Original Article

The predictive value of urinary kidney injury molecular-1 for long-term graft function in kidney transplant patients: a prospective study

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Background: Monitoring allograft function during the early stages is crucial, and therefore requires biomarkers more sensitive than serum creatinine (Scr). Kidney injury molecular-1 (KIM-1) is a potent biomarker; however, disparities exist in the literature concerning its predictive value in allograft function. Therefore, this study aimed to evaluate its predictive value for the long-term prognosis of kidney transplantation patients.

Methods: A prospective study with a cohort comprising 160 patients scheduled for kidney transplantation was conducted to evaluate the predictive power of urinary KIM-1 (uKIM-1) and other renal ischemia-reperfusion biomarkers including urinary L-type fatty acid binding protein (uL-FABP), urinary N-acetyl-β-D glucosaminidase (uNAG), and urinary neutrophil gelatinase-related lipoprotein (uNGAL) for allograft prognosis.

Results: One hundred and forty kidney recipients who were admitted to our hospital between September 2014 and December 2017 with a median follow-up of 30.3 months were included. Thirty-seven recipients had functional delayed graft function (fDGF) in the first week post transplantation, and 42 recipients had progressed to allograft dysfunction [estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2] by the end of the study, while nine recipients deteriorated into allograft loss (defined by the initiation of dialysis). The levels of uKIM-1 in the fDGF group were higher than those in the immediate graft function (IGF) recipients (P<0.05) at 0 hour post transplantation [5.885 (4.420–7.913) *vs.* 4.605 (3.417–5.653) ng/mmol], and on the first day post transplantation [5.569 (4.181–6.722) *vs.* 4.002 (3.222–6.488) ng/mmol]. The levels of uL-FABP in the fDGF group were also higher than those in the IGF group at 0 hour post transplantation (89.818±39.332 *vs.* 69.187±37.926 µg/mmol) and on the third day post transplantation [77.835 (60.368–100.678) *vs.* 66.841 (28.815–89.783) µg/mmol]. Multivariate Cox regression analysis demonstrated that recipients with higher uKIM-1 levels on the first day post transplantation had a 23.5% increase in the risk of developing fDGF and a 27.3% increase in the risk of prolonged renal allograft dysfunction.

Conclusions: uKIM-1 on the first day post transplantation can predict short-term graft function and is a potent biomarker for the long-term prognosis of graft function.

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Introduction

Despite surgical techniques and immunotherapy having progressed rapidly in recent years, the incidence of delayed graft function (DGF) and the occurrence of long-term adverse events after kidney transplantation are still increasing (1,2). One of the reasons for this is the expansion of the donor pool through donation after cardiac death (DCD) in the last few decades, accompanied by the increased incidences of DGF and long-term graft loss caused by ischemia-reperfusion injury (3,4). Elderly donors, prolonged cold ischemia time, and recipient characteristics [including age, human leukocyte antigen (HLA) matches, panel-reactive antibody (PRA) percentages] have also been reported to be factors affecting the incidences of DGF and long-term graft survival (2,5). Thus, monitoring allograft function at an early stage is of paramount importance. The two clinical indicators that currently exist are not adequate for this purpose (6); serum creatinine (Scr) shows inadequate sensitivity, while obtaining surveillance allograft biopsy is invasive. Therefore, there is growing interest in exploring blood and urine biomarkers for predicting the prognosis of kidney transplantation patients.

To predict both short- and long-term prognosis of patients with renal transplantation, non-invasive and diagnostic urinary or serum biomarkers are needed for early detection. The existing method for assessing allograft function requires invasive renal puncture, which can cause hemorrhage and other complications. Studies focused on kidney tubular injury-related proteins, namely neutrophil gelatinase-related lipoprotein (NGAL), kidney injury molecule-1 (KIM-1), and interleukin-18 (IL-18), as potential markers for monitoring DGF are under investigation (6-8). Nevertheless, these biomarker candidates still lack clinical validation or positive predictive value for long-term allograft function (7). Moreover, NGAL and IL-18 are also produced by immune cells during urinary tract infections and sepsis. The confounding effect of infections or sepsis on the predictive value of NGAL and IL-18 for allograft prognosis has not been clarified in those studies.

KIM-1 is a transmembrane immunoglobulin that is not detectable in healthy kidneys. It is dramatically upregulated in damaged tubular epithelial cells in multiple types of kidney injury. Disparities exist within the literature on the predictive value of KIM-1 in allograft function. For instance, in a cohort study of 145 renal transplantation recipients, KIM-1 was reported to be a potent predictor of transplantation failure (9); however, another study found that KIM-1 could not effectively predict DGF (10).

Therefore, to determine the predictive value of KIM-1 for both the short-term and long-term prognosis of kidney transplantation, we conducted a cohort study of renal transplantation recipients. We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/atm-20-2215a).

Methods

Study subjects

This study was a prospective, single-center cohort study from September 2014 to December 2017. The inclusion criteria were as follows: (I) end-stage renal disease (ESRD) patients aged 18–65 years old who were scheduled to receive kidney transplantation through DCD in the hospital within 3 days who (II) had an HLA mismatch (ABDR) at no more than 3 sites, and (III) had received anti-thymocyte globulin (ATG) as induction therapy, and tacrolimus with mycophenolate mofetil as the maintenance immunosuppression regimen. The exclusion criteria were as follows: (I) recipients who had received transplantation before or who had been infected with human immunodeficiency virus (HIV) or the hepatitis C virus; (II) recipients who were infected or had sepsis during follow-up.

The patients were followed up every week within the first month post transplantation, every month within the first year post transplantation, and every 3 months afterwards. Each enrolled patient had been followed up for at least 1 year. With reference to Boom *et al.* (11), functional delayed graft function (fDGF) was defined as a recovery of Scr of <70% in the first week after transplantation, regardless of dialysis. Conversely, immediate graft function (IGF) was characterized by a recovery of Scr of >70% in the first week after transplantation

(10,12). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Renji Hospital, School of Medicine, Shanghai Jiao Tong University (14140903200). All patients provided informed consent.

Endpoint definitions

The primary endpoint for the analysis was allograft loss (marked by the initiation of dialysis). Time to event was calculated from enrollment to either allograft loss or the end of the study (13). The secondary endpoint was graft dysfunction [estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2].

Sampling

Fresh blood samples were collected from all patients within the 1 week before transplantation; specifically, immediately after the perfusion of the donor kidney (0 hour), and at 24 and 72 hours after transplantation. Urine samples were obtained before transplantation if the patient was able to urinate, and collected from the bag and catheter of each patient after the perfusion of the donor kidney during transplantation, and at 24 and 72 hours after transplantation. All samples were submitted to the biochemical laboratory for analysis shortly after collection. The rate of centrifugation was 3,500 ×g for 15 min for fresh urine (10 mL) and serum (500 µL). The supernatant was then transferred to Eppendorf tubes and stored at –80 ℃ until further analysis.

Biomarker measurement

The levels of KIM-1, L-type fatty acid binding protein (L-FABP), NGAL, and N-acetyl-β-D glucosaminidase (NAG) in the patients' serum and urine were examined using enzyme-linked immunosorbent assay (ELISA) kits from American R&D Corporation (Minneapolis, MN, USA). A standard curve was generated to calculate the concentration of the samples. The results were corrected through synchronous normalization to urinary creatinine (uCr), which was measured by sarcosine oxidase method (14,15).

Other measurements

Patient characteristics assessed at enrollment included demographic features (age and sex), physical examination findings [body mass index (BMI)], medical history (hypertension and diabetes mellitus), and transplantation features (duration of dialysis and mode of dialysis before transplantation). Routine pathology tests were also performed 1 day before transplantation, and included the following markers: Scr, hemoglobin, serum albumin, serum globulin, white blood cells, serum potassium, serum sodium, serum bicarbonate, pre-albumin, glutamate pyruvic transaminase, aspartate aminotransferase, γ-glutamyl transpeptidase, alkaline phosphatase, serum total cholesterol, serum triglyceride, serum high density lipoprotein, serum low-density lipoprotein, serum calcium ion, serum phosphorus, and immunoreactive serum parathyroid hormone.

Scr was measured by sarcosine oxidase method pre-transplantation, and at 0 hour, 1, 3, 5, 7, 10, 14, 21 days, every 1 month within the first year post transplantation, and every 3 months thereafter (4). eGFR was calculated using the modification of diet in renal disease (MDRD) formula: eGFR $=186 \times (Scr/88.4)^{-1.154} \times age^{-0.203}$ (×0.742, female) (16,17). BMI was calculated by weight (kg)/height (m)^2. Medical history was self-reported at admission.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics (version 25, SPSS Inc., Chicago, IL, USA), GraphPad Prism (version 8.0, GraphPad Software, San Diego, CA, USA), and survival package in R, version 6.0.1. Before analysis, all data were tested for normal distribution. Student's t-test was applied to parametric continuous variables, the Mann-Whitney U test was performed for non-parametric data, and a chi-squared test was used for categorical variables. Comparisons between multiple groups were performed using analysis of variance (ANOVA) for parametric variables and the Kruskal-Wallis test for nonparametric variables. Kaplan-Meier survival analysis was carried out to compare allograft survival between the fDGF and IGF groups, together with the log-rank test for calculation. Receiver operating characteristic (ROC) analysis was performed to determine the biomarker thresholds that were best associated with fDGF development, and Spearman's correlation analysis was used to measure the degree of association between variables. Univariate and multivariate linear regression analyses were performed to examine the relationships between fDGF and independent variables. Univariate and multivariate Cox
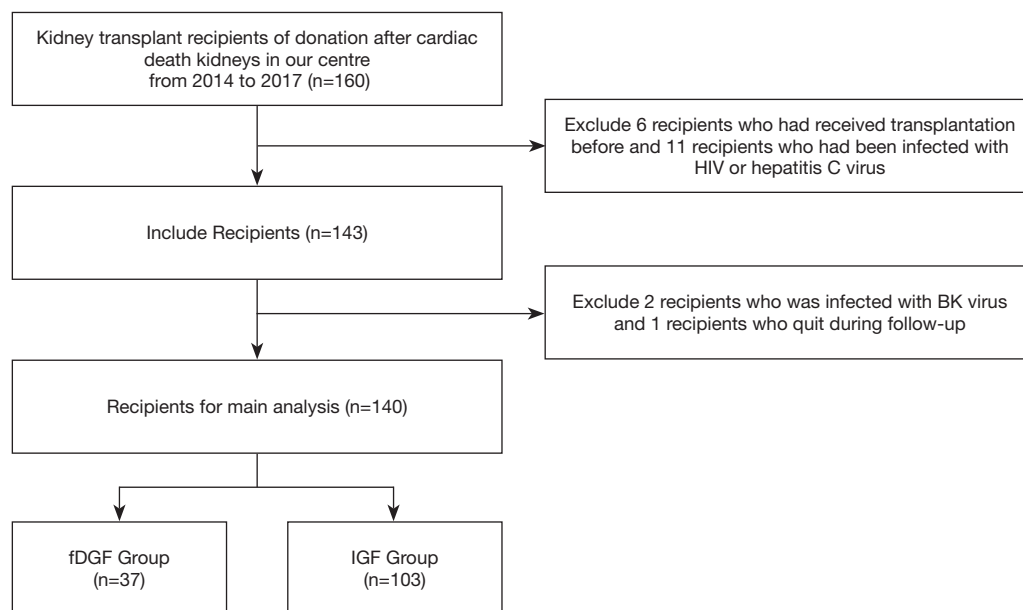
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Figure 1 Flow diagram of the study design. fDGF, functional delayed graft function; IGF, immediate graft function.

regression analyses were performed to assess the hazard ratios (HRs) and 95% confidence intervals (CIs). For all tests, statistical significance was considered as a two-sided P value of ≤0.05. Normally distributed data were expressed as mean ± standard deviation (SD), non-normally distributed data were expressed as median (interquartile range), and categorical data were expressed as frequencies (percentages).

Results

Patient characteristics

A total of 160 kidney transplantation recipients through DCD were included in this study between September 2014 to December 2017. A total of 17 transplant recipients who had HIV or hepatitis C virus infection were excluded. Subsequently, 143 patients were followed up for a median duration of 30.3 months (IQR, 9.18–44.25 months) after transplantation. Three recipients were excluded during follow-up due to BK virus infection or loss to follow-up. Finally, a total of 140 patients were enrolled for the final analysis (*Figure 1*).

At baseline, the median age of the 140 enrolled recipients was 41.0 years old (32.0–50.0 years old). A total of 37 recipients progressed to fDGF during the first week after transplantation; 7 of these fDGF cases were elicited by allograft rejection and another 3 by surgical reasons.

A total of 3 recipients in the IGF group had allograft rejection during the follow-up after the first week post transplantation. There were no statistically significant differences between fDGF and IGF recipients in terms of HLA mismatches, hot and cold ischemia time, creatinine clearance rate, donor age, or medical history before transplantation (*Table 1* & Tables S1-S3).

Increased long-term graft dysfunction in patients with fDGF

A total of 42 recipients exhibited impaired graft function (eGFR ≤60 mL/min/1.73 m^2) (17, 45.90% in fDGF group *vs*. 25, 24.20% in IGF group, P=0.004) during the last follow-up visit (Table S3). Nine recipients deteriorated into allograft loss by the end of this study (Table S4). The fDGF group included 7 (18.9%) allograft loss recipients, which was more than the 2 (1.9%) in the IGF group (P=0.000). Among the 9 recipients who deteriorated into allograft loss, 3 died during dialysis, due to allograft rejection, infection, and cardiac arrest, respectively, although this was not statistically significant.

Comparison of biomarkers between recipients with and without fDGF

Both urinary KIM-1 (uKIM-1)/uCr and urinary L-FABP

Table 1 Characteristics of transplant recipients at baseline

Characteristics	Total (n=140)	fDGF (n=37)	IGF (n=103)	P value
Age (years)	41.0 (32.0–50.0)	40.0 (31.5–48.5)	42.0 (32.0–50.0)	0.607
Sex male, n (%)	85 (60.7)	18 (48.6)	67 (65.0)	0.080
BMI (kg/m^2)	21.35 (19.24–23.91)	22.86 (19.29–25.40)	21.88 (19.89–25.31)	0.978
Pre-Scr (μmol/L)	880.20 (690–1172.25)	904.00 (739.15–1,165.00)	874.00 (685.00–1,175.00)	0.789
Pre-eGFR (mL/min/1.73 m^2) preoperative	5.207 (4.025–7.154)	4.797 (3.737–6.885)	5.349 (4.112–7.272)	0.345
Hb (g/L)	108.00 (96.50–120.00)	113.00 (99.50–126.00)	110.00 (99.00–125.00)	0.355
Alb (g/L)	46.00 (42.00–48.80)	44.75 (41.73–48.28)	47.90 (44.80–51.00)	0.077
TC (mmol/L)	1.54 (1.09–3.06)	1.54 (1.00–3.21)	1.62 (1.30–2.93)	0.494
TG (mmol/L)	4.55 (3.55–5.28)	4.62 (3.95–5.08)	4.70 (4.00–5.48)	0.637
HDL (mmol/L)	1.13 (0.87–1.55)	1.07 (0.90–1.78)	1.05 (0.82–1.31)	0.485
LDL (mmol/L)	2.37 (1.93–3.14)	2.35 (1.93–2.75)	2.47 (2.02–3.22)	0.332
Ca^{2+} (mmol/L)	2.36 (2.23–2.51)	2.36 (2.26–2.50)	2.36 (2.22–2.51)	0.650
P (mmol/L)	1.79 (1.31–2.27)	2.09 (1.41–2.55)	1.74 (1.27–2.35)	0.779
iPTH (pg/L)	206.45 (90.10–412.43)	165.75 (110.88–320.83)	228.70 (105.10–552.00)	0.716
Duration time of dialysis (m)	10.00 (3.00–24.00)	12.00 (5.50–31.00)	10.00 (2.00–24.00)	0.155
Mode of dialysis, n (%)				0.069
HD	73 (52.1)	19 (51.4)	57 (52.4)	
PD	42 (30.0)	16 (43.2)	29 (28.2)	
Hypertension, n (%)	106 (75.7)	32 (86.5)	74 (71.8)	0.075
Diabetes, n (%)	7 (5.0)	1 (2.7)	6 (5.8)	0.455
Neutrophil (%)	68.80 (63.65–73.40)	69.20 (67.00–74.30)	67.60 (63.40–73.50)	0.121
Lymphocyte (%)	21.40 (17.85–26.05)	20.50 (15.00–24.50)	23.00 (18.20–27.10)	0.079
WBC (×10^9/L)	7.29 (6.09–8.24)	7.56 (5.91–8.76)	7.18 (6.00–8.61)	0.972
K (mmol/L)	4.10 (3.55–4.70)	4.10 (3.40–4.70)	4.10 (3.60–4.60)	0.397
Na$^+$ (mmol/L)	139.00 (137.00–141.00)	139.00 (137.00–140.00)	139.00 (137.00–141.00)	0.944
HCO$_3^-$ (mmol/L)	23.25 (21.80–25.63)	24.50 (21.80–28.20)	23.20 (21.80–25.60)	0.290
Globulin (g/L)	29.322±5.673	28.017±4.705	29.767±5.927	0.152
Prealbumin (mg/L)	408.475±84.416	399.207±96.139	411.877±80.087	0.492
ALT (U/L)	13.10 (11.00–19.35)	13.00 (11.00–19.00)	14.00 (10.00–20.00)	0.982
AST (U/L)	14.00 (12.00–17.70)	13.90 (11.00–18.00)	14.90 (11.95–18.05)	0.716
γ-GT (U/L)	20.00 (14.00–31.50)	19.00 (13.00–28.00)	20.00 (13.70–31.40)	0.637
HLA mismatches (ABDR), n (%)				0.357
0	135 (96.4)	35 (94.6)	100 (97.1)	
1–2	4 (2.9)	1 (2.7)	3 (2.9)	
3	1 (0.7)	1 (2.7)	0 (0.0)	

Table 1 (*continued*)

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Table 1 (*continued*)

Characteristics	Total (n=140)	fDGF (n=37)	IGF (n=103)	P value
Hot ischemia time (min)	5.00 (4.73–5.70)	4.90 (4.70–5.20)	5.10 (4.80–5.60)	0.057
Cold ischemia time (hours)	4.60 (2.50–5.10)	4.30 (2.25–4.95)	4.70 (3.40–5.10)	0.067

Data that conform to a normal distribution are represented by the mean ± standard deviation (SD), and non-normally distributed data are represented by the median (interquartile range). Frequency of occurrence of the event is expressed as the frequency (percentage). fDGF, functional delayed graft function; IGF, immediate graft function; BMI, body mass index; pre-Scr, pre-operative serum creatinine; pre-eGFR, pre-operative estimated glomerular filtration rate; Hb, hemoglobin; Alb, serum albumin; TC, serum total cholesterol; TG, serum triglyceride; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; Ca^{2+}, serum total calcium; P, serum phosphorus; iPTH, immunoreactive serum parathyroid hormone; HD, hemodialysis; PD, peritoneal dialysis; WBC, white blood cells; K, serum potassium; Na^+, serum sodium; HCO_3^-, serum bicarbonate; ALT, glutamate pyruvic transaminase; AST, aspartate aminotransferase; γ-GT, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; HLA, human leukocyte antigen.

(uL-FABP)/uCr were higher in the fDGF group than in the IGF group immediately after transplantation, at 0 hour [uKIM-1/uCr: 5.885 (4.420–7.913) *vs.* 4.605 (3.417–5.653) ng/mmol, P<0.05; uL-FABP/uCr: 89.818±39.332 *vs.* 69.187±37.926 µg/mmol, P<0.05; *Figure 2*, *Table 2*]. On the first day after transplantation, only uKIM-1/uCr was significantly different between the two groups [5.569 (4.181–6.722) *vs.* 4.002 (3.222–6.488) ng/mmol, P<0.05]. However, on the third day after transplantation, only uL-FABP/uCr was significantly different between the two groups [77.835 (60.368–100.678) *vs.* 66.841 (28.815–89.783) µg/mmol, P<0.05]. Other biomarkers did not show significant differences between the two groups. The levels of serum biomarkers were also tested, but no differences were observed (Table S5).

Correlation between uKIM-1 and fDGF

Correlation between uKIM-1 and fDGF was shown in *Figure 3*. On both the seventh day post transplantation and at the last follow-up visit, Scr was significantly higher and eGFR significantly lower in the fDGF group than in the IGF group (*Figure 3A,B*). Spearman's correlation analysis indicated that uKIM-1 on the first day post-surgery was positively correlated with eGFR on the seventh day post-surgery (r²=0.1092, P<0.0001) and at the last visit (r²=0.1045, P<0.0001), respectively (*Figure 3E,F*). The correlation between uKIM-1 (first day post-surgery) and Scr started from the third day post-surgery (r=0.256, P<0.05, Table S6). Univariate and multivariate cox regression analyses demonstrated a 23.5% increase in the risk for fDGF for each 1 ng/mmol increase in uKIM-1 on the first day post-surgery while uL-FABP demonstrate no statistical significance in the effect for fDGF in the multivariate cox regression analyses

(Figure S1).

Assessment of short-term and long-term graft function on the basis of uKIM-1 levels on the first day post transplantation

Figure S2 displays the ROC curves of uKIM-1/uCr at multiple timepoints for predicting the diagnosis of fDGF. On the first day post-surgery, uKIM-1/uCr exhibited a sensitivity of 81.10%, which was the highest among all biomarkers, together with a specificity of 52.40% for fDGF. The combination of uKIM-/uCr and Scr on the first day post-surgery had the second highest area under curve (AUC) for fDGF at 0.761 (95% CI, 0.664–0.857).

By reclassifying recipients into the KIM-1-high group (uKIM-1/uCr ≥ cutoff value for fDGF, 4.08 µg/mmol) or the KIM-1-low group (uKIM-1/uCr < cutoff value for fDGF, 4.08 µg/mmol), the median Scr were higher, and eGFR were lower in the uKIM-1-high group than in the uKIM-1-low group on the seventh day post transplantation and during the last follow-up visit (*Figure 3C,D*).

uKIM-1 can predict long-term graft prognosis

To determine whether uKIM-1 is related to long-term allograft prognosis, recipients who once presented with increased Scr due to surgical reasons, allograft rejection, infection, or cardiac arrest during the follow-up period were excluded. Ultimately, 125 recipients were included in the Kaplan-Meier analysis. The Kaplan-Meier plots indicated that recipients in the KIM-1-low group had a higher graft function survival rate (eGFR >60 mL/min/1.73m²) than those in the KIM-1-high group (log-rank test, P<0.0001; *Figure 4*).
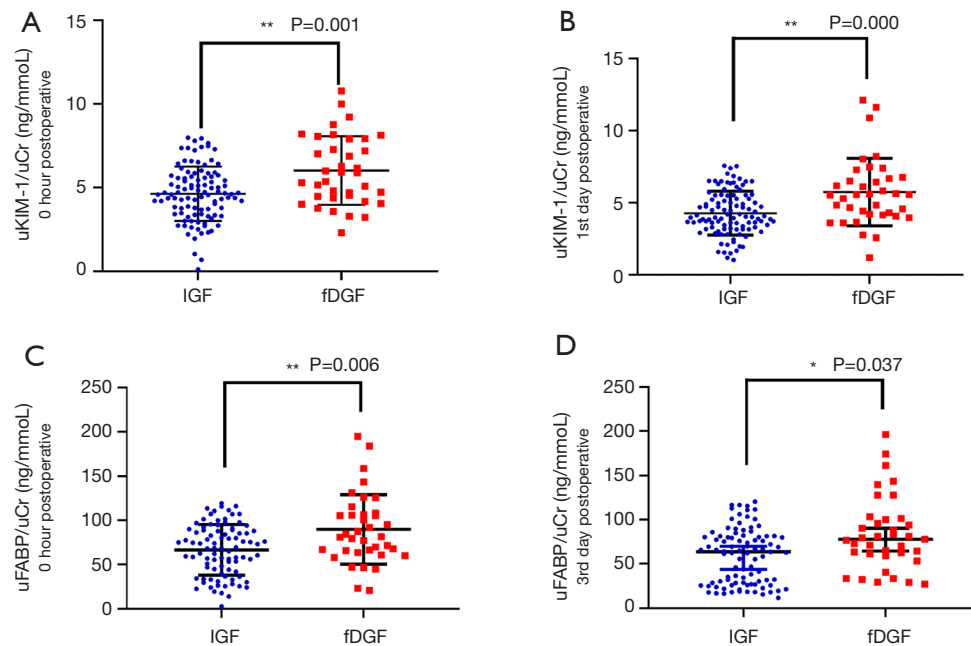
Figure 2 Levels of uKIM-1/uCr (at 0 hour and day 1 post transplantation) and uL-FABP/uCr (at 0 hour and day 3 post-transplantation) in the fDGF group were both significantly higher than those in the IGF group after kidney transplantation. (A) uKIM-1/uCr expression at 0 hour post-surgery in the fDGF and the IGF group; (B) uKIM-1/uCr expression on day 1 post-surgery in the fDGF and the IGF group; (C) uL-FABP/uCr expression at 0 hour post-surgery in the fDGF and the IGF group; (D) uL-FABP/uCr expression on day 3 post-surgery in the fDGF and the IGF group. *, P<0.05; **, P<0.01. fDGF, functional delayed graft function; IGF, immediate graft function; uKIM-1, urinary kidney injury molecular 1; uL-FABP, urinary L-type fatty acid binding protein; uCr, urinary creatinine; uKIM-1/uCr, the ratio of uKIM-1 to uCr; uL-FABP/uCr, the ratio of uL-FABP to uCr.

ROC curves revealed that uKIM-1/uCr on the first day post-surgery had a sensitivity of 90.00% and a specificity of 51.60% for diagnosing long-term graft dysfunction (eGFR <60 mL/min/1.73m^2, *Figure 5*). Univariate and multivariate Cox regression analyses demonstrated that uKIM-1 on the first day post-surgery increased the risk of developing allograft dysfunction by 27.3% in for each 1 ng/mmol increase (*Figure 6*).

Discussion

DGF is a critical risk factor for long-term allograft function (11,18). Despite controversy over its definition, DGF fundamentally refers to the absence of allograft function following kidney transplantation (11,19,20). An increasing number of studies have demonstrated that 30-day re-admission, poorer graft function, acute rejection, allograft loss, and death occur more frequently in patients with DGF compared to non-DGF patients (21-23). Acute tubular necrosis is an important marker of the deterioration stage

towards DGF (24); however, its prognostic significance for long-term graft outcome remains unclear. The broadening criteria for DCD kidney donors accounts for the growing incidence of DGF (21). Hence, biomarkers for the early detection of DGF are urgently needed. In this study, to consider the impact of slow graft function, we applied the definition of fDGF as a decrease of Scr of <70% in the first week after transplantation, regardless of dialysis, with reference to Boom *et al.* and Hall *et al.* (6,11).

During a median follow-up of 30.3 months, we discovered that high concentrations of uKIM-1 were strongly associated with fDGF and future allograft loss, while other acute kidney injury (AKI) biomarkers including L-FABP, NGAL, and NAG were not. These associations were independent of uCr, Scr, and acute allograft rejection situations where immunity might be a confounding factor. These results provide insights for graft function monitoring of patients after transplantation, as well as a foundation for future studies in this field.

In our previous work, we studied the diagnostic value

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Table 2 Comparison of urinary biomarker levels between fDGF group and IGF group

Biomarker	Time after transplant	Total (n=140)	fDGF (n=37)	IGF (n=103)	P value
uKIM-1/uCr (ng/mmol)	0 hour postoperative	4.741 (3.770–6.011)	5.885 (4.420–7.913)	4.605 (3.417–5.653)	0.001**
	1st day postoperative	4.452 (3.602–5.705)	5.569 (4.181–6.722)	4.002 (3.222–6.488)	0.000**
	3rd day postoperative	4.099 (2.241–6.5913)	4.362 (3.501–6.950)	3.696 (1.879–6.547)	0.175
uL-FABP/uCr (µg/mmol)	0 hour postoperative	74.639±39.237	89.818±39.332	69.187±37.926	0.006**
	1st day postoperative	74.630±43.795	82.197±44.773	71.912±43.336	0.222
	3rd day postoperative	69.705 (32.723–92.533)	77.835 (60.368–100.678)	66.841 (28.815–89.783)	0.037*
uNGAL/uCr (ng/mmol)	0 hour postoperative	0.720 (0.500–1.483)	0.640 (0.440–0.915)	0.760 (0.550–1.540)	0.060
	1st day postoperative	0.740 (0.513–1.268)	0.700 (0.500–0.895)	0.820 (0.510–1.390)	0.147
	3rd day postoperative	0.775 (0.500–1.275)	0.610 (0.510–1.140)	0.810 (0.480–1.350)	0.357
uNAG/uCr (ng/mmol)	0 hour postoperative	12.435 (8.650–17.525)	13.697 (10.262–19.575)	11.671 (8.136–17.317)	0.109
	1st day postoperative	12.395 (8.040–16.535)	12.542 (10.280–17.933)	12.254 (7.379–16.031)	0.220
	3rd day postoperative	12.125 (7.248–1.155)	13.341 (11.142–17.158)	11.554 (6.453–17.237)	0.083

Data that conform to a normal distribution are represented by the mean ± standard deviation (SD), and non-normally distributed data are represented by the median (interquartile range). *, $P<0.05$; **, $P<0.01$. fDGF, functional delayed graft function; IGF, immediate graft function; uKIM-1, urinary kidney injury molecular 1; uL-FABP, urinary fatty acid-binding protein; uCr, urinary creatinine; uNGAL, urinary neutrophil gelatinase-related lipoprotein; uNAG, N-acetyl-β-D glucosaminidase; uKIM-1/uCr, the ratio of uKIM-1 to uCr; uL-FABP/uCr, the ratio of uL-FABP to uCr; uNGAL/uCr, the ratio of uNGAL to uCr; uNAG/uCr, the ratio of uNAG to uCr.

of novel and non-invasive markers for AKI (25-27); in the current study, we extended our investigation to renal transplantation. KIM-1 has diverse characteristics that make it an ideal biomarker for the prediction of graft function. Specifically, as a transmembrane protein, KIM-1 is significantly upregulated in urine by injured proximal tubular cells after various types of kidney injury, but it remains undetectable in healthy kidneys (28). Serum KIM-1 level testing revealed no difference between the fDGF and IGF groups, supporting KIM-1 as an independent urinary tubular injury marker. Also, the existence of an immunoglobulin and mucin domain allows KIM-1 to participate in innate immunity downregulation by mediating epithelial cell phagocytosis of apoptotic cells, which subsequently reduces inflammation and innate immunity (29,30). Furthermore, KIM-1 is also essential in maladaptive repair between AKI and progressive chronic kidney disease (CKD) by promoting monocyte chemotactic protein-1 (MCP-1)-dependent kidney fibrosis (31). Additionally, KIM-1 exhibits sensitivity-enhanced responses to AKI compared to the traditional marker Scr (32,33), making it an ideal candidate as an early biomarker of graft function.

Our previous work examined the relationship between

AKI and CKD. In a case-control study of 201 participants with AKI, we observed that 98 patients did not recover renal function during 1 year of follow-up, whereas 50% of patients presented with renal function deterioration. A higher level of uKIM-1 was associated with an elevated risk of renal function deterioration (HR, 1.018; 95% CI, 1.004–1.031) (16). Our studies in early and obstructive nephropathy demonstrated similar results (26,34).

Few prior studies have evaluated the association between uKIM-1 and the long-term outcomes of kidney transplantation patients. While Hall *et al.* (6) proposed that uKIM-1 did not predict allograft failure in 91 patients, another 1-year follow-up cohort study led by Szeto *et al.* (35) showed that uKIM-1 predicted the rate of graft function decline through the detection of messenger RNA (mRNA), which is in accordance with our conclusions. van Timmeren *et al.* (9) also reported recipients graded by uKIM-1 tertiles as 0.72 (interquartile range, 0.49–1.09) or 1.69 (interquartile range, 1.15–10.04) ng/24 h had a HR of 3.6 (95% CI, 0.9–13.5) or 5.1 (95% CI, 1.5–17.8) for predicting graft loss, respectively. Discrepancies in findings among different studies may be the consequence of diverse cohort population sizes, complication conditions, and the possibility of residual confounding, necessitating further
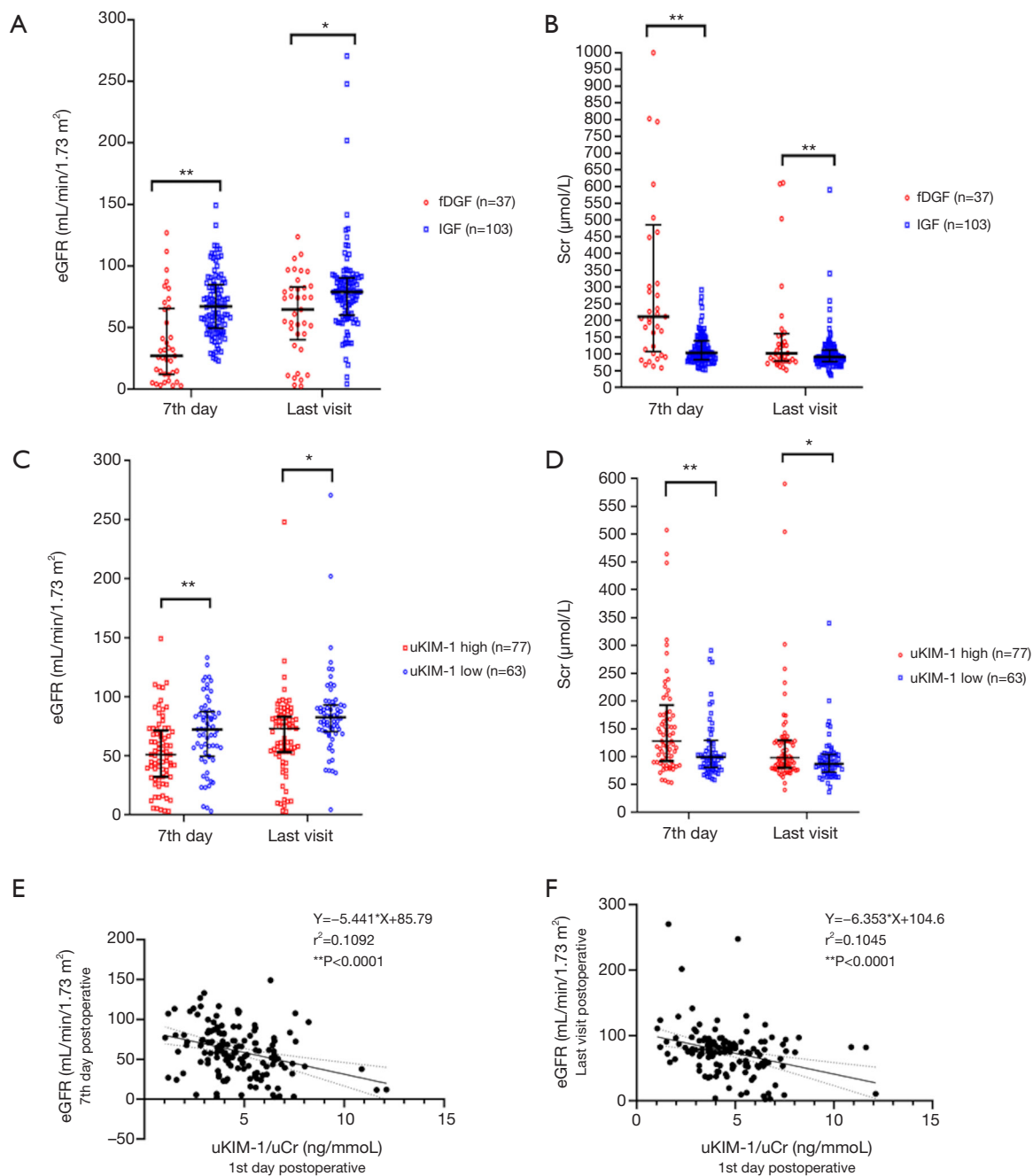
Figure 3 Association between uKIM-1/uCr and graft function at different time points post transplantation. (A,B,C,D) Medians and interquartile ranges for Scr and eGFR after kidney transplantation on day 7 post-surgery and at the last follow-up visit. (E,F) Spearman's correlation analysis between uKIM-1/uCr (1st day post-operation) and Scr (days 3 and 7 post-surgery). *, P<0.05; **, P<0.01. fDGF, functional delayed graft function; IGF, immediate graft function; uKIM-1, urinary kidney injury molecular 1; uCr, urinary creatinine; uKIM-1/uCr, the ratio of uKIM-1 to uCr; Scr, serum creatinine; uKIM-1/uCr low, uKIM-1/uCr <4.08 (μg/mmol); uKIM-1/uCr high, uKIM-1/uCr ≥4.08 (μg/mmol); r, regression coefficient.

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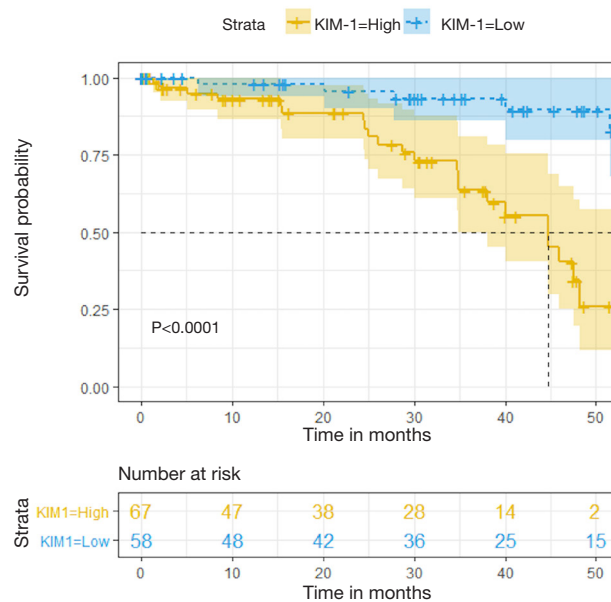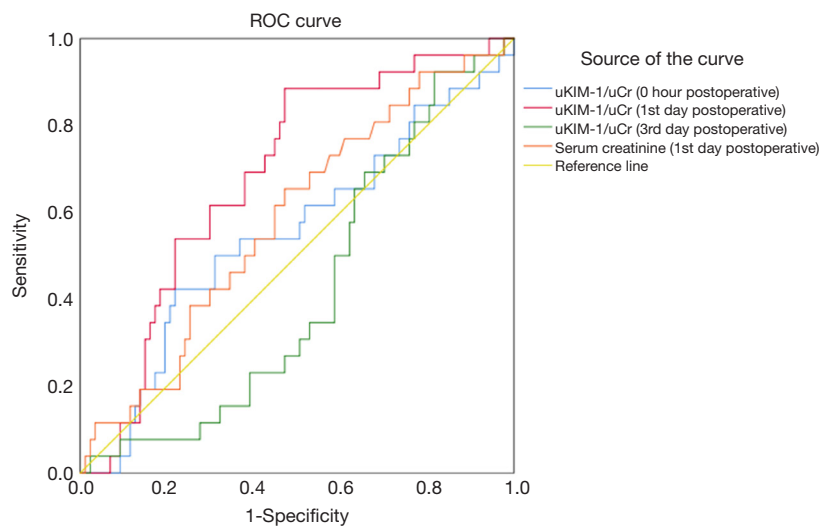

Figure 4 Kaplan-Meier plot of long-term graft dysfunction. Graft dysfunction: eGFR <60 (mL/min/1.73 m^2); KIM-1 = low: uKIM-1/uCr <4.08 µg/mmol (n=58); KIM-1 = high: uKIM-1/uCr ≥4.08 µg/mmol (n=67). eGFR, estimated glomerular filtration rate; uKIM-1, urinary kidney injury molecular 1; uCr, urinary creatinine.

Potential biomarker	Time after transplant	AUC (95% CI)	P-value	Cutoff	Sensitivity	Specificity
uKIM-1/uCr (ng/mmol)	0 hour postoperative	0.549 (0.427–0.670)	0.422			
uKIM-1/uCr (ng/mmol)	1st day postoperative	0.682 (0.581–0.782)	0.003*	3.98	90.00%	51.60%
uKIM-1/uCr (ng/mmol)	3rd day postoperative	0.430 (0.318–0.543)	0.268			
Serum creatinine (µmol/L)	1st day postoperative	0.592 (0.478–0.706)	0.139			

Figure 5 Receiver operating characteristic (ROC) curves and areas under the curves for predicting long-term graft dysfunction. *, P<0.05. AUC, area under the curve; 95% CI, 95% confidence interval; uKIM-1/uCr, the ratio of uKIM-1 to uCr; uKIM-1, urinary kidney injury molecular 1; uCr, urinary creatinine.
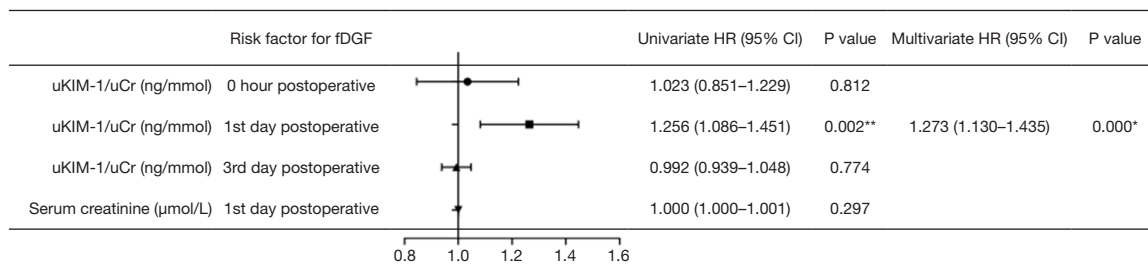
	Risk factor for fDGF		Univariate HR (95% CI)	P value	Multivariate HR (95% CI)	P value
uKIM-1/uCr (ng/mmol)	0 hour postoperative		1.023 (0.851–1.229)	0.812		
uKIM-1/uCr (ng/mmol)	1st day postoperative		1.256 (1.086–1.451)	0.002**	1.273 (1.130–1.435)	0.000*
uKIM-1/uCr (ng/mmol)	3rd day postoperative		0.992 (0.939–1.048)	0.774		
Serum creatinine (µmol/L)	1st day postoperative		1.000 (1.000–1.001)	0.297		

Figure 6 Forest plot of Cox regression analysis in long-term graft function among recipients. *, P<0.05; **, P<0.01. HR, hazard ratio; 95% CI, 95% confidence interval; fDGF, functional delayed graft function.

investigation.

Based on the aforementioned literature, our study further detected uKIM-1 at 3 time points after transplantation in a larger cohort of 140 recipients. We demonstrated that on the first day post-surgery, uKIM-1 predicted a 23.5% increase in the risk of fDGF and a 27.3% increase in the risk of long-term allograft dysfunction.

Recent studies focusing on DGF have uncovered several promising biomarkers, including NGAL, IL-18, KIM-1, and NAG in particular (22,24). The present study also included L-FABP, a fatty acid-binding protein excreted from the cytoplasm of proximal renal tubular cells in response to acute injury (36,37), NGAL, a siderophore binding protein expressed by distal epithelial cells which is sensitive to AKI (38,39), and NAG, a lysosomal enzyme of proximal tubular cells that is sensitive to proximal tubular injury caused by drugs, environmental toxicants, contrast-induced toxicity, and ischemia (40,41). We found no statistically significant differences between the fDGF group and the IGF group in terms of either NGAL or NAG in the first 3 days post transplantation. Further regression analysis reinforced this result. These findings support those of an American study which indicated that urine NGAL/Cr was independently associated with cardiovascular events rather than fDGF or long-term allograft outcomes (42). Additionally, another report from Nauta *et al.* (15) confirmed that NAG could not predict graft loss after adjustment with albuminuria, which further validates our conclusion.

Multivariate regression analysis showed that uL-FABP/Cr on the first day post transplantation was also not related to fDGF. Research by Bansal *et al.* (42) produced comparable results. Despite being strongly associated with AKI, urinary biomarkers for tubular injury may not be applicable for fDGF or long-term allograft outcomes. A probable explanation may be the slower responses of these biomarkers compared to KIM-1, or there may be a unique mechanism underlying ischemia tubular injury and long-term allograft outcomes that involves KIM-1 but not other markers. Our findings suggest that proximal tubular injury, which is more closely related to fibrosis, contributes to long-term allograft loss, regardless of kidney filtration.

There were also some limitations to this study. Firstly, it is a single-center study. Bias in patient selection might therefore have been introduced. Moreover, extracorporeal membrane oxygenation (ECMO) was not performed during the acquirement of all DCD kidneys in our center. The relationships between uKIM-1, fDGF, allograft loss, and all-cause mortality may be further explored with longer follow-ups in the future. Secondly, our findings require validation in separate cohorts, due to our cohort only covering recipients with a deceased donor kidney. Thirdly, we only tested biomarker levels during the first 3 days after transplantation. Whether uKIM-1 can be restored to a normal level, or if there are other biomarkers of AKI related to allograft outcomes at later time points post-surgery remains uncertain.

Conclusions

In this cohort of 140 renal transplantation recipients, we found that high uKIM-1 expression on the first day post-surgery predicts short-term fDGF and is a potential biomarker for long-term graft function. This study therefore proposes a potent biomarker for early monitoring and graft deterioration interventions due to graft ischemia-reperfusion injury, and a feasible therapeutic target for chronic allograft nephropathy.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-2215a). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Renji Hospital, School of Medicine, Shanghai Jiao Tong University (14140903200). All participants had signed informed consent.

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