Technische Universität München
TUM School of Medicine and Health

Serum Uromodulin as marker for graft function after kidney transplantation

Helene Sandberger

Vollständiger Abdruck der von der TUM School of Medicine and Health der Technischen Universität München zur Erlangung einer

Doktorin der Medizin (Dr. med.)

genehmigten Dissertation.

Vorsitz: Prof. Dr. Susanne Kossatz

Prüfende der Dissertation:

1. apl. Prof. Dr. Christoph Schmaderer
2. apl. Prof. Dr. Matthias Heck

Die Dissertation wurde am 18.03.2024 bei der Technischen Universität München eingereicht und durch die TUM School of Medicine and Health am 03.07.2024 angenommen.

Table of contents

1. Introduction:

In order to understand the content and relevance of this study, it is important to know some key facts and definitions. These are briefly explained on the subsequent pages.

1.1 Chronic kidney disease (CKD)

"CKD is defined as abnormalities of kidney structure or function, present for >3 months, with implications for health" [1]. These abnormalities include for example an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m^2 body surface area (BSA) or eGFR >60 ml/min/m^2 and albuminuria >30 mg/day and/or urine sediment pathologies [1]. Kidney failure is defined by an eGFR of <15 ml/min/1.73m^2 and/or renal replacement therapy [1, 2].

In the year 2006 in Germany diabetic nephropathy and glomerulonephritis were the most frequent underlying disease of prevalent kidney failure treated with dialysis. Further relevant primary diseases of kidney failure under dialysis therapy in Germany were for example vascular nephropathy and interstitial nephritis [3].

In the year 2016 the mean prevalence of CKD amounted to approximately 11-13% worldwide [4]. The aging of populations and the growing number of patients with diabetes and cardiovascular disease lead to a rising global occurrence of CKD, which results in an enormous economic burden [5, 6]. Therefore, CKD in general and specifically kidney failure (meaning terminal renal insufficiency) represents a crucial medical and economic issue nowadays.

Concerning the prevalence of kidney failure in Germany reliable data is scarce. In 2014 the estimated number of patients suffering from kidney failure (patients needing renal replacement therapy or renal transplantation) amounted to between 50.000 and 83.000 people (approximately 0.05 to 0.1%) of the German population [7].

1.2 Renal replacement therapy

Kidney failure is a severe complication of CKD which can be treated by renal replacement therapy, namely dialysis and kidney transplantation. The main indications for renal replacement therapy are eGFR <7 ml/min, metabolic acidosis (pH <7.2 or Base Excess >-10 mmol/l), hyperkalemia (>6.5 mmol/l) and hyperurikemia (>200 mg/dl). In simplified terms dialysis is a method to eliminate water and substances, which are subjected to urinary excretion, and to counteract acid-base imbalances. [8]

Hemodialysis (HD) and peritoneal dialysis (PD) are common techniques of dialysis. HD is the most frequently used method of dialysis. PD can either be carried out mechanically supported or without mechanical support. The latter is also known as continuous ambulatory peritoneal dialysis (CAPD).

Kidney transplantation is currently the optimal therapy for end-stage renal disease [8-10]. Despite being the preferable therapy, Germany is

registering a downward-trend concerning the frequency of renal transplantation over the last few years. The prevailing shortage of donor organs in Germany is reflected in the remarkable number of patients on the waiting list for kidney transplantation. At the end of the year 2020, 11.903 patients have been on this waiting list. In the same year the total number of kidney transplantations carried out in Germany amounted to 1.909. Thereof 1.459 transplantations were carried out with organs of deceased donors and 450 with organs of living donors. [11]

1.3 Kidney transplantation

The two types of kidney transplantation in clinical practice are, as already briefly mentioned above, transplantation with post-mortal donation and transplantation with living donation [8].

Living donation entails some advantages compared to deceased donation [8]. Living donor kidney transplantation was shown to lead to superior survival of the recipient in comparison to deceased donor kidney transplantation [12]. One further pivotal benefit of living donation is that it results in better rates of graft survival compared to deceased donation [9, 13]. An explanation for the superior graft survival rates might be the lower degree of ischemic injury and the reduced waiting time for transplantation while receiving dialysis, meaning fewer comorbidities such as cardiovascular disease which are associated with the time being on dialysis [14, 15].

In spite of all advantages of living donor transplantation, kidney donation from post-mortal donors is a valuable tool to enlarge the donor pool and an attempt to better meet the growing demand for donor organs. In 1999 Wolfe and his colleagues showed that even deceased donor kidney transplantation considerably improves the long-term outcome of patients with kidney failure compared to receiving dialysis [16].

After kidney transplantation the average life expectancy is higher compared to the one of dialysis patients listed for transplantation (annual death rate 3,8 per 100 patient-years compared to 6,3 per 100 patient-years) [16]. In 2017 the United States recorded an adjusted mortality rate of dialysis patients of 165 per 1000 patient-years [17]. This was significantly higher than the adjusted mortality rate of transplant patients, which amounted to only 29 per 1000 patient-years [17]. Dialysis patients are not only exposed to higher mortality rates compared to transplant patients, but dialysis also implies enormous economic expenditures while transplantation is by far more cost effective [18].

As Germany has to deal with the great discrepancy of kidney transplantation representing the recommended therapy of kidney failure and at the same time having a shortage of donor organs, it seems particularly important to improve the success rate of those transplantations which are actually carried out. This study was conducted to contribute to this improvement.

1.4 Delayed Graft Function (DGF) and Primary Non-Function (PNF)

DGF deserves special consideration since it has significant disadvantageous, harmful influence on patient and graft survival [19]. Unfortunately, there is no uniform definition of DGF [20]. Instead, various different definitions are used in research [21]. According to the most popular definition DGF is described as the need of dialysis within the first seven days after transplantation [21].

PNF is another possible adverse event after renal transplantation. This term is used to describe an insufficient kidney function three months after transplantation with the consequent need for continuous dialysis or retransplantation, respectively a graft that never worked at all after renal transplantation [10, 22].

1.5 Uromodulin (Umod)

DGF is difficult to predict or to detect on the basis of invasive methods, especially at an early stage. Tubular biomarkers could be useful for this purpose since ischemic reperfusion injury leading to tubular dysfunction, is a key factor in the pathophysiology of DGF [23].

At present the standard tool for assessing kidney function post-transplant is the measurement of serum creatinine level, despite it can be biased by factors such as age, sex, diet and muscle mass and also primarily reflects glomerular function instead of kidney function as whole [24]. Thus, significant effort is dedicated to research on alternative or additional possible biomarkers for monitoring and predicting renal function and DGF in the post-transplant phase.

A considerable biomarker candidate for this endeavor is neutrophil gelatinase-associated lipocalin (NGAL). NGAL obtained from urine and plasma is promising for the assessment of DGF-risk post-transplant. However, it has significant drawbacks such as its poor specificity. Besides up to date we are lacking a conclusive and reliable cut-off. Furthermore, most studies on NGAL in this context include only a low number of patients, reducing its statistical power [25, 26].

Besides NGAL also Interleukin 18 (IL-18) is proposed as non-invasive biomarker of DGF respectively graft function post-transplant in general. As well as NGAL, IL-18 is not specific for a certain type of kidney damage. Since NGAL and IL-18 are also synthesized by other cells than kidney-specific cells, for example immune cells, conditions such as urinary tract infections and systemic inflammatory processes like sepsis may influence urinary NGAL- and IL-18-levels. [27-30]

Furthermore, donor plasma mitochondrial DNA (mtDNA) has shown to be a promising predictor of DGF and for allograft function 6 months after kidney transplantation. As well as serum Uromodulin (sUmod) it is a non-invasive marker and is easily detected. Nevertheless, there are some limitations, which should be considered when using donor plasma mtDNA

as a biomarker for DGF and for graft function post-transplant in general. [31]
Other studies have proven that systemic mtDNA levels are influenced by the experience of trauma before the measurement [32]. Especially cadaveric donors might have experienced significant trauma before donation of the graft, which could distort the association between mtDNA levels and DGF-risk/-occurrence [32]. Also, we are lacking large, multicenter cohort studies investigating the association between donor plasma mtDNA and DGF [31].
Besides the above mentioned, there are many other potential biomarkers for predicting and/or monitoring allograft function and DGF after kidney transplantation. However, an ideal biomarker for this purpose with ideal characteristics has not yet been found. Umod is of great interest in this context. The reason for this, as well as some background information about Umod are mentioned in the subsequent sections.
Umod is an approximately 85-kDa glycoprotein produced by the epithelial cells of the thick ascending limb (TAL) of the loop of Henle and of the early distal tubule [33-36]. It is exclusively expressed in renal tissue [37-39]. Besides, it is the most abundantly occurring protein in human urine and the principal component of hyaline casts [38, 40]. This protein is composed of a high carbohydrate ratio (approximately 30%) and a high amount of cysteine residues [34, 41]. Due to its chemical properties Umod is prone to aggregate to large clusters [34, 41].
In 1950 Igor Tamm and Frank Horsfall were the first to describe Umod in urine of healthy test persons and called it “Tamm-Horsfall protein” [42]. Tamm and Horsfall demonstrated that this protein exhibits strong inhibitory potential concerning viral hemagglutination and precipitates in sodium-chloride solutions [42]. 35 years later Muchmore and Decker isolated a protein from the urine of pregnant women, which they termed Uromodulin [34]. Interestingly Pennica et al.’s study in 1987 revealed that Umod is identical to the earlier discovered Tamm-Horsfall protein [43].
Umod is thought to have multiple different functions. Among these is the defense against urinary tract infections by inhibiting the attachment of uropathogenic type I fimbriated E.coli to and the uptake by the urothelium [44].
Furthermore, Umod is involved in water and electrolyte homeostasis [45-47]. It seems to be an influencing factor concerning the water impermeability of the TAL of the loop of Henle [48, 49]. This implies a considerable impact on the ability to concentrate urine.
Umod also modulates the activity of the NKCC2 co-transporter (sodium potassium chloride co-transporter) and the ROMK channel (renal outer medullary potassium channel) in tubular cells [46, 47]. As the renal sodium chloride and water reabsorption is of considerable relevance for the regulation of blood pressure, the mentioned findings point out Umod’s

significance in the regulation of systemic phenomena such as blood pressure regulation.
When speaking of Umod's versatile properties its immunomodulatory characteristics should also be considered. Next to its already stated in vitro inhibition of viral hemagglutination, it binds to various different cytokines and thereby influences their activity [42, 50].
Besides acting as an inhibitor of inflammatory processes, this protein also exhibits pro-inflammatory properties. It can trigger an inflammatory reaction of the innate immune system. Via a TLR4-dependent (Toll-like-receptor-4-dependent) mechanism Umod induces the production and secretion of cytokines such as TNF-α (tumor necrosis factor alpha). Moreover, it stimulates the maturation of dendritic cells, which are antigen-presenting cells and hence are essential players of the innate immune system. [51]
El-Achkar, Micanovic and their colleagues have published some notable papers on Umod's role in ischemic kidney injury. A lack in Umod has shown to cause systemic neurtophilia via an Interleukin 23/Interleukin 17 (IL-23/IL-17) pathway [52]. Also, some results suggest that Umod could be a major inhibitory regulator of systemic oxidative stress [53]. Furthermore, there are substantial hints that Umod is involved in the regulation of mononuclear phagocyte function and number in the kidney [54]. In the same study, which revealed the lastly mentioned results, the administration of exogenic Umod could ameliorate the course of kidney injury [54]. The authors suggested that Umod might be a sort of "renal stress hormone" [54]. Moreover, El-Achkar et al. stated that Umod generally has a protective function in ischemic kidney injury [55]. They demonstrated that animals with Umod deficiency show enhanced inflammation, necrosis as well as an inferior renal function [55]. The expression of Umod after acute kidney injury follows biphasic dynamics [56]. At the maximum point of injury Umod seems to be down-regulated, whereas its expression increases 48 hours after ischemic reperfusion injury [56]. These mentioned findings by El-Achkar, Micanovic and their colleagues are especially relevant for the research in the field of renal transplantation. This becomes particularly clear when considering that acute kidney injury respectively ischemic reperfusion injury is an important phenomenon in the post-transplant phase.
Considering the immunological processes taking place after renal transplantation and their involvement in adverse events such as DGF or PNF, it is especially interesting to investigate Umod in that context within this study.

1.6 Motivation for this study

Long-term organ survival after transplantation is critical for both, the individual patient and the healthcare system as a whole (regarding e.g. economic expenses). Factors influencing long-term graft survival are

among others donor gender and donor age, recipient gender and recipient age, occurrence of acute rejection and DGF after transplantation [57-59]. DGF is induced by factors with different degrees of severity and can be regarded as a pronounced form of acute kidney injury (AKI) [60]. The degree of recovery from AKI is dependent on the extent respectively the severity of injury [61]. Incomplete recovery from DGF is associated with lower rates of long-term death-censored graft survival [60].
In order to determine the grade of injury respectively the extent of acute tubular injury (ATI) a biopsy with histological evaluation is required. Biopsy is invasive and therefore inherently implicates various possible complications. Currently the indication for biopsy after renal transplantation is based on clinical features such as oliguria. However, thereby subclinical forms of ATI are overseen which is worrying when considering that ATI is associated with inferior long-term graft outcomes after kidney transplantation [62]. A reliable non-invasive biomarker is needed to detect DGF at an early stage allowing timely therapeutic intervention when chances for complete recovery (and therefore avoidance of graft loss) are still given. DGF is mainly the clinical manifestation of ischemia reperfusion injury with consequent acute tubular necrosis (ATN) [63]. In this context Uromodulin is interesting as it is a tubular marker and because it exerts immunomodulatory effects in the setting of ischemia reperfusion [55]. Also, Uromodulin is uniquely synthesized by renal cells which facilitates the interpretation of changes in sUmod levels [37-39].
As assessment of Uromodulin in urine has inherent limitations such as chemical instability and stability in serum samples was proven to be reasonable, this study investigated Umod as a serum biomarker [64, 65].

1.7 Aims and hypotheses

To further comprehend the value of sUmod regarding the topics referred to above, the following aims and hypotheses have evolved for this study. The primary aim of this study is to evaluate the association between sUmod after renal transplantation and DGF in renal transplant recipients:

- **Aim 1**: Evaluation of the association of sUmod post-transplant with DGF
  1. Hypothesis 1: sUmod levels are significantly associated with post-transplant DGF-rates.

Secondary aims of this study are to evaluate the association between sUmod post-transplant and ATI of the graft at baseline, the association between sUmod post-transplant and long-term graft function as well as the association between sUmod pre-transplant, sUmod post-transplant and DGF, and various demographic and laboratory parameter:

- **Aim 2**: Evaluation of the association of sUmod post-transplant with ATI at the time of transplantation
  2. Hypothesis 2: The increase in sUmod levels post-transplant is significantly associated with rates of severe graft ATI at the time of transplantation.
- **Aim 3**: Evaluation of the association of sUmod levels 1-3 months (30-120 days) post-transplant with long-term graft function
  3. Hypothesis 3: Lower sUmod levels 1-3 months post-transplant are significantly associated with worse graft function 1 year post-transplant.
- **Aim 4**: Evaluation of the association of sUmod levels pre-transplant, 1st day post-transplant, 1-3 months post-transplant and sUmod ratio x/0 (x = 1st day post-transplant or 1-3 months post-transplant, 0 = pre-transplant) with demographic and laboratory parameters in transplant patients
  4. Hypothesis 4: Early post-transplant changes of serum Uromodulin are significantly associated with different demographic and laboratory parameters in transplant patients

2. Patients and Methods:

2.1 Patients and study design

To address the aims and hypotheses descried above, we conducted a single-center retrospective, observational cohort study. The cohort consisted of 186 patients undergoing renal or simultaneous renal-pancreas-transplantation between the years 2007 and 2017 at “Klinikum Rechts der Isar”, University Hospital of the Technical University of Munich (Germany). Inclusion criteria were a written informed consent and the age of over 18 years at the time of transplantation. Exclusion criteria were different for each individual analysis.

For aim one, patients were excluded if information on the incidence of DGF/PNF is missing and/or no sUmod measurements on post-transplant day 1 and 2 are available.

For aim two, patients were excluded if no information on sUmod measurements pre-transplant is available and/or no sUmod measurements on post-transplant day 1 and 2 are available and/or no donor kidney biopsy was performed or no histological evaluation of the donor kidney at the time of transplantation is available.

For aim three, patients were excluded if the sUmod measurement pre-transplant is missing and/or no sUmod measurement 1-3 months post-transplant is available and/or no eGFR measurement 1 year post-transplant is available.

For aim four, patients were excluded in the case of missing information on three or more sUmod measurements from post-transplant day 1 to day 30 and/or missing information on creatinine measurements at the time when sUmod measurements are available.

2.2 Variables

The data was collected from electronic and paper chart review as well as Eurotransplant database. The endpoint of data acquisition was the 20th of February 2018.

2.2.1 Independent predictor variable

Serum samples for the Umod measurements had been taken at definite time points. Shortly afterwards they had been centrifuged, aliquoted and frozen at -80°C until they had been analyzed. The ideal time points have been directly pre-transplant (meaning the day before transplantation or the actual day of transplantation), the first day post-transplant, 30 days post-transplant and 90 days post-transplant. If it had not been possible to take samples at these exact time points, the samples had been taken at the nearest possible time point.

sUmod measurements were carried out with a commercially obtainable assay (Euroimmun AG, Lübeck, Germany). According to the ELISA's short performance characteristics stated by the producer: the detection limit for plasma/serum samples is 2ng/ml; mean linearity recovery 97% (83% to 107% at 59-379ng/ml); intraassay precision 1.8-3.2% (at 30-214ng/ml); intraassay precision 6.6% to 7.8% (at 35-228ng/ml); and interlot precision 7,2%-10.1% (at 37-227ng/ml). 1:101 dilution was achieved with a dilution buffer. Afterwards 100µl of controls, calibrators or diluted samples were pipetted into coated wells of the microtiter plate. After this an amount of 100µl of biotinylated antibodies for detection was added. The concentration ultimately amounted to 50ng/ml. After covering the microtiter plate with a foil 2-hour incubation (450 rotations/minute) at room temperature followed. Next washing of the microtiter plate using 300µl of washing buffer done. This step was repeated three times. Having added 100µl of steptavidin-polyperoxidase (SPO) to each well (final concentration of 67ng/ml) further incubation over half an hour (again 450 rotations/minute) followed. After this again 3 times of washing with 300µl of washing buffer was carried out. Subsequently, 100µl of the substrate solution (comprised of the chromogen tetramethylbenzidin and hydrogen peroxide as substrate for SPO) was added to each well. Next 15-minute-incubation of the MTP in the dark at room temperature followed. After adding 100µl of stop solution color change from blue to yellow indicated termination of the reaction. Ultimately photometric measurement of the substrate solution at a wavelength of 450nm (reference 620nm) was performed. [66]

2.2.2 Outcomes/Dependent variables

DGF is the dependent variable of this study's primary aim. As already mentioned in the introduction above there is no uniform definition in research or clinical practice up to date [21]. For this study the most frequent definition was used, namely the need of dialysis within the first seven days post-transplant [21].
A further dependent variable of this study's analyses is severe ATI. This was evaluated in histological samples. In 105 cases a usable biopsy of the donor kidney had been obtained at the time of transplantation (so-called zero-time biopsy). External specialists of a working group in Erlangen (Germany) had performed the histological evaluation of these biopsy samples. They had assessed them according to a standardized protocol including the parameters acute tubular necrosis (ATN), interstitial fibrosis (IF), tubular atrophy (TA), glomerulosclerosis and arteriosclerosis. Severe ATI is defined as ATN in >75% of tubuli in the sample. These experts had also decided whether a biopsy was usable for histological evaluation or

not (i.e. if a sufficient amount of relevant tissue was available or not).
The dependent variable of this study's third aim is long-term graft function. As up to date the standard parameter of kidney function respectively graft function is still eGFR, this outcome was evaluated by using eGFR 1 year post-transplant. eGFR was estimated by the standard CKD-EPI-formula.

2.2.3 Co-variables

Table 1: Overview of parameters used in this study

Parameters	Description	Unit/Categories
Age	-	years
Gender recipient	biological gender of recipient	female or male
Diabetes recipient	diagnosis of diabetes mellitus pre-Tx according to WHO-criteria	diabetes mellitus type I, diabetes mellitus type II or no diabetes mellitus prevalent
Hypertension (HT) recipient	diagnosis of arterial hypertension pre-Tx according to WHO-criteria	arterial hypertension prevalent or no arterial hypertension prevalent
Coronary heart disease (CHD) recipient	diagnosis of coronary heart disease pre-Tx according to WHO-criteria	coronary heart disease prevalent or no coronary heart disease prevalent
Cardiovascular disease (CVD) recipient	diagnosis of cardiovascular disease pre-Tx according to WHO-criteria (coronary heart disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, deep vein thrombosis and pulmonary embolism)	cardiovascular disease prevalent or no cardiovascular disease prevalent
Underlying disease	disease that lead to the need of kidney transplantation	19 defined categories
Nephrectomy	nephrectomy pre-Tx	yes or no
Dialysis pre-transplant	dialysis therapy pf recipient pre-Tx	non, HD or PD
Dialysis vintage	total duration of dialysis the recipient received pre-Tx	days
Recipient death	-	dead or still alive at study endpoint
Type of transplantation	-	living or deceased donor transplantation
Number of transplant	count of current transplantation	natural numbers
ABO-compatibility transplantation	compatibility of recipient and donor according to ABO-blood groups	compatible or incompatible transplantation
Cold ischemic time (CIT)	time between complete cooling of organ after harvesting and implantation in recipient (start of anastomosis)	min
Warm ischemic time (WIT)	time between clipping of renal artery and complete cooling of organ during harvesting	min
Panel-reactive antibodies (PRA)	assessed pre-Tx	0-100%
Mismatch	Number of mismatching HLAs	natural numbers
Residual diuresis (RD)	last residual diuresis of recipient pre-Tx	ml

Table 1: Overview of parameters used in this study

Parameters	Description	Unit/Categories
Primary non-function (PNF)	never-working transplant and consequent need of continuous dialysis post-Tx	PNF or no PNF
Delayed graft function (DGF)	need of dialysis (HD, PD, HF or HDF) within first week post-Tx (incl. post-transplant day 0), independent of reason for dialysis	DGF or non-DGF
Donor kidney biopsy at time of transplant	useful biopsy of donor kidney (graft) obtained at time of transplantation	obtained or not obtained
Acute tubular necrosis (ATN)	quantified by standardized ATN-score; assessed in biopsy samples, which were taken 10min after reperfusion (at time of transplantation)	relative number of tubuli affected: non/minimally, <25%, 25–50%, 50–75% or >75%
ATN 50	quantified by standardized ATN-score; acute atrophy of tubules in ≥50% of tubules in biopsy, which was taken 10min after reperfusion (at time of transplantation)	yes or no
Interstitial fibrosis (IF)	standardized histological assessment criteria; assessed in biopsy samples, which were taken 10min after reperfusion (at time of transplantation)	5%-steps
Tubular atrophy (TA)	standardized histological assessment criteria; assessed in biopsy samples, which were taken 10min after reperfusion (at time of transplantation)	5%-steps
Glomeruli count	in biopsy samples, which were taken 10min after reperfusion (at time of transplantation)	natural numbers
Glomerulosclerosis	standardized histological assessment criteria; assessed in biopsy samples, which were taken 10min after reperfusion (at time of transplantation)	natural numbers or %
Arteriosclerosis	standardized histological assessment criteria; assessed in biopsy samples, which were taken 10min after reperfusion (at time of transplantation)	BANFF classification
Umod	quantitative Umod measured in serum samples	ng/ml
Umod_0	Umod pre-Tx (at time 0), measured in serum samples	ng/ml
Umod x/0	ratio of Umod at time x and sUmod at time 0, measured in serum samples	ng/ml
Creatinine	measured in serum samples	mg/dl
Blood-urea-nitrogen (BUN)	measured in serum samples	mg/dl
eGFR (CKD-EPI)	estimated Glomerular Filtration Rate (Chronic Kidney Disease Epidemiology Collaboration)	ml/min/1.73m^2

Table 1: Overview of parameters used in this study

Parameters	Description	Unit/Categories
Leucocyte count	measured in serum samples	count/µl
Sodium	measured in serum samples	mmol/l
Potassium	measured in serum samples	mmol/l
Phosphate	measured in serum samples	mg/dl
Protein	measured in serum samples	g/dl
C-reactive protein (CRP)	measured in serum samples	mg/dl
Hemoglobin	measured in serum samples	g/dl
Proteinuria	measured in urine samples	mg/g creatinine
Serum Protein	measured in serum samples	g/dl
Use of calcineurin inhibitors	use of calcineurin inhibitor medication by recipient post-Tx	Cyclosporine, Tacrolimus or no use of calcineurin inhibitor
Use of mycofenolat mofetile (MMF)	use of MMF medication by recipient post-Tx	yes, no or not registered
MPA	level of mycofenolic acid	g/day
Use of steroids	use of steroid medication by recipient post-Tx	yes or no
Donor age	-	years
Donor gender	biological gender of donor	female or male
Donor BMI	Body-Mass-Index of donor	kg/m^2
Diabetes donor	diagnosis of diabetes mellitus pre-nephrectomy according to WHO-criteria	diabetes mellitus type I, diabetes mellitus type II or no diabetes mellitus prevalent
Hypertension (HT) donor	diagnosis of arterial hypertension pre-nephrectomy according to WHO-criteria	arterial hypertension prevalent, no arterial hypertension prevalent or unknown
Smoking status donor	smoking status of donor pre-nephrectomy according to anamnesis	smoker, non-smoker or unknown
Last serum creatinine donor	last registered serum creatinine level of donor pre-nephrectomy	mg/dl

Table 1: Overview of parameters used in this study. WHO, World Health Organization; pre-Tx, pre-transplant; post-Tx, post-transplant; HD, hemodialysis; PD, peritoneal dialysis; HF, hemoflitration; HDF, hemodiafiltration; HLAs, human leucocyte antigens; ml, milliliter; dl, deciliters; µl, microliters; ng, nanogram; mg, milligrams; kg, kilograms, m^2, square meters; mmol, millimoles; %, percent.

2.3 Statistical analysis

2.3.1 General remarks

Results were considered to be significant if their p-value was <0.05 or if their 95%-confidence interval did not include the value 1 for odds ratio respectively the value 0 for regression coefficients. Adjustment for multiple testing was not done. The statistical analysis was carried out with the data processing program ‘SPSS’ (SPSS Inc., Stanford, USA) and ‘R’ (Gentleman and Ihaka, Auckland, New Zealand). Visual depictions presenting the data (i.e. graphs, figures, tables etc.) were created with the programs ‘apple pages’ (Apple Inc., California, USA) and ‘R’ (Gentleman and Ihaka, Auckland, New Zealand). For data acquisition and storage ‘Excel’ (Microsoft, Redmond, USA) was used.

2.3.2 Statistical application and details

For the descriptive analysis (Aim 4) stratification by the variable DGF (DGF vs. non-DGF) or according to quartiles of sUmod levels (pre-transplant, post-transplant day 1 or post-transplant month 1–3) respectively of their ratio at definite time points (sUmod x/0; x = post-transplant day 1 or post-transplant month 1–3; 0 = pre-transplant) was done. Categorical variables are presented in counts and percentages, continuous variables in median and interquartile range due to small sample size and questionable normal distribution.

For all other analyses sUmod was not only used as an absolute value but also as sUmod ratio composed of the sUmod level at a certain time post-transplant and the pre-transplant level. The rationale for this is that there is always a residual synthesis of sUmod in the native kidney, but this study is interested in the graft function (respectively the tubular function of the graft). By using the ratio between post-transplant sUmod and pre-transplant sUmod levels the sole graft function can better be analyzed than by using only an absolute post-transplant value.

To assess the association of sUmod with DGF as the binary outcome uni- and multivariable logistic regression analyses were performed. In nested models sUmod was entered as a continuous variable and categorized into quartiles. sUmod levels were used as an absolute value on post-translant day 1 and once as sUmod ratio x/0 (x = post-transplant day 1; 0 = pre-transplant). In case of violation of logistic regression assumptions, sUmod values were transformed into a natural logarithmic scale. Co-variable selection

was based on clinical expertise and published data [19, 20, 67]. The following nested models were applied:
M0: sUmod
M1: M0 + demographic parameters (recipient age, recipient sex, recipient BMI and prevalence of diabetes in recipient)
M2: M1 + eGFR (CKD-EPI)
M3: M2 + transplant-associated parameters (cold ischemic time and living transplantation)

To evaluate sUmod's value as predicting factor for DGF a Receiver-Operator-Characteristic (ROC) curve analysis was performed. As already briefly mentioned before, in one model the absolute sUmod value on post-transplant day 1 was used. In a second model the sUmod ratio x/0 (x = post-transplant day 1; 0 = pre-transplant) was used. For both of these two analyses three different ROC curves were created: One univariable ROC curve with sUmod respectively the sUmod ratio x/0 as a predictor, a second ROC curve with only the covariables as predictors (excluding sUmod) and a third ROC curve with the covariables and additionally sUmod respectively additionally the sUmod ratio x/0 as a predictor for DGF. The covariables used for these ROC curves included recipient age, recipient sex, recipient BMI, the prevalence of diabetes mellitus in the recipient as well as the eGFR-change from pre-transplant to post-transplant day 1.
For the evaluation of the association of sUmod with histological features of the donor kidney, uni- and multivariable logistic regression analysis and ROC-analysis were performed. sUmod was entered as the predictor variable both as continuous and ordinal scale. sUmod was used as an absolute value on post-transplant day 1 and also as the sUmod ratio x/0 (x = post-transplant day 1; 0 = pre-transplant). In case of violation of logistic regression assumptions, sUmod were transformed into a natural logarithmic scale. Again, the most important potential confounding factors were selected for adjustment according based in clinical expertise and published data [23, 68-71]. Thus, the following nested models were applied:
M0: sUmod
M1: M0 + demographic parameters of donor (donor age, donor sex, prevalence of diabetes mellitus in donor and last serum creatinine level of donor) and transplant-related parameters (cold ischemic time and living donor transplantation)

For the assessment of sUmod's value as a predicting factor for acute histological changes in the donor kidney at the time of transplantation, a ROC curve analysis was performed. In one model

the absolute sUmod value on post-transplant day 1 was used. In a second model the sUmod ratio x/0 (x = post-transplant day 1; 0 = pre-transplant) was entered. For the basic ROC curve sUmod respectively the sUmod ratio x/0 was used as a single predictor. For a further ROC curve only the covariables (excluding sUmod respectively the sUmod ratio) were used as a predictor. Lastly, a ROC curve depicting the covariables and additionally sUmod respectively the sUmod ratio x/0 as predictors for acute histological changes in the donor kidney at the time of transplantation, was created. The covariables mentioned include the parameters donor age, donor sex, prevalence of diabetes mellitus in the donor, last pre-transplant serum creatinine level of the donor, cold ischemic time, type of transplantation as well as the absolute eGFR-change from pre-transplant to post-transplant day 1.

For the evaluation of the association of sUmod with long-term graft function regression modeling was performed as a linear regression analysis. sUmod represented the principal continuous predictor in the model. In a further model sUmod was entered as an ordinal variable categorized into quartiles. sUmod was either entered as an absolute value 1–3 months post-transplant or again as sUmod ratio x/0, with 0 = pre-transplant, but this time x = 1–3 months post-transplant. In case of violation of linear regression assumptions, sUmod values were transformed into a natural logarithmic scale. Once more the most important potential confounding factors were selected for adjustment based on clinical expertise and published data [72-75]. Consequently, the following nested models were applied:

M0: sUmod

M1: M0 + demographic parameters (recipient age, recipient sex, recipient BMI and prevalence of diabetes in the donor)

M2: M1 + eGFR (CKD-EPI)

M3: M2 + transplant-related parameters (living donor transplantation, cold ischemic time, dialysis vintage and DGF)

3. Results:

3.1 Descriptive data

3.1.1 Overall cohort

The total cohort consisted of 186 patients. Of these, 123 (66.1%) patients received an organ from a deceased donor and 63 (33.9%) patients from a living donor. The total population was composed of 124 (66.7%) male persons and 62 (33.3%) female persons. All patients included were Caucasians. Median recipient age was 54 years [43.00, 62.75]. 23 recipients (12.4%) have had nephrectomy before the examined transplantation. Median recipient BMI was 25.18 kg/m^2 [22.27, 28.72]. 38 recipients (20.4%) had suffered from diabetes mellitus pre-transplant. 147 recipients (79%) had suffered from arterial hypertension, 35 recipients (18.8%) from coronary heart disease and 72 recipients (38.7%) from cardiovascular disease pre-transplant. 147 recipients (79%) have had hemodialysis, 20 recipients (10.8%) peritoneal dialysis and 19 recipients (10.2%) no dialysis at all pre-transplant. Median dialysis vintage of the recipients was 1712 days [711.00, 2719.50]. Median cold ischemic time was 420 min [120.00, 780.00] and median warm ischemic time 20 min [20.00, 30.00]. Median mismatch was 4 HLAs [2.00, 5.00]. Median recipient residual diuresis was 500 ml [0.00, 1500.00]. 8 recipients (4.3%) experienced PNF, while 67 recipients (36.0%) experienced DGF. 45 biopsy samples (77.6%) taken at the time of transplantation showed ≥ 50% acute tubular atrophy. Interstitial fibrosis was not at all observed in the biopsy samples taken at the time of transplantation. Median recipient sUmod pre-transplant was 7.13 ng/ml [3.11, 14.12]. Median recipient pre-transplant serum creatinine was 6.95 mg/dl [5.70, 9.10], BUN was 45.00 mg/dl [33.00, 58.00] and potassium was 5.30 mmol/l [4.70, 5.70]. Median recipient pre-transplant eGFR (CKD-EPI) was 7.26 ml/min/1.73m^2 [5.26, 9.52]. Median recipient pre-transplant leucocyte count was 7.17/µ [5.89, 8.60], median hemoglobin was 12.10 g/dl [11.20, 13.28], median sodium level was 139.00 mmol/l [137.00, 141.00], median serum protein was 7.20 g/dl [6.60, 7.60], median CRP was 0.30 mg/dl [0.00, 0.60] and median blood pH was 4.90 [4.20, 6.05]. Referring to immunosuppressive medication, 145 recipients (78.4%) received Tacrolimus and 40 recipients (21.5%) received Cyclosporin A. Furthermore 182 recipients (98.4%) received Mycophenolate Mofetil (MMF), 2 patients did not receive MMF and for a single patient MMF intake was unknown. Only one recipient (0.5%) received steroids. Median recipient proteinuria 3 months post-transplant was 169.00 mg/g creatinine, 6 months post-transplant it was 146.50 mg/g creatinine [96.00, 263.25] and 12

months post-transplant it was 127.00 mg/g creatinine [100.75, 265.00]. Median recipient eGFR (CKD-EPI) 3 months post-transplant was 43.80 ml/min/1.73m^2 [27.70, 55.00], 6 months post-transplant it was 43.00 ml/min/1.73m^2 [31.00, 55.00] and 12 months post-transplant it was 49.60 ml/min/1.73m^2 [31.98, 62.85].
88 donors (47.3%) were female. The median donor age was 52 years [43.00, 63.00]. Median donor BMI was 26 kg/m^2 [23.00, 28.75]. 39 donors (21%) suffered from diabetes mellitus type 2, 13 donors (7%) from diabetes mellitus type 1 and 134 donors (72%) did not suffer from diabetes mellitus at all. 70 donors (37.6%) suffered from arterial hypertension, 94 donors (50.5%) did not suffer from arterial hypertension and for 22 donors (11.8%) it was unknown if they suffered from arterial hypertension. 53 donors (28%) were smokers, 83 donors (44.6%) were non-smokers and for 51 donors (27.4%) the smoking status was unknown. Median last pre-transplant donor serum creatinine was 0.90 mg/dl [0.70, 1.10].

3.1.2 sUmod pre-transplant

The three most prominent significant associations when stratification of descriptive data by the sUmod level pre-transplant was done are briefly mentioned here. All details and further associations with the corresponding p-values are found in Table 2 below.
In this analysis a significant association between the sUmod level pre-transplant and living donor transplantation type was found. Most recipients are found in sUmod quartile 4, while least are found in sUmod quartile 1.
Furthermore, a significant association between dialysis pre-transplant and the sUmod level pre-transplant was found. Most recipients who did not at all receive pre-transplant dialysis are found in sUmod quartile 4, while least are found in sUmod quartile 1 and 2. Also the type of dialysis (HD or PD) was significantly associated with pre-transplant sUmod.
Moreover, a significant association between DGF and the pre-transplant sUmod level was found. Most recipients who experienced DGF post-transplant are found in sUmod quartile 1, while least are found in quartile 3 and 4.

Table 2: Stratification by sUmod pre-transplant

Characteristics	Total	sUmod quartile 1	sUmod quartile 2	sUmod quartile 3	sUmod quartile 4	p-value
n	184	46	46	46	46	
Living donation	62 (33.7)	6 (13.0)	11 (23.9)	20 (43.5)	25 (54.3)	**<0.001**
Female gender donor	88 (47.8)	18 (39.1)	23 (50.0)	22 (47.8)	25 (54.3)	0.519
Age donor	52.00 [43.00, 63.00]	54.00 [41.50, 60.75]	55.50 [44.00, 63.00]	54.00 [44.00, 63.00]	51.00 [40.25, 62.75]	0.783
BMI donor	26.00 [23.00, 28.75]	26.50 [23.25, 28.00]	25.00 [23.00, 28.75]	25.50 [23.00, 28.75]	26.00 [24.00, 28.00]	0.918
Diabetes donor						0.093
No	132 (71.7)	32 (69.6)	36 (78.3)	26 (56.5)	38 (82.6)	
Yes	13 (7.0)	4 (8.7)	4 (8.7)	4 (8.7)	1 (2.2)	
Unknown	39 (21.1)	10 (21.7)	6 (13.0)	16 (34.8)	7 (15.2)	
HT donor						0.698
No	93 (50.5)	25 (54.3)	25 (54.3)	18 (39.1)	25 (54.3)	
Yes	69 (37.5)	16 (34.8)	17 (37.0)	20 (43.5)	16 (34.8)	
Unknown	22 (12.0)	5 (10.9)	4 (8.7)	8 (17.4)	5 (10.9)	
Smoking status donor						**0.024**
No	83 (45.1)	28 (60.9)	21 (45.7)	17 (37.0)	17 (37.0)	
Yes	50 (27.2)	12 (26.1)	16 (34.8)	12 (26.1)	10 (21.7)	
Unknown	51 (27.7)	6 (13.0)	9 (19.6)	17 (37.0)	19 (41.3)	
Last serum creatinine donor	0.90 [0.70, 1.10]	0.80 [0.70, 1.10]	0.80 [0.70, 1.10]	0.90 [0.70, 1.00]	0.90 [0.70, 1.00]	0.954
Female gender recipient	62 (33.7)	17 (37.0)	15 (32.6)	14 (30.4)	16 (34.8)	0.922
Age recipient	54.00 [43.00, 62.75]	54.00 [41.50, 60.75]	55.50 [44.00, 61.50]	54.00 [44.00, 63.00]	51.00 [40.25, 62.75]	0.783
Nephrectomy	23 (12.5)	14 (30.4)	3 (6.5)	2 (4.3)	4 (8.7)	**<0.001**
BMI recipient	25.18 [22.72, 28.72]	24.61 [22.58, 28.69]	24.74 [22.99, 28.94]	25.86 [23.32, 28.65]	24.47 [22.41, 27.95]	0.654
Diabetes recipient	38 (20.7)	6 (13.0)	14 (30.4)	11 (23.9)	7 (15.2)	0.142
HT recipient	145 (78.8)	36 (78.3)	37 (80.4)	37 (80.4)	35 (76.1)	0.949
CHD recipient	35 (19.0)	11 (23.9)	13 (28.3)	7 (15.2)	4 (8.7)	0.076

Table 2: Stratification by sUmod pre-transplant

Characteristics	Total	sUmod quartile 1	sUmod quartile 2	sUmod quartile 3	sUmod quartile 4	p-value
CVD recipient	71 (38.6)	23 (50.0)	20 (43.5)	17 (37.0)	11 (23.9)	0.065
Dialysis pre-Tx						**<0.001**
Non	19 (10.3)	1 (2.2)	1 (2.2)	5 (10.9)	12 (26.1)	
HD	145 (78.8)	43 (93.5)	43 (93.5)	33 (71.7)	26 (56.5)	
PD	20 (10.9)	2 (4.3)	2 (4.3)	8 (17.4)	8 (17.4)	
Dialysis vintage	1712.00 [711.00, 2719.50]	2756.00 [1620.00, 3402.00]	1809.00 [852.00, 2649.00]	998.00 [559.00, 2314.00]	973.00 [574.75, 2174.25]	**<0.001**
CIT	420.00 [120.00, 780.00]	600.00 [360.00, 840.00]	480.00 [326.25, 840.00]	360.00 [120.00, 600.00]	165.00 [120.00, 456.00]	**<0.001**
WIT	20.00 [20.00, 30.00]	20.00 [20.00, 24.50]	20.00 [20.00, 30.00]	20.00 [20.00, 30.00]	20.00 [20.00, 20.00]	0.230
PRA	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.259
Mismatch	4.00 [2.00, 5.00]	3.50 [2.00, 5.00]	4.00 [3.00, 5.00]	3.00 [2.00, 4.00]	3.45 [2.00, 4.00]	0.737
RD	500.00 [0.00, 1500.00]	0.00 [0.00, 175.00]	400.00 [0.00, 800.00]	1000.00 [450.00, 1500.00]	1500.00 [400.00, 2000.00]	**<0.001**
PNF	8 (4.3)	3 (6.5)	1 (2.2)	2 (4.3)	2 (4.3)	0.790
DGF	66 (35.9)	24 (52.2)	18 (39.1)	12 (26.1)	12 (26.1)	**0.025**
ATN	N = 103	n = 32	n = 25	n = 19	n = 27	0.273
Non/minimally	4 (3.9)	0 (0.0)	1 (4.0)	2 (10.5)	1 (3.7)	
<25%	9 (8.7)	3 (9.4)	1 (4.0)	1 (5.3)	4 (14.8)	
25–50%	17 (16.5)	4 (12.5)	4 (16.0)	5 (26.3)	4 (14.8)	
50–75%	28 (27.2)	5 (15.6)	10 (40.0)	4 (21.1)	9 (33.3)	
>75%	45 (43.7)	20 (62.5)	9 (36.0)	7 (36.8)	9 (33.3)	
ATN 50	73 (70.9)	25 (78.1)	19 (76.0)	11 (57.9)	18 (66.7)	0.405
IF/TA	0.00 [0.00, 5.00]	0.00 [0.00, 5.00]	5.00 [0.00, 5.00]	0.00 [0.00, 7.50]	0.00 [0.00, 5.00]	0.573
Glomerulo-sclerosis	1.00 [1.00, 2.00]	1.00 [1.00, 3.00]	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	0.835
Glomerulo-sclerosis (%)	20.00 [11.11, 40.00]	20.00 [14.29, 34.09]	20.00 [11.11, 50.00]	14.29 [9.40, 39.23]	16.67 [9.17, 38.89]	0.726
Arteroscleroses	1.00 [0.00, 2.00]	1.00 [0.00, 2.00]	1.50 [0.00, 3.00]	0.00 [0.00, 1.00]	1.00 [0.00, 1.00]	0.197
Umod_0	7.13 [3.11, 14.12]	1.67 [0.66, 2.43]	5.00 [3.96, 5.79]	9.96 [8.55, 11.24]	25.01. [17.09, 56.05]	**<0.001**

Table 2: Stratification by sUmod pre-transplant

Characteristics	Total	sUmod quartile 1	sUmod quartile 2	sUmod quartile 3	sUmod quartile 4	p-value
Creatinine	6.95 [5.70, 9.10]	8.10 [6.43, 9.43]	7.75 [6.50, 9.80]	7.05 [5.55, 9.10]	6.20 [4.60, 7.45]	**0.001**
BUN	45.00 [33.00, 58.00]	41.50 [31.25, 50.50]	48.00 [33.00, 61.00]	45.00 [34.00, 58.00]	46.00 [33.50, 65.00]	0.526
eGFR (CKD-EPI)	7.26 [5.26, 9.52]	6.32 [4.98, 8.07]	6.59 [4.93, 8.28]	7.43 [5.56, 9.73]	8.26 [6.83, 11.64]	**0.001**
Leucocyte count	7.17 [5.89, 8.60]	6.44 [5.60, 8.06]	7.18 [5.92, 8.19]	7.32 [6.44, 8.60]	7.48 [5.90, 9.34]	0.173
Hemoglobin	12.10 [11.20, 13.28]	13.00 [11.62, 13.57]	12.15 [11.43, 13.07]	12.00 [11.00, 13.10]	11.65 [10.45, 13.12]	0.062
Sodium	139.00 [137.00, 141.00]	139.50 [138.00, 141.00]	139.00 [138.00, 141.00]	139.00 [137.00, 141.00]	139.00 [137.00, 142.00]	0.750
Potassium	5.30 [4.70, 5.70]	5.40 [5.12, 5.90]	5.40 [4.90, 5.90]	5.20 [4.70, 5.50]	4.80 [4.50, 5.38]	**0.001**
Phosphate	4.90 [4.20, 6.05]	5.10 [4.38, 6.50]	4.95 [3.97, 5.75]	5.80 [4.43, 6.47]	4.70 [3.70, 5.00]	0.169
Protein	7.20 [6.60, 7.60]	7.30 [6.75, 7.80]	7.45 [6.88, 7.73]	7.20 [6.70, 7.50]	6.90 [5.90, 7.35]	**0.015**
CRP	0.30 [0.00, 0.60]	0.30 [0.05, 0.60]	0.45 [0.20, 0.90]	0.30 [0.00, 0.60]	0.20 [0.00, 0.50]	0.152
Tacrolimus	143 (77.7)	37 (80.4)	35 (76.1)	33 (71.7)	38 (84.4)	0.493
Cyclosporin A	40 (21.7)	9 (19.6)	10 (21.7)	14 (30.4)	7 (15.2)	0.345
MMF	n = 184	n = 46	n = 46	n = 46	n = 46	0.174
No	2 (1.1)	0 (0.0)	2 (4.3)	0 (0.0)	0 (0.0)	
Yes	180 (97.8)	45 (100.00)	44 (95.7)	46 (100.0)	45 (97.8)	
Not registered	2 (1.1)	1 (2.2)	0 (0.0)	0 (0.0)	1 (2.2)	
Steroids	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.2)	0.392
Proteinuria post-Tx month 3	169.00 [113.50, 297.50]	161.50 [99.25, 298.75]	221.00 [137.50, 318.50]	171.00 [131.00, 235.50]	150.00 [107.75, 233.00]	0.287
Proteinuria post-Tx month 6	146.50 [96.00, 263.25]	159.00 [109.00, 334.00]	161.50 [117.25, 242.50]	147.00 [88.00, 262.00]	115.00 [83.00, 255.00]	0.547
Proteinuria month 12	127.00 [100.75, 265.00]	159.00 [111.00, 337.00]	128.00 [111.00, 272.00]	127.00 [95.00, 202.00]	111.00 [73.50, 227.50]	0.277
eGFR (CKD-EPI) post-Tx month 3	43.80 [27.70, 55.00]	35.55 [23.00, 45.95]	39.70 [27.90, 53.80]	44.70 [36.12, 55.70]	47.45 [30.93, 58.23]	0.056
eGFR (CKD-EPI) post-Tx month 6	43.00 [31.00, 55.00]	34.70 [27.70, 48.00]	39.30 [26.50, 58.90]	44.50 [32.30, 53.00]	45.55 [36.67, 57.03]	0.166
eGFR (CKD-EPI) post-Tx month 12	49.60 [31.98, 62.85]	39.70 [30.30, 61.80]	56.20 [29.70, 65.80]	47.00 [33.15, 60.40]	52.05 [37.55, 62.95]	0.879

Table 2: Descriptive data stratified by sUmod pre-transplant. Laboratory parameters also including sUmod refer to their pre-transplant value. n, number of cases; post-Tx, post-transplant; pre-Tx, pre-transplant; HT, arterial hypertension, BMI, Body-Mass-Index, CHD, coronary heart disease; CVD, cardiovascular disease; CIT, cold ischemic time; WIT, warm ischemic time; PRA, panel.reactive antibodies; RD, residual diuresis; PNF, primary non-function; DGF, delayed graft function; ATN, acute tubular necrosis; IF/TA, interstitial fibrosis/tubular atrophy; Umod_0, serum Uromodulin pre-transplant; MMF, Mycofenolat Mofetil.

3.1.3 DGF vs. non-DGF

In the non-DGF-group donors were significantly more often living donors, female donors and younger donors compared to the DGF-group. Also, in the non-DGF-group donors had a significantly lower BMI and did significantly less often suffer from diabetes mellitus.
Also concerning recipient characteristics significant differences between the DGF- and the non-DGF-group were found. In the non-DGF-group recipients were more often female, were younger, had a lower BMI and less often suffered from diabetes mellitus, coronary heart disease or cardiovascular disease.
Dialysis vintage and cold ischemic time were significantly shorter in the non-DGF-group, while residual diuresis was greater in the non-DGF-group. Furthermore, the non-DGF-group showed a significantly higher pre-transplant sUmod level, while the sUmod level at first day post-transplant was significantly lower than in the DGF-group.
Interestingly proteinuria at post-transplant moths 6 and 12 were significantly higher in the DGF-group and eGFR (CKD-EPI) at post-transplant months 3, 6 and 12 was significantly lower in the DGF-group compared to the non-DGF-group. Further significant and insignificant results are shown in Table 3 below.

Table 3: Stratification by DGF

Characteristics	Total	DGF	non-DGF	p-value
n	184	63	121	
Living donation	64 (34.8)	11 (17.5)	53 (43.8)	**0.001**
Female gender donor	86 (46.7)	18 (28.6)	68 (56.2)	**0.001**
Age donor	52.00 [43.00, 63.00]	59.00 [51.00, 67.50]	50.00 [39.00, 61.00]	**0.001**
BMI donor	26.00 [23.00, 28.75]	27.00 [24.50, 30.00]	25.00 [23.00, 28.00]	**0.002**
Diabetes donor				**0.001**
No	132 (71.7)	41 (65.1)	91 (75.2)	
Yes	14 (7.6)	11 (17.5)	3 (2.5)	
Unknown	38 (20.7)	11 (17.5)	27 (22.3)	

Table 3: Stratification by DGF

Characteristics	Total	DGF	non-DGF	p-value
HT donor				0.219
No	93 (50.5)	28 (44.4)	65 (53.7)	
Yes	69 (37.5)	29 (46.0)	40 (33.1)	
Unknown	22 (12.0)	6 (9.5)	16 (13.2)	
Smoking status donor				**0.024**
No	82 (44.6)	35 (55.6)	47 (38.8)	
Yes	51 (27.7)	18 (28.6)	33 (27.3)	
Unknown	51 (27.7)	10 (15.9)	41 (33.9)	
Last serum creatinine donor	0.90 [0.70, 1.10]	0.90 [0.80, 1.10]	0.80 [0.70, 1.00]	**0.001**
Female gender recipient	60 (32.6)	13 (20.6)	47 (38.8)	**0.020**
Age recipient	54.00 [43.00, 63.00]	59.00 [49.00, 65.50]	49.00 [40.00, 59.00]	**<0.001**
Nephrectomy	22 (12.0)	8 (12.7)	14 (11.6)	1.000
BMI recipient	25.04 [22.14, 28.47]	27.78 [24.99, 31.34]	23.77 [21.23, 26.70]	**<0.001**
Diabetes recipient	39 (21.2)	20 (31.7)	19 (15.7)	**0.019**
HT recipient	147 (79.9)	49 (77.8)	98 (81.0)	0.747
CHD recipient	36 (19.6)	23 (36.5)	13 (10.7)	**<0.001**
CVD recipient	73 (39.7)	40 (63.5)	33 (27.3)	**<0.001**
Dialysis pre-Tx				**0.003**
Non	20 (10.9)	1 (1.6)	19 (15.7)	
HD	144 (78.3)	58 (92.1)	86 (71.1)	
PD	20 (10.9)	4 (6.3)	16 (13.2)	
Dialysis vintage	1629.50 [697.50, 2690.75]	1863.50 [1100.75, 2747.50]	1338.50 [572.50, 2648.25]	**0.027**
CIT	420.00 [120.00, 780.00]	600.00 [345.00, 960.00]	360.00 [120.00, 660.00]	**<0.001**
WIT	20.00 [20.00, 30.00]	20.00 [20.00, 30.00]	20.00 [20.00, 27.00]	0.615
PRA	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.899
Mismatch	3.90 [2.00, 5.00]	4.00 [2.00, 5.00]	3.45 [2.00, 4.25]	0.557
RD	500.00 [0.00, 1500.00]	100.00 [0.00, 700.00]	750.00 [0.00, 1500.00]	**0.001**

Table 3: Stratification by DGF

Characteristics	Total	DGF	non-DGF	p-value
PNF	8 (4.3)	7 (11.1)	1 (0.8)	**0.004**
ATN	n = 100	n = 36	n = 64	**0.028**
Non/minimally	4 (4)	0 (0.0)	4 (6.3)	
<25%	9 (9)	2 (5.6)	7 (10.9)	
25–50%	18 (18)	8 (22.2)	10 (15.6)	
50–75%	26 (26)	15 (41.7)	11 (17.2)	
>75%	43 (43)	11 (30.6)	32 (50.0)	
ATN 50	69 (69)	26 (72.2)	43 (67.2)	0.766
IF/TA	0.00 [0.00, 5.00]	5.00 [0.00, 10.00]	0.00 [0.00, 5.00]	0.068
Glomerulosclerosis	1.00 [1.00, 2.00]	1.00 [1.00, 3.00]	1.00 [1.00, 2.00]	0.177
Glomerulosclerosis (%)	20.00 [11.11, 38.85]	20.00 [11.60, 41.54]	20.00 [11.11, 37.74]	0.630
Arteriosclerosis	1.00 [0.00, 2.00]	1.00 [0.00, 2.00]	1.00 [0.00, 1.00]	0.162
Umod_0	7.35 [3.15, 13.84]	5.77 [2.13, 10.17]	9.00 [3.99, 15.31]	**0.005**
Umod	36.41 [22.38, 67.12]	44.92 [26.00, 72.40]	31.85 [21.00, 61.38]	**0.039**
Umod ratio x/0	4.55 [2.33, 12.24]	8.43 [4.25, 23.37]	3.21 [2.06, 7.86]	**<0.001**
ΔUmod	22.21 [12.50, 52.34]	42.95 [19.65, 70.74]	19.12 [11.41, 33.45]	**<0.001**
Creatinine	5.95 [4.40, 7.50]	7.00 [5.80, 8.40]	5.60 [4.10, 7.10]	**<0.001**
BUN	48.00 [38.00, 60.00]	43.00 [35.00, 52.00]	49.50 [39.25, 60.00]	0.122
eGFR (CKD-EPI)	9.04 [6.66, 13.12]	7.20 [6.27, 9.26]	10.00 [7.67, 15.13]	**<0.001**
Leucocyte count	10.65 [8.59, 14.12]	12.11 [8.52, 15.52]	10.49 [8.73, 13.66]	0.188
Hemoglobin	10.60 [9.38, 11.53]	10.60 [9.75, 11.55]	10.60 [9.30, 11.50]	0.529
Sodium	141.00 [138.00, 144.00]	139.00 [136.00, 142.50]	141.00 [139.00, 144.00]	**0.005**
Potassium	4.80 [4.20, 5.40]	5.40 [4.90, 5.90]	4.50 [4.00, 5.10]	**<0.001**
Phosphate	6.70 [5.60, 7.75]	6.00 [4.95, 6.80]	6.80 [5.88, 7.93]	**0.038**
Protein	5.55 [5.20, 6.00]	5.70 [5.30, 6.00]	5.50 [5.20, 5.95]	0.791
CRP	3.15 [1.90, 4.45]	3.25 [2.10, 3.75]	2.90 [1.83, 4.72]	0.829
Tacrolimus	142 (77.2)	45 (71.4)	97 (80.2)	0.207
Cyclosporin A	42 (22.8)	20 (31.7)	22 (18.2)	0.058

Table 3: Stratification by DGF

Characteristics	Total	DGF	non-DGF	p-value
Proteinuria post-Tx month 3	169.00 [113.00, 296.50]	215.00 [114.50, 434.00]	159.00 [113.00, 226.25]	0.119
Proteinuria post-Tx month 6	148.00 [97.50, 262.00]	209.00 [125.00, 370.50]	128.50 [83.50, 245.00]	**0.011**
Proteinuria post-Tx month 12	144.00 [101.50, 272.00]	272.00 [131.50, 506.25]	117.00 [95.00, 202.00]	**0.001**
eGFR (CKD-EPI) post-Tx month 3	44.80 [27.90, 56.30]	33.30 [22.02, 46.45]	47.90 [35.60, 59.90]	**<0.001**
eGFR (CKD-EPI) post-Tx month 6	43.65 [31.60, 56.17]	33.95 [22.98, 48.63]	47.55 [35.65, 59.18]	**0.001**
eGFR (CKD-EPI) post-Tx month 12	49.90 [31.85, 62.90]	35.40 [27.08, 56.97]	53.80 [36.95, 65.70]	**0.002**

Table 3: Descriptive data stratified by delayed graft function. Laboratory parameters also including serum Uromodulin refer to their value at first day post-transplant. n, summ of cases; post-Tx, post-transplant; pre-Tx, pre-transplant; HT, arterial hypertension, BMI, Body-Mass-Index, CHD, coronary heart disease; CVD, cardiovascular disease; CIT, cold ischemic time; WIT, warm ischemic time; PRA, panel reactive antibodies; RD, residual diuresis; PNF, primary non-function; DGF, delayed graft function; ATN, acute tubular necrosis; IF/TA, interstitial fibrosis/tubular atrophy; Umod_0, serum Uromodulin pre-transplant; MMF, Mycofenolat Mofetil; Umod ratio x/0, serum Uromodulin at post-transplant day 1/serum Uromodulin pre-transplant, ΔUmod, difference between serum Uromodulin at post-transplant day 1 and serum Uromodulin pre-transplant.

3.1.4 sUmod first day post-transplant

Stratification by the sUmod level at post-transplant day 1 revealed significant associations with various different descriptive parameters. For example, the donors' smoking status was significantly associated with the sUmod level at the first day post-transplant. The highest number of smoking donors is found in the sUmod quartile 2, the least in sUmod quartile 4. Furthermore, the number of mismatching HLAs was significantly associated with the sUmod level at first day post-transplant with the highest mismatch being found in sUmod quartile 4 and the lowest in quartile 1. Further significant and insignificant associations are shown in Table 4 below.

Table 4: Stratification by sUmod at first day post-transplant

Characteristics	Total	sUmod quartile 1	sUmod quartile 2	sUmod quartile 3	sUmod quartile 4	p-value
n	183	46	45	46	46	
Living donation	63 (34.4)	19 (41.3)	20 (44.4)	15 (32.6)	9 (19.6)	0.057
Female gender donor	86 (47.0)	28 (60.9)	22 (48.9)	19 (41.3)	17 (37.0)	0.108

Table 4: Stratification by sUmod at first day post-transplant

Characteristics	Total	sUmod quartile 1	sUmod quartile 2	sUmod quartile 3	sUmod quartile 4	p-value
Age donor	52.00 [43.00, 63.00]	51.00 [38.50, 59.50]	52.00 [43.00, 63.00]	57.00 [46.00, 65.75]	53.50 [43.00, 68.75]	0.387
BMI donor	26.00 [23.00, 28.75]	26.00 [22.25, 28.00]	26.00 [23.00, 29.00]	26.00 [23.00, 29.00]	26.00 [24.00, 28.00]	0.676
Diabetes donor						0.053
No	131 (71.6)	29 (63.0)	35 (77.8)	33 (71.7)	34 (73.9)	
Yes	14 (7.7)	1 (2.2)	3 (6.7)	7 (15.2)	3 (6.5)	
Unknown	38 (20.8)	16 (34.8)	7 (15.6)	6 (13.0)	9 (19.6)	
HT donor						0.161
No	92 (50.3)	27 (58.7)	23 (51.1)	22 (47.8)	20 (43.5)	
Yes	69 (37.7)	10 (21.7)	18 (40.0)	21 (45.7)	20 (43.5)	
Unknown	22 (12.0)	9 (19.6)	4 (8.9)	3 (6.5)	6 (13.0)	
Smoking status donor						**0.034**
No	82 (44.8)	18 (39.1)	13 (28.9)	22 (47.8)	29 (63.0)	
Yes	50 (27.3)	11 (23.9)	18 (40.0)	12 (26.1)	9 (19.6)	
Unknown	51 (27.9)	17 (37.0)	14 (31.1)	12 (26.1)	8 (17.4)	
Last serum creatinine donor	0.90 [0.70, 1.10]	0.80 [0.70, 1.00]	0.80 [0.70, 1.00]	0.90 [0.70, 1.08]	0.93 [0.80, 1.17]	0.051
Female gender recipient	60 (32.8)	15 (32.6)	14 (31.1)	14 (30.4)	17 (37.0)	0.911
Age recipient	54.00 [43.00, 63.00]	53.50 [44.00, 59.75]	55.00 [39.00, 63.00]	53.50 [45.25, 63.75]	53.00 [43.00, 64.50]	0.744
Nephrectomy	22 (12.0)	8 (17.4)	2 (4.4)	7 (15.2)	5 (10.9)	0.241
BMI recipient	25.04 [22.14, 28.47]	25.59 [22.83, 28.22]	25.83 [22.11, 30.96]	24.88 [22.21, 28.01]	23.75 [21.45, 27.85]	0.448
Diabetes recipient	39 (21.3)	5 (10.9)	15 (33.3)	5 (10.9)	14 (30.4)	**0.007**
HT recipient	146 (79.8)	38 (82.6)	41 (91.1)	35 (76.1)	32 (69.6)	0.067
CHD recipient	36 (19.7)	8 (17.4)	12 (26.7)	6 (13.0)	10 (21.7)	0.400
CVD recipient	73 (39.9)	17 (37.0)	17 (37.8)	19 (41.3)	20 (43.5)	0.911
Dialysis pre-transplant						0.112
Non	20 (10.9)	2 (4.3)	6 (13.3)	8 (17.4)	4 (8.7)	

Table 4: Stratification by sUmod at first day post-transplant

Characteristics	Total	sUmod quartile 1	sUmod quartile 2	sUmod quartile 3	sUmod quartile 4	p-value
HD	143 (78.1)	42 (91.3)	33 (73.3)	30 (65.2)	38 (82.6)	
PD	20 (10.9)	2 (4.3)	6 (13.3)	8 (17.4)	4 (8.7)	
Dialysis vintage	1629.50 [697.50, 2690.75]	1825.00 [498.25, 2825.00]	998.00 [561.00, 2406.50]	1975.50 [823.50, 2834.00]	1728.00 [1193.75, 2682.00]	0.118
CIT	420.00 [120.00, 780.00]	345.00 [120.00, 669.00]	330.00 [120.00, 636.00]	420.00 [120.00, 742.50]	570.00 [270.00, 885.00]	**0.043**
WIT	20.00 [20.00, 30.00]	20.00 [20.00, 30.00]	20.00 [20.00, 30.00]	20.00 [20.00, 22.25]	20.00 [18.50, 20.00]	**0.007**
PRA	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 34.00]	**0.017**
Mismatch	3.90 [2.00, 5.00]	2.00 [2.00, 4.00]	3.00 [2.00, 4.00]	3.00 [2.00, 5.00]	4.00 [3.00, 5.00]	**0.017**
RD	500.00 [0.00, 1500.00]	400.00 [0.00, 1500.00]	700.00 [450.00, 1500.00]	400.00 [0.00, 1500.00]	200.00 [0.00, 1000.00]	0.063
PNF	8 (4.4)	2 (4.3)	1 (2.2)	3 (6.5)	2 (4.3)	0.800
DGF	62 (33.9)	10 (21.7)	15 (33.3)	18 (39.1)	19 (41.3)	0.193
ATN	n = 100	n = 19	n = 23	n = 25	n = 33	0.630
Non/minimally	4 (4)	1 (5.3)	1 (4.3)	1 (4.0)	1 (3.0)	
<25%	9 (9)	3 (15.8)	1 (4.3)	4 (16.0)	1 (3.0)	
25–50%	18 (18)	2 (10.5)	4 (17.4)	6 (24.0)	6 (18.2)	
50–75%	26 (26)	3 (15.8)	7 (30.4)	8 (32.0)	8 (24.2)	
>75%	43 (43)	10 (52.6)	10 (43.5)	6 (24.0)	17 (51.5)	
ATN 50	69 (69)	13 (68.4)	17 (73.9)	14 (56.0)	25 (75.8)	0.401
IF/TA	0.00 [0.00, 5.00]	0.00 [0.00, 5.00]	0.00 [0.00, 5.00]	0.00 [0.00, 5.00]	5.00 [0.00, 5.00]	0.681
Glomerulo-sclerosis	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	0.905
Glomerulo-sclerosis (%)	20.00 [11.11, 38.85]	22.22 [17.14, 48.08]	16.67 [12.14, 25.00]	20.00 [7.14, 44.44]	16.67 [12.50, 38.46]	0.474
Arteriosclerosis	1.00 [0.00, 2.00]	0.00 [0.00, 1.00]	0.00 [0.00, 2.00]	1.50 [0.75, 3.00]	1.00 [0.00, 2.00]	**0.033**
Umod_0	7.35 [3.15, 13.84]	5.12 [2.59, 10.17]	8.65 [5.26, 11.98]	9.95 [3.81, 19.34]	5.84 [2.01, 14.94]	**0.022**
Umod	36.41 [22.38, 67.12]	16.62 [13.71, 19.77]	28.30 [25.89, 31.20]	48.60 [39.35, 55.94]	97.85 [76.77, 126.11]	**<0.001**
Umod ratio x/0	4.55 [2.33, 12.24]	3.07 [2.06, 6.55]	3.36 [2.45, 5.06]	4.84 [2.48, 10.68]	18.96 [6.31, 66.12]	**<0.001**

Table 4: Stratification by sUmod at first day post-transplant

Characteristics	Total	sUmod quartile 1	sUmod quartile 2	sUmod quartile 3	sUmod quartile 4	p-value
ΔUmod	22.21 [12.50, 52.34]	11.41 [7.07, 13.62]	19.95 [17.17, 22.79]	34.40 [26.59, 46. 88]	78.94 [64.78, 120.76]	**<0.001**
Creatinine	5.95 [4.40, 7.50]	6.45 [4.82, 7.83]	5.70 [4.10, 7.90]	6.10 [4.43, 7.40]	5.95 [4..60, 7.10]	0.790
BUN	48.00 [38.00, 60.00]	48.00 [39.75, 52.00]	51.00 [40.00, 64.00]	49.00 [38.00, 55.00]	44.50 [33.00, 55.00]	0.398
eGFR (CKD-EPI)	9.04 [6.66, 13.12]	8.20 [6.90, 12.05]	9.81 [6.74, 13.32]	9.48 [6.23, 13.83]	8.75 [7.02, 12.45]	0.758
Leucocyte count	10.65 [8.59, 14.12]	10.19 [8.23, 13.58]	11.58 [8.90, 15.18]	10.56 [8.79, 14.67]	10.62 [8.63, 13.60]	0.407
Hemoglobin	10.60 [9.38, 11.53]	10.60 [9.72, 11.55]	10.60 [9.60, 11.50]	10.45 [9.45, 11.67]	10.40 [9.30, 11.50]	0.709
Sodium	141.00 [138.00, 144.00]	142.00 [139.00, 145.00]	141.00 [138.00, 145.00]	138.50 [137.00, 143.00]	140.00 |137.00, 142.00]	0.083
Potassium	4.80 [4.20, 5.40]	4.75 [4.30, 5.27]	4.80 [4.40, 5.30]	4.85 [3.90, 5.47]	4.85 [4.20, 5.47]	0.906
Phosphate	6.70 [5.60, 7.75]	6.70 [6.25, 7.80]	6.75 [5.62, 7.15]	6.10 [5.10, 7.10]	6.50 [5.80, 8.07]	0.667
Protein	5.55 [5.20, 6.00]	5.50 [5.20, 6.30]	5.70 [5.20, 6.20]	5.80 [5.47, 6.03]	5.40 [5.00, 5.80]	0.131
CRP	3.15 [1.90, 4.45]	2.30 [1.90, 3.40]	3.50 [2.67, 4.88]	1.90 [1.67, 3.32]	4.10 [2.20, 5.55]	0.199
Tacrolimus	141 (77.0)	32 (69.6)	32 (71.1)	37 (80.4)	40 (87.0)	0.186
Cyclosporin A	42 (23.0)	13 (28.3)	13 (28.9)	9 (19.6)	7 (15.2)	0.323
Proteinuria post-Tx month 3	169.00 [113.00, 296.50]	171.00 [109.50, 224.00]	198.00 [149.00, 412.50]	165.00 [122.00, 271.00]	145.00 [101.50, 235.00]	0.101
Proteinuria post-Tx month 6	148.00 [97.50, 262.00]	123.00 [68.00, 195.00]	212 [119.00, 286.00]	129.00 [98.00, 250.00]	154.00 [106.50, 322.00]	0.139
Proteinuria post-Tx month 12	144.00 [101.50, 272.00]	121.00 [95.00, 317.00]	163.50 [104.75, 248.50]	127.00 [92.00, 312.00]	149.00 [111.00, 256.00]	0.974
eGFR (CKD-EPI) post-Tx month 3	44.80 [27.90, 56.30]	41.00 [28.50, 56.90]	44.40 [31.00, 58.10]	45.40 [32.40, 53.60]	42.05 [25.63, 56.22]	0.947
eGFR (CKD-EPI) post-Tx month 6	43.65 [31.60, 56.17]	47.55 [32.67, 55.42]	43.00 [27.40, 52.80]	43.40 [31.75, 55.48]	39.40 [31.38, 60.55]	0.786
eGFR (CKD-EPI) post-Tx month 12	49.90 [31.85, 62.90]	44.90 [29.00, 61.95]	47.55 [29.45, 57.70]	49.90 [35.00, 62.55]	53.20 [33.40, 81.00]	0.581

Table 4: Descriptive data stratified by serum Uromodulin at first day post-transplant. Laboratory parameters also including serum Uromodulin refer to their value at first day post-transplant. n, summ of cases; post-Tx, post-transplant; pre-Tx, pre-transplant; HT, arterial hypertension, BMI, Body-Mass-Index, CHD, coronary heart disease; CVD, cardiovascular disease; CIT, cold ischemic time; WIT, warm ischemic time; PRA, panel reactive antibodies; RD, residual diuresis; PNF, primary non-function; DGF, delayed graft function; ATN, acute tubular necrosis; IF/TA, interstitial fibrosis/tubular atrophy; Umod_0, serum Uromodulin pre-transplant; MMF, Mycofenolat Mofetil; Umod ratio x/0, serum Uromodulin at post-transplant day 1/serum Uromodulin pre-transplant, ΔUmod, difference between serum Uromodulin at post-transplant day 1 and serum Uromodulin pre-transplant.

3.1.5 sUmod ratio (post-transplant day 1/pre-transplant)

Interestingly significant associations between renal retention markers (BUN, potassium) respectively eGFR (CKD-EPI) at first day post-transplant and the sUmod ratio were found. The highest BUN level was found in sUmod ratio quartile 1, while smallest BUN level was found in quartile 4. Strikingly for potassium this was the exact other way around. eGFR (CKD-EPI) at first day post-transplant was highest in sUmod ratio quartile 1, and smallest in quartiles 3 and 4. All significant and insignificant associations are shown in Table 5 below.

Table 5: Stratification by sUmod ratio (post-Tx day 1/pre-transplant)

Characteristics	Total	sUmod ratio quartile 1	sUmod ratio quartile 2	sUmod ratio quartile 3	sUmod ratio quartile 4	p-value
n	179	45	44	45	45	
Living donation	62 (34.6)	26 (57.8)	20 (45.5)	13 (28.9)	3 (6.7)	**<0.001**
Female gender donor	85 (47.5)	27 (60.0)	23 (52.3)	23 (51.1)	12 (26.7)	**0.010**
Age donor	52.00 [43.00, 63.00]	51.00 [40.00, 60.00]	55.00 [44.00, 68.00]	52.00 [43.00, 63.00]	51.00 [43.00, 63.00]	0.692
BMI donor	26.00 [23.00, 28.75]	26.00 [23.00, 28.00]	27.00 [23.00, 29.00]	25.00 [23.59, 28.00]	26.00 [23.00, 29.00]	0.826
Diabetes donor						0.876
No	128 (71.5)	33 (73.3)	32 (72.7)	31 (68.9)	32 (71.1)	
Yes	13 (7.3)	2 (4.4)	2 (4.5)	4 (8.9)	5 (11.1)	
Unknown	38 (21.2)	10 (22.2)	10 (22.7)	10 (22.2)	8 (17.8)	
HT donor						0.739
No	90 (50.3)	23 (51.1)	22 (50.0)	22 (48.9)	23 (51.1)	
Yes	68 (38.0)	17 (37.8)	14 (31.8)	20 (44.4)	17 (37.8)	
Unknown	21 (11.7)	5 (11.1)	8 (18.2)	3 (6.7)	5 (11.1)	
Smoking status donor						**0.002**
No	79 (44.1)	14 (31.1)	15 (34.1)	20 (44.4)	30 (66.7)	
Yes	49 (27.4)	10 (22.2)	14 (31.8)	14 (31.1)	11 (24.4)	
Unknown	51 (28.5)	21 (46.7)	15 (34.1)	11 (24.4)	4 (8.9)	

Table 5: Stratification by sUmod ratio (post-Tx day 1/pre-transplant)

Characteristics	Total	sUmod ratio quartile 1	sUmod ratio quartile 2	sUmod ratio quartile 3	sUmod ratio quartile 4	p-value
Last serum creatinine donor	0.90 [0.70, 1.10]	0.80 [0.70, 1.00]	0.85 [0.70, 1.10]	0.80 [0.70, 1.00]	0.90 [0.70, 1.20]	0.354
Female gender recipient	60 (33.5)	12 (26.7)	16 (36.4)	18 (40.0)	14 (31.1)	0.557
Age recipient	54.00 [43.00, 63.00]	49.00 [40.00, 63.00]	54.00 [46.00, 63.25]	55.00 [43.00, 62.00]	52.00 [43.00, 61.00]	0.580
Nephrectomy	21 (11.7)	2 (4.4)	2 (4.5)	6 (13.3)	11 (24.4)	**0.009**
BMI recipient	25.04 [22.14, 28.47]	24.71 [22.53, 27.16]	25.23 [22.66, 28.48]	26.60 [22.84, 30.96]	23.51 [20.70, 27.78]	0.090
Diabetes recipient	37 (20.7)	3 (6.7)	12 (27.3)	13 (28.9)	9 (20.0)	**0.038**
HT recipient	142 (79.3)	33 (73.3)	40 (90.9)	40 (88.9)	29 (64.4)	**0.004**
CHD recipient	34 (19.0)	5 (11.1)	10 (22.7)	9 (20.0)	10 (22.2)	0.466
CVD recipient	69 (38.5)	14 (31.1)	16 (36.4)	16 (35.6)	23 (51.1)	0.230
Dialysis pre-Tx						**0.020**
Non	20 (11.2)	11 (24.4)	5 (11.4)	3 (6.7)	1 (2.2)	
HD	139 (77.7)	28 (62.2)	33 (75.0)	37 (82.2)	41 (91.1)	
PD	20 (11.2)	6 (13.3)	6 (13.6)	5 (11.1)	3 (6.7)	
Dialysis vintage	1629.50 [697.50, 2690.75]	798.50 [385.00, 2198.50]	979.00 [435.00, 2406.50]	1737.50 [942.75, 2642.75]	2552.00 [1424.25, 3341.75]	**<0.001**
CIT	420.00 [120.00, 780.00]	130.00 [120.00, 420.00]	307.50 [120.00, 604.00]	505.00 [150.00, 840.00]	720.00 [480.00, 900.00]	**<0.001**
WIT	20.00 [20.00, 30.00]	20.00 [20.00, 20.00]	20.00 [20.00, 30.00]	20.00 [20.00, 30.00]	20.00 [20.00, 23.00]	0.113
PRA	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 5.00]	0.055
Mismatch	3.90 [2.00, 5.00]	3.00 [2.00, 4.00]	3.00 [2.00, 4.00]	4.00 [3.00, 5.00]	4.00 [2.00, 5.00]	0.158
RD	500.00 [0.00, 1500.00]	1400.00 [200.00, 2000.00]	700.00 [50.00, 1500.00]	500.00 [150.00, 1500.00]	0.00 [0.00, 200.00]	**<0.001**
PNF	7 (3.9)	1 (2.2)	1 (2.3)	2 (4.4)	3 (6.7)	0.660
DGF	60 (33.5)	8 (17.8)	10 (22.7)	18 (40.0)	24 (53.3)	**0.001**
ATN	n = 99	n = 24	n = 21	n = 24	n = 30	0.741
Non/minimally	4 (4.0)	2 (8.3)	1 (4.8)	0 (0.0)	1 (3.3)	
<25%	9 (9.1)	3 (12.5)	2 (9.5)	2 (8.3)	2 (6.7)	

Table 5: Stratification by sUmod ratio (post-Tx day 1/pre-transplant)

Characteristics	Total	sUmod ratio quartile 1	sUmod ratio quartile 2	sUmod ratio quartile 3	sUmod ratio quartile 4	p-value
25–50%	18 (18.2)	5 (20.8)	2 (9.5)	6 (25.0)	5 (16.7)	
50–75%	26 (26.3)	6 (25.0)	7 (33.3)	8 (33.3)	5 (16.7)	
>75%	42 (42.4)	8 (33.3)	9 (42.9)	8 (33.3)	17 (56.7)	
ATN 50	68 (68.7)	14 (58.3)	16 (76.2)	16 (66.7)	22 (73.3)	0.553
IF/TA	0.00 [0.00, 5.00]	2.50 [0.00, 5.00]	0.00 [0.00, 5.00]	2.50 [0.00, 6.25]	0.00 [0.00, 5.00]	0.770
Glomerulo-sclerosis	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	1.00 [1.00, 3.00]	1.00 [1.00, 2.00]	0.912
Glomerulo-sclerosis (%)	20.00 [11.11, 38.85]	18.33 [8.17, 30.80]	20.00 [14.29, 50.00]	20.00 [9.58, 40.38]	20.00 [11.27, 25.00]	0.592
Arteriosclerosis	1.00 [0.00, 2.00]	0.00 [0.00, 1.00]	0.00 [0.00, 2.00]	1.00 [0.25, 2.00]	1.00 [0.00, 2.00]	0.247
Umod_0	7.35 [3.15, 13.84]	20.75 [11.78, 44.63]	9.02 [5.92, 12.77]	5.30 [3.20, 7.95]	1.87 [0.66, 3.15]	**<0.001**
Umod	36.41 [22.38, 67.12]	31.85 [20.46, 51.31]	28.73 [20.74, 38.45]	31.15 [24.42, 55.54]	76.22 [54.24, 120.41]	**<0.001**
Umod ratio x/0	4.55 [2.33, 12.24]	1.49 [0.98, 1.92]	3.11 [2.67, 3.84]	6.77 [5.47, 8.26]	40.57 [19.82, 109.56]	**<0.001**
ΔUmod	22.21 [12.50, 52.34]	8.24 [-0.44, 14.30]	19.65 [13.05, 26.98]	27.35 [19.13, 45.58]	71.55 [51.14, 113.92]	**<0.001**
Creatinine	5.95 [4.40, 7.50]	5.50 [3.90, 6.80]	5.85 [4.10, 8.33]	6.30 [4.80, 7.90]	6.30 [5.40, 7.70]	0.113
BUN	48.00 [38.00, 60.00]	54.50 [42.25, 62.00]	49.00 [39.00, 61.00]	48.00 [40.00, 60.00]	41.00 [31.00, 52.00]	**0.012**
eGFR (CKD-EPI)	9.04 [6.66, 13.12]	10.15 [8.31, 15.85]	9.52 [6.21, 13.40]	8.03 [6.42, 11.68]	8.22 [7.25, 10.47]	**0.027**
Leucocyte count	10.65 [8.59, 14.12]	10.75 [8.99, 13.36]	11.15 [9.32, 12.87]	10.90 [8.53, 15.10]	10.13 [8.10, 13.25]	0.535
Hemoglobin	10.60 [9.38, 11.53]	10.60 [9.30, 11.40]	10.35 [9.20, 10.88]	10.70 [9.80, 11.90]	10.60 [9.70, 11.90]	0.278
Sodium	141.00 [138.00, 144.00]	142.00 [139.00, 145.00]	140.00 [138.00, 145.00]	140.00 [137.00, 142.00]	139.00 [137.00, 143.00]	0.153
Potassium	4.80 [4.20, 5.40]	4.40 [4.00, 4.90]	4.75 [4.20, 5.30]	4.90 [4.40, 5.50]	5.20 [4.70, 5.80]	**<0.001**
Phosphate	6.70 [5.60, 7.75]	7.20 [6.00, 8.00]	6.80 [5.75, 7.27]	6.50 [6.00, 7.20]	5.80 [4.80, 7.10]	0.299
Protein	5.55 [5.20, 6.00]	5.50 [5.10, 5.90]	5.50 [5.20, 6.00]	5.80 [5.30, 6.45]	5.65 [5.20, 5.90]	0.451
CRP	3.15 [1.90, 4.45]	2.70 [1.78, 3.57]	3.65 [2.50, 4.88]	2.65 [1.82, 3.62]	3.40 [2.00, 5.20]	0.519
Tacrolimus	138 (77.1)	36 (80.0)	31 (70.5)	34 (75.6)	37 (82.2)	0.494
Cyclosporin A	40 (22.3)	8 (17.8)	12 (27.3)	11 (24.4)	9 (20.0)	0.702

Table 5: Stratification by sUmod ratio (post-Tx day 1/pre-transplant)

Characteristics	Total	sUmod ratio quartile 1	sUmod ratio quartile 2	sUmod ratio quartile 3	sUmod ratio quartile 4	p-value
Proteinuria post-Tx month 3	169.00 [113.00, 296.50]	157.00 [113.50, 217.50]	176.00 [133.00, 298.00]	164.50 [112.50, 308.50]	169.00 [101.50, 345.50]	0.895
Proteinuria post-Tx month 6	148.00 [97.50, 262.00]	122.00 [82.00, 258.00]	142.00 [101.00, 250.00]	201.00 [92.50, 274.50]	154.00 [109.75, 302.50]	0.777
Proteinuria post-Tx month 12	144.00 [101.50, 272.00]	106.00 [74.25, 204.25]	145.50 [111.00, 202.00]	230.00 [117.00, 394.00]	158.00 [111.00, 313.25]	0.072
eGFR. (CKD-EPI) post-Tx month 3	44.80 [27..90, 56.30]	52.10 [30.85, 62.15]	46.05 [36.12, 52.63]	43.85 [27.45, 51.55]	33.90 [23.15, 51.45]	0.080
eGFR. (CKD-EPI) post-Tx month 6	43.65 [31.60, 56.17]	48.60 [33.70, 58.60]	44.10 [33.05, 51.85]	43.50 [32.05, 59.95]	35.30 [28.05, 58.40]	0.524
eGFR. (CKD-EPI) post-Tx month 12	49.90 [31.85, 62.90]	49.55 [33.40, 62.95]	47.45 [28.50, 57.00]	56.25 [30.52, 66.33]	48.35 [32.65, 82.28]	0.663

Table 5: Descriptive data stratified by serum Uromodulin ratio (sUmod at first day post-transplant/sUmod pre-transplant). Laboratory parameters also including serum Uromodulin refer to their value at first day post-transplant. n, summ of cases; post-Tx, post-transplant; pre-Tx, pre-transplant; HT, arterial hypertension, BMI, Body-Mass-Index, CHD, coronary heart disease; CVD, cardiovascular disease; CIT, cold ischemic time; WIT, warm ischemic time; PRA, panel reactive antibodies; RD, residual diuresis; PNF, primary non-function; DGF, delayed graft function; ATN, acute tubular necrosis; IF/TA, interstitial fibrosis/tubular atrophy; Umod_0, serum Uromodulin pre-transplant; MMF, Mycofenolat Mofetil; Umod ratio x/0, serum Uromodulin at post-transplant day 1/serum Uromodulin pre-transplant, ΔUmod, difference between serum Uromodulin at post-transplant day 1 and serum Uromodulin pre-transplant.

3.1.6 sUmod post-transplant month 1-3

Also, the stratification by sUmod at post-transplant month 1–3 revealed some significant associations with descriptive parameters. For example, CRP levels 1-3 months post-transplant were significantly highest in sUmod quartiles 1 and 2, while they were lowest in quartile 4. Furthermore, PNF was significantly associated with sUmod at post-transplant month 1–3. The highest number of recipients who experienced PNF was found in sUmod quartile 1, followed by quartile 4, while in median non are found in quartiles 2 and 3. Further significant and insignificant results are shown in Table 6 below.

Table 6: Stratification by sUmod post-transplant month 1–3

Characteristics	Total	sUmod quartile 1	sUmod quartile 2	sUmod quartile 3	sUmod quartile 4	p-value
n	160	40	40	40	40	
Living donation	53 (33.1)	8 (20.0)	19 (47.5)	11 (27.5)	15 (37.5)	0.051
Female gender donor	71 (44.4)	13 (32.5)	24 (60.0)	18 (45.0)	16 (40.0)	0.087
Age donor	52.00 [43.00, 63.00]	58.50 [43.00, 67.25]	57.00 [48.75, 62.00]	54.00 [44.75, 68.00]	47.00 [39.75, 57.25]	0.094
BMI donor	26.00 [23.00, 28.75]	26.50 [24.00, 30.25]	27.00 [23.00, 28.25]	25.00 [23.75, 28.25]	26.00 [24.00, 29.25]	0.503
Diabetes donor						0.678
No	114 (71.25)	29 (72.5)	25 (62.5)	28 (70.0)	32 (80.0)	
Yes	10 (6.25)	3 (7.5)	4 (10.0)	2 (5.0)	1 (2.5)	
Unknown	36 (22.5)	8 (20.0)	11 (27.5)	10 (25.0)	7 (17.5)	
HT donor						0.673
No	81 (50.6)	20 (50.0)	18 (45.0)	19 (47.5)	24 (60.0)	
Yes	59 (36.9)	17 (42.5)	16 (40.0)	14 (35.0)	12 (30.0)	
Unknown	20 (12.5)	3 (7.5)	6 (15.0)	7 (17.5)	4 (10.0)	
Smoking status donor						0.224
No	76 (47.5)	23 (57.5)	17 (42.5)	16 (40.0)	20 (50.0)	
Yes	41 (25.6)	10 (25.0)	8 (20.0)	15 (37.5)	8 (20.0)	
Unknown	43 (26.9)	7 (17.5)	15 (37.5)	9 (22.5)	12 (30.0)	
Last serum creatinine donor	0.90 [0.70, 1.10]	0.90 [0.70, 1.10]	0.80 [0.70, 1.00]	0.85 [0.70, 1.02]	0.80 [0.78, 1.00]	0.603
Female gender recipient	58 (36.3)	15 (37.5)	9 (22.5)	16 (40.0)	18 (45.0)	0.182
Age recipient	54.00 [43.00, 63.00]	56 [42.75, 62.75]	59.00 [43.75, 65.00]	53.50 [43.75, 63.00]	49.00 [41.75, 58.00]	0.323
Nephrectomy	20 (12.5)	5 (12.5)	5 (12.5)	6 (15.0)	4 (10.0)	0.928
BMI recipient	25.31 [21.95, 28.56]	25.11 [22.17, 29.10]	25.33 [23.25, 27.57]	26.29 [23.12, 29.30]	22.78 [20.43, 27.49]	0.124
Diabetes recipient	32 (20.0)	6 (15.0)	7 (17.5)	11 (27.5)	8 (20.0)	0.534
HT recipient	126 (78.8)	34 (85.0)	31 (77.5)	28 (70.0)	33 (82.5)	0.371
CHD recipient	28 (17.5)	9 (22.5)	7 (17.5)	7 (17.5)	5 (12.5)	0.709

Table 6: Stratification by sUmod post-transplant month 1–3

Characteristics	Total	sUmod quartile 1	sUmod quartile 2	sUmod quartile 3	sUmod quartile 4	p-value
CVD recipient	63 (39.4)	22 (55.0)	13 (32.5)	14 (35.0)	14 (35.0)	0.137
Dialysis pre-Tx						0.055
Non	17 (10.6)	3 (7.5)	2 (5.0)	4 (10.0)	8 (20.0)	
HD	126 (78.8)	34 (85.0)	37 (92.5)	32 (80.0)	25 (62.5)	
PD	15 (9.4)	3 (7.5)	1 (2.5)	4 (10.0)	7 (17.5)	
Dialysis vintage	1821.50 [793.75, 2730.50]	2672.00 [1639.00, 3339.00]	1468.00 [443.50, 2587.00]	1729.50 [912.00, 2659.25]	1529.00 [643.00, 2344.00]	**0.005**
CIT	480.00 [120.00, 780.00]	450.00 [240.00, 780.00]	187.50 [120.00, 609.00]	480.00 [167.50, 795.00]	510.00 [120.00, 840.00]	0.148
WIT	20.00 [20.00, 30.00]	20.00 [20.00, 27.75]	20.00 [20.00, 30.00]	20.00 [20.00, 30.00]	20.00 [20.00, 20.00]	0.241
PRA	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 10.25]	0.074
Mismatch	4.00 [2.00, 5.00]	3.95 [3.00, 5.00]	4.00 [2.00, 5.00]	4.00 [2.00, 5.00]	3.00 [2.00, 4.25]	0.765
RD	500.00 [0.00, 1500.00]	200.00 [0.00, 850.00]	500.00 [0.00, 1500.00]	500.00 [0.00, 1000.00]	500.00 [0.00, 1575.00]	0.240
PNF	7 (4.4)	6 (15.0)	0 (0.0)	0 (0.0)	1 (2.5)	**0.002**
DGF	60 (37.5)	20 (50.0)	15 (37.5)	16 (40.0)	9 (22.5)	0.085
ATN	n = 92	n = 24	n = 20	n = 22	n = 26	0.118
Non/minimally	4 (4.3)	1 (4.2)	2 (10.0)	0 (0.0)	1 (3.8)	
<25%	7 (7.6)	1 (4.2)	3 (15.0)	0 (0.0)	3 (11.5)	
25–50%	17 (18.5)	6 (25.0)	3 (15.0)	6 (27.3)	2 (7.7)	
50–75%	24 (26.1)	8 (33.3)	7 (35.0)	6 (27.3)	3 (11.5)	
>75%	40 (43.5)	8 (33.3)	5 (25.0)	10 (45.5)	17 (65.4)	
ATN 50	64 (69.6)	16 (66.7)	12 (60.0)	16 (72.7)	20 (76.9)	0.631
IF/TA	0.00 [0.00, 5.00]	0.00 [0.00, 5.00]	0.00 [0.00, 5.00]	5.00 [0.00, 5.00]	0.00 [0.00, 5.00]	0.680
Glomerulo-sclerosis	1.00 [1.00, 2.00]	1.00 [1.00, 2.25]	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	1.00 [1.00, 1.75]	0.713
Glomerulo-sclerosis (%)	20.00 [11.11, 38.46]	21.11 [12.28, 40.71]	26.79 [14.29, 61.67]	14.29 [7.69, 39.62]	14.29 [11.11, 23.75]	0.146
Arteriosclerosis	1.00 [0.00, 2.00]	1.50 [0.00, 3.00]	1.00 [0.00, 3.00]	0.50 [0.00, 1.00]	1.00 [0.00, 1.00]	0.184
Umod_0	7.28 [3.02, 14.27]	4.75 [2.09, 9.79]	6.43 [3.12, 10.58]	9.66 [4.86, 15.44]	9.48 [2.69, 15.98]	0.098

Table 6: Stratification by sUmod post-transplant month 1–3

Characteristics	Total	sUmod quartile 1	sUmod quartile 2	sUmod quartile 3	sUmod quartile 4	p-value
Umod	45.21 [28.96, 66.25]	19.80 [17.00, 26.00]	38.54 [36.06, 42.21]	54.39 [50.89, 61.15]	87.06 [75.20, 100.05]	**<0.001**
Umod ratio x/0	6.03 [3.39, 13.63]	3.69 [1.66, 8.92]	5.64 [3.44, 11.89]	5.95 [3.69, 11.39]	10.04 [5.20, 29.52]	**0.011**
ΔUmod	34.61 [17.92, 52.34]	14.97 [8.58, 20.27]	31.29 [25.37, 34.49]	45.26 [37.79, 49.10]	75.33 [60.47, 92.01]	**<0.001**
Creatinine	1.70 [1.30, 2.30]	2.05 [1.60, 3.25]	2.00 [1.50, 2.40]	1.70 [1.20, 2.10]	1.40 [1.20, 1.65]	**<0.001**
BUN	31.50 [23.00, 42.25]	35.50 [29.00, 52.25]	34.00 [26.25, 46.00]	30.00 [21.50, 36.50]	23.00 [19.50, 33.00]	**0.001**
eGFR (CKD-EPI)	41.45 [26.79, 54.82]	34.63 [19.27, 47.03]	34.01 [25.48, 51.54]	41.45 [29.82, 55.12]	51.31 [39.34, 63.90]	**<0.001**
Leucocyte count	8.24 [5.90, 10.34]	7.50 [4.96, 8.77]	8.81 [5.86, 10.55]	8.64 [6.68, 11.15]	7.97 [5.89, 10.37]	0.147
Hemoglobin	11.10 [10.20, 12.30]	10.30 [9.10, 11.80]	11.75 [10.75, 12.90]	11.05 [10.70, 12.30]	11.40 [10.60, 12.30]	**0.005**
Sodium	140.00 [138.00, 142.00]	139.00 [137.00, 141.00]	140.00 [137.00, 143.50]	140.00 [138.00, 141.00]	141.00 [139.25, 142.75]	**0.029**
Potassium	4.50 [4.10, 4.90]	4.50 [4.10, 5.00]	4.55 [4.03, 4.97]	4.55 [4.10, 4.80]	4.40 [4.03, 4.77]	0.788
Phosphate	2.90 [2.50, 3.40]	3.10 [2.80, 4.10]	3.00 [2.40, 3.42]	2.70 [2.45, 3.25]	2.80 [2.40, 3.20]	**0.048**
Protein	6.40 [5.90, 6.85]	6.20 [5.80, 6.50]	6.40 [6.00, 6.90]	6.55 [5.93, 6.88]	6.50 [6.00, 6.90]	0.176
CRP	0.20 [0.00, 0.55]	0.20 [0.00, 0.65]	0.20 [0.08, 0.75]	0.15 [0.00, 0.60]	0.00 [0.00, 0.20]	**0.009**
Tacrolimus	125 (78.1)	28 (70.0)	30 (75.0)	32 (82.1)	35 (87.5)	0.238
Cyclosporin A	35 (21.9)	12 (30.0)	11 (27.5)	7 (17.5)	5 (12.5)	0.176
Steroids	156 (97.5)	39 (98.0)	39 (98.0)	39 (98.0)	39 (98.0)	0.400
Proteinuria post-Tx month 3	170.00 [113.00, 307.00]	226.00 [160.50, 339.75]	144.00 [111.50, 236.50]	170.00 [116.50, 312.50]	159.00 [106.00, 210.00]	0.063
Proteinuria post-Tx month 6	148.00 [97.50, 295.50]	240.00 [148.50, 428.75]	166.00 [95.00, 254.00]	125.50 [97.75, 280.25]	134.00 [88.00, 176.00]	0.079
Proteinuria post-Tx month 12	135.00 [97.50, 272.00]	264.00 [111.00, 519.50]	125.00 [96.75, 255.50]	111.00 [74.00, 317.00]	139.50 [111.00, 185.50]	0.093
eGFR (CKD-EPI) post-Tx month 3	44.10 [27.30, 54.85]	32.10 [22.00, 47.30]	40.30 [24.90, 52.00]	41.40 [28.30, 55.15]	50.40 [44.30, 60.50]	**0.001**
eGFR (CKD-EPI) post-Tx month 6	42.35 [30.62, 54.22]	35.10 [24.40, 47.85]	37.90 [27.48, 51.43]	41.75 [31.60, 57.50]	47.65 [39.47, 59.43]	**0.018**

Table 6: Stratification by sUmod post-transplant month 1–3

Characteristics	Total	sUmod quartile 1	sUmod quartile 2	sUmod quartile 3	sUmod quartile 4	p-value
eGFR (CKD-EPI) post-Tx month 12	47.05 [31.28, 62.15]	33.80 [28.30, 53.45]	39.70 [29.10, 56.85]	46.60 [33.38, 61.75]	56.55 [49.72, 81.83]	**0.007**

Table 6: Descriptive data stratified by serum Uromodulin post-transplant month 1–3. Laboratory parameters also including serum Uromodulin refer to their value post-transplant month 1–3. n, summ of cases; post-Tx, post-transplant; pre-Tx, pre-transplant; HT, arterial hypertension, BMI, Body-Mass-Index, CHD, coronary heart disease; CVD, cardiovascular disease; CIT, cold ischemic time; WIT, warm ischemic time; PRA, panel reactive antibodies; RD, residual diuresis; PNF, primary non-function; DGF, delayed graft function; ATN, acute tubular necrosis; IF/TA, interstitial fibrosis/tubular atrophy; Umod_0, serum Uromodulin pre-transplant; MMF, Mycofenolat Mofetil; Umod ratio x/0, serum Uromodulin post-transplant month 1–3/serum Uromodulin pre-transplant, ΔUmod, difference between serum Uromodulin post-transplant month 1–3 and serum Uromodulin pre-transplant.

3.1.7 sUmod ratio (post-transplant month 1-3/pre-transplant)

After stratification by sUmod ratio (post-transplant month 1–3/pre-transplant), also some mentionable significant associations evolved. The significantly highest number of recipients who received living donation was found in sUmod ratio quartile 2 and the least in quartile 4. Moreover, the highest number of recipients who had nephrectomy pre-transplant was found in sUmod ratio quartile 4 and least in quartile 2. Interestingly also cold ischemic time was significantly associated with the sUmod ratio (post-transplant month 1–3/pre-transplant). The longest cold ischemic time was found in sUmod ratio quartile 4, while the shortest was found in quartiles 1 and 2. All significant and insignificant associations are shown in Table 7 below.

Table 7: Stratification by sUmod ratio (post-Tx month 1–3/pre-transplant)

Characteristics	Total	sUmod ratio quartile 1	sUmod ratio quartile 2	sUmod ratio quartile 3	sUmod ratio quartile 4	p-value
n	159	40	39	40	40	
Living donation	53 (33.3)	16 (40.0)	18 (46.2)	13 (32.5)	6 (15.0)	**0.021**
Female gender donor	71 (44.7)	18 (45.0)	18 (46.2)	20 (50.0)	15 (37.5)	0.722
Age donor	52.00 [43.00, 63.00]	51.00 [40.75, 64.75]	56.00 [47.50, 64.00]	56.50 [41.50, 68.00]	51.00 [45.25, 61.00]	0.636
BMI donor	26.00 [23.00, 28.75]	26.00 [23.00, 28.00]	26.00 [23.95, 29.00]	27.00 [23.75, 29.00]	26.00 [23.90, 29.00]	0.846
HT donor						0.315
No	81 (50.9)	18 (45.0)	23 (59.0)	15 (37.5)	25 (62.5)	

Table 7: Stratification by sUmod ratio (post-Tx month 1–3/pre-transplant)

Characteristics	Total	sUmod ratio quartile 1	sUmod ratio quartile 2	sUmod ratio quartile 3	sUmod ratio quartile 4	p-value
Yes	58 (36.5)	16 (40.0)	13 (33.3)	18 (45.0)	11 (27.5)	
Unknown	20 (12.6)	6 (15.0)	3 (7.7)	7 (17.5)	4 (1.0)	
Smoking donor						0.068
No	76 (47.8)	16 (40.0)	16 (41.0)	23 (57.5)	21 (52.5)	
Yes	40 (25.2)	14 (35.0)	7 (17.9)	6 (15.0)	13 (32.5)	
Unknown	43 (27.0)	10 (25.0)	16 (41.0)	11 (27.5)	6 (15.0)	
Last serum creatinine donor	0.90 [0.70, 1.10]	0.90 [0.80, 1.10]	0.80 [0.70, 1.00]	0.80 [0.70, 1.02]	0.80 [0.70, 1.10]	0.578
Female gender recipient	58 (36.5)	12 (30.0)	15 (38.5)	13 (32.5)	18 (45.0)	0.509
Age recipient	54.00 [43.00, 63.00]	51.50 [42.50, 63.00]	54.00 [42.00, 63.00]	58.00 [43.00, 65.00]	53.00 [43.50, 61.00]	0.775
Nephrectomy	20 (12.6)	4 (10.0)	1 (2.6)	5 (12.5)	10 (25.0)	**0.024**
BMI recipient	25.31 [21.95, 28.56]	23.49 [21.91, 26.24]	25.78 [22.47, 28.38]	26.21 [23.18, 29.89]	25.79 [21.45, 28.93]	0.168
Diabetes recipient	32 (20.1)	6 (15.0)	7 (17.9)	13 (32.5)	6 (15.0)	0.156
HT recipient	125 (78.6)	31 (77.5)	30 (76.9)	32 (80.0)	32 (80.0)	0.980
CHD recipient	28 (17.6)	5 (12.5)	6 (15.4)	9 (22.5)	8 (20.0)	0.644
CVD recipient	62 (39.0)	10 (25.0)	13 (33.3)	22 (55.0)	17 (42.5)	**0.040**
Dialysis pre-Tx						0.153
Non	17 (10.7)	8 (20.0)	3 (7.7)	5 (12.5)	1 (2.5)	
HD	127 (79.9)	27 (67.5)	31 (79.5)	33 (82.5)	36 (90.0)	
PD	15 (9.4)	5 (12.5)	5 (12.8)	2 (5.0)	3 (7.5)	
Dialysis vintage	1821.50 [793.75, 2730.50]	1858.00 [926.50, 2617.50]	769.00 [330.75, 2361.75]	1891.00 [945.50, 2837.00]	2407.00 [1503.50, 3376.00]	**<0.001**
CIT	480.00 [120.00, 780.00]	315.00 [120.00, 627.00]	315.00 [120.00, 618.00]	418.50 [120.00, 795.00]	666.00 [480.00, 840.00]	**0.020**
WIT	20.00 [20.00, 30.00]	20.00 [20.00, 23.25]	20.00 [20.00, 30.00]	20.00 [20.00, 30.00]	20.00 [20.00, 20.00]	0.495
PRA	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 57.25]	**0.001**
Mismatch	4.00 [2.00, 5.00]	4.00 [2.00, 4.25]	3.00 [3.00, 4.00]	4.00 [2.00, 5.00]	3.50 [2.00, 5.00]	0.599

Table 7: Stratification by sUmod ratio (post-Tx month 1–3/pre-transplant)

Characteristics	Total	sUmod ratio quartile 1	sUmod ratio quartile 2	sUmod ratio quartile 3	sUmod ratio quartile 4	p-value
RD	500.00 [0.00, 1500.00]	600.00 [0.00, 1500.00]	1000.00 [500.00, 2000.00]	400.00 [0.00, 1000.00]	0.00 [0.00, 212.50]	**<0.001**
PNF	7 (4.4)	3 (7.5)	1 (2.6)	2 (5.0)	1 (2.5)	0.659
DGF	60 (37.7)	14 (35.0)	12 (30.8)	16 (40.0)	18 (45.0)	0.589
ATN	n = 91	n = 22	n = 19	n = 21	n = 29	0.086
Non/minimally	4 (4.4)	2 (9.1)	1 (5.3)	0 (0.0)	1 (3.4)	
<25%	7 (7.7)	2 (9.1)	2 (10.5)	0 (0.0)	3 (10.3)	
25–50%	17 (18.7)	4 (18.2)	6 (31.6)	5 (23.8)	2 (6.9)	
50–75%	24 (26.4)	10 (45.5)	3 (15.8)	6 (28.6)	5 (17.2)	
>75%	39 (42.9)	4 (18.2)	7 (36.8)	10 (47.6)	18 (62.1)	
ATN 50	41 (45.1)	14 (63.6)	9 (47.4)	11 (52.4)	7 (24.1)	0.204
IF/TA	0.00 [0.00, 5.00]	0.00 [0.00, 5.00]	0.00 [0.00,7.50]	5.00 [0.00, 5.00]	0.00 [0.00, 5.00]	0.756
Glomerulo-sclerosis	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	1.00 [1.00, 2.00]	1.00 [1.00, 3.00]	0.942
Glomerulo-sclerosis (%)	20.00 [11.11, 38.46]	20.00 [13.12, 62.50]	20.00 [10.56, 35.90]	14.81 [10.00, 36.36]	20.00 [12.50, 33.33]	0.786
Arteriosclerosis	1.00 [0.00, 2.00]	1.00 [0.00, 1.00]	0.50 [0.00, 2.75]	1.50 [0.00, 2.25]	1.00 [0.00, 1.75]	0.661
Umo_0	7.28 [3.02, 14.27]	28.22 [14.70, 59.82]	10.24 [7.84, 12.82]	5.16 [3.14, 7.64]	1.82 [0.65, 2.82]	**<0.001**
Umod	45.21 [28.96, 66.25]	39.75 [22.00, 60.42]	43.49 [32.27, 62.98]	43.54 [29.81, 58.05]	59.40 [44.00, 92.50]	**0.005**
Umod ratio x/0	6.03 [3.39, 13.63]	1.40 [0.88, 2.18]	4.72 [3.72, 5.17]	8.42 [7.19, 10.40]	29.63 [18.66, 67.71]	**<0.001**
ΔUmod	34.61 [17.92, 52.34]	8.95 [-7.63, 20.92]	34.22 [24.35, 48.50]	39.56 [26.97, 48.72]	56.97 [42.21, 89.31]	**<0.001**
Creatinine	1.70 [1.30, 2.30]	1.70 [1.37, 2.50]	1.60 [1.20, 2.10]	1.80 [1.48, 2.40]	1.80 [1.30, 2.35]	0.617
BUN	31.50 [23.00, 42.25]	29.00 [21.75, 39.75]	31.00 [25.00, 46.00]	32.50 [23.00, 41.25]	31.00 [23.00, 45.00]	0.968
eGFR (CKD-EPI)	41.45 [26.79, 54.82]	41.10 [27.67, 58.72]	47.40 [32.60, 55.48]	37.51 [27.13, 50.87]	40.54 [24.97, 54.18]	0.635
Leucoctye count	8.24 [5.90, 10.34]	6.76 [5.23, 9.66]	8.24 [6.98, 10.46]	8.34 [6.88, 9.46]	9.14 [5.99, 11.33]	0.291
Hemoglobin	11.10 [10.20, 12.30]	10.90 [9.20, 11.88]	11.70 [10.70, 12.75]	10.90 [10.30, 12.00]	11.40 [10.30, 12.50]	0.084
Sodium	140.00 [138.00, 142.00]	140.00 [138.00, 141.00]	140.00 [138.25, 142.00]	140.50 [139.00, 141.75]	140.00 [138.00, 142.00]	0.835

Table 7: Stratification by sUmod ratio (post-Tx month 1–3/pre-transplant)

Characteristics	Total	sUmod ratio quartile 1	sUmod ratio quartile 2	sUmod ratio quartile 3	sUmod ratio quartile 4	p-value
Potassium	4.50 [4.10, 4.90]	4.60 [4.00, 4.80]	4.55 [4.10, 5.00]	4.30 [4.10, 4.95]	4.50 [4.10, 4.90]	0.774
Phosphate	2.90 [2.50, 3.40]	2.90 [2.40, 3.30]	2.95 [2.50, 3.55]	2.95 [2.40, 3.20]	2.90 [2.60, 3.50]	0.911
Protein	6.40 [5.90, 6.85]	6.10 [5.73, 6.60]	6.50 [6.27, 6.82]	6.40 [5.80, 6.70]	6.40 [5.95, 7.00]	0.233
CRP	0.20 [0.00, 0.55]	0.00 [0.00, 0.40]	0.10 [0.00, 0.40]	0.20 [0.00, 0.60]	0.20 [0.00, 0.60]	0.567
Tacrolimus	124 (78.0)	28 (70.0)	30 (77.0)	33 (82.5)	33 (82.5)	0.480
Cyclosporin A	35 (22.0)	12 (30.0)	8 (20.5)	8 (20.0)	7 (17.5)	0.573
Steroids	155 (97.4)	38 (95.0)	39 (100)	39 (97.5)	39 (97.5)	0.404
Proteinuria post-Tx month 3	170.00 [113.00, 307.00]	155.50 [110.75, 220.75]	181.00 [117.50, 329.00]	201.00 [132.25, 327.25]	163.50 [107.25, 250.50]	0.326
Proteinuria post-Tx month 6	148.00 [97.50, 295.50]	119.00 [81.50, 272.00]	123.00 [97.00, 262.00]	166.00 [134.00, 320.00]	154.00 [108.75, 263.75]	0.274
Proteinuria post-Tx month 12	135.00 [97.50, 272.00]	111.00 [76.00, 217.00]	161.00 [88.50, 252.00]	185.00 [111.00, 331.00]	155.00 [111.00, 271.75]	0.345
eGFR (CKD-EPI) post-Tx month 3	44.10 [27.30, 54.85]	41.40 [27.50, 54.60]	47.00 [36.70, 56.40]	37.50 [27.70, 50.35]	44.80 [23.95, 53.60]	0.465
eGFR (CKD-EPI) post-Tx month 6	42.35 [30.62, 54.22]	40.55 [33.33, 54.03]	49.30 [35.10, 55.50]	36.60 [28.25, 45.78]	43.00 [28.00, 59.40]	0.345
eGFR (CKD-EPI) post-Tx month 12	47.05 [31.28, 62.15]	44.10 [30.30, 63.00]	50.50 [33.22, 60.02]	40.50 [28.90, 65.80]	52.25 [33.07, 66.48]	0.852

Table 7: Descriptive data stratified by serum Uromodulin ratio (sUmod post-transplant month 1–3/sUmod pre-transplant). Laboratory parameters also including serum Uromodulin refer to their value post-transplant month 1–3. n, summ of cases; post-Tx, post-transplant; pre-Tx, pre-transplant; HT, arterial hypertension, BMI, Body-Mass-Index, CHD, coronary heart disease; CVD, cardiovascular disease; CIT, cold ischemic time; WIT, warm ischemic time; PRA, panel reactive antibodies; RD, residual diuresis; PNF, primary non-function; DGF, delayed graft function; ATN, acute tubular necrosis; IF/TA, interstitial fibrosis/tubular atrophy; Umod_0, serum Uromodulin pre-transplant; MMF, Mycofenolat Mofetil; Umod ratio x/0, serum Uromodulin at post-transplant month 1–3/serum Uromodulin pre-transplant, ΔUmod, difference between serum Uromodulin post-transplant month 1–3 and serum Uromodulin pre-transplant.

3.2 Regression analysis

3.2.1 sUmod post-transplant day 1 and DGF

The regression anaylsis with sUmod as a continuous variable did not show any significant results.

In the regression analysis with sUmod as a categorical parameter, the odds of having DGF significantly decreased by 61% when

having a sUmod level within the first quartile compared to a sUmod level within quartile 4 (OR 0.39, 95%-CI 0.15–0.97, Table 8).
In the multivariable analysis (after full adjustment) sUmod quartile 1 showed a significant decrease in the odds of 72% compared to quartile 4 (OR 0.28, 95%-CI 0.09–0.86, Table 8).

Table 8: Association of sUmod at post-transplant day 1 with DGF

sUmod (ng/ml)	No. events	Univariable OR (95%-CI)	Plus adjusted for demographics OR (95%-CI)	Plus adjusted for eGFR (CKD-EPI) OR (95%-CI)	Plus adjusted for CIT and living donation OR (95%-CI)
DGF (n = 183)	62	1.03 (0.97–1.09)	1.06 (0.99–1.14)	1.05 (0.98–1.13)	1.04 (0.97–1.12)
Quartile 1		0.39 (0.15–0.97)	0.26 (0.09–0.75)	0.24 (0.08–0.71)	0.28 (0.09–0.86)
Quartile 2		0.71 (0.30–1.66)	0.50 (0.18–1.34)	0.54 (0.18–1.51)	0.69 (0.23–2.05)
Quartile 3		0.91 (0.39–2.11)	0.78 (0.30–2.05)	0.84 (0.30–2.34)	0.87 (0.30–2.51)
Quartile 4		1	1	1	1

Table 8: Logistic regression analysis for the association of serum Uromodulin (sUmod) at post-transplant day 1 with delayed graft function (DGF). sUmod was once entered as a continuous variable (see first row) and once as a categorical variable (see rows 2-5) into the regression model. For the categorical analysis quartile 4 serves as reference. Odds ratios (OR) are given per 10 units sUmod (for continuous variable analysis) respectively per 1 unit sUmod (for categorical variable analysis). eGFR (CKD-EPI), estimated glomerular filtration rate; CIT, cold ischemic time, CI, confidence interval. Demographics are age recipient, gender recipient, BMI recipient and diabetes recipient.

3.2.2 sUmod ratio (post-transplant day 1/pre-transplant) and DGF

In the regression analysis with the sUmod ratio as a continuous variable, the univariable model did not show significant results. However, multivariable analysis (after full adjustment) showed that the odds of experiencing DGF significantly increased by 7% per 10 units increase in the sUmod ratio (OR 1.07, 95%-CI 1.01–1.14, Table 9).
In the regression analysis with the sUmod ratio as a categorical parameter, the odds of experiencing DGF significantly decreased by 81% when having a sUmod ratio within quartile 1 compared to a sUmod ratio within quartile 4 (OR 0.19, 95%-CI 0.07–0.48, Table 9). For quartile 2 a significant 74%-reduction in the odds of experiencing DGF compared to quartile 4 (OR 0.26, 95%-CI 0.10–0.63, Table 9) was observed. In the multivariable analysis (after full adjustment) sUmod ratio quartile 1 showed a significant decrease in the odds of 81% compared to quartile 4 (OR, 0.19, 95%-CI 0.05–0.63, Table 9). When having a sUmod ratio within quartile 2 the odds significantly decreased by 85% compared to a sUmod ratio within quartile 4 (OR 0.15, 95%-CI 0.04–0.47, Table 9). For quartile 3 a significant 66%-reduction in the odds of experiencing DGF

compared to quartile 4 (OR 0.34, 95%-CI 0.11–0.99, Table 9) was observed.

Table 9: Association of sUmod ratio (post-transplant day 1/pre-transplant) with DGF

sUmod ratio	No. events	Univariable OR (95%-CI)	Plus adjusted for demographics OR (95%-CI)	Plus adjusted for eGFR (CKD-EPI) OR (95%-CI)	Plus adjusted for CIT and living donation OR (95%-CI)
DGF (n = 179)	60	1.04 (0.99–1.10)	1.08 (1.02–1.15)	1.08 (1.02–1.16)	1.07 (1.01–1.14)
Quartile 1		0.19 (0.07–0.48)	0.12 (0.04 –0.35)	0.14 (0.04–0.44)	0.19 (0.05–0.63)
Quartile 2		0.26 (0.10–0.63)	0.11 (0.03–0.33)	0.12 (0.04–0.36)	0.15 (0.04–0.47)
Quartile 3		0.58 (0.25–1.34)	0.28 (0.09–0.78)	0.29 (0.09–0.83)	0.34 (0.11–0.99)
Quartile 4		1	1	1	1

Table 9: Logistic regression analysis for the association of serum Uromodulin (sUmod) ratio (post-transplant day 1/pre-transplant) with delayed graft function (DGF). sUmod was once entered as a continuous variable (see first row) and once as a categorical variable (see rows 2-5) into the regression model. For the categorical analysis quartile 4 serves as reference. Odds ratios (OR) are given per 10 units sUmod (for continuous variable analysis) respectively per 1 unit sUmod (for categorical variable analysis). eGFR (CKD-EPI), estimated glomerular filtration rate; CIT, cold ischemic time, CI, confidence interval. Demographics are age recipient, gender recipient, BMI recipient and diabetes recipient.

3.2.3 sUmod post-transplant day 1 and severe acute tubular injury

The univariable as well as the multivariable analysis with sUmod entered as a continuous as well as a categorical variable did not show any significant results.

Table 10: Association of sUmod post-transplant day 1 with severe acute tubular injury

sUmod (ng/ml)	No. events	Univariabe model OR (95%-CI)	Multivariable model OR (95%-CI)
ATN >75% (n = 100)	69	1.08 (0.99–1.22)	1.07 (0.97–1.23)
Quartile 1		0.69 (0.20–2.50)	0.92 (0.23–3.70)
Quartile 2		0.91 (0.27–3.20)	1.07 (0.30–4.04)
Quartile 3		0.41 (0.13–1.24)	0.46 (0.14–1.49)
Quartile 4		1	1

Table 10: Logistic regression analysis for the association of serum Uromodulin (sUmod) at post-transplant day 1 with sever acute tubular injury (ATN >75%) in biopsy taken at time of transplantation. sUmod was once entered as a continuous variable (see first row) and once as a categorical variable (see rows 2-5) into the regression model. For the categorical analysis quartile 4 serves as reference. Odds ratios (OR) are given per 10 units sUmod (for continuous variable analysis) respectively per 1 unit sUmod (for categorical variable analysis). ATN, acute tubular necrosis; CI, confidence interval. In multivariable model adjustment for variables cold ischemic time, living donor transplantation, age donor, gender donor, diabetes donor and last serum creatinine donor was performed.

3.2.4 sUmod ratio (post-transplant day 1/pre-transplant) and severe acute tubular injury

In the regression analysis with sUmod as a continuous variable, the univariable model showed that per 10 units increase in the sUmod ratio the odds for a severe acute tubular injury in the zero-time biopsy significantly increased by 18% (OR 1.18, 95%-CI 1.03–1-52, Table 11). In multivariable analysis the odds for having sever acute tubular injury in the zero-time biopsy again significantly increased by 18% per 10 units increase in the sUmod ratio (OR 1.18, 95%-CI 1.03–1.52, Table 11).

The regression analysis with sUmod as a categorical parameter did not show any significant results.

Table 11: Association of sUmod ratio (post-transplant day 1/pre-transplant) with severe acute tubular injury

sUmod ratio	No. events	Univariable model OR (95%-CI)	Multivariable model OR (95%-CI)
ATN >75% (n = 99)	68	1.18 (1.03–1.52)	1.18 (1.03–1.52)
Quartile 1		0.51 (0.16–1.59)	0.82 (0.22–3.18)
Quartile 2		1.16 (0.32–4.47)	1.53 (0.37–6.96)
Quartile 3		0.73 (0.22–2.37)	0.78 (0.22–2.73)
Quartile 4		1	1

Table 11: Logistic regression analysis for the association of serum Uromodulin (sUmod) ratio (post-transplant day 1/pre-transplant) with sever acute tubular injury (ATN >75%) in biopsy taken at time of transplantation. sUmod was once entered as a continuous variable (see first row) and once as a categorical variable (see rows 2-5) into the regression model. For the categorical analysis quartile 4 serves as reference. Odds ratios (OR) are given per 10 units sUmod ratio (for continuous variable analysis) respectively per 1 unit sUmod ratio (for categorical variable analysis). ATN, acute tubular necrosis; CI, confidence interval. In multivariable model adjustment for variables cold ischemic time, living donor transplantation, age donor, gender donor, diabetes donor and last serum creatinine donor was performed.

3.2.5 sUmod 30-120 days post-transplant and eGFR (CKD-EPI) 1 year post-transplant

Linear regression modeling showed that sUmod 1-3 months post-transplant as a continuous variable can significantly predict graft function 1 year post-transplant independent of recipient age, recipient gender, recipient BMI and recipient diabetes. Besides the

analysis shows a positive linear relationship between sUmod as a continuous variable and 1-year-eGFR (CKD-EPI).
Also, linear regression modeling with sUmod 1-3 months post-transplant as a categorical variable partly achieves significant results for prediction of post-transplant 1-year-eGFR (CKD-EPI), namely the univariable model as well as the multivariable model which adjusted for demographic recipient parameters. In the univariable model quartile 1 shows the least increase in 1-year eGFR (CKD-EPI) per unit sUmod increase compared to quartile 4. After adjustment for demographic recipient parameters the results are similar to the ones of the univariable model.

Table 12: Association of sUmod 30-120 days post-transplant and eGFR (CKD-EPI) 1 year post-transplant

sUmod (ng/ml)	Univariable regression coefficient (95%-CI)	Plus adjusted for demographics regression coefficient (95%-CI)	Plus adjusted for eGFR (CKD-EPI) regression coefficient (95%-CI)	Plus adjusted for transplant-related parameters regression coefficient (95%-CI)
1-year-eGFR (CKD-EPI) (n = 161)	0.35 (0.17–0.52)	0.32 (0.14–0.50)	0.12 (-0.06–0.30)	0.13 (-0.06–0.32)
Quartile 1	-20.67 (-33.97–-7.37)	-18.05 (-31.55–-4.54)	-4.96 (-17.73–7.81)	-6.24 (-19.79–7.31)
Quartile 2	-16.79 (-29.72–-3.87)	-16.56 (-29.91–-3.21)	-7.91 (-20.22–4.40)	-10.37 (-23.15–2.40)
Quartile 3	-10.93 (-23.64–1.79)	-8.12 (-21.21–4.97)	-1.39 (-13.39–10.62)	-1.28 (-13.88–11.32)
Quartile 4	1	1	1	1

Table 12: Linear regression analysis for the association of serum Uromodulin (sUmod) 1-3 months post-transplant with estimated glomerular filtration rate (eGFR) 1 year post-transplant. sUmod was once entered as a continuous variable (see first row) and once as a categorical variable (see rows 2-5) into the regression model. For the categorical analysis quartile 4 serves as a reference. CI, confidence interval. Demographics adjusted for in 1st multivariable model are recipient age, recipient gender, recipient BMI and recipient diabetes. Transplant-related parameters adjusted for in the 3rd multivariable model are living donor transplantation, cold ischemic time, dialysis vintage and delayed graft function.

3.2.6 sUmod ratio (30-120 days post-transplant/pre-transplant) and eGFR (CKD-EPI) 1 year post-transplant

Linear regression modeling with the sUmod ratio (1-3 months post-transplant/pre-transplant) as an independent variable shows a positive linear relationship between the sUmod ratio and eGFR (CKD-EPI) 1 year post-transplant, although the results fail to reach statistical significance. Also, the analysis with the sUmod ratio as a categorical variable could not reach statistical significance.

Table 13: Association of sUmod ratio (30-120 days post-transplant/pre-transplant) and eGFR (CKD-EPI) 1 year post-transplant

sUmod ratio	Univariable regression coefficient (95%-CI)	Plus adjusted for demographics regression coefficient (95%-CI)	Plus adjusted for eGFR (CKD-EPI) regression coefficient (95%-CI)	Plus adjusted for transplant-related parameters regression coefficient (95%-CI)
1-year-eGFR (CKD-EPI) (n = 161)	0.02 (-0.05–0.09)	0.01 (-0.07–0.08)	0.00 (-0.06–0.07)	0.00 (-0.06–0.07)
Quartile 1	-4.80 (-18.60–9.00)	-5.06 (-18.80–8.67)	-3.46 (-15.31–8.39)	-7.17 (-20.46–6.13)
Quartile 2	-8.06 (-21.51–5.40)	-6.80 (-20.17–6.56)	-8.07 (-19.88–3.74)	-6.52 (-19.83–6.79)
Quartile 3	-1.14 (-14.94–12.66)	0.30 (-13.78–14.37)	0.89 (-11.29–13.07)	2.68 (-10.15–15.51)
Quartile 4	1	1	1	1

Table 13: Linear regression analysis for the association of serum Uromodulin ratio (sUmod ratio) 1-3 months post-transplant /pre-transplant with estimated glomerular filtration rate (eGFR) 1 year post-transplant. sUmod ratio was once entered as a continuous variable (see first row) and once as a categorical variable (see rows 2-5) into the regression model. For the categorical analysis quartile 4 serves as a reference. CI, confidence interval. Demographics adjusted for in 1st multivariable model are recipient age, recipient gender, recipient BMI and recipient diabetes. Transplant-related parameters adjusted for in the 3rd multivariable model are living donor transplantation, cold ischemic time, dialysis vintage and delayed graft function.

3.3 Receiver-Operator-Characteristics curves (ROC curves) and Area-Under-the-Curves (AUC)

The ROC curves which correspond to the previously mentioned regression models are shown below (see Figure 1-4 below).

In the ROC curve analyses depicted in Figure 1 the model with sUmod at post-transplant day 1 as a predictor works moderately well to predict DGF (AUC 0.593 [95%-CI 0.507–0.680]). In multivariable ROC curve analysis, the model with only co-variables as predictor for DGF as well as the model with co-variables additionally to sUmod shows increased accuracy in the prediction of DGF (AUC 0.815 [95%-CI 0.752–0.879], AUC 0.821 [0.758–0.883]). Notably the two lastly mentioned multivariable models show a quite similar predictive accuracy for DGF, while the AUC of the univariable model (with sUmod as singular predictor for DGF) is significantly smaller.

Figure 1: ROC curves for regression analyses sUmod post-transplant day 1 and DGF

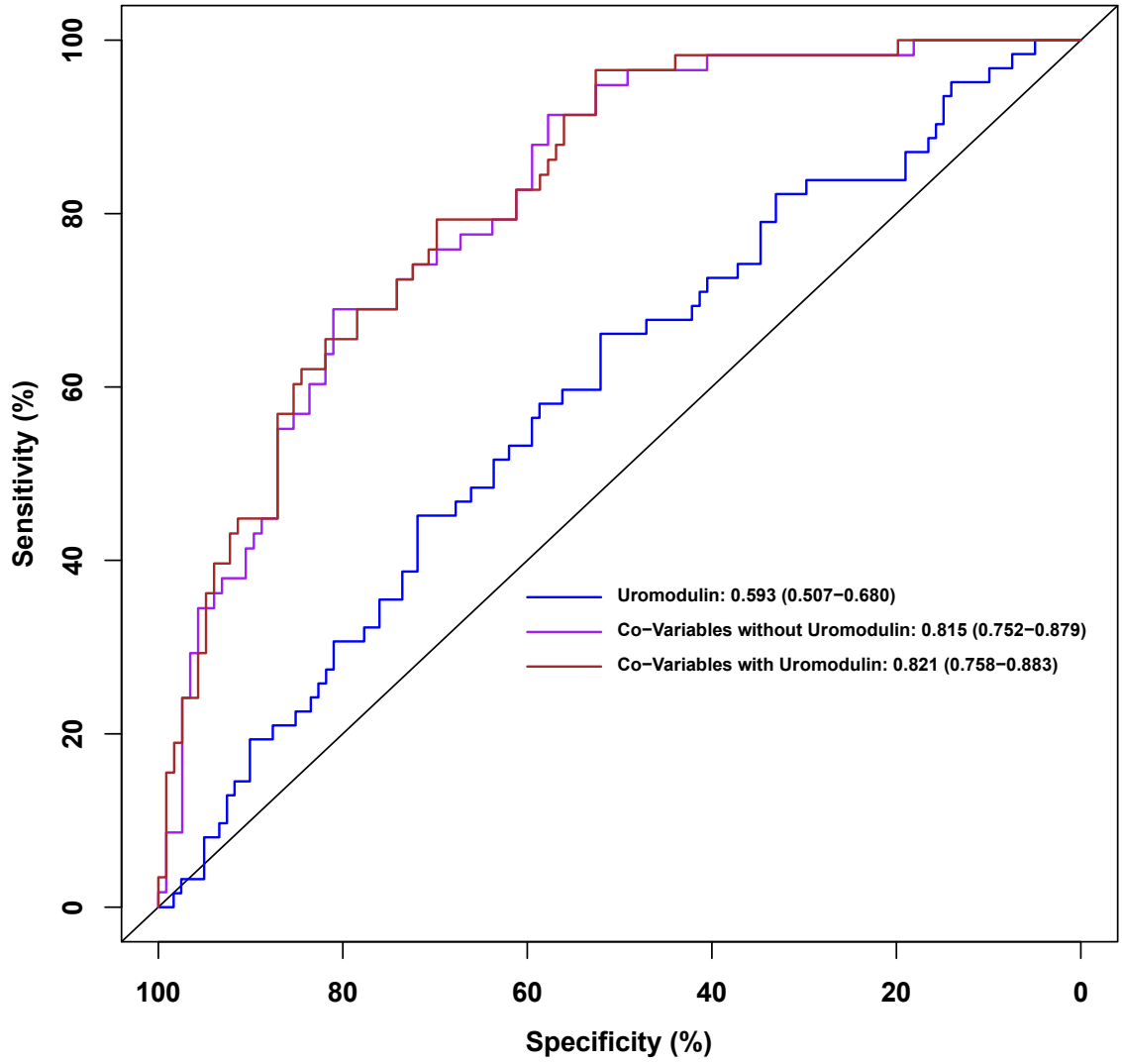

Figure 1: Receiver-Operator-Characteristic curves (ROC) for regression analyses with serum Uromodulin (sUmod) at post-transplant day 1 as predictor for delayed graft function (DGF). Co-variables include recipient age, recipient gender, recipient BMI, recipient diabetes mellitus, eGFR (CKD-EPI), cold ischemic time and living donor transplantation.

In the ROC curve analyses shown in Figure 2 the model with the sUmod ratio (post-transplant day 1/pre-transplant) as a singular predictor works moderately well to predict DGF (AUC 0.695 [95%-CI 0.612–0.778]). Again, when a multivariable model (co-variables alone as predictor or co-variables additionally to the sUmod ratio as predictor) is used, the predictive accuracy for DGF-prediction is increased (AUC 0.815 [95%-CI 0.752–0.879|, AUC 0.830 [95%-CI 0.768–0.891]). Also, now the two multivariable models show a quite similar predictive accuracy, while the AUC of the univariable model (with the sUmod ratio as a singular predictor for DGF) is significantly smaller.

Figure 2: ROC curves for regression analyses sUmod ratio (post-transplant day 1/pre-transplant) and DGF

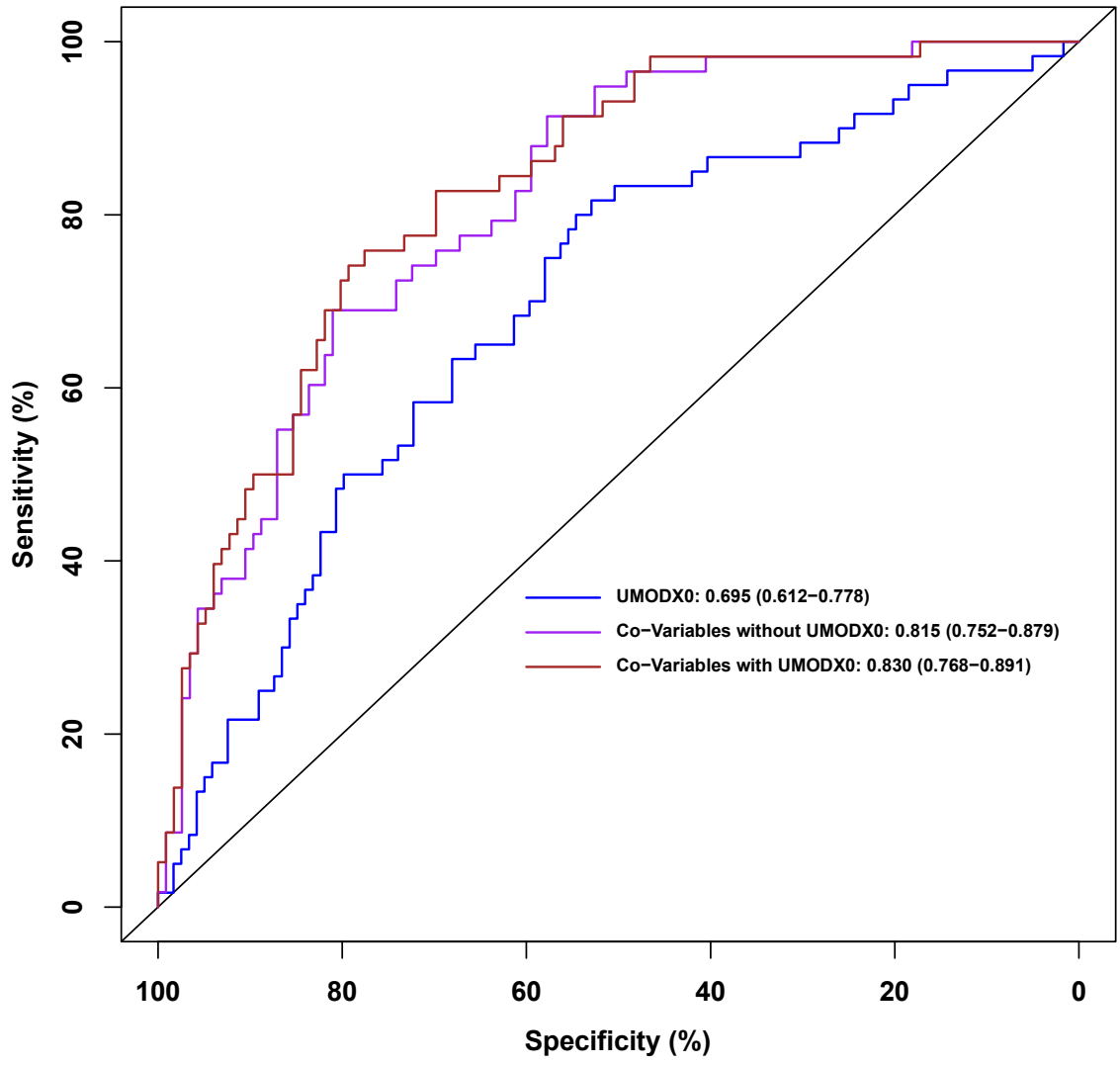

Figure 2: Receiver-Operator-Characteristic (ROC) curves for regression analyses with serum Uromodulin (sUmod) ratio (post-transplant day 1/pre-transplant) as a predictor for delayed graft function (DGF). Co-variables include recipient age, recipient gender, recipient BMI, recipient diabetes mellitus, eGFR (CKD-EPI), cold ischemic time and living donor transplantation.

In the ROC curve analyses shown in Figure 3 the model with sUmod at post-transplant day 1 as a solitary predictor performs worse (AUC 0.534 [95%-CI 0.398–0.669]) than if multivariable models are (with co-variables as predictor or with co-variables additionally to sUmod as predictor) used as predictor for acute tubular injury (AUC 0.620 [95%-CI 0.503–0.736], AUC 0.628 [0.511–0.745]). The two mentioned multivariable models show a very similar AUC with a slightly larger AUC for the model with co-variables and additionally sUmod as predictor.

Figure 3: ROC curves for regression analyses sUmod post-transplant day 1 and severe acute tubular injury

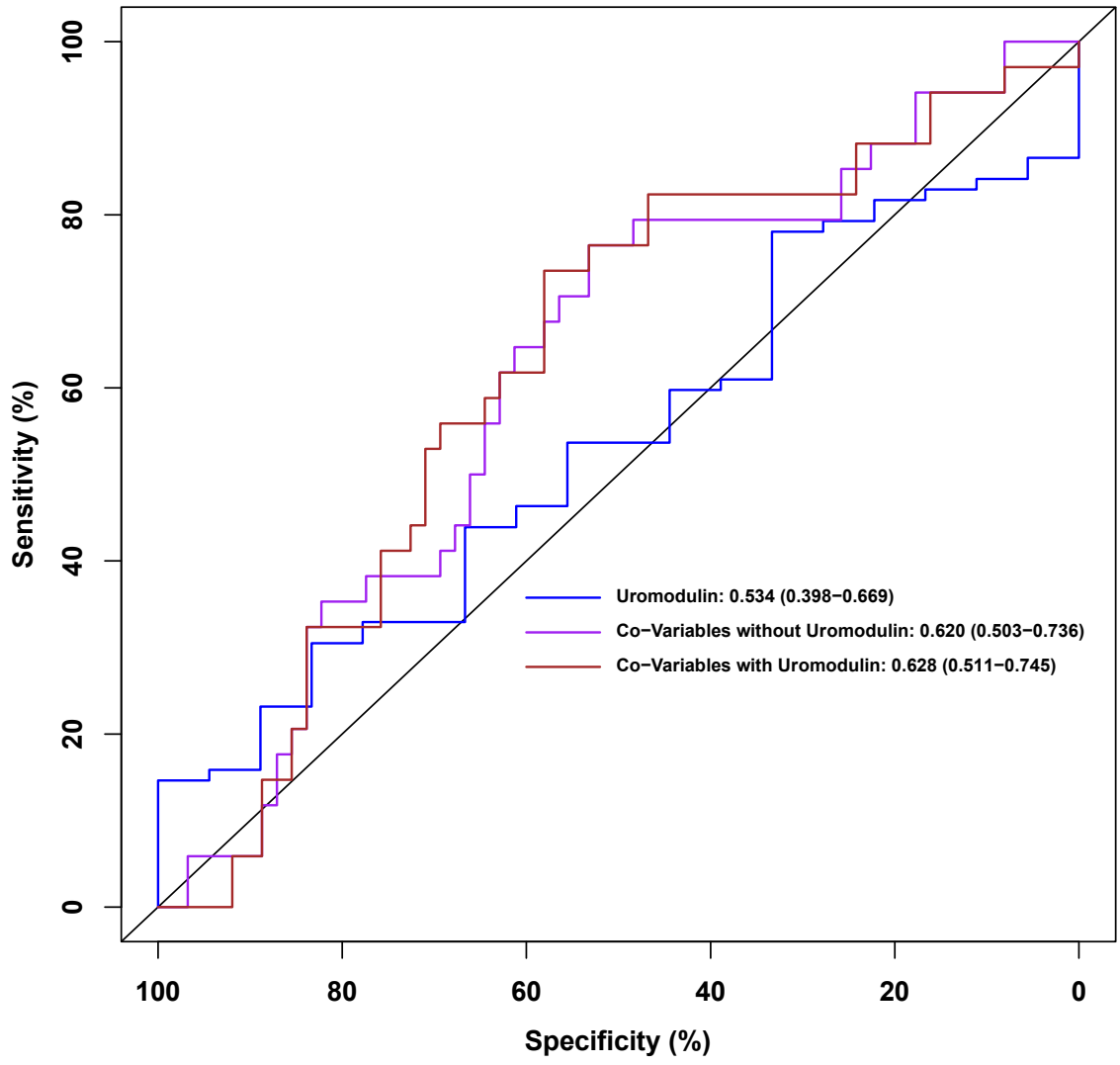

Figure 3: Receiver-Operator-Characteristic (ROC) curves for regression analyses with serum Uromodulin (sUmod) on post-transplant day 1 as predictor and severe acute tubular injury in zero-time biopsy as outcome. Co-variables include cold ischemic time, living donor transplantation, donor age, donor gender, donor diabetes mellitus and last donor creatinine level.

Lastly ROC curve analyses depicted in Figure 4 show that again the sUmod ratio as predictor alone does not very well predict acute tubular injury (AUC 0.534 [95%-CI 0.387–0.681]), while the two multivariable models show greater predictive accuracy. The AUC of the multivariable model with co-variables additionally to the sUmod ratio as predictor provides a greater AUC (AUC 0.650 [95%-CI 0.533–0.767]) than the multivariable model with co-variables alone as predictor (AUC 0.620 [95%-CI 0.503–0.736]).

Figure 4: ROC curves for regression analyses sUmod ratio (post-transplant day 1/pre-transplant) and acute tubular injury

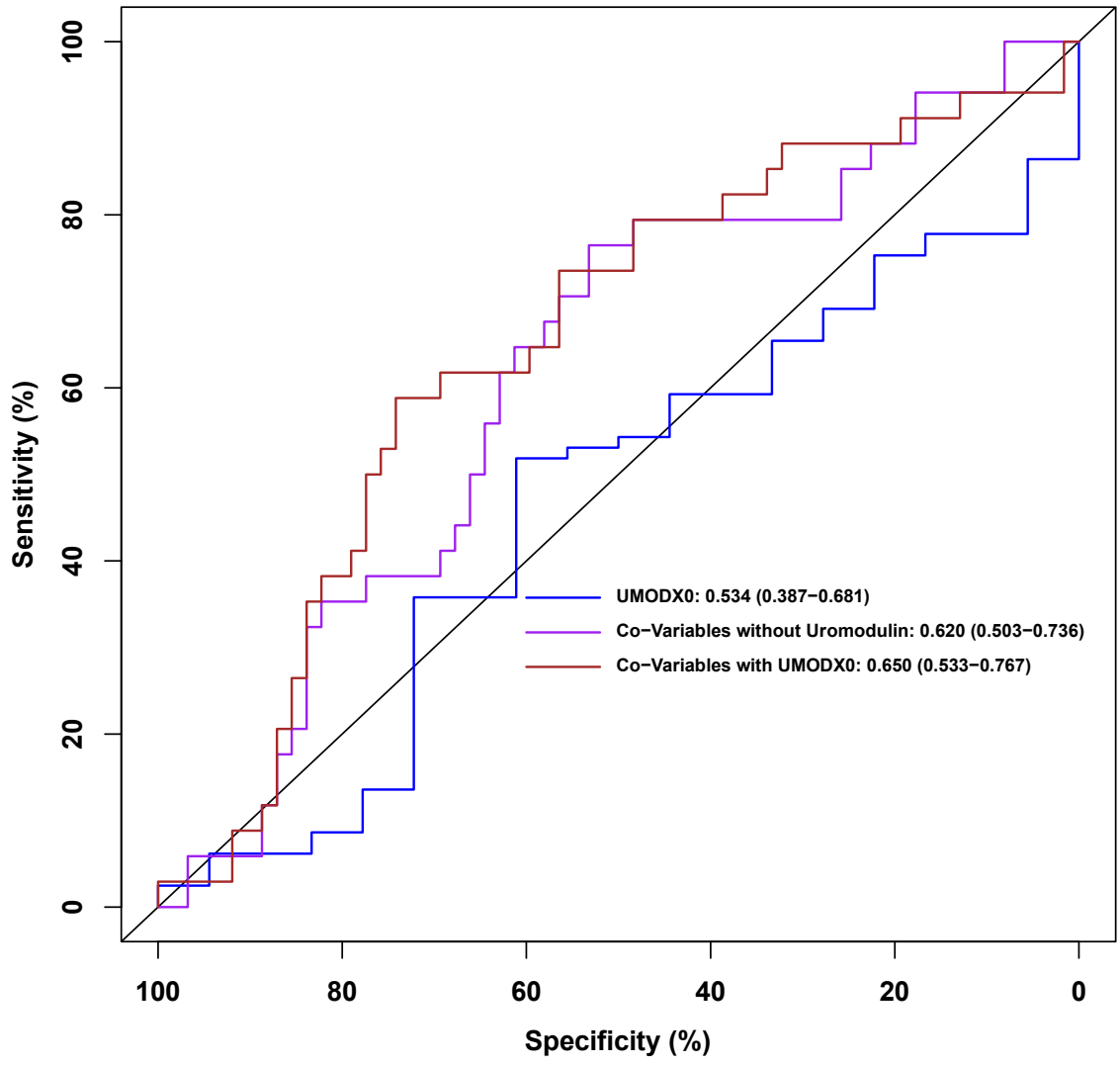

Figure 4: Receiver-Operator-Characteristic (ROC) curves for regression analyses with serum Uromodulin (sUmod) ratio (post-transplant day 1/pre-transplant) as a predictor and sever acute tubular injury in zero-time biopsy as outcome. Co-variables include cold ischemic time, living donor transplantation, donor age, donor gender, donor diabetes mellitus and last donor creatinine level.

4. Discussion:

4.1 Discussion of main results

Generally speaking, this study shows that sUmod could be a reliable marker for renal and transplant function. In the pre-transplant as well as in the medium-/long-term post-transplant phase it, broadly speaking, behaves like already established markers for renal function such as serum creatinine (higher sUmod levels correlate with better renal/graft function, lower retention markers etc.). However, in the short-term post-transplant context sUmod's role seems to be more complicated, presumably due to its immunological function and the immunological/inflammatory processes taking place at that time.

Regarding hypothesis four the results show that different demographic and laboratory parameters related to kidney function (respectively functional renal mass) such as pre-transplant nephrectomy, residual diuresis, dialysis vintage and dialysis modality, are significantly associated with early post-transplant sUmod increase (ratio post-transplant day 1/pre-transplant). Pre-transplant nephrectomy and lower residual diuresis are shown to be significantly associated with a higher increase in sUmod at first day post-transplant. Already previous research has demonstrated that systemic Umod levels positively correlate with renal function and functioning nephron mass [6, 65, 66]. Furthermore, pre-transplant PD as dialysis modality is significantly associated with a smaller increase in sUmod, while HD is significantly associated with a higher sUmod increase. This can be explained by the fact that PD better preserves residual kidney function than HD [76]. Concerning pre-transplant dialysis also longer dialysis vintage is shown to be significantly associated with a higher increase in sUmod, which is comprehensible when considering that kidney function declines over time being on dialysis and that systemic Umod positively correlates with functioning tubular mass [66, 76]. At first sight it seems surprising that this study shows a significant association between lower eGFR (CKD-EPI) at first day post-transplant and higher sUmod increase, as in general a positive correlation between systemic Umod levels and kidney function has to be assumed [65]. Yet this inverse relationship makes sense in the early post-transplant phase when immunological and inflammatory processes occur, taking El-Achkar et al.'s in 2008 published research into account [55]. They have shown that Tamm-Horsfall protein levels increase in ischemic injury and also other past research has demonstrated that Umod is involved in immunological and inflammatory processes [54, 55, 77]

Parameters related to inflammatory/immunological processes, such as cold ischemic time and DGF are shown to be significantly associated with

sUmod increase in the immediate post-transplant phase (1st day). Longer cold ischemic time, representing a greater ischemic stimulus, as well as DGF, essentially representing the clinical manifestation of ischemia reperfusion, are significantly associated with a higher increase in sUmod from pre-transplant to the first day post-transplant. Already past research has shown evidence on sUmod's protective role in ischemic kidney injury as well as its relevance as mediator in immune processes. [23, 55, 63, 77]

Although, this study demonstrates that higher pre-transplant sUmod levels in kidney transplant recipients are significantly associated with a lower DGF-risk, no significant correlation between sUmod levels at first day post-transplant and DGF was found. When comparing predictive models, sUmod at post-transplant day 1 increased the accuracy of DGF-prediction when being added to a model of established DGF-risk factors. Interestingly a significant positive association between the sUmod ratio (post-transplant day 1/pre-transplant) and DGF was found in the regression analysis. Also, the predictive accuracy increased when the sUmod ratio was added to established DGF-risk factors.
According to current knowledge, no study which examines the association between serum Umod pre-transplant or precisely at first post-transplant day and DGF as its main issue has been published before. Yet, Scherberich et al. had shown in 2018 that sUmod measured in renal transplant recipients rises to higher post-transplant levels and could possibly distinguish between immediate and delayed graft function [65]. Taking into account that higher plasma Umod levels indicate a greater amount of functioning nephron mass, that Umod is synthesized by tubular cells as well as the fact that DGF is above all the result of ischemia reperfusion injury and tubular cells are especially susceptible to ischemia, it is comprehensible hat this study shows a significant association between higher sUmod levels pre-transplant and lower DGF rates [33-36, 63, 66]. In line with the before mentioned, higher sUmod levels could imply a greater 'buffer' for ischemic injury until the threshold when ischemia reperfusion injury becomes manifest (in the form of DGF), is reached. Considering, that DGF primarily results from ischemia reperfusion injury which pathophysiologically involves the activation of the innate and adaptive immune system and that Umod is a relevant mediator in immunological processes, it seems surprising that this study could not show a significant association between sUmod levels at first day post-transplant and DGF [23, 63, 77]. A possible explanation might be that significance could not be reached due to under powering and with a larger sample size significance would be reached, as the basic trend is already evident now. However, why is the sUmod ratio (first day post-transplant/pre-transplant) significantly associated with DGF? El-Achkar et al. showed in 2008 in a murine model that Umod expression is increased

after ischemia and suggested that this could have a protective function [55]. Higher pre-transplant sUmod levels indicate a greater functioning nephron mass, which in turn leads to a greater, possibly protective physiological, increase in sUmod after ischemia [55, 66].

No significant association between post-transplant (day 1) sUmod and severe acute tubular injury in the zero-time biopsy could be found. On the other hand, a 10-unit increase in the sUmod ratio (post-transplant day 1/pre-transplant) was significantly associated with the occurrence of severe acute tubular injury. The post-transplant (day 1) sUmod level respectively the sUmod ratio (post-transplant day 1/pre-transplant) was of additional predictive value, when being added to a model of established risk factors for acute tubular injury.
Two interesting studies by El-Achkar and his colleagues have been published, which showed similar results [55, 56]. In their 2008 published work they showed in a murine model that 15min-clamping of the renal pedicles resulted in a profound increase in Tamm-Horsfall protein (THP) [55]. Also, their 5 years later published work showed THP changes after an ischemic stimulus was applied to mice kidneys [56]. Even though THP mRNA was significantly downregulated 24h post-ischemia, the serum THP levels were significantly increased and also the location of THP detection in the kidney was shifted from apical to basolateral and consequently to the interstitial and vascular space (while polarity of the TAL cells was maintained) [56]. They also showed that THP might be important for suppression of inflammation after ischemia and thereby for a quick recovery after ischemic acute kidney injury [56]. Having all these results in mind it seems conclusive that an increase in the sUmod ratio (POD1/pre-transplant) is significantly associated with sever acute tubular injury (with tubular cells being especially prone to ischemic damage) in the biopsy taken about 10min after reperfusion. A conceivable explanation for the insignificant association of sUmod at first post-transplant day and severe acute tubular injury, might be that sUmod changes after ischemia in a highly dynamic fashion, so it might already be relevant if sUmod was measured e.g. 2h or 12h post-transplant. The exact timing of serum sampling was not defined more precisely than “first day post-transplant”, meaning quite heterogeneous time points are possible.

Furthermore, a significant positive relationship between sUmod level 30-120 days post-transplant and eGFR (CKD-EPI) 1 year post-transplant was found. sUmod 30-120 days post-transplant could significantly predict 1-year-eGFR, independent of recipient age, recipient gender, recipient BMI and recipient diabetes. Interestingly, no significant linear relationship between the sUmod ratio (30-120 days post-transplant/pre-transplant) and 1-year-eGFR could be found.

The 1-year-eGFR is frequently used as one of the standard parameters for assessing the success of kidney transplantation. Up to date no data on the association between post-transplant sUmod and 1-year-eGFR is available. Yet it was shown that in chronic kidney disease patients and healthy controls systemic Umod positively correlates with eGFR, systemic Umod represents a solid biomarker of kidney function and enables the identification of early stages of chronic kidney disease when established markers of kidney function fail to do so (within “creatinine blind range”) [65, 66, 78, 79].
Returning to the kidney transplant setting sUmod has been studied as a predictor of allograft failure/loss. Bostom et al. had shown in 2018 a significant association between baseline sUmod and risk of allograft failure (lower baseline sUmod associated with greater allograft failure risk) [80]. Two years before, Steubl and his colleagues published evidence that sUmod measured 1-3 months post-transplant can predict graft loss at least equivalently to conventional biomarkers [81]. Up to date no single biomarker has been identified which can predict long-term graft function (especially eGFR 1 year post-transplant) alone with a significant accuracy and feasible applicability. It seems that this goal might only be reached by using predictive models which include a whole biomarker panel rather than a singular biomarker. Yet robust and reliable biomarkers are needed to be part of such panels and sUmod might be one of them.

4.2 Strength and Limitations

One of this study's major strengths is the use of serum Uromodulin as the main predictor in this analysis. sUmod shows exceptional stability, while urinary Uromodulin is only poorly stable and therefore urinary measurements of Uromodulin are problematic with regards to reproducibility and reliability [64, 65]. Also due to extremely reduced or even absent residual diuresis in transplant recipients at the pre-transplant time urinary measurements would have probably reduced the sample size significantly, while serum measurements are broadly available (and independent of residual diuresis) in end-stage kidney disease patients. Additionally circulatory Uromodulin seems to outperform Uromodulin measured in urine concerning correlation with glomerular filtration rate [66].

Furthermore, data on Uromodulin (especially circulatory Uromodulin) in the transplant setting is rare and this study is up to date the first one evaluating sUmod's association with severe acute tubular injury in zero-time biopsies as well as 1-3 months post-transplant sUmod and its association with 1-year graft function.

A general aspect, which increases the relevance of this study is the prevailing shortage of donor-organs in Germany [11]. As already stressed above these circumstances make it necessary to improve the outcome of those transplants that are actually transplanted. Therefore, research on potential renal biomarkers, such as Uromodulin, for monitoring graft outcome is urgently needed. Conclusively this study's topic/relevance should also be regarded as a major strength of this work.

One limitation of this study is the moderate sample size. While the total collective had a quite reasonable size, exclusion of patients had to be done for the individual analyses. Especially, the analysis involving histological evaluation could only use a rather small number of samples, since zero-time biopsy is no standard procedure in renal transplantation at Klinikum Rechts der Isar and some biopsies had to be excluded by the external specialists (who assessed them) due to inadequate quality. Reasons for exclusion of histological samples were for instance that the biopsy-section was not representative for the whole kidney (defined criteria) or that the biopsy section did not include renal tissue at all (instead e.g. only fatty tissue). Small sample sizes often lead to the problem of statistical underpowering. This is especially relevant when the investigated association is rather weak (but still true and relevant). As only a limited number of transplantations are done per hospital/transplant-center and also not all of the patients consent to participating in such a study, it is difficult to conduct large studies in the field of transplantation medicine. A possible solution would be to conduct a multi-center study by cooperating with other transplant centers and thereby achieving a greater sample size. However, this is associated with great logistic and organizational effort.

A further limitation is the already beforehand briefly mentioned issue of defining delayed graft function. In this study DGF was solely defined by receiving dialysis treatment within the first week post-transplant. Despite being the most frequently used definition by other researchers, it neither takes the reasons for dialysis (e.g. hyperkalemia, rejection) into account, nor includes a standardized manual for the indication of dialysis treatment. Indication for dialysis is often an individual decision, especially in very sick, multimorbid patients. Due to considerable inconsistency in defining DGF and due to possibly differing criteria for indication of dialysis treatment among different hospitals, it remains difficult to compare studies using DGF as an outcome [21]. Yet DGF should not be neglected in research as it is a frequent phenomenon, which significantly influences the outcome after renal transplantation [20]. Rather a universal definition respectively a gold standard in the diagnosis of delayed graft function is needed [21].
Moreover, the fact that the cohort consisted exclusively of Caucasians limits the generalizability of the results. Past studies have shown that there are differences in the risk of renal graft adverse outcomes among different ethnicities [58, 82]. Such adverse outcomes include among others DGF, which is a major parameter in the present study's analysis [82]. Non-Caucasian was no specific exclusion criterion. It was rather a coincidence that only Caucasians were transplanted and investigated at the time the study's data was collected.
A limitation of the descriptive analysis is the circumstance that no multivariable adjustment was done. This has to be considered when interpreting these results, since multivariable adjustment would have changed the level of significance.
Lastly, up to date it still remains unclear how sUmod is eliminated and if it is surely not at all glomerularily filtered, as one might expect when taking the protein size into account. It certainly should be mentioned that not only the production but also the elimination of Uromodulin could influence serum levels. To elucidate the molecular mechanisms of this glycoprotein's elimination/degradation further studies have to be conducted.

5. Summary and Conclusion:

5.1 Background

Viewed globally CKD is a frequent illness related to a significant economic and social burden [4, 7]. Terminal CKD can be treated by renal replacement therapy namely dialysis or kidney transplantation, the latter representing the optimal therapy [8-10]. Since several years Germany has to deal with a pronounced discrepancy between organ supply and demand resulting in a significant shortage of donor organs [11].

Therefore, it is important to improve the success rates of those transplantations which are carried out. For this, careful allograft surveillance is inevitable. Up to date the standard monitoring tools are eGFR and transplant biopsy, which are both not ideal due to various limiting factors (for eGFR e.g. 'creatinine-blind-range' and influence of muscle mass, for biopsy e.g. invasiveness with bleeding and infection risk as well as lacking feasibility for sequential evaluation) [63, 83]. For monitoring graft function practical and reliable biomarkers are needed. Uromodulin, an approx. 85-kDA large protein, was first described in 1950 by Igor Tamm and Frank Horsfall and was rediscovered 35 years later by Muchmore and Decker [34, 42]. It seems promising in the context of graft function monitoring as it is exclusively expressed in renal tissue, shows good stability in serum samples, is associated with renal function and able to detect early decline in renal function in CKD patients when established markers fail to do so. [33-39, 64-66]

Besides these, Uromodulin has versatile other functions (e.g. in electrolyte and water homeostasis, immune modulation, defense of urinary tract infections [42, 45-47, 50, 51].

The major outcome examined in this study was delayed graft function (DGF), which can essentially be regarded as a pronounced form of acute kidney injury resulting from ischemic reperfusion injury [63]. DGF exerts significant negative effects on patient and graft survival [19]. There is no uniform definition of DGF [20, 21]. For this study the most frequently one, namely the need of dialysis within the first post-transplant week, was chosen [21].

5.2 Research questions

The main objective of this work was to examine the association between sUmod and DGF as well as sUmod's predictive value for DGF. Furthermore, the association between acute tubular injury (ATI) in zero-time biopsy and sUmod should be clarified. Moreover, the association between sUmod 1-3 months post-transplant and 1-year-eGFR post-transplant should be evaluated. Lastly, the association between early post-transplant sUmod changes (sUmod ratio x/0 (x= 1st day post-transplant, 1-3 months post-transplant, 0= pre-transplant)) and various demographic as

well as laboratory parameters in transplant recipients and donors should be examined.

5.3 Patients and Methods

A retrospective single-center, observational cohort study was conducted, in which 186 patients who received kidney or simultaneous kidney-pancreas transplantation between 2007 and 2017 at Klinikum Rechts der Isar were included. Serum samples for sUmod measurements were centrifuged, aliquoted and frozen at -80°C until analysis (ELISA). For the ATI analysis 105 usable histological samples were obtained at the time of transplantation (approx. 10min after reperfusion). External specialists (Erlangen, Germany) performed histological evaluation according to a standardized protocol. Severe ATI was defined as >75% of in the sample included tubuli showing signs of acute tubular necrosis. Long-term graft function was evaluated at 1 year post-transplant as eGFR (CKD-EPI).
For evaluating the association between sUmod and demographic/laboratory parameters stratification by sUmod at different time points (pre-transplant, 1st day post-transplant, 1-3 months post-transplant, ratio x/0) was done. For all other analyses (outcomes DGF, ATI, 1-year-eGFR) regression analyses were performed. For each analysis a univariable model and multivariable models with adjustment for co-variables was done. sUmod, the independent variable, was entered in each outcome analysis once as a continuous variable and once as a categorical variable (divided into quartiles). Lastly ROC-analysis with AUC-calculation was performed for the outcomes DGF and severe ATI.

5.4 Results

Higher pre-transplant sUmod in recipients were significantly associated with a lower risk of DGF. No significant correlation between sUmod at first day post-transplant and DGF was found. sUmod at post-transplant day 1 increased the accuracy of DGF-prediction when being added to a model of established DGF-risk factors. Also, a significant association between the sUmod ratio (post-transplant day 1/pre-transplant) and DGF was found. The predictive accuracy increased when the sUmod ratio was added to established DGF-risk factors.
No significant association between post-transplant (day 1) sUmod and severe ATI in the zero-time biopsy could be found. Yet, a 10-unit increase in the sUmod ratio (post-transplant day 1/pre-transplant) was significantly associated with the occurrence of severe ATI. The post-transplant (day 1) sUmod level respectively the sUmod ratio (post-transplant day 1/pre-transplant) was of additional predictive value, when being added to a model of established risk factors for ATI.
A significant positive relationship between sUmod 30-120 days post-transplant and eGFR 1 year post-transplant was found. This was independent of recipient age, gender, BMI and diabetes. However, the

sUmod ratio (30-120 days post-transplant/pre-transplant) did not significantly correlate with eGFR 1 year post-transplant.
The analysis of the association between sUmod and demographic respectively laboratory parameters in transplant patients, generally speaking, showed that sUmod could be a reliable maker of transplant function in the pre-transplant and medium-/long-term post-transplant phase. Factors influencing or representing kidney/transplant function or functional renal mass, such as living donation, pre-transplant nephrectomy, dialysis vintage, residual diuresis, serum creatinine level or eGFR (CKD-EPI) were significantly associated with sUmod pre-transplant and early post-transplant sUmod increase. On post-transplant day 1 alone this relationship could not be shown, probably due to sUmod's role in immunological processes. In line with this for example longer cold ischemic time and higher grades of HLA mismatch were significantly associated with higher sUmod levels on first day post-transplant. Also, DGF was significantly associated with a higher increase in sUmod from pre-transplant to first day post-transplant.

5.5 Conclusion and Outlook

Serum Uromodulin is easily obtained, measured, shows good stability in serum samples, is uniquely synthesized by renal cells and shows promising potential for predicting transplant outcomes (DGF, ATI, long-term graft function) as demonstrated in this work. Therefore, sUmod might be an important part of future transplant monitoring and could help to increase the success rates of kidney transplantation by enabling a timely detection of transplant function decline and consequently offering the opportunity of early medical intervention. Thereby sUmod could be one component of improving the issue of renal transplant organ shortage in Germany.

Bibliography

1. *Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group.Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease*. 2013, International Society of Nephrology: Kidney International supplements. p. 150.
2. Hashmi, M.F., O. Benjamin, and S.L. Lappin, *End-Stage Renal Disease*, in *StatPearls*. 2023, StatPearls Publishing

Copyright © 2023, StatPearls Publishing LLC.: Treasure Island (FL).

3. Frei, U. and H.-J. Schober-Halstenberg, *Nierenersatztherapie in Deutschland, Bericht über Dialysebehandlung und Nierentransplantation in Deutschland 2006/2007*, in *QuaSi-Niere*, Q.-N. gGmbH, Editor. 2008. p. 60.
4. Hill, N.R., et al., *Global Prevalence of Chronic Kidney Disease - A Systematic Review and Meta-Analysis.* PLoS One, 2016. **11**(7): p. e0158765.
5. James, M.T., B.R. Hemmelgarn, and M. Tonelli, *Early recognition and prevention of chronic kidney disease.* Lancet, 2010. **375**(9722): p. 1296-309.
6. Jha, V., A.Y. Wang, and H. Wang, *The impact of CKD identification in large countries: the burden of illness.* Nephrol Dial Transplant, 2012. **27 Suppl 3**: p. iii32-8.
7. Bless, H.H., et al., *Status Quo Und Zukunft Der Heimdialyse*. 2014: Nomos Verlagsgesellschaft.
8. Herold, G., *Innere Medizin*. 2019, Herold: Köln. p. 640-647.
9. Hariharan, S., et al., *Improved graft survival after renal transplantation in the United States, 1988 to 1996.* N Engl J Med, 2000. **342**(9): p. 605-12.
10. Tapiawala, S.N., et al., *Delayed graft function and the risk for death with a functioning graft.* J Am Soc Nephrol, 2010. **21**(1): p. 153-61.
11. *Jahresbericht Organspende und Transplantation in Deutschland 2020*. 2021: Frankfurt/Main. p. 115.
12. Medin, C., et al., *Survival of patients who have been on a waiting list for renal transplantation.* Nephrol Dial Transplant, 2000. **15**(5): p. 701-4.
13. Gjertson, D.W. and J.M. Cecka, *Living unrelated donor kidney transplantation.* Kidney Int, 2000. **58**(2): p. 491-9.
14. Cosio, F.G., et al., *Patient survival after renal transplantation: I. The impact of dialysis pre-transplant.* Kidney Int, 1998. **53**(3): p. 767-72.

15. Sapir-Pichhadze, R., A. Young, and S. Joseph Kim, *Living donor age and kidney transplant outcomes: an assessment of risk across the age continuum.* Transpl Int, 2013. **26**(5): p. 493-501.
16. Wolfe, R.A., et al., *Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant.* N Engl J Med, 1999. **341**(23): p. 1725-30.
17. *US Renal Data System 2019 Annual Data Report: Epidemiology of Kidney Disease in the United States* Am J Kidney Dis, 2019.
18. Wong, G., et al., *Comparative survival and economic benefits of deceased donor kidney transplantation and dialysis in people with varying ages and co-morbidities.* PLoS One, 2012. **7**(1): p. e29591.
19. Siedlecki, A., W. Irish, and D.C. Brennan, *Delayed graft function in the kidney transplant.* Am J Transplant, 2011. **11**(11): p. 2279-96.
20. Perico, N., et al., *Delayed graft function in kidney transplantation.* Lancet, 2004. **364**(9447): p. 1814-27.
21. Yarlagadda, S.G., et al., *Marked variation in the definition and diagnosis of delayed graft function: a systematic review.* Nephrol Dial Transplant, 2008. **23**(9): p. 2995-3003.
22. Hoogland, E.R., et al., *Improvements in kidney transplantation from donors after cardiac death.* Clin Transplant, 2013. **27**(3): p. E295-301.
23. Salvadori, M., G. Rosso, and E. Bertoni, *Update on ischemia-reperfusion injury in kidney transplantation: Pathogenesis and treatment.* World J Transplant, 2015. **5**(2): p. 52-67.
24. Slocum, J.L., M. Heung, and S. Pennathur, *Marking renal injury: can we move beyond serum creatinine?* Transl Res, 2012. **159**(4): p. 277-89.
25. Cappuccilli, M., et al., *Neutrophil Gelatinase-Associated Lipocalin as a Biomarker of Allograft Function After Renal Transplantation: Evaluation of the Current Status and Future Insights.* Artif Organs, 2018. **42**(1): p. 8-14.
26. Maier, H.T., et al., *Prediction of delayed graft function and long-term graft survival by serum and urinary neutrophil gelatinase-associated lipocalin during the early postoperative phase after kidney transplantation.* PLoS One, 2018. **13**(1): p. e0189932.
27. Schmidt-Ott, K.M., et al., *Dual action of neutrophil gelatinase-associated lipocalin.* J Am Soc Nephrol, 2007. **18**(2): p. 407-13.
28. Yilmaz, A., et al., *Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin.* Pediatr Nephrol, 2009. **24**(12): p. 2387-92.

29. Hall, I.E., et al., *IL-18 and urinary NGAL predict dialysis and graft recovery after kidney transplantation.* J Am Soc Nephrol, 2010. **21**(1): p. 189-97.
30. Fonseca, I., et al., *Neutrophil gelatinase-associated lipocalin in kidney transplantation is an early marker of graft dysfunction and is associated with one-year renal function.* J Transplant, 2013. **2013**: p. 650123.
31. Han, F., et al., *Donor Plasma Mitochondrial DNA Is Correlated with Posttransplant Renal Allograft Function.* Transplantation, 2019. **103**(11): p. 2347-2358.
32. Lo, Y.M., et al., *Plasma DNA as a prognostic marker in trauma patients.* Clin Chem, 2000. **46**(3): p. 319-23.
33. McKenzie, J.K. and E.G. McQueen, *Immunofluorescent localization of Tamm-Horsfall mucoprotein in human kidney.* J Clin Pathol, 1969. **22**(3): p. 334-9.
34. Muchmore, A.V. and J.M. Decker, *Uromodulin: a unique 85-kilodalton immunosuppressive glycoprotein isolated from urine of pregnant women.* Science, 1985. **229**(4712): p. 479-81.
35. Bachmann, S., R. Metzger, and B. Bunnemann, *Tamm-Horsfall protein-mRNA synthesis is localized to the thick ascending limb of Henle's loop in rat kidney.* Histochemistry, 1990. **94**(5): p. 517-23.
36. Lee, J.W., C.L. Chou, and M.A. Knepper, *Deep Sequencing in Microdissected Renal Tubules Identifies Nephron Segment-Specific Transcriptomes.* J Am Soc Nephrol, 2015. **26**(11): p. 2669-77.
37. Hoyer, J.R. and M.W. Seiler, *Pathophysiology of Tamm-Horsfall protein.* Kidney Int, 1979. **16**(3): p. 279-89.
38. Kumar, S. and A. Muchmore, *Tamm-Horsfall protein--uromodulin (1950-1990).* Kidney Int, 1990. **37**(6): p. 1395-401.
39. Devuyst, O., E. Olinger, and L. Rampoldi, *Uromodulin: from physiology to rare and complex kidney disorders.* Nat Rev Nephrol, 2017. **13**(9): p. 525-544.
40. McQueen, E.G., *The nature of urinary casts.* J Clin Pathol, 1962. **15**(4): p. 367-73.
41. Fletcher, A.P., A. Neuberger, and W.A. Ratcliffe, *Tamm-Horsfall urinary glycoprotein. The chemical composition.* Biochem J, 1970. **120**(2): p. 417-24.
42. Tamm, I. and F.L. Horsfall, Jr., *Characterization and separation of an inhibitor of viral hemagglutination present in urine.* Proc Soc Exp Biol Med, 1950. **74**(1): p. 106-8.
43. Pennica, D., et al., *Identification of human uromodulin as the Tamm-Horsfall urinary glycoprotein.* Science, 1987. **236**(4797): p. 83-8.

44. Pak, J., et al., *Tamm-Horsfall protein binds to type 1 fimbriated Escherichia coli and prevents E. coli from binding to uroplakin Ia and Ib receptors.* J Biol Chem, 2001. **276**(13): p. 9924-30.

45. Bachmann, S., et al., *Renal effects of Tamm-Horsfall protein (uromodulin) deficiency in mice.* Am J Physiol Renal Physiol, 2005. **288**(3): p. F559-67.

46. Mutig, K., et al., *Activation of the bumetanide-sensitive Na+,K+,2Cl- cotransporter (NKCC2) is facilitated by Tamm-Horsfall protein in a chloride-sensitive manner.* J Biol Chem, 2011. **286**(34): p. 30200-10.

47. Renigunta, A., et al., *Tamm-Horsfall glycoprotein interacts with renal outer medullary potassium channel ROMK2 and regulates its function.* J Biol Chem, 2011. **286**(3): p. 2224-35.

48. Wiggins, R.C., *Uromucoid (Tamm-Horsfall glycoprotein) forms different polymeric arrangements on a filter surface under different physicochemical conditions.* Clin Chim Acta, 1987. **162**(3): p. 329-40.

49. Bleyer, A.J., M. Zivná, and S. Kmoch, *Uromodulin-associated kidney disease.* Nephron Clin Pract, 2011. **118**(1): p. c31-6.

50. Hession, C., et al., *Uromodulin (Tamm-Horsfall glycoprotein): a renal ligand for lymphokines.* Science, 1987. **237**(4821): p. 1479-84.

51. Säemann, M.D., et al., *Tamm-Horsfall glycoprotein links innate immune cell activation with adaptive immunity via a Toll-like receptor-4-dependent mechanism.* J Clin Invest, 2005. **115**(2): p. 468-75.

52. Micanovic, R., et al., *Tamm-Horsfall Protein Regulates Granulopoiesis and Systemic Neutrophil Homeostasis.* J Am Soc Nephrol, 2015. **26**(9): p. 2172-82.

53. LaFavers, K.A., et al., *Circulating uromodulin inhibits systemic oxidative stress by inactivating the TRPM2 channel.* Sci Transl Med, 2019. **11**(512).

54. Micanovic, R., et al., *Tamm-Horsfall Protein Regulates Mononuclear Phagocytes in the Kidney.* J Am Soc Nephrol, 2018. **29**(3): p. 841-856.

55. El-Achkar, T.M., et al., *Tamm-Horsfall protein protects the kidney from ischemic injury by decreasing inflammation and altering TLR4 expression.* Am J Physiol Renal Physiol, 2008. **295**(2): p. F534-44.

56. El-Achkar, T.M., et al., *Tamm-Horsfall protein translocates to the basolateral domain of thick ascending limbs, interstitium, and circulation during recovery from acute kidney injury.* Am J Physiol Renal Physiol, 2013. **304**(8): p. F1066-75.

57. Cole, E., et al., *An analysis of predictors of long-term cadaveric renal allograft survival.* Clin Transplant, 1995. **9**(4): p. 282-8.

58. Ojo, A.O., et al., *Delayed graft function: risk factors and implications for renal allograft survival.* Transplantation, 1997. **63**(7): p. 968-74.

59. Głyda, M., et al., *Influence of donor and recipient gender as well as selected factors on the five-year survival of kidney graft.* Pol Przegl Chir, 2011. **83**(4): p. 188-95.

60. Lee, J., et al., *The recovery status from delayed graft function can predict long-term outcome after deceased donor kidney transplantation.* Sci Rep, 2017. **7**(1): p. 13725.

61. Chawla, L.S., et al., *The severity of acute kidney injury predicts progression to chronic kidney disease.* Kidney Int, 2011. **79**(12): p. 1361-9.

62. Gwinner, W., et al., *Acute tubular injury in protocol biopsies of renal grafts: prevalence, associated factors and effect on long-term function.* Am J Transplant, 2008. **8**(8): p. 1684-93.

63. Schröppel, B. and C. Legendre, *Delayed kidney graft function: from mechanism to translation.* Kidney Int, 2014. **86**(2): p. 251-8.

64. Youhanna, S., et al., *Determination of uromodulin in human urine: influence of storage and processing.* Nephrol Dial Transplant, 2014. **29**(1): p. 136-45.

65. Scherberich, J.E., et al., *Serum uromodulin-a marker of kidney function and renal parenchymal integrity.* Nephrol Dial Transplant, 2018. **33**(2): p. 284-295.

66. Steubl, D., et al., *Plasma Uromodulin Correlates With Kidney Function and Identifies Early Stages in Chronic Kidney Disease Patients.* Medicine (Baltimore), 2016. **95**(10): p. e3011.

67. Al Otaibi, T., et al., *Delayed Graft Function in Living-Donor Kidney Transplant: A Middle Eastern Perspective.* Exp Clin Transplant, 2016. **14**(1): p. 1-11.

68. Levey, A.S., R.D. Perrone, and N.E. Madias, *Serum creatinine and renal function.* Annu Rev Med, 1988. **39**: p. 465-90.

69. Lechevallier, E., et al., *Posttransplantation acute tubular necrosis: risk factors and implications for graft survival.* Am J Kidney Dis, 1998. **32**(6): p. 984-91.

70. Fekete, A., et al., *Sex differences in heat shock protein 72 expression and localization in rats following renal ischemia-reperfusion injury.* Am J Physiol Renal Physiol, 2006. **291**(4): p. F806-11.

71. Muratsubaki, S., et al., *Suppressed autophagic response underlies augmentation of renal ischemia/reperfusion injury by type 2 diabetes.* Sci Rep, 2017. **7**(1): p. 5311.

72. Salvadori, M., et al., *Estimated one-year glomerular filtration rate is the best predictor of long-term graft function following renal transplant.* Transplantation, 2006. **81**(2): p. 202-6.
73. Klein, R., et al., *Transplantation with kidneys retrieved from deceased donors with acute renal failure.* Transplantation, 2013. **95**(4): p. 611-6.
74. Eide, I.A., et al., *Associations Between Posttransplantation Diabetes Mellitus and Renal Graft Survival.* Transplantation, 2017. **101**(6): p. 1282-1289.
75. Huang, Y., et al., *Understanding Trends in Kidney Function 1 Year after Kidney Transplant in the United States.* J Am Soc Nephrol, 2017. **28**(8): p. 2498-2510.
76. Jansen, M.A., et al., *Predictors of the rate of decline of residual renal function in incident dialysis patients.* Kidney Int, 2002. **62**(3): p. 1046-53.
77. Wu, T.H., et al., *Tamm-Horsfall Protein is a Potent Immunomodulatory Molecule and a Disease Biomarker in the Urinary System.* Molecules, 2018. **23**(1).
78. Risch, L., et al., *The serum uromodulin level is associated with kidney function.* Clin Chem Lab Med, 2014. **52**(12): p. 1755-61.
79. Fedak, D., et al., *Serum uromodulin concentrations correlate with glomerular filtration rate in patients with chronic kidney disease.* Pol Arch Med Wewn, 2016. **126**(12): p. 995-1004.
80. Bostom, A., et al., *Serum Uromodulin: A Biomarker of Long-Term Kidney Allograft Failure.* Am J Nephrol, 2018. **47**(4): p. 275-282.
81. Steubl, D., et al., *Serum uromodulin predicts graft failure in renal transplant recipients.* Biomarkers, 2017. **22**(2): p. 171-177.
82. Kwan, J.M., et al., *Donor and Recipient Ethnicity Impacts Renal Graft Adverse Outcomes.* J Racial Ethn Health Disparities, 2018. **5**(5): p. 1003-1013.
83. Sirolli, V., L. Piscitani, and M. Bonomini, *Biomarker-Development Proteomics in Kidney Transplantation: An Updated Review.* Int J Mol Sci, 2023. **24**(6).

List of tables and figures

Acknowledgements

Special thanks go to my mentor PD Dr. med. Dominik Steubl whose professional advice and assistance helped me to realize this project. Thank you for your honest feedback and constructive criticism. Furthermore, I would also like to thank my supervisor Prof. Dr. med. Christoph Schmaderer for giving me the opportunity to conduct this work in his working group and under his supervision.
Finally, I would like to deeply thank my family. My boyfriend owes special thanks. Without your support and encouragement in the past years this would not have been possible.