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RESEARCH ARTICLE

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Urinary neutrophil gelatinase-associated lipocalin rapidly decreases in the first week after kidney transplantation

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Abstract

Background: Recipient delayed graft function, which is defined as dialysis in the first week after transplantation, is one of the most common early complications after kidney transplantation. This study aimed to evaluate the daily changes in renal function-related biomarkers in the first week post-transplant.

Methods: A total of 72 kidney transplant recipients were retrospectively included in this study. Clinical and laboratory data were collected daily during the first week post-transplant, including urinary concentrations of neutrophil gelatinase-associated lipocalin (NGAL), serum concentrations of NGAL, creatinine, urea nitrogen, uric acid (UA), β2-microglobulin, cystatin C, and estimated glomerular filtration rate (eGFR).

Results: There were no significant differences in urea nitrogen (P = .375), UA (P = .090), and cystatin C (P = .691), while urinary NGAL (P < .0001), serum NGAL (P < .0001), creatinine (P < .0001), β2-microglobulin (P < .0001), and eGFR (P < .0001) were statistically significant in the first week post-transplant. In comparison with serum NGAL (P < .0001), creatinine (P < .0001), β2-microglobulin (P = .001), and eGFR (P = .001), the change ratios of urinary NGAL changed the most between day 1 and day 2 after renal transplantation, while the changing degree of urinary NGAL showed no significant difference compared with these indicators between day 1 and day 7 after kidney transplantation.

Conclusion: Urinary NGAL is a sensitive marker for indicating renal function. Urinary NGAL combined with other markers can be more helpful for evaluating renal function in the first week following kidney transplantation.

KEYWORDS
renal function markers, renal transplantation, urinary NGAL

1 | INTRODUCTION

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa protein belonging to the lipocalin superfamily[1] and is released by activated neutrophils and renal tubular cells.[2] NGAL is freely filtered through the glomerulus and reabsorbed in the proximal tubule.[3] The NGAL concentration is very low in normal physiologic conditions. NGAL is synthesized in large amounts during the course of kidney injury and was explored as

a marker for compromised organ function.[4,5] Urine-related biomarkers during kidney injury, such as NGAL, kidney injury molecule-1, IL-18, and liver fatty acid-binding protein, have been confirmed as accurate markers of kidney injury through clinical experimentation.[6,7]

Delayed graft function (DGF) is one of the most common complications of kidney transplantation, defined as the need for dialysis within one week of transplantation.[8] Additionally, DGF is associated with decreased short- and long-term graft survival rates.[9] The current evaluation of renal function in renal transplantation recipients still depends on serum creatinine (SCr), glomerular filtration rate, serum urea nitrogen, and other clinical indicators.[10,11] In this retrospective study, we investigated daily changes in renal function-related biomarkers in the first week after transplantation and evaluated the role of NGAL in the functional recovery of renal transplantation.

2 | METHODS AND METHODS

2.1 | Patient selection

This was a retrospective study that analyzed patients who received a kidney transplantation in the First Affiliated Hospital of Zhengzhou University. The inclusion criteria included the following: (a) patients who were first time recipients of renal transplantation; (b) the follow-up period was at least 3 months. The exclusion criteria were as follows: (a) patients with any malignant diseases; (b) outpatients and patients without complete medical records; (c) patients with immunodeficiency; (d) patients with chronic and acute inflammatory diseases; (e) patients with genetic disease; and (f) loss of follow-up during observation. All study subjects submitted informed consent. The study procedures are in accordance with the Declaration of Helsinki and were approved by the Medical Research and Research Ethics Committee of the First Affiliated Hospital of Zhengzhou University. We reviewed the information of 72 patients who received a kidney transplantation from February 2017 to October 2018, including clinical demographic characteristics and daily renal function markers within one week after kidney transplantation.

2.2 | Sample collection and analysis

Postoperative serum and urine samples were collected from patients on an empty stomach in the morning. Samples on postoperative day 1 were collected nearly 3 to 12 hours after renal transplantation. The samples were collected daily from day 1 to day 7 after kidney transplantation.

Serum creatinine was measured by the creatinine oxidase method; blood urea nitrogen and uric acid (UA) were detected by the colorimetric method; cystatin C (Cys C) and β2-microglobulin (β2-MG) were measured by the immunoturbidimetric method; estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease formula.[12] Roche cobas 8000 automatic biochemical analyzer (Roche Diagnostics, Switzerland) and test kits (Roche Diagnostics, Switzerland) were used to detect daily renal function markers, and all experimental procedures strictly followed the manufacturer's instructions. Immunological scattering turbidity was used to detect serum and urinary NGAL using the PA8800 special protein analyzer (Beijing Prom Medical, China), according to the neutrophil gelatinase-related lipocalin detection kit (Beijing Prom Medical). Urinary NGAL >100 ng/mL or serum NGAL concentration >106 ng/mL was judged as positive, according to the reference value provided by the manufacturer.

2.3 | Statistical analysis

Statistical analyses were performed using the SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). The data in each group were tested for normality using the Kolmogorov-Smirnov (KS) test. The data of each group were non-normally distributed and are represented by the median and quartile [M (P25~P75)]. The Kruskal-Wallis H test was used for multiple group comparisons, and the Mann-Whitney *U* test was used for pairwise comparisons. Student's *t* test was used to compare the change ratios between independent quantitative data. A $P < .05$ indicated statistical significance. GraphPad Prism 5.0 software was used for image drawing.

3 | RESULTS

3.1 | Patient characteristics

Basic clinical characteristics of the renal transplantation patients are shown in Table 1. Seventy-two patients were included in the study,

TABLE 1 Clinical data of 72 kidney transplant recipients

Characteristics	All Patients n = 72, n (%)
Age (y), median (range)	35 (13-57)
Gender	
Male	53 (74)
Female	19 (26)
Underlying diseases	
IgA nephropathy (%)	1 (1)
Gout nephropathy (%)	1 (1)
Comorbidities	
Renal hypertension (%)	41 (57)
Renal anemia (%)	38 (53)
Preoperative dialysis mode	
Hemodialysis status (%)	38 (53)
Peritoneal dialysis status (%)	7 (10)
Operation	
Allogeneic kidney transplantation (%)	70 (97)
Allogeneic kidney transplantation & peritoneal dialysis catheter resection (%)	2 (3)

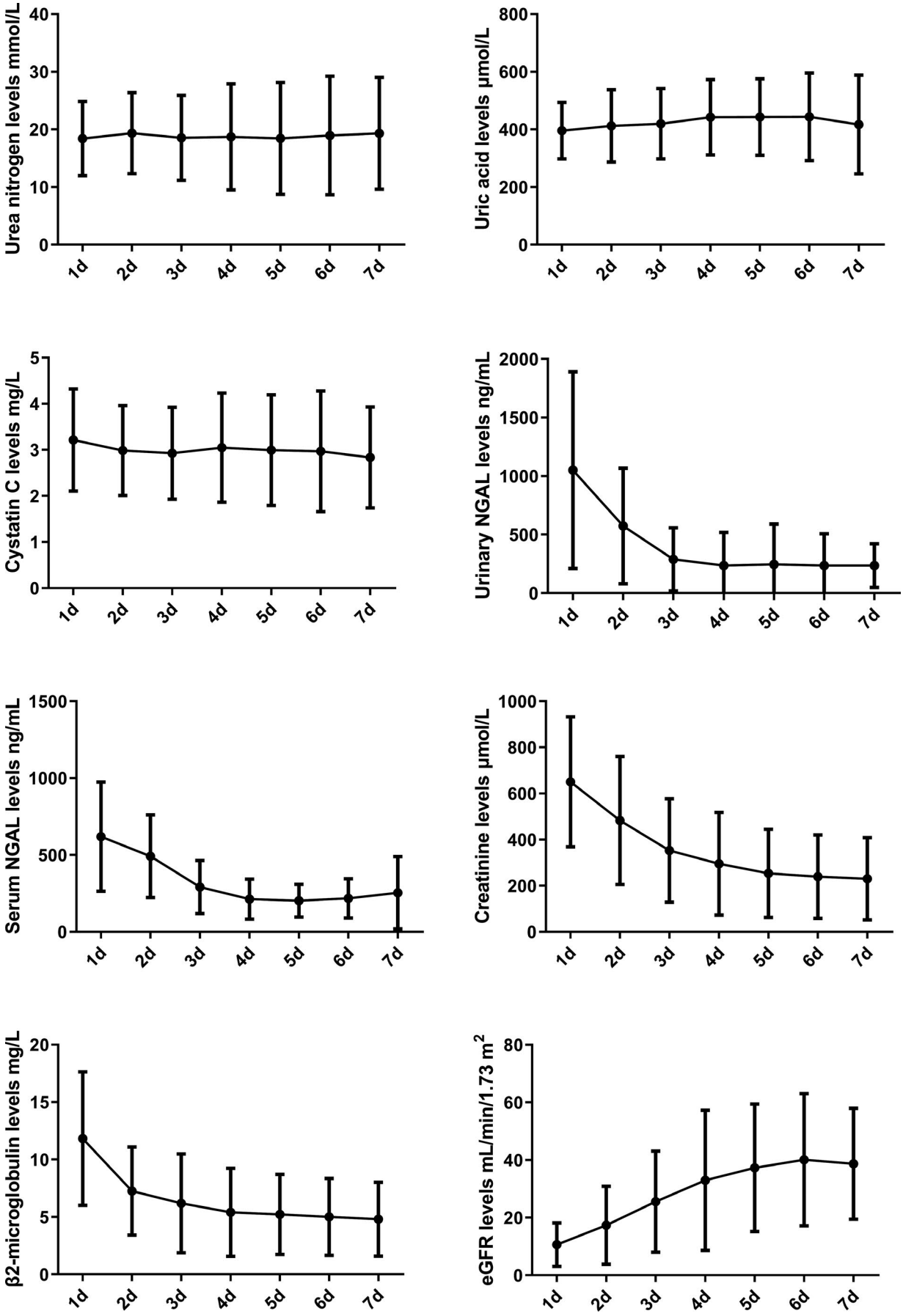

FIGURE 1 Changing trends in kidney functional tests within 1 week after kidney transplantation

TABLE 2 Whole variation of renal function tests in the first week after kidney transplantation

	1d	2d	3d	4d	5d	6d	7d	*P*-value
UN (mmol/L)	18.41 (14.75, 22.05)	19.34 (14.12, 23.35)	18.55 (13.43, 22.73)	18.72 (13.80, 20.90)	18.44 (12.0, 20.20)	18.94 (11.62, 22.13)	19.32 (12.60, 22.20)	.375
UA (μmol/L)	395.69 (326.0, 435.50)	412.5 (319.25, 461.75)	419.86 (347.25, 477.0)	442.57 (368.75, 517.25)	443.1 (350.0, 521.0)	443.88 (308.50, 540.50)	416.79 (316.0, 493.0)	.090
Cys C (mg/L)	3.21 (2.31, 3.98)	2.99 (2.20, 3.66)	2.93 (2.20, 3.44)	3.05 (2.22, 3.41)	3.00 (2.07, 3.31)	2.97 (1.95, 3.50)	2.84 (2.08, 3.61)	.691
Urinary NGAL (ng/mL)	1051.5 (382.59, 1580.43)	573.74 (223.63, 791.74)	289.98 (95.49, 422.82)	235.23 (91.72, 241.59)	245.82 (57.90, 362.89)	236.26 (85.91, 332.19)	235.4 (76.96, 337.63)	<.0001
Serum NGAL (ng/mL)	619.81 (330.20, 18.70)	491.29 (267.48, 655.96)	291.48 (180.65, 347.28)	212.62 (114.83, 252.44)	212.62 (112.93, 262.51)	218.7 (103.17, 289.80)	253.85 (110.57, 309.28)	<.0001
Cr (μmol/L)	650.79 (401.0, 857.25)	483.21 (263.5, 630.75)	352.86 (183.0, 441.25)	295.3 (162.25, 326.75)	254.12 (138.75, 284.0)	239.46 (127.25, 249.75)	230.76 (133.25, 240.25)	<.0001
β_2-MG (mg/L)	11.82 (7.10, 15.53)	7.26 (3.87, 9.57)	6.19 (3.37, 7.43)	5.41 (2.91, 6.32)	5.23 (2.80, 6.80)	5.00 (2.71, 6.08)	4.82 (2.49, 6.94)	<.0001
eGFR (mL/min/1.73 m^2)	10.61 (5.90, 13.16)	17.36 (8.15, 22.88)	25.54 (11.45, 34.58)	32.99 (15.02, 42.42)	37.32 (19.95, 49.93)	40.11 (21.95, 55.55)	38.69 (22.70, 53.65)	<.0001

Note: The data were expressed as median and quartile (M [P25 ~ P75]).

Abbreviations: Cr, creatinine; Cys C, cystatin C; eGFR, estimated glomerular filtration rate; serum NGAL, serum neutrophil gelatinase-associated lipocalin; UA, uric acid; UN, urea nitrogen; urinary NGAL, urinary neutrophil gelatinase-associated lipocalin; β2-MG, β2-microglobulin.

including 53 males and 19 females, and the median age was 35 years (range 13–57). Among the 72 patients, renal hypertension (57%) and renal anemia (53%) were the most common complications. All patients had end-stage renal disease before transplantation. Few patients had IgA nephropathy (1%) or gout nephropathy (1%).

3.2 | Trends of renal function-related indicators within one week after renal transplantation

Our study included 72 patients who underwent renal transplantation. Data showed that within one week after kidney transplantation, urinary NGAL, serum NGAL, SCr, and β2-MG showed a significant decline, while eGFR increased significantly. There were no significant differences observed in UA, urea nitrogen, and cystatin C (Figure 1). Table 2 shows the laboratory data of the kidney-related indicators within one week after transplantation. Within the first week post-transplantation, there were no significant differences in the daily concentration variation of urea nitrogen ($P = .375$), UA ($P = .090$), and cystatin C ($P = .691$). Conversely, urinary NGAL ($P < .0001$), serum NGAL ($P < .0001$), SCr ($P < .0001$), β2-microglobulin ($P < .0001$), and GFR ($P < .0001$) showed statistically significant changes in the first week after kidney transplantation.

3.3 | Comparison of daily concentrations of indicators within the first week of post-transplantation

Within the first week after kidney transplantation, the concentrations of urinary NGAL, β2-MG, and eGFR were statistically different on day 1 and day 2 compared with concentrations on other days. There were statistical differences in the concentrations of serum NGAL, creatinine at day 1, day 2, and day 3 compared with other days. Additionally, the concentration of creatinine on day 4 showed significant differences compared with day 6 and day 7 after surgery, and statistical significance was observed in the concentrations of β2-MG on day 3 compared with day 6 and day 7. The eGFR concentration was statistically significant on day 3 compared with day 5, day 6, and day 7 after surgery, and there were significant differences in eGFR concentrations between day 4 and day 6 after transplantation. Moreover, there were no differences in pairwise comparisons at other times. We also found there were no significant differences in the daily concentration changes in UA, urea nitrogen, and cystatin C within the first week after surgery (Figure 2).

3.4 | Comparison of indicator change ratios between day 1 and day 2, day 1 and day 7 after renal transplantation

The comparison of change ratios of urinary NGAL, serum NGAL, creatinine, β2-MG, and eGFR between day 1 and days 2 to 7 after kidney transplantation are shown in Table 3.

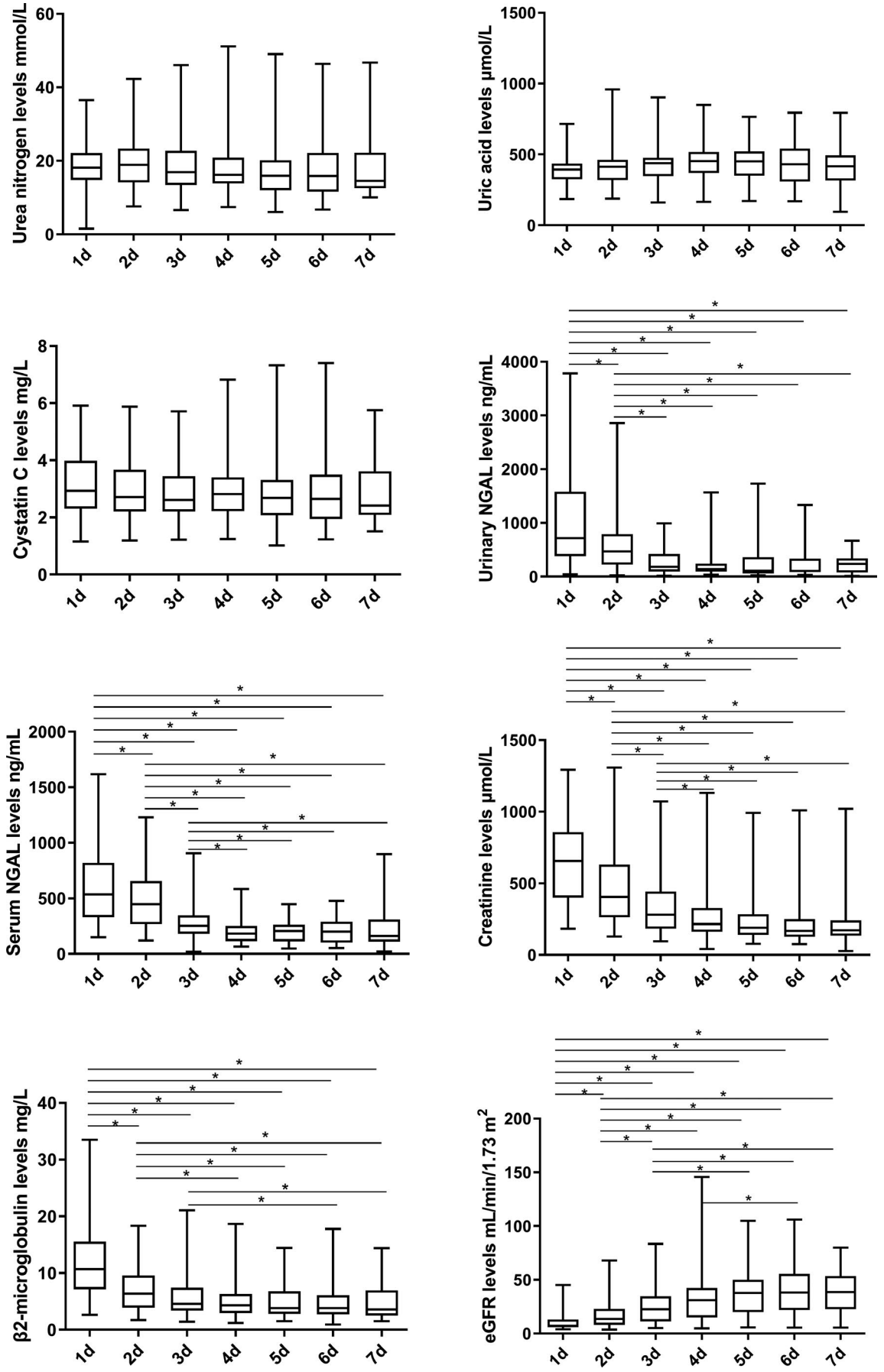

FIGURE 2 Daily concentration changes in kidney functional indexes in the first week after kidney transplantation. *$P < .05$

TABLE 3 Change ratios of urinary NGAL, serum NGAL, creatinine, β2-MG, and eGFR on day 1 compared with the rest days within the first week after kidney transplantation (%)

	Urinary NGAL	Serum NGAL	Creatinine	β2-MG	eGFR
2d vs 1d	45.44%	20.74%	25.75%	38.54%	38.86%
3d vs 1d	72.42%	52.97%	45.78%	47.58%	58.43%
4d vs 1d	77.63%	65.70%	54.62%	54.19%	67.82%
5d vs 1d	76.62%	67.15%	60.95%	55.72%	71.55%
6d vs 1d	77.53%	64.71%	63.20%	57.67%	73.53%
7d vs 1d	77.61%	59.04%	64.54%	59.26%	72.56%

TABLE 4 Change ratios of urinary NGAL compared with serum NGAL, creatinine, β2-MG, and eGFR after kidney transplantation (%)

	2d vs 1d	*P*-value	7d vs 1d	*P*-value
Urinary NGAL vs serum NGAL	45.44% vs 20.74%	<.0001	77.61% vs 59.04%	.096
Urinary NGAL vs creatinine	45.44% vs 20.76%	<.0001	77.61% vs 64.54%	.183
Urinary NGAL vs β2-MG	45.44% vs 38.54%	.001	77.61% vs 59.26%	.125
Urinary NGAL vs eGFR	45.44% vs 38.86%	.001	77.61% vs 72.56%	.117

In the comparison of the change ratios between day 1 and day 2 after renal transplantation in urinary NGAL with serum NGAL ($P < .0001$), creatinine ($P < .0001$), β2-MG ($P = .001$), and eGFR ($P = .001$), the change in urinary NGAL was statistically significant. In the comparison of changes between day 1 and day 7 after renal transplantation, urinary NGAL showed no significant difference in the proportion of change compared with serum NGAL ($P = .096$), creatinine ($P = .183$), β2-MG ($P = .125$), and eGFR ($P = .117$) (Table 4).

4 | DISCUSSION

Delayed graft function, defined as the requirement for dialysis in the first week after transplantation, is one of the most common early complications in kidney transplantation recipients. In a previous study, the incidence of DGF in kidney transplant recipients was 24.3% when the source of the kidney was from a cadaver, and the incidence of DGF was 4% to 10% in kidneys from living donors.[13-15] At present, the most common indicators used to evaluate renal function after transplantation are creatinine, urea nitrogen, UA, cystatin C, and eGFR. However, these indicators are insensitive and susceptible to non-renal factors, which may lead to inaccurate assessments, unsatisfactory curative effects, and poor prognosis. This also may be one of the important factors that seriously affect the recovery of renal function in kidney transplantation. NGAL is used as a biomarker for the diagnosis of early renal injury. When acute renal injury occurs in non-renal transplant patients, the concentration of NGAL in serum and urine increases at the early stage, and this rapid change in NGAL occurs much earlier than changes in blood creatinine.[16] In contrast, NGAL detection is less affected by other factors and has a unique advantage in reflecting various acute and chronic kidney injuries.[17] In this study, we analyzed the common renal function indicators and NGAL within one week after kidney transplantation to evaluate the role of urinary NGAL in renal function recovery after kidney transplantation.

According to our analysis of changes in renal function-related indicators within one week after transplantation, we found that both serum and urinary NGAL have the same downward trend as creatinine and β2-MG, and they have the opposite trend of eGFR, which was consistent with other studies.[18-20] In the first week after renal transplantation, serum and urinary NGAL, creatinine, and β2-MG increased, while eGFR decreased. This demonstrates these indicators are effective for the early evaluation of renal function after renal transplantation. Conversely, obvious changes in UA, urea nitrogen, and cystatin C were not observed, which suggests they are poor indexes for the early evaluation of renal function. However, Li et al[21] showed that cystatin C, serum NGAL, and urinary NGAL could accurately reflect renal function after renal transplantation. The results of our study showed that cystatin C did not change significantly after kidney transplantation, which was not consistent with Li et al's findings. This discrepancy could be attributed to the low sample size in our study. We will increase the sample size to investigate the role of cystatin C in future studies. However, based on our findings, we suggest that urinary NGAL has an advantage over cystatin C in evaluating renal function injury and recovery.

After analyzing the concentration changes in renal function-related indicators, we found that there were significant differences in serum and urinary NGAL, creatinine, β2-MG, and eGFR within one week after renal transplantation, especially on day 1 and day 2. Out of all the indicators, we observed that urinary NGAL exhibited the most drastic change in the two days after transplantation. Urinary NGAL was significantly higher than the other indicators with a change rate of 45%, indicating that urinary NGAL changed the most quickly in response to renal function recovery after surgery. Although serum

NGAL also changed significantly in the two days after the operation, there were no significant differences in the proportion of changes compared with creatinine and other indicators. This shows that urinary NGAL may be more accurate than serum NGAL at indicating rapid changes in renal function after renal transplantation.

Though urinary NGAL changed the most in the 2 days after surgery, the concentration did not decrease significantly during the rest of the week. This trend was not observed in serum NGAL and other indexes. Regarding creatinine and eGFR, obvious differences were observed on day 4 after the kidney transplantation, which indicates creatinine and eGFR may be more appropriate indexes for the continuous assessment of renal function. Within one week after kidney transplantation, the declining proportion of urinary NGAL was the largest as a whole, but statistical analysis showed there was no statistical difference in the change in urinary NGAL on day 7 after surgery when compared with other indicators. Taken together, the role of NGAL needs further investigation in order to determine its accuracy as an indicator during long-term renal function assessment.

This study preliminary analyzed the relationship between NGAL and commonly used renal function indicators by retrospectively evaluating the clinical and laboratory data of 72 patients after renal transplantation. Our study had a few limitations, such as an inadequate sample size and short follow-up time. In future studies, we plan to expand the number of patients and extend the follow-up time in order to further explore whether NGAL has a predictive role in evaluating renal function and related complications after renal transplantation.

In general, this study has clearly demonstrated that the concentration of NGAL rapidly decreases after kidney transplantation, especially urinary NGAL. Therefore, urinary NGAL combined with common renal function markers may be helpful in the early evaluation of renal function after kidney transplantation.

CONFLICT OF INTEREST

There is no conflict of interest.

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REFERENCES

1. Flower DR. The lipocalin protein family: structure and function. *Biochem J*. 1996;318(Pt 1):1-14.
2. Devarajan P. Review: neutrophil gelatinase-associated lipocalin: a troponin-like biomarker for human acute kidney injury. *Nephrology*. 2010;15(4):419-428.
3. Bolignano D, Lacquaniti A, Coppolino G, Campo S, Arena A, Buemi M. Neutrophil gelatinase-associated lipocalin reflects the severity of renal impairment in subjects affected by chronic kidney disease. *Kidney Blood Press Res*. 2008;31(4):255-258.
4. Yigit IP, Celiker H, Dogukan A, et al. Can serum NGAL levels be used as an inflammation marker on hemodialysis patients with permanent catheter? *Ren Fail*. 2015;37(1):77-82.
5. Schmidt-Ott KM, Mori K, Li JY, et al. Dual action of neutrophil gelatinase-associated lipocalin. *J Am Soc Nephrol*. 2007;18(2):407-413.
6. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis*. 2009;54(6):1012-1024.
7. Siew ED, Ware LB, Ikizler TA. Biological markers of acute kidney injury. *J Am Soc Nephrol*. 2011;22(5):810-820.
8. Hall IE, Yarlagadda SG, Coca SG, et al. IL-18 and urinary NGAL predict dialysis and graft recovery after kidney transplantation. *J Am Soc Nephrol*. 2010;21(1):189-197.
9. Boom H, Mallat MJ, de Fijter JW, Zwinderman AH, Paul LC. Delayed graft function influences renal function, but not survival. *Kidney Int*. 2000;58(2):859-866.
10. Myers GL, Miller WG, Coresh J, et al. Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. *Clin Chem*. 2006;52(1):5-18.
11. Seo MS, Kim HJ, Kim H, Park SW. Ethyl pyruvate directly attenuates active secretion of HMGB1 in proximal tubular cells via induction of heme oxygenase-1. *J Clin Med*. 2019;8(5):629.
12. Ma YC, Zuo L, Chen JH, et al. Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. *J Am Soc Nephrol*. 2006;17(10):2937-2944.
13. Halloran PF, Hunsicker LG. Delayed graft function: state of the art, November 10–11, 2000. Summit meeting, Scottsdale, Arizona, USA. *Am J Transplant*. 2001;1(2):115-120.
14. El-Maghraby TA, Boom H, Camps JA, et al. Delayed graft function is characterized by reduced functional mass measured by (99m) Technetium-mercaptoacetyltriglycine renography. *Transplantation*. 2002;74(2):203-208.
15. Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. *Lancet*. 2004;364(9447):1814-1827.
16. Shang W, Wang Z. The update of NGAL in acute kidney injury. *Curr Protein Pept Sci*. 2017;18(12):1211-1217.
17. Haase-Fielitz A, Haase M, Devarajan P. Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury: a critical evaluation of current status. *Ann Clin Biochem*. 2014;51(Pt 3):335-351.
18. Lei L, Li LP, Zeng Z, et al. Value of urinary KIM-1 and NGAL combined with serum Cys C for predicting acute kidney injury secondary to decompensated cirrhosis. *Sci Rep*. 2018;8(1):7962.
19. Mitsnefes MM, Kathman TS, Mishra J, et al. Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in children with chronic kidney disease. *Pediatr Nephrol*. 2007;22(1):101-108.
20. Maas RJ, van den Brand JA, Waanders F, et al. Kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin as prognostic markers in idiopathic membranous nephropathy. *Ann Clin Biochem*. 2016;53(Pt 1):51-57.
21. Li F, Hu L, Zhao X, et al. The value of cystatin C and urinary and serum neutrophil gelatinase-associated lipocalin during the perioperative period of renal transplantation. *Transl Androl Urol*. 2019;8(5):432-441.

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