ORIGINAL ARTICLE

Kidney transplant recipients after nonrenal solid organ transplantation show low alloreactivity but an increased risk of infection

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SUMMARY

The number of kidney transplant recipients (KTRs) after nonrenal solid organ transplantation (SOT) has increased to almost 5%. Knowledge on patient and allograft outcomes, infections, and alloreactivity, however, remains scarce. We studied 40 KTRs after nonrenal SOT. Seven hundred and twenty primary KTRs and 119 repeat KTRs were used for comparison. Samples were collected pretransplantation, at +1, +2, and +3 months post-transplantation. Alloreactive and CMV-specific T cells were measured by interferon-γ ELISPOT assay. Patient survival in KTRs after SOT, primary and repeat KTRs was comparable. While death-censored allograft survival was comparable between KTRs after SOT and primary KTRs, KTRs after SOT showed superior 5-year death-censored allograft survival of 92.5% compared to 81.2% in repeat KTRs. Interestingly, KTRs after SOT show less preformed panel-reactive antibodies, frequencies of alloreactive T cells, and acute rejections compared to repeat KTRs. KTRs after SOT, however, show higher incidences of EBV viremia and PTLD, sepsis, and death from sepsis. Impaired CMV-specific cellular immunity was associated with more CMV replication compared to repeat KTRs. Our results suggest comparable patient and allograft outcomes in KTRs after SOT and primary KTRs. The observed low alloreactivity may contribute to excellent allograft outcomes. Caution should be taken in KTRs after SOT regarding infectious complications due to overimmunosuppression.

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Introduction

As outcomes of nonrenal solid organ transplantation (SOT) have significantly improved, mainly due to advances in immunosuppression and perioperative management, the number of such patients subsequently developing end-stage renal disease (ESRD) has dramatically increased [1–6]. During the last two decades, the number of recipients of a previous nonrenal SOT being evaluated and wait-listed for a subsequent kidney transplantation has disproportionately increased to 5% of all wait-listed patients [2,7]. Besides, patients who most likely progress to chronic kidney disease stage 4 or 5 after nonrenal SOT should be commonly listed for combined organ transplantation. The rate of progression to ESRD after nonrenal SOT may be multifactorial and depend on the

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pretransplant stage of chronic kidney disease, type of SOT, perioperative complications, maintenance immunosuppression, and common comorbid conditions associated with chronic kidney disease [4,8–10]. Here, nephrotoxicity of calcineurin inhibitors, histologically characterized by tubular vacuolization, arteriolar hyalinosis, and striped interstitial fibrosis/tubular atrophy has been suggested to be the most important risk factor [11–13].

In the context, that ESRD represents the leading cause of morbidity and mortality among recipients of a nonrenal SOT, and many previous studies showed that KTRs after previous nonrenal SOT show superior patient survival compared to those on dialysis [7,14–17]. However, outcomes following repeat transplantation of any kind have been demonstrated to be inferior to primary transplants due to allogeneic presensitization, impaired infection control, and more severe comormid conditions [18,19].

The impact of a sequential kidney transplantation after previous nonrenal SOT on infectious complications and alloreactivity, that in turn may impact long-term patient and allograft outcomes, however, remains poorly characterized. Therefore, the aims of this study were to assess patient and allograft outcomes among KTRs after nonrenal SOT compared with primary and repeat KTRs. We attempted to address the following questions: (i) What impact does previous nonrenal SOT have on patient and allograft outcomes? (ii) What impact does previous nonrenal SOT have on infectious complications and infection control? (iii) What impact does previous nonrenal SOT have on alloreactivity and risk of acute rejection? Here, we quantified CMV-specific cellular immunity directed to CMV-IE1 and CMV-pp65, and alloreactive T cells.

Materials and methods

Patients

This study was approved by our local ethical review committee in compliance with the declaration of Helsinki and Istanbul (Ethic Committee Charité University Medicine Berlin, Germany, 126/2001, 07/30/2001). Informed consent was obtained from all patients included in our immune monitoring.

We retrospectively examined 879 KTRs between 2004 and 2014. Forty KTRs underwent a previous nonrenal SOT, 15 KTRs (37.5%) previous liver transplantation, 20 KTRs (50%) previous heart transplantation, 4 KTRs (10%) previous lung transplantation, and 1 KTR (2.5%) a combined heart–lung transplantation. Four KTRs underwent repeat previous liver transplantations and 2 KTRs repeat previous heart transplantations. Only KTRs of a primary kidney allograft after nonrenal SOT were included. For comparison, a control group of 119 repeat KTRs and a control group of 720 primary KTRs were used. Four KTRs who received a primary and repeat kidney transplantation during the study period were included in the primary KTR group only.

Recipients of an ABO-incompatible transplant were excluded. Estimated glomerular filtration rate (eGFR) was calculated by the abbreviated MDRD equation.

Immunosuppressive therapy

Primary immunosuppression in all KTRs was a triple-drug regimen with calcineurin inhibitor, mycophenolate mofetil (MMF), and steroids tapered to a maintenance dose of 4 mg methylprednisolone after 4 weeks. All patients received induction with IL-2R antagonist, except presensitized KTRs. Presensitized KTRs were characterized as KTRs (i) undergoing repeat kidney transplantation plus (ii) having panel-reactive antibodies (PRA) >10%. Those KTRs received a lymphocyte-depleting agent (OKT 3, antithymocyte globulin) for induction. KTRs after nonrenal SOT who received a kidney allograft from a deceased donor were switched from cyclosporine to tacrolimus, whereas KTRs after nonrenal SOT who received a kidney from a living donor were kept on cyclosporine. All presensitized KTRs after nonrenal SOT were switched from cyclosporine to tacrolimus.

If acute rejection was suspected, a kidney biopsy was performed and the rejection classified according to the Banff classification [20]. Rejections were treated with intravenous steroid for three to 5 days. Kidney biopsies grade Banff IIA or higher were treated with a lymphocyte-depleting agent.

Infection monitoring and prophylaxis

Screening for BKV, EBV, and CMV load was performed pretransplantation, bimonthly until +6 months, then 3-monthly until +12 months, and yearly thereafter. All patients with a high-risk CMV constellation (D+R−) received a prophylaxis with valganciclovir for 3 months. Oral prophylaxis for pneumocystis jirovecii pneumonia with trimethoprim/sulfamethoxazole was administered for 6 months.

Collection of samples for immune monitoring

Five hundred and nine KTRs between 2007 and 2014 were enrolled in our immune monitoring. Twenty KTRs

after nonrenal SOT, 53 repeat KTRs, and 436 primary KTRs were successfully enrolled. Blood samples were collected at the following time points: pretransplantation, at +1, +2, and +3 months. Peripheral blood mononuclear cells were isolated from 10 to 20 ml of heparinized blood using standard Ficoll–Hypaque density gradient technique.

Isolation of mononuclear cells (splenocytes) from donor spleen

In case of deceased donation, stimulator cells for ELISPOT assay are prepared from donor spleen [21]. Five-millimeter spleen pieces are gently pressed through a sterile 100- and 40-μm cell strainer sieve and diluted with sterile phosphate-buffered saline (PBS). Splenocytes were isolated using standard Ficoll–Hypaque density gradient technique. A total of 7.5×10^7 splenocytes are used for CD2 depletion (EasySep Human CD2 selection kit Stemcell, Cat. No. 18657) using 112.5 μl EasySep-positive selection mix incubated for 15 min at room temperature. After resuspension 56.3 μl magnetic particle is added to the cell suspension and incubated for 10 min at room temperature. Depletion buffer is added to a final volume of 2.5 ml and transferred into the EasySep magnet. After cell separation, donor splenocytes are resuspended in complete media, cryopreserved with dimethyl sulfoxide (DMSO).

ELISPOT assay for IFNg detection of CMV-specific and alloreactive T cells

Cytomegalovirus (CMV)-specific and alloreactive T-cells were determined by measuring IFNg upon stimulation of PBMC [22,23]. PBMC were isolated from 30 to 40 ml of citrate blood using standard Ficoll–Hypaque density gradient technique. For ELISPOT assay, 96-well multiscreen filter plates (Millipore (Billerica, MA, USA), MAIPS 4510) were coated with 100 μl of primary IFNG monoclonal antibody at a concentration of 3 μg/ml (ahu-IFNG-Endogen M700A) and incubated overnight at 4 °C. A standardized responder T-cell number of 3.0×10^5 PBMC per well were added in duplicate wells with peptides (1 μg/ml), 3.0×10^5 T-cell-depleted donor splenocytes or PBMC, and with Staphylococcus enterotoxin B (SEB; SIGMA (St. Louis, MO, USA), 1 μg/ml) as positive control and incubated for 24 h at 37 °C. Negative controls were run in parallel using responder cells plus medium and DMSO. Plates were incubated overnight at 4 °C with 100 μl (1 μl/ml) biotinylated detection IFNG antibody (ahu-IFNG biotin-Endogen M701). After adding streptavidin (1μg/ml) for 2 h at room temperature, spots were developed by adding 200 μl visualization solution, AEC (3-amino-9-ethylcarbazole; SIGMA) in acetate buffer supplemented with H_2O_2 30% for 3–5 min. Resulting spots were counted using a computer-assisted ELISPOT reader (Immunospot; Cellular Technologies Ltd., Cleveland, OH, USA). Positive ELISPOT signals were predefined as containing 25 spot-forming units per well.

Statistical methods

Statistical tests were performed using SPSS version 22 (SPSS, Chicago, IL, USA). For comparisons of study groups, two-sided Mann–Whitney *U*-test for nonparametric independent samples was used. For comparisons between paired samples two-sided Wilcoxon signed-rank test for nonparametric dependent samples were used. Outcomes were measured with Kaplan–Meier models and overall strata comparisons measured by log-rank tests. Clinical characteristics were compared across groups using Fisher's exact test for categorical variables. Box plots show median, interquartile range, and 90th percentile. Two-sided *P*-values less than 0.05 were considered statistical significant with Bonferroni adjustment to less than 0.017 for pairwise comparisons.

Results

Clinical characteristics

Forty KTRs after nonrenal SOT were analyzed. Median follow-up after kidney transplantation was 66 months (range 0–142 months), during which 10 patients died (25.0%), and three returned to dialysis (7.5%). Four of 40 KTRs (10.0%) had ESRD at the time of SOT. Two of 40 KTRs (5.0%) developed ESRD in the perioperative period of nonrenal SOT. KTRs after liver transplantation were older at the time of SOT, older at the time of kidney transplantation, and more likely to receive a deceased kidney donation compared to KTRs after heart/lung transplantation ($P = 0.036$; $P = 0.023$; $P = 0.040$). Patient characteristics by type of nonrenal SOT are shown in Table 1.

The control group consisted of 119 repeat KTRs with a median follow-up of 64 months (range 0–143). Eighteen patients died (15.1%) and 20 returned to dialysis (16.9%). In addition, 720 primary KTRs with a median follow-up of 69 months (range 0–143). Ninety patients died (13.2%) and 66 returned to dialysis (9.2%). KTRs

Transplant International 2016; 29: 1296–1306

after nonrenal SOT were more likely to undergo living kidney donation compared to repeat and primary KTRs and therefore had a shorter time on dialysis ($P < 0.001$; $P = 0.012$). Patient characteristics are shown in Table 2. Table S1 shows patient characteristics for KTRs of deceased donation only.

Table 1. Patient characteristics and outcomes in KTRs after nonrenal SOT and by type of SOT.

	KTRs after nonrenal SOT ($n = 40$)	KTRs after liver Tx ($n = 15$)	KTRs after heart/lung Tx ($n = 25$)	*P* value
Characteristics				
Age at KT, year*	53 (18–72)	55 (24–72)	43 (18–69)	0.036*
Age at SOT, year*	44 (4–67)	53 (16–67)	37 (4–64)	0.023*
Male sex, *n* (%)	25 (63)	10 (67)	15 (60)	0.746
Donor age, year*	53 (13–76)	48 (19-76)	55 (13–76)	0.502
Deceased donation, *n* (%)	26 (65)	13 (87)	13 (52)	0.040*
Time of KT after nonrenal SOT, month*	98 (4–262)	61 (4–209)	103 (25–262)	0.184
Causes of ESRD, *n* (%)				
CNI toxicity	26 (65)	7 (47)	19 (76)	0.089
CNI toxicity + diabetes	9 (23)	4 (27)	5 (20)	0.705
Acute kidney failure due to SOT	3 (7)	2 (13)	1 (4)	0.544
ADPKD	1 (3)	1 (7)	–	–
Uropathy	1 (3)	1 (7)	0 (0)	0.375
Diabetes mellitus, *n* (%)	10 (25)	5 (33)	5 (20)	0.457
Cold ischemia time, h:min*	8:31 (1:50–23:00)	12:01 (2:00–23:00)	7:05 (1:50–16:22)	0.021*
BMI >30, *n* (%)	3 (7)	3 (20)	0 (0)	0.046*
Time on dialysis, month*	37 (0–97)	45 (3–83)	26 (0–97)	0.167
CMV seropositivity, *n* (%)	31 (78)	14 (93)	17 (68)	0.117
	5 (13)	1 (7)	4 (16)	0.633
CMV D+R–, *n* (%)				
HBV seropositivity, *n* (%)	1 (3)	1 (7)	0 (0)	0.375
HCV seropositivity, *n* (%)	6 (15)	6 (40)	0 (0)	0.001*
Maintenance IS, *n* (%)				
Tacrolimus, MMF, steroids	34 (85)	15 (100)	19 (76)	0.067
Cyclosporine, MMF, steroids	6 (15)	0 (0)	6 (24)	0.067
Induction therapy, *n* (%)				
Lymphocyte depletion	4 (10)	1 (7)	3 (12)	1
IL-2 receptor antagonist	36 (90)	14 (93)	22 (88)	1
Total HLA mismatch, *n* (%)				
4–6 HLA mismatch	12 (30)	6 (40)	6 (24)	0.311
PRA, *n* (%)				
0–10%	40 (100)	15 (100)	25 (100)	1
>10%	0 (0)	0 (0)	0 (0)	1
Outcomes				
Delayed graft function, *n* (%)	14 (35)	7 (47)	7 (28)	0.310
Acute rejection, *n* (%)	12 (30)	3 (20)	9 (36)	0.477
IA/IB	8 (20)	3 (20)	5 (20)	1
IIA/IIB/III	4 (10)	0 (0)	4 (16)	0.278
CMV infection, *n* (%)	15 (38)	4 (27)	11 (44)	0.329
BK viremia/BKVN, *n* (%)	6 (15)/1 (3)	3 (20)/1 (7)	3 (12)/0 (0)	0.654
EBV viremia/PTLD, *n* (%)	16 (40)/3 (7)	2 (13)/1 (7)	14 (56)/2 (8)	0.010*
Septic events, *n* (%)	13 (33)	7 (47)	6 (24)	0.175
Severe sepsis/septic shock	8 (20)	5 (33)	3 (12)	0.126
Cancer	5 (13)	1 (7)	4 (16)	0.633
Nonmelanoma skin tumor, *n* (%)	2 (5)	0 (0)	2 (8)	0.519

KTRs, kidney transplant recipients; SOT, solid organ transplantation.

*Median (range).

Table 2. Patient characteristics and outcomes of KTRs after nonrenal SOT compared to repeat KTRs and primary KTRs.

	KTRs after nonrenal SOT (*n* = 40)	Repeat KTRs (*n* = 119)	*P* value	KTRs after nonrenal SOT (*n* = 40)	Primary KTRs (*n* = 720)	*P* value
Characteristics						
Age, year*	53 (18–72)	47 (19–76)	0.297	53 (18–72)	54 (18–80)	0.324
Male sex, *n* (%)	25 (63)	67 (56)	0.580	25 (63)	456 (63)	1
Donor age, year*	53 (13–76)	51 (12–84)	0.185	53 (13–76)	54 (0–85)	0.651
Deceased donation, *n* (%)	26 (65)	104 (87)	0.004†	26 (65)	544 (76)	0.137
Diabetes mellitus, *n* (%)	10 (25)	13 (11)	0.038	10 (25)	161 (22)	0.698
Cold ischemia time, h:min*	8:31 (1:50–23:00)	12:19 (2:25–28:20)	0.031	8:31 (1:50–23:00)	10:17 (2:20–29:15)	0.142
BMI >30, *n* (%)	3 (7)	11 (10)	1	3 (7)	115 (16)	0.182
Time on dialysis, month*	37 (0–97)	66 (0–261)	<0.001†	37 (0–97)	58 (0–239)	0.019
CMV seropositivity, *n* (%)	31 (78)	98 (82)	0.491	31 (78)	447 (62)	0.063
	5 (13)	9 (8)	0.344	5 (13)	114 (16)	0.822
CMV D+R−, *n* (%)						
HBV seropositivity, *n* (%)	1 (3)	10 (8)	0.293	1 (3)	22 (3)	1
HCV seropositivity, *n* (%)	6 (15)	25 (21)	0.494	6 (15)	11 (2)	<0.001†
Maintenance IS, *n* (%)						
Tacrolimus, MMF, steroids	34 (85)	109 (92)	0.235	34 (85)	549 (76)	0.251
Cyclosporine, MMF, steroids	6 (15)	10 (8)	0.235	6 (15)	171 (24)	0.251
Induction therapy, *n* (%)						
Lymphocyte depletion	4 (10)	57 (48)	<0.001†	4 (10)	54 (7)	0.537
IL-2 receptor antagonist	36 (90)	62 (52)	<0.001†	36 (90)	666 (93)	0.537
Total HLA mismatch, *n* (%)						
4–6 HLA mismatch	12 (30)	32 (27)	0.688	12 (30)	250 (35)	0.611
PRA, *n* (%)						
0–10%	40 (100)	78 (66)	<0.001†	40 (100)	720 (100)	1
>10%	0 (0)	39 (34)	<0.001†	0 (0)	0 (0)	1
Outcomes						
Delayed graft function, *n* (%)	14 (35)	50 (42)	0.462	14 (35)	215 (30)	0.483
Acute rejection, *n* (%)	12 (30)	58 (49)	0.044	12 (30)	223 (31)	1
IA/IB	8 (20)	34 (29)	0.407	8 (20)	163 (23)	0.846
IIA/IIB/III	4 (10)	24 (20)	0.229	4 (10)	60 (8)	0.767
Multiple rejection episodes, *n* (%)	3 (7)	28 (24)	0.036	3 (7)	91 (13)	0.462
CMV infection, *n* (%)	15 (38)	26 (22)	0.061	15 (38)	258 (36)	0.866
BK viremia/BKVN, *n* (%)	6 (15)/1 (3)	13 (11)/4 (3)	0.574	6 (15)/1 (3)	84 (12)/22 (3)	0.458
EBV viremia/PTLD, *n* (%)	16 (40)/3 (8)	15 (13)/1 (1)	<0.001†	16 (40)/3 (7)	66 (9)/4 (1)	<0.001†
Septic events, *n* (%)	13 (33)	15 (13)	0.007†	13 (33)	65 (9)	<0.001†
Severe sepsis/septic shock	8 (20)	5 (4)	0.004†	8 (20)	15 (2)	<0.001†
Cancer	5 (13)	8 (7)	0.315	5 (13)	48 (7)	0.190
Nonmelanoma skin tumor, *n* (%)	2 (5)	9 (7)	0.732	2 (5)	40 (6)	1

KTRs, kidney transplant recipients; SOT, solid organ transplantation.

*Median (range).

†Bonferroni adjustment was applied for pairwise comparisons with two-sided *P*-values <0.017 being considered statistical significant.

Patient and allograft outcomes

Overall, no differences were observed for 1- and 5-year patient survival rates between KTRs after nonrenal SOT (1 year: 100%; 5 year: 85.1%), repeat KTRs (1 year: 96.5%; 5 year: 83.5%), and primary KTRs (1 year: 96.7%; 5 year: 88.4%; Fig. 1a,b). However, KTRs after nonrenal SOT were more likely to die from septic complications (seven KTRs; 70%) compared to repeat KTRs (two KTRs; 17%; $P = 0.003$) and primary KTRs (23 KTRs; 24%; $P = 0.005$). Two KTRs after nonrenal SOT died from organ failure of the nonrenal SOT.

No differences were observed for 1- and 5-year death-censored allograft survival rates between KTRs after nonrenal SOT and primary KTRs (1 year: 92.5% vs. 95.3%; 5 year: 92.5% vs. 92.0%; Fig. 2a,b). While KTRs after nonrenal SOT show comparable 1-year death-censored allograft survival rates with repeat KTRs (1 year: 92.5% vs. 89.9%), KTRs after nonrenal SOT show superior 5-year death-censored allograft survival compared to repeat KTRs (5 year: 92.5% vs. 81.2%; Fig. 2a,b). No differences, however, were observed for primary nonfunction between KTRs after nonrenal SOT (two KTRs; 5%), repeat KTRs (five KTRs; 4%), and primary KTRs (15 KTRs; 2%; $P = 0.229$).

Kidney transplant recipients after nonrenal SOT showed comparable allograft function compared with repeat KTRs and primary KTRs (Fig. 3a,b). KTRs after nonrenal SOT had a lower incidence of acute cellular rejections compared to repeat KTRs (Table 2; $P = 0.044$). No differences were observed for the incidence of acute cellular rejections between KTRs after nonrenal SOT and primary KTRs ($P = 1$).

Kidney transplant recipients after nonrenal SOT showed a significantly higher incidence of EBV viremia after kidney transplantation compared with repeat KTRs and primary KTRs ($P < 0.001$). While three of 40 KTRs after nonrenal SOT (7.5%) developed post-transplant lymphoproliferative disorder, only one of 119 repeat KTRs (0.8%), and four of 720 primary KTRs (0.6%) developed post-transplant lymphoproliferative disorder ($P = 0.004$). KTRs after nonrenal SOT showed a higher incidence of CMV viremia compared to repeat KTRs (38% vs. 22%; $P = 0.061$).

Kidney transplant recipients after nonrenal SOT showed a higher incidence of sepsis and severe sepsis/septic shock compared to repeat KTRs ($P = 0.007$) and primary KTRs ($P < 0.001$). The median time of diagnosis was 25 months (range 0–102 months) post-transplantation and later compared to repeat KTRs 15 months (range 0–95 months) and primary KTRs 12 months (range 0–108 months). KTRs after nonrenal SOT were more likely to develop sepsis from pneumonia (nine of 13 KTRs; 69%) compared to repeat KTRs (five of 13 KTRs; 38%) and primary KTRs (14 of 65 KTRs; 22%). KTRs after nonrenal SOT were more likely to develop sepsis from Gram-positive microorganisms (eight of 13 KTRs; 62%) compared to repeat (five of 13 KTRs; 38%) and primary KTRs (13 of 65 KTRs; 20%).

No differences were observed between KTRs after liver transplantation and KTRs after heart/lung transplantation concerning patient survival, death-censored

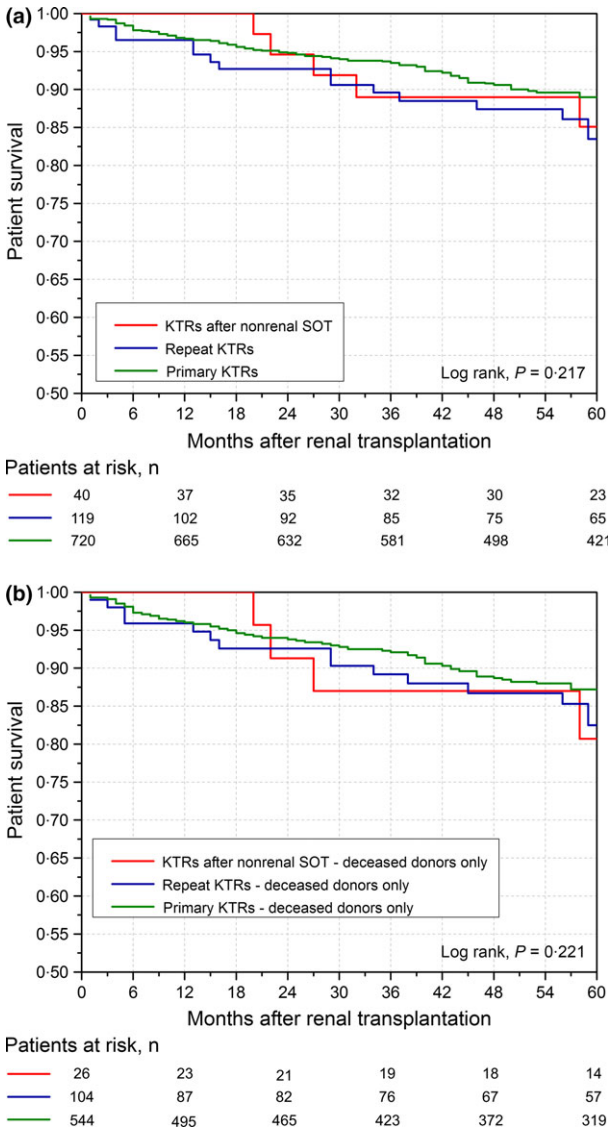

Figure 1 (a) Kaplan–Meier plot of patient survival by type of transplantation. No differences were observed between kidney transplant recipients (KTRs) after nonrenal solid organ transplantation (SOT), primary KTRs, and repeat KTRs (Log Rank, $P = 0.217$). (b) Kaplan–Meier plot of patient survival by type of transplantation (deceased donors only). No differences were observed between KTRs after nonrenal SOT, primary KTRs, and repeat KTRs (log rank, $P = 0.221$).

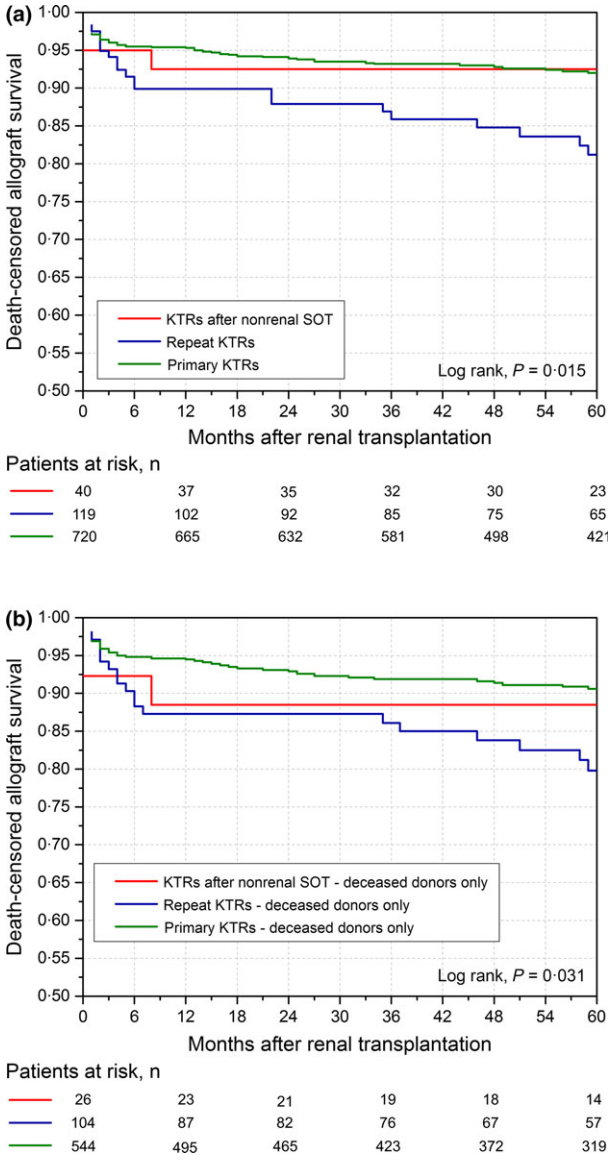

Figure 2 (a) Kaplan–Meier plot of death-censored allograft survival by type of transplantation. Significantly inferior death-censored allograft survival in repeat kidney transplant recipients (KTRs) compared to primary KTRs (Log Rank, $P = 0.005$). Superior death-censored allograft survival in KTRs after nonrenal solid organ transplantation (SOT) compared to repeat KTRs (log rank, $P = 0.078$). (b) Kaplan–Meier plot of death-censored allograft survival by type of transplantation (deceased donors only). Significantly inferior death-censored allograft survival in repeat KTRs compared to primary KTRs (log rank, $P = 0.008$). Comparable death-censored allograft survival in KTRs after nonrenal SOT compared to repeat KTRs (log rank, $P = 0.133$).

allograft survival, and allograft function (Fig. S1a,b). KTRs after heart/lung transplantation showed a significantly higher incidence of EBV viremia after kidney transplantation compared to KTRs after liver transplantation ($P = 0.010$). No differences were observed between KTRs after nonrenal SOT, repeat KTRs, and primary KTRs who underwent living kidney donation concerning patient survival, death-censored allograft survival, and allograft function (Fig. S2a,b).

Immunological characteristics

CMV-specific immunity

Kidney transplant recipients after nonrenal SOT were more likely not to have CMV-specific T-cells pretransplantation and post-transplantation compared to repeat KTRs. While nine of 20 KTRS after nonrenal SOT (45%) show detectable CMV-specific T cells directed to CMV-IE1 and 10 of 20 KTRs after nonrenal SOT (50%) show detectable CMV-specific T cells directed to CMV-pp65 pretransplantation, 36 of 53 repeat KTRs (68%) show CMV-specific T cells directed to CMV-IE1, and 37 of 53 repeat KTRs (70%) show CMV-specific T cells directed to CMV-pp65 pretransplantation ($P = 0.105$; $P = 0.170$). Three KTRs after nonrenal SOT lost CMV-specific T cells from pre- to post-transplantation. While six of 20 KTRS after nonrenal SOT (30%) show detectable CMV-specific T cells directed to CMV-IE1 and seven of 20 KTRs after nonrenal SOT (35%) show detectable CMV-specific T cells directed to CMV-pp65 at +1 month post-transplantation, 36 of 53 repeat KTRs (68%) show CMV-specific T cells directed to CMV-IE1, and 37 of 53 repeat KTRs (70%) show CMV-specific T cells directed to CMV-pp65 pretransplantation ($P = 0.007$; $P = 0.014$). KTRs after nonrenal SOT show lower CMV-specific T cells directed to CMV-IE1 and CMV-pp65 compared to the repeat KTRs. In this context, KTRs after nonrenal SOT show a higher incidence of CMV replication (38% vs. 22%), higher incidence of CMV disease (20% vs. 9%), and more need for ganciclovir therapy (23% vs. 9%) compared to repeat KTRs.

No differences were observed for the presence and frequencies of CMV-specific T-cells pre- and post-transplantation, or the incidence and severity of CMV replication between KTRs after nonrenal SOT and primary KTRs ($P > 0.05$).

Alloreactivity

Kidney transplant recipients after nonrenal SOT were more likely not to have alloreactive T-cells pretransplantation and post-transplantation compared to repeat KTRs. Five of 20 KTRs after nonrenal SOT (25%), 22 of 53 repeat KTRs (42%), and 144 of 436 primary KTRs (33%) showed detectable alloreactive T-cells pretransplantation. KTRs after nonrenal SOT showed lower alloreactive T cells compared to repeat KTRs (Fig. 4). In

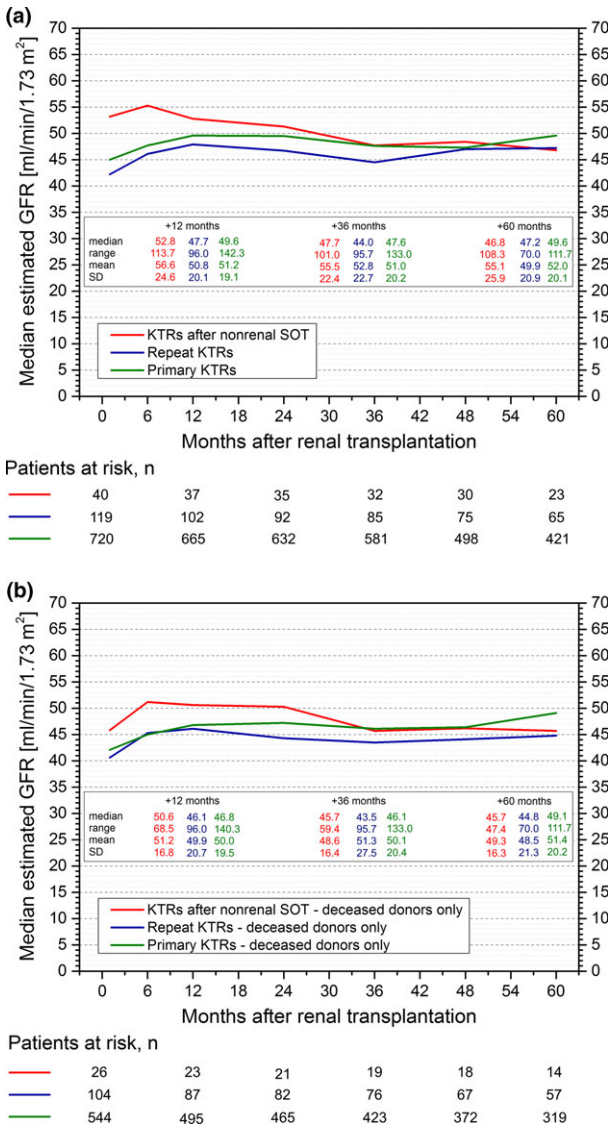

Figure 3 (a) Median eGFR by type of transplantation. No differences were observed between kidney transplant recipients (KTRs) after nonrenal solid organ transplantation (SOT), primary KTRs, and repeat KTRs in long-term follow-up at any time ($P > 0.05$). (b) Median eGFR by type of transplantation (deceased donors only). No differences were observed between KTRs after nonrenal SOT, primary KTRs, and repeat KTRs in long-term follow-up ($P > 0.05$).

addition, KTRs after nonrenal SOT showed less panel-reactive antibodies (PRA) pretransplantation compared to repeat KTRs ($P < 0.001$) and were less likely to develop de-novo donor-specific antibodies (DSA) post-transplantation. In this context, KTRs after nonrenal SOT showed significantly less acute cellular rejection episodes compared to repeat KTRs ($P = 0.044$).

No differences were observed for the presence of preformed alloreactive T cells by type of the previous SOT. Interestingly, while two KTRs after liver transplantation with preformed alloreactive T cells showed a decline of alloreactive T-cells post transplantation, three KTRs after heart/lung transplantation showed stable alloreactive T cells from pre- to post-transplantation. Zero KTR after liver transplantation, but three KTRs after heart/lung transplantation developed alloreactive T-cells post-transplantation.

No differences were observed for the presence and frequencies of alloreactive T cells or PRA/DSA pre- and post-transplantation, or the incidence and severity of acute cellular rejection between KTRs after nonrenal SOT and primary KTRs (Table 2).

Discussion

Kidney transplantation in patients after previous nonrenal SOT, who don't qualify for an initial simultaneous transplantation, is becoming increasingly common due to improved patient survival of recipients of SOT. Here, our aims were to refine the understanding of outcomes of KTRs after nonrenal SOT, to identify the incidence, etiology and mortality of infection, and the impact on alloreactivity.

Firstly, our results suggest comparable patient and allograft outcomes compared with primary KTRs and superior death-censored allograft survival compared to repeat KTRs. Here, the difference in death-censored allograft survival compared to repeat KTRs may be attributed to the observed lower alloreactivity with associated lower rates of acute cellular rejection.

Our results are in line with previous findings that have demonstrated inferior allograft survival among patients retransplanted with the same organ [24]. Comparable allograft outcomes of KTRs after nonrenal SOT compared with primary KTRs have been suggested previously; however, underlying mechanisms remain unclear [25]. Our observations with comparable patient survival in KTRs after nonrenal SOT compared with primary KTRs may reflect coexisting comorbidities in both groups as hypertension, diabetes, and atherosclerosis associated with prolonged dialysis treatment. Our promising results regarding patient and allograft outcomes in KTRs after nonrenal SOT may be beneficial in advising potential living donors considering donation to a patient after previous nonrenal SOT and encourage evaluation of those patients for subsequent kidney transplantation.

Secondly, our results suggest a higher risk of infectious complications as EBV replication, PTLD, sepsis, and death from sepsis in KTRs after nonrenal SOT compared to repeat KTRs and primary KTRs. The increased incidence of EBV viremia, CMV viremia, and

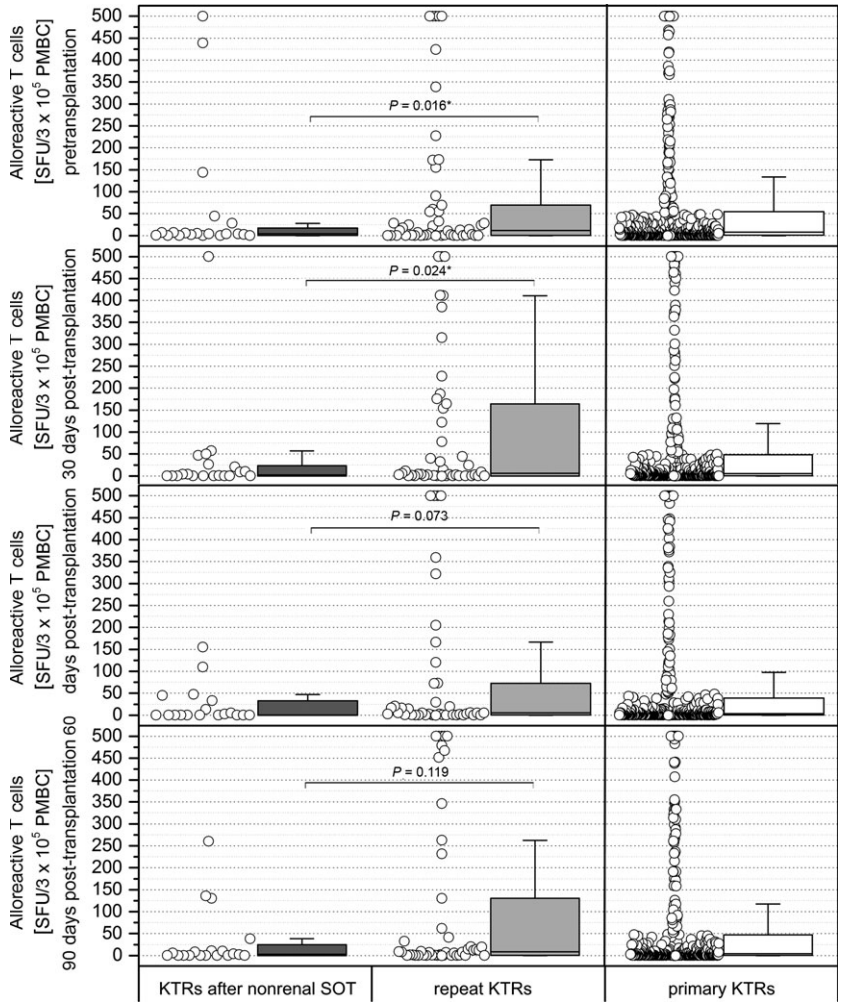

Figure 4 Lower pretransplant and post-transplant frequencies of alloreactive T cells in kidney transplant recipients (KTRs) after nonrenal solid organ transplantation (SOT) compared to repeat KTRs.

severe bacterial infection with progression to sepsis suggests a state of impaired overall immunity in KTRs after nonrenal SOT most likely related to pre-existing maintenance immunosuppression plus a summation effect of induction immunosuppression, immunomodulating comorbidities as diabetes, and impaired immune control due to primary viral infections in the setting of immunosuppression.

The site of infection is an important determinant of outcome after SOT. Here, our data suggest pneumonia as the most common site of infection in KTRs after nonrenal SOT. Here, our own observations in KTRs show mortality rates of severe sepsis and septic shock due to pneumonia of 70% and 85% [26]. These increased mortality rates may result from the severely immunocompromised state, substantially more subtile manifestations, the inability to withdraw maintenance immunosuppression, and in particular, a high proportion of infections with Gram-positive microorganisms. Here, any intensification of immunosuppression in the case of acute rejection must be strictly evaluated and linked to an efficient infection monitoring and risk-adjusted prophylaxis for opportunistic infections.

The increased risk of EBV viremia among KTRs after nonrenal SOT most likely results from impaired immune control due to a state of overimmunosuppression and impaired EBV-specific immunity due to primary infection after SOT. Our own work very recently suggested an increased risk of viral complications after simultaneous pancreas–kidney transplantation [20]. Our results call for EBV-load monitoring in KTRs after nonrenal SOT conducted on a regular basis. Here, preemptive treatment to reduce the risk of PTLD needs to be evaluated individually. In addition, impaired CMV-specific cellular immunity may predispose KTRs after nonrenal SOT to severe CMV infection which further alters the immunocompromised state with CMV-induced neutropenia and an increased risk of septic

complications. Here, stratification of KTRs by the presence of CMV-specific T cells may prove useful to guide CMV prophylaxis and the need for monitoring and preemptive treatment in these high-risk KTRs.

Thirdly, our results suggest less alloreactivity and an associated lower risk of acute cellular rejections in KTRs after nonrenal SOT compared to repeat KTRs. Less cellular alloimmunity may contribute to superior death-censored allograft survival in KTRs after nonrenal SOT compared to repeat KTRs. It can be speculated that exposure to long-term immunosuppression at the time of kidney transplantation increases the acceptance of the new allograft. Previous data suggest a lower incidence of renal allograft rejection in KTRs after heart transplantation compared to single KTRs [27]. Another study comparing kidney after liver transplantation and combined liver/kidney transplantation showed no differences in death-censored allograft survival between both groups [28]. However, KTRs after previous liver transplantation showed a higher incidence of acute cellular rejection compared to patients who underwent combined liver/kidney transplantation. These findings suggested an immunoprotection by the liver on the kidney allograft that may be HLA-specific and present only in combined transplantation. However, the underlying mechanisms remain controversially discussed.

One mechanism suggests that the liver has a unique ability to protect other organs. Here, recent studies suggested that hepatocytes and nonparenchymal liver cells are able to neutralize cytotoxic antibodies and degradate circulating cytotoxic T cells [29–31]. A comprehensive analysis of the UNOS data, however, showed that not only the liver, but even heart, lung, and kidney were able to protect each other, and result in lower rates of acute cellular rejection [32]. Here, previous reports hypothesized that high doses of antigen, meaning high organ mass, but not the type of transplanted organ induces organ tolerance by exhaustion of immune responses due to overstimulation [33]. Despite a lack of data on patients after combined transplantation, our results suggest less alloreactivity and acute cellular rejection among KTRs after previous liver transplantation that may be attributed to a unique protection by the liver. Here, stratification of KTRs by the presence of alloreactive T cells may prove useful to tailor individual immunosuppression to reduce rates of acute cellular rejection.

The most important limitation of our study is the retrospective nature of our data collection. Missing septic events in those lost to follow-up or treated in other hospitals cannot be excluded.

In summary, KTRs after nonrenal SOT show comparable patient survival, death-censored allograft survival, and function compared with primary KTRs. The observed low alloreactivity, that may be attributed to pre-existing maintenance immunosuppression and perhaps the presence of an allograft liver, results in less acute cellular rejection and superior death-censored allograft survival compared to repeat KTRs. Due to higher incidences of EBV viremia, PTLD, sepsis, and death from sepsis, caution should be taken in KTRs after nonrenal SOT regarding overimmunosuppression.

Authorship

Thomas Schachtner: designed research/study, performed research/study, collected data, analyzed data, wrote paper. Maik Stein: performed research/study. Petra Reinke: designed research/study, analyzed data, wrote paper.

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Conflicts of interest

The authors declare that they have no competing financial interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. (a) Kaplan–Meier plot of patient survival by type of previous nonrenal solid organ transplantation (SOT). No differences were observed between kidney transplant recipients (KTRs) after liver transplantation and KTRs after heart/lung transplantation (Log Rank, $P = 0.764$). (b) Kaplan–Meier plot of death-censored allograft survival by type of previous nonrenal SOT.

Figure S2. (a) Kaplan–Meier plot of patient survival by type of transplantation (living donors only). No differences were observed between kidney transplant recipients (KTRs) after nonrenal solid organ transplantation

(SOT), primary KTRs, and repeat KTRs (Log Rank, $P = 0.645$). (b) Kaplan–Meier plot of death-censored allograft survival by type of transplantation (living donors only).

Table S1. Patient characteristics and outcomes in kidney transplant recipients (KTRs) after nonrenal solid organ transplantation (SOT) compared to repeat KTRs (deceased only).

REFERENCES

1. Chandrakantan A, de Mattos AM, Naftel D, Crosswy A, Kirklin J, Curtis JJ. Increasing referral for renal transplant evaluation in recipients of nonrenal solid-organ transplants: a single-center experience. *Clin J Am Soc Nephrol* 2006; **1**: 832.
2. Srinivas TR, Stephany BR, Budev M, *et al.* An emerging population: kidney transplant candidates who are placed on the waiting list after liver, heart, and lung transplantation. *Clin J Am Soc Nephrol* 2010; **5**: 1881.
3. O'Riordan A, Wong V, McCormick PA, Hegarty JE, Watson AJ. Chronic kidney disease post-liver transplantation. *Nephrol Dial Transplant* 2006; **21**: 2630.
4. Ojo AO, Held PJ, Port FK, *et al.* Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 2003; **349**: 931.
5. Shiba N, Chan MC, Kwok BW, Valantine HA, Robbins RC, Hunt SA. Analysis of survivors more than 10 years after heart transplantation in the cyclosporine era: Stanford experience. *J Heart Lung Transplant* 2004; **23**: 155.
6. Mason DP, Solovera-Rozas M, Feng J, *et al.* Dialysis after lung transplantation: prevalence, risk factors and outcome. *J Heart Lung Transplant* 2007; **26**: 1155.
7. Cassuto JR, Reese PP, Sonnad S, *et al.* Wait list death and survival benefit of kidney transplantation among nonrenal transplant recipients. *Am J Transplant* 2010; **10**: 2502.
8. Bennett WM, DeMattos A, Meyer MM, Andoh T, Barry JM. Chronic cyclosporine nephropathy: the Achilles' heel of immunosuppressive therapy. *Kidney Int* 1996; **50**: 1089.
9. Lindelow B, Bergh CH, Herlitz H, Waagstein F. Predictors and evolution of renal function during 9 years following heart transplantation. *J Am Soc Nephrol* 2000; **11**: 951.
10. Hamour AM, Omar F, Lyster HS, Palmer A, Banner NR. Chronic kidney disease after heart transplantation. *Nephrol Dial Transplant* 2009; **24**: 1655.
11. Puschett JB, Greenberg A, Holley J, McCauley J. The spectrum of ciclosporin nephrotoxicity. *Am J Nephrol* 1990; **10**: 296.
12. Bertani T, Ferrazzi P, Schieppati A, *et al.* Nature and extent of glomerular injury induced by cyclosporine in heart transplant patients. *Kidney Int* 1991; **40**: 243.
13. Schwarz A, Haller H, Schmitt R, *et al.* Biopsy-diagnosed renal disease in patients after transplantation of other organs and tissues. *Am J Transplant* 2010; **10**: 2017.
14. Wolfe RA, Ashby VB, Milford EL, *et al.* Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med* 1999; **341**: 1725.
15. Coopersmith CM, Brennan DC, Miller B, *et al.* Renal transplantation following previous heart, liver, and lung transplantation: an 8-year single-center experience. *Surgery* 2001; **130**: 457.
16. Gupta J, Amaral S, Mahle WT. Renal transplantation after previous pediatric heart transplantation. *J Heart Lung Transplant* 2008; **27**: 217.
17. Merion RM, Ashby VB, Wolfe RA, *et al.* Deceased-donor characteristics and the survival benefit of kidney transplantation. *JAMA* 2005; **294**: 2726.
18. Srivastava R, Keck BM, Bennett LE, Hosenpud JD. The results of cardiac retransplantation: an analysis of the Joint International Society for Heart and Lung Transplantation/United Network for Organ Sharing Thoracic Registry. *Transplantation* 2000; **70**: 606.
19. Topkara VK, Dang NC, John R, *et al.* A decade experience of cardiac retransplantation in adult recipients. *J Heart Lung Transplant* 2005; **24**: 1745.
20. Schachtner T, Zaks M, Kahl A, Reinke P. Simultaneous pancreas/kidney transplantation presents with late-onset BKV-associated nephropathy. *Nephrol Dial Transplant* 2016; **31**: 1174.
21. Bestard O, Crespo E, Stein M, *et al.* Cross-validation of IFN-γ Elispot assay for measuring alloreactive memory/effector T cell responses in renal transplant recipients. *Am J Transplant* 2013; **13**: 1880.
22. Schachtner T, Stein M, Reinke P. ABO desensitization affects cellular immunity and infection control after renal transplantation. *Transpl Int* 2015; **28**: 1179.
23. Schachtner T, Stein M, Babel N, Reinke P. The loss of BKV-specific immunity from pretransplantation to posttransplantation identifies kidney transplant recipients at increased risk of BKV replication. *Am J Transpl* 2015; **15**: 2159.
24. Magee JC, Barr ML, Basadonna GP, *et al.* Repeat organ transplantation in the United States, 1996–2005. *Am J Transplant* 2007; **7**: 1424.
25. Lonze BE, Warren DS, Stewart ZA, *et al.* Kidney transplantation in previous heart or lung recipients. *Am J Transplant* 2009; **9**: 578.
26. Schachtner T, Reinke P. Sepsis after renal transplantation: clinical, microbiological, and immunological characteristics [abstract]. *Am J Transplant* 2013; **13**(Suppl. 5): 188.
27. Kuo PC, Luikart H, Busse-Henry S, *et al.* Clinical outcome of interval cadaveric renal transplantation in cardiac allograft recipients. *Clin Transplant* 1995; **9**: 92.
28. Simpson N, Cho YW, Cicciarelli JC, Selby RR, Fong TL. Comparison of renal allograft outcomes in combined liver-kidney transplantation versus subsequent kidney transplantation in liver transplant recipients: analysis of UNOS database. *Transplantation* 2006; **10**: 1298.
29. Benseler V, Warren A, Vo M, *et al.* Hepatocyte entry leads to degradation of autoreactive CD8 T cells. *Proc Natl Acad Sci USA* 2011; **108**: 16735.
30. Bowen DG, Zen M, Holz L, Davis T, McCaughan GW, Bertolino P. The site of primary T cell activation is a determinant of the balance between intrahepatic tolerance and immunity. *J Clin Invest* 2004; **114**: 701.
31. Steger U, Denecke C, Sawitzki B, Karim M, Jones ND, Wood KJ. Exhaustive differentiation of alloreactive CD8+ T cells: critical for determination of graft acceptance or rejection. *Transplantation* 2008; **85**: 1339.
32. Rana A, Robles S, Russo MJ, *et al.* The combined organ effect: protection against rejection? *Ann Surg* 2008; **248**: 871.
33. Bishop GA, Sun J, Sheil AG, McCaughan GW. High-dose/activation-associated tolerance: a mechanism for allograft tolerance. *Transplantation* 1997; **64**: 1377.