Soluble Urokinase-Type Plasminogen Activator Receptor (suPAR) is a Risk Indicator for eGFR Loss in Kidney Transplant Recipients

Ulrich Jehn (ulrich.jehn@ukmuenster.de)
University Hospital of Muenster, Departmentof Medicine D, Division of General INternal Medicine, Nephrology and Rheumatology

Katharina Schütte-Nütgen
University Hospital of Muenster, Departmentof Medicine D, Division of General INternal Medicine, Nephrology and Rheumatology

Ute Henke
University Hospital of Muenster, Departmentof Medicine D, Division of General INternal Medicine, Nephrology and Rheumatology

Hermann Pavenstädt
University Hospital of Muenster, Departmentof Medicine D, Division of General INternal Medicine, Nephrology and Rheumatology

Barbara Suwelack
University Hospital of Muenster, Departmentof Medicine D, Division of General INternal Medicine, Nephrology and Rheumatology

Stefan Reuter
University Hospital of Muenster, Departmentof Medicine D, Division of General INternal Medicine, Nephrology and Rheumatology

Research Article

Keywords: Ly6/alpha-neurotoxin, suPAR, glomerular barrier, podocyte membrane

Posted Date: December 7th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-114837/v1

Version of Record: A version of this preprint was published at Scientific Reports on February 12th, 2021.
See the published version at https://doi.org/10.1038/s41598-021-83333-7.

Abstract

Background suPAR is a signaling protein of the Ly6/alpha-neurotoxin family. In the kidney, suPAR contributes to podocyte foot process effacement and glomerular barrier function disruption via activating αvβ3 integrin on the podocyte membrane. Its role in allograft function or transplant-specific outcomes needs clarification. Therefore, we prospectively investigated the prognostic relevance of suPAR in patients before and one year after kidney transplantation (KTx).

Methods We included 100 patients who had received a kidney transplantation between 2013 and 2015. The plasma concentration of suPAR was measured using an uPAR ELISA assay.

Results In patients who had received a living donation (LD), pre-transplant suPAR levels were significantly lower than those who had received a deceased donation (DD). After KTx, suPAR levels significantly declined in LD and DD recipients, without a detectable difference between LD and DD recipients. Higher suPAR levels in recipients one year after KTx were associated with a more severe eGFR loss in the following three years ($n = 82$, $p = 0.021$).

Conclusions After KTx, suPAR levels drop significantly. Nevertheless, suPAR-levels above 6,212 pg/ml one year after KTx are independently associated with a nearly twice as fast loss of renal function > 30% ($p < 0.001$). Therefore, suPAR level at one year mark might be a risk indicator of increased eGFR loss.

Introduction

The soluble urokinase-type plasminogen activator receptor (suPAR) and its membrane-bound form uPAR are signaling proteins of the Ly6/alpha-neurotoxin family. They are secreted by immature myeloid cells, neutrophils, endothelial cells, smooth muscle cells, and podocytes, amongst others[1], [2]. Notably, neutrophils can also serve as a major suPAR source in the bloodstream, at least in inflammatory conditions, when uPAR sheds from the neutrophil surface [3]. Both circulating and membrane-bound forms of suPAR are directly linked to cell adhesion and migration through binding integrins [2]. In the kidney, suPAR binds to and activates αvβ3 integrin on the podocyte membrane. Thereby, it contributes to podocyte foot process effacement and glomerular barrier function disruption, resulting in proteinuria [4], [5]. Interestingly, it was found that uPAR-deficient mice are protected from lipopolysaccharide (LPS)-induced injury of the kidney filtration barrier. However, this protection can be reverted by the constitutive expression of αvβ3 integrin [5].

An experimental study by Hahm et al. identified bone marrow (BM)-derived immature myeloid cells as a significant source of suPAR in LPS-treated mice. Knockout mice with uPAR deficiency were irradiated and reconstituted by BM cells of uPAR wild-type mice. These chimeric mice that selectively express uPAR within hematopoietic cells showed elevated suPAR levels upon stimulation with LPS, both in the blood and urine, as well as proteinuria [1].

The expression of uPAR in the kidney and the concentrations of suPAR in the blood are consistently elevated in patients with kidney diseases, especially with focal segmental glomerulosclerosis (FSGS) and diabetic nephropathy [6], [7], [8]. In patients experiencing autosomal dominant polycystic kidney disease (ADPKD), higher suPAR levels indicate unfavorable disease courses, even though this type of kidney disease is not caused by podocyte injury [9]. In congruence, Hayek et al. also showed that elevated plasma levels of suPAR indicate emerging chronic kidney disease (CKD) in persons with normal kidney functions at the baseline [10]. The association of suPAR with outcome measures has recently been shown to persist in hemodialysis patients [11], [12].

The elevation of suPAR is not solely associated with kidney diseases but was also linked to inflammatory and diverse pathologic conditions such as rheumatologic diseases [13], [14], acute respiratory distress syndrome (ARDS) [15], or different types of cancer [16].

It is still unclear as to whether suPAR has a pathophysiologic or prognostic role in kidney transplant patients. Moreover, Harel et al. could not find a significant correlation between suPAR levels and FSGS recurrence after renal transplantation (KTx) [17]. However, its role in kidney function or transplant specific outcomes needs further clarification. Therefore, we prospectively investigated the prognostic relevance of suPAR in patients immediately before and one year after KTx.

Materials And Methods

Study population:

In this study, we prospectively included 100 consented patients (age ≥ 18 years) who had received a kidney transplant at our transplant center between April 2013 and October 2015.

The suPAR levels in their blood samples were measured in a timeframe of 24 hours before and one year after KTx. The initial immunosuppressive regimen consisted of basiliximab, tacrolimus (target trough 6–12 ng/mL), mycophenolate mofetil, and prednisolone. Anti-thymocyte globulin was administered to re-transplanted or highly immunized patients (PRA > 85%). AB0-incompatible patients received rituximab four weeks before KTx. Three patients with atypical hemolytic uremic syndrome (aHUS) as an underlying disease were given eculizumab in addition to basiliximab. Oral CMV-prophylaxis with valganciclovir was administered for 100 days in R+ and 200 days in the D+/R− constellation [18].

ELISA:

Plasma and urine concentrations of suPAR were measured using the Quantikine Human uPAR ELISA assay (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. The assay range is 62.5–4,000 pg/ml, with a sensitivity of 33 pg/ml to suPAR. The samples above the concentration limit of the test were re-measured after 10-fold dilution in Calibrator Diluent RD 6-10 reagent according to the manufacturer’s directions. Blood and urine samples were collected after gaining informed consent at hospital admission within 24 hours prior to KTx. The baseline and one-year samples were collected from

each patient and immediately sent to the research core laboratory. The serum was obtained by centrifugation for 10 minutes at 2,000 g using a refrigerated centrifuge, transferred into clean polypropylene tubes, and stored at -80°C until time of assay.

Patient characteristics were taken from the hospital's electronic patient records. The data of all the participating patients was made anonymous prior to conducting an analysis. Moreover, written informed consent was obtained from all the participants. All experiments were performed in accordance with the current transplantation guidelines and the declarations of Istanbul and Helsinki. This study was approved by the local ethics committee, Ethik Kommission der Ärztekammer Westfalen-Lippe und der Medizinischen Fakultät der Westfälischen Wilhelms-Universität (No. 2013-364-f-S and No. 2019-109-f-S).

Outcome measures:

The main outcome measures involved renal function (eGFR calculated using the CKD-EPI equation and urine-protein/creatinine ratio (UPCR)) at years one to four after KTx. Other outcome parameters were patient and overall graft survival. Patient survival was defined as the time from KTx to death (due to any cause) or the last contact with a patient. Overall graft survival was defined as the time from KTx to death (from any cause), graft failure, or the last contact, whichever occurred first. Re-initiation of dialysis or re-transplantation was considered as graft failure. Recipients after LD and DD were considered separately [18].

Patients were subjected to kidney biopsy in case of increased creatinine levels (≥ 0.3 mg/dL) and/or a significant increase in proteinuria. The kidney biopsies were evaluated by two independent pathologists. The whole blood was analyzed for creatinine (enzymatic assay; Creatinine-Pap, Roche Diagnostics, Mannheim, Germany). Organ rejections were diagnosed as per the BANFF classification [19].

Statistical analysis:

Data was analyzed using IBM SPSS Statistics 26 (IBM Corp., Armonk, New York, USA). Normally distributed continuous variables have been presented as mean ± standard deviation (SD), and non-normally distributed continuous variables were presented as median and 1^{st} and 3^{rd} quartiles (interquartile range, IQR). Absolute and relative frequencies have been provided for categorical variables [18].

Pairs of independent groups were compared using the Student's t-test for normally distributed data, the Mann–Whitney U test for non-normal data, and the Fisher's exact test or Chi-Quadrat-Test for categorical variables. To compare the paired data, we used the Wilcoxon test for continuous variables and the McNemar test for categorical variables.

The cumulative probability of developing eGFR loss > 30% in our KTx cohort was calculated using the Kaplan-Meier analysis, and the curves were compared using the log-rank test. A cut-off for suPAR after

one year for patients at higher risk for eGFR-loss was identified by calculating Youden-indices based on a ROC-analysis.

To evaluate independent risk factors for the onset of eGFR loss > 30%, we performed multivariable Cox-regression analyses.

Results

Demographic and clinical characteristics have been presented in Table 1. 56 patients (56%) received living donations (LD), and 44 (44%) had deceased donations (DD). 17 (30.4%) of the LD were AB0-incompatible transplantations. 64 recipients (64%), the majority, were male. The mean age of the recipients at the time of KTx was 49.3 years (SD = 15.62).

Patients' outcome data have been presented in Table 2.

In patients who received LD, pre-transplant suPAR levels were significantly lower compared to those receiving DD (suPAR 6,839 (4,887, 10,183) pg/ml, vs. 9,117 (6,891, 12,211) pg/ml, p=0.008, Figure 1).

Dialysis vintage and cold ischemic time were significantly shorter in LD than DD recipients (19.7 months vs. 77.5 months, $p < 0.001$; 2.47 hours vs. 9.96 hours, $p < 0.001$).

It was found that kidney function improved one year after KTx to a mean eGFR of 57.3 ± 20.1 ml/min/1.73 m^2 (Table 1). Although LD recipients showed a tendency toward a higher eGFR compared to DD recipients (60.0 ± 20.3 ml/min/1.73 m^2 vs. 53.9 ± 19.5 ml/min/1.73 m^2, $p = 0.075$), the difference was not statistically significant. In contrast, suPAR levels significantly declined in LD and DD recipients after KTx (8,095 (5,818, 11,192) pg/ml before KTx vs. 4,376 (2,757, 5,612) pg/ml one year after KTx; Fig. 2), ($n = 100$, $p < 0.001$).

One year after KTx, the suPAR levels of recipients aligned without any detectable difference between those who had LD and DD (LD 4,332 (2,914, 5,567) pg/ml vs. DD 4,413 (2,575, 6,049) pg/ml, n=100, p=0.879, Fig. 3).

Dialysis vintage tended to be associated with suPAR levels prior to KTx ($n = 100$, $p = 0.067$,). Upon a closer analysis, it was seen that preemptive recipients who never underwent dialysis had significantly lower suPAR levels (suPAR 5,249 (2,302, 7,806) pg/ml, $n = 8$) compared to patients on any mode of dialysis before transplantation (suPAR 8,392 (6,011, 11,503) pg/ml, $n = 92$) ($p = 0.006$) (see Figure 4 A). The suPAR levels of patients on hemodialysis (suPAR 8,322 pg/ml (5,956, 11,475), $n = 71$) and patients treated with peritoneal dialysis (8,075 (6,936, 12,492) pg/ml, $n = 12$) were comparable ($p = 0.928$) (see Fig. 4 A). One year after KTx, the suPAR levels of patients on any mode of dialysis prior to transplant compared with preemptively transplanted patients became equal (Fig. 4 B).

The suPAR levels in patients one year after KTx were not correlated to the eGFR at the same time (p=0.24, r= -0.119). However, it was associated with the development of the eGFR between the second and fourth

year after KTx. Higher suPAR levels one year after KTx could be associated with a higher eGFR-loss in the following three years (Figure 5, n = 82 (18 patients lost of follow-up), p = 0.021, r = -0.255).

In our study, a correlation between the suPAR levels and the incidence or the number of biopsy-proven allograft rejections could not be detected.

We found that only four patients experienced terminal graft failures by the time of the follow-up. Moreover, two patients died during the course of the study, and two patients died without losing their graft before. Due to these small number of events, we did not perform survival analyses with these endpoints.

Instead, we took a loss of renal function in terms of more than 30% of eGFR loss from year one as an endpoint [20], [21]. eGFR-loss >30% was stated, when it was constant for at least one month and did not increase subsequently. Our patient collective was divided into two groups based on a cut-off for suPAR below and above 6,212 pg/ml by calculating the Youden-index for a ROC-curve according to suPAR measured after one year. (n = 82 vs. n = 18). Patients with allograft loss were defined as eGFR-loss >30%, patients who died with unimpaired allograft function were handled as negative for eGFR-loss > 30%. The Kaplan-Meier analysis and Log-rank test showed a significant ten-month reduced time to eGFR-loss > 30% for patients with suPAR levels above 6,212 pg/ml (33.5 vs. 61.9 months, Figure 6).

The suPAR levels after one year as an independent risk factor for eGFR-loss > 30% subsequently

To evaluate whether suPAR after one year is independently associated with accelerated eGFR-loss survival, apart from other known risk factors, we performed a multivariable Cox-regression analysis that included several known risk factors causing inferior allograft survival (Table 3).

Besides the well-known risk factors for kidney allograft failure such as age at the time of KTx (p = 0.012, HR 1.052), previous KTx (p = 0.036, HR 0.214), and the occurrence of acute rejection episodes (p = 0.033, HR 0.265), the Cox-regression analysis confirmed suPAR one year after transplantation as an independent risk factor for eGFR-loss > 30% (p = 0.001, HR 1.000).

Discussion

It is known that suPAR levels are high in patients with chronic proteinuric and non-proteinuric kidney diseases and may predict disease courses [7], [10]. Wei et al. described a mechanism for suPAR leading to podocyte foot process effacement through the activation of αvβ3 integrin in podocytes [5], thereby acting as a driver of kidney injury. Moreover, Hahm et al. reported that BM-derived immature myeloid cells are responsible for the elevated pathological levels of suPAR in the case of LPS-treated mice with proteinuric kidney disease and thus, a key contributor to glomerular dysfunction [1].

In summary, whether suPAR acts as an originator of kidney injury, is produced and/or released as a consequence of (kidney) injury, or both can occur concurrently has not been completely elucidated yet [22].

Data on suPAR and outcome after KTx is still limited. To identify a potential prognostic role of suPAR, we performed this prospective study and assessed the suPAR serum levels in KTx recipients prior and post KTx.

We found that, after KTx, suPAR levels improved significantly (Fig. 2). The decrease of suPAR was in parallel with an increase in renal function (Table 1). Notably, there were distinct differences between LD and DD recipients. The suPAR levels in patients receiving DD were significantly higher compared to those receiving LD (Fig. 1). This could perhaps be related to the fact that eight (16%) of the LD were preemptive. These patients had significantly lower suPAR levels compared to those on dialysis prior to transplantation (Fig. 4 A). Moreover, the dialysis vintage of LD recipients was shorter, and these patients were younger than DD recipients, (Table 1). In our data, dialysis vintage was significantly correlated to suPAR levels prior to transplantation ($p = 0.013$, $r = 0.258$). In congruence, among other factors, dialysis vintage as well as the age of the patient have recently been linked to the suPAR levels in dialysis patients [11], [12]. After KTx, the suPAR levels decreased to a comparable level at the one-year mark (Fig. 3).

Similarly, morbidity and mortality of DD recipients are usually higher that is perhaps related to longer dialysis vintage [23], [24]. Notably, Torino and Drechsler et al. demonstrated that higher suPAR levels even translated into all-cause and both CVD and non-CVD mortality in hemodialysis patients [11], [12].

Thus, one may only speculate that lower suPAR levels in preemptive LD might contribute to preferable outcomes of LD KTx [23]. However, our study sample was too small to allow us to draw such conclusions.

Otherwise, as suPAR is excreted by the kidneys, end-stage renal disease may cause a reduction in the absolute amount of suPAR excreted with urine, leading to an accumulation in the serum [25].

The measurement of suPAR levels can indicate CKD prior to measurable function loss [10]. As the suPAR levels decline after KTx and as the native recipient kidneys usually remain in-situ after transplantation, the pathologically altered kidneys seem to be ineligible as a relevant suPAR source (due to progressive loss of function); otherwise, suPAR could be excreted by the working graft after transplantation, hence, kidneys seemed to have cleared suPAR from the circulation, at least in healthy volunteers [26]. However, our data confirmed the prognostical value of suPAR to predict the decline in functionality of the allograft (Fig. 5 and 6).

We observed that the suPAR level one year after KTx is predictive of future graft function (eGFR) to some extent, and suPAR levels above a cut-off of 6,212 pg/ml serve as a risk factor for a significantly accelerated decrease of allograft function. This is in line with the observations made in CKD patients for a kidney-transplant cohort [9], [10].

In contrast, we could not detect a significant correlation between suPAR and UPCR in our kidney transplant cohort. Interestingly, it was found that suPAR is not always associated with proteinuria. In an animal model, the suPAR application per se was not related to the development of proteinuria, at least in

the absence of kidney disease [27]. Further, in patients with paroxysmal nocturnal hemoglobinuria (and normal kidney function), who have extremely high suPAR levels, suPAR was also not related to the development of proteinuria [28]. In African Americans with CKD, suPAR was only associated with worsening proteinuria with two APOL-I risk alleles, at the same time, it was linked to CKD progression, end-stage renal disease, and all-cause mortality in all patients [29]. It seems that suPAR may develop its damaging effect if it hits an already damaged kidney. Interestingly, there are interferences in the αvβ3 integrin pathway, which is induced by suPAR and calcineurin-mediated processes. However, these are suppressed in KTx patients through the administration of calcineurin inhibitors (CNI) such as tacrolimus or cyclosporine. CNI exhibit antiproteinuric effects by stabilizing the actin cytoskeleton and stress fibers of podocytes through synaptopodin re-storage [30], [31]. Furthermore, in lupus nephritis, a combination therapy of tacrolimus and mycophenolate mofetil (a standard of care after KTx [32], had an additive protective effect for the podocyte actin cytoskeleton by tacrolimus-induced synaptopodin-mediated activation of RhoA and mycophenolate mofetil-mediated VAV1 inhibition of Rac1 [31].

However, our study has limitations, as it is a single-center study and observational in nature. Further, for our study, we analyzed data of a relatively small cohort, which did not include data on non-renal causes for eGFR decline or proteinuria such as infections, CNI toxicity, or surgical problems.

In conclusion, on the one hand, our observations implicate that elevated suPAR is essentially rather a consequence of than a cause for kidney disease. It was also found that, after resolving the state of end-stage renal disease by KTx, suPAR levels dropped significantly. On the other hand, suPAR seems capable as an early marker for allograft dysfunction after KTx.

Declarations

Funding This study was supported by a grant from Innovative Medizinische Forschung, Medical Faculty, University of Münster, to UJ and a grant from the Interdisciplinary Centre for Clinical Research (IZKF), the University of Münster to KSN.

Author contributions: Jehn U did research and wrote the paper; Schütte-Nütgen K did research and wrote the paper; Henke U did research, Suwelack B, Pavenstädt H supervised the project and revised the paper; Reuter S designed the research, collected samples, and wrote the paper.

Jehn U and Schütte-Nütgen K contributed equally.

Conflict-of-interest statement: The authors have declared that no competing interests exist.

Acknowledgments

The present authors would like to express their gratitude to Birgit Jaxy, Ute Neugebauer, and Rita Schröter for their excellent technical assistance.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Hahm, E. *et al.* Bone marrow-derived immature myeloid cells are a main source of circulating suPAR contributing to proteinuric kidney disease. *Nature medicine* **23,** 100–106 (2017).
2. Thunø, M., Macho, B. & Eugen-Olsen, J. suPAR: the molecular crystal ball. *Disease markers* **27,** 157–172 (2009).
3. Gussen, H. *et al.* Neutrophils are a main source of circulating suPAR predicting outcome in critical illness. *Journal of intensive care* **7,** 26 (2019).
4. Wei, C. *et al.* uPAR isoform 2 forms a dimer and induces severe kidney disease in mice. *The Journal of clinical investigation* **129,** 1946–1959 (2019).
5. Wei, C. *et al.* Modification of kidney barrier function by the urokinase receptor. *Nature medicine* **14,** 55–63 (2008).
6. Yoo, T.-H. *et al.* Sphingomyelinase-like phosphodiesterase 3b expression levels determine podocyte injury phenotypes in glomerular disease. *Journal of the American Society of Nephrology : JASN* **26,** 133–147 (2015).
7. Wei, C. *et al.* Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nature medicine* **17,** 952–960 (2011).
8. Alachkar, N. *et al.* Podocyte effacement closely links to suPAR levels at time of posttransplantation focal segmental glomerulosclerosis occurrence and improves with therapy. *Transplantation* **96,** 649–656 (2013).
9. Hayek, S. S. *et al.* Soluble Urokinase Plasminogen Activator Receptor and Decline in Kidney Function in Autosomal Dominant Polycystic Kidney Disease. *Journal of the American Society of Nephrology : JASN* **30,** 1305–1313 (2019).
10. Hayek, S. S. *et al.* Soluble Urokinase Receptor and Chronic Kidney Disease. *The New England journal of medicine* **373,** 1916–1925 (2015).
11. Torino, C. *et al.* Soluble Urokinase Plasminogen Activator Receptor (suPAR) and All-Cause and Cardiovascular Mortality in Diverse Hemodialysis Patients. *Kidney international reports* **3,** 1100–1109 (2018).
12. Drechsler, C. *et al.* Soluble Urokinase Plasminogen Activator Receptor and Outcomes in Patients with Diabetes on Hemodialysis. *Clinical journal of the American Society of Nephrology : CJASN* **12,** 1265–1273 (2017).
13. Saylam Kurtipek, G. *et al.* Plasma-soluble urokinase plasminogen activator receptor (suPAR) levels in Behçet's disease and correlation with disease activity. *International journal of rheumatic diseases* **21,** 866–870 (2018).

14. Pliyev, B. K. & Menshikov, M. Y. Release of the soluble urokinase-type plasminogen activator receptor (suPAR) by activated neutrophils in rheumatoid arthritis. *Inflammation* **33,** 1–9 (2010).
15. Geboers, D. G. P. J. *et al.* Plasma suPAR as a prognostic biological marker for ICU mortality in ARDS patients. *Intensive care medicine* **41,** 1281–1290 (2015).
16. Liu, K. L., Fan, J. H. & Wu, J. Prognostic Role of Circulating Soluble uPAR in Various Cancers: a Systematic Review and Meta-Analysis. *Clinical laboratory* **63,** 871–880 (2017).
17. Harel, E. *et al.* Identifying a potential biomarker for primary focal segmental glomerulosclerosis and its association with recurrence after transplantation. *Clinical transplantation* **33,** e13487 (2019).
18. Jehn, U. et al. Prognostic Value of Growth Differentiation Factor 15 in Kidney Donors and Recipients. *Journal of Clinical Medicine*. **9** (2020).
19. Mengel, M., Sis, B. & Halloran, P. F. SWOT analysis of Banff: strengths, weaknesses, opportunities and threats of the international Banff consensus process and classification system for renal allograft pathology. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* **7,** 2221–2226 (2007).
20. Levey, A. S. *et al.* GFR decline as an end point for clinical trials in CKD: a scientific workshop sponsored by the National Kidney Foundation and the US Food and Drug Administration. *American journal of kidney diseases : the official journal of the National Kidney Foundation* **64,** 821–835 (2014).
21. Mol, P. G. M., Maciulaitis, R. & Vetter, T. GFR decline as an end point for clinical trials in CKD: a view from Europe. *American journal of kidney diseases : the official journal of the National Kidney Foundation* **64,** 838–840 (2014).
22. Saleem, M. A. What is the Role of Soluble Urokinase-Type Plasminogen Activator in Renal Disease? *Nephron* **139,** 334–341 (2018).
23. Hart, A. *et al.* OPTN/SRTR 2017 Annual Data Report: Kidney. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* **19 Suppl 2,** 19–123 (2019).
24. Reuter, S. *et al.* A Comparison of Different Algorithms for the Assessment of Cardiovascular Risk in Patients at Waiting List for Kidney Transplantation. *PloS one* **11,** e0161927 (2016).
25. Franco Palacios, C. R. *et al.* Urine but not serum soluble urokinase receptor (suPAR) may identify cases of recurrent FSGS in kidney transplant candidates. *Transplantation* **96,** 394–399 (2013).
26. Chew-Harris, J., Appleby, S., Richards, A. M., Troughton, R. W. & Pemberton, C. J. Analytical, biochemical and clearance considerations of soluble urokinase plasminogen activator receptor (suPAR) in healthy individuals. *Clinical biochemistry* **69,** 36–44 (2019).
27. Cathelin, D. *et al.* Administration of recombinant soluble urokinase receptor per se is not sufficient to induce podocyte alterations and proteinuria in mice. *Journal of the American Society of Nephrology : JASN* **25,** 1662–1668 (2014).
28. Mesnard, L., Luque, Y. & Rondeau, E. Experimental concerns regarding suPAR-related proteinuria. *Nature reviews. Nephrology* **13,** 593 (2017).

29. Luo, S. *et al.* Soluble Urokinase-Type Plasminogen Activator Receptor in Black Americans with CKD. *Clinical journal of the American Society of Nephrology : CJASN* **13,** 1013–1021 (2018).
30. Faul, C. *et al.* The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. *Nature medicine* **14,** 931–938 (2008).
31. Fu, J. *et al.* Transcriptomic analysis uncovers novel synergistic mechanisms in combination therapy for lupus nephritis. *Kidney international* **93,** 416–429 (2018).
32. KDIGO clinical practice guideline for the care of kidney transplant recipients. *American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons* **9 Suppl 3,** S1-155 (2009).

Tables

Table 1: Baseline characteristics of the recipients

-	All (n = 100)	Living (n = 56)	Deceased (n = 44)	p-value
Age (years, mean ± SD)	49.31 (15.62)	42.76 (15.06)	57.65 (12.02)	0.000[a]
Male, n (%)	64 (64)	35 (62.5)	29 (65.9)	0.728[a]
Diagnosis of ESRD, n (%)				0.498[b]
Hypertension	13 (13)	7 (12.5)	6 (13.6)	
Diabetes	3 (3)	0 (0)	3 (6.8)	
Polycystic Kidney Disease	13 (13)	6 (10.7)	7 (15.9)	
Obstructive Nephropathy	4 (4)	3 (5.3)	1 (2.3)	
Glomerulonephritis	33 (33)	21 (37.5)	12 (27.3)	
FSGS	7 (7)	5 (8.9)	2 (4.5)	
Interstitial Nephritis	3 (3)	1 (1,8)	2 (4.5)	
Vasculitis	2 (2)	2 (3.6)	0 (0)	
Other	22 (22)	11 (19.6)	11 (25)	
Mode of dialysis, n (%)				0.009[b]
Hemodialysis	71 (71)	40 (71.4)	31 (70.4)	
Peritoneal Dialysis	12 (12)	6 (10.7)	6 (13.6)	
Both	9 (9)	2 (3.6)	7 (16)	
Preemptive Donation	8 (8)	8 (14.3)	0 (0)	
Time on Dialysis (months, mean (1st & 3rd quartile))	44.9 (11.3, 79.2)	19.7 (0.9, 26.2)	77.5 (41.6, 102.2)	0.000[c]
Immunized	38 (38)	21 (37.5)	17 (39)	0.907[d]
European Senior Program (ESP)	14 (14)	0 (0)	14 (32)	0.000[d]
≥ 1 Prior Kidney Transplantat, n (%)	14 (14)	8 (14.3)	6 (13.6)	0.926[d]
Current PRA, n (%)				0.567[b]
0–20%	73 (73)	41 (73.2)	32 (73)	
> 20%	27 (73)	15 (26.8)	12 (27.3)	
Induction, n (%)				0.000[b]

Basiliximab	75 (75)	38 (67.8)	37 (84.1)	
Thymoglobuline	5 (5)	0 (0)	5 (11.3)	
Basiliximab + Thymoglobuline	1 (1)	0 (0)	1 (2.3)	
Rituximab + Thymoglobuline	1 (1)	1 (1.8)	0 (0)	
Rituximab + Basiliximab	16 (16)	16 (28.6)	0 (0)	
Eculizumab + Basiliximab	2 (2)	1 (2.6)	1 (2.3)	
Cold ischemia time (hours, mean ± SD)	5.77 (7.8)	2.47 (0.64)	9.96 (3.3)	0.000[a]
Warm ischemia time (min, mean ± SD)	34.3 (4.35)	33.46 (8.03)	35.4 (7.36)	0.211[a]
AB0i	17 (17)	17 (30.4%)	0 (0)	0.000[b]
eGFR 365 days (ml/min/1.73 m², mean ± SD)	57.32 (20.06)	60.01 (20.25)	53.9 (19.5)	0.131[a]

Demographic characteristics of the study population. The results have been presented as mean ± standard deviation or median and 1st and 3rd quartile, respectively, or as absolute and relative frequencies.

ESRD—end-stage renal disease; FSGS—focal segmental glomerulosclerosis; ESP—European Senior Program; HLA—human leukocyte antigen; PRA—panel reactive antibodies. eGFR—estimated glomerular filtration rate; SD—standard deviation

[a] Mann-Whitney U test

[b] Fisher's exact test

[c] Kruskal-Wallis test

Table 2: Outcomes of the recipients

	All (n = 100)	Living (n = 56)	Deceased (n = 44)	p-value
suPAR pre KTx median (1st & 3rd quartile)	8,095 (5,818, 11,192)	6,839 (4.887, 10.183)	9,117 (6,891, 12,211)	0.008[a]
suPAR day 365 median (1st & 3rd quartile)	4,376 (2,757, 5,612)	4,332 (2,914, 5,567)	4,413 (2,575, 6,049)	0.879[a]
eGFR day 365 (ml/min/1.73m², mean ± SD)	57.3 ± 20.1	60.0 ± 20.3	53.9 ± 19.5	0.075[a]
eGFR day 720 (ml/min/1.73m², mean ± SD)	54.7 ± 20.1	56.4 ± 18.8	52.6 ± 21.6	0.165[a]
eGFR day 1080 (ml/min/1.73m², mean ± SD)	53.9 ± 17.6	55.5 ± 17.6	52.0 ± 17.7	0.153[a]
eGFR day 1440 (ml/min/1.73m², mean ± SD)	51.8 ± 15.9	53.8 ± 14.3	49.5 ± 17.4	0.287[a]
UPCR day 365 (mg/g crea, mean ± SD)	284 ± 906	308 ± 1,124	101 ± 519	0.884[a]
UPCR day 720 (mg/g crea, mean ± SD)	165 ± 218	157 ± 190	174 ± 255	0.597[a]
UPCR day 1,080 (mg/g crea, mean ± SD)	163 ± 187	167 ± 212	158 ± 151	0.726[a]
UPCR day 1,440 (mg/g crea, mean ± SD)	169 ± 201	176 ± 240	162 ± 156	0.885[a]
eGFR-loss > 30% from year one, n (%)	20 (20%)	14 (25%)	6 (13.6%)	0.210[a]
Rejection, n (%)	46 (46%)	29 (51.8%)	17 (38.6%)	0.069[b]
- Antibody-mediated rejection	14 (14%)	10 (17.9%)	4 (9.1%)	
- T-cellular rejection	5 (5%)	4 (7.1)	1 (2.3%)	
- Combined rejection	4 (4%)	2 (3.6)	2 (4.5%)	
- T-cellular borderline rejection	22 (22%)	12 (21.4)	10 (22.7%)	
CMV viremia, n (%)	21 (21%)	5 (8.9%)	16 (36.4%)	0.001[b]
BKPyV viremia, n (%)	20 (20%)	7 (12.5%)	13 (29.5%)	0.043[b]
NODAT, n (%)	20 (20%)	7 (12.5%)	13 (29.5%)	0.045[b]

suPAR: Soluble urokinase-type plasminogen activator receptor; eGFR: estimated glomerular filtration rate, calculated using CKD-EPI formula; UPCR: urine protein creatinine ratio; CMV: cytomegalovirus; BKPyV: BK-Polyomavirus; NODAT: New-onset diabetes after transplantation; crea: creatinine

[a] Mann–Whitney U test

[b] Fisher's exact test

Table 3: Cox-regression analysis for determining risk factors associated with *eGFR loss > 30%*

Variable	Hazard Ratio	95% CI	P-value
Age at KTx	1.052	1.011 – 1.094	0.012
Previous KTx	0.214	0.050 – 0.906	0.036
CMV-viremia	1.4223	0.323 – 6.273	0.641
BKV-viremia	4.806	0.653 – 35.353	0.123
Postmortal donation	0.647	0.063 – 6.658	0.715
NODAT	2.867	0.478 – 17.213	0.249
Time on dialysis	1.013	0.995 – 1.031	0.170
Cold ischemia time	0.799	0.560 – 1.139	0.214
Delayed graft function	1.533	0.127 – 18.454	0.736
Acute rejection	0.265	0.078 – 0.896	0.033
suPAR pre KTx	1.000	1.000 – 1.000	0.064
suPAR day 365	1.000	1.000 – 1.001	0.001

* Abbreviations: KTx: kidney transplantation; CMV: cytomegalovirus; BKV: BK-Polyomavirus; NODAT: new onset of diabetes after transplantation; suPAR: soluble urokinase-type plasminogen activator receptor

Figures

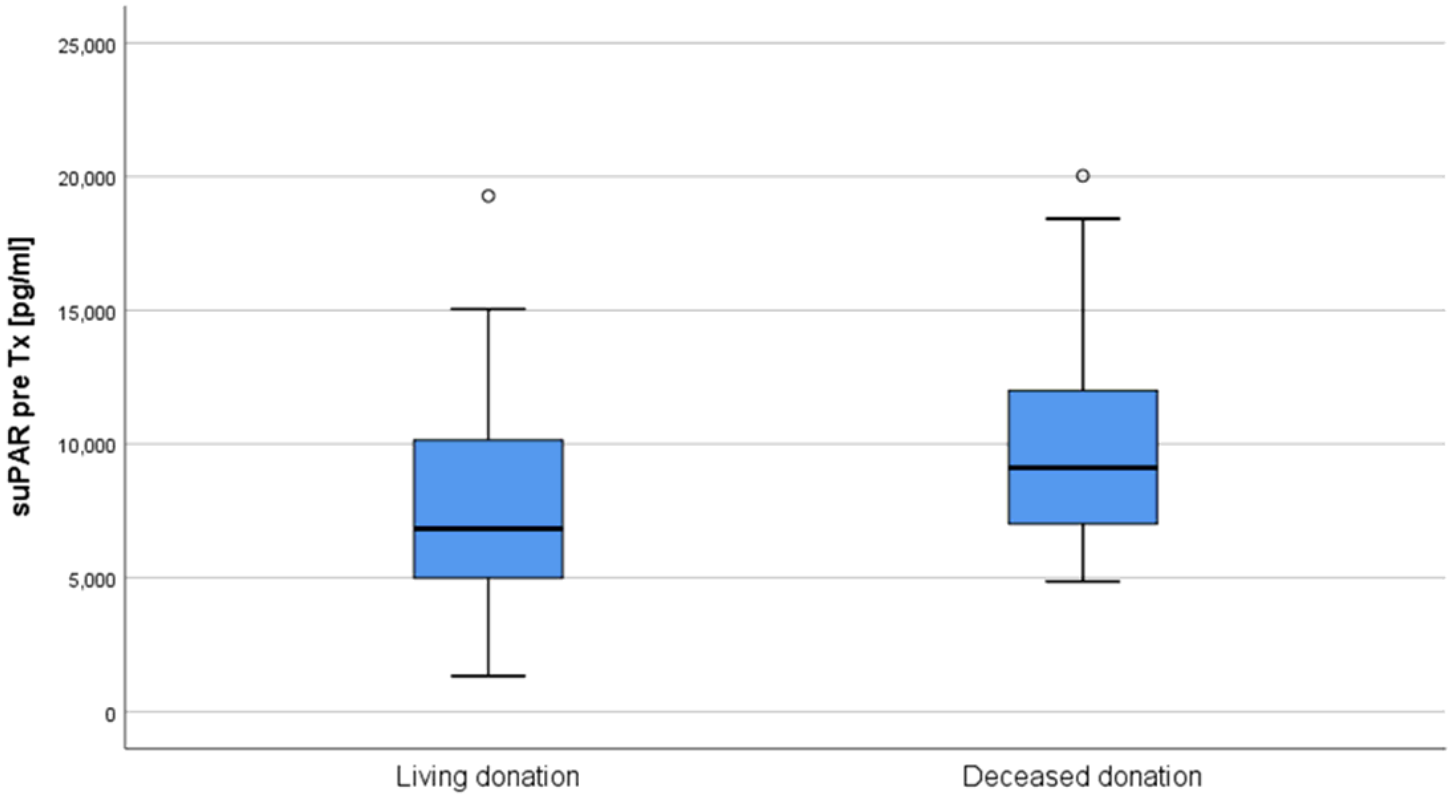


Figure 1

Recipients of living donation (n = 56) had significantly lower suPAR levels prior to transplantation compared to recipients of deceased donation (n = 44, p = 0.008).

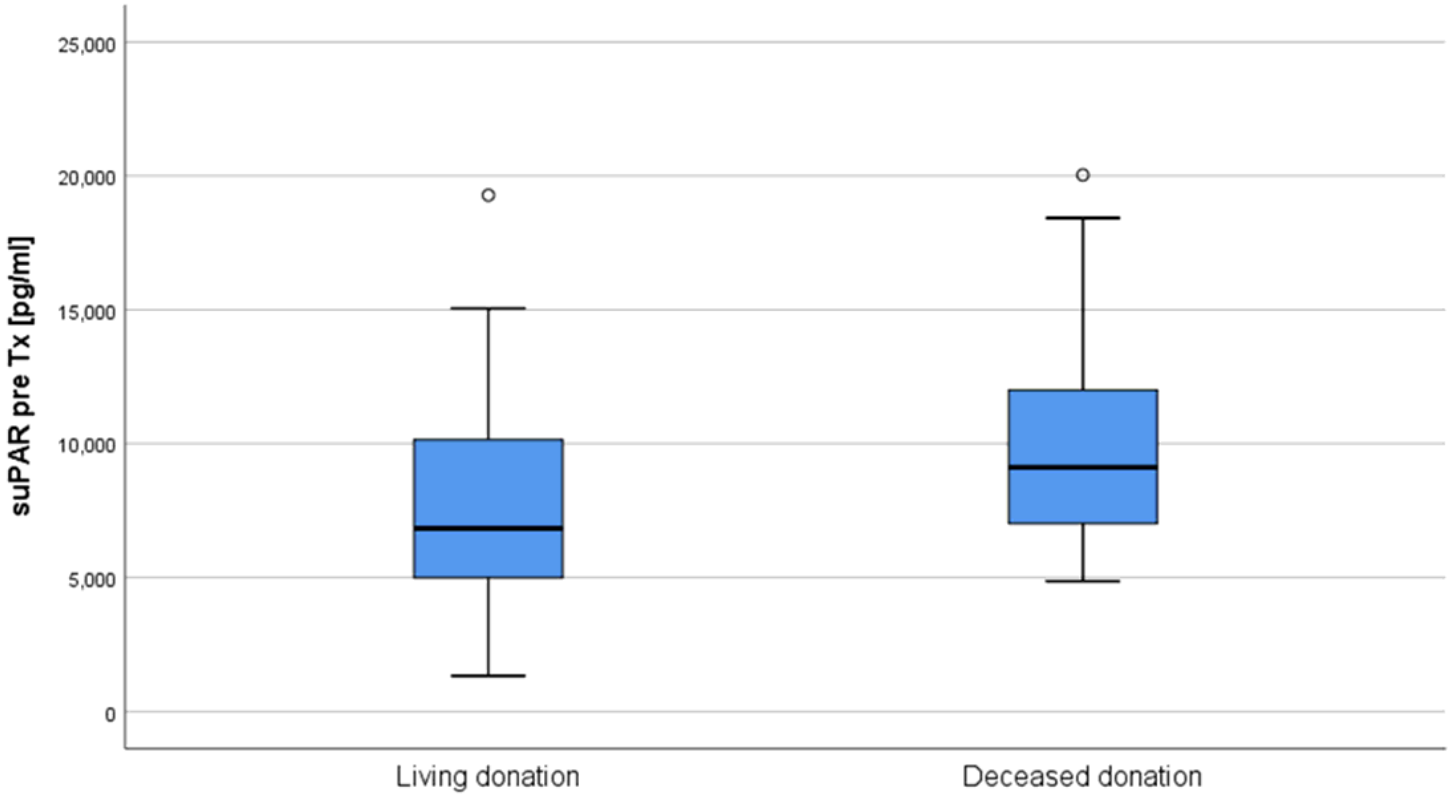


Figure 1

Recipients of living donation (n = 56) had significantly lower suPAR levels prior to transplantation compared to recipients of deceased donation (n = 44, p = 0.008).

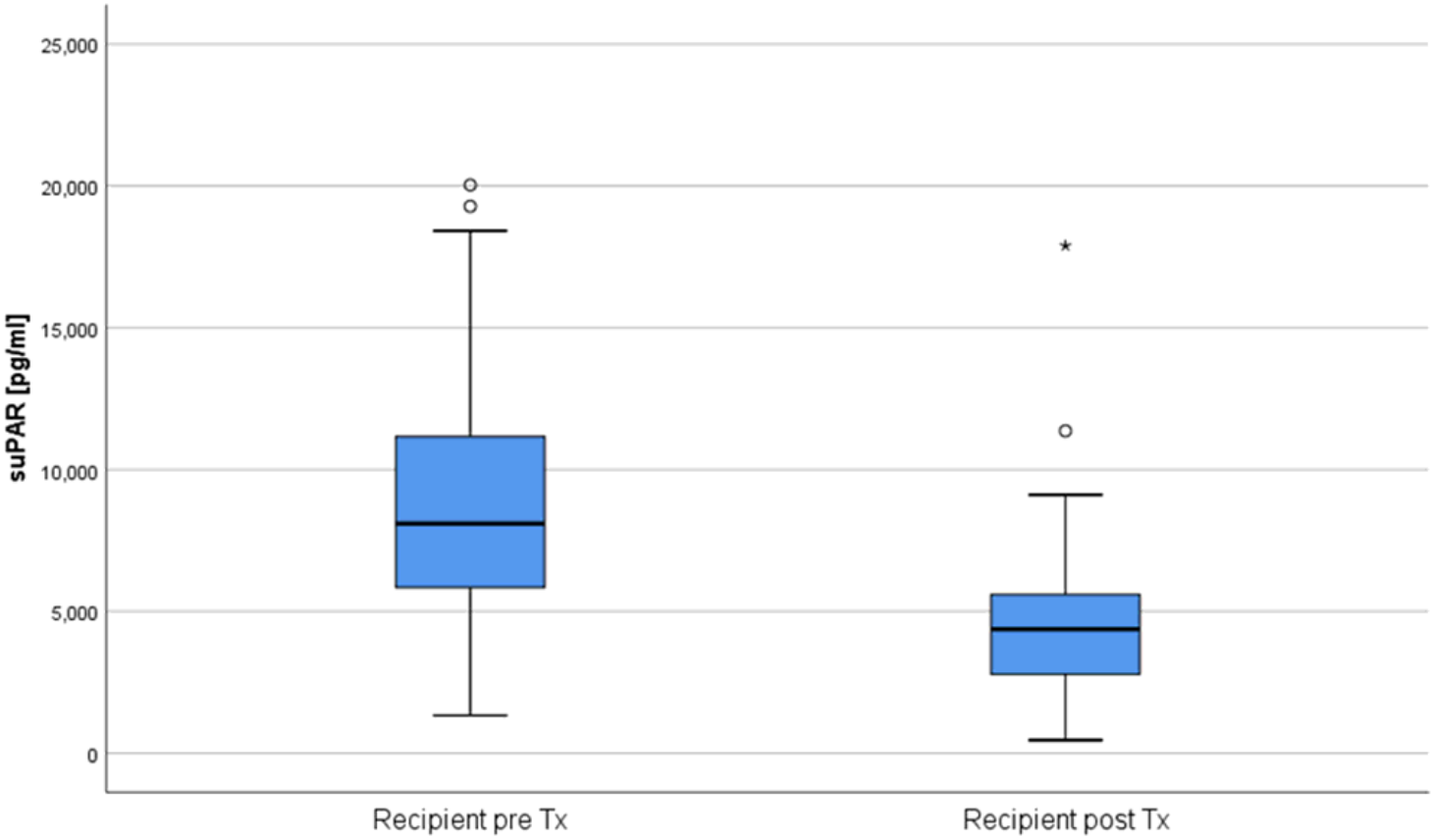


Figure 2

Recipients had significantly lower suPAR levels one year after KTx compared to suPAR levels before KTx.

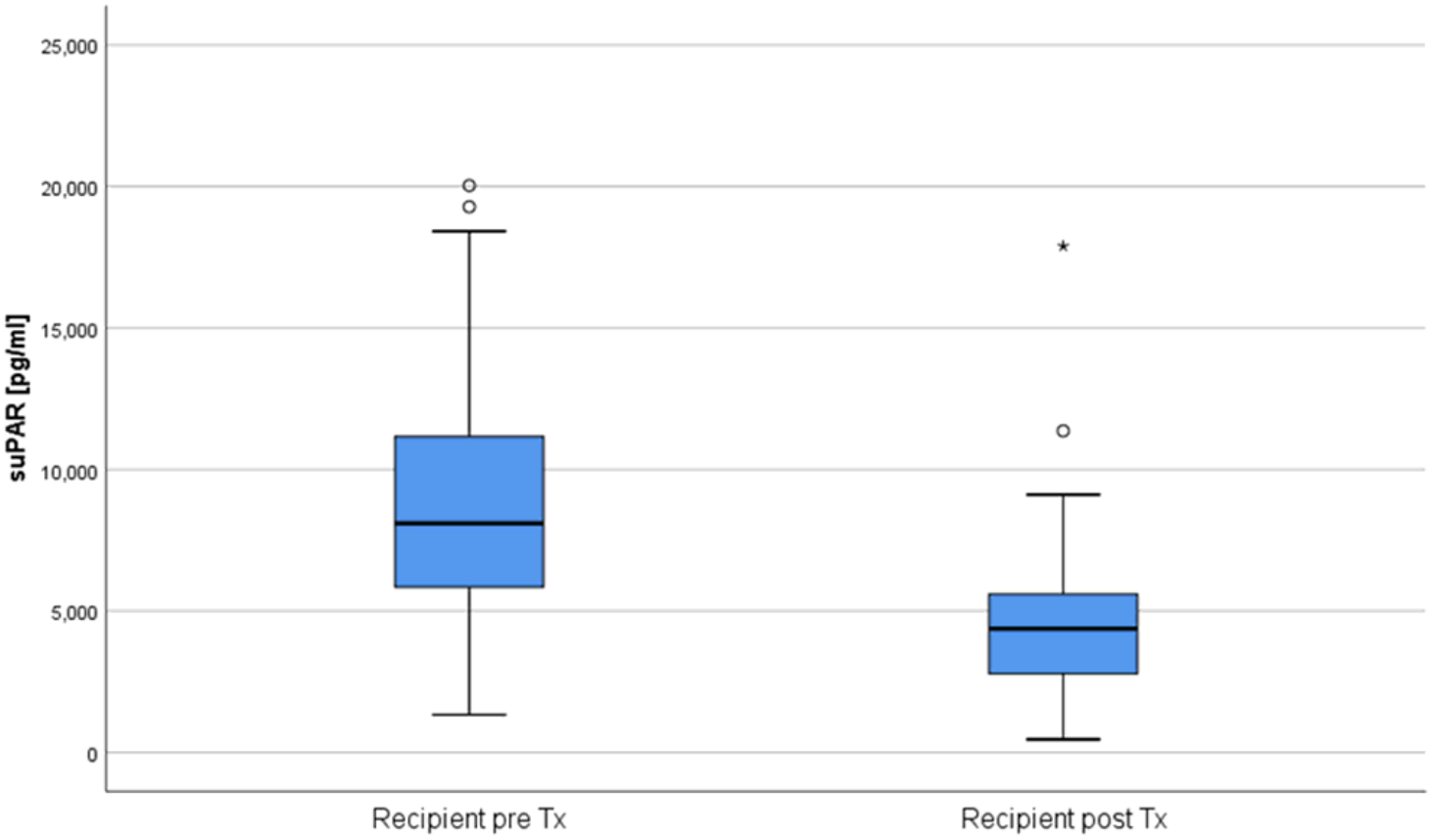


Figure 2

Recipients had significantly lower suPAR levels one year after KTx compared to suPAR levels before KTx.

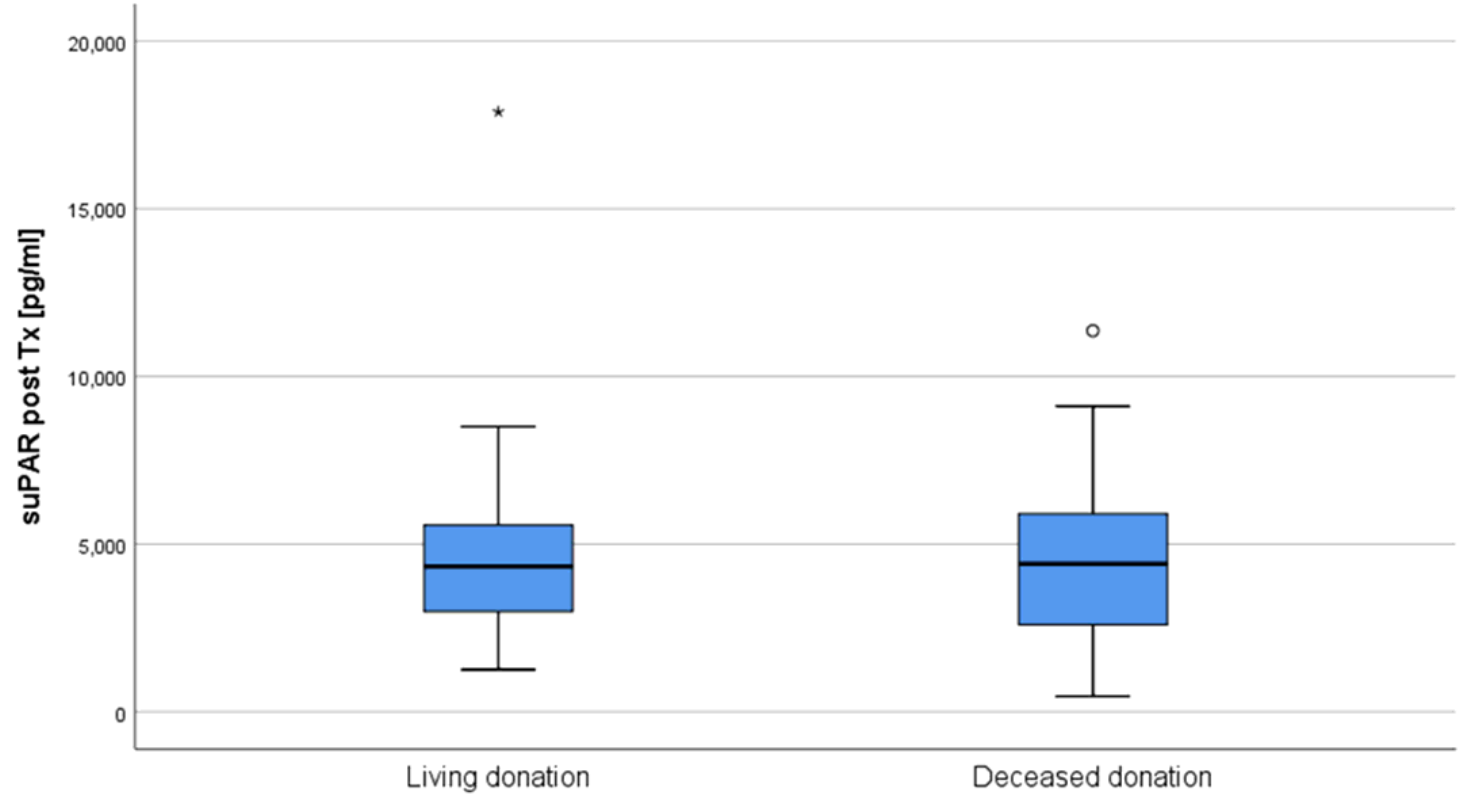


Figure 3

Recipients’ suPAR levels after LD and DD aligned one year after KTx.

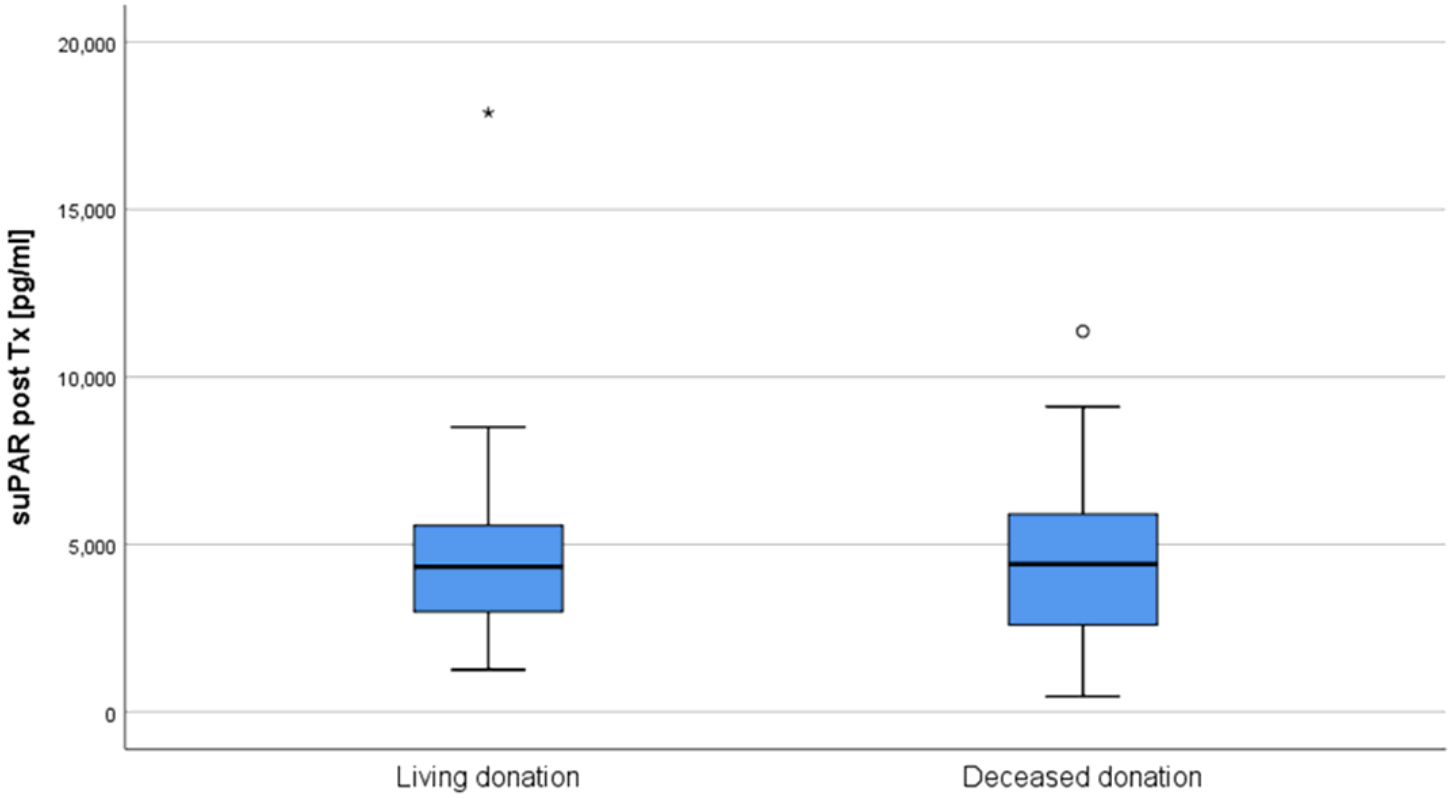


Figure 3

Recipients’ suPAR levels after LD and DD aligned one year after KTx.

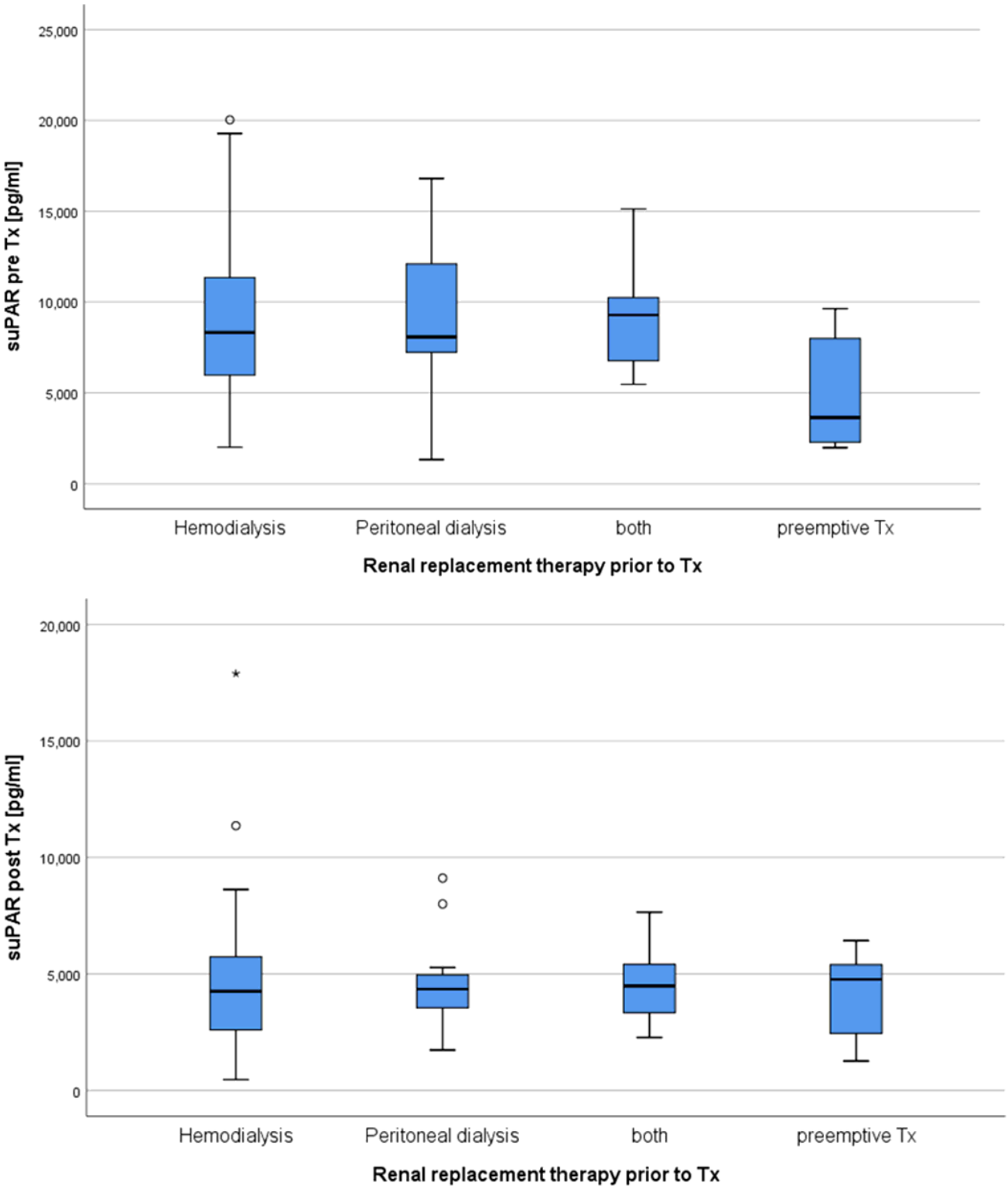


Figure 4

The suPAR levels in recipients receiving preemptive donations are significantly lower compared to the patients receiving hemo- or peritoneal dialysis. One year after transplantation, the suPAR levels of both cases were comparable.

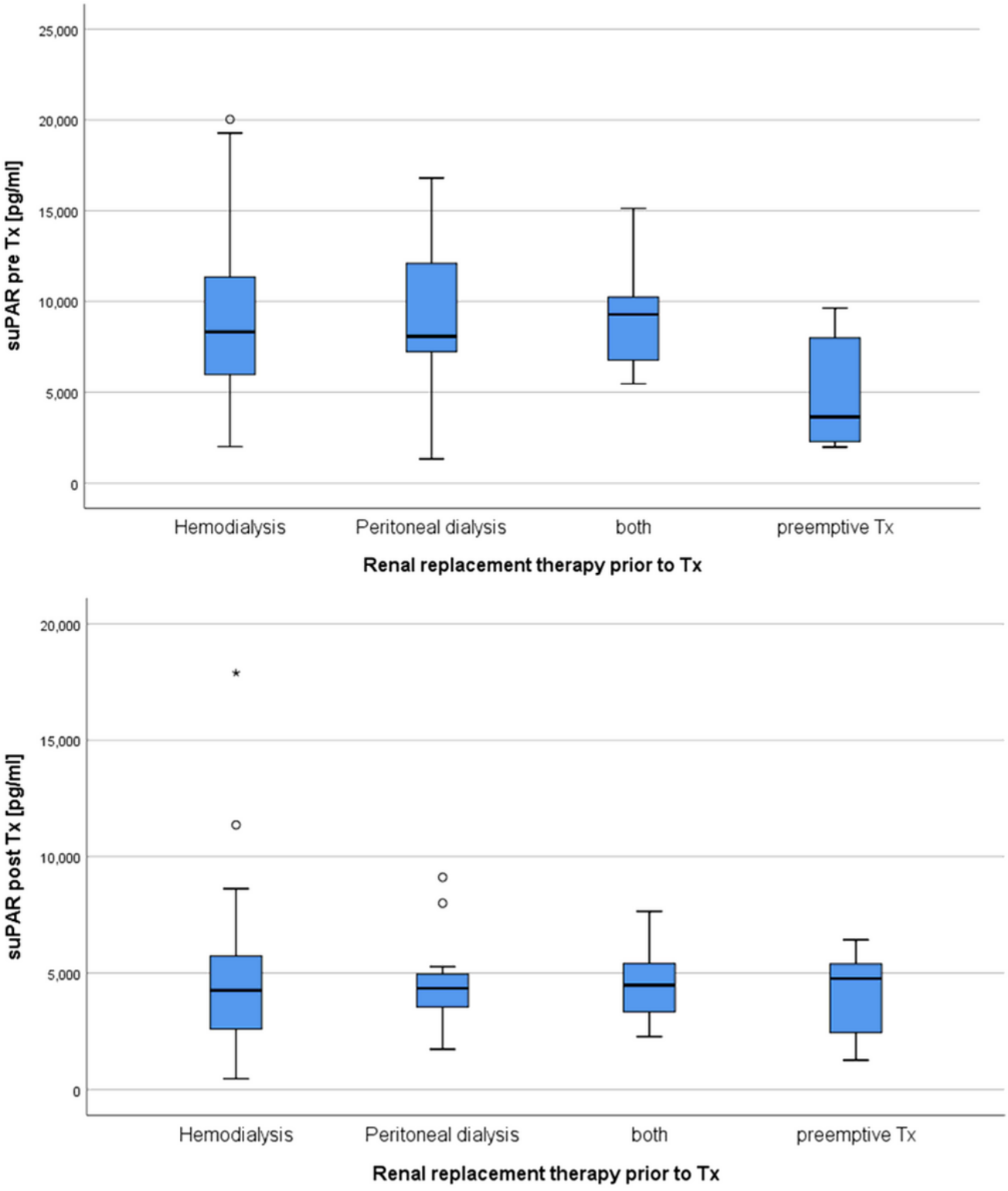


Figure 4

The suPAR levels in recipients receiving preemptive donations are significantly lower compared to the patients receiving hemo- or peritoneal dialysis. One year after transplantation, the suPAR levels of both cases were comparable.

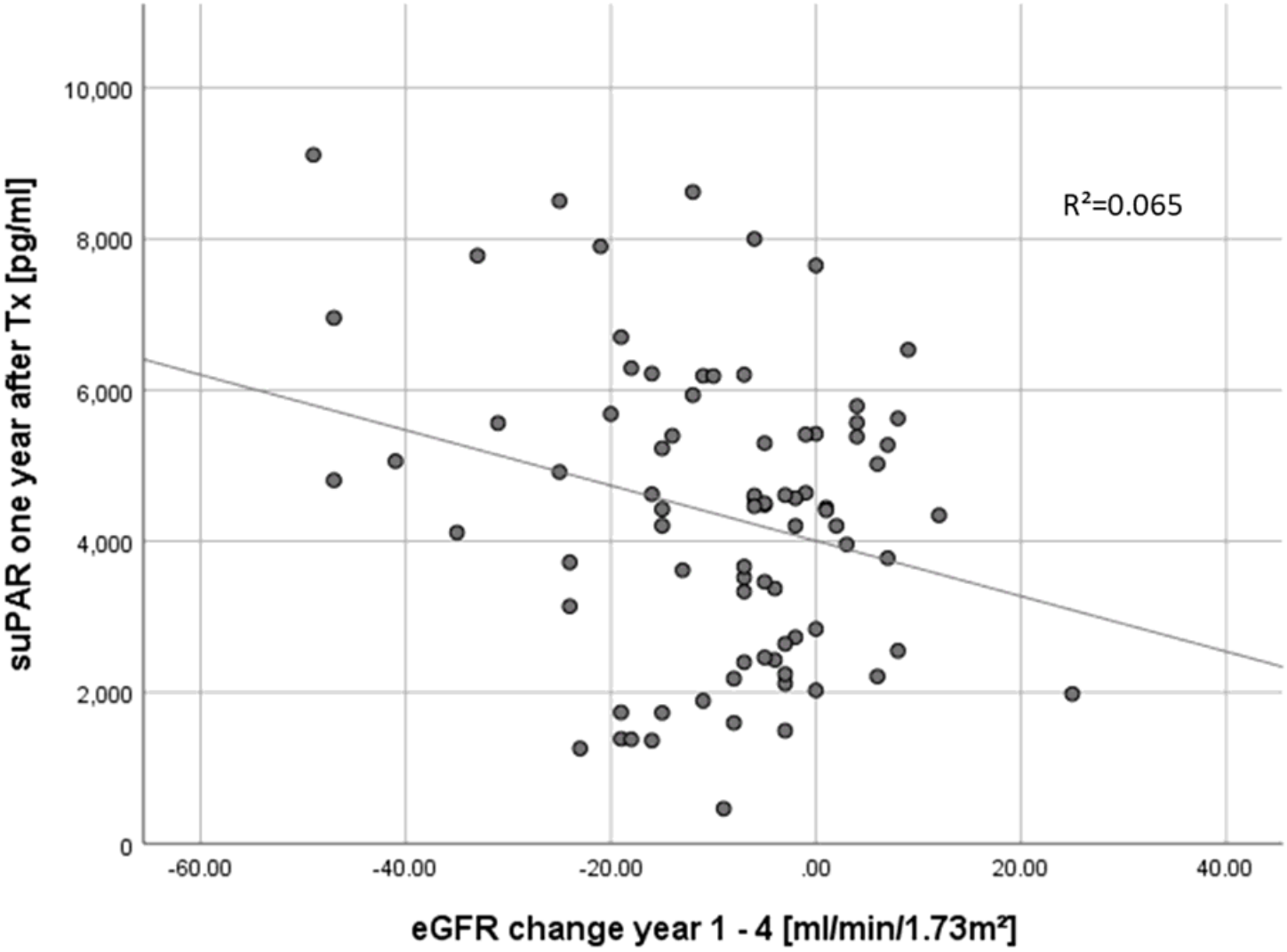


Figure 5

The correlation between the recipients' suPAR levels at day 365 and eGFR decline in the following three years. Higher suPAR-levels preceded a higher eGFR-loss in the following three years after KTx.

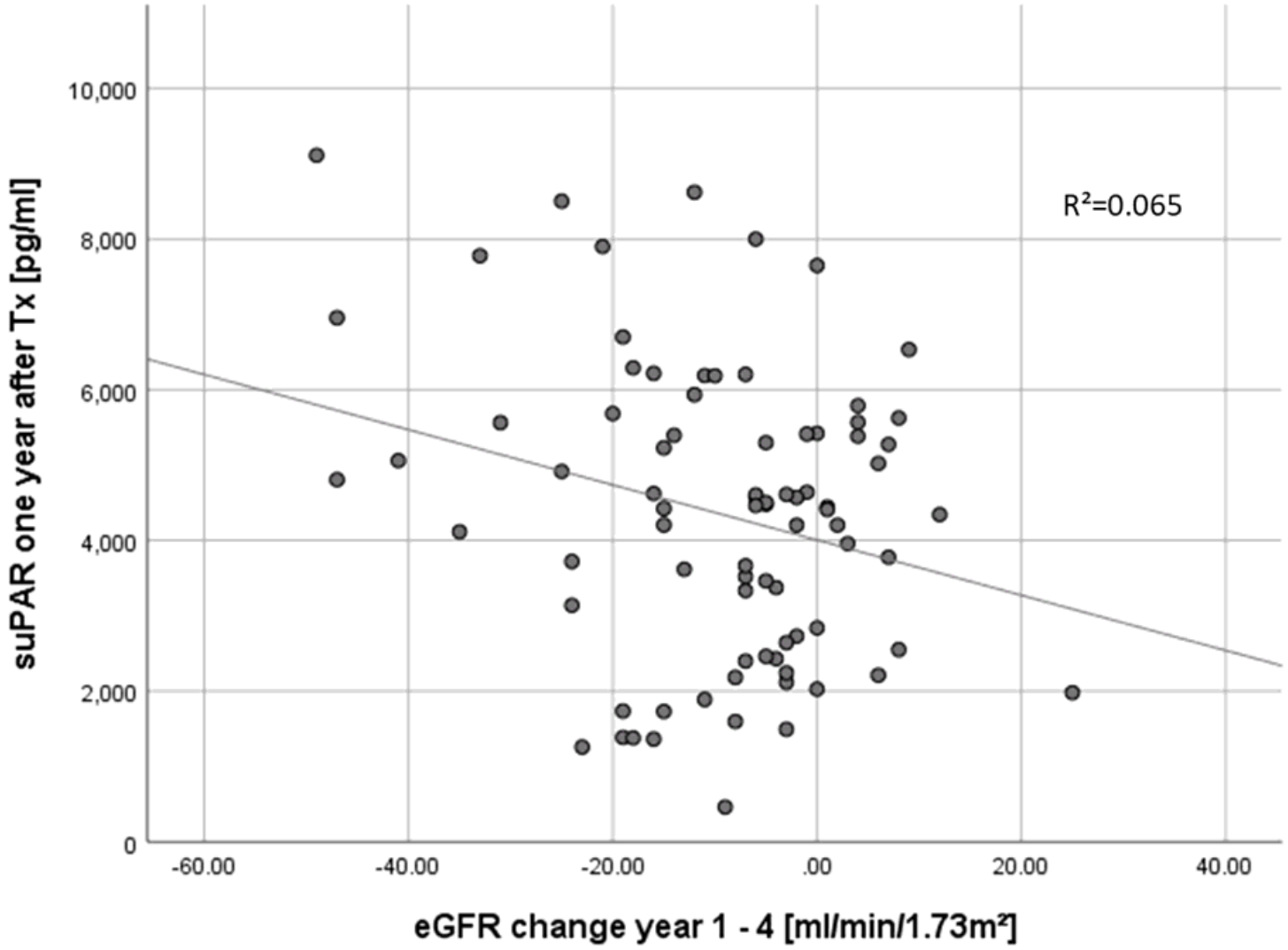


Figure 5

The correlation between the recipients' suPAR levels at day 365 and eGFR decline in the following three years. Higher suPAR-levels preceded a higher eGFR-loss in the following three years after KTx.

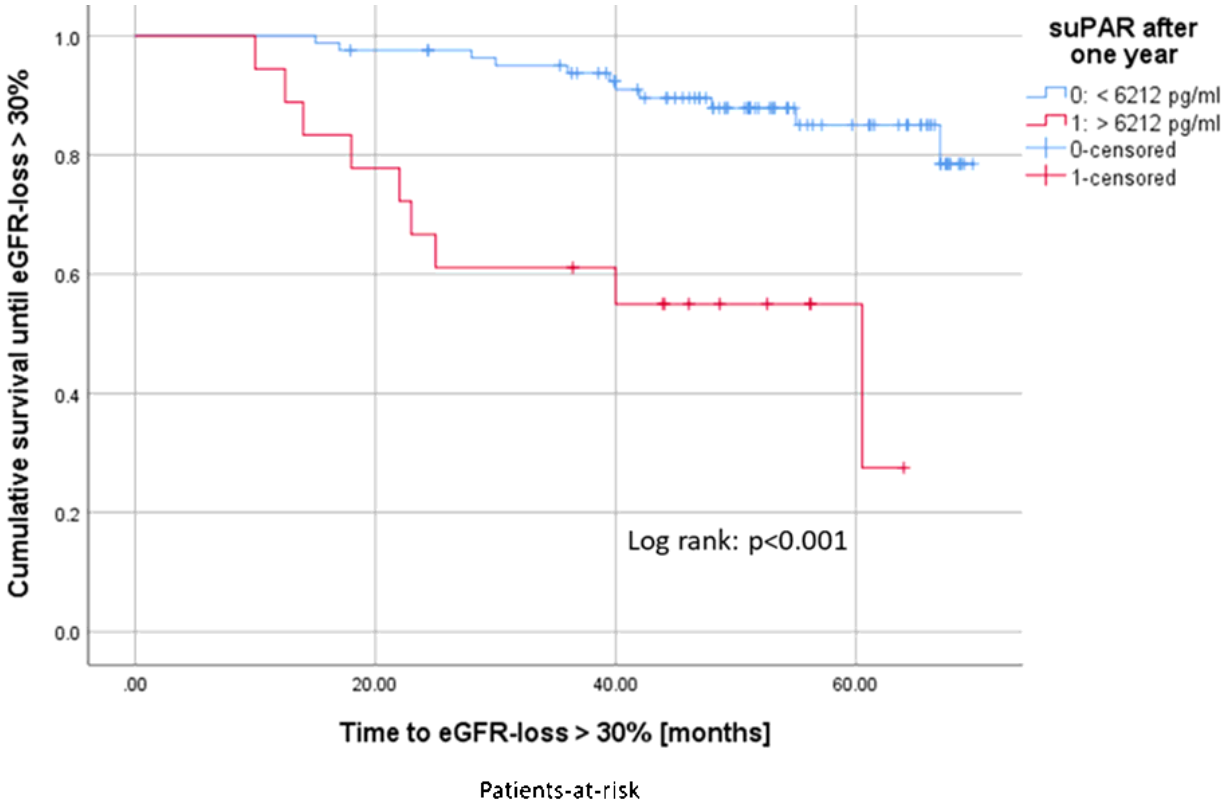
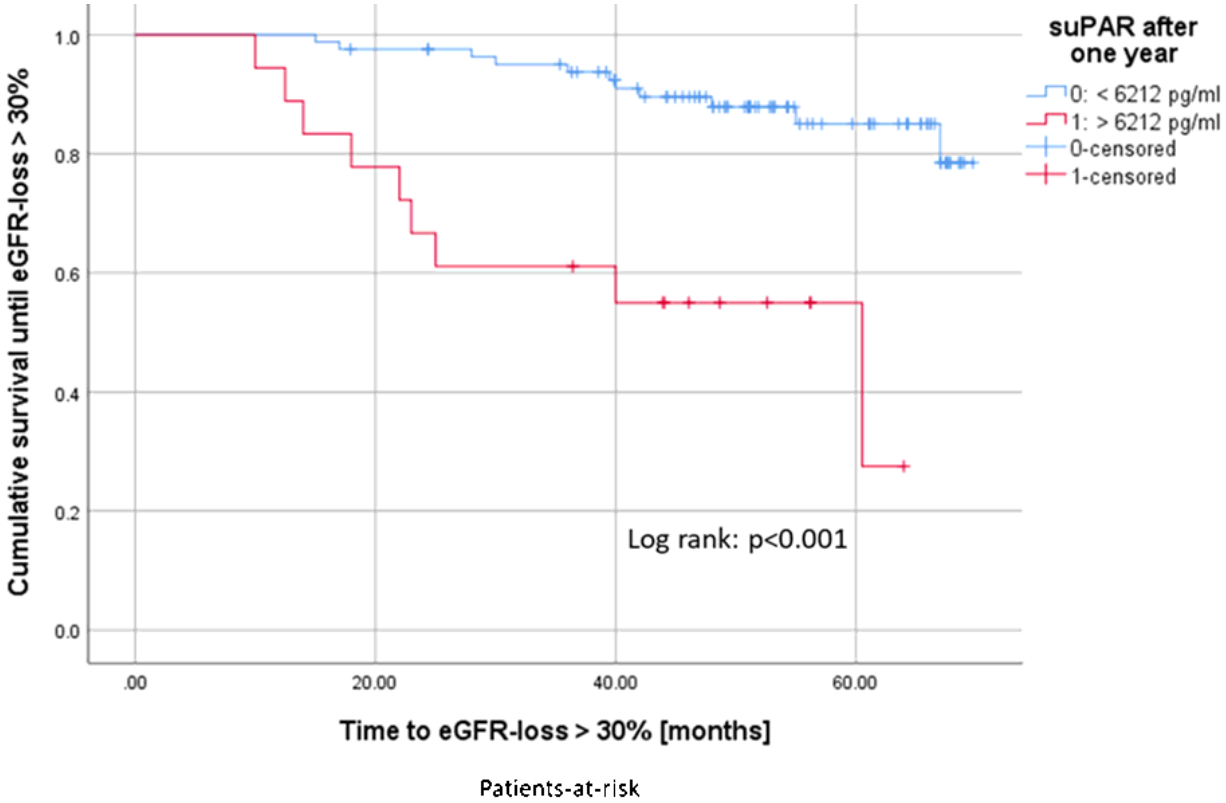


Figure 6

The suPAR-levels above 6,212 pg/ml measured one year after KTx are significantly associated with an increased loss of allograft function.



Patients-at-risk

suPAR < 6212 pg/ml	82	79	66	25
suPAR > 6212 pg/ml	18	14	10	2

Figure 6

The suPAR-levels above 6,212 pg/ml measured one year after KTx are significantly associated with an increased loss of allograft function.