overall outcomes, but with differing adverse event profile; ABFM resulting in higher hepatotoxicity, pancreatitis and osteonecrosis whereas HyperCVAD resulting in more bone marrow suppression related toxicity. Of note, the 5-year OS was 60% in both groups and around 10% of patients underwent HCT in CR1. Collectively, it remains unclear which treatment modality is preferred and further studies are needed to resolve this debate. The heterogeneity within the inclusion criteria among studies is the likely result in such discrepant outcomes.

Our aim with this analysis was to ascertain outcome of patients whom underwent HCT for ALL/LBL at our center. The patients presented herein were all those with high risk features, *i.e.*, conventional risk factors, positive MRD or those with relapsed

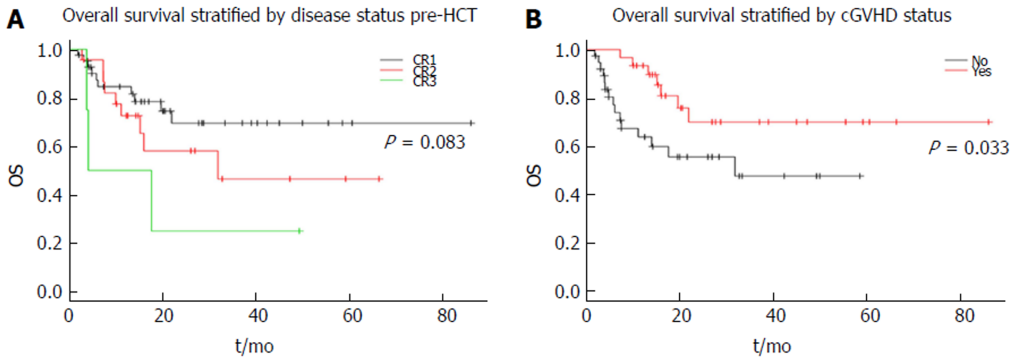

Figure 2 Overall survival of high risk acute lymphoblastic leukemia. A: Stratified by remission status prior to transplantation; B: Stratified by chronic graft *vs* host disease status HCT: Hematopoietic stem cell transplant; OS: Overall survival; cGVHD: Chronic graft *vs* host disease.

disease in second or subsequent remissions. We observed an OS of 62.8% at 2-year for the entire cohort which is quite promising. Furthermore, the CIR at 2-years was 34.1% for the entire cohort irrespective of the remission status at HCT. The majority of patients in this cohort underwent HCT utilizing MAC intensity conditioning and a MSD. Previously, the largest prospective trial in ALL, *i.e.*, the ECOG/MRC trial cohort reported a 5-year OS of 41% for high risk patients undergoing HCT in CR1[7]. Interestingly, the relapse rate observed within this trial was 37% for the high risk group and 24% within the standard risk which was comparable to our cohort. However, the incidence of NRM within the high risk cohort was 35.8% at 2-years which is substantially higher than what we observed despite having similar HCT criteria. We have two plausible observations that could have resulted in such higher NRM; first, the median age within our cohort was younger, and as such the expected complications post HCT are likely to be lower. This was reported previously where younger patients were reported to fare better than their older counterparts which was largely driven by higher incidence of NRM, whereas disease control with HCT is the same[20]. Second, the changes in supportive care over the last 1-2 decades, particularly with the use of antimicrobials for prophylaxis and management could have led to a reduction in post HCT complications[21].

Subsequently, we analyzed the cohort to ascertain factors influencing outcome at the multivariable analysis stage. We included typical patient, disease and transplant variables that may impact outcome. We observed that acute and chronic GVHD predicted for OS. There was a trend towards significance for B-cell subtype and CR1 remission status for OS and perhaps a larger sample size could have identified such variables as significant as well. Interestingly in our cohort, presence of Ph chromosome did not portend a negative prognostic marker and is likely due to the use of dasatinib as targeted TKI therapy during induction and as post HCT maintenance.

Allogeneic HCT is favored as post remission therapy due to relatively potent graft *vs* leukemia effect. Although difficult to measure or quantify, it is felt that cGVHD is a surrogate for such GVL effect[22,23]. Such effect is felt to be mediated by a number of donor factors but perhaps largely T-lymphocytes that exhibit their role by targetting any residual leukemia cells and prolonging patient's remission. However, this is a double edged sword as significant GVHD can augment the NRM effect and lead to more detrimental outcomes. Our patients experienced largely mild to moderate cGVHD, possibly due to majority of donors being MRD and we observed a favorable effect of such cGVHD on OS. aGVHD on the other hand had a detrimental impact on OS with a high case fatality ratio due to organ toxicity or infectious complications. Lastly, all B-ALL/LBL within this cohort received the monoclonal antibody rituximab, if CD20 positive, and it is possible that this has contributed to the trend of improved OS seen within our cohort. Previously, multiple studies reported on the favorable impact of rituximab on the outcome of ALL including Burkitt type ALL[24-26].

This analysis has some inherent limitations, particularly with its retrospective single center design and sample size. However, a number of important observations were noted; First, conventional high risk features of ALL/LBL can be overcome by the conditioning effect of the transplant coupled by the GVL effect. This is evident as the survival curve has plateaued indicating the curative potential of this therapy. Second, cGVHD leads to enhanced OS likely as it represents a surrogate for GVL. Third, aGVHD can be detrimental to outcome as it causes significant morbidity and mortality mainly due to infectious complications. In conclusion, allogeneic-HCT for high risk ALL/LBL results in promising remissions in high risk disease and early

Table 2 Univariable and multivariable risk factors influencing post hematopoietic stem cell transplant outcome

		Univariable HR (95%CI; *P* value)	Multivariable HR (95%CI; *P* value)
PFS	Age at HCT	1.5 (0.27-6; *P* = 0.6)	
	B-cell *vs* T-cell	0.53 (0.25-1.17; *P* = 0.11)	
	ALL *vs* LBL	0.6 (0.27-1.45; *P* = 0.24)	
	Female D → male R	0.87 (0.25-2.26; *P* = 0.79)	
	Donorgender mismatch	0.53 (0.22-1.17; *P* = 0.12)	
	MSD *vs* other	0.5 (0.22-1.28; *P* = 0.14)	
	TBI regimen	1.1 (0.41-3.67; *P* = 0.89)	
	MAC *vs* RIC/NMA	1.37 (0.41-8.5; *P* = 0.65)	
	CR1 *vs* other	0.59 (0.28-1.28; *P* = 0.18)	
	aGVHD	2.1 (0.95-4.5; *P* = 0.066)	3.14 (1.36-7.1; *P* = 0.008)
	cGVHD	0.43 (0.18-0.94; *P* = 0.033)	0.38 (0.15-0.89; *P* = 0.026)
OS	Age at HCT	1.02 (0.98-1.05; *P* = 0.28)	
	B-cell *vs* T-cell	0.57 (0.24-1.37; *P* = 0.2)	
	ALL *vs* LBL	0.44 (0.19-1.11; *P* = 0.08)	
	Female D → male R	1.15 (0.33-3.1; *P* = 0.8)	
	Donorgender mismatch	0.62 (0.23-1.48; *P* = 0.29)	
	MSD *vs* other	1.27 (0.43-5.4; *P* = 0.69)	
	TBI regimen	1.99 (0.58-12.5; *P* = 0.31)	
	MAC *vs* RIC/NMA	0.69 (0.23-2.92; *P* = 0.56)	
	CR1 *vs* other	0.5 (0.21-1.17; *P* = 0.11)	
	aGVHD	3.35 (1.42-7.9; *P* = 0.006)	4.9 (1.99-12; *P* = 0.0007)
	cGVHD	0.4 (0.15-0.97; *P* = 0.043)	0.29 (0.1-0.67; *P* = 0.0044)

HCT: Hematopoietic stem cell transplant; ALL: Acute lymphoblastic leukemia; LBL: Lymphoblastic lymphoma; Ph: Philadelphia chromosome; MSD: Matched sibling donor; TBI: Total body irradiation; CR: Complete remission; MAC: Myeloablative conditioning; RIC/NMA: Reduced intensity conditioning/no myeloablative; a/cGVHD: Acute/chronic graft *vs* host disease.

referral for HCT to be considered for young and fit patients.

ARTICLE HIGHLIGHTS

Research background

Allogeneic hematopoietic stem cell transplantation (HCT) is a potentially curative therapy for patients with high risk acute lymphoblastic leukemia (ALL). The indications for HCT have evolved over time with the introduction of pediatric inspired protocols and minimal residual disease (MRD) monitoring. Our aim from this study is to examine the outcome and prognostic factors for high risk ALL patients at our center.

Research motivation

Identifying the prognostic factors that may facilitate patient selection and select the ideal candidate for transplantation.

Research objectives

Our aim from this study is to examine the outcome and prognostic factors for high risk ALL patients.

Research methods

After due institutional review board approval, patients with high risk ALL/ lymphoblastic lymphoma (LBL) post HCT were identified and included. All records were retrospectively collected. Time to event analysis, was calculated from the date of HCT until event of interest or last follow up with KM means. Cox regression model was used for multivariable analysis calculation.

Research results

A total of 69 patients were enrolled and examined with a median age of 21 (14-61). After a median follow up of 15 mo (2-87.3), the 2-year cumulative incidence of relapse (CIR), cumulative incidence of non-relapse mortality (CI-NRM), progression free survival (PFS) and overall survival (OS) were 34.1%, 10.9%, 54.9% and 62.8%, respectively. In a multivariable analysis for OS; acute graft *vs* host disease (GVHD) and chronic GVHD were significant with corresponding

HR 4.9 (1.99-12; P = 0.0007) and 0.29 (0.1-0.67; P = 0.0044), respectively.

Research conclusions

Allogeneic-HCT for high risk ALL/LBL results in promising remissions and early referral for HCT is to be considered for young and fit patients.

Research perspectives

We identified that acute and chronic graft *vs* host diseases were prognostic for overall survival. We also observed that patients with Philadelphia positive ALL whom were given tyrosine kinase inhibitor therapy fared better than expected. Post HCT outcome of patients with ALL is expected to improve over time with the changing therapeutic landscape. We wished to examine the outcome of ALL patients treated in a contemporary era and identify prognostic factors for outcome. Our findings warrant confirmation in a larger cohort of patients.

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