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ORIGINAL ARTICLE

Development and validation of a risk score for the prediction of cardiovascular disease in living donor kidney transplant recipients

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ABSTRACT

Background. Cardiovascular disease (CVD) is a major cause of death in kidney transplant (KT) recipients. To improve their long-term survival, it is clinically important to estimate the risk of CVD after living donor KT via adequate pre-transplant CVD screening.
Methods. A derivation cohort containing 331 KT recipients underwent living donor KT at Kyushu University Hospital from January 2006 to December 2012. A prediction model was retrospectively developed and risk scores were investigated via a Cox proportional hazards regression model. The discrimination and calibration capacities of the prediction model were estimated via the c-statistic and the Hosmer–Lemeshow goodness of fit test. External validation was estimated via the same statistical methods by applying the model to a validation cohort of 300 KT recipients who underwent living donor KT at Tokyo Women's Medical University Hospital.
Results. In the derivation cohort, 28 patients (8.5%) had CVD events during the observation period. Recipient age, CVD history, diabetic nephropathy, dialysis vintage, serum albumin and proteinuria at 12 months after KT were significant predictors of CVD. A prediction model consisting of integer risk scores demonstrated good discrimination (c-statistic 0.88) and goodness of fit (Hosmer–Lemeshow test $P = 0.18$). In a validation cohort, the model demonstrated moderate discrimination (c-statistic 0.77) and goodness of fit (Hosmer–Lemeshow test $P = 0.15$), suggesting external validity.
Conclusions. The above-described simple model for predicting CVD after living donor KT was accurate and useful in clinical situations.

Keywords: dialysis vintage, external validation, nutritional status, proteinuria, risk score

INTRODUCTION

In recent years elucidation of the pathologic aspects of immunological rejection and immunosuppression in kidney transplant (KT) recipients has improved short-term graft survival. There is now a focus on improving long-term survival. Cardiovascular disease (CVD) is the most frequent cause of death in KT recipients with functioning kidney allografts [1]. In deceased donor KTs cardiovascular mortality within 3 months

KEY LEARNING POINTS

What is already known about this subject?

- as kidney transplant (KT) recipients have improved short-term graft survival, there is now a focus on improving long-term survival and cardiovascular disease (CVD) is the most frequent cause of death in KT recipients with functioning kidney allografts;
- various preparations including pre-transplant CVD screening in living donor KT have reduced the prevalence of CVD in the early phase after KT, but it is still important to prevent new onset of CVD in the long-term phase after living donor KT; and
- one risk calculator was previously published, but the definition of outcomes was restricted to cardiac events and the proportion of cases involving deceased donors was high, and therefore the new CVD prediction model in living donor KT was developed in this study.

What this study adds?

- in this study, a new CVD prediction model with simple integer points risk score incorporating CVD history, diabetic nephropathy, recipient age, dialysis vintage, serum albumin level and proteinuria was developed for use in living donor KT recipients;
- the new prediction model had statistically high robustness because the model showed good discrimination and calibration in derivation cohort, and external validity was verified via another independent validation cohort; and
- when the previously published risk calculator was applied to the derivation cohort of this study, it performed moderately good external validation but not as well as the present risk model, suggesting that the difference may be attributed to the difference of background between the two studies.

What impact this may have on practice or policy?

- this risk score is useful for estimating long-term CVD prevalence, particularly in living donor KT recipients who have undergone adequate pre-transplant CVD screening, and KT recipients with highly weighted risk factors should be diligently followed up with CVD estimation;
- the simple integer points risk score utilized is readily amenable to clinical application because the simple integer risk score clearly shows how the risk factors affect CVD risk at a glance; and
- among risk factors, serum albumin level may be indicative of a nutrition status, and if KT recipients exhibit low serum albumin 12 months after KT, inadequate nutrition should be investigated.

was very high [2], suggesting that the potential for pre-transplant CVD was associated with high mortality. In contrast, CVD mortality in living donor KTs was lower in the early phase than it was in deceased donor KTs [2], and it increased gradually after the second year. In cases of living donor KT there is usually sufficient time for various advantageous preparations, including pre-transplant CVD screening. While such preparations have substantially reduced the prevalence of CVD in the early phase after KT , it is still important to prevent new onset of CVD in order to improve the long-term survival of KT recipients.

Various risk factors are associated with CVD in KT recipients [3]. A risk calculator pertaining to CVD was previously developed based on patients who participated in the Assessment of LEscol in Renal Transplantation (ALERT) study [4, 5]. In the ALERT trial, patients with unstable angina or hospital-confirmed myocardial infarction <6 months before randomization were excluded and the outcome was restricted to cardiac events. In addition, the proportion of cases involving deceased donors was extremely high. Thus, it is unclear whether indications derived from that prediction model can reliably predict any CVD events or be extrapolated to living donor KT recipients. In this study, a simple new score-based model for predicting CVD in living donor KT recipients was developed, then its external validity was assessed using a second independent cohort.

MATERIALS AND METHODS

Study population and design

Derivation cohort. From January 2006 to December 2012 a total of 375 patients underwent living donor KT at Kyushu University Hospital in Japan. Of these, 23 were excluded because they were aged <16 years. A further 21 were excluded because they were lost to follow-up within 12 months after KT, including 12 who changed hospitals, 3 who reached end-stage kidney disease and 6 who died. The causes of death among the six dead patients were four infections, one hypoglycemia of unknown cause and one subarachnoid hemorrhage. The remaining 331 patients were enrolled in accordance with a registered study protocol.

Validation cohort. From January 2006 to December 2012 a total of 518 patients underwent a living donor KT at Tokyo Women's Medical University Hospital in Japan. A total of 218 of these patients were subsequently excluded from analysis in this study, 139 whose serum albumin data were missing, 78 who were aged <16 years and 1 who died within 12 months after KT. The remaining 300 patients were enrolled in the study.

This observational study was performed in accordance with the guidelines of the Declaration of Helsinki. Data were

extracted from the Japan Academic Consortium of KT (JACK) study-II. Written informed consent to the collection of data from their medical records was obtained from all patients at the time of KT. The study was registered at the University Hospital Medical Information Network clinical trial registry (ID: UMIN000033449) and was approved by the Institutional Review Boards of all participating institutions.

Clinical measures and definitions

Demographic and clinical data were retrospectively obtained from medical records. Twelve months after the KT was defined as the start of observation because graft function stabilizes and the incidences of hazardous complications such as infection and acute rejection fall at the period. To develop a risk prediction model, 25 potentially predictive factors were selected; recipient age, sex, body mass index (BMI), smoking history, CVD history, diabetic nephropathy, number of transplantations, pre-emptive/non-pre-emptive KT, dialysis vintage, donor age, ABO compatibility, post-transplant diabetes mellitus, history of rejection, BK polyomavirus (BKPyV) infection and cytomegalovirus (CMV) infection, hypertension, dyslipidemia, proteinuria, estimated glomerular filtration rate (eGFR) and serum levels of total cholesterol, uric acid, calcium, phosphate, albumin and C-reactive protein (CRP). 'Smoking history' was defined as present or past history of smoking. 'CVD history' was defined as CVD onset before and within 12 months after KT. Post-transplant diabetes mellitus was defined as the first diagnosis of diabetes mellitus after KT [6]. Hypertension was defined as blood pressure $\geq$140/90 mmHg or the current use of antihypertensive agents. Dyslipidemia was defined as a total cholesterol level $\geq$220 mg/dL or the use of lipid-modifying agents. Proteinuria was defined as a urinary protein-to-creatinine ratio $\geq$0.15 g/gCr or a urine qualitative test result of $\geq$1+. Corrected calcium levels in serum were calculated using Payne's formula [7], and eGFR was calculated using the appropriate equation for Japanese chronic kidney disease (CKD) patients [8]. CMV infection was defined as the presence of pp65 antigenemia. BKPyV nephropathy was diagnosed in graft biopsies with tubular cytopathic changes and positive staining of simian virus 40 large antigens. Rejection was restricted to biopsy-proven cases. Protocol biopsies at 3 and 12 months after KT or episode biopsies were performed at both institutions, with reference to the Banff 2013 working classification [9].

Outcome

As a primary outcome, 'CVD event' was based on the onset of three endpoints; cardiac event, stroke and peripheral arterial disease. A cardiac event was defined as congestive heart disease with New York Heart Association functional classification III or IV, coronary artery disease treated with percutaneous coronary intervention or coronary artery surgery, myocardial infarction with ST elevation on electrocardiography, severe valvular disease or sudden unexplained death. Stroke was defined as brain infarction or hemorrhage with any symptoms recorded. Peripheral arterial disease was defined as arteriosclerotic obliterans treated with revascularization or lower limb amputation.

Statistical analysis

Data were expressed as frequency and percentage, mean and standard deviation or median and interquartile range, as appropriate. Categorical variables in the derivation cohort and the validation cohort were compared via the chi-square test, and continuous variables were compared via the *t*-test or the Mann–Whitney U test. Univariable analyses were performed in the derivation cohort to identify risk factors for CVD in living donor KT recipients.

To generate a prediction model, independent CVD risk factors were selected using multivariable Cox proportional hazards regression analysis with backward elimination at a threshold of $P < 0.05$. The risk score for each selected variable was weighted according to the estimated regression coefficient of the final Cox proportional hazards model. For these analyses, patients were censored at the date of graft failure, their non-cardiac death, change hospitals or at the end of follow-up for those still alive. This methodology was based on procedures described by Sullivan *et al.* [10]. In order to facilitate use in clinical practice, a simple integer point score was created for each variable. We rounded off regression coefficients divided by the smallest coefficient in the model to the nearest integer. For internal and external validation of the prediction model, its discriminative capacity was assessed via the c-statistic, and the calibration 6-year timeframe via the Hosmer–Lemeshow and whole-time calibration via the Hosmer–May goodness of fit test were performed [11, 12]. Trends in categorical values across predictive risk scores were assessed using the Cochran–Armitage test. JMP version 13.0.0 (SAS Institute, Cary, NC, USA) and the R software package version 3.4.1 (R Development Core Team) were used for all statistical analyses. A two-tailed P-value of 0.05 was deemed to indicate statistical significance.

RESULTS

Baseline characteristics in the study population

Baseline characteristics at 12 months after KT are shown in Table 1. The mean age of the 331 recipients in the derivation cohort was 43.0 years, and 61.3% of them were male. The mean age of the 300 recipients in the validation cohort was 46.0 years, and 64.0% of them were male. The median follow-up times after the start of observation (12 months after KT) were 6.6 years in the derivation cohort and 7.0 years in the validation cohort. BMI, diabetic nephropathy, pre-emptive KT, BKPyV infection, serum phosphate level, proteinuria, use of renin–angiotensin–aldosterone system (RAS) inhibitors, statin, cyclosporine and mycophenolate mofetil were all significantly higher in the derivation cohort than in the validation cohort. Dialysis vintage, serum total cholesterol, uric acid and CRP levels and use of tacrolimus were all significantly lower in the derivation cohort than in the validation cohort. Neither CVD history nor serum albumin level differed significantly in the two cohorts.

Development of the prediction model for renal outcome in the derivation cohort

In the derivation cohort, 28/331 patients (8.5%) had a CVD outcome during the observation period. The median time to

Table 1. Baseline clinical characteristics of the living donor KT recipients at the time of 12 months after KT in the derivation cohort and the validation cohort

Characteristics	Derivation cohort ($n = 331$)	Validation cohort ($n = 300$)	P-value
Recipient age (years)	43.0 (33–56)	46.0 (34–58)	0.22
Male recipient (%)	61.3	64.0	0.51
Follow-up (years)	6.6 (5.6–8.1)	7.0 (6.0–9.0)	0.062
BMI (kg/m^2)	21.4 (19.4–24.0)	20.6 (18.8–22.9)	0.004
Smoking history (%)	37.4	33.2	0.070
CVD history (%)	15.7	13.3	0.43
Diabetic nephropathy (%)	20.5	14.3	0.047
First transplantation (%)	96.4	92.3	0.39
Pre-emptive KT (%)	21.8	4.7	<0.001
Dialysis vintage (months)	24 (4–71)	33 (14–66)	0.002
Donor age (years)	57 (49–64)	58.5 (52–64)	0.060
Male donor (%)	37.8	31.7	0.11
ABO incompatible (%)	29.0	30.7	0.66
Post-transplant diabetes mellitus (%)	5.1	9.3	0.38
Rejection history (%)	20.5	22.7	0.56
BKPyV infection history (%)	2.7	0.3	0.022
CMV infection history (%)	32.3	NA	NA
Hypertension (%)	79.8	60.3	0.74
Dyslipidemia (%)	44.1	44.6	0.94
SBP (mmHg)	127.2 ± 15.5	120.6 ± 15.1	0.15
DBP (mmHg)	74.7 ± 10.5	75.3 ± 11.3	0.19
Serum total cholesterol (mg/dL)	188.1 ± 34.3	203.8 ± 39.0	<0.001
Serum HDL cholesterol (mg/dL)	57.8 ± 16.9	NA	NA
Serum LDL cholesterol (mg/dL)	100.0 ± 26.6	NA	NA
Serum uric acid (mg/dL)	5.8 ± 1.2	6.0 ± 1.4	0.022
Serum correction calcium (mg/dL)	9.6 (9.3–9.9)	9.6 (9.3–10.0)	0.66
Serum phosphate (mg/dL)	3 (2.6–3.4)	2.8 (2.4–3.3)	0.020
Serum albumin (g/dL)	4.3 (4.1–4.5)	4.3 (4.0–4.5)	0.64
Serum CRP (mg/dL)	0.04 (0.02–0.1)	0.08 (0.04–0.24)	<0.001
eGFR (mL/min/1.73 m^2)	48.8 (40.1–58.2)	46.8 (38.9–57.6)	0.29
UPCR (g/gCr)	0.10 (0.06–0.18)	NA	NA
Proteinuria (%)	31.7	10.3	<0.001
Antiplatelet agents use (%)	24.5	NA	NA
RAS inhibitors use (%)	56.4	28.3	<0.001
Statin use (%)	31.7	23.1	0.021
Tacrolimus use (%)	90.9	99.0	<0.001
Cyclosporine use (%)	8.8	1.0	<0.001
Mycophenolate mofetil use (%)	98.1	95.0	0.043
Mizoribine use (%)	1.5	4.3	0.053
Everolimus use (%)	2.4	0.5	0.18

Values represent the percentage, mean ± standard deviation or the median followed by the interquartile range in brackets.
SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; UPCR, urinary-protein to urinary-creatinine ratio; NA, not available.

CVD was 58.2 (27.3–75.0) months in derivation cohort. Results of the univariable and multivariable Cox proportional hazards analyses with backward elimination for CVD onset in the derivation cohort are summarized in Table 2. CVD history, diabetic nephropathy, long dialysis vintage, low serum albumin level and proteinuria were significantly associated with a higher risk of CVD onset in multivariable analysis with backward elimination. Recipient age had no significant effect, but we included it as a factor in the risk score because of its clinical significance.

The cases of graft failure and non-cardiac death during observational period were likely to be the competing risk, but hazards regression analysis with backward elimination by Fine and Gray methods showed that the same risk factors were selected as Cox proportional hazards regression analysis (Supplementary data, Table S1). Moreover, the difference between cumulative incidences of Kaplan–Meier and competing risk method was negligibly small (Supplementary data, Figure S1). Therefore, the Cox proportional hazards model was adopted.

Selected continuous variables were converted into categorical variables. Recipient age was categorized based on the median: <45 years and ≥45 years. Dialysis vintage and pre-emptive KT were categorized based on clinical suitability: pre-emptive KT, <5 years and ≥5 years. Serum albumin level was categorized based on standard clinical values: <4.0 g/dL and ≥4.0 g/dL. Lastly, a score-based prediction model containing six variables including CVD history, diabetic nephropathy, recipient age, dialysis vintage, serum albumin level and proteinuria was created using the regression coefficients obtained via the relevant Cox proportional hazards model (Table 3).

Table 2. Unadjusted and multivariable adjusted hazard ratios (HRs) for the development of CVD

Characteristics	Patients (*n*)	Events (*n*)	Unadjusted		Multivariable adjusted	
			HR (95% CI)	P-value	HR (95% CI)	P-value
Recipient age (1-year increase)			1.06 (1.03–1.09)	<0.001	1.03 (0.99–1.07)	0.13
Male recipient (versus women)	203	21	1.85 (0.79–4.36)	0.139	–	–
BMI (1 kg/m^2 increase)	–	–	1.09 (1.00–1.17)	0.060	–	–
Smoking history	124	14	1.97 (0.93–4.16)	0.075	–	–
CVD history	52	13	5.95 (2.81–12.6)	<0.001	3.42 (1.42–8.22)	0.006
First transplantation	319	26	0.42 (0.099–1.77)	0.2366	–	–
Diabetic nephropathy	68	14	5.28 (2.49–11.2)	<0.001	4.28 (1.67–11.0)	0.002
Pre-emptive KT	72	1	0.15 (0.02–1.11)	0.011	–	–
Dialysis vintage (1-year increase)	–	–	1.01 (1.00–1.01)	0.018	1.01 (1.00–1.01)	0.004
Donor age (1-year increase)	–	–	1.00 (0.97–1.04)	0.83	–	–
ABO incompatible	96	8	1.20 (0.52–2.73)	0.67	–	–
Post-transplant diabetes mellitus	17	1	0.67 (0.09–4.91)	0.69	–	–
Rejection history	68	8	1.44 (0.63–3.26)	0.40	–	–
BKPyV infection history	9	1	1.19 (0.16–8.75)	0.87	–	–
CMV infection history	107	9	1.10 (0.50–2.45)	0.80	–	–
Hypertension	264	25	2.06 (0.62–6.84)	0.20	–	–
Dyslipidemia	146	17	2.05 (0.96–4.39)	0.060	–	–
Serum total cholesterol (10 mg/dL increase)	–	–	0.96 (0.85–1.06)	0.42	–	–
Serum uric acid (1 mg/dL increase)	–	–	1.19 (0.86–1.69)	0.30	–	–
Serum correction calcium (1 mg/dL increase)	–	–	1.32 (0.74–2.23)	0.34	–	–
Serum phosphate (1 mg/dL increase)	–	–	1.20 (0.78–1.54)	0.35	–	–
Serum albumin (1 g/dL increase)	–	–	0.12 (0.05–0.31)	<0.001	0.18 (0.06-–0.52)	0.001
Proteinuria	105	14	2.44 (1.16–5.13)	0.020	1.47 (1.06–2.01)	0.023
eGFR (10 mL/min/1.73 m^2 increase)	–	–	0.79 (0.60–1.02)	0.079	–	–
CRP (1 mg/dL increase)	–	–	1.38 (1.03–1.82)	0.030	–	–

Table 3. Multivariable adjusted hazard ratios (HRs) for the development of CVD using categorical variables

Characteristics	Patients (*n*)		Multivariable adjusted		
			HR (95% CI)	P-value	β
CVD history	279	Absence	1	–	–
	52	Presence	3.4 (1.34–8.64)	0.010	1.2252
Diabetic nephropathy	263	Absence	1	–	–
	68	Presence	2.6 (0.98–6.81)	0.056	0.9471
Recipient age	171	<45 years	1	–	–
	160	≥45 years	1.5 (0.63–3.62)	0.35	0.4133
Dialysis vintage	72	Pre-emptive KT	1	–	–
	167	<5 years	2.4 (0.31–19.20)	0.40	0.8897
	92	≥5 years	6.8 (0.88–53.40)	0.066	1.9241
Serum albumin	276	≥4 g/dL	1	–	–
	55	<4 g/dL	2.8 (1.26–6.38)	0.012	1.0440
Proteinuria	226	Absence	1	–	–
	105	Presence	3.3 (1.41–7.54)	0.005	1.1832

The smallest regression coefficient associated with any of the aforementioned six variables was 0.413 for recipient age, and it was assigned one point. A simple predictive risk score for CVD onset is shown in Table 4, and the orange bars on the histogram in Figure 1 show the risk scores for derivation cohort. Figure 2 shows observed CVD incidence rates based on risk scores. Clear increases in incidence rates were associated with increases in risk scores in the derivation cohort ($P < 0.001$). The predicted 6-year absolute risks of CVD onset per one-point increase in the total prediction rule are shown in Table 5. This simple score-based prediction model of the six variables performed extremely well in terms of discrimination via the c-statistic [0.88; 95% confidence interval (CI) 0.79–0.97] (Figure 3A) and moderately well in terms of calibration 6 years via the Hosmer–Lemeshow test (chi-square statistic with eight degrees of freedom = 11.4; $P = 0.18$) (Figure 4A). During the timeframe of 6 years, the censored cases including 12 of graft failure, 5 of non-cardiac death and 28 of change hospitals were not considered. Therefore, the whole-time goodness of fit analysis considering those censored cases by Hosmer–May test was performed, and it showed well calibration (chi-square statistic with eight degrees of freedom = 8.62, $P = 0.47$).

External validation of the prediction model in the validation cohort

The prediction model was externally validated in a population independent of the derivation cohort. In this validation cohort containing 300 KT recipients, 21 (7.0%) had a CVD outcome.

The median time to CVD was 51.0 (39.0–91.0) months in validation cohort. The purple bars on the histogram in Figure 1 show the risk scores for validation cohort. The prediction model had moderate discriminative capacity (c-statistic 0.77; 95% CI 0.67–0.87) (Figure 3B), and it also exhibited good calibration 6 years in the validation cohort (chi-square statistic with eight degrees of freedom = 11.9; P = 0.15) (Figure 4B). During the timeframe of 6 years, the censored cases included 10 of graft failure, 5 of non-cardiac death and 9 of change hospitals. The whole-time analysis of goodness of fit by Hosmer–May test showed moderately well calibration (chi-square statistic with eight degrees of freedom statistic = 12.5, P = 0.19).

Variations of the prediction model

Three prediction models were created in other ways. First, the participants were categorized into three subgroups according to their serum albumin concentration and age, instead of the dichotomized groups. The prediction model using these subgroups is shown in Supplementary data, Tables S2 and S3. For the derivation and validation cohorts, the discrimination and calibration capacities were similar to those of the original model (Supplementary data, Figures S2 and S3). Second, continuous variables were converted to categorical variables using cut-off values derived from receiver operating characteristic analyses (Supplementary data, Figure S4) and used to create another model (Supplementary data, Tables S4 and S5) that showed similar discrimination and calibration capacities (Supplementary data, Figures S5 and S6) to those of the original

Table 4. Risk scores for CVD development in living donor KT recipients

Variables	Scores
CVD history	
Absence	0
Presence	3
Diabetic nephropathy	
Absence	0
Presence	2
Recipient age	
<45 years	0
≥45 years	1
Dialysis vintage	
Preemptive KT	0
<5 years	2
≥5 years	5
Serum albumin	
≥4 g/dL	0
<4 g/dL	3
Proteinuria	
Absence	0
Presence	3
Maximum total risk scores	17

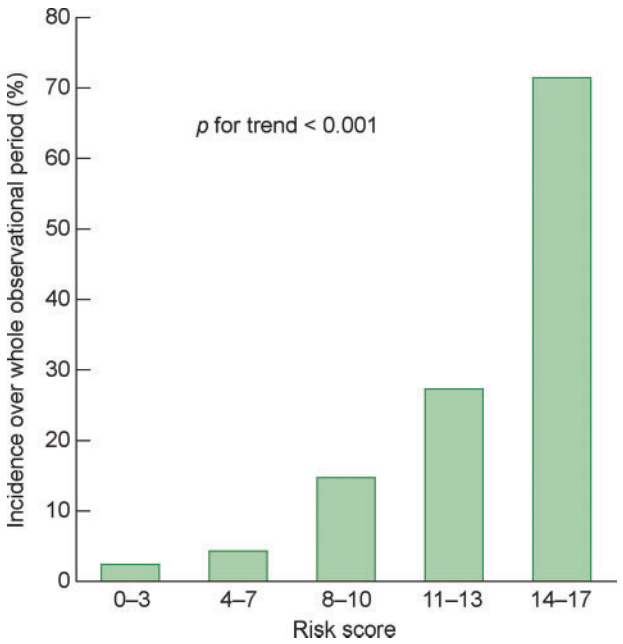

FIGURE 2: CVD incidence rates at simple prediction risk scores in derivation cohort. Clear increases in incidence rates were associated with increases in risk scores.

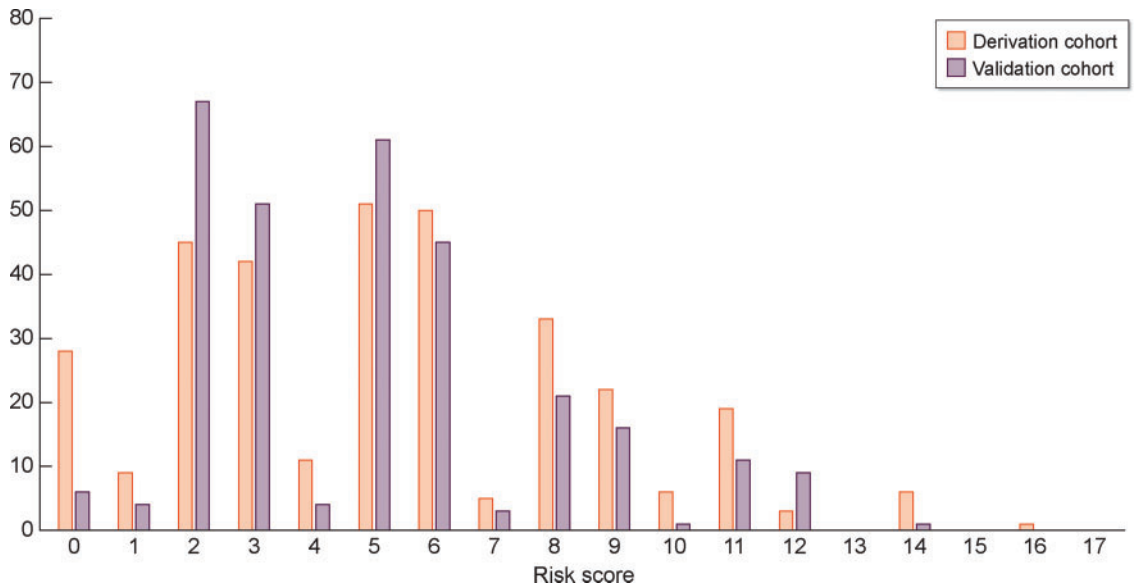

FIGURE 1: Histogram for each risk score. Orange bars: derivation cohort; purple bars: validation cohort.

Table 5. Predicted 6-year absolute risks of CVD development in living donor KT recipients according to total risk score

Total risk score	Predicted 6-year absolute risk (%)
0	0.51
1	0.76
2	1.13
3	1.66
4	2.46
5	3.63
6	5.33
7	7.81
8	11.37
9	16.39
≥10	≥23.32

model for the derivation and validation cohorts. Third, hypertension and dyslipidemia were selected automatically as risk factors, and the prediction model created (Supplementary data, Tables S6 and S7) showed slightly worse discrimination than the original model (Supplementary data, Figure S7). Therefore, these additional three models were not superior to the original model.

Comparisons with previously published models

To compare the utility of the present model with those of other previously reported CVD prediction models, the prediction risk calculator derived from the ALERT trial [5] and the Framingham score for the general population [13] were applied to our derivation cohort. The risk calculator derived from

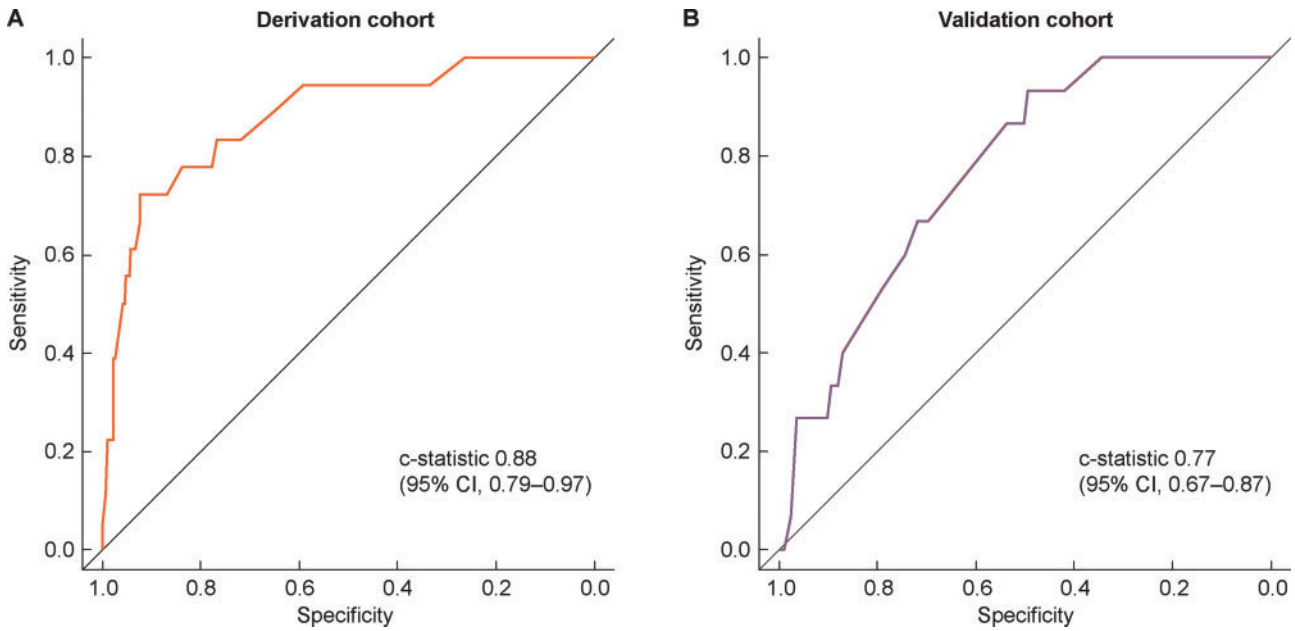

FIGURE 3: Receiver operating characteristic curves plotted by the prediction model showing that the c-statistics yielded extremely good discrimination in the derivation cohort (A) and moderate discrimination in the validation cohort (B).

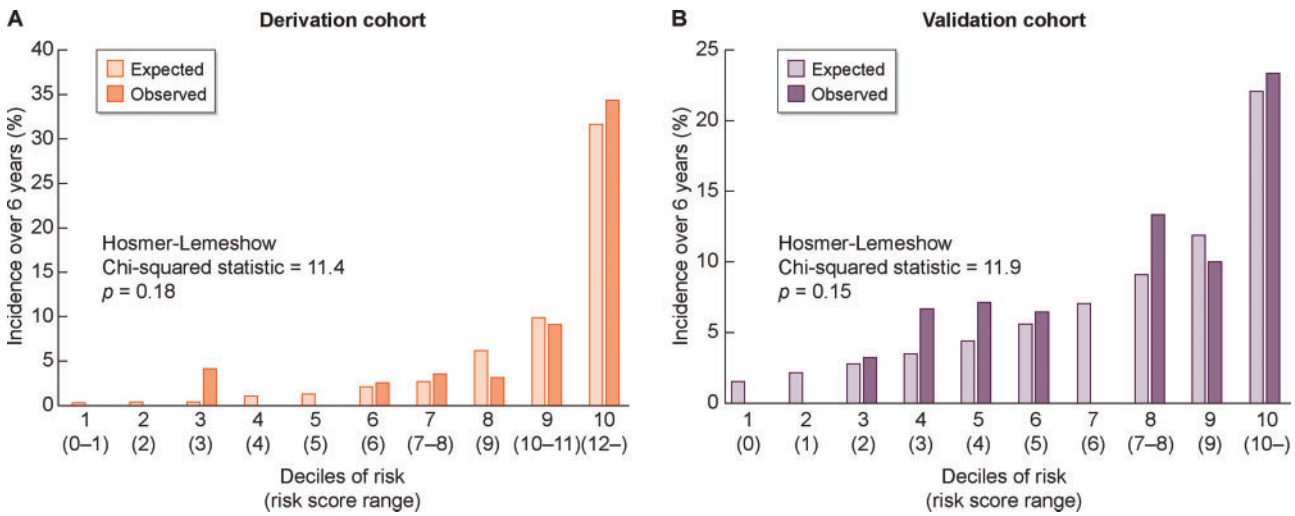

FIGURE 4: Observed and predicted 6-year absolute risk for CVD onset by deciles of risk in the derivation cohort and in the validation cohort. Calibration was moderate in the derivation cohort (A), as was external validity (B) in Hosmer–Lemeshow testing.

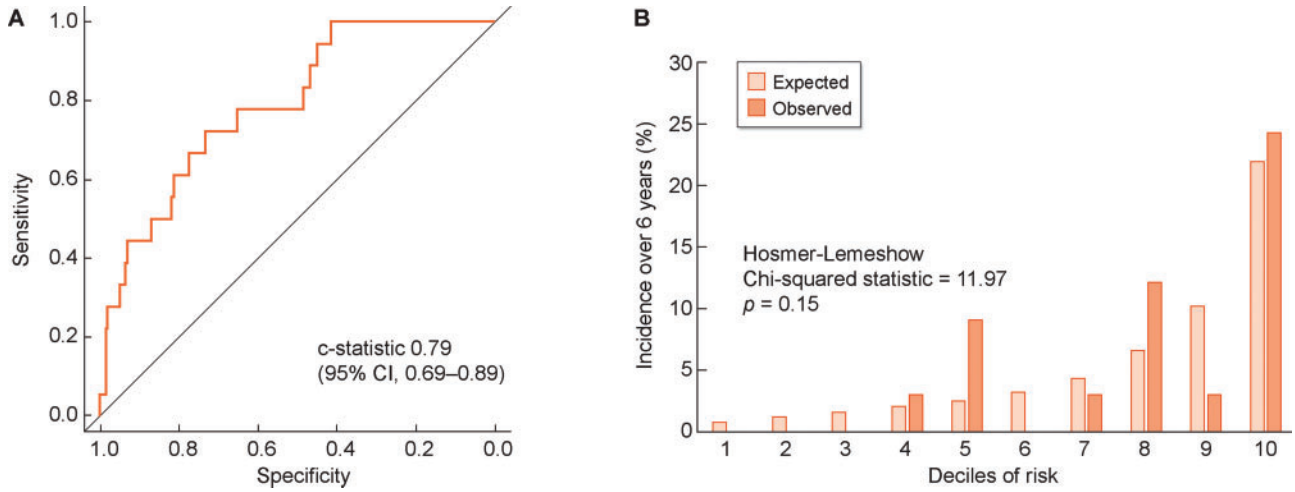

FIGURE 5: Use of the risk calculator derived from the ALERT trial for analysis of the derivation cohort. The risk calculator showed good discrimination capacity according to the c-statistic (A), and moderate calibration according to the Hosmer–Lemeshow test (B). However, the values of each were lower than those achieved using our original risk score.

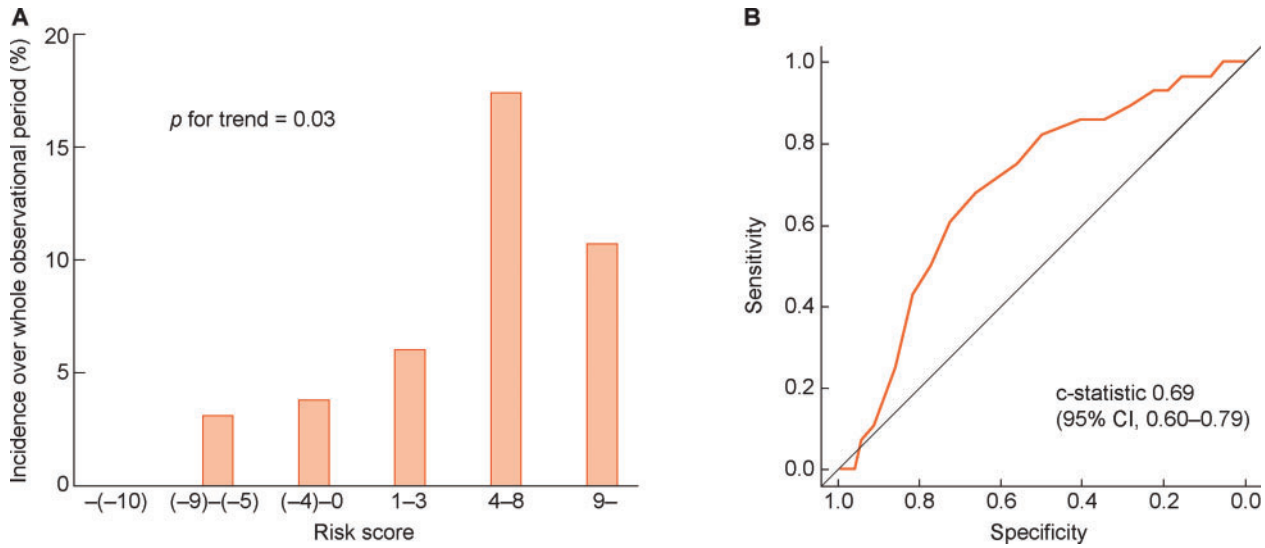

FIGURE 6: Use of the Framingham score for analysis of the derivation cohort. Increases in the observed incidence were associated with increases in the Framingham risk score in our derivation cohort (A). However, the discrimination capacity was poor, according to the c-statistic (B).

the ALERT trial performed well in our derivation cohort, but not as well as our risk score in terms of discrimination, according to the c-statistic (0.79; 95% CI 0.69–0.89) (Figure 5A), and moderately well in terms of calibration, according to the Hosmer–Lemeshow test (Figure 5B). When the Framingham risk score was applied to our derivation cohort the increases in observed incidence rates were associated with increases in Framingham risk scores (P = 0.03) (Figure 6A), but its discrimination was poor via c-static (0.69; 95% CI 0.60–0.79) (Figure 6B). The visual summary of c-statistics was shown (Figure 7).

DISCUSSION

In this study, a new CVD prediction model was developed for use in living donor KT recipients, and the first practical risk score for total CVD in living donor KT recipients was verified externally using another independent cohort based at a different hospital. We believe that this risk score is useful for estimating long-term CVD prevalence, particularly in living donor KT recipients who have undergone adequate pre-transplant CVD screening.

Recipient age had no significant effect in multi-variable Cox regression. The reason for this might be that the cohorts studied were relatively young, with median ages of 43 and 46 years in the derivation and validation cohorts, respectively, but the median ages of CVD cases were high (56.5 and 56.0 years, respectively). CVD history and diabetic nephropathy were reasonably foreseeable factors, whereas dialysis vintage, serum albumin level and proteinuria had more significant influences than expected. Dialysis vintage was associated with CVD prevalence in previous studies [2, 14]. Coronary artery calcification tends

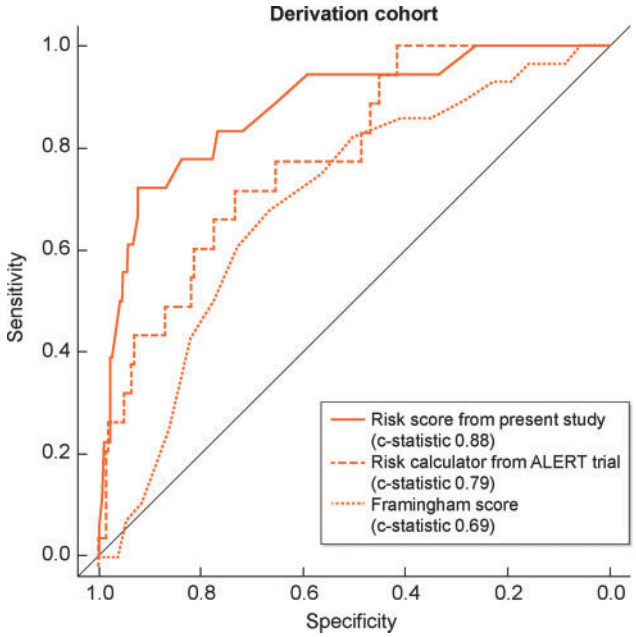

FIGURE 7: The visual summary of c-statistics in derivation cohort by risk models from this study and previous study.

to progress in KT recipients with a long dialysis vintage [15], and artery calcification is reportedly an independent predictor of long-term CVD outcome [16].

An association between serum albumin level and CVD incidence has been reported previously [17]. Hypoalbuminemia is an independent predictor of CVD in every stage of CKD patients [18–20]. Hypoalbuminemia is caused by hepatic synthetic disability, nephrotic syndrome, inflammation and malnutrition. The cohorts in this study included few liver cirrhosis patients and three patients with nephrotic syndrome. Serum CRP was associated with CVD onset in unadjusted data analysis, but not in multivariable-adjusted analysis. Therefore, hypoalbuminemia could be attributed to nutritional status and inflammation. Pre-transplant malnutrition is an independent predictor of post-transplant CVD [21], but little is known about post-transplant nutrition. There are only limited guidelines pertaining to post-transplant malnutrition [22]. Serum albumin level, 12 months after KT, may be indicative of a patient's nutrition status in the early phase after a KT, which may contribute to CVD events in the late phase. Inflammation is associated with hypoalbuminemia [23] and proteinuria [24]. To estimate the effects of inflammation on CVD, further biomarker measurements such as high sensitivity CRP may be necessary.

The risk calculator derived from the ALERT trial showed good external validation in our derivation cohort, but our risk score was more accurate and robust for living donor KT recipients. Smoking history was not selected for use in our cohort, despite its significance in the ALERT trial data. One of the reasons for this might be that the number of current smokers in our derivation cohort was very small (only 25 people), because living-donor KT is usually performed after patients stop smoking. The difference might suggest the significance of stopping smoking before KT for preventing CVD. Serum cholesterol concentration was also not selected in our cohort. This may be attributable to the definition of the outcome used. Although the ALERT trial restricted the outcome to cardiac events, the outcome of this study was any CVD event, including stroke, which was not associated with cholesterol concentration or statin use. Furthermore, the comparison with the Framingham score showed that a distinct CVD risk model should be used for KT recipients, because they have a different set of characteristics. Unfortunately, we could not apply these other risk models to our validation cohort because we had no data about serum high- and low-density lipoprotein.

This study had several strengths. A new prediction model with statistically high robustness was developed. The model was derived from a real living donor KT recipient cohort and exhibited good discrimination and calibration. Moreover, external validity was verified via another independent cohort. These indications of statistical robustness support the reliability of the model and suggest its broader applicability in other KT recipient populations. Notably, the model described herein is the first designed for predicting CVD risk in living donor KT recipients. Lastly, the simple integer points risk score utilized is readily amenable to clinical application. Our simple integer risk score clearly shows how the risk factors affect CVD risk at a glance. Calculating a risk score facilitates more accurate prediction of CVD incidence, and KT recipients with highly weighted risk factors should be diligently followed up with CVD estimation.

This study had several limitations. It was retrospective and observational, thus there is an inevitable possibility that immeasurable confounders may have affected the results. Another potential limitation was that the study participants were all members of the Japanese population. The differences between the present model and the risk model derived from the ALERT trial may be accounted for by differences in the characteristics of the populations studied. Many of the Japanese living donor KT recipients had a history of long-term dialysis, and the risk of CVD in Asian populations may differ from that in Western populations. Thus, whether the prediction formulas are readily applicable in Western populations remains to be determined.

In conclusion, in this study, a new prediction model and associated risk score for predicting CVD events in living donor KT recipients was developed. The model's internal and external validity were assessed using two independent cohorts. The prediction risk score is a simple and useful means of estimating CVD risks in the clinical setting. Further investigations in cohorts of other ethnicities should be conducted.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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AUTHORS' CONTRIBUTIONS

K.U. designed the study, evaluated histopathology, collected and analyzed data, and wrote the initial draft of the manuscript. A.T. designed the study, evaluated histopathology, collected and analyzed data and assisted in the preparation of the manuscript. Y.M. and K.N. contributed to evaluation of histopathology and data interpretation. H.T. and S.T. contributed to analysis and interpretation of data. K.K., H.N., Y.O. Y.K. and M.O. performed operations and collected data. K.U. contributed to data collection and interpretation. K.M., M.N., K.Tsuruya, T.N., K.Tanabe and T.K. oversaw and revised the manuscript. All authors contributed to critical review of the manuscript and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

None declared.

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