Anti-donor T cell responses are equivalent between elderly and non-elderly kidney transplant recipients

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Abstract

Although the number of kidney transplantations (KTs) among elderly patients has been increasing, no specific recommendations have been established for managing elderly patients. In this study, we investigated the influence of aging on anti-donor T-cell responses by comparing elderly (aged ≥ 60 years) and non-elderly KT recipients with cyclosporine-based immunosuppressive regimens. We performed a serial mixed lymphocyte reaction assay to evaluate the status of T-cell immunity in KT recipients.

During the observation period, a certain number of patients in both groups presented with hyper T-cell responses to donor stimuli. Notably, the proportion of patients showing anti-donor hyper-responsiveness in the elderly group gradually increased over time with a reduced dosage of immunosuppressants.

In conclusion, anti-donor T cell responses were equivalent between elderly and non-elderly recipients. Thus, caution is required regarding the imprudent reduction of immunosuppressants in elderly recipients. A rigorously designed, large-scale, prospective study is needed to validate these results.

Introduction

Due to a globally aging population with end-stage kidney disease and improved survival on dialysis, the number of elderly patients requiring kidney transplantation (KT) is continuously increasing worldwide [1–4]. Previous studies have shown that KT in elderly patients can significantly reduce mortality compared with dialysis [5,6]. Under such circumstances, similar to other countries [7,8], the number of KTs for patients > 60 years old in Japan has increased over the past decade, from 286 in 2010 (21.5% of the total) to 468 in 2020 (30.4% of the total).

Understandably, transplantation in elderly recipients is more complicated because of pre-existing comorbidities, frailty, changes in pharmacokinetics (PK) and pharmacodynamics (PD) of immunosuppressive drugs, and changes in immunoreactivity. Several studies has demonstrated that elderly patients have increased susceptibility to infectious diseases and cancer [9–11]. Moreover, immunosenescence affects all immune compartments, with the most striking changes observed in T-cell functions [12].

While the number of KT among elderly patients has increased, no specific recommendations have been established for managing elderly KT recipients. Recent findings from a United States transplant registry-based study suggest that risk-adjusted death-censored graft failure is higher among elderly patients who received antimetabolite avoidance, mammalian target of rapamycin inhibitor-based, and cyclosporine (CsA)-based regimens [8].

In general, elderly recipients appear to have a lower risk of cellular rejection than younger recipients and may require less intense immunosuppression. Previous studies have reported that elderly recipients have improved short-term death-censored graft survival compared to younger patients, probably due to immunosenescence and decreased acute rejection risk [13,14]. Recently, the Japan Academic Consortium of

Kidney Transplantation (JACK) reported that although elderly (aged ≥ 60 years) patients had similar graft survival as younger patients, elderly patients had inferior patient survival and more complications such as sepsis, hepatic disorder, post-transplant diabetes mellitus, malignancy, and cardiovascular disease than younger patients [15]. Notably, higher chronic T-cell mediated rejection (TCMR) was observed in the elderly group [15]. Such incongruous opinions prompted us to investigate the influence of aging on anti-donor T-cell responses by comparing elderly (aged ≥ 60 years) and non-elderly KT recipients. To evaluate anti-donor T cell responses, mixed lymphocyte reaction (MLR) assays using the intracellular fluorescent dye carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeling technique (CFSE-MLR) were applied [16]. The objective of this study was to elucidate whether elderly KT recipients have reduced anti-donor T cell responses compared to non-elderly recipients based on CFSE-MLR.

Results

Baseline characteristics of elderly patients versus non-elderly recipients

The baseline characteristics of the 67 KT recipients according to age are summarized in Table 1. Regarding donor characteristics, elderly recipients were more likely to undergo transplants with donations from spouses than non-elderly recipients. The number of mismatches at the HLA-B, and -DRB1 loci was significantly higher in the elderly group than in the non-elderly group. Moreover, elderly recipients were more likely to have preformed DSAs than were non-elderly recipients. Other demographic variables, including sex, primary diseases, donor age, donor sex, and ABO-blood type compatibility, were comparable between the two groups.

Table 1
Baseline characteristics of elderly patients versus non-elderly recipients

	Elderly (n = 18)	Non-elderly (n = 49)	P-value
Recipient age, years (median, IQR)	66.5 (63.8–70.3)	42 (36.5–53.0)	< 0.0001
Recipient gender, male/female	13 / 5	30 / 19	0.41
Primary disease, n (%)			0.18
CGN	3 (16.7)	8 (16.3)	
DMN	6 (33.3)	11 (22.5)	
IgAN	1 (5.6)	9 (18.4)	
Nephrosclerosis	3 (16.7)	3 (6.1)	
PKD	2 (11.1)	1 (2.0)	
Others	3 (16.7)	17 (34.7)	
Body mass index, kg/m^2 (median, IQR)	22.5 (21.0–24.1)	20.8 (19.4–23.8)	0.25
Modality of RRT, n (%)			0.99
Pre-emptive	7 (38.9)	19 (38.8)	
Dialysis	11 (61.1)	30 (61.2)	
Dialysis period, years (median, IQR)	0.6 (0–2.7)	0.4 (0–1.4)	0.43
Donor age, years (median, IQR)	60.5 (55.0–70.8)	57 (48.0–63.5)	0.10
Donor sex, male/female	4 / 14	20 / 29	0.16
Donor type, living/deceased	16 / 2	46 /3	0.49
Relationship of living donor, n (%)			0.0028
Child	1 (6.3)	1 (2.2)	
Parents	0 (0)	23 (50.0)	
Sibling	2 (12.5)	6 (13.0)	
Spouse	13 (81.3)	16 (34.8)	
HLA allele MM			

CGN, chronic glomerulonephritis; DMN, diabetic nephropathy; IgAN, immunoglobulin A nephropathy; PKD, polycystic kidney disease; others, other diseases including focal glomerular sclerosis, lupus nephritis, gestational toxicosis, gouty nephropathy, and other unknown diseases; RRT, renal replacement therapy; MM, mismatch; DSA, donor-specific anti-human leukocyte antigen antibody

	Elderly (n = 18)	Non-elderly (n = 49)	P-value
A (0:1:2)	2: 8: 8	5: 34: 10	0.13
B (0:1:2)	2: 1: 15	5: 27: 17	0.0008
DRB1 (0:1:2)	2: 4: 12	8: 28: 13	0.0096
ABO-incompatible, n (%)	8 (44.4)	18 (36.7)	0.57
Preformed DSA, n (%)	2 (3.0)	0 (0)	0.018
Observation period, years (median, IQR)	3.5 (2.4–6.0)	5.0 (2.4–9.0)	0.19

CGN, chronic glomerulonephritis; DMN, diabetic nephropathy; IgAN, immunoglobulin A nephropathy; PKD, polycystic kidney disease; others, other diseases including focal glomerular sclerosis, lupus nephritis, gestational toxicosis, gouty nephropathy, and other unknown diseases; RRT, renal replacement therapy; MM, mismatch; DSA, donor-specific anti-human leukocyte antigen antibody

Comparison of anti-donor T cell immune responses between elderly and non-elderly recipients

Five years after transplantation, serum creatinine levels were comparable between the two groups (Supplemental Figure 1). Although the trough levels of CsA were comparable between the two groups, the dosage of CsA was significantly lower in the elderly group than in the non-elderly group (Figure 1). The dosages of MP and MMF were also comparable, except in the early period after KT (Supplemental Figure 2). During the first year after transplantation, there were no significant differences in the anti-donor SI values for $CD4^+$ and CD8+ T cells between the two groups (Figure 2). Although not statistically significant, the proportion of patients showing anti-donor hyperresponsiveness in the elderly group gradually increased over time (Figure 3).

Post-transplant complications and patient survival

Table 2 shows the post-transplant complications of the elderly and non-elderly groups for the following incidences: CMV antigenemia positivity, UTI, TCMR, dnDSA, cardiovascular disease, and malignancy. There were no significant between-group differences in the incidence. In this study, death with a functioning graft was the leading cause of graft loss. The between-group patient survival rates were also compatible (Figure 4).

Table 2
Post-transplant complications of elderly patients versus non-elderly recipients

	Elderly (n = 18)	Non-elderly (n = 49)	P-value
CMV, n (%)	12 (66.7)	22 (44.9)	0.11
UTI, n (%)	8 (44.4)	16 (32.7)	0.37
TCMR, n (%)	1 (5.6)	2 (4.1)	0.80
dnDSA, n (%)	2 (11.1)	3 (6.1)	0.49
CVD, n (%)	3 (16.7)	3 (6.1)	0.18
Malignancy, n (%)	3 (16.7)	7 (10.5)	0.31

CMV, cytomegalovirus antigenemia; UTI, urinary tract infection; TCMR, T cell mediated rejection; dnDSA, *de novo* donor-specific anti-human leukocyte antigen antibody; CVD, cardiovascular disease

Discussion

Immunosenescence encompasses a series of aging-induced modifications in the immune system that are primarily characterized by dysfunctional immune responses and increased systemic inflammation, termed as inflamm-aging [17,18]. Immunosenescence affects all immune compartments, with the most striking changes observed in the phenotypes and functions of $CD4^+$ and $CD8^+$ T cell components and is less frequently observed in components of innate immunity [12]. Based on the idea that the elderly patients may have a reduced immune response to the transplanted organ, the immunosuppressive load in elderly recipients tends to be reduced after KT. However prospective multicenter randomized controlled trials assessing immunosuppressive agents in elderly recipients are currently unavailable because they are usually excluded from clinical trials [19,20].

After KT, combined treatment with calcineurin inhibitors (CNIs, CsA or tacrolimus), mycophenolic acid (MPA), MP, and basiliximab induction therapy is most frequently used across all age groups [21,22]. However, age-related changes in both the PK and PD of immunosuppressants may result in different outcomes in KT recipients. It has been reported that the PK of MPA and basiliximab is not affected by the physiological changes in elderly patients [23,24]. In contrast, serum CNI trough levels, independent of the choice of CNI, were 50% higher in elderly KT recipients when normalized for dose and weight [25]. In other words, elderly recipients may require lower doses of CNIs to obtain the same therapeutic levels because of a decrease in the metabolism of CYP3A4 isozymes and reduced P-glycoprotein activity, resulting in enhanced bioavailability [25]. In this study, consistent with previous reports [25,26], the dosages of CsA

required to maintain comparable trough levels were significantly lower among elderly recipients than among non-elderly recipients. In contrast, the dosages of MP and MMF were comparable between the two groups.

We performed a serial CFSE-MLR assay to evaluate the status of T-cell immunity in KT recipients. During the observation period, regardless of CFSE-MLR-based optimization of immunosuppressive therapy, a certain number of patients presented hyper T cell responses to donor stimuli in both groups. Notably, the proportion of patients showing anti-donor hyper-responsiveness in the elderly group gradually increased over time with a reduced dosage of immunosuppressants. Fritsche et al. showed a strong relationship between the degree of HLA mismatch and acute rejection in elderly KT recipients [27]. In this study, elderly recipients were more likely to be transplanted from spouses with a high number of HLA mismatches. Moreover, sensitized elderly recipients might have preformed donor-reactive T cells, resulting in T cell hyperresponsiveness. Therefore, caution is required regarding the imprudent reduction of immunosuppressants in elderly recipients.

Our study had several limitations. First, this study reported single-center retrospective data with a relatively small sample size and the associated risk of type II error; therefore, risk quantification should be interpreted cautiously and validated in a larger independent cohort. Second, we selected the age of 60 years as the cut-off point to discriminate between the groups, while in many studies, the age of 65 is used. However, there is no medical justification to use the age of 65, especially considering that, according to the World Health Organization's arbitrary definitions, old age begins at 60 [28]. Finally, our protocol is a CsA-based immunosuppressive regimen, whereas a tacrolimus (TAC) -based immunosuppressive regimen is preferred worldwide because of a better patient outcome [29].

Further validation studies with TAC-based immunosuppressive regimens are required to confirm our results. Despite the above-mentioned limitations, to our knowledge, this study is the first to investigate the influence of aging on anti-donor T-cell responses by comparing elderly and non-elderly KT recipients. We believe that these observations will potentially be of immense value in tailoring personalized immunosuppressive treatment protocols.

In conclusion, anti-donor T cell responses are equivalent between elderly and non-elderly kidney transplant recipients receiving a CsA-based immunosuppressive regimen. Thus, caution is required regarding the imprudent reduction of immunosuppressants in elderly recipients. A rigorously designed, large-scale, prospective study is needed to validate these results.

Material And Methods

Patients

Between January 2010 and December 2020, 72 patients underwent KT with a CsA-based immunosuppressive regimen at the Hiroshima University Hospital. Of these, five were excluded from the

study because of incomplete immune monitoring data caused by a limited volume of stored lymphocytes from donors for in vitro MLR assays. The remaining 67 patients (41 ABO-blood-type-compatible recipients [ABO-C] and 26 ABO-blood-type-incompatible recipients [ABO-I]) were enrolled in the study. A complete clinical history, including age, sex, primary disease, body mass index, human leukocyte antigen (HLA) mismatch, relationship, and dialysis period, was recorded at the time of transplantation.

Elderly recipients were defined as those aged ≥ 60 years (≥ 75th percentile; 49 non-elderly vs. 18 elderly patients). This study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board of the Hiroshima University Hospital (No. Hi-77), and informed consent was obtained from all patients.

HLA typing and anti-HLA antibody testing

HLA (-A, -B, -C, -DRB1, and -DQB1) typing of donors and recipients was performed by xMAP R Technology of Luminex Corp., using polymerase chain reaction–sequence-specific oligonucleotide probes at high resolution (Wakunaga, Hiroshima, Japan). Anti-HLA antibodies in all recipients were analyzed before transplantation and monitored annually following transplantation. Serum samples were examined for IgG antibodies against HLA class I or II using methodologies such as WAKFlow (Wakunaga) or LABScreen Mixed (One Lambda Inc. Canoga CA). All positive evaluations were re-screened, and donor-specific anti-human leukocyte antigen antibodies (DSAs) were identified using the LABScreen Single Antigen (One Lambda). Mean fluorescence intensity values above 1,000 for DSAs against the two fields for HLA-A, -B, -C, -DRB1, and -DQB1 were considered positive.

Desensitization protocol and immunosuppressive regimen

Preoperative desensitization was performed in ABO-blood-type incompatible cases. Two weeks before transplantation, a single dose of rituximab (375 mg/m^2 body surface area) was administered to the patients. Subsequently, they received CsA (target trough level: 80-100 ng/mL) and mycophenolate mofetil (MMF; 20 mg/kg/day) and underwent between zero and five plasmapheresis sessions to achieve at least a 16-fold preoperative reduction in anti-blood group isoagglutinin titers. The basic CsA-based immunosuppressive regimen following KT has been previously described [16].

Immune monitoring via *in vitro* mixed lymphocyte reaction assays

To evaluate the anti-donor immune reactivity of the patients, T cell responses to alloantigens were evaluated via MLR assays using an intracellular CFSE labeling technique. The detailed regimens and calculation of the stimulation indices (SIs) have been described previously [16]. To evaluate the immune reactivity of recipients, CFSE-MLR assays were performed at 1, 2, and 3 weeks, after 1, 3, 6, and 12

months, and annually after KT. After analyzing the proliferation of $CD4^+$ and $CD8^+$ T cell subsets in response to anti-donor and comparing it with that in response to anti-third party stimuli in protocoled MLR, we categorized the immune status as hypo-, normo-, or hyperresponsive [30]. Therapeutic adjustments for immunosuppressants were determined by tapering dosages in cases exhibiting anti-donor hyporesponsiveness in both T cell subsets and increasing them for anti-donor hyperresponsiveness.

Definitions and other laboratory data

Serum creatinine levels were monitored every day until postoperative week 2 and at least every other day until postoperative week 4. TCMR was defined as graft dysfunction as evidenced by elevated serum creatinine levels in the absence of vascular or urinary complications or infection. Vascular and urinary complications were identified using Doppler ultrasonography (US). The clinical suspicion of TCMR was supported by the protocoled MLR assay, which can rigorously monitor rejection [16]. TCMR diagnosis was based on the Banff criteria in episode biopsies. Episodes of rejection were initially treated with either mini pulse (125-250 mg intravenous methylprednisolone (MP) for ≥ 2-3 days) or steroid pulse (500 mg intravenous MP for ≥ 3 days) according to the clinical severity of TCMR, with a gradual tapering of the dose and return to the previous oral triple-drug regimen.

The criteria for urinary tract infections (UTIs) were the presence of microbes at a concentration of $> 10^4$ CFU/mL of urine or $> 10^3$ CFU/mL after culture with clinical signs and symptoms and the use of antibacterial agents. Cytomegalovirus (CMV) antigenemia-positive was defined as the detection of ≥ 3/50,000 CMVpp65-positive cells. Clinical and laboratory data were extracted from the patient' medical charts.

Statistical analysis

Statistical analyses were performed using the JMP version 16 (SAS Institute, Cary, NC, USA). Quantitative variables are expressed as mean ± standard deviation or median and range. Student's t-test, Wilcoxon-Mann-Whitney test, chi-squared test, and Fischer's exact test were used to compare variables between the two groups. Kaplan-Meier analysis was used to compare the time-to-event variables. The differences between the curves were examined using the log-rank test. Statistical significance was set at $P < 0.05$.

Declarations

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Author contributions

YS, KI, AT, YT, and HO participated in the research design. YS, KI, and HO wrote the manuscript. YS, KI, AT, SA, YT, HT, MO, NT, and DM participated in the research. YS, KI, TA, YT, and HO participated in data analysis.

Declarations of interest:

The authors declare that they have no known competing financial interests or personal relationships that could have influenced to influence the work reported in this study.

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Figures

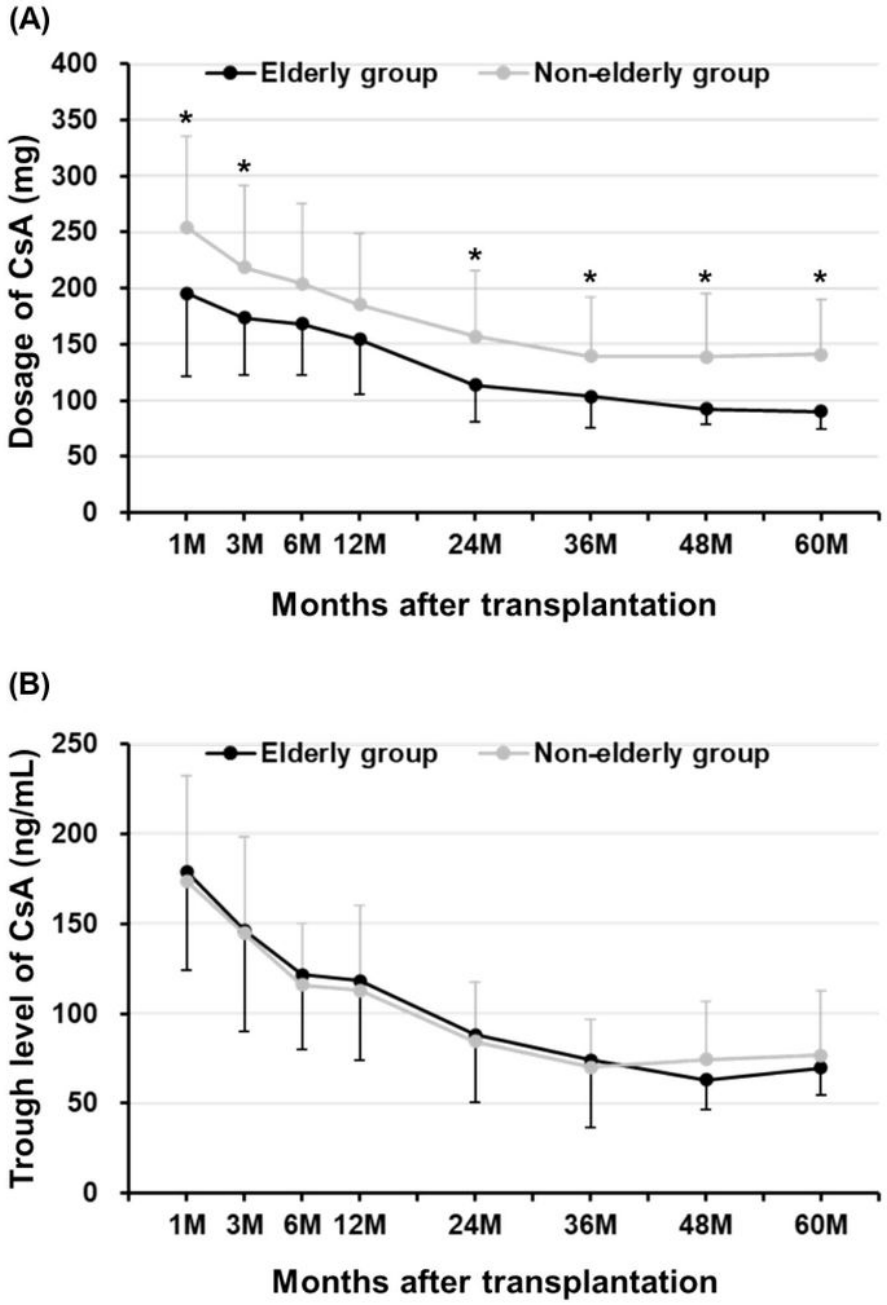

Figure 1

Comparison of the dosages of CsA and the trough levels of CsA between elderly and non-elderly recipients.

Kinetics of the dosages of CsA (A) and trough levels of CsA (B) in elderly (aged ≥ 60 years) and non-elderly recipients during the 5 years after kidney transplantation are shown. Data are presented as the

mean ± standard deviation. Black line, elderly group; gray line, non-elderly group. * P < 0.05.

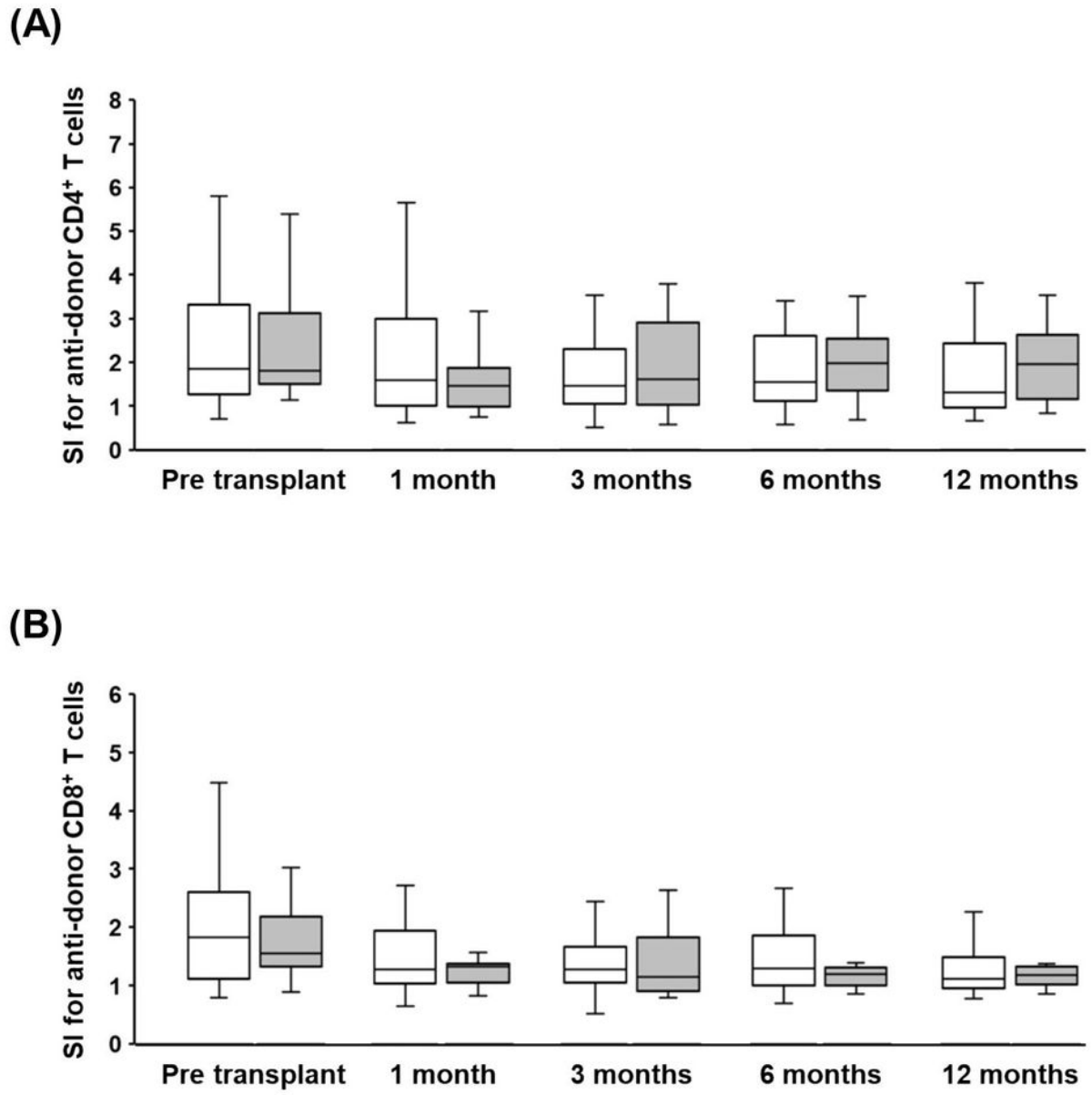

Figure 2

Comparison of the anti-donor T cell responses between elderly and non-elderly recipients.

Anti-donor T-cell responses based on CFSE-MLR were analyzed in elderly (aged ≥ 60 years) and non-elderly recipients during the first years after kidney transplantation. The stimulation index (SI) values for

anti-donor $CD4^+$ T cells (A) and $CD8^+$ T cells (B) are shown. Gray box, elderly group; white box, non-elderly group. Data are shown as the median, 25th and 75th percentiles, and range. The Wilcoxon-Mann-Whitney test was used to test the differences between the elderly and non-elderly groups.

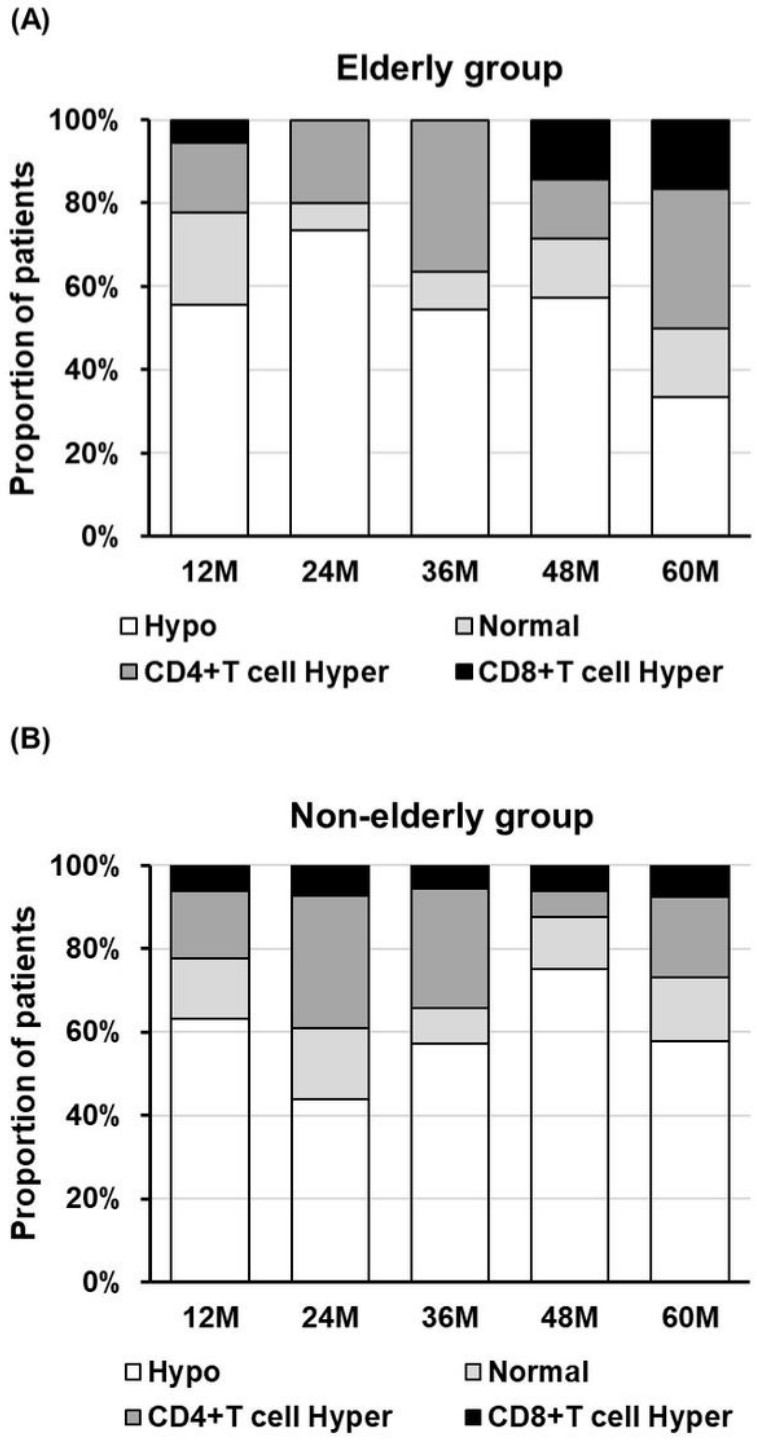

Figure 3

Comparison of immune status after kidney transplantation between elderly and non-elderly recipients.

The immune status of the patients after kidney transplantation was classified into four categories. By analyzing the proliferation and CD25 expression of $CD4^+$ and $CD8^+$ T cell subsets in response to anti-donor and anti-third-party stimuli, immune status was categorized as hypo-, normo-, or hyperresponsiveness. The proportions of patients categorized according to their immune status during the 5 years after kidney transplantation are shown. White box, hypo-responsiveness; light gray box, normo-responsiveness; dark gray box, hyper-responsiveness of $CD4^+$ T cells; black box, hyper-responsiveness of $CD8^+$ T cells. (A) Elderly group (aged ≥60 years); (B) non-elderly group.

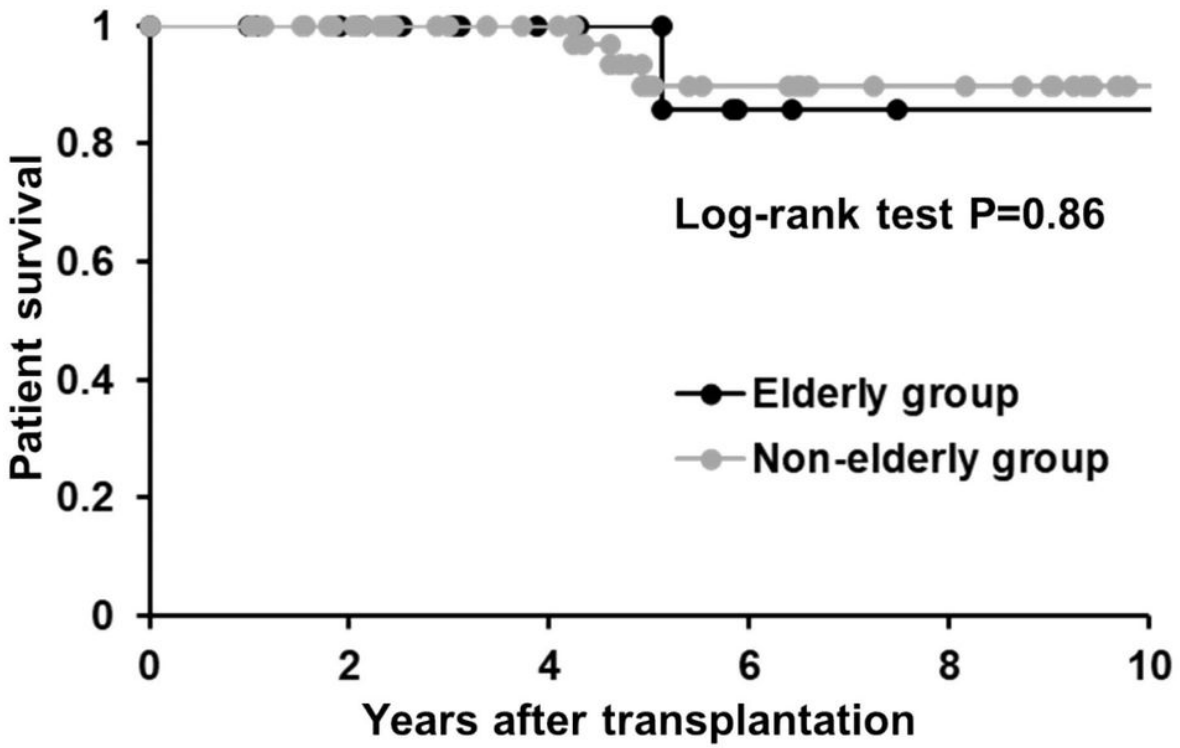

Figure 4

Comparison of Kaplan-Meier survival rates between elderly and non-elderly recipients.

Kaplan–Meier survival curves with log-rank statistics for 10 years of patient survival are shown. Black line, elderly group (aged ≥60 years); gray line, non-elderly group.

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