ISSN 2220-3230 (online)

World Journal of *Transplantation*

World J Transplant 2018 February 24; 8(1): 1-37

Published by Baishideng Publishing Group Inc

WJT World Journal of Transplantation

Contents

Bimonthly Volume 8 Number 1 February 24, 2018

Contents

World Journal of Transplantation
Volume 8 Number 1 February 24, 2018

ABOUT COVER

Editorial Board Member of *World Journal of Transplantation*, Robert M Aris, MD, Professor, Division of Pulmonary and Critical Care Medicine, University of North Carolina, Chapel Hill, NC 27599, United States

AIM AND SCOPE

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

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

AIM AND SCOPE

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

NAME OF JOURNAL
World Journal of Transplantation

ISSN
ISSN 2220-3230 (online)

LAUNCH DATE
December 24, 2011

FREQUENCY
Bimonthly

World Journal of Transplantation
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
February 24, 2018

World Journal of Transplantation

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

World J Transplant 2018 February 24; 8(1): 1-12

DOI: 10.5500/wjt.v8.i1.1

ISSN 2220-3230 (online)

REVIEW

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

Marianne Yardley, Lars Gullestad, Kari Nytrøen

Marianne Yardley, Lars Gullestad, Kari Nytrøen, Department of Cardiology, Oslo University Hospital Rikshospitalet, Oslo 0424, Norway

Marianne Yardley, Lars Gullestad, Kari Nytrøen, Faculty of Medicine, University of Oslo, Oslo 0316, Norway

ORCID number: Marianne Yardley (0000-0002-6411-6665); Lars Gullestad (0000-0002-5932-6641); Kari Nytrøen (0000-0002-4827-4700).

Author contributions: Gullestad L and Nytrøen K designed the research; Yardley M and Nytrøen K has performed different parts of the research, analyzed the data and drafted the paper; all authors equally contributed to the final analyses, further drafting and critical revision and editing, and final approval of the final version.

Conflict-of-interest statement: No potential conflicts of interest. No financial support.

Manuscript source: Invited manuscript

Correspondence to: Dr. Kari Nytrøen, PhD, Senior Postdoctoral Fellow, Department of Cardiology, Oslo University Hospital Rikshospitalet, Postbox 4950 Nydalen, Oslo 0424, Norway. kari.nytroen@medisin.uio.no
Telephone: +47-951-89935
Fax: +47-230-73917

Received: November 17, 2017
Peer-review started: November 17, 2017
First decision: November 30, 2017
Revised: December 12, 2017
Accepted: December 28, 2017

Article in press: December 28, 2017
Published online: February 24, 2018

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 immuno-suppression 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 $\leqslant$ 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.

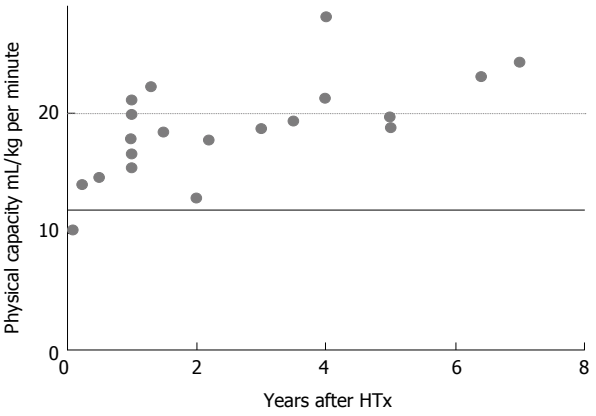

Figure 1 Physical capacity, as assessed by VO_{2peak} after heart transplantation from published studies, illustrated by years after surgery. Black line at 12 mL/kg per minute, show the threshold to be candidates for HTx, dotted line at 20 mL/min per kilogram, show the start of Weber function class A, 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.

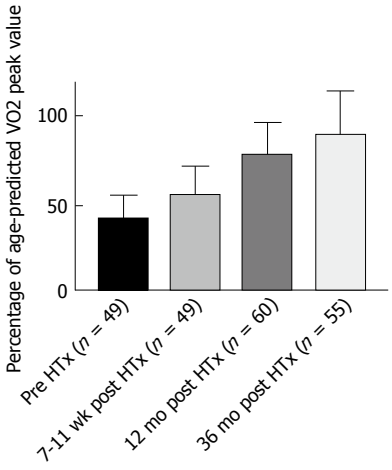

Figure 2 Illustration of the increase in peak VO_2 from pre heart transplantation to 36 mo post heart transplantation. The data presented in the figure is from the unpublished Schedule trial.

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

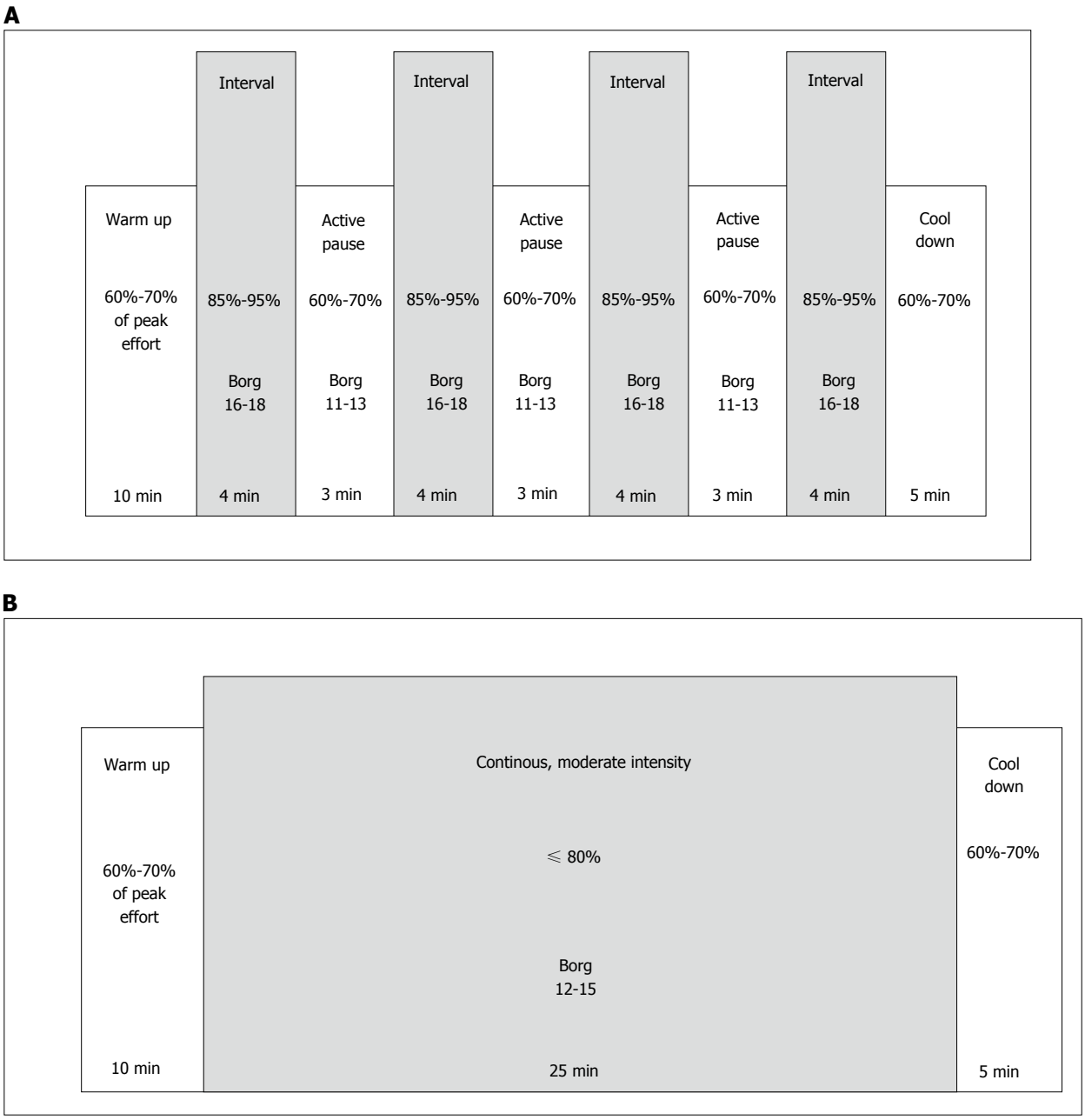

Figure 3 Illustration of two different exercise modalities. A: Illustration of a session with high-intensity interval training (HIT). HIT is an exercise strategy with alternating short periods of intense endurance exercise with less-intense recovery periods. A usual HIT session may include 4 × 4 min 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

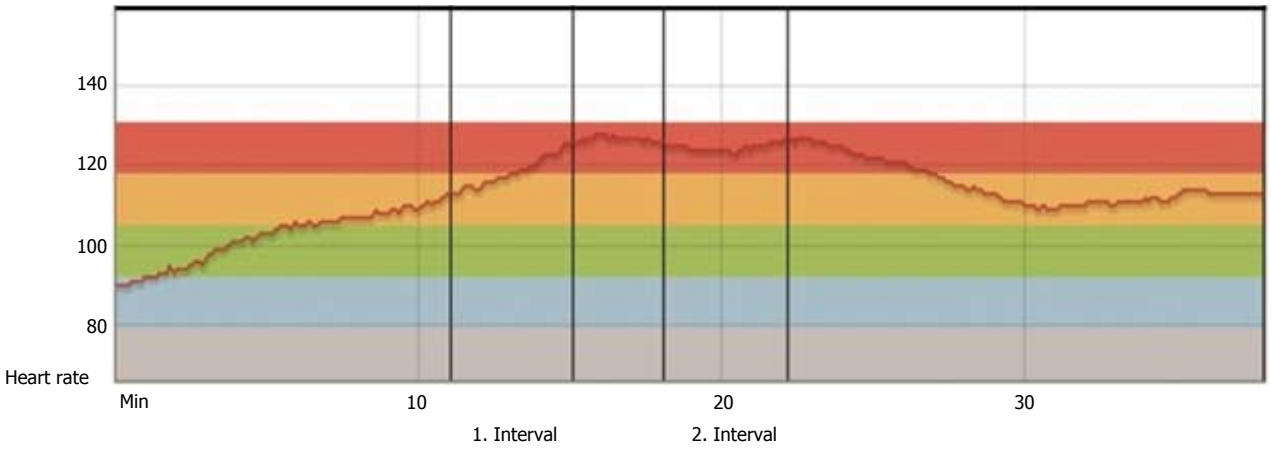

Figure 4 Heart rate during exercise 3 mo post heart transplantation. Patient from our hospital, 3 mo post-HTx: HR curve during warm-up, two high-intensity intervals divided by one recovery period and cool-down. The curve shows a typical pattern of impaired HR responses in the early stage after HTx. HTx: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

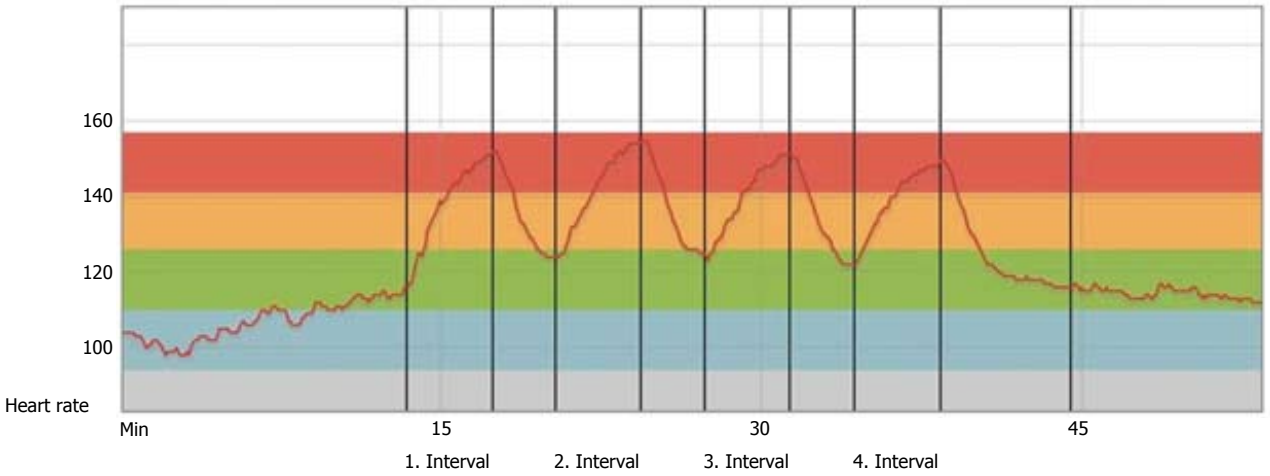

Figure 5 Heart rate during exercise 12 mo post heart transplantation. The same patient 12 mo post-HTx: Heart rate curve during warm-up four high-intensity intervals divided by 3 recovery periods and cool-down. The curve shows a largely normalized HR, with immediate HR adaptions to exercise intensity.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

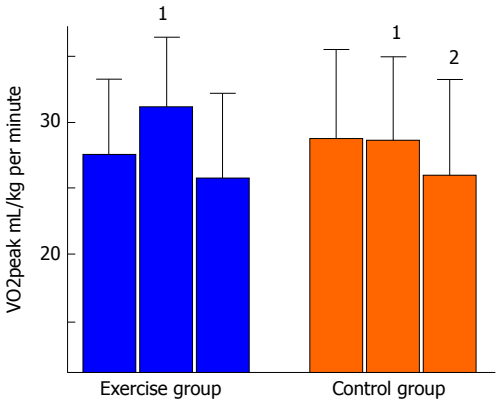

Figure 6 Measurements of VO_{2peak} at baseline, 1-year and 5-year follow-up. [1]Significant changes between groups; [2]Significant changes from baseline to 5-year follow-up within group.

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.

REFERENCES

1 **Ho KK**, Anderson KM, Kannel WB, Grossman W, Levy D. Survival after the onset of congestive heart failure in Framingham Heart Study subjects. *Circulation* 1993; **88**: 107-115 [PMID: 8319323 DOI: 10.1161/01.CIR.88.1.107]

2 **Zannad F**, Mebazaa A, Juillière Y, Cohen-Solal A, Guize L, Alla F, Rougé P, Blin P, Barlet MH, Paolozzi L, Vincent C, Desnos M, Samii K; EFICA Investigators. Clinical profile, contemporary management and one-year mortality in patients with severe acute heart failure syndromes: The EFICA study. *Eur J Heart Fail* 2006; **8**: 697-705 [PMID: 16516552 DOI: 10.1016/j.ejheart.2006.01.001]

3 **Taylor CJ**, Ryan R, Nichols L, Gale N, Hobbs FR, Marshall T. Survival following a diagnosis of heart failure in primary care. *Fam Pract* 2017; **34**: 161-168 [PMID: 28137979 DOI: 10.1093/fampra/cmw145]

4 **Stehlik J**, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI, Dobbels F, Kirk R, Rahmel AO, Hertz MI; International Society of Heart and Lung Transplantation. The Registry of the International Society for Heart and Lung Transplantation: 29th official adult heart transplant report--2012. *J Heart Lung Transplant* 2012; **31**: 1052-1064 [PMID: 22975095 DOI: 10.1016/j.healun.2012.08.002]

5 **Osada N**, Chaitman BR, Donohue TJ, Wolford TL, Stelken AM, Miller LW. Long-term cardiopulmonary exercise performance after heart transplantation. *Am J Cardiol* 1997; **79**: 451-456 [PMID: 9052349 DOI: 10.1016/S0002-9149(96)00785-0]

6 **Weber KT**, Kinasewitz GT, Janicki JS, Fishman AP. Oxygen utilization and ventilation during exercise in patients with chronic cardiac failure. *Circulation* 1982; **65**: 1213-1223 [PMID: 6804111 DOI: 10.1161/01.CIR.65.6.1213]

7 **Soumagne D**. Weber classification in cardiac rehabilitation. *Acta Cardiol* 2012; **67**: 285-290 [PMID: 22870735 DOI: 10.2143/ac.67.3.2160716]

8 **Janaudis-Ferreira T**, Mathur S, Konidis S, Tansey CM, Beaurepaire C. Outcomes in randomized controlled trials of exercise interventions in solid organ transplant. *World J Transplant* 2016; **6**: 774-789 [PMID: 28058230 DOI: 10.5500/wjt.v6.i4.774]

9 **Dennis C**. Rehabilitation of patients with coronary artery disease. In: Braunwald's Heart disease, a textbook of cardiovascular medicine, 9th ed. Philadelphia: Elsevier Saunders, 2012: 19611-19656

10 **Kodama S**, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, Sugawara A, Totsuka K, Shimano H, Ohashi Y, Yamada N, Sone H. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *JAMA* 2009; **301**: 2024-2035 [PMID: 19454641 DOI: 10.1001/jama.2009.681]

11 **Arena R**, Myers J, Guazzi M. The future of aerobic exercise testing in clinical practice: is it the ultimate vital sign? *Future Cardiol* 2010; **6**: 325-342 [PMID: 20462339 DOI: 10.2217/fca.10.21]

12 **Kavanagh T**, Mertens DJ, Hamm LF, Beyene J, Kennedy J, Corey P, Shephard RJ. Prediction of long-term prognosis in 12 169 men referred for cardiac rehabilitation. *Circulation* 2002; **106**: 666-671 [PMID: 12163425 DOI: 10.1161/01.CIR.0000024413.15949.ED]

13 **Cahalin LP**, Chase P, Arena R, Myers J, Bensimhon D, Peberdy MA, Ashley E, West E, Forman DE, Pinkstaff S, Lavie CJ, Guazzi M. A meta-analysis of the prognostic significance of cardiopulmonary exercise testing in patients with heart failure. *Heart Fail Rev* 2013; **18**: 79-94 [PMID: 22733204 DOI: 10.1007/s10741-012-9332-0]

14 **Mehra MR**, Kobashigawa J, Starling R, Russell S, Uber PA, Parameshwar J, Mohacsi P, Augustine S, Aaronson K, Barr M. Listing criteria for heart transplantation: International Society for Heart and Lung Transplantation guidelines for the care of cardiac transplant candidates--2006. *J Heart Lung Transplant* 2006; **25**: 1024-1042 [PMID: 16962464 DOI: 10.1016/j.healun.2006.06.008]

15 **Jaramillo N**, Segovia J, Gómez-Bueno M, García-Cosío D, Castedo E, Serrano S, Burgos R, García Montero C, Ugarte J, Martínez Cabeza P, Alonso-Pulpón L. Characteristics of patients with survival longer than 20 years following heart transplantation. *Rev Esp Cardiol* (Engl Ed) 2013; **66**: 797-802 [PMID: 24773860 DOI: 10.1016/j.rec.2013.05.016]

16 **Tallaj JA**, Pamboukian SV, George JF, Kirklin JK, Brown RN, McGiffin DC, Acharya D, Loyaga-Rendon R, Melby SJ, Bourge RC, Naftel DC. Have risk factors for mortality after heart transplantation changed over time? Insights from 19 years of Cardiac Transplant Research Database study. *J Heart Lung Transplant* 2014; **33**: 1304-1311 [PMID: 25443871 DOI: 10.1016/j.healun.2014.08.014]

17 **Yardley M**, Havik OE, Grov I, Relbo A, Gullestad L, Nytrøen K. Peak oxygen uptake and self-reported physical health are strong predictors of long-term survival after heart transplantation. *Clin Transplant* 2016; **30**: 161-169 [PMID: 26589579 DOI: 10.1111/ctr.12672]

18 **Grigioni F**, Specchia S, Maietta P, Potena L, Bacchi-Reggiani ML, Ghetti G, Boriani G, Foschi E, Corazza I, Ionico T, Magnani G, Zannoli R, Tentoni C, Branzi A. Changes in exercise capacity induced by heart transplantation: prognostic and therapeutic implications. *Scand J Med Sci Sports* 2011; **21**: 519-525 [PMID: 20459480 DOI: 10.1111/j.1600-0838.2009.01065.x]

19 **Chang AC**, Shyr Y, Groves J, Chomsky DB, Davis SF, Wilson JR, Drinkwater DC, Pierson RN, Merrill WH. The utility of exercise testing after cardiac transplantation in older patients. *J Surg Res* 1999; **81**: 48-54 [PMID: 9889057 DOI: 10.1006/jsre.1998.5484]

20 **Rosenbaum AN**, Kremers WK, Schirger JA, Thomas RJ, Squires

RW, Allison TG, Daly RC, Kushwaha SS, Edwards BS. Association Between Early Cardiac Rehabilitation and Long-term Survival in Cardiac Transplant Recipients. *Mayo Clin Proc* 2016; **91**: 149-156 [PMID: 26848001 DOI: 10.1016/j.mayocp.2015.12.002]

21 **ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories**. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002; **166**: 111-117 [PMID: 12091180 DOI: 10.1164/ajrccm.166.1.at1102]

22 **Lee IM**, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT; Lancet Physical Activity Series Working Group. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet* 2012; **380**: 219-229 [PMID: 22818936 DOI: 10.1016/s0140-6736(12)61031-9]

23 **Holme I**, Anderssen SA. [Physical activity, smoking and mortality among men who participated in the Oslo studies of 1972 and 2000]. *Tidsskr Nor Laegeforen* 2014; **134**: 1743-1748 [PMID: 25273247 DOI: 10.4045/tidsskr.14.0073]

24 **Eriksen L**, Curtis T, Grønbæk M, Helge JW, Tolstrup JS. The association between physical activity, cardiorespiratory fitness and self-rated health. *Prev Med* 2013; **57**: 900-902 [PMID: 24099878 DOI: 10.1016/j.ypmed.2013.09.024]

25 **Yardley M**, Gullestad L, Bendz B, Bjørkelund E, Rolid K, Arora S, Nytrøen K. Long-term effects of high-intensity interval training in heart transplant recipients: A 5-year follow-up study of a randomized controlled trial. *Clin Transplant* 2017; **31**: Epub ahead of print [PMID: 27865004 DOI: 10.1111/ctr.12868]

26 **Hsieh PL**, Wu YT, Chao WJ. Effects of exercise training in heart transplant recipients: a meta-analysis. *Cardiology* 2011; **120**: 27-35 [PMID: 22094922 DOI: 10.1159/000332998]

27 **Wisløff U**, Støylen A, Loennechen JP, Bruvold M, Rognmo Ø, Haram PM, Tjønna AE, Helgerud J, Slørdahl SA, Lee SJ, Videm V, Bye A, Smith GL, Najjar SM, Ellingsen Ø, Skjaerpe T. Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study. *Circulation* 2007; **115**: 3086-3094 [PMID: 17548726 DOI: 10.1161/CIRCULATIONAHA.106.675041]

28 **Elliott AD**, Rajopadhyaya K, Bentley DJ, Beltrame JF, Aromataris EC. Interval training versus continuous exercise in patients with coronary artery disease: a meta-analysis. *Heart Lung Circ* 2015; **24**: 149-157 [PMID: 25306500 DOI: 10.1016/j.hlc.2014.09.001]

29 **Tjønna AE**, Lee SJ, Rognmo Ø, Stølen TO, Bye A, Haram PM, Loennechen JP, Al-Share QY, Skogvoll E, Slørdahl SA, Kemi OJ, Najjar SM, Wisløff U. Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. *Circulation* 2008; **118**: 346-354 [PMID: 18606913 DOI: 10.1161/CIRCULATIONAHA.108.772822]

30 **Wisløff U**, Ellingsen Ø, Kemi OJ. High-intensity interval training to maximize cardiac benefits of exercise training? *Exerc Sport Sci Rev* 2009; **37**: 139-146 [PMID: 19550205 DOI: 10.1097/JES.0b013e3181aa65fc]

31 **Borg G**. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med* 1970; **2**: 92-98 [PMID: 5523831]

32 **Anderssen SA**, Strømme SB. [Physical activity and health--recommendations]. *Tidsskr Nor Laegeforen* 2001; **121**: 2037-2041 [PMID: 11875902]

33 **Piña IL**, Apstein CS, Balady GJ, Belardinelli R, Chaitman BR, Duscha BD, Fletcher BJ, Fleg JL, Myers JN, Sullivan MJ; American Heart Association Committee on exercise, rehabilitation, and prevention. Exercise and heart failure: A statement from the American Heart Association Committee on exercise, rehabilitation, and prevention. *Circulation* 2003; **107**: 1210-1225 [PMID: 12615804 DOI: 10.1161/01.CIR.0000055013.92097.40]

34 **Nytrøen K**, Myers J, Chan KN, Geiran OR, Gullestad L. Chronotropic responses to exercise in heart transplant recipients: 1-yr follow-up. *Am J Phys Med Rehabil* 2011; **90**: 579-588 [PMID: 21765276 DOI: 10.1097/PHM.0b013e31821f711d]

35 **Dall CH**, Snoer M, Christensen S, Monk-Hansen T, Frederiksen M, Gustafsson F, Langberg H, Prescott E. Effect of high-intensity training versus moderate training on peak oxygen uptake and chronotropic response in heart transplant recipients: a randomized crossover trial. *Am J Transplant* 2014; **14**: 2391-2399 [PMID: 25135383 DOI: 10.1111/ajt.12873]

36 **Hermann TS**, Dall CH, Christensen SB, Goetze JP, Prescott E, Gustafsson F. Effect of high intensity exercise on peak oxygen uptake and endothelial function in long-term heart transplant recipients. *Am J Transplant* 2011; **11**: 536-541 [PMID: 21219582 DOI: 10.1111/j.1600-6143.2010.03403.x]

37 **Nytrøen K**, Rustad LA, Aukrust P, Ueland T, Hallén J, Holm I, Rolid K, Lekva T, Fiane AE, Amlie JP, Aakhus S, Gullestad L. High-intensity interval training improves peak oxygen uptake and muscular exercise capacity in heart transplant recipients. *Am J Transplant* 2012; **12**: 3134-3142 [PMID: 22900793 DOI: 10.1111/j.1600-6143.2012.04221.x]

38 **Mezzani A**, Hamm LF, Jones AM, McBride PE, Moholdt T, Stone JA, Urhausen A, Williams MA; European Association for Cardiovascular Prevention and Rehabilitation; American Association of Cardiovascular and Pulmonary Rehabilitation; Canadian Association of Cardiac Rehabilitation. Aerobic exercise intensity assessment and prescription in cardiac rehabilitation: a joint position statement of the European Association for Cardiovascular Prevention and Rehabilitation, the American Association of Cardiovascular and Pulmonary Rehabilitation and the Canadian Association of Cardiac Rehabilitation. *Eur J Prev Cardiol* 2013; **20**: 442-467 [PMID: 23104970 DOI: 10.1177/2047487312460484]

39 **Nytrøen K**, Rustad LA, Erikstad I, Aukrust P, Ueland T, Lekva T, Gude E, Wilhelmsen N, Hervold A, Aakhus S, Gullestad L, Arora S. Effect of high-intensity interval training on progression of cardiac allograft vasculopathy. *J Heart Lung Transplant* 2013; **32**: 1073-1080 [PMID: 23906899 DOI: 10.1016/j.healun.2013.06.023]

40 **Nytrøen K**, Yardley M, Rolid K, Bjørkelund E, Karason K, Wigh JP, Dall CH, Arora S, Aakhus S, Lunde K, Solberg OG, Gustafsson F, Prescott EI, Gullestad L. Design and rationale of the HITTS randomized controlled trial: Effect of High-intensity Interval Training in de novo Heart Transplant Recipients in Scandinavia. *Am Heart J* 2016; **172**: 96-105 [PMID: 26856221 DOI: 10.1016/j.ahj.2015.10.011]

41 **Piepoli MF**, Davos C, Francis DP, Coats AJ; ExTraMATCH Collaborative. Exercise training meta-analysis of trials in patients with chronic heart failure (ExTraMATCH). *BMJ* 2004; **328**: 189 [PMID: 14729656 DOI: 10.1136/bmj.37938.645220.EE]

42 **Sagar VA**, Davies EJ, Briscoe S, Coats AJ, Dalal HM, Lough F, Rees K, Singh S, Taylor RS. Exercise-based rehabilitation for heart failure: systematic review and meta-analysis. *Open Heart* 2015; **2**: e000163 [PMID: 25685361 DOI: 10.1136/openhrt-2014-000163]

43 **Anderson L**, Nguyen TT, Dall CH, Burgess L, Bridges C, Taylor RS. Exercise-based cardiac rehabilitation in heart transplant recipients. *Cochrane Database Syst Rev* 2017; **4**: CD012264 [PMID: 28375548 DOI: 10.1002/14651858.CD012264]

44 **Mathur S**, Janaudis-Ferreira T, Wickerson L, Singer LG, Patcai J, Rozenberg D, Blydt-Hansen T, Hartmann EL, Haykowsky M, Helm D, High K, Howes N, Kamath BM, Lands L, Marzolini S, Sonnenday C. Meeting report: consensus recommendations for a research agenda in exercise in solid organ transplantation. *Am J Transplant* 2014; **14**: 2235-2245 [PMID: 25135579 DOI: 10.1111/ajt.12874]

45 **Moholdt T**, Aamot IL, Granøien I, Gjerde L, Myklebust G, Walderhaug L, Hole T, Graven T, Stølen T, Mølmen-Hansen HE, Støylen A, Skogvoll E, Slørdahl SA, Wisløff U. Long-term follow-up after cardiac rehabilitation: a randomized study of usual care exercise training versus aerobic interval training after myocardial infarction. *Int J Cardiol* 2011; **152**: 388-390 [PMID: 21907424 DOI: 10.1016/j.ijcard.2011.08.025]

46 **Fleg JL**, Morrell CH, Bos AG, Brant LJ, Talbot LA, Wright JG, Lakatta EG. Accelerated longitudinal decline of aerobic capacity in healthy older adults. *Circulation* 2005; **112**: 674-682 [PMID: 16043637 DOI: 10.1161/circulationaha.105.545459]

47 **Betik AC**, Hepple RT. Determinants of VO2 max decline with aging: an integrated perspective. *Appl Physiol Nutr Metab* 2008; **33**: 130-140 [PMID: 18347663 DOI: 10.1139/h07-174]

48 **Ismail H**, McFarlane JR, Nojoumian AH, Dieberg G, Smart NA. Clinical outcomes and cardiovascular responses to different exercise training intensities in patients with heart failure: a systematic review and meta-analysis. *JACC Heart Fail* 2013; **1**: 514-522 [PMID: 24622004 DOI: 10.1016/j.jchf.2013.08.006]

49 **Kemi OJ**, Wisloff U. High-intensity aerobic exercise training improves the heart in health and disease. *J Cardiopulm Rehabil Prev* 2010; **30**: 2-11 [PMID: 20040880 DOI: 10.1097/HCR.0b013e3181c56b89]

50 **Rustad LA**, Nytrøen K, Amundsen BH, Gullestad L, Aakhus S. One year of high-intensity interval training improves exercise capacity, but not left ventricular function in stable heart transplant recipients: a randomised controlled trial. *Eur J Prev Cardiol* 2014; **21**: 181-191 [PMID: 23185084 DOI: 10.1177/2047487312469477]

51 **Dall CH**, Gustafsson F, Christensen SB, Dela F, Langberg H, Prescott E. Effect of moderate- versus high-intensity exercise on vascular function, biomarkers and quality of life in heart transplant recipients: A randomized, crossover trial. *J Heart Lung Transplant* 2015; **34**: 1033-1041 [PMID: 25840503 DOI: 10.1016/j.healun.2015.02.001]

52 **Yardley M**, Ueland T, Aukrust P, Michelsen A, Bjørkelund E, Gullestad L, Nytrøen K. Immediate response in markers of inflammation and angiogenesis during exercise: a randomised cross-over study in heart transplant recipients. *Open Heart* 2017; **4**: e000635 [PMID: 29225901 DOI: 10.1136/openhrt-2017-000635]

53 **Wallén NH**, Goodall AH, Li N, Hjemdahl P. Activation of haemostasis by exercise, mental stress and adrenaline: effects on platelet sensitivity to thrombin and thrombin generation. *Clin Sci* (Lond) 1999; **97**: 27-35 [PMID: 10369791 DOI: 10.1042/cs0970027]

54 **Crimi E**, Ignarro LJ, Cacciatore F, Napoli C. Mechanisms by which exercise training benefits patients with heart failure. *Nat Rev Cardiol* 2009; **6**: 292-300 [PMID: 19352333 DOI: 10.1038/nrcardio.2009.8]

55 **Hambrecht R**, Adams V, Erbs S, Linke A, Kränkel N, Shu Y, Baither Y, Gielen S, Thiele H, Gummert JF, Mohr FW, Schuler G. Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation* 2003; **107**: 3152-3158 [PMID: 12810615 DOI: 10.1161/01.cir.0000074229.93804.5c]

56 **Wang Y**, Li M, Dong F, Zhang J, Zhang F. Physical exercise-induced protection on ischemic cardiovascular and cerebrovascular diseases. *Int J Clin Exp Med* 2015; **8**: 19859-19866 [PMID: 26884896]

57 **Kilian Y**, Wehmeier UF, Wahl P, Mester J, Hilberg T, Sperlich B. Acute Response of Circulating Vascular Regulating MicroRNAs during and after High-Intensity and High-Volume Cycling in Children. *Front Physiol* 2016; **7**: 92 [PMID: 27014090 DOI: 10.3389/fphys.2016.00092]

58 **Delavar H**, Nogueira L, Wagner PD, Hogan MC, Metzger D, Breen EC. Skeletal myofiber VEGF is essential for the exercise training response in adult mice. *Am J Physiol Regul Integr Comp Physiol* 2014; **306**: R586-R595 [PMID: 24523345 DOI: 10.1152/ajpregu.00522.2013]

59 **Olfert IM**, Baum O, Hellsten Y, Egginton S. Advances and challenges in skeletal muscle angiogenesis. *Am J Physiol Heart Circ Physiol* 2016; **310**: H326-H336 [PMID: 26608338 DOI: 10.1152/ajpheart.00635.2015]

60 **Hoier B**, Hellsten Y. Exercise-induced capillary growth in human skeletal muscle and the dynamics of VEGF. *Microcirculation* 2014; **21**: 301-314 [PMID: 24450403 DOI: 10.1111/micc.12117]

61 **Avery RK**. Cardiac-allograft vasculopathy. *N Engl J Med* 2003; **349**: 829-830 [PMID: 12944567 DOI: 10.1056/NEJMp038124]

62 **Jansen MA**, Otten HG, de Weger RA, Huibers MM. Immunological and Fibrotic Mechanisms in Cardiac Allograft Vasculopathy. *Transplantation* 2015; **99**: 2467-2475 [PMID: 26285017 DOI: 10.1097/tp.0000000000000848]

63 **Tuzcu EM**, Kapadia SR, Sachar R, Ziada KM, Crowe TD, Feng J, Magyar WA, Hobbs RE, Starling RC, Young JB, McCarthy P, Nissen SE. Intravascular ultrasound evidence of angiographically silent progression in coronary atherosclerosis predicts long-term morbidity and mortality after cardiac transplantation. *J Am Coll Cardiol* 2005; **45**: 1538-1542 [PMID: 15862431 DOI: 10.1016/j.jacc.2004.12.076]

64 **Kobashigawa JA**, Tobis JM, Starling RC, Tuzcu EM, Smith AL, Valantine HA, Yeung AC, Mehra MR, Anzai H, Oeser BT, Abeywickrama KH, Murphy J, Cretin N. Multicenter intravascular ultrasound validation study among heart transplant recipients: outcomes after five years. *J Am Coll Cardiol* 2005; **45**: 1532-1537 [PMID: 15862430 DOI: 10.1016/j.jacc.2005.02.035]

65 **Potena L**, Masetti M, Sabatino M, Bacchi-Reggiani ML, Pece V, Prestinenzi P, Dall'Ara G, Taglieri N, Saia F, Fallani F, Magnani G, Rapezzi C, Grigioni F. Interplay of coronary angiography and intravascular ultrasound in predicting long-term outcomes after heart transplantation. *J Heart Lung Transplant* 2015; **34**: 1146-1153 [PMID: 25843518 DOI: 10.1016/j.healun.2015.01.990]

66 **Kobashigawa JA**, Katznelson S, Laks H, Johnson JA, Yeatman L, Wang XM, Chia D, Terasaki PI, Sabad A, Cogert GA. Effect of pravastatin on outcomes after cardiac transplantation. *N Engl J Med* 1995; **333**: 621-627 [PMID: 7637722 DOI: 10.1056/nejm199509073331003]

67 **Arora S**, Andreassen AK, Andersson B, Gustafsson F, Eiskjaer H, Bøtker HE, Rådegran G, Gude E, Ioanes D, Solbu D, Sigurdardottir V, Dellgren G, Erikstad I, Solberg OG, Ueland T, Aukrust P, Gullestad L; SCHEDULE (SCandinavian HEart transplant everolimus De novo stUdy with earLy calcineurin inhibitors avoidancE) Investigators. The Effect of Everolimus Initiation and Calcineurin Inhibitor Elimination on Cardiac Allograft Vasculopathy in De Novo Recipients: One-Year Results of a Scandinavian Randomized Trial. *Am J Transplant* 2015; **15**: 1967-1975 [PMID: 25783974 DOI: 10.1111/ajt.13214]

68 **Andreassen AK**, Andersson B, Gustafsson F, Eiskjaer H, Rådegran G, Gude E, Jansson K, Solbu D, Karason K, Arora S, Dellgren G, Gullestad L; SCHEDULE investigators. Everolimus Initiation With Early Calcineurin Inhibitor Withdrawal in De Novo Heart Transplant Recipients: Three-Year Results From the Randomized SCHEDULE Study. *Am J Transplant* 2016; **16**: 1238-1247 [PMID: 26820618 DOI: 10.1111/ajt.13588]

69 **DeCampli WM**. Of mice and men ... does exercise decrease progression of transplant coronary vasculopathy? *J Thorac Cardiovasc Surg* 2015; **149**: 337-339 [PMID: 25524688 DOI: 10.1016/j.jtcvs.2014.10.107]

70 **Madssen E**, Moholdt T, Videm V, Wisløff U, Hegbom K, Wiseth R. Coronary atheroma regression and plaque characteristics assessed by grayscale and radiofrequency intravascular ultrasound after aerobic exercise. *Am J Cardiol* 2014; **114**: 1504-1511 [PMID: 25248813 DOI: 10.1016/j.amjcard.2014.08.012]

71 **Lavie CJ**, Arena R, Swift DL, Johannsen NM, Sui X, Lee DC, Earnest CP, Church TS, O'Keefe JH, Milani RV, Blair SN. Exercise and the cardiovascular system: clinical science and cardiovascular outcomes. *Circ Res* 2015; **117**: 207-219 [PMID: 26139859 DOI: 10.1161/circresaha.117.305205]

72 **Lavie CJ**, Church TS, Milani RV, Earnest CP. Impact of physical activity, cardiorespiratory fitness, and exercise training on markers of inflammation. *J Cardiopulm Rehabil Prev* 2011; **31**: 137-145 [PMID: 21427600 DOI: 10.1097/HCR.0b013e3182122827]

73 **Cornish AK**, Broadbent S, Cheema BS. Interval training for patients with coronary artery disease: a systematic review. *Eur J Appl Physiol* 2011; **111**: 579-589 [PMID: 20972578 DOI: 10.1007/s00421-010-1682-5]

74 **Arora S**, Erikstad I, Ueland T, Sigurdardottir V, Ekmehag B, Jansson K, Eiskjaer H, Bøtker HE, Mortensen SA, Saunamaki K, Gude E, Ragnarsson A, Solbu D, Aukrust P, Gullestad L. Virtual histology assessment of cardiac allograft vasculopathy following introduction of everolimus--results of a multicenter trial. *Am J Transplant* 2012; **12**: 2700-2709 [PMID: 22958738 DOI: 10.1111/j.1600-6143.2012.04234.x]

75 **Grady KL**, Naftel DC, Kobashigawa J, Chait J, Young JB, Pelegrin D, Czerr J, Heroux A, Higgins R, Rybarczyk B, McLeod M, White-Williams C, Kirklin JK. Patterns and predictors of

quality of life at 5 to 10 years after heart transplantation. *J Heart Lung Transplant* 2007; **26**: 535-543 [PMID: 17449426 DOI: 10.1016/j.healun.2007.01.042]

76 **Karam VH**, Gasquet I, Delvart V, Hiesse C, Dorent R, Danet C, Samuel D, Charpentier B, Gandjbakhch I, Bismuth H, Castaing D. Quality of life in adult survivors beyond 10 years after liver, kidney, and heart transplantation. *Transplantation* 2003; **76**: 1699-1704 [PMID: 14688519 DOI: 10.1097/01.tp.0000092955.28529.1e]

77 **Wu YT**, Chien CL, Chou NK, Wang SS, Lai JS, Wu YW. Efficacy of a home-based exercise program for orthotopic heart transplant recipients. *Cardiology* 2008; **111**: 87-93 [PMID: 18376119 DOI: 10.1159/000119695]

78 **Karapolat H**, Eyigor S, Durmaz B, Nalbantgil S, Yagdi T, Zoghi M. The effect of functional performance, respiratory function and osteopenia on the quality of life after heart transplantation. *Int J Cardiol* 2008; **124**: 381-383 [PMID: 17395310 DOI: 10.1016/j.ijcard.2006.12.053]

79 **Christensen SB**, Dall CH, Prescott E, Pedersen SS, Gustafsson F. A high-intensity exercise program improves exercise capacity, self-perceived health, anxiety and depression in heart transplant recipients: a randomized, controlled trial. *J Heart Lung Transplant* 2012; **31**: 106-107 [PMID: 22153554 DOI: 10.1016/j.healun.2011.10.014]

80 **Dew MA**, DiMartini AF. Psychological disorders and distress after adult cardiothoracic transplantation. *J Cardiovasc Nurs* 2005; **20**: S51-S66 [PMID: 16160585 DOI: 10.1097/00005082-200509001-00007]

81 **Sivertsen B**, Relbo A, Gullestad L, Hellesvik M, Grov I, Andreassen A, Simonsen S, Geiran O, Havik OE. [Self-assessed health and psychological symptoms after heart transplantation]. *Tidsskr Nor Laegeforen* 2007; **127**: 3198-3201 [PMID: 18084359]

82 **Dew MA**, Rosenberger EM, Myaskovsky L, DiMartini AF, DeVito Dabbs AJ, Posluszny DM, Steel J, Switzer GE, Shellmer DA, Greenhouse JB. Depression and Anxiety as Risk Factors for Morbidity and Mortality After Organ Transplantation: A Systematic Review and Meta-Analysis. *Transplantation* 2015; **100**: 988-1003 [PMID: 26492128 DOI: 10.1097/tp.0000000000000901]

83 **Karapolat H**, Eyigor S, Durmaz B, Yagdi T, Nalbantgil S, Karakula S. The relationship between depressive symptoms and anxiety and quality of life and functional capacity in heart transplant patients. *Clin Res Cardiol* 2007; **96**: 593-599 [PMID: 17593317 DOI: 10.1007/s00392-007-0536-6]

84 **Ulubay G**, Ulasli SS, Sezgin A, Haberal M. Assessing exercise performance after heart transplantation. *Clin Transplant* 2007; **21**: 398-404 [PMID: 17488391 DOI: 10.1111/j.1399-0012.2007.00658.x]

85 **Bernardi L**, Radaelli A, Passino C, Falcone C, Auguadro C, Martinelli L, Rinaldi M, Viganò M, Finardi G. Effects of physical training on cardiovascular control after heart transplantation. *Int J Cardiol* 2007; **118**: 356-362 [PMID: 17050012 DOI: 10.1016/j.ijcard.2006.07.032]

86 **Carter R**, Al-Rawas OA, Stevenson A, Mcdonagh T, Stevenson RD. Exercise responses following heart transplantation: 5 year follow-up. *Scott Med J* 2006; **51**: 6-14 [PMID: 16910044 DOI: 10.1258/RSMSMJ.51.3.6]

87 **Ewert R**, Wensel R, Bruch L, Mutze S, Bauer U, Plauth M, Kleber FX. Relationship between impaired pulmonary diffusion and cardiopulmonary exercise capacity after heart transplantation. *Chest* 2000; **117**: 968-975 [PMID: 10767226 DOI: 10.1378/chest.117.4.968]

88 **Givertz MM**, Hartley LH, Colucci WS. Long-term sequential changes in exercise capacity and chronotropic responsiveness after cardiac transplantation. *Circulation* 1997; **96**: 232-237 [PMID: 9236439 DOI: 10.1161/01.CIR.96.1.232]

89 **Gullestad L**, Myers J, Edvardsen T, Kjekshus J, Geiran O, Simonsen S. Predictors of exercise capacity and the impact of angiographic coronary artery disease in heart transplant recipients. *Am Heart J* 2004; **147**: 49-54 [PMID: 14691418 DOI: 10.1016/j.ahj.2003.07.013]

90 **Habedank D**, Ewert R, Hummel M, Wensel R, Hetzer R, Anker SD. Changes in exercise capacity, ventilation, and body weight following heart transplantation. *Eur J Heart Fail* 2007; **9**: 310-316 [PMID: 17023206 DOI: 10.1016/j.ejheart.2006.07.001]

91 **Haykowsky M**, Taylor D, Kim D, Tymchak W. Exercise training improves aerobic capacity and skeletal muscle function in heart transplant recipients. *Am J Transplant* 2009; **9**: 734-739 [PMID: 19344465 DOI: 10.1111/j.1600-6143.2008.02531.x]

92 **Hognestad A**, Holm T, Simonsen S, Kjekshus J, Andreassen AK. Serial measurements of peripheral vascular reactivity and exercise capacity in congestive heart failure and after heart transplantation. *J Card Fail* 2005; **11**: 447-454 [PMID: 16105636 DOI: 10.1016/j.cardfail.2005.01.008]

93 **Karapolat H**, Eyigor S, Zoghi M, Yagdi T, Nalbantgil S, Durmaz B, Ozbaran M. Effects of cardiac rehabilitation program on exercise capacity and chronotropic variables in patients with orthotopic heart transplant. *Clin Res Cardiol* 2008; **97**: 449-456 [PMID: 18317667 DOI: 10.1007/s00392-008-0648-7]

94 **Kavanagh T**, Mertens DJ, Shephard RJ, Beyene J, Kennedy J, Campbell R, Sawyer P, Yacoub M. Long-term cardiorespiratory results of exercise training following cardiac transplantation. *Am J Cardiol* 2003; **91**: 190-194 [PMID: 12521633 DOI: 10.1016/S0002-9149(02)03108-9]

95 **Kemp DL**, Jennison SH, Stelken AM, Younis LT, Miller LW. Association of resting heart rate and chronotropic response. *Am J Cardiol* 1995; **75**: 751-752 [PMID: 7900681 DOI: 10.1016/S0002-9149(99)80674-2]

96 **Kobashigawa JA**, Leaf DA, Lee N, Gleeson MP, Liu H, Hamilton MA, Moriguchi JD, Kawata N, Einhorn K, Herlihy E, Laks H. A controlled trial of exercise rehabilitation after heart transplantation. *N Engl J Med* 1999; **340**: 272-277 [PMID: 9920951 DOI: 10.1056/NEJM199901283400404]

97 **Renlund DG**, Taylor DO, Ensley RD, O'Connell JB, Gilbert EM, Bristow MR, Ma H, Yanowitz FG. Exercise capacity after heart transplantation: influence of donor and recipient characteristics. *J Heart Lung Transplant* 1996; **15**: 16-24 [PMID: 8820079]

98 **Schwaiblmair M**, von Scheidt W, Uberfuhr P, Reichart B, Vogelmeier C. Lung function and cardiopulmonary exercise performance after heart transplantation: influence of cardiac allograft vasculopathy. *Chest* 1999; **116**: 332-339 [PMID: 10453859 DOI: 10.1378/chest.116.2.332]

99 **Squires RW**. Exercise therapy for cardiac transplant recipients. *Prog Cardiovasc Dis* 2011; **53**: 429-436 [PMID: 21545929 DOI: 10.1016/j.pcad.2011.03.010]

100 **Tegtbur U**, Busse MW, Jung K, Pethig K, Haverich A. Time course of physical reconditioning during exercise rehabilitation late after heart transplantation. *J Heart Lung Transplant* 2005; **24**: 270-274 [PMID: 15737752 DOI: 10.1016/j.healun.2003.12.010]

P- Reviewer: Cheungpasitporn W, Kita K, Therapondos G
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Li D

World Journal of Transplantation

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

World J Transplant 2018 February 24; 8(1): 13-22

DOI: 10.5500/wjt.v8.i1.13

ISSN 2220-3230 (online)

ORIGINAL ARTICLE

Clinical Trials Study

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

Giulio Sergio Roi, Giovanni Mosconi, Valentina Totti, Maria Laura Angelini, Erica Brugin, Patrizio Sarto, Laura Merlo, Sergio Sgarzi, Michele Stancari, Paola Todeschini, Gaetano La Manna, Andrea Ermolao, Ferdinando Tripi, Lucia Andreoli, Gianluigi Sella, Alberto Anedda, Laura Stefani, Giorgio Galanti, Rocco Di Michele, Franco Merni, Manuela Trerotola, Daniela Storani, Alessandro Nanni Costa

Giulio Sergio Roi, Department of Education and Research, Isokinetic Medical Group, Bologna 40123, Italy

Giovanni Mosconi, Maria Laura Angelini, Department of Nephrology and Dialysis, Morgagni-Pierantoni Hospital, Forlì 47121, Italy

Valentina Totti, Rocco Di Michele, Franco Merni, Department of Biomedical & Neuromotor Sciences, University of Bologna, Bologna 40121, Italy

Valentina Totti, Associazione Nazionale Emodializzati, Dialisi e Trapianto, Milano 20121, Italy

Erica Brugin, Department of Cardiovascular, Sports Medicine, Noale 30033, Italy

Patrizio Sarto, Laura Merlo, Sports Medicine, ULSS Company 9, Treviso 31100, Italy

Sergio Sgarzi, Michele Stancari, Sports Medicine, Regional Hospital of Bologna, Bologna 40121, Italy

Paola Todeschini, Gaetano La Manna, Department of Nephrology and Dialysis, S. Orsola Hospital, Bologna 40121, Italy

Andrea Ermolao, Sports Medicine Unit DIMED, Department of Medicine, University of Padua, Padua 35100, Italy

Ferdinando Tripi, Lucia Andreoli, Sports Medicine, Regional Hospital of Modena, Modena 41100, Italy

Gianluigi Sella, Sports Medicine, Regional Hospital of Ravenna, Ravenna 48121, Italy

Alberto Anedda, Sports Medicine, Regional Hospital of Parma, Parma 43121, Italy

Laura Stefani, Giorgio Galanti, Department of Experimental and Clinical Medicine, School of Sports Medicine, University of Florence, Sports Medicine Centre, Florence 50100, Italy

Manuela Trerotola, Daniela Storani, Alessandro Nanni Costa, Italian National Transplant Centre, Rome 00161, Italy

ORCID number: Giulio Sergio Roi (0000-0002-0738-4460); Giovanni Mosconi (0000-0002-1027-1619); Valentina Totti (0000-0003-0646-778X); Maria Laura Angelini (0000-0002-5606-7218); Erica Brugin (0000-0001-8377-9467); Paola Todeschini (0000-0003-0528-4527); Gaetano La Manna (0000-0001-5473-8551); Andrea Ermolao (0000-0002-0546-1514); Alberto Anedda (0000-0002-1235-0716); Laura Stefani (0000-0002-2981-0424); Giorgio Galanti (0000-0002-2054-8650); Rocco Di Michele (0000-0003-2771-7661); Franco Merni (0000-0002-6012-4596); Alessandro Nanni Costa (0000-0002-9350-9521).

Author contributions: Roi GS and Mosconi G have participated in research design, drafting the paper, performance of the research and critical revision of the paper; Totti V and Angelini ML has participated in drafting the paper, performance of the research and critical revision of the paper; Brugin E, Sarto P, Merlo L, Sgarzi S, Stancari M, Todeschini P, La Manna G, Ermolao A, Capone S, Tripi F, Andreoli L, Sella G, Anedda A, Stefani L, Galanti G and Trerotola M have participated in the performance of the research and data collection; Di Michele R and Merni F have participated in drafting the paper, data analysis and statistics; Nanni Costa A has participated in research design and in the performance of the research.

Institutional review board statement: 118/2010/O/Sper approved 20-07-2010.

Clinical trial registration statement: This study is registered at http://www.isrctn.com/ISRCTN66295470. The registration identification number is ISRCTN66295470.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: Dr. Nanni Costa has nothing to disclose.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited Manuscript

Correspondence to: Alessandro Nanni Costa, MD, Doctor, Italian National Transplant Centre, Istituto Superiore di Sanità, Viale Regina Elena 299, Roma 00161, Italy. daniela.storani@iss.it
Telephone: +39-06-49904040
Fax: +39-06-49904101

Received: October 31, 2017
Peer-review started: November 1, 2017
First decision: November 30, 2017
Revised: December 6, 2017
Accepted: December 28, 2017
Article in press: December 28, 2017
Published online: February 24, 2018

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).

ACKNOWLEDGMENTS

The authors gratefully acknowledge Paola Di Ciaccio, Claudia Carella, Claudia Ferraro and Carlo Donati for their cooperation in the project.

REFERENCES

1 **Varghese T**, Schultz WM, McCue AA, Lambert CT, Sandesara PB, Eapen DJ, Gordon NF, Franklin BA, Sperling LS. Physical activity in the prevention of coronary heart disease: implications for the clinician. *Heart* 2016; **102**: 904-909 [PMID: 26941396 DOI: 10.1136/heartjnl-2015-308773]

2 **Samitz G**, Egger M, Zwahlen M. Domains of physical activity and all-cause mortality: systematic review and dose-response meta-analysis of cohort studies. *Int J Epidemiol* 2011; **40**: 1382-1400 [PMID: 22039197 DOI: 10.1093/ije/dyr112]

3 **Kodama S**, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, Sugawara A, Totsuka K, Shimano H, Ohashi Y, Yamada N, Sone H. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *JAMA* 2009; **301**: 2024-2035 [PMID: 19454641 DOI: 10.1001/jama.2009.681]

4 **Riess KJ**, Haykowsky M, Lawrance R, Tomczak CR, Welsh R, Lewanczuk R, Tymchak W, Haennel RG, Gourishankar S. Exercise training improves aerobic capacity, muscle strength, and quality of life in renal transplant recipients. *Appl Physiol Nutr Metab* 2014; **39**: 566-571 [PMID: 24766239 DOI: 10.1139/apnm-2013-0449]

5 **Kohl HW**, Craig CL, Lambert EV, Inoue S, Alkandari JR, Leetongin G, Kahlmeier S; Lancet Physical Activity Series Working Group. The pandemic of physical inactivity: global action for public health. *Lancet* 2012; **380**: 294-305 [PMID: 22818941 DOI: 10.1016/S0140-6736(12)60898-8]

6 **Painter PL**, Hector L, Ray K, Lynes L, Dibble S, Paul SM, Tomlanovich SL, Ascher NL. A randomized trial of exercise training after renal transplantation. *Transplantation* 2002; **74**: 42-48 [PMID: 12134097]

7 **Painter P**. Exercise following organ transplantation: A critical part of the routine post transplant care. *Ann Transplant* 2005; **10**: 28-30 [PMID: 17037085]

8 **Roi GS**, Mosconi G, Capelli I, Cuna V, Persici E, Parigino M, Pisoni D, Todeschini P, Costa AN, Stefoni S. Alpine skiing and anaerobic performance in solid organ transplant recipients. *Transplant Proc* 2010; **42**: 1029-1031 [PMID: 20534216 DOI: 10.1016/j.transproceed.2010.03.094]

9 **Clunk JM**, Lin CY, Curtis JJ. Variables affecting weight gain in renal transplant recipients. *Am J Kidney Dis* 2001; **38**: 349-353 [PMID: 11479161 DOI: 10.1053/ajkd.2001.26100]

10 **McKenzie KJ**, McKenzie DC, Yoshida EM. Solid organ transplant recipients: clinical considerations in the application of exercise. *Br J Sports Med* 2015; **49**: 76-78 [PMID: 25115810 DOI: 10.1136/bjsports-2014-093583]

11 **O'Brien T**, Hathaway D. An Integrative Literature Review of Physical Activity Recommendations for Adult Renal Transplant Recipients. *Prog Transplant* 2016; **26**: 381-385 [PMID: 27555071 DOI: 10.1177/1526924816664079]

12 **Mosconi G**, Cuna V, Tonioli M, Totti V, Roi GS, Sarto P, Stefoni S, Trerotola M, Costa AN. Physical activity in solid organ transplant recipients: preliminary results of the Italian project. *Kidney Blood Press Res* 2014; **39**: 220-227 [PMID: 25118112 DOI: 10.1159/000355800]

13 **Roi GS**, Stefoni S, Mosconi G, Brugin E, Burra P, Ermolao A, Granito M, Macini P, Mastrosimone S, Nacchia F, Pegoraro C, Rigotti P, Sella G, Sgarzi S, Tamè MR, Totti V, Trerotola M, Tripi F, Nanni Costa A. Physical activity in solid organ transplant recipients: organizational aspects and preliminary results of the Italian project. *Transplant Proc* 2014; **46**: 2345-2349 [PMID: 25242784 DOI: 10.1016/j.transproceed.2014.07.055]

14 **Kindermann W**, Simon G, Keul J. The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. *Eur J Appl Physiol Occup Physiol* 1979; **42**: 25-34 [PMID: 499194]

15 **American College of Sports Medicine**. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc* 2009; **41**: 687-708 [PMID: 19204579 DOI: 10.1249/MSS.0b013e3181915670]

16 **American College of Sports Medicine**, Chodzko-Zajko WJ, Proctor DN, Fiatarone Singh MA, Minson CT, Nigg CR, Salem GJ, Skinner JS. American College of Sports Medicine position stand. Exercise and physical activity for older adults. *Med Sci Sports Exerc* 2009; **41**: 1510-1530 [PMID: 19516148 DOI: 10.1249/MSS.0b013e3181a0c95c]

17 **Craig CL**, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003; **35**: 1381-1395 [PMID: 12900694 DOI: 10.1249/01.MSS.0000078924.61453.FB]

18 **Taylor JD**, Fletcher JP. Correlation between the 8-repetition maximum test and isokinetic dynamometry in the measurement of muscle strength of the knee extensors: A concurrent validity study. *Physiother Theory Pract* 2013; **29**: 335-341 [PMID: 23035768 DOI: 10.3109/09593985.2012.727529]

19 **Jackson AS**, Pollock ML. Generalized equations for predicting body density of men. *Br J Nutr* 1978; **40**: 497-504 [PMID: 718832]

20 **Ware JE**, Kosinski M. Interpreting SF-36 summary health measures: a response. *Qual Life Res* 2001; **10**: 405-413; discussion 415-420 [PMID: 11763203]

21 **van Adrichem EJ**, van de Zande SC, Dekker R, Verschuuren EA, Dijkstra PU, van der Schans CP. Perceived Barriers to and Facilitators of Physical Activity in Recipients of Solid Organ Transplantation, a Qualitative Study. *PLoS One* 2016; **11**: e0162725 [PMID: 27622291 DOI: 10.1371/journal.pone.0162725]

22 **Greenwood SA**, Koufaki P, Mercer TH, Rush R, O'Connor E, Tuffnell R, Lindup H, Haggis L, Dew T, Abdulnassir L, Nugent E, Goldsmith D, Macdougall IC. Aerobic or Resistance Training and Pulse Wave Velocity in Kidney Transplant Recipients: A 12-Week Pilot Randomized Controlled Trial (the Exercise in Renal Transplant [ExeRT] Trial). *Am J Kidney Dis* 2015; **66**: 689-698 [PMID: 26209542 DOI: 10.1053/j.ajkd.2015.06.016]

23 **O'Connor EM**, Koufaki P, Mercer TH, Lindup H, Nugent E, Goldsmith D, Macdougall IC, Greenwood SA. Long-term pulse wave velocity outcomes with aerobic and resistance training in kidney transplant recipients - A pilot randomised controlled trial. *PLoS One* 2017; **12**: e0171063 [PMID: 28158243 DOI: 10.1371/journal.pone.0171063]

24 **Schoo E**, Gustaw T, Barbalinardo C, Rodrigues N, Zameni Y,

Mathur S, Janaudis-Ferreira T. Solid Organ Transplant Recipients' Opinions of Pre- and Post-Transplant Supervised Exercise Programmes: A Brief Report. *Physiother Can* 2017; **69**: 178-183 [PMID: 28539698 DOI: 10.3138/ptc.2016-18EP]

25 **Rhodes RE**, Warburton DE, Murray H. Characteristics of physical activity guidelines and their effect on adherence: a review of randomized trials. *Sports Med* 2009; **39**: 355-375 [PMID: 19402741]

26 **van den Ham EC**, Kooman JP, Schols AM, Nieman FH, Does JD, Akkermans MA, Janssen PP, Gosker HR, Ward KA, MacDonald JH, Christiaans MH, Leunissen KM, van Hooff JP. The functional, metabolic, and anabolic responses to exercise training in renal transplant and hemodialysis patients. *Transplantation* 2007; **83**: 1059-1068 [PMID: 17452896 DOI: 10.1097/01.tp.0000259552.55689.fd]

27 **Romano G**, Simonella R, Falleti E, Bortolotti N, Deiuri E, Antonutto G, De Vita S, Ferraccioli GF, Montanaro D. Physical training effects in renal transplant recipients. *Clin Transplant* 2010; **24**: 510-514 [PMID: 19788449 DOI: 10.1111/j.1399-0012.2009.01098.x]

28 **Kempeneers G**, Noakes TD, van Zyl-Smit R, Myburgh KH, Lambert M, Adams B, Wiggins T. Skeletal muscle limits the exercise tolerance of renal transplant recipients: effects of a graded exercise training program. *Am J Kidney Dis* 1990; **16**: 57-65 [PMID: 2368706]

29 **Painter PL**, Luetkemeier MJ, Moore GE, Dibble SL, Green GA, Myll JO, Carlson LL. Health-related fitness and quality of life in organ transplant recipients. *Transplantation* 1997; **64**: 1795-1800 [PMID: 9422422]

30 **Kouidi E**, Vergoulas G, Anifanti M, Deligiannis A. A randomized controlled trial of exercise training on cardiovascular and autonomic function among renal transplant recipients. *Nephrol Dial Transplant* 2013; **28**: 1294-1305 [PMID: 23129823 DOI: 10.1093/ndt/gfs455]

31 **Leong DP**, Teo KK, Rangarajan S, Lopez-Jaramillo P, Avezum A Jr, Orlandini A, Seron P, Ahmed SH, Rosengren A, Kelishadi R, Rahman O, Swaminathan S, Iqbal R, Gupta R, Lear SA, Oguz A, Yusoff K, Zatonska K, Chifamba J, Igumbor E, Mohan V, Anjana RM, Gu H, Li W, Yusuf S; Prospective Urban Rural Epidemiology (PURE) Study investigators. Prognostic value of grip strength: findings from the Prospective Urban Rural Epidemiology (PURE) study. *Lancet* 2015; **386**: 266-273 [PMID: 25982160 DOI: 10.1016/S0140-6736(14)62000-6]

32 **Leal VO**, Mafra D, Fouque D, Anjos LA. Use of handgrip strength in the assessment of the muscle function of chronic kidney disease patients on dialysis: a systematic review. *Nephrol Dial Transplant* 2011; **26**: 1354-1360 [PMID: 20709742 DOI: 10.1093/ndt/gfq487]

33 **Lee DC**, Sui X, Artero EG, Lee IM, Church TS, McAuley PA, Stanford FC, Kohl HW 3rd, Blair SN. Long-term effects of changes in cardiorespiratory fitness and body mass index on all-cause and cardiovascular disease mortality in men: the Aerobics Center Longitudinal Study. *Circulation* 2011; **124**: 2483-2490 [PMID: 22144631 DOI: 10.1161/CIRCULATIONAHA.111.038422]

34 **Didsbury M**, McGee RG, Tong A, Craig JC, Chapman JR, Chadban S, Wong G. Exercise training in solid organ transplant recipients: a systematic review and meta-analysis. *Transplantation* 2013; **95**: 679-687 [PMID: 23364480 DOI: 10.1097/TP.0b013e31827a3d3e]

35 **Roi GS**, Parigino M, Pisoni D, Mosconi G, Nanni Costa A, Stefoni S. Energy expenditure during a day of sport competitions in kidney transplant recipients. *Transplantation* 2010; **90**: 1136-1138 [PMID: 21427637 DOI: 10.1097/TP.0b013e3181f8699d]

P- Reviewer: Pham PT, Stavroulopoulos A **S- Editor**: Cui LJ
L- Editor: A **E- Editor**: Li D

World Journal of Transplantation

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

World J Transplant 2018 February 24; 8(1): 23-37

DOI: 10.5500/wjt.v8.i1.23

ISSN 2220-3230 (online)

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

Francisco Boix, Santiago Llorente, Jorge Eguía, Gema Gonzalez-Martinez, Rafael Alfaro, Jose A Galián, Jose A Campillo, María Rosa Moya-Quiles, Alfredo Minguela, Jose A Pons, Manuel Muro

Francisco Boix, Jorge Eguía, Gema Gonzalez-Martinez, Rafael Alfaro, Jose A Galián, Jose A Campillo, María Rosa Moya-Quiles, Alfredo Minguela, Manuel Muro, Department of Immunology, Clinical University Hospital Virgen de la Arrixaca-IMIB, Clinical University Hospital 'Virgen Arrixaca', Murcia 30120, Spain

Santiago Llorente, Department of Nephrology, Clinical University Hospital 'Virgen de la Arrixaca-IMIB', Clinical University Hospital 'Virgen Arrixaca', Murcia 30120, Spain

Jose A Pons, Digestive Medicine Service, Clinical University Hospital 'Virgen de la Arrixaca-IMIB', Clinical University Hospital 'Virgen Arrixaca', Murcia 30120, Spain

ORCID number: Francisco Boix (0000-0002-3926-7920); Santiago Llorente (0000-0002-4933-8243); Jorge Eguía (0000-0002-6702-992X); Gema Gonzalez-Martinez (0000-0002-3369-9028); Rafael Alfaro (0000-0002-8298-8918); Jose A Galián (0000-0002-1415-3767); Jose A Campillo (0000-0002-6541-0700); María Rosa Moya-Quiles (0000-0003-3677-6372); Alfredo Minguela (0000-0003-2472-5893); Jose A Pons (0000-0003-4606-1557); Manuel Muro (0000-0001-9987-0994).

Author contributions: Muro M planned the experiments, overviewed the research project and reviewed the manuscript; Eguía J, Gonzalez-Martinez G, Alfaro R and Galián JA performed the experimental worked supervised by Boix F; Boix F, Campillo JA and Moya-Quiles MR analysed the data; Minguela A reviewed the manuscript; Llorente S and Pons JA provided clinical data and reviewed the manuscript; Boix F and Muro M equally participated in the writing of the manuscript.

Supported by Instituto de Salud Carlos Ⅲ, Spanish Ministry of Economy and Competitiveness, No. PI15/01370; Co-funding of the European Union with European Fund of Regional Development (FEDER) with the principle of "A manner to build Europe".

Institutional review board statement: The study protocol, standard operating procedures and good manufacturing practice protocols used in this research were approved by the institutional ethical committee.

Informed consent statement: Formal informed consent was obtained from both patients and healthy controls.

Conflict-of-interest statement: Authors have no relevant conflicts of interest to disclose.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Francisco Boix, PhD, Doctor, Senior Scientist, Department of Immunology, Clinical University Hospital 'Virgen de la Arrixaca-IMIB', Clinical University Hospital 'Virgen Arrixaca', Murcia 30120, Spain. francisco.boix-giner@nhsbt.nhs.uk
Telephone: +34-968-369599
Fax: +34-968-369678

Received: November 6, 2017
Peer-review started: November 7, 2017
First decision: November 20, 2017
Revised: January 13, 2018
Accepted: February 4, 2018
Article in press: February 5, 2018
Published online: February 24, 2018

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

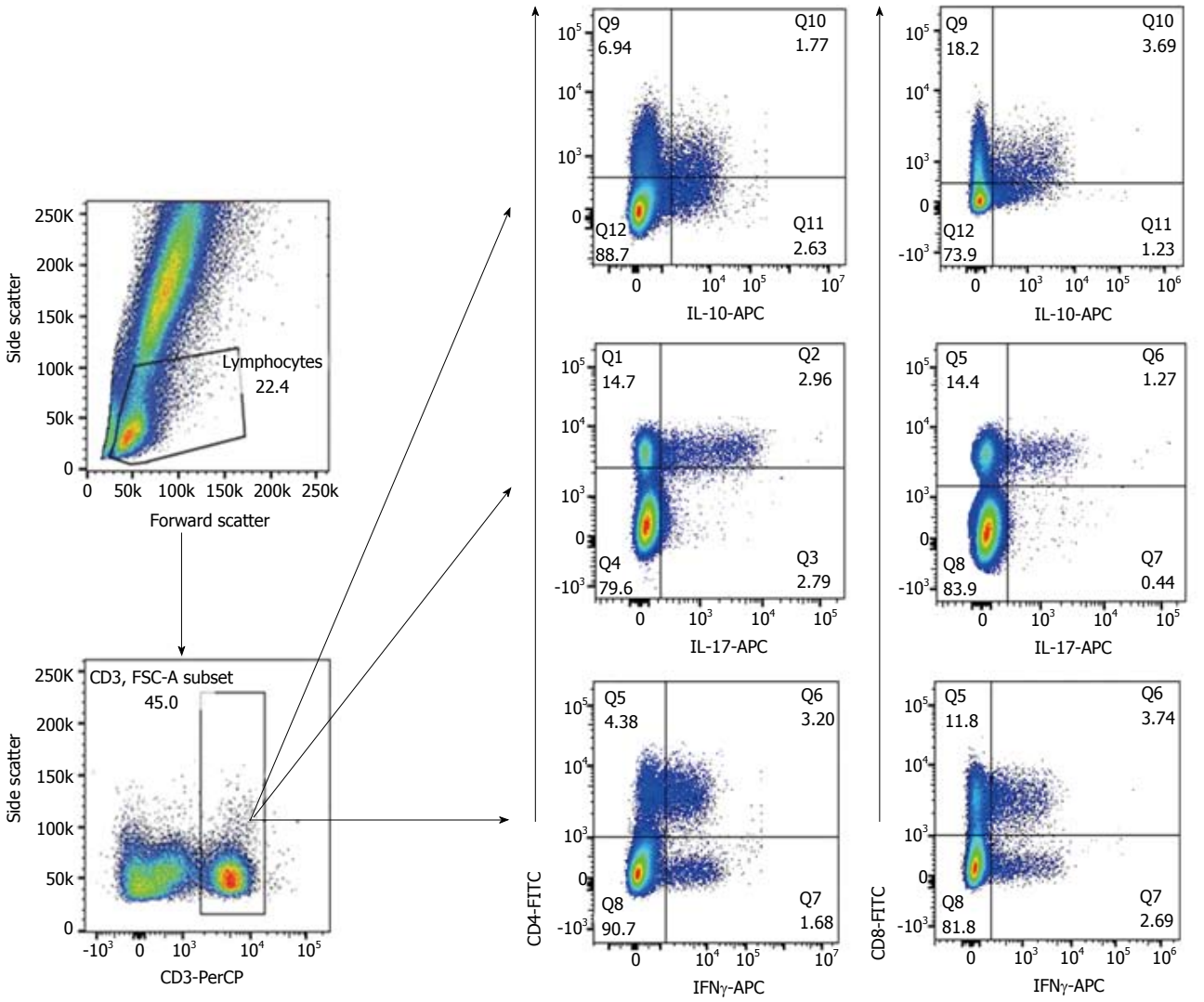

Figure 1 FACS analysis of cultured CD3$^+$ T lymphocyte. Whole blood peripheral CD4$^+$ and CD8$^+$ T lymphocytes from individual ESLF and ESRF patients as well as HC subjects were stain with anti-CD3, anti-CD8, anti-CD69, anti-IL-17, anti-IL-10 and anti-IFNγ monoclonal antibodies following manufacture's 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 TH1, TH2 and TH17 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 TH1, TH2 and TH17 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, TH17 (Figure 3B) and TH2 (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$^+$

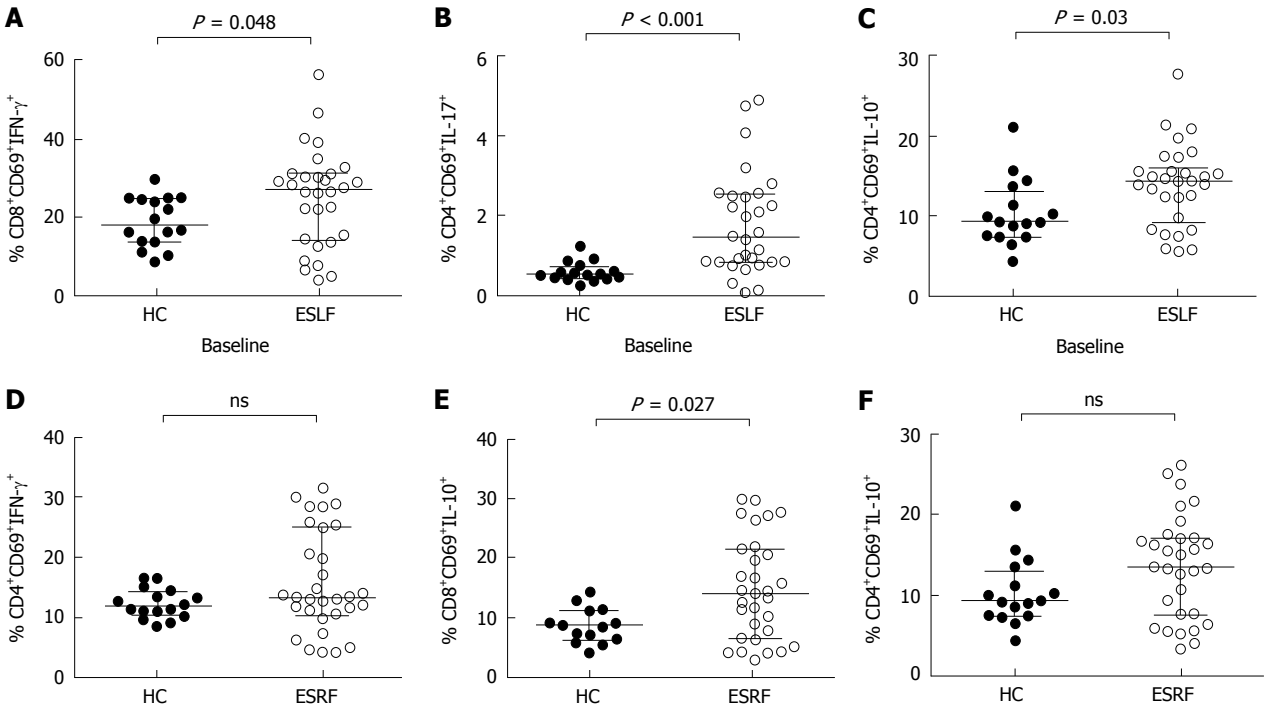

Figure 2 Quantitative analysis of cultured CD4$^+$ and CD8$^+$ T lymphocytes from individual end-stage liver failure, end-stage renal failure and healthy control subjects. A: % CD4$^+$CD69$^+$INFγ$^+$; B: CD4$^+$CD69$^+$IL-17$^+$; C: CD4$^+$CD69$^+$IL-10$^+$ cells in ESLF patients and HC individuals; 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

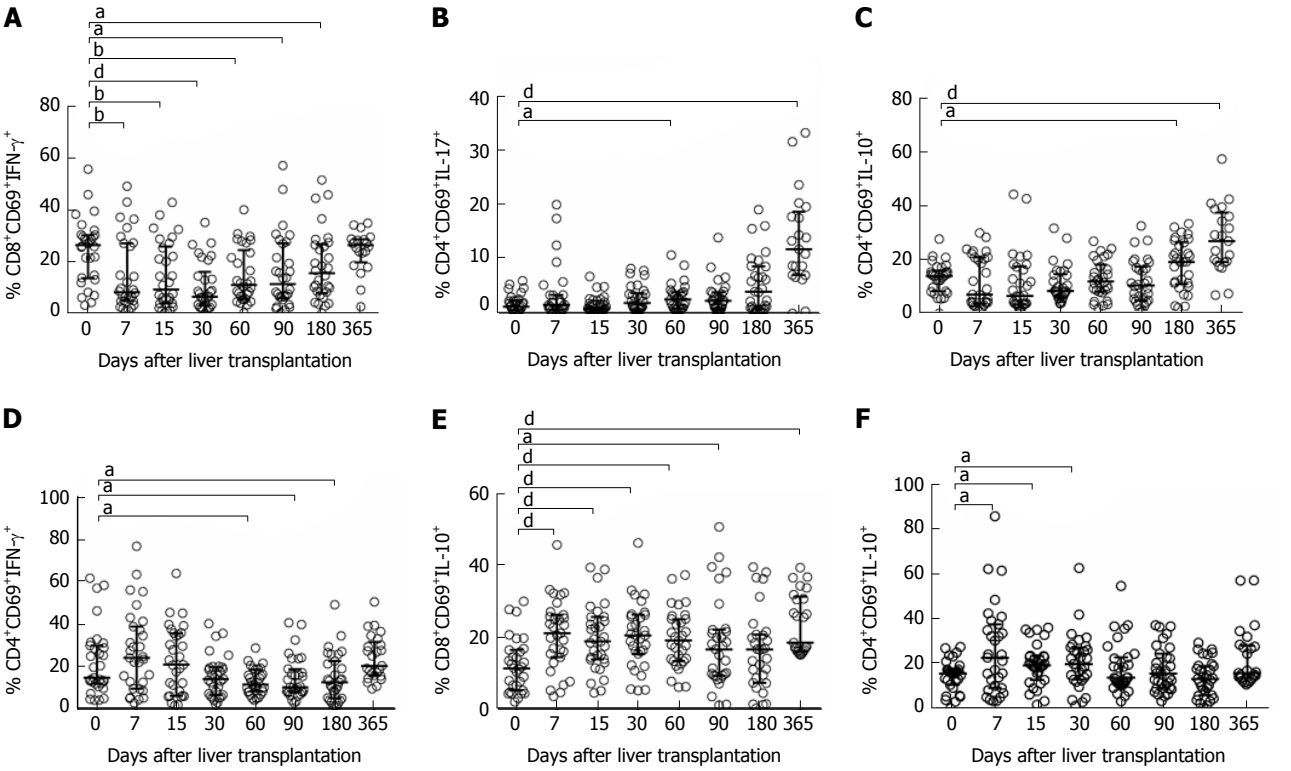

Figure 3 Cytokine-producing CD4$^+$ and CD8$^+$ T cells follow-up along first year after liver and kidney transplantation. A: % of cultured IFNγ-producing CD8$^+$ lymphocytes; B: % of cultured TH17 lymphocytes; C: % of cultured TH2 lymphocytes in LTr; D: % of cultured TH1 lymphocytes; E: % of cultured 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$^+$ $\leqslant$ 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$^+$ $\leqslant$ 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$^+$ $\leqslant$ 14.95% and CD4$^+$CD69$^+$IFNγ$^+$ $\leqslant$ 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 $\geqslant$ 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$^+$ $\geqslant$ 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 $\geqslant$ 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 $\geqslant$ 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 $\geqslant$ 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 $\geqslant$ 9.35% in LTr (Figure 5E; $P = 0.003$, Long Rang test) and $\geqslant$ 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

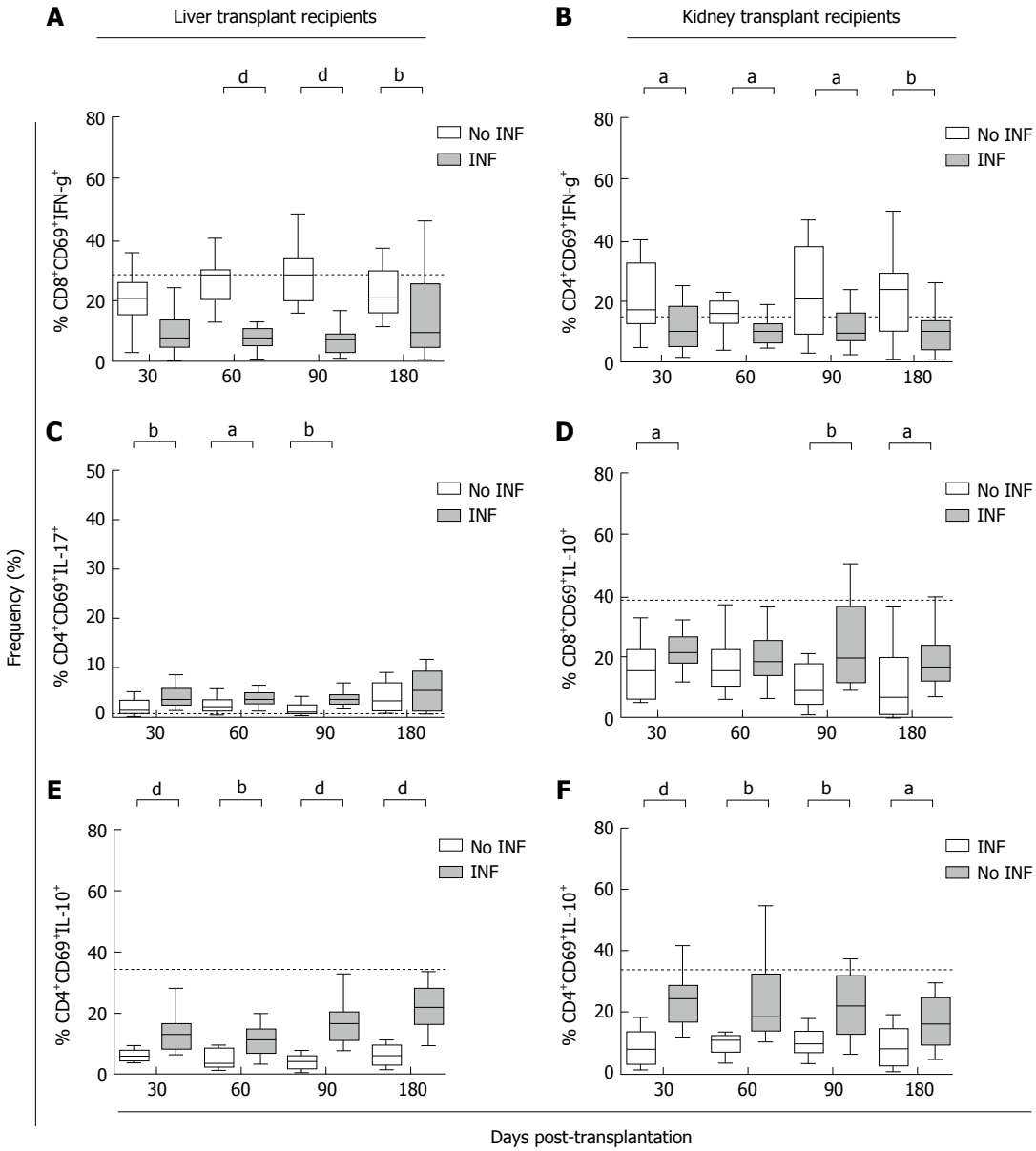

Figure 4 Stratification analysis of the percentage for the intracellular cytokine production capacity of CD4$^+$ and CD8$^+$ T lymphocytes between the 1st and 6th month post-transplantation. A: % of CD8$^+$CD69$^+$IFNγ^+ T lymphocytes in LTr with and without OI; B: % of CD4$^+$CD69$^+$IFNγ^+ T lymphocytes 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. $^aP < 0.05$, $^bP < 0.01$, $^dP < 0.001$.

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 =

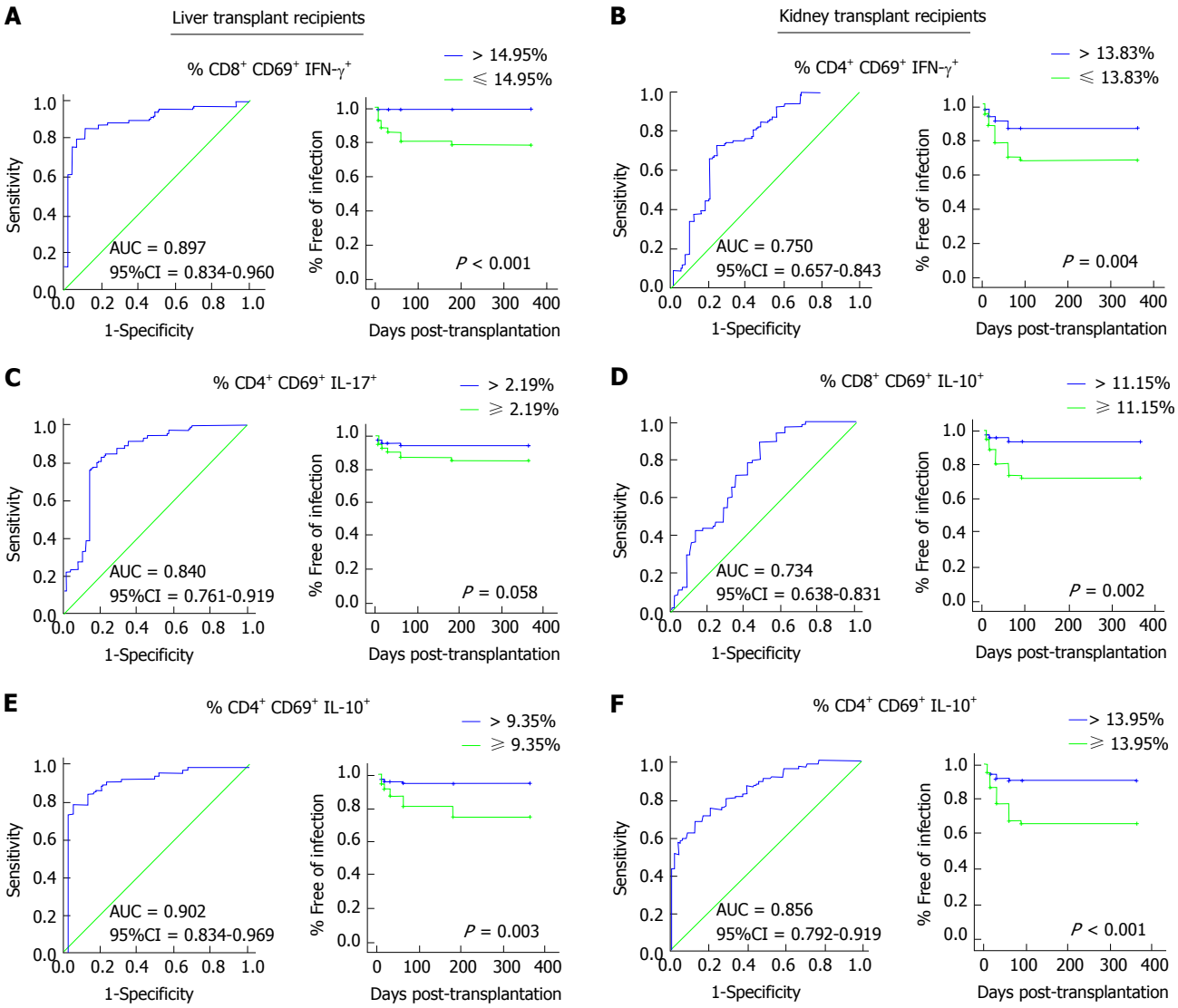

Figure 5 Post-transplantation receiver operating characteristic curve for the intracellular cytokine production capacity and the effect of the % of intracellular cytokine production capacity in stimulated T lymphocytes cut-off values for the discrimination of liver and kidney recipients likely to develop 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.

ACKNOWLEDGMENTS

We, Dr. Francisco Boix and Dr. Manuel Muro want to pay a special mention to Cristabel Trujillo who kindly reviewed this manuscript, which without her contribution this manuscript would have not been possible.

REFERENCES

1 **Fishman JA**. Infection in solid-organ transplant recipients. *N Engl J Med* 2007; **357**: 2601-2614 [PMID: 18094380 DOI: 10.1056/NEJMra064928]

2 **Khurana A,** Brennan DC. Current concepts of immunosuppression and side effects. In: Liapis H, Wang HL, eds. Pathology of Solid Organ Transplantation. Berlin, Germany: Springer-Verlag, 2011: 11–30

3 **Fishman JA**; AST Infectious Diseases Community of Practice. Introduction: infection in solid organ transplant recipients. *Am J Transplant* 2009; **9** Suppl 4: S3-S6 [PMID: 20070692 DOI: 10.1111/j.1600-6143.2009.02887.x]

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

5 **Cooper JE**, Wiseman AC. Novel immunosuppressive agents in kidney transplantation. *Clin Nephrol* 2010; **73**: 333-343 [PMID: 20420793 DOI: 10.5414/CNP73333]

6 **Brunet M**, Shipkova M, van Gelder T, Wieland E, Sommerer C, Budde K, Haufroid V, Christians U, López-Hoyos M, Barten MJ, Bergan S, Picard N, Millán López O, Marquet P, Hesselink DA, Noceti O, Pawinski T, Wallemacq P, Oellerich M. Barcelona Consensus on Biomarker-Based Immunosuppressive Drugs Management in Solid Organ Transplantation. *Ther Drug Monit* 2016; **38** Suppl 1: S1-20 [PMID: 26977997 DOI: 10.1097/FTD.0000000000000287]

7 **Boix F**, Mrowiec A, Muro M. Cytokine Expression Profile as Predictive Surrogate Biomarkers for Clinical Events in the Field of Solid Organ Transplantation. *Curr Protein Pept Sci* 2017; **18**: 240-249 [PMID: 27593089 DOI: 10.2174/1389203717666160902130001]

8 **Boix F**, Bolarín JM, Mrowiec A, Eguía J, Gonzalez-Martinez G, de la Peña J, Galian JA, Alfaro R, Moya-Quiles MR, Legaz I, Campillo JA, Ramírez P, García-Alonso A, Pons JA, Sánchez-Bueno F, Minguela A, Llorente S, Muro M. CD28 biomarker quantification and expression level profiles in CD4+ T-lymphocytes in solid organ transplantation. *Transpl Immunol* 2017; **42**: 9-17 [PMID: 28392336 DOI: 10.1016/j.trim.2017.04.001]

9 **Millán O**, Rafael-Valdivia L, San Segundo D, Boix F, Castro-Panete MJ, López-Hoyos M, Muro M, Valero-Hervás D, Rimola A, Navasa M, Muñoz P, Miras M, Andrés A, Guirado L, Pascual J, Brunet M. Should IFN-γ, IL-17 and IL-2 be considered predictive biomarkers of acute rejection in liver and kidney transplant? Results of a multicentric study. *Clin Immunol* 2014; **154**: 141-154 [PMID: 25088788 DOI: 10.1016/j.clim.2014.07.007]

10 **Boix-Giner F**, Millan O, San Segundo D, Muñoz-Cacho P, Mancebo E, Llorente S, Rafael-Valdivia L, Rimola A, Fábrega E, Mrowiec A, Allende L, Minguela A, Bolarín JM, Paz-Artal E, López-Hoyos M, Brunet M, Muro M. High frequency of central memory regulatory T cells allows detection of liver recipients at risk of early acute rejection within the first month after transplantation. *Int Immunol* 2016; **28**: 55-64 [PMID: 26270267 DOI: 10.1093/intimm/dxv048]

11 **Fernández-Ruiz M**, López-Medrano F, Allende LM, Andrés A, García-Reyne A, Lumbreras C, San-Juan R, Morales JM, Paz-Artal E, Aguado JM. Kinetics of peripheral blood lymphocyte subpopulations predicts the occurrence of opportunistic infection after kidney transplantation. *Transpl Int* 2014; **27**: 674-685 [PMID: 24650360 DOI: 10.1111/tri.12321]

12 **Fernández-Ruiz M**, Parra P, López-Medrano F, Ruiz-Merlo T, González E, Polanco N, Origüen J, San Juan R, Andrés A, Aguado JM. Serum sCD30: A promising biomarker for predicting the risk of bacterial infection after kidney transplantation. *Transpl Infect Dis* 2017; **19**: Epub ahead of print [PMID: 28122147 DOI: 10.1111/tid.12668]

13 **Fernández-Ruiz M**, López-Medrano F, San-Juan R, Aguado JM. Post-transplant hypogammaglobulinemia and risk of infection after kidney transplantation: Magnitude matters. *Transpl Infect Dis* 2017; **19**: Epub ahead of print [PMID: 27792293 DOI: 10.1111/tid.12628]

14 **Fernández-Ruiz M**, Silva JT, López-Medrano F, Allende LM, San Juan R, Cambra F, Justo I, Paz-Artal E, Jiménez C, Aguado JM. Post-transplant monitoring of NK cell counts as a simple approach to predict the occurrence of opportunistic infection in liver transplant recipients. *Transpl Infect Dis* 2016; **18**: 552-565 [PMID: 27260953 DOI: 10.1111/tid.12564]

15 **Sun HY**, Singh N. Opportunistic infection-associated immune reconstitution syndrome in transplant recipients. *Clin Infect Dis* 2011; **53**: 168-176 [PMID: 21690625 DOI: 10.1093/cid/cir276]

16 **Khader SA**, Gaffen SL, Kolls JK. Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. *Mucosal Immunol* 2009; **2**: 403-411 [PMID: 19587639 DOI: 10.1038/mi.2009.100]

17 **Lieberman LA**, Cardillo F, Owyang AM, Rennick DM, Cua DJ, Kastelein RA, Hunter CA. IL-23 provides a limited mechanism of resistance to acute toxoplasmosis in the absence of IL-12. *J Immunol* 2004; **173**: 1887-1893 [PMID: 15265921 DOI: 10.4049/jimmunol.173.3.1887]

18 **van de Veerdonk FL**, Marijnissen RJ, Kullberg BJ, Koenen HJ, Cheng SC, Joosten I, van den Berg WB, Williams DL, van der Meer JW, Joosten LA, Netea MG. The macrophage mannose receptor induces IL-17 in response to Candida albicans. *Cell Host Microbe* 2009; **5**: 329-340 [PMID: 19380112 DOI: 10.1016/j.chom.2009.02.006]

19 **Szabo SJ**, Sullivan BM, Peng SL, Glimcher LH. Molecular mechanisms regulating Th1 immune responses. *Annu Rev Immunol* 2003; **21**: 713-758 [PMID: 12500979 DOI: 10.1146/annurev.immunol.21.120601.140942]

20 **Couper KN**, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol* 2008; **180**: 5771-5777 [PMID: 18424693 DOI: 10.4049/jimmunol.180.9.5771]

21 **Youden WJ**. Index for rating diagnostic tests. *Cancer* 1950; **3**: 32-35 [PMID: 15405679 DOI: 10.1002/1097-0142(1950)3:13.0.CO;2-3]

22 **Hanley JA**, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982; **143**: 29-36 [PMID: 7063747 DOI: 10.1148/radiology.143.1.7063747]

23 **Ma L**, Zhang H, Hu K, Lv G, Fu Y, Ayana DA, Zhao P, Jiang Y. The imbalance between Tregs, Th17 cells and inflammatory cytokines among renal transplant recipients. *BMC Immunol* 2015; **16**: 56 [PMID: 26400627 DOI: 10.1186/s12865-015-0118-8]

24 **Erol A**, Arpali E, Murat Yelken B, Kocak B, Calıskan YK, Nane I, Turkmen A, Savran Oguz F. Evaluation of TH17 and TH1 Immune Response Profile in Patients After Renal Transplant. *Transplant Proc* 2017; **49**: 467-471 [PMID: 28340814 DOI: 10.1016/j.transproceed.2017.01.017]

25 **Loverre A**, Divella C, Castellano G, Tataranni T, Zaza G, Rossini M, Ditonno P, Battaglia M, Palazzo S, Gigante M, Ranieri E, Schena FP, Grandaliano G. T helper 1, 2 and 17 cell subsets in renal transplant patients with delayed graft function. *Transpl Int* 2011; **24**: 233-242 [PMID: 21281362 DOI: 10.1111/j.1432-2277.2010.01157.x]

26 **van de Berg PJ**, Hoevenaars EC, Yong SL, van Donselaar-van der Pant KA, van Tellingen A, Florquin S, van Lier RA, Bemelman FJ, ten Berge IJ. Circulating lymphocyte subsets in different clinical situations after renal transplantation. *Immunology* 2012; **136**: 198-207 [PMID: 22321054 DOI: 10.1111/j.1365-2567.2012.03570.x]

27 **Hernandez Mdel P**, Martin P, Simkins J. Infectious Complications After Liver Transplantation. *Gastroenterol Hepatol (NY)* 2015; **11**: 741-753 [PMID: 27134589]

28 **Jha V**. Post-transplant infections: An ounce of prevention. *Indian J Nephrol* 2010; **20**: 171-178 [PMID: 21206677 DOI: 10.4103/0971-4065.73431]

29 **Antas PR**, Sales JS, Pereira KC, Oliveira EB, Cunha KS, Sarno EN, Sampaio EP. Patterns of intracellular cytokines in CD4 and CD8 T cells from patients with mycobacterial infections. *Braz J Med Biol Res* 2004; **37**: 1119-1129 [PMID: 15273814 DOI: 10.1590/S0100-879X2004000800003]

30 **Kwak DJ**, Augustine NH, Borges WG, Joyner JL, Green WF, Hill

HR. Intracellular and extracellular cytokine production by human mixed mononuclear cells in response to group B streptococci. *Infect Immun* 2000; **68**: 320-327 [PMID: 10603404 DOI: 10.1128/IAI.68.1.320-327.2000]

31 **Dharnidharka VR**, Agodoa LY, Abbott KC. Risk factors for hospitalization for bacterial or viral infection in renal transplant recipients--an analysis of USRDS data. *Am J Transplant* 2007; **7**: 653-661 [PMID: 17250559 DOI: 10.1111/j.1600-6143.2006.01674.x]

32 **van Hoek B**, de Rooij BJ, Verspaget HW. Risk factors for infection after liver transplantation. *Best Pract Res Clin Gastroenterol* 2012; **26**: 61-72 [PMID: 22482526 DOI: 10.1016/j.bpg.2012.01.004]

33 **McClelland EE**, Smith JM. Gender specific differences in the immune response to infection. *Arch Immunol Ther Exp* (Warsz) 2011; **59**: 203-213 [PMID: 21442309 DOI: 10.1007/s00005-011-0124-3]

34 **Demopoulos L**, Polinsky M, Steele G, Mines D, Blum M, Caulfield M, Adamkovic A, Liu Q, Harler MB, Hahn C, Singh A. Reduced risk of cytomegalovirus infection in solid organ transplant recipients treated with sirolimus: a pooled analysis of clinical trials. *Transplant Proc* 2008; **40**: 1407-1410 [PMID: 18589118 DOI: 10.1016/j.transproceed.2008.03.084]

35 **Issa NC**, Fishman JA. Infectious complications of antilymphocyte therapies in solid organ transplantation. *Clin Infect Dis* 2009; **48**: 772-786 [PMID: 19207081 DOI: 10.1086/597089]

P- Reviewer: Cheungpasitporn W, Kute VB, Lu K, Sugawara Y, Taheri S
S- Editor: Wang JL **L- Editor**: A **E- Editor**: Li D

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com