The Predictive value of serum NGAL for the diagnosis of Delayed Graft Function in kidney transplantation

CURRENT STATUS: UNDER REVIEW

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DOI: 10.21203/rs.2.24586/v1

SUBJECT AREAS
Urology & Nephrology

KEYWORDS
kidney transplantation, delay graft function, serum NGAL, predictive biomarkers
Abstract
Background and aims the role of serum neutrophil gelatinase-associated lipocalin (NGAL) in predicting delayed graft function (DGF) after kidney transplantation is poorly defined. The objective of this study was to evaluate the serum NGAL expression in the early postoperative phase after kidney transplantation and compare it with serum creatinine (Cr).

Methods We studied 32 patients who received kidney transplantation from deceased (n=24) and live (n=5) donors during October 2017 to December 2018 at the Urology Research Center, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran. Serum NGAL, Serum Cr and urine output were measured at 1 to 7 day after transplantation. The need for dialysis in one week after transplantation was evaluated.

Results Among 29 recipients with serum biomarkers measurements, 8 (27.5%) developed DGF (need to hemodialysis within 1 week of transplantation). Resulted in areas under ROC curves (AUCs) for serum NGAL at early hours following transplantation was (0.839, 95% CI: 0.69-0.98, P =0.005) that could accurately predict DGF compared to urine output (0.747, 95% CI: 0.55-0.93, P =0.045) and serum Cr (0.607, 95% CI: 0.34-0.86, P =0.398) at 24 hours after transplantation. Multivariate analysis revealed that only serum NGAL was significant independent predictor of DGF (OR: 0.996, 95% CI: 0.993-1.000, P =0.039).

Conclusion Serum NGAL at early hours of post-transplantation was valuable biomarker for early accurate predictor of DGF in kidney transplantation compare with tradition biomarkers such as serum Cr and urine output.

Introduction
According to the Global Observatory Donation and Transplantation (GODT) data, 90,306 kidney transplants were performed worldwide in 2017 (1). Kidney transplantation is the highest potential treatment for long-term survival for patients with end-stage renal disease (ESRD) (2). However, in some cases acute kidney injury (AKI) can lead to allograft and early renal dysfunction, which can increases the risk of acute or chronic rejection as well as chronic allograft nephropathy and consequently graft loss (3). Delayed graft function (DGF) is one the complications after kidney transplantation.
transplantation, which is defined as the necessity of dialysis during the first week after renal transplantation (4, 5). The cause of DGF is related to donor and recipient parameters such as ischemia-reperfusion injury, immunological response and immunosuppressive medications (6). The prevalence of DGF is significantly higher in deceased donor (5–50%) than live donor (4–10%) kidney transplant (7). Unfortunately there is no effective treatment for DGF, however early detection of DGF and intervention treatment may improve outcome. Due to serious possible consequences of DGF there is a necessity of identifying early biomarkers that will rapidly and reliably detect acute and chronic allograft rejection as well as DGF.

Serum creatinine (Cr) levels are currently being measured to determine the acute renal failure (AFR) (8). Unfortunately, Cr is an unreliable indicator during AKI. Because, it is affected by many factors such as body weight, age, sex and muscle metabolism (9, 10). In addition, a detectable increase in Cr serum occurs at a stage where significant allograft damage has occurred. (11). Thus, neutrophil gelatinase-associated lipocalin (NGAL) has been introduced as an early marker protein for kidney dysfunction in various clinical settings (12, 13).

The clinical value of NGAL in predicting AKI associated with cardiac surgery and regular AKI with radiologic contrast has been demonstrated in several studies (14, 15). Hall et al. (16) showed that NGAL levels following kidney transplantation could predict the DGF whereas serum Cr could not. Mishra et al. (17) demonstrated that NGAL expression was significantly increased in patients who developed DGF after transplantation and they found a strong correlation between NGAL staining intensity and cold ischemia time as well as serum Cr. Most of previous studies used urinary NGAL as biomarker to predict DGF, thus there is not enough information on serum NGAL as biomarker. Therefore, we conducted this study to evaluate the serum NGAL expression in the early postoperative phase after kidney transplantation and to examine the role of serum NGAL as biomarker in predicting DGF and acute allograft rejection.

Material And Methods
Study design
In this prospective cohort study, 32 adult patients who underwent first time kidney transplantation at
the Urology Research Center, Sina Hospital, Tehran, Iran, from deceased and live donors from October 2017 to December 2018 were enrolled. Patients less than 18 years old, re-transplantation or combined transplantation with another organ and with graft impairment caused by renal artery thrombosis or bleeding from the graft vascular anastomosis were excluded from the study. Development of DGF which is defined a need for dialysis within the first week after transplantation, was evaluated in all patients (18). All participants received the same immunosuppressive regimen, consisting thymoglobulin induction and prednisolone, mycophenolate mofetil and tacrolimus. The study was approved by the Ethics Committee of Tehran University of Medical Sciences (Code: XXX) and the patients were enrolled after giving written informed consents.

Data collection
Demographic and clinical characteristics of recipients and donors were collected from the patient’s medical records. Age, sex, height, weight, cause of end-stage renal disease, duration of dialysis before transplantation, comorbid condition such as diabetes, hypertension and ischemic heart disease were collected. In addition, serum NGAL, serum Cr and urine output were measured preoperatively and postoperatively. All patients were followed within six months of transplantation and all aspects of graft function, serum Cr, other complications and mortality were recorded. Preoperative samples were taken prior to surgery. Postoperative samples of serum NGAL were collected at first and seven day following the transplantation. Samples were immediately processed and stored at 70 °C. We utilized a commercial double antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory, Shanghai, China) for measuring plasma NGAL concentrations. Post-transplantation samples of urine output and serum Cr were measured at 1 to 14 days after transplantation and the day of patient’s discharge from the hospital. Moreover, serum creatinine was measured at 1, 3, and 6 months after transplantation in order to evaluate the glomerular filtration rate (\( \text{Serum creatinine was measured by Jaffe method (Roche Diagnostics).} \)

Statistical analysis
Qualitative variables were reported as number or percentage and quantitative variables as mean ± standard deviation (SD). Normality distribution of data was tested using Kolmogorov-Smirnov test.
Demographic and clinical characteristics of the patients with and without DGF were compared by two-sample student’s t test or Mann-Whitney test in quantitative variables and by chi-square test or Fisher exact tests in qualitative variables. The association between biomarkers and DGF were evaluated in a multivariate logistic analysis while adjusting the variables selected by univariate analysis and risk factors for DGF. Correlation between NGAL and other variables were evaluated by Pearson’s correlation coefficients. Receiver-operating characteristic (ROC) curve was used to determine the cutoff point for serum NGAL and creatinine level to predict DGF. The statistical analysis was conducted with SPSS version 21.0 (SPSS Inc., IMB Corporation, and Chicago, Illinois, USA). For all analyses, a p-value < 0.05 (two-tailed) was defined as statistically significant. Confidence intervals (CI) were calculated on a 95% level.

Results

Recipients and donors’ characteristics
A total of 32 patients were enrolled in this study, but in three patients was not possible to recognize the DGF because of early acute rejection and mortality. Demographic and clinical characteristics of the recipients and donors, as well as findings in patients DGF and non-DGF are shown in Table 1. The mean age of patients at transplantation time was 45.21 ± 13.86 years (range: 17–69 years), and the majority of them 21 (72.4%) were men. In 13 (44.8%) patients the cause of end-stage renal disease (ESRD) was unknown. Diabetes (DM) and hypertension (HTN) were the cause in 7 (24.1%) and 6 (20.7%) patients, respectively. The source of kidney was from deceased and live donors in 24 (82.8%) and 5 (17.2%) patients, respectively. The mean age of donors at transplantation time was 37 ± 11.5 years (range: 17–59 years) and 21 of them (72.4%) were male. Eight (27.6%) recipients developed DGF in the study. No significant difference was found between “DGF” and “non-DGF” patients in terms of demographic and clinical characteristics before transplantation (P > 0.05). However in term of findings, the mean duration of hospitalization was significantly lower in the “non-DGF” group compared to patients in “DGF” group (18.2 ± 7.05 vs. 27.6 ± 15.2 days, P = 0.029).
Table 1
Demographic and clinical characteristics of recipients and donors in DGF and non-DGF groups

| Variables                      | Total (n = 29) | Non-DGF (n = 21) | DGF (n = 8) | P-value |
|--------------------------------|----------------|------------------|-------------|---------|
| **Recipient characteristics**  |                |                  |             |         |
| Age (year)                     | 45.21 ± 13.86  | 45.14 ± 13.29    | 45.38 ± 16.23 | 0.969∞  |
| Sex (male)/ n (%)              | 21 (72.4)      | 15 (71.4)        | 6 (75)      | 0.615≠   |
| BMI (kg/m²)                    | 24.79 ± 4.66   | 23.87 ± 3.67     | 27.21 ± 6.25 | 0.084∞  |
| Diabetes/ n (%)                | 7 (24.1)       | 4 (19.0)         | 3 (37.5)    | 0.357≠   |
| Hypertension/ n (%)            | 13 (44.8)      | 9 (42.9)         | 4 (50)      | 0.526≠   |
| Ischemic heart diseases/ n (%) | 3 (10.3)       | 1 (4.8)          | 2 (25.0)    | 0.176≠   |
| Smoking / n (%)                | 6 (20.7)       | 5 (23.8)         | 1 (12.5)    | 0.475≠   |
| Duration of dialysis (months)  | 21.34 ± 15.42  | 20.90 ± 16.60    | 22.50 ± 12.72 | 0.809∞  |
| **Serum NGAL pre-transplant**  | 951.25 ± 332.29| 944.42 ± 379.51  | 974.37 ± 171.22 | 0.822∞  |
| **Serum Cr pre-transplant**    | 7.15 ± 2.50    | 6.42 ± 2.01      | 9.15 ± 2.94 | 0.059∞   |
| **Donor characteristics**      |                |                  |             |         |
| Type of donor (deceased)/ n (%)| 24 (82.7)      | 17 (80.9)        | 7 (87.5)    | 0.575≠   |
| Age (year)                     | 37 ± 11.5      | 37.56 ± 11.5     | 35.57 ± 12.43 | 0.708∞  |
| Sex (male)/ n (%)              | 21 (72.4)      | 15 (71.4)        | 6 (75)      | 0.618≠   |
| **Findings**                   |                |                  |             |         |
| Hospitalization (day)          | 20.79 ± 10.58  | 18.19 ± 7.05     | 27.63 ± 15.23 | 0.029*∞ |
| Mortality                      | 3 (10.3)       | 3 (14.3)         | 0           | 0.364≠   |

Data are expressed as number of total (%), mean ± standard division, DGF: delayed graft function, BMI: body mass index, NGAL: neutrophil gelatinase-associate lipocalin, Cr: creatinine, ≠ chi-square test or Fisher’s exact test, ∞ Student t-test, * P < 0.05 was considered as statistical significant

Postoperative biomarkers
To demonstrate differences in the expression of biomarkers; serum NGAL, serum Cr and urine output was measured postoperative day 1 to 7. Table 2 shows mean ± SD values of serum NGAL, serum Cr and urine output levels from postoperative day 1 to 7, as well as findings of serum Cr in months 1, 3 and 6 following transplantation in DGF and non-DGF groups. Plasma NGAL level at early hours of post-transplantation (1143.4 ± 231.6 vs. 761.3 ± 356.1 ng/ml, P = 0.009) and day 7 (574.13 ± 290.71 vs. 220.45 ± 67.78 ng/ml, P = 0.007) was significantly higher in patients with DGF than those without DGF; while, it was not significantly different among the groups before transplantation (P = 0.822). The serum Cr levels from postoperative day 1 to 3 were not significantly different between the two groups of patients. However, significant higher serum Cr was observed in post-transplantation day 4 and after that in patients with DGF than those without DGF (P < 0.05). In addition, urine output at first day after transplantation was significantly lower in “DGF” group than those patients in “non-DGF” group.
(1658.7 ± 1398.7 vs. 3746.5 ± 2610.7 ml P = 0.043). No statistically significant difference was found between the groups in terms of serum Cr level in months 1, 3 and 6 following transplantation (P > 0.05).

Table 2

| Biomarkers               | Total (n = 29) | Non-DGF (n = 21) | DGF (n = 8) | 95%CI                | P-value |
|--------------------------|---------------|-----------------|-------------|----------------------|---------|
| Serum NGAL (ng/ml)       |               |                 |             |                      |         |
| Day 1                    | 866.72 ± 366.32 | 761.32 ± 356.11 | 1143.40 ± 231.59 | 102.12-662.03 | 0.009*  |
| Day 7                    | 316.91 ± 217.74 | 220.45 ± 67.78  | 574.13 ± 290.71 | 124.7-582.66  | 0.007*  |
| Serum Cr (mg/dl)         |               |                 |             |                      |         |
| Day 1                    | 5.42 ± 2.55    | 5.01 ± 1.84     | 6.31 ± 3.64  | 0.95–3.54            | 0.245   |
| Day 2                    | 5.08 ± 2.42    | 4.61 ± 2.29     | 6.26 ± 2.49  | 0.37-3.66            | 0.105   |
| Day 3                    | 3.48 ± 2.22    | 2.97 ± 1.98     | 4.88 ± 2.46  | 0.03-3.83            | 0.053   |
| Day 4                    | 3.11 ± 2.28    | 2.42 ± 1.72     | 4.75 ± 2.73  | 0.548-4.11           | 0.012*  |
| Day 5                    | 2.87 ± 2.08    | 2.21 ± 1.42     | 4.52 ± 2.62  | 0.742-3.88           | 0.006*  |
| Day 6                    | 2.87 ± 2.21    | 2.04 ± 1.03     | 5.01 ± 3.02  | 1.32-4.61            | 0.001*  |
| Day 7                    | 2.46 ± 1.72    | 1.84 ± 0.84     | 3.77 ± 2.38  | 0.61-3.26            | 0.006*  |
| Urine output (ml)        |               |                 |             |                      |         |
| Day 1                    | 3150.0 ± 2495.2 | 3746.5 ± 2610.7 | 1658.7 ± 1398.7 | 69.75-4105.74 | 0.043*  |
| Day 2                    | 3748.2 ± 2611.4 | 4100.0 ± 2484.9 | 2868.7 ± 2882.0 | 1002.5–3465.0 | 0.268   |
| Day 3                    | 3469.6 ± 1955.8 | 3815.1 ± 1929.8 | 2606.2 ± 1858.4 | 434.3-2851.8 | 0.143   |
| Day 4                    | 3211.1 ± 1461.5 | 3365.7 ± 1292.5 | 2843.7 ± 1899.0 | 753.7-1797.7 | 0.407   |
| Day 5                    | 3265.3 ± 1463.3 | 3377.5 ± 1379.5 | 2985.1 ± 1722.8 | 8879.9-1664.9 | 0.532   |
| Day 6                    | 3012.5 ± 1226.6 | 3020.0 ± 983.2  | 2939.7 ± 1782.9 | 1048.5-1101.1 | 0.960   |
| Day 7                    | 2677.8 ± 1088.5 | 2752.5 ± 1002.1 | 2491.2 ± 1337.2 | 686.7-1209.2 | 0.576   |

Prediction of DGF by serum NGAL

In order to assess the predictive value of serum NGAL vs. serum Cr to detect DGF we compared the area under the ROC curves (AUCs). Table 3 shows the AUC and cutoff point of serum NGAL, Cr and urine output at day 1 to 7 following transplantation for predicting DGF. ROC analysis showed that the AUC for predicting DGF using serum NGAL at early hours of post-transplantation were (0.839, 95% CI: 0.69–0.98, P = 0.005) compared to serum Cr (0.607, 95% CI: 0.34–0.86, P = 0.398) and urine output (0.747, 95% CI: 0.555–0.939, P = 0.045) at the first day following transplantation. However, the AUC for Serum Cr after operative is highest at day 4 to 7 with highly accurate prediction of DGF. The cutoff point of 884.64 (ng/ml) for serum NGAL at postoperative had a sensitivity 100% and specificity of 76.5%, as well as 232.98 (ng/ml) at day 7 had a sensitivity 100% and specificity of 75%. While, The cutoff point of 1075 (ml) for urine output at day 1 following transplantation had a sensitivity 80% and
specificity of 50%. Regarding the AUC, serum NGAL could predict DGF highly accurate at postoperative day compared to serum Cr (Fig. 1).

### Table 3
Area under the receiver-operating curves for DGF prediction

| Biomarkers       | AUC (95% CI)     | P-value | Cut-off | Sensitivity (%) | Specificity (%) |
|------------------|------------------|---------|---------|-----------------|-----------------|
| Serum NGAL (ng/ml) |                  |         |         |                 |                 |
| post-transplant  | 0.839 (0.694–0.985) | 0.005*  | 884.64  | 100             | 76.5            |
| Day 7            | 0.917 (0.731–1.000) | 0.041*  | 232.98  | 100             | 75.0            |
| Serum Cr (mg/dl) |                  |         |         |                 |                 |
| Day 1            | 0.607 (0.346–0.868) | 0.398   |         |                 |                 |
| Day 2            | 0.725 (0.491–0.959) | 0.067   | 4.39    | 88.0            | 65.0            |
| Day 3            | 0.714 (0.463–0.966) | 0.099   | 3.18    | 83.0            | 62.0            |
| Day 4            | 0.770 (0.562–0.977) | 0.029*  | 2.02    | 83.0            | 55.0            |
| Day 5            | 0.794 (0.597–0.991) | 0.017*  | 1.79    | 83.0            | 54.0            |
| Day 6            | 0.810 (0.584–1.000) | 0.018*  | 3.94    | 67.0            | 85.0            |
| Day 7            | 0.768 (0.558–0.979) | 0.033*  | 3.05    | 67.0            | 77.0            |
| Urine output (ml) |                  |         |         |                 |                 |
| Day 1            | 0.747 (0.555–0.939) | 0.045*  | 1075.0  | 80.0            | 50.0            |
| Day 2            | 0.653 (0.410–0.896) | 0.213   |         |                 |                 |
| Day 3            | 0.659 (0.432–0.887) | 0.195   |         |                 |                 |
| Day 4            | 0.520 (0.262–0.777) | 0.873   |         |                 |                 |
| Day 5            | 0.481 (0.226–0.736) | 0.897   |         |                 |                 |
| Day 6            | 0.463 (0.189–0.736) | 0.760   |         |                 |                 |
| Day 7            | 0.556 (0.300–0.813) | 0.647   |         |                 |                 |

AUC: area under the curve, CI: confidence interval, NGAL: neutrophil gelatinase-associate lipocalin, *P < 0.05 was considered as statistical significant

### Multivariate analysis for predicting DGF

In order to determine independent parameters for DGF with regard to NGAL expression, univariate and multivariate analyses were performed. Univariate analysis showed that the age (P = 0.967), sex (P = 0.848), duration of dialysis (P = 0.933), type of donor (P = 0.607), serum Cr and urine output at day 1 after transplantation (P = 0.252 and 0.064) were not predictive of DGF. However, post transplantation serum NGAL and level of serum Cr at day 4 following transplantation were independent predictors of DGF (Table 4). However, in multivariate analysis, only post transplantation serum NGAL was significant independent predictor of DGF (OR: 0.996, 95% CI: 0.993-1.000, P = 0.039).
Table 4

| Variables            | Univariate                  | Multivariate              |
|----------------------|-----------------------------|---------------------------|
|                      | OR  | 95% CI        | P-value | OR  | 95% CI        | P-value |
| Age                  | 0.999 | 0.941–1.060 | 0.969   | -   | -              | -       |
| Sex                  | 0.833 | 0.130–5.350 | 0.848   | -   | -              | -       |
| Duration of dialysis | 0.993 | 0.942–1.047 | 0.800   | -   | -              | -       |
| Type of donor        | 0.679 | 0.155–17.47 | 0.679   | -   | -              | -       |
| Serum NGAL           | 0.996 | 0.992–0.999 | 0.022*  | 0.996 | 0.993–1.000 | 0.039*0.018* |
| Serum Cr, day 1      | 0.811 | 0.568–1.160 | 0.252   | -   | -              | -       |
| Serum Cr, day 4      | 0.627 | 0.415–0.948 | 0.027*  | 0.667 | 0.417–1.064 | 0.089   |
| Urine output, day 1  | 1.000 | 1.000-1.001 | 0.064   | -   | -              | -       |

DGF: delayed graft function, CI: confidence interval, OR: odds ratio, NGAL: neutrophil gelatinase-associate lipocalin, * P < 0.05 was considered as statistical significant

Discussion

Diagnosis of DGF is usually made by elevated serum Cr, which is a late signal of kidney injury. Early predict and treatment is essential for better outcome. Therefore, it would be ideal to find a marker better than Cr to early predict the acute kidney injury (19). In the study, we compared the serum NGAL with serum Cr as predictive biomarkers for DGF following kidney transplantation. The study showed that values of serum NGAL after transplantation was more useful than absolute or percentage of serum Cr decrease in predicting DGF. In both ROC and multivariable analyses, serum NGAL was superior to serum Cr in predicting early DGF. We have shown that absolute values of serum NGAL on the first hours of post-transplantation correspond with DGF, whereas Serum Cr values at these times poorly differentiate between groups. In addition, the results revealed that better accuracy and predictive power of serum Cr occurred at 4 or more days after transplantation. The absolute value of serum Cr on the post-transplantation day 1 and 4 for predicting DGF resulted in AUCs was (0.607, 95%CI: 0.346–0.868, P = 0.398) and (0.770, 95%CI: 0.562–0.977, P = 0.029), respectively. Therefore, serum NGAL detects DGF 1 to 3 days earlier than serum Cr after transplantation. Furthermore, urine output at 24 hours after transplantation had modest diagnostic performance AUC (0.747, 95% CI: 0.555–0.939, P = 0.045).

The accuracy and predictive power of serum Cr was affected by many of parameters such as age, sex and muscle mass, protein metabolism, volume of distribution, and drugs (20). Furthermore, the ability of creatinine to detect functional impairment is less than 50% (21). Several studies have been
evaluated the role of urine or serum NGAL in predicting DGF after kidney transplantation, compare with traditional biomarkers such as serum Cr and urine output (22–24). Similar to our study they showed that NGAL level was more useful than serum Cr in early predicting DGF. In contrast, Mahdavi-Mazdeh et al. (25) reported ROC curve and AUCs of serum NGAL and serum Cr levels on the first post-transplantation day had similar significance in predicting DGF. However, they showed that the highest AUC (0.82) was attributed to serum NGAL at 24 hour (P = 0.002). Parikh et al. (23) in 53 transplant patients from living and deceased donor found a better AUC (0.9) for urine NGAL in prediction of DGF than serum Cr. While, Mojtahedzadeh et al. (26) in 69 transplant patients from deceased donor found a relative fall in serum Cr had higher AUC (0.821 vs. 0.790) compared to urine NGAL at 24 hour post-transplantation in prediction of DGF. In contrast to our findings, some of previous studies showed that NGAL level in early hours of postoperative were not able to differentiate patients with DGF from those with non-DGF (25, 26). Lebkowska, et al. (27) found a significant decrease in serum NGAL as early as 24 hours after transplantation, before the serum Cr level decreases. Two studies demonstrated that 48 hours urine NGAL showed larger AUCs and better sensitivity and specificity than those of early hours after transplantation (28, 29).

Plasma and urine NGAL were evaluated and compare in some studies to investigate their performance to predict DGF (21, 22, 30). In some studies plasma NGAL showed an obviously higher sensitivity and a slightly higher specificity than those of urine NGAL, which supported the superiority of plasma NGAL over urine NGAL in predicting DGF within 24 hours following kidney transplantation (30). Hollmen et al. (21) found a higher AUC value of serum NGAL (AUC, 0.85) compared to urine NGAL (AUC, 0.74). However, Maier et al. (22) reported higher AUC (0.74) of urine NGAL compare to serum NGAL (0.73) at 24 hour post-transplantation to predict DGF. These contradictory findings may be due to differences in study designs or related to sample size. Therefore, large prospective kidney transplantation cohort with different sampling time points is required to illustrate the features of NGAL in predicting the risk of DGF.

The small sample size is the main limitation of this study, so further studies in larger cohorts is necessary to confirm our results. The small sample size also limited our ability to evaluate
meaningfully between different variables and to examine important patient subgroups. In addition, due to the low sample size, a multivariate analysis of the combination of the two biomarkers was not possible. Another limitation of this study was the unavailability of cold ischemia time.

In conclusion, the study showed that the superior of serum NGAL to serum Cr as an early predictor of graft function and DGF. Serum NGAL at the early hours after transplantation also demonstrates excellent potential for predicting DGF with fair sensitivity and specificity. While, measuring serum Cr at 24 hour post-transplantation did not help in the diagnosis of DGF. Our findings emphasize the promising role that NGAL serum can play as an early indicator of DGF.

Declarations

Ethics approval and consent to participate: An informed consent was taken before surgery based on the ethical code of Tehran University of Medical Sciences ethics committee.

Consent for publication: The information is published without the name of patients.

Availability of data and material: Information, data, and photos will be provided upon request.

Competing interests: All authors claim that there is not any potential competing or conflict of interest

Funding: Urology research center, Tehran University of medical sciences.

Authors’ contributions: SMK.A. principal investigator. B.E., S.G., F.N., A.M., and S.D., were responsible to run the project and collecting data. R.M., M.A., F.B., F.H., run the laboratory test and entering data from patient’s documents to the SPSS software. F.KH. analysis the data and edit manuscript and S.A manuscript preparation.

Acknowledgments: Special thanks to the Urology Research Center (URC), Sina Hospital.

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Figures
Diagonal segments are produced by ties.

Figure 1
Receiver-operating characteristics (ROC) curve for serum NGAL post-transplant and serum Cr at post-transplant day 1, 2, 4, 6 and 8 for predicting graft delayed Function.