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*Research article*

# Novel Biomarkers Predictive for Graft Dysfunction – A Pilot Study using Machine Learning as an AI Tool

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**Abstract:** Renal transplantation ensures advantages for patients with end-stage kidney disease. Howev-er, in some cases early complications can lead to allograft dysfunction and consequently graft loss. Creatinine is not an ideal indicator of kidney injury, mainly because it cannot reliably de-tect early acute renal failure and cannot distinguish between different underlying causes. New-er kidney damage biomarkers—such as kidney injury molecule-1 (KIM-1), neutrophil gelati-nase-associated lipocalin (NGAL), and beta-2 microglobulin (B2MG)—may improve the early identification of graft dysfunction. Aim: This study aimed to evaluate serum levels of these bi-omarkers in comparison with traditional kidney function markers (creatinine and cystatin C) and to examine their potential as early predictors of kidney injury using artificial intelligence–based methods. We included 19 patients who had their first kidney transplanta-tion (5 females, 14 males), without prior immunization, having complete HLA typing and a negative cross-match test before transplantation. Serum concentrations of creatinine, cystatin C, and the biomarkers KIM-1, NGAL, and B2MG were measured 24 hours after transplantation. These data were used to develop a Random Forest Classifier (RFC) to predict renal injury. The RFC model, built using two or three key input variables—specifically KIM-1 and cystatin C, and alternatively KIM-1, NGAL, and B2MG—accurately identified patients at risk of graft dysfunction. According to the RFC model, serum KIM-1, NGAL, and B2MG may serve as useful early markers of renal impairment following kidney transplan-tation. However, the small sample size limits the broader applicability of these findings.

**Keywords:** kidney injury, random forest classifier, artificial intelligence, KIM1, NGAL, beta2microglobulin

## 1. Introduction

Kidney transplantation (KT) is currently the elective approach for patients with end-stage renal disease. While it is considered a safe option for these patients, early complications may result in graft dysfunction. Creatinine is a poor marker to establish kidney injury, and its reliability is affected by several factors [1, 2]. Different serum and urine proteins are being studied as possible predictive biomarkers for graft dysfunction. Studies have focused on kidney injury molecules such as neutrophil gelatinase-related lipoprotein (NGAL), beta 2 microglobulin (B2MG), kidney injury molecule 1 (KIM1), and others, as the potential markers for the prognosis of graft durability [3].

Artificial intelligence (AI) refers to computer-based systems that use algorithms to simulate and process human-like thinking and behavior. Machine learning (ML), one of the major branches of AI, is the study of algorithms that are learned from sample data

without being specifically programmed to perform a particular task [4]. Machine learning (ML), a branch of AI, is increasingly being incorporated across various fields to address clinical and health-related challenges. ML approaches typically fall into four categories: supervised, unsupervised, semi-supervised, and reinforcement learning. Unlike supervised models, unsupervised learning seeks to uncover hidden structures or patterns within datasets without relying on labeled training data [5]. The most widely used supervised learning methods such as neural networks and classification-based models recognize patterns in the training data set and help make predictions by identifying similar patterns in future data sets. In contrast, unsupervised models aim to identify hidden patterns in a data set and are not trained in a previous data set. Semi-supervised learning acts as a middle ground between supervised and unsupervised learning that is trained using a smaller fraction of labeled data and a significantly larger set of unlabeled data. Reinforcement learning is a technique in which the algorithm learns autonomously from feedback in a dataset in a trial-and-error manner, closely mimicking human learning. Widely used ML models include neural network–based architectures such as artificial neural networks (ANN) and convolutional neural networks (CNN), as well as decision trees (DT), random forests (RF), and support vector machines (SVM) [6,7]. With the progression of machine learning, its role in organ transplantation highlights notable potential; nevertheless, the small cohort size represents a key limitation, alongside ongoing challenges in validating model predictions and confirming their clinical utility.

Despite the advances in KT, accurately predicting graft survival (GS) after transplantation using standard statistical models remains a significant challenge [8]. Existing risk prediction models have a limited ability to predict outcomes for kidney transplant recipients. Although ML has been applied to predicting graft survival (GS) and other outcomes following solid organ transplantation, the reported performance and reliability of these predictive models remain highly variable [9].

The aim of the present study was to evaluate serum levels of kidney injury biomarkers (KIM1, NGAL, and B2MG) in kidney transplant recipients and compare these findings with traditional markers of renal function, including creatinine and cystatin C Another goal was to analyze the potential value of serum KIM1, NGAL and B2MG concentrations as predictors of kidney damage after kidney transplantation with the use of artificial intelligence tools.

## 2. Results

### 2.1. Serum KIM1, NGAL, B2MG, Cystatin C, Creatinine

The serum KIM1 and NGAL concentrations were significantly higher in the patients undergoing KT versus the control group (Figure 1, Figure 2). Contrary B2MG was lower in the Tx patients compared with the control group, but with no statistical significance.

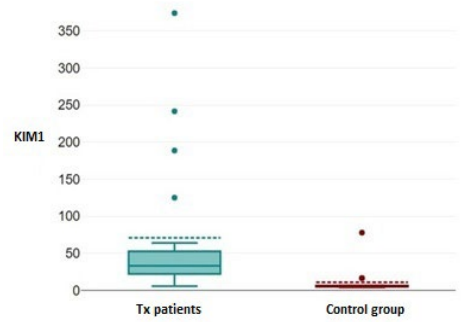


**Figure 1**. Box-plot showing the KIM-1 values between the Tx patients and the control group (74.5 ±98.45 vs 10.54±17.19, p = 0.01).

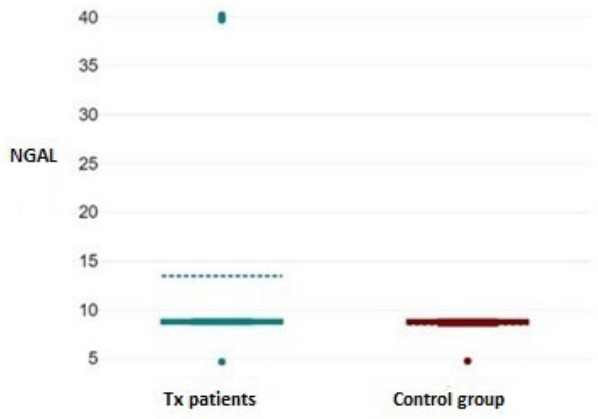


**Figure 2.** Box-plot showing the NGAL values between the Tx patients and the control group (13.48 ±11.81 vs 8.54±1.06, p = 0.08).

The creatinine value at 24h postTx was 5.81 ± 3.6, respectively 1.58 ±0.54 at 12 months postTx. The correlation between creatinine value at 24h postTx and creatinine at 12 months postTx was R2=0.53, p =0.016. The correlation between Cystatin C at 24h postTx and creatinine value at 12 months post Tx was R2=0.51, p =0.025, respectively R2=0.4, p =0.08 for KIM1 at 24h postTx and creatinine at 12 months postTx.

## 2.2. The Random Forest Classifier (RFC) Model

6 patients experienced graft dysfunction (5 patients with a creatinine value >5 mg/dl at 24h postTx). The RFC model was designed using core laboratory results, including serum cystatin C, and the analyzed damage markers (KIM1, NGAL and B2MG). Only KIM1 and Cystatin C showed an important accuracy related to the graft dysfunction (Figure 3).

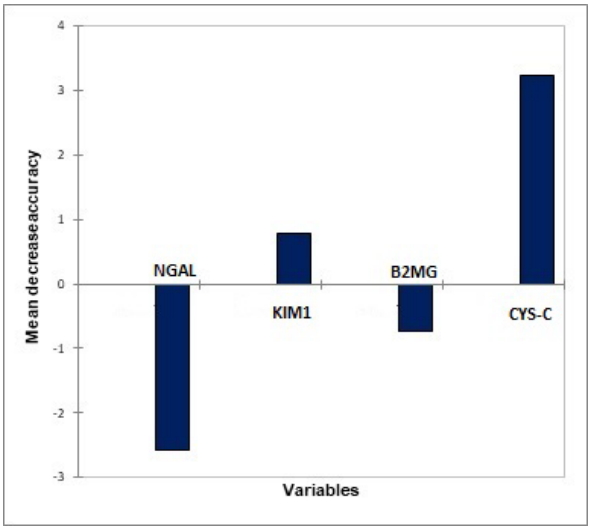


**Figure 3**: Chart showing the accuracy of the variables in order to predict the graft dysfunction.

All input variables were assessed in the fixed time point at 24h after transplantation. The subsequent observations were completed in 19 patients and served as the database for establishing RFC. The recursively selected subsets of variables constituted the input of a random forest classifier (RFC). The best models were taken into account. The RFC model for KIM1 achieved the AUROC of 0.981, NGAL achieved the AUROC of 0.756

respectively 0.846 for B2MG. Finally, we built models based on 2 or 3 input variables: the model based on 2 input variables (Cystatin C and KIM-1) and the one built based on 3 input variables (KIM1, NGAL and B2MG) were effective in identifying the proportion of patients experiencing graft dysfunction (the exemplary decision trees are shown in Figure 4-5), with an AUROC value of 1 for the 2 variables model and 0.875 for the 3 variables model (Figure 6).

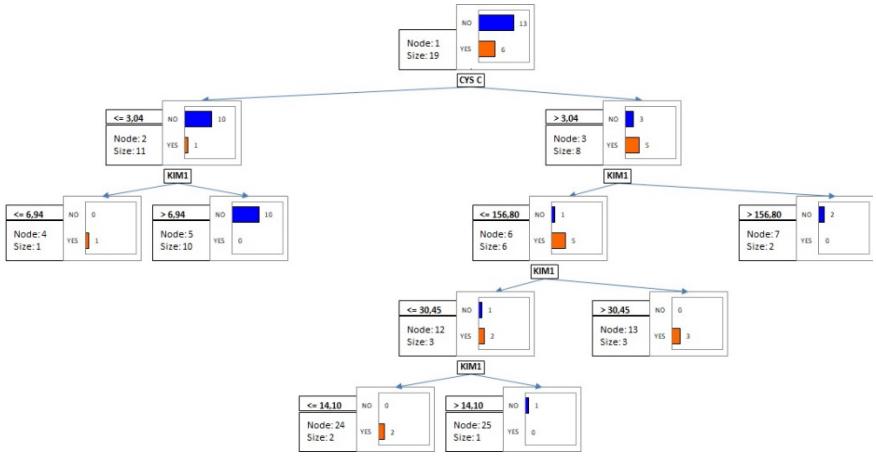


**Figure 4:** The decision tree from RFC model based on 2 variables (Cystatin C and KIM-1)

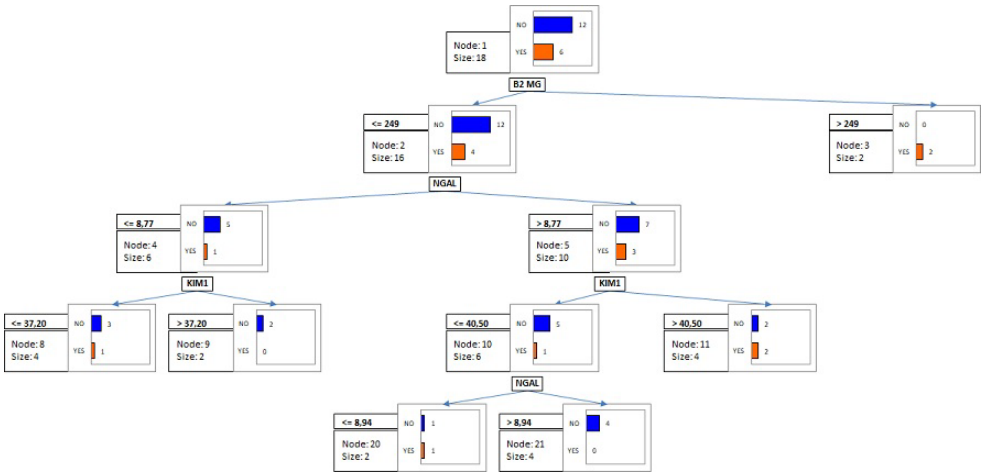


**Figure 5:** The decision tree from RFC model based on 3 variables (KIM-1, NGAL and B2MG)

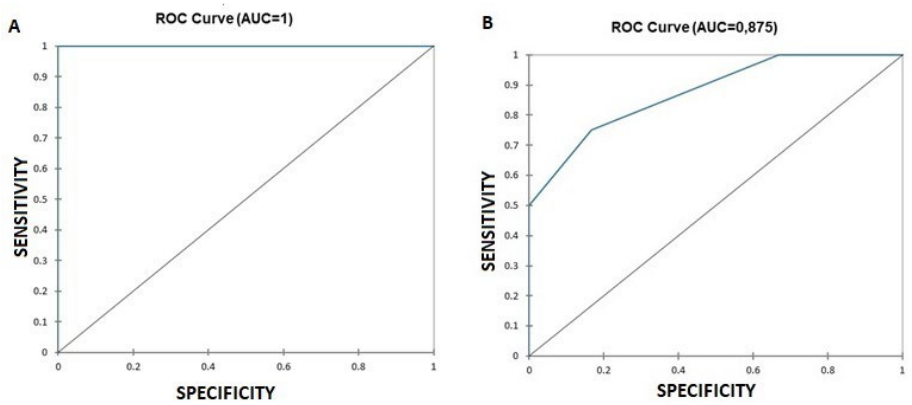


**Figure 6:** (A) ROC curve for 2 variables model; (B) ROC curve for 3 variables model

## 3. Discussion

In this study, we investigated the relevance of three specific biomarkers of kidney injury in the context of kidney transplantation: KIM1, NGAL and B2MG. All of them are classified as early biomarkers since they can be elevated before serum creatinine in some clinical scenarios [10]. The predictive utility of these biomarkers has already been described, both in early detection and in differentiating the nature and severity of lesions, providing prognostic information in the disease course [11]. It is important to note that in our study the values for KIM1 at day 1 post-transplantation were six times higher than in healthy individuals. However, the values for NGAL, a very sensitive biomarker at the beginning of the transplant period [12], or B2MG did not exhibit the same trend. There was also a very strong correlation between functional renal tests such as Cystatin C and creatinine levels at 24h and 12 months postTx. At 12 months after KT, 6 patients (31.57%) experienced graft dysfunction, 5 of them having high creatinine levels (> 5mg/dl) at day 1.

Our next step was to verify the predictive potential of AI tools in relation to graft dysfunction development, acknowledging the preliminary nature of the research among renal transplant patients and the limited strength of its conclusions. Although there is considerable interest in applying machine learning to predict graft dysfunction, debate continues regarding its practical value in routine clinical decision-making. This uncertainty has made some clinicians cautious about adopting modern statistical and computational approaches [13]. Using the Machine Learning Prediction tools we used the 4 biomarkers: KIM1, NGAL, B2MG and Cystatin C as input variables in accordance with graft dysfunction, and we noticed that only KIM1 and Cystatin C can be used as accurate variables to predict renal impairment. Using the random forest classifier algorithm (RFC) we generated a decision tree for each variable (KIM1, NGAL and B2MG), the response variable being graft dysfunction as a qualitative input, observing that KIM1 had the best AUROC value of 0.981 suggesting that between these 3 biomarkers, KIM1 is the most predictive for renal injury. After that, we generated RFC models using 2 and 3 input variables. The model using 2 variables such as Cystatin C and KIM1 had the best accuracy to predict graft dysfunction with an AUROC value of 1. On the other hand, the 3 variable model using all 3 renal damage biomarkers showed also a good predictive value, with an AUROC 0.875, lower than the 2 variable model and KIM1 model, but higher than NGAL and B2MG model. This observation is very significant because of its importance, giving KIM1 the best predictive value among renal damage biomarkers.

There are several articles using machine learning and AI to predict the graft survival in kidney transplantation patients. Aksoy et al [14] published an article in 2025 showing the utility of machine learning and several models such as Naive Bayes, logistic regression, support vector machine (SVM), multi-layer perceptron, and XGBoost. They performed a retrospective study and they used 48 input variables, using 80% of the patients for training and 20% of the patients for testing. A study by Minato et al. [15] involving 1,225 kidney transplant recipients found that transplantation from deceased donors, underlying glomerulopathy, and donor use of vasoactive medications each contributed more than 20% importance as predictors of rejection risk. The factors with the strongest predictive power were thymoglobulin induction therapy and delayed graft function. Similarly, Senanayake et al. [16] analyzed a large cohort consisting of 3,758 living-donor and 7,365 deceased-donor kidney transplants from the Australia and New Zealand Dialysis and Transplant Registry. Using three ML approaches—survival trees, random survival forests, and survival support vector machines—alongside traditional Cox proportional hazards modeling, they developed separate prediction models for living and deceased donor transplants. In 2024, Ali et al. [17,18] conducted a retrospective analysis using data from the UK Transplant Registry, encompassing 29,713 transplant recipients. Their results showed that the XGBoost algorithm per-

formed exceptionally well, achieving the highest concordance index (0.74) and consistently maintaining an area under the curve (AUC) above 0.73. Furthermore, the model demonstrated strong calibration accuracy, reflected by an Integrated Brier Score (IBS) of 0.14, indicating precise survival probability predictions. Considering the examples above, the published articles used machine learning and predictive models, and almost all of them were retrospective studies and they used common functional renal assessment tests. Upon further review of the literature, Ravindhran B [19] et al published an article in 2023 presenting a meta-analysis of original studies reporting machine learning model-based prediction of graft survival in kidney transplant patients. Although there is still no consensus on the single best ML method for forecasting graft outcomes, this body of evidence suggests that hybrid models, SVM variants, and RF-based approaches tend to yield superior performance in predicting graft survival. Despite variability in reported accuracy among different ML models, many outperform traditional gold-standard tools, such as the Kidney Donor Risk Index (KDRI), which has a reported C-index of 0.63.

However, the main aspect restricting the AI application is the difficulty of training a model. In our study, we successfully trained a model despite the limited size of the dataset and confirmed its predictive value through multiple performance metrics. Additionally, the random forest classifier (RFC) is a relatively simple model, useful even if the number of variables is limited. However, it is clear that the value of our analysis would benefit from validation on larger datasets. This work is the first attempt in the literature to predict the risk of renal damage in grafted patients using biomarkers such as KIM1, NGAL or B2MG, and the prospective manner of observing the evolution of the patients gives it originality. The creation of a predictive model with data on the current renal function such as Cystatin C and injury biomarkers such as KIM1, as input variables sounds promising. Our preliminary findings indicate that the Random Forest Classifier may serve as a promising approach for predicting kidney dysfunction using injury-related biomarkers. In our view, this method warrants further investigation and should be validated in larger cohorts of kidney transplant recipients. Another argument for its utility is the clinical importance of kidney damage prediction in the renal transplantation. The preemptive stratification of patients vulnerable to graft dysfunction offers the promise of personalized medicine, with the therapy tailored to both current kidney function and future scenarios of its impairment.

Several limitations of this study should be considered. First, the study cohort was relatively small and heterogeneous, which limited the statistical power of the AI-based analyses and may have affected the robustness and generalizability of the findings. Small sample sizes can increase the risk of overfitting in machine learning models, potentially inflating predictive performance within the study population while reducing applicability to broader kidney transplant cohorts. Second, not all potential confounding variables influencing renal impairment—such as differences in immunosuppressive regimens, comorbid conditions, lifestyle factors, and genetic predispositions—were accounted for, which may have introduced bias and affected the observed associations. Third, the follow-up period was relatively short, limiting the ability to assess the long-term predictive value of the proposed biomarkers and their correlation with clinically significant graft outcomes. Finally, while AI tools offer powerful predictive capabilities, their interpretation in clinical practice requires caution, particularly in small or heterogeneous datasets. Despite these limitations, the findings provide important preliminary insights and establish a foundation for future studies involving larger, more homogeneous patient populations, longer follow-up, and comprehensive confounder adjustment, which are essential to confirm the clinical utility of these biomarkers in predicting graft dysfunction.

## 4. Materials and Methods

### 4.1. Study Design and Settings

A prospective cohort study was developed, in which 19 patients who underwent renal transplantation between June 2023 and December 2023 were consecutively enrolled. The study group contained 5 females and 14 males, with the mean age 47.71 ± 13.02 years, without prior immunization, having complete HLA typing and a negative cross match test before transplantation. We determined serum creatinine and cystatin C and several biomarkers (NGAL, KIM1, beta2microglobulin) at 24h post-transplantation. The control group comprised 18 age-matched healthy individuals with normal kidney function, confirmed by standard biochemical parameters, and without any known history of renal impairment, hypertension, diabetes, or other systemic diseases affecting kidney function. The characteristics of the cohort is described in Table 1.

***Table 1.*** *Cohort characteristics (n=19)*

| | |
|---|---|
| **Sex (F/M)** | **5/14** |
| **Mean age at transplantation (years)** | 47.71 ± 13.02 |
| **No. brain dead donors/ No. living donors** | 16/3 |
| **Type of renal clearance (PD/HD)** | 1/14 |
| **Pre-emptive transplantation** | 4 (21.05%) |

### 4.2. Methods

The blood samples were drawn from peripheral veins after an overnight fast. The samples were left to clot for 30 min, centrifuged at 4 ◦C, 1000× g for 15 min, and then serum was stored at −80 ◦C until analysis. The serum concentrations of KIM1, NGAL, B2MG and cystatin C, were evaluated by Bio-Rad ELISA kits. Standards and serum samples were transferred to 96 well microplates pre-coated with recombinant antibodies to human KIM1, NGAL, B2MG and cystatin C. Captured proteins were then detected using monoclonal antibodies against KIM1, NGAL, B2MG and cystatin C, conjugated to horseradish peroxidase. The assay was then developed using a tetramethylbenzidine substrate, producing a blue coloration proportional to the amount of protein bound in the wells. Adding an acidic stop solution halted the reaction and converted the color to yellow. The final absorbance was read at 450 nm on a microplate reader, with a reference correction at 550/650 nm. All measurements followed the manufacturer's protocol, and concentrations were derived from standard calibration curves.The intra-assay and inter-assay coefficients of variation for all tested parameters were below 10%, indicating good reproducibility. Data processing and interpretation were carried out using parametric logistic regression, and plasma KIM-1 levels were quantified using the Bio-Plex 200 system.

Kidney function was evaluated based on serum creatinine levels measured at predetermined intervals. Routine automated laboratory methods were used to analyze serum chemistry parameters. The serum concentrations of all parameters were measured 24 hours after transplantation, and then creatinine at 12 months after transplantation.

### 4.3. Construction of the Random Forest Classifier Model

The purpose of the classifier [8] is to categorize a heterogeneous dataset according to predefined labels—such as clinical status—based on the values contained in each record, including laboratory measurements. Essentially, the algorithm identifies the most probable label for a set of variables. Within a single decision tree, each node partitions the data according to a specific condition. When a split results in a subset containing

only one label type, the impurity of that node becomes zero, and no additional splits occur. Ideally, each successive node reduces class impurity. Randomness is introduced by selecting a random subset of input variables when constructing each tree. This reduces the complexity encoded in any single tree, minimizes overfitting, and improves the model's ability to generalize to new data. Combining multiple randomized trees in a forest enhances coverage of the dataset and improves predictive performance. The Random Forest Classifier (RFC) is also robust with respect to variable scaling and demonstrates stability even when many input variables are included. Redundant or non-informative variables do not significantly impair model performance and can be readily removed during optimization for clinical application.

### 4.4. Statistical Analysis

All obtained data were compiled into a database created using Microsoft Office Excel Version 16.78.3 and subsequently analyzed with IBM SPSS Statistics Version 20. For the continuously distributed variables, we applied descriptive statistics to calculate the averages and standard deviations. To assess the statistical relevance of these variables, we utilized Student's t-test. To assess the statistical significance of the correlations between the qualitative variables, we utilized the Pearson Chi-square test. Additionally, we calculated the Pearson correlation coefficient (Pearson r). Across all analyses, a p-value of less than 0.05 was deemed statistically significant. For p-values less than 0.01, we considered the statistical significance to be good, and for p-values less than 0.001, the statistical significance was regarded as extremely important, indicating a very high level of confidence in the results with an error margin of just 0.1%. In order to create RFC we used the XLSTAT 2024 version and Machine learning application had been launched (classification and regression random forest).

## 5. Conclusions

KIM-1, NGAL and B2MG can be useful biomarkers in assessing subclinical kidney dysfunction in renal transplantation. The serum cystatin C concentration outperforms serum creatinine in the reliable assessment of kidney function. For grafted patients, an ideal diagnostic strategy for graft dysfunction would involve simultaneous evaluation of both functional markers and indicators of cellular injury. The RFC developed in this study appears to provide a clinically meaningful method for identifying individuals at increased risk of persistent post-transplant kidney dysfunction. Further validation in a larger cohort and over an extended follow-up period is warranted to confirm its utility.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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