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ORIGINAL RESEARCH ARTICLE

Impact of tacrolimus versus cyclosporin A on renal function during the first year after heart transplant

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Abstract

Aims Nephrotoxicity of calcineurin inhibitors (CNIs) is associated with adverse events in patients undergoing heart transplant (HTx), although studies directly comparing tacrolimus (TAC) versus cyclosporin A (CsA), especially in combination with everolimus and low-dose CNIs approach, are limited. Thus, we sought to investigate the associations of TAC and CsA with clinical outcomes in HTx recipients, with specific focus on renal function.

Methods and results From August 2007 to February 2017, 72 consecutive patients (39 treated with TAC vs. 33 with CsA) receiving *de novo* HTx in a single transplant centre were retrospectively evaluated. We used the instrumental variable method to account for unmeasured confounding. The study outcomes were percentage change in estimated glomerular filtration rates (eGFR) (safety endpoint) and biopsy-proven acute rejection (efficacy endpoint) within the first year after HTx. The enrolled patients (median age 40 years) were predominantly men (68%). There were no significant differences in baseline characteristics, including eGFR (64.8 [45.7–96.4] mL/min/1.73 m^2 in TAC vs. 65.6 [57.9–83.0] mL/min/1.73 m^2 for CsA; $P = 0.48$), other than sex (male, 49% for TAC vs. 91% for CsA; $P < 0.001$) between the two groups. Within the first year after HTx, 23 (59%) in the TAC group switched mycophenolate mofetil to everolimus, whereas 16 (48%) in the CsA group ($P = 0.52$). At 12 months, the rates of mortality and end-stage renal disease requiring renal replacement therapies were both 0%. In the instrumental variable analysis, no differences in renal function as well as graft rejection for 1 year after HTx existed between the TAC and CsA groups. These results were similar when taking into account of everolimus use.

Conclusions Irrespective of everolimus use with low-dose CNIs, our analysis using the instrumental variable method showed no differences in renal function as well as graft rejection during the first year after HTx between HTx recipients who received TAC or CsA.

Keywords Calcineurin inhibitor; Graft rejection; Heart transplant; Instrumental variable; Renal dysfunction

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Introduction

Heart transplant (HTx) is the gold standard therapy for end-stage heart failure.[1] Despite HTx has greatly improved long-term prognosis, post-HTx patients are still facing several problems such as renal dysfunction. Renal dysfunction is frequently observed prior to HTx and typically followed by a progressive decline over time after HTx,[2–4] which contributes late mortality in HTx recipients. Therefore, renal protection strategies are warranted to further improve patients' outcomes after HTx.[5,6]

Renal dysfunction after HTx is derived from pre-operative, intraoperative, and post-operative factors. Among the post-operative factors, the use of calcineurin

inhibitors (CNIs) has a marked effect on renal function. Indeed, CNI, including tacrolimus (TAC) or cyclosporin A (CsA), as a part of immunosuppressive regimens has successfully increased graft survival and life expectancy of organ transplant recipients, but they often trigger renal dysfunction, which is associated with adverse events.[7] However, data comparing TAC-based and CsA-based immunosuppression regimens and focusing on nephrotoxicity remain limited,[8,9] especially in the era where mammalian target of rapamycin (mTOR) inhibitor everolimus (EVL) is available and results in reducing doses of CNIs. Herein, using data of HTx patients who use EVL frequently within 1 year after HTx, we sought to investigate the association of TAC-based and CsA-based immunosuppression regimens with renal function as well as graft rejection after HTx.

Methods

Study sample

From August 2007 to February 2017, 74 consecutive patients who underwent *de novo* HTx in a single transplant center in Japan were enrolled in the present study. All patients, except for one patient, underwent HTx following left ventricular assist device (LVAD) bridging. After excluding two patients who died during a perioperative period, 72 patients completed the month 12 hospital visit. The study protocol was approved by the institutional review board at the University of Tokyo, and research was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject before the study.

Immunosuppression regimen

All recipients initially received the standard triple therapy with CNI (TAC or CsA) and mycophenolate mofetil (MMF) in addition to low dose prednisolone. In general, the trough levels of TAC were maintained at 10–15 ng/mL until 3 months, approximately 10 ng/mL until 6 months, 5–8 ng/mL until 1 year and approximately 5 ng/mL after 1 year. On the other hand, the target trough levels of CsA during the first 3 months were 300–400 ng/mL, with reduction to 250–300 ng/mL until 6 months, 200–250 ng/mL until 1 year, and 150–200 ng/mL after 1 year. MMF was started <1 week after transplant and maintained at a dose of 1500–2000 mg/day. Prednisolone was initially administered at 1 mg/kg and then tapered off gradually until 1 year.

The initiation of EVL was determined based on the following institutional criteria: conversion from MMF to EVL because of neutropenia or digestive symptoms; progression in coronary artery disease (cardiac allograft vasculopathy); repeated episodes of cytomegalovirus infection; and repeated acute cellular rejection with the International Society of Heart and Lung Transplantation 2004 grade $\geq$ 2R.[10] One patient started EVL in addition to MMF because of repeated graft rejection, and other patients switched MMF to EVL around 6 months after HTx. Complete healing of the surgical wound was confirmed before the initiation of EVL. The target trough levels of CNI were maintained with 30% reduction in the standard levels during EVL treatment. The trough levels of EVL were maintained within 3–8 ng/mL.

Study outcomes and follow-up assessment

The study outcomes were (i) percentage change in estimated glomerular filtration rate (eGFR) between baseline (the day prior to HTx) and 1 year after HTx and (ii) frequencies of biopsy-proven acute rejection within 1 year after HTx. Percentage change in eGFR was calculated by the following formula: (eGFR at 1 year after HTx − eGFR at baseline)/(eGFR at baseline) × 100 (%).

Endomyocardial biopsies were performed weekly during the first month after HTx, biweekly during months 1 to 3, monthly during months 3 to 6, at month 12 and then yearly, and when clinically indicated. Rejection episodes were graded according to the revised International Society of Heart and Lung Transplantation classification and an episode of acute rejection was defined as $\geq$2R.[10] Trough levels of immunosuppressants as well as laboratory data, including serum creatinine, were regularly measured during the study period. The eGFR was calculated using the Modification of Diet in Renal Disease Equation for Japanese Patients, proposed by the Japanese Society of Nephrology.[11]

Statistical analysis

Results were expressed as mean with standard deviation or median with interquartile range for continuous variables and as frequency and percentages for categorical variables. Patients were divided into two groups based on the type of CNIs (TAC vs. CsA), and their baseline characteristics were compared using the unpaired *t*-test or Mann–Whitney U-test for continuous variables and the Pearson's chi-squared test or Fisher's exact test for categorical variables.

To mitigate not only measured but also unmeasured confounding biases, we adopted the instrumental variable (IV) method. The choice between TAC and CsA largely depended on the preference of the transplant centres or treating physicians, as well as on the side effect profiles of each medication, and was individually tailored to the recipient. We used sex as the IV, and our choice was

informed by clinical knowledge because CsA tend to avoid for adverse effects such as hypertrichosis among female patients. We hypothesized that sex (men or women) as an instrument would behave similarly to physician prescribing preferences. There are three important assumptions for the validity of the IV.[12] The first assumption is the existence of correlation between the IV and the intervention (selection of CNIs). The second assumption is no expected confounders between the IV and the intervention. The third assumption is no correlation between the IV and the outcomes (renal function and rejection). To confirm the validity of the first assumption, we made binominal distribution model (hypothesis: the immunosuppressant selection was influenced by sex) and calculated the polychoric correlation, a correlation indicator between categorical variables. For the second assumption, we evaluated the differences in the baseline characteristics between men and women. If there were no statistically significant differences in inferred confounders, the assumption was not collapsed. Finally, we constructed gamma distribution model for the primary outcome (hypothesis: the change in eGFR was not influenced by sex when adjusted with other confounders). We also constructed a binominal model for the secondary outcome (rejection). We found that the IV was a good predictor of CNI selection (odds ratio [OR], 2.35, 95% confidence interval [CI], 1.67–3.04, $P < 0.001$; polychoric correlation coefficient, 0.70), was well balanced across patient characteristics (*Table* S1 in the Supplementary Appendix), and was independent of the patient outcomes (percentage change in eGFR; OR 1.00, 95% CI, 0.99–1.01, $P = 0.82$, frequencies of biopsy-proven acute rejection, OR, 1.05, 95% CI, 0.76–1.34, $P = 0.87$) to meet the required assumptions as a valid instrument.

We used a two-stage least square method for these IV analysis. This model is well established and widely accepted in economy, biology, epidemiology, and medical research field.[13] This method needs to construct two models. The first-stage model is simple association between the selection of CNIs and sex. The second-stage model is association between outcomes and the IV (sex) adjusting with age, diabetes mellitus, dose of loop diuretics, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker use after transplant, mineralocorticoid receptor antagonist use after transplant, haemoglobin level, and ischemic aetiology. When the observation period of the patients is same, the frequency of biopsy-proven acute rejection follows the Poisson distribution.[14] We adopted the Poisson distribution model for the second stage model for the frequency of biopsy-proven acute rejection. In addition, we made different second-stage models that include adding EVL within 1 year as a confounding. All probability values were two-tailed, and values of $P < 0.05$ were considered statistically significant. All statistical analyses were performed with RSTUDIO software, Version 3.2.3.

Results

Baseline characteristics

In the total cohort, patients were predominantly men (68%) with a median age of 40 (interquartile range, 30–52) years. Of these, 39 (54%) received TAC after HTx, while 33 (46%) patients received CsA. Baseline characteristics between patients who received TAC and CsA are shown in *Table* 1. Compared with the TAC group, no significant differences in patient characteristics were observed in those with CsA, including baseline eGFR (64.8 [45.7–96.4] mL/min/1.73 m^2 in the TAC group vs. 65.6 [57.9–83.0] mL/min/1.73 m^2 in the CsA group, $P = 0.48$), other than sex, body mass index, and haemoglobin levels; the TAC group comprised more females and had a lower body mass index and haemoglobin level (each $P < 0.05$). With respect to induction therapy, two patients were treated with an anti-CD25 receptor monoclonal antibody (two in the TAC group). In three patients who were cytomegalovirus antibody negative, preventive treatment with ganciclovir was performed for 3 months after HTx.

Within the first year after HTx, 23 (59%) in the TAC group initiated EVL, whereas 16 (48%) in the CsA group did ($P = 0.52$) (Table 2). The trough levels of each CNI were well controlled within the target level at the time of 1 year after HTx. In addition, the trough level of EVL was also controlled within the target level in both groups. At 12 months, the rates of mortality and end-stage renal disease requiring renal replacement therapies were both 0%. During the median follow-up period of 6.0 (interquartile range, 4.2–7.4) years, six patients died in the TAC group and one in the CsA group ($P > 0.05$ for log-rank test).

Impact of calcineurin inhibitors on renal function and graft rejection

Overall, there was no significant difference in eGFR 1 year after HTx between the TAC and CsA groups (57.5 [39.7–82.4] mL/min/1.73 m^2 in the TAC group vs. 51.3 [37.4–67.0] mL/min/1.73m^2 in the CsA group, $P = 0.19$). *Figure* 1 shows the percentage change in eGFR from baseline to 1 year after HTx that are normalized by baseline eGFR, indicating that CsA was associated with a higher risk of worsening renal function when compared with TAC ($P = 0.02$). In addition, use of loop diuretics before HTx was significantly associated with a decreased risk of worsening renal function ($P < 0.05$) among several other factors, including diabetes mellitus and use of renin-angiotensin-aldosterone-system inhibitors.

In contrast, there was no difference in the frequencies of biopsy-proven acute rejection between the two groups (0.8 ± 1.3 times in the TAC group vs. 0.7 ± 0.9 times in the CsA group; $P = 0.91$) (Figure 2). When comparing the total

Table 1 Baseline characteristics according to calcineurin inhibitors

Characteristic	TAC $n = 39$	CsA $n = 33$	P value
Age, years	39.4 ± 13.7	40.9 ± 12.6	0.65
Male, *n* (%)	19 (49)	30 (91)	<.001
Body mass index, kg/m^2	19.0 ± 5.5	20.7 ± 6.3	0.03
Aetiology, *n* (%)			0.27
DCM	28 (72)	24 (73)	
ICM	3 (8)	5 (15)	
Others	8 (20)	4 (12)	
Comorbidities, *n* (%)			
Hypertension	1 (3)	0 (0)	1
Diabetes mellitus	2 (5)	2 (6)	1
Dyslipidaemia	3 (8)	7 (21)	0.19
Laboratory findings before HTx			
Haemoglobin, g/dL	11.2 ± 2.1	12.3 ± 1.9	0.02
BUN, mg/dL	16.9 ± 11.1	15.6 ± 5.2	0.90
Albumin, mg/dL	4.0 ± 0.6	4.2 ± 0.5	0.16
eGFR, ml/min/1.73 m^2			
Before HTx	64.8 (45.7–96.4)	65.6 (57.9–83.0)	0.48
1 month after HTx	72.4 (47.9–84.1)	68.9 (55.7–88.0)	0.60
BNP, pg/mL			
Before LVAD implantation[b]	857 (456–1,326)	920 (612–2,666)	0.41
Before HTx	242 (91–397)	144 (89–297)	0.46
Medication before HTx, *n* (%)			
ACEI or ARB	18 (46)	19 (58)	0.47
Beta blocker	34 (87)	31 (94)	0.57
MRA	25 (64)	19 (58)	0.75
Loop diuretics	13 (33)	16 (49)	0.29
Furosemide equivalent, mg [a]	12.8 ± 29.7	13.0 ± 20.4	0.29
Medication after HTx, *n* (%)			
ACEI or ARB	28 (72)	27 (82)	0.47
Beta blocker	22 (56)	18 (55)	1
MRA	9 (23)	13 (39)	0.22
CCB	10 (26)	9 (27)	0.88
Statin	33 (85)	32 (97)	0.11
Loop diuretics	11 (28)	11 (33)	0.83
Furosemide equivalent, mg[a]	7.7 ± 12.9	7.6 ± 12.0	0.92

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; BUN, blood urea nitrogen; CCB, calcium channel blocker; CsA, cyclosporin A; DCM, dilated cardiomyopathy; eGFR, estimated glomerular filtration rate; HTx, heart transplantation; ICM, ischemic cardiomyopathy; LVAD, left ventricular assist device; MRA, mineralocorticoid receptor antagonist; TAC, tacrolimus.
[a]Furosemide 20 mg = Azosemide 30 mg = Torsemide 10 mg.
[b]In the 43 patients (20 in the TAC group and 23 in the CsA group), BNP levels at the time of LVAD implantation were available.

Table 2 Dose and trough level of immunosuppressants

Dose and trough level	TAC $n = 39$	CsA $n = 33$	P value
CNIs, dose at 1 year (mg)	3.0 (2.2–4.0)	180 (123–220)	NA
CNIs, trough level at 1 year (ng/mL)	7.4 (5.4–9.4)	206 (132–247)	NA
Everolimus[a], number (%)	23 (59)	16 (49)	0.52
Everolimus, dose at 1 year (mg)	1.25 (1.0–1.5)	1.0 (1.0–1.0)	<0.001
Everolimus, trough level at 1 year (ng/ml)	3.5 (3.0–5.0)	8.0 (5.6–9.9)	<0.001

CNIs, calcineurin inhibitors; CsA, cyclosporin A; NA, not assessed; TAC, tacrolimus.
[a]indicates switching from mycophenolate mofetil to everolimus within 1 year after heart transplantation.

number of biopsy-proven acute rejection episodes, irrespective of the grade of acute rejection (i.e., from grade 1R to 3), no significant difference was observed between the two groups (4.4 ± 2.5 episodes in the TAC group vs. 4.2 ± 2.5 episodes in the CsA group; $P = 0.75$). During the study period of 1 year after HTx, there were no patients who experienced acute antibody-mediated rejection, were hemodynamically unstable or experienced acute heart failure. In addition, two patients were newly diagnosed with diabetes mellitus and were treated with oral hypoglycemic drugs 1 year after HTx (one and one each in both groups).

After adjustment with using the IV analysis, the type of CNIs did not remain to be linked with worsening renal function at 1 year after HTx (OR, 1.01, 95% CI 0.99–1.02, $P = 0.89$). In addition, there was also no relationship between the type of CNIs and the frequencies of biopsy-proven acute rejection (OR, 1.01, 95% CI 0.78–1.25, $P = 0.87$). Even if added use of EVL in the IV method, the differences in the impacts of

Figure 1 Distribution of normalized change in estimated glomerular filtration rate (eGFR) by calcineurin inhibitors. There was a significant difference in normalized change in eGFR between the two groups (P = 0.019). Normalized change in eGFR was calculated as the following equation: (eGFR at 1 year after heart transplant − eGFR at baseline) / (eGFR at baseline) × 100. eGFR, estimated glomerular filtration rate.

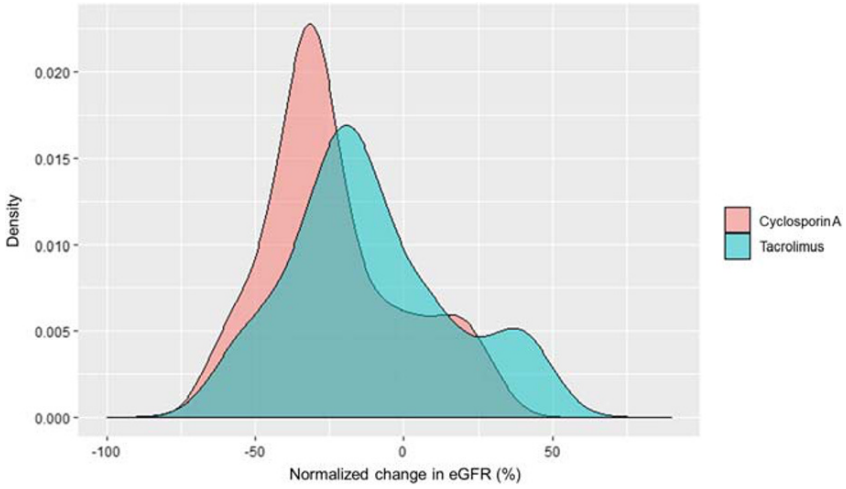
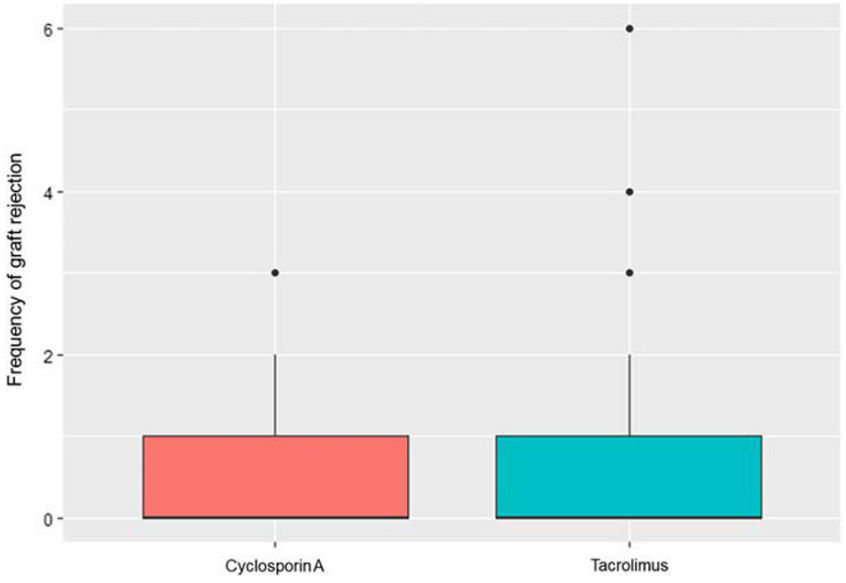


Figure 2 Frequencies of biopsy-proven acute rejection by calcineurin inhibitors. There was no difference in frequencies of biopsy-proven acute rejection between the two groups (P = 0.91).



TAC versus CsA on renal function as well as graft rejection were not observed.

Discussion

Our research found that (i) almost a half of the patients initiated EVL within 1 year after HTx and (ii) the type of CNIs was not associated with a risk of worsening renal function as well as biopsy-proven acute rejection in the IV method. Our results suggest that alternative immunosuppression strategies, irrespective of the type of CNIs, are needed to protect renal function and further improve long-term outcomes in HTx recipients, and several approaches minimizing renal damage are under investigation.

Immunosuppression regimens combined with CNIs are widely available and accepted in the current HTx. The advantage of CNIs compared with cytotoxic immunosuppressants is that they act specifically on targeted sites in the immune

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system, not affecting other rapidly proliferating cells. The main mechanism of CNIs involves binding to specific proteins to form complexes that inhibit gene transcription for the expression of molecules playing a key role in the immune responses, thereby blocking the signal transduction pathway responsible for T-cell and B-cell activation.[15,16] Several prospective studies have confirmed that TAC and CsA have a similar efficacy in preventing acute graft rejection in HTx recipients and show similar survival rates for up to 5 years.[8,17,18] Conversely, CNIs cause renal vasoconstriction of afferent and efferent glomerular arterioles via several mechanisms (e.g., endothelin, thromboxane, renin-angiotensin system, and nitric oxide) that predisposes patients to acute and chronic kidney injury.[19–21] Decreased GFR in association with reduced renal blood flow, elevated mean arterial pressure, increased renal vascular resistance, and albumin excretion have been observed in patients treated with CNIs, which can lead to progressive arteriolopathy, and glomerular ischemic collapse.[22] Nephrotoxicity triggered by CNIs is well established, and its incidence is similar between TAC and CsA,[8,23] although there is limited information on dosing patterns (i.e., low-dose CNIs with mTOR inhibitors) and renal function. Kobashigawa et al previously reported a randomized, open-label, parallel three-group trial comparing TAC/sirolimus, TAC/MMF, with CsA/MMF in HTx patients.[24] The study showed that TAC/MMF was superior to CsA/MMF in terms of graft rejection during the first year after HTx (23.4% vs. 36.8%, P = 0.029) and TAC/sirolimus was likely to be associated with progressing renal dysfunction, which was the primary reason for withdrawn from sirolimus.[24] In our study, no differences in renal impairment as well as graft rejection at 1 year were observed between TAC and CsA, although the patients who initiated EVL during the first year after HTx tended to have a lower eGFR at baseline and worsen renal function at the time of 1 year after HTx, compared with those without EVL, irrespective of the types of CNIs (data not shown).

The alternative approaches involving adjunctive renal protection strategies are needed to avoid CNI nephrotoxicity in HTx recipients. One of the alternatives is the minimization or complete withdrawal of CNIs in combination with mTOR inhibitors or MMF. Despite several trials have been performed to compare immunosuppression regimens with low-dose CNIs to standard-dose CNIs, their results remained controversial.[25–29] In addition, there are few studies directly comparing low-dose TAC and low-dose CsA, especially in combination with mTOR inhibitors. Using a retrospective observational data, Fuchs et al previously showed no significant difference in renal function between EVL with low-dose TAC and with low-dose CsA for up to 5 years after HTx, while a higher risk for graft rejection were observed in patients receiving EVL/CsA compared with EVL/TAC.[30] In the present study, we performed the IV analysis to adjust unmeasured confounders to investigate the impact of TAC versus CsA on clinical outcomes, and showed no differences in renal function as well as graft rejection during the first year after HTx. Further large-scale studies are warranted to confirm these results to tailor immunosuppression strategies for renal protection in organ transplant recipients.

Although the current situation for HTx varies internationally, the shortage of donors remains an important issue, even in the non-Western countries. In Japan, the number of HTx procedures is small with an average of 30–40 procedures performed annually and the mean waiting period for HTx exceeding 3 years.[31] Thus, almost all patients are pretreated with implantation of an LVAD as a long delay leads to low levels of B-type natriuretic peptide, as was seen in our cohort. We also found that diuretic use before HTx was paradoxically associated with a lower risk of worsening renal function 1 year after HTx, which indicates the presence of right heart failure under LVAD support related to renal congestion, resulting in renal dysfunction at baseline. This may indicate improved renal function after HTx. In addition, it is of note that none of the patients died at 1 year after HTx, indicating a marked improvement in prognosis after HTx, which is in line with national reports from Japan. In Japan, the survival rates at 5, 10, and 15 years after HTx are 92.7%, 89.6%, and 81.8%, respectively, regardless of the underlying heart diseases.[31]

In any analysis targeting on unselected populations, that is, real-world settings, there are a variety of conditions leading to biased estimates. Sample selection bias arises when there are unmeasured confounders that influence both treatment selection and outcomes. Failure to account for these unmeasured variables results in biased estimates of treatment effects because the parameter estimates of the treatment variable also reflects the effects of the missing variables on outcomes. To avoid biases and establish causal effects of specific treatment strategies, a randomized controlled trial is needed to overcome these issues. However, conducting randomized controlled trials is always a challenge. Thus, a well-designed observational study, including adequate criteria and information, is warranted to investigate the relationship between the treatment and outcomes. The IV analysis enables to account for unmeasured variables despite there are strict constraints when it is applied. In our analysis, sex is considered a relevant IV; however, our results should be treated with caution because it is difficult to find ideal situations to use the IV method.

Limitations

There were some limitations in our study. There is a possibility that unknown confounders influenced our results because of the nature of the study design. Although the IV analysis adjusted for confounding by indication, residual confounding is probable. Second, these results are derived from a single transplant centre, and the sample size of the present study

is small. In general, patient background can highly influence the results of clinical studies. Compared with studies in Western countries, the decline in renal function for 1 year after HTx was slightly worse or almost the same in the present study.[32–34] Furthermore, although the protocols of immunosuppressive regimens vary among each affiliation of HTx centres, the data in the present study were similar to those in the national database.[31] Despite differences in the impact of polymorphisms on TAC and CsA pharmacokinetics, studies comparing Japanese patients with other ethnicities with regard to renal function and other clinical outcomes are scarce. Our results need to be confirmed in large-scale datasets. Third, the follow-up periods are relatively short and there is a possibility not to clarify the effects of each CNIs on the outcomes for long-term periods. Finally, we have no detailed data on other adverse effects of CNIs, including hypertension, dyslipidaemia, and hyperuricemia/gout, which can influence the trajectories of HTx recipients, including renal dysfunction and prognosis.

Conclusions

In the context of the absence of robust evidence confirming the best combination of immunosuppressants, it is valuable to perform an investigation to clarify such effects in observational studies. Under sufficient adjustment for biases using the IV method, we showed no significant differences in renal function and acute biopsy-proven rejection between TAC and CsA. In addition, among patients who initiated EVL and reduced doses of CNIs within the first year after HTx, these results were similar between the TAC and CsA group. Our findings can provide an important insight into future trials to identify the best effective immunosuppressive treatment in HTx recipients.

Conflict of interests

Dr Shiraishi has received an honorarium from Otsuka Pharmaceutical Co. Ltd. Dr Amiya and Dr Hatano belong to the Department of Therapeutic Strategy for Heart Failure, Graduate School of Medicine, University of Tokyo, which is endowed by Actelion Pharmaceuticals Japan Ltd., Otsuka Pharmaceutical, NIPRO CORPORATION, Terumo Corp., Senko Medical Instrument Mfg., Century Medical Inc., Kinetic Concepts Inc., St. Jude Medical, and has received honoraria from Takeda Pharmaceutical Co. Ltd., Bayer Yakuhin. Ltd., Otsuka Pharmaceutical Co. Ltd. Other authors have no conflicts of interest to disclose. There are no patents, products in development, or marketed products to declare.

Authorships

YS and EA contributed to the conception and design, data acquisition, and interpretation of the data, and writing and drafting of the manuscript. TK contributed to the analysis of the study. Remaining authors helped review the draft manuscript and supervised the study. All authors approved the final manuscript.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Baseline characteristics according to men and women

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