Urinary Tissue Inhibitor of Metalloproteinase and Insulin-like Growth Factor–7 as Early Biomarkers of Delayed Graft Function After Kidney Transplantation

J. Yang[a], S.Y. Lim[a], M.-G. Kim[a], C.-W. Jung[b], W.-Y. Cho[a], and S.-K. Jo[a,*]

[a]Department of Nephrology, Korea University Anam Hospital, Seoul, Korea; and [b]Department of Transplantation and Vascular Surgery, Korea University Anam Hospital, Seoul, Korea

ABSTRACT

Background. Recently, urinary tissue inhibitor of metalloproteinase–2 (TIMP-2) and insulin-like growth factor–7 (IGFBP-7), markers for G1 cell cycle arrest, have been identified and validated in predicting the development of acute kidney injury in critically ill patients. It is unknown, however, whether these two biomarkers could predict the development of delayed graft function (DGF) after kidney transplantation (KT).

Methods. This is a single-center, prospective, observational study. We enrolled 74 patients who underwent KT between August 2013 and December 2016. Urine sample were collected immediately after the operation. The primary outcome was development of DGF as defined by need for dialysis of more than 1 session within 7 days of KT.

Results. Twenty-three patients (31%) were diagnosed with DGF. In univariate analysis, kidneys from expanded criteria donors, higher donor serum creatinine, lower donor estimated glomerular filtration rate, antithymoglobulin exposure, neutrophil gelatinase associated lipocalin, and urinary [TIMP-2]·[IGFBP7] were significantly different between early graft function and DGF. However, in multivariate analysis adjusting other factors, deceased donor and urinary [TIMP-2]·[IGFBP7] at 0 hours post-transplantation could predict the development of DGF. The receiver operating characteristic curve for prediction of DGF showed an area under the curve of 0.867 (sensitivity 0.86, specificity 0.71) for a cutoff value of 1.39.

Conclusions. Our results indicate that urine [TIMP-2]·[IGFBP7] immediately after transplantation could be an early, predictive biomarker of DGF in kidney transplantation.

DELAYED graft function (DGF) is most often the result of perioperative ischemic injury, and it can have adverse effects on both short-term and long-term graft outcomes [1–4]. Recognition of DGF has increased as more kidneys from expanded-criteria donors have been transplanted to overcome the relative shortage of organs. Kidneys from suboptimal donors, including older and hypertensive donors, are known to be more susceptible to ischemic or nephrotoxic injuries, and may be associated with impaired recovery [5].

Several urine biomarkers, including neutrophil gelatinase associated lipocalin (NGAL), liver type fatty acid binding protein, and interleukin 18, have been shown to be more useful than serum creatinine for predicting DGF [2,6–8]. These biomarkers have also been validated in a number of patient populations, including patients who have undergone cardiothoracic surgery or experienced contrast-induced nephropathy, intensive care management, or intra-abdominal surgery. In addition, attempts to predict DGF early by assessing the urine biomarkers of donors have also been studied recently in different centers, yielding conflicting results.

Insulin like growth factor–7 (IGFBP-7) and tissue inhibitor of metalloproteinase-2 (TIMP-2) are G1 cell cycle arrest markers

*Address correspondence to Sang-kyung Jo, PhD, Korea University, 02841, Seongbuk-gu Inchon-ro 73, Seoul, Korea. E-mail: sang-kyung@korea.ac.kr

0041-1345/17
https://doi.org/10.1016/j.transproceed.2017.09.023

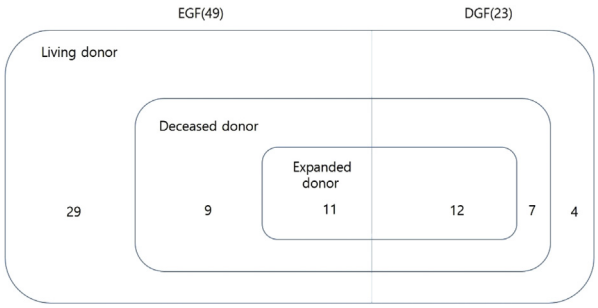

Fig 1. Donor category and short term outcome.

that are upregulated after various types of kidney injury. The NephroCheck Test (Astute Medical, San Diego, California, United States), which measures both of these markers simultaneously, was approved by the Food and Drug Administration (FDA) in 2014. The NephroCheck Test can determine the risk of developing moderate to severe acute kidney injury (AKI) within 12 hours in high-risk patients [9–14], but the utility of cell cycle arrest markers for predicting DGF in transplantation patients has not been shown or compared with other biomarkers. In this prospective observational study, we evaluated whether immediate post-transplantation urine [TIMP-2]·[IGFBP7] could be a useful novel biomarker for the early prediction of DGF. We also compared its predictive accuracy with that of NGAL after kidney transplantation (KT).

MATERIALS AND METHODS

Patients

This was a single-center, prospective, observational study. We enrolled patients who underwent KT in Korea University's Anam Hospital between August 2013 and December 2016. A total of 74 patients were enrolled. This study was approved by the Korea University Institutional Review Board (ED 12151). All of the patients were provided with written informed consent. A similar immunosuppressive regimen was used for all of the patients that consisted of tacrolimus with prednisone and mycophenolate mofetil. Basiliximab or antithymoglobulin antibody was infused in cases involving high-risk donors, ABO-incompatible KT, and with high panel-reactive antibody titer. Spot urine samples were collected immediately after the operation, centrifuged at 20,000 G for 5 minutes, and the supernatants were stored in aliquots at −80°C. The primary study outcome was the development of DGF, with DGF defined by the need for at least one dialysis session within 7 days of KT [5]. Prolonged DGF was defined as having a duration longer than 15 days [3]. Early graft function (EGF) was defined as a non-DGF patient who showed good graft function at the beginning of the post-transplantation period. Slow graft function was defined as a serum creatinine reduction ratio between 0 hours and on day 7 divided by the serum creatinine at 0 hours that was less than 0.75, as we described in our previous study [8].

Enzyme-linked Immunosorbent Assay for IGFBP7, TIMP-2, and NGAL

The urine samples were pipetted into wells that were pre-coated with a mouse monoclonal antibody raised against human IGFBP-7 (human IGFBP7 enzyme-linked immunosorbent assay [ELISA] kit, Biorbyt, Cambridge, United Kingdom), TIMP-2 (human TIMP2 ELISA kit, abcam, Cambridge, United Kingdom), and NGAL (Bioporto, Gentofte, Denmark). We measured each protein level according to the manufacturer's instructions. The wells pre-coated with antibodies specific for each protein were blocked with a buffer containing 1% bovine serum antigen, and 100 μL of samples (urine) or standards were added and incubated with a biotinylated monoclonal antibody. TMB substrate was added and the color developed in proportion to the amount of protein bound. The color intensity was measured after 30 minutes at 450 nm with a microplate reader. The ELISA process was completed within 4 hours. The laboratory facilities did not have any information on the sample sources and clinical outcomes until the end of the process. Urine NGAL was expressed in ng/mg creatinine to standardize for changes in urine concentration.

Statistics

The results were expressed as the mean ± SD when they showed standard distribution or the median with ranges for elements with skewed distribution. The SPSS software (IBM, version 22.0, Armonk, New York, USA) was used for the statistical analyses. Comparisons between two groups were performed using the Student t test for numerical data, and the χ^2 test for categorical data. Categorical variables were expressed as numbers with proportions. Comparisons between multiple groups were analyzed with analysis of variance, followed by the Bonferroni post hoc test. We conducted univariate and multivariate logistic regression analyses to assess the predictors of DGF. A conventional receiver operating characteristic (ROC) curve was generated for urine [TIMP-2]·[IGFBP7] and NGAL at day 0 post-transplantation and DGF. We used the MedCalc software Version 17.7.2 (MedCalc Software, Ostend, Belgium) DeLong method to compare the effectiveness of ROC between [TIMP-2]·[IGFBP7] and NGAL. $P < .05$ was considered statistically significant.

RESULTS

Patient Characteristics and Outcomes

Of the 74 patients enrolled, 32 received kidneys from deceased donors (43.2%). In deceased donor transplantation, 23 were from donors from expanded donor criteria (EDC) (71.8%). All living donor transplantation was conducted from standard criteria donors (Fig 1). Twenty-three patients developed DGF or prolonged DGF (31%). The median follow-up period was 20 months (minimum 3 months, maximum 91 months) and 2 patients who died of unexpected new onset myocardial infarction and uncontrolled bleeding after transplantation were excluded from the analysis. The baseline characteristics of the 72 recipients and information on the donors are listed in Table 1. There were no differences in age, gender, causes of end-stage renal disease, ischemic time, or degree of HLA mismatch between the patients with EGF and DGF. However, percent hemodialysis and transplantation from deceased or EDC donors were significantly higher in the DGF group. Donor serum creatinine levels and estimated glomerular filtration rates (eGFRs) were significantly higher. During the follow-up period, there was no difference in the incidence of humoral or cellular mediated acute rejection. However, patients who developed DGF showed significantly worse graft function at 1, 3, 6, and 12 months after KT (Table 1).