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Early prediction of graft outcomes after kidney transplantation from donors after circulatory death: biomarkers and transplantation characteristics

Anne-Sophie Truche^{1,4} M.D., Candice Trocme² M.D., Sabrina Vergnaud² M.D., Bénédicte Janbon¹ M.D., Diane Giovannini⁵ M.D., Paolo Malvezzi¹ M.D., Xavier Moreau-Gaudry³ M.D., Lionel Rostaing^{1,4} M.D. PhD., Rachel Tetaz¹, M.D.

¹Nephrology Hemodialysis Apheresis, and Kidney Transplantation, Grenoble University Hospital, La Tronche, France

²Department of Biochemistry Toxicology and Pharmacology, Grenoble University Hospital, La Tronche, France

³Grenoble Dialysis Association AGDUC, La Tronche, France

⁴Université Grenoble-Alpes, France

⁵Laboratory of histopathology, Grenoble University Hospital, La Tronche, France

Author email addresses: astruche@chu-grenoble.fr ; ctrocme@chu-grenoble.fr ; svergnaud@chu-grenoble.fr ; bjanbon@chu-grenoble.fr ; dgiovannini@chu-grenoble.fr ; pmalvezzi@chu-grenoble.fr ; xmoreau-gaudry@agdud.com ; rtetaz@chu-grenoble.fr

Authors' contributions:

Study design: A-S. Truche, R. Tetaz

Biological measurements: C. Trocme, S. Vergnaud

Data collection: A-S. Truche

Statistical analysis: A-S. Truche, X. Moreau-Gaudry

Paper redaction: A-S. Truche, R. Tetaz , L. Rostaing

Patients' care: B. Janbon, P. Malvezzi, R. Tetaz

Histopathology reading: D. Giovannini

Critical review of the paper: all

Corresponding author

Pr Lionel Rostaing

CHU Grenoble-Alpes

Avenue Maquis du Grésivaudan

38700 La Tronche

Tel: +33 4 76 76 55 23

E-mail: lrostaing@chu-grenoble.fr

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Abbreviations

AUC, area under the curve

CKD-EPI, Chronic Kidney Disease - Epidemiology Collaboration

DBD, donor after brain death

DCD, donor after circulatory death

DGF, delayed graft function

DSA, donor-specific antibody

HLA, human leukocyte antigen

IGFBP7, insulin-like growth factor-binding protein 7

KT, kidney transplantation

LD, living donor

ROC, receiver operating characteristics

sLDH, serum lactate dehydrogenase

TIMP2, tissue inhibitor of metalloproteinases 2

uNAG, urinary N-acetyl beta-D glucosaminidase

uNGAL, urinary neutrophil gelatinase-associated lipocalin

Introduction

A shortage of kidney allografts has resulted in extending the pool of potential donors, i.e. for example living-kidney transplantation to non-related donors, and organs from donors after circulatory death (DCD), which has been made possible by the development of hypothermic-perfusion machines that improve organ preservation. Kidney transplantation using DCDs has shown similar results to those from donors after brain death (DBD) in terms of patient- and graft-survival rates [1], and is associated with enhanced survival compared to patients that remain on dialysis while awaiting a DBD [2]. However, DCD recipients suffer from a much higher rate of delayed graft function (DGF) [3].

DGF is defined as a requirement for dialysis in the first week post-transplantation [4] and is a risk factor in DBD-kidney transplantation (DBD KT) for acute and chronic allograft rejection [5] and decreased long-term graft function [6]. However, the outcome following DGF in DCD KT is uncertain [7]. DGF is mainly caused by ischemic reperfusion injury and its severity depends on factors linked to the donor, the recipient, and graft-storage characteristics (8).

Patients with more serious kidney lesions caused by ischemic reperfusion injury need to be identified to optimize their management: this is a major early post-transplantation objective. The use of baseline characteristics alone (donor age, serum creatinine at graft removal, cold-ischemia time) has limited accuracy to predict early and late allograft outcomes. Unfortunately, serum creatinine cannot be considered as a perfect marker for kidney injury.

An increase in serum creatinine is an indication of extensive kidney damage, whereas tubular damage alone, without subsequent renal dysfunction, is associated with a worse prognosis [9]. In addition, the increase in serum creatinine is always delayed with regards to the injury occurrence: therefore it can be used to diagnose rather than predict DGF [10].

Early biomarkers are needed to improve predicting graft outcomes. Specific urinary markers of tubular injury could be of potential interest. Among these markers, neutrophil gelatinase-

associated lipocalin (NGAL) has been the most widely studied in living-donors (LD) and DBD KT [11]. N-acetyl β -D glucosaminidase (NAG), a lysosomal enzyme, could also be potentially useful for predicting graft outcome [12]. However, these markers have not been used routinely; in patients with DCD KT, the prognostic use of these markers has not been investigated. Two urinary biomarkers for cell-cycle arrest, the tissue inhibitor of metalloproteinases 2 (TIMP2) and insulin-like growth-factor binding protein 7 (IGFBP7), have shown promising results in an acute kidney-injury setting and could be transposed to kidney transplantation [13-15]. Finally, serum lactate dehydrogenase (sLDH), a non-specific marker for cell injury has been little studied following kidney transplantation, despite the availability of a cheap test.

The aim of this study was to identify the prognostic performance of injury-associated urinary biomarkers and sLDH to predict DGF and subsequent 3-month persistent graft dysfunction in patients with a DCD KT. In order to assess the association between these markers and ischemia-reperfusion injuries, values were compared with those from patients with a LD KT (“gold standard”) performed within the same period.

Methods

Study design and population

This was a prospective, observational, single-center cohort study. Fifty-four consecutive patients receiving either LD KT or DCD KT between February 2014 and July 2016 in our center were included in this study. Exclusion criteria were patients aged <18 years and ABO and/or human leukocyte antigen (HLA) incompatible KT. Six patients with LD KT had major graft dysfunction in the early post-transplant period (i.e. within less than three months posttransplant) secondary to complications: thus, they could no longer be considered as reference KT patients and their data were excluded (of the six: one died, two had graft-artery

stenosis, one had acute kidney injury, one had graft rejection on day 11 post-transplant, and one had recurrence of nephropathy). The study was performed in accordance with the ethical standards laid down in the 2000 Declaration of Helsinki and the Declaration of Istanbul 2008. Each included participant signed an informed-consent form before enrollment. This study is an ancillary study to another that aims to identify the urinary proteomic profile of the accommodation phenomenon in the presence of donor-specific antibodies (DSAs) among KT recipients (Grenoble Institutional Review Board number 6705).

DCD KT

All patients, except one, received a Maastricht II category graft; the exception was Maastricht III category [16]. Inclusion and exclusion of both donors and recipients followed the French Biomedicine Agency protocols [17]. Normothermic regional circulation was used in all cases. All kidneys were subsequently machine perfused (LifePort® Kidney Transporter 1.1, Organ Recovery Systems) and kidneys with end-perfusion resistive indices greater than 0.3 mmHg/mL/min were discarded [18]. No routine biopsy was performed on the graft before transplantation.

Management of patients and follow-up

In our center, standard immunosuppression consisted of induction treatment using anti-thymocyte globulin (DCD KT and LD KT) or basiliximab (in some LD KT), with a maintenance immunosuppressive regimen consisting of calcineurin inhibitors (tacrolimus) introduced at day 4, and mycophenolate mofetil and prednisone at day 0. During the first month following KT, a trough tacrolimus concentration of 8--12 µg/L and then 5--8 µg/L was targeted.

After discharge of a patient, follow-up visits were scheduled at day 15, at 6 weeks, and at 3 months. A graft biopsy was systematically performed at 3 months to detect any subclinical acute rejection. All biopsies were scored according to the semi-quantitative 2013--2015 Banff classification [19]. Routine urine and serum biological monitoring was performed according to our clinical protocol.

Data collection

Demographic data (patient gender, age, initial nephropathy, body-mass index, prior anti-HLA sensitization), KT characteristics (donor age and serum creatinine, cold-ischemia time, resistive indices when placing the kidney on a perfusion machine and at 30 minutes after perfusion on that machine, number of HLA mismatches), and patient evolution (dialysis requirement, presence of *de novo* DSA, biopsy results at 3 months, BK virus infection) were recorded.

Serum creatinine, sLDH, urinary creatinine, uNGAL and uNAG were measured at days 1 and 3, and uNGAL and serum creatinine were assessed at day 7, at 6 weeks, and at 3 months. The initial protocol did not include urinary TIMP2 and IGFBP7, which were added after patient inclusion as this was considered to be a novel but promising biomarker [20]. Serum creatinine, LDH, and urine creatinine analyses were performed on the sample day, whereas urine samples for the other markers were centrifuged at room temperature for 10 min at 2000g, then decanted, aliquoted, frozen, and stored at -20°C. uNGAL was measured using an ELISA test (Human NGAL ELISA kit 036CE, Bioporto Diagnostics, Hellerup, Denmark), uNAG was measured using a colorimetric test (10875406001 kit, Roche Life Science), and [TIMP2]·[IGFBP7] was measured using a sandwich immunoassay technique (NephroCheck™, Astute Medical, San Diego, CA, USA). uNAG and uNGAL are presented as a ratio, i.e., they were divided by the urinary creatinine value.

Outcomes

The primary outcome was the occurrence of DGF, defined as the requirement of at least one dialysis session within the first week post-transplantation.

Secondary outcomes were: persistent kidney dysfunction at 3 months in DCD KT patients, defined as an estimated glomerular filtration rate $<45 \text{ mL/min/1.73 m}^2$ (Chronic Kidney Disease - Epidemiology Collaboration (CKD-EPI) equation [21]) or the need for dialysis at 3 months. Sub-clinical rejection was assessed from a biopsy performed at 3 months post-transplantation.

Missing data

Patients that did not have complete uNAG, uNGAL, creatinine or sLDH values at day 1 or 3 were excluded from the analysis (i.e., six DCD KTs and one LD KT). It was decided to conserve the data from patients when [TIMP2].[IGFBP7] measurement was absent because this dosage was added afterwards. A complete case analysis was performed for each outcome; imputation was not performed. It was hypothesized that missing data followed a random pattern.

Statistics

Quantitative variables are described as their medians and interquartile ranges and were compared using the Wilcoxon-rank or Kruskal--Wallis test, as appropriate. Qualitative variables are presented as their frequencies and percentages and were compared using Fisher's exact test (because of the small population size).

Spearman's correlation coefficients between urine and serum biomarkers and transplantation characteristics (i.e., cold-ischemia time, resistive index, donor age, creatinine value at graft removal) were assessed. A strong correlation was defined as a coefficient of >0.5 .

Receiver operating characteristics (ROC) analysis was performed to assess uNAG, uNGAL, [TIMP2]·[IGFBP7], and sLDH on the day-1 respective predictive performances of the primary outcome. No logistic regression for multivariate analyses was performed due to the small population size. A p -value of 0.05 was considered to be statistically significant. All analyses were conducted using R software, version 3.3.1.

Results

Patients' characteristics

Of the 54 patients screened, 41 were included in the analysis: 15 (36.6%) DCD KT and 26 (63.4%) LD KT (Figure 1). Nine (60.0%) of the DCD KT patients experienced DGF.

The patients' baseline characteristics are presented in Table 1. Median age was 54.0 years [range: 49.0-58.5] and 36.5 years [range: 30.0-54.25] for DCD KT and LD KT patients, respectively ($p = 0.02$). Concerning the donors' characteristics, DCDs were slightly younger without reaching statistical difference (46.0 years [range: 38.0-48.0] vs. 50.0 years [range: 39.5-55.0]) ($p = 0.07$) and had higher creatinine values at graft removal (111 $\mu\text{mol/L}$ [range: 83.5-134.5] vs. 74.5 $\mu\text{mol/L}$ [range: 64.5-79.75], $p = <0.01$).

There was no statistically significant difference between DCD KT patients with or without DGF regarding donor age, creatinine value at graft removal, cold-ischemia time, or number of HLA mismatches, including DQ mismatches (data not shown).

Primary endpoint: delayed graft function

A total of 34 patients with complete data were included in the analysis, including 12 with DCD KT, six of whom had DGF, with a median of 16 days between transplantation procedure and the last dialysis session.

Among the biomarkers measured at day 1, there was a statistically significant difference between LD and DCD KT for sLDH, serum creatinine, uNAG, and uNGAL (sLDH: 221 IU/L [184.5; 247] vs. 531 IU/L [310.2; 828], $p < 0.01$; serum creatinine: 358.5 $\mu\text{mol/L}$ [243.5; 430.2] vs. 562 $\mu\text{mol/L}$ [513.5; 710.5], $p < 0.01$; uNAG: 0.52 IU/mmol [0.38; 0.9] vs. 0.96 [0.69; 2.6], $p = 0.02$; uNGAL: 45.62 $\mu\text{g/mmol}$ [15.14; 103.24] vs. 126.58 $\mu\text{g/mmol}$ [52.38; 285.36], $p = 0.01$), and between DCD KT with DGF vs. DCD KT without DGF (sLDH: 954 IU/L [633.8; 1487.2] vs. 310.5 IU/L [289.2; 416.5], $p = 0.04$; serum creatinine: 742 $\mu\text{mol/L}$ [634.8; 1048.8] vs. 506 $\mu\text{mol/L}$ [470; 541.2], $p < 0.01$; uNAG: 3.30 IU/mmol [1.37; 6.10] vs. 0.68 IU/mmol [0.42; 0.77], $p < 0.01$; uNGAL: 298.9 $\mu\text{g/mmol}$ [248; 442.75] vs. 59.87 $\mu\text{g/mmol}$ [52.13; 108.27], $p = 0.04$) (Figure 2).

Similarly, there was a statistically significant difference between DCD KT without DGF compared to LD KT for sLDH and serum creatinine (sLDH: 310.5 IU/L [289.2; 416.5] vs. 221 IU/L [184.5; 247], $p < 0.01$; serum creatinine: 506 $\mu\text{mol/L}$ [470; 541.2] vs. 385.5 $\mu\text{mol/L}$ [243.5; 430.2], $p = 0.01$), whereas there was no difference for uNAG and uNGAL (uNAG: 0.68 IU/mmol [0.42; 0.77] vs. 0.52 IU/mmol [0.38; 0.90], $p = 0.76$; uNGAL: 59.87 $\mu\text{g/mmol}$ [52.13; 108.27] vs. 45.62 $\mu\text{g/mmol}$ [15.14; 103.24], $p = 0.53$).

For patients with data available for [TIMP2].[IGFBP7] ($n=32$, including four DCD KT patients with DGF), there was a statistically significant difference between LD KT and DCD KT ($0.07 \text{ (ng/mL)}^2/1000$ [0.05; 0.14] vs. $0.31 \text{ (ng/mL)}^2/1000$ [0.13; 0.7], $p = 0.01$) and between DCD KT with DGF and DCD KT without DGF ($1.56 \text{ (ng/mL)}^2/1000$ [0.70; 2.61] vs. $0.17 \text{ (ng/mL)}^2/1000$ [0.07; 0.29], $p = 0.01$). In contrast, the difference between DCD KT without DGF and LD KT was not statistically significant ($0.17 \text{ (ng/mL)}^2/1000$ [0.07; 0.29] vs. $0.07 \text{ (ng/mL)}^2/1000$ [0.05; 0.14], $p = 0.23$). When performing ROC analysis on the 10 DCD KT, the biomarkers with the highest area under the curve (AUC) were uNGAL and [TIMP2].[IGFBP7] with a value of 1. The AUC for sLDH was 0.92 (95%CI: 0.73-1.0) and

was 0.96 (95%CI: 0.84-1) for uNAG (Figure 3). A comparison between AUCs was not performed due to the small population size.

Of the transplantation characteristics ($n=12$), only 30-minute resistive index was significantly higher for DCD KTs with DGF compared to DCD KTs without DGF (0.26 mmHg/mL/min [0.20; 0.32] vs. 0.14 mmHg/mL/min [0.12; 0.16], $p = 0.05$). It is notable that the resistive index, when the kidney was placed on the perfusion machine, did not differ between groups (0.43 mmHg/mL/min [0.34; 0.51] vs. 0.34 mmHg/mL/min [0.31; 0.35], $p = 0.38$). When studying correlations between biomarkers and transplantation characteristics ($n=10$), a strong correlation for all the biomarkers (uNGAL, uNAG, sLDH and [TIMP2].[IGFBP7]) was only found with the 30-minute resistive index ($\rho= 0.84, 0.62, 0.54$, and 0.79 , respectively).

Graft function at 3 months

Of the 12 DCD KT patients with complete day-3 values, three suffered from persistent graft dysfunction at 3 months, without dialysis dependency. Median creatinine clearance among DGF DCD KT was 49 mL/min/1.73 m² [42; 65] and 65 mL/min/1.73 m² [62; 66] among DCD KTs without DGF ($p = 0.22$). Day 3 uNAG, uNGAL and [TIMP2].[IGFBP7] were not associated with the 3-month graft outcomes ($p = 0.27, 0.6, 0.15$, respectively), whereas sLDH was associated ($p = 0.02$). When considering median day-3 sLDH value (400 IU/L), median creatinine clearance was 54.5 mL/min/1.73 m² [41; 68.75] in patients with higher sLDH values vs. 61 mL/min/1.73 m² [60 – 65] in patients with lower values. Day-3 biomarker values appeared to be related to short-term graft outcomes and notably the occurrence of DGF (Figure 4). Similarly, 30-minute resistive index did not differ between the two groups ($p = 0.08$). For the 12 patients with complete day-1 values (i.e., two persistent dysfunctions), none of the biomarkers was statistically associated with 3-month persistent dysfunction.

Biopsy-proven graft rejection at 3 months

Of the 41 patients included, 36 underwent a biopsy at 3 months. Of these, two DCD KT (15.38%) and four LD KT (17.39%) experienced subclinical graft rejection ($p = 1.00$). Of the 31 patients with complete day-1 values, five had a subclinical graft rejection. Neither the overall population nor the patient subgroups (i.e., DCD or LD KT status), based on uNAG, sLDH, uNGAL, or [TIMP2].[IGFBP7] values on day 1, was associated with subsequent subclinical graft rejection. Results were similar for the 32 patients with complete day-3 values.

uNGAL kinetics

The kinetics for uNGAL over the first 3 months post-transplantation are presented in Figure 5. Although uNGAL was high in both groups on day 1, it then decreased substantially by day 3 in the LD KT group. At 6 weeks and 3 months, uNGAL values were low in both groups.

Discussion

This is the first study to extensively assess the prognostic performance of several urinary biomarkers of tubular injury in addition to serum LDH to predict short- and mid-term graft outcomes in DCD KT patients using LD KT patients as the “gold standard”. uNGAL, uNAG, [TIMP2].[IGFBP7] and sLDH showed promising results to predict DGF, defined as requiring dialysis in the first week post-transplantation. Interestingly, the 30-minute resistive index on the perfusion machine appeared to be associated with DGF. Measured markers were poorly associated with the 3-month outcomes.

Traditionally, graft dysfunction has been diagnosed based on increased serum creatinine, in association with urine output, despite its obvious limitations. Increased serum creatinine is only evident 24--48 h after kidney injury, which is the time required to reach a new steady state between constant creatinine generation and its elimination via the new glomerular-

filtration rate [9]. Hence, although it remains the reference method to assess acute changes in renal function [22], serum creatinine cannot be considered as a "gold standard" [23]. In the setting of kidney transplantation, diagnosis of DGF requires a kinetic approach rather than a unique measurement, which leads, in most cases, to a 48-hour delay.

In order to predict DGF, uNGAL values on day 1 were analyzed. In previous studies, uNGAL values appeared to reach a maximum at 24--48 hours after kidney transplantation [24, 25], therefore, the timing used to measure it in this study seems appropriate. Moreover, we considered that values measured at 48--72 hours post-surgery were of little benefit because they were mostly performed after the first dialysis session, thus, providing no further information other than serum-creatinine level. In our study, uNGAL measured at day 1 in DCD KT patients seemed to both sensitively and specifically predict DGF. These results are in agreement with previous studies conducted on LD and DBD KT patients [11, 24, 25]. Based on the current results, uNGAL dosage may reflect lesion severity. [TIMP2].[IGFBP7] is associated with cell cycle arrest of tubular cells in G1 stage when an injury occurs, a mechanism that could be protective [26]. It has emerged in recent years as the most promising acute kidney-injury biomarker [20, 27] in various settings, without being modified by patient comorbidities [28]. Studies on its association with kidney transplantation are few [13-15] and no study has been conducted specifically in DCD KTs. In this current study, it has been confirmed that [TIMP2].[IGFBP7] could be a useful biomarker in this context, allowing the early identification of patients at risk for DGF. Similarly, the day-1 sLDH, showed an ability to predict DGF and thus merits further study, considering that it is a routine, convenient and very cheap measurement and is easy to implement in every-day practice. Finally, in agreement with several studies indicating the potential of NAG to predict short- and long-term graft outcomes [12], the results presented for uNAG also appear interesting in this setting. It should be noted that the perfect uNGAL and [TIMP2].[IGFBP7] AUCs in the current study is

probably linked to the small population size, thus not enabling direct comparison with the sLDH and uNAG AUCs.

Perfusion machines have improved the short-term outcomes after DCD KT. However, the longer term benefits are uncertain [29, 30], reflecting the severity of ischemic injuries inherent in this type of donor. Currently, resistive index on the perfusion machine have been used to assess graft viability, in particular at the end of perfusion [31]. A high index indicates grafts that have a high risk of primary non-function [32] and has been used as an indicator for decision making as to whether to discard a graft [33]. Interestingly, in our study, the 30-minute resistive index on the perfusion machine was marginally associated with DGF in DCD KT patients.

Other baseline characteristics (donor and recipient ages, donor serum creatinine, cold-ischemia time) [34, 35], which are classically associated with DGF, were not significant in this study. This could be due to the homogeneity of donor pre-conditioning, graft conditioning, or the surgery, as well as to the monocentric nature of our study. Furthermore, kidney grafts were only sourced from a single center in this study.

Neither uNGAL, uNAG, [TIMP2].[IGFBP7] nor the 30-minute resistive index were predictive of 3-month persisting dysfunction. It is notable that sLDH was associated with the 3-month outcome. However, this single significant result was probably due to multiple comparison and should be interpreted with caution. These findings are in contrast with previous studies on other kidney-transplant populations [11, 24]. The levels of these markers at days 1 and 3 were strongly associated with DGF in the current study. In previous studies, the DGF impact on DCD KT patients' long-term outcomes appeared to be moderate compared to other types of transplantation [36, 37], however, this remains controversial [7]. In our cohort, 3-month persistent dysfunction in DCD KT patients appears to be linked to subsequent events rather than to transplantation characteristics or DGF. The lack of a

predictive performance of our marker could confirm the lower impact of DGF on long-term graft outcomes after DCD KT, which is in agreement with DCD KT satisfying outcomes despite a higher incidence of DGF [1]. A possible explanation could be milder up-regulation of inflammatory and injury genes in comparison to DBD [38]. We also acknowledge that the small size of our population, and thus the low incidence of events of interest, may have prevented us obtaining a significant result.

The kinetics of uNGAL in the first 3 months post-transplantation was of interest. On day 1, uNGAL was elevated in patients undergoing LD KT, suggesting the existence of tubular injury, even for this type of transplantation. However, the earlier decrease in uNGAL in LD KT could be explained by less extensive or less severe lesions compared to those encountered with DCD KT. At both 6 weeks and 3 months, the uNGAL level indicated the disappearance of tubular injuries in both groups.

This study has several strengths. Firstly, only one previous study has studied biomarker performance specifically within a DCD KT population but was limited to serum NGAL [39]. Based on our results, a score associating uNGAL, sLDH, uNAG, [TIMP2].[IGFBP7], and possibly the 30-minute resistive index could help identify patients at higher risk for DGF. Specific therapeutic measures could then be implemented at an early stage, similar to the QPI-1002 study (Quark Pharmaceuticals), where the anti-apoptotic siRNA targeting *p53* gene was investigated [40].

Our study has some limitations. Firstly, the small population size may have prevented definitive conclusions to be drawn. However, it should be noted that DCD KT is relatively rare in France (62 cases reported in 2015). Secondly, sample collection could have been better standardized during the early post-transplantation period. The delay between transplantation and sampling time on day 1 varied between patients and, consequently, these comparisons

may not be viable. However, the time before sampling only ranged between 18 and 33 hours, a time difference which seems unlikely to have influenced the results.

In conclusion, in the DCD KT population, clinical (30-minute resistive index on a perfusion machine) and biological markers (uNGAL, uNAG, [TIMP2].[IGFBP7] and sLDH) could provide a predictive score for DGF: they could thus identify patients at risk and allow modification of their management accordingly. In particular, due to its availability and low cost, sLDH could be recommended as a routine measurement in transplant centers. Our findings should be investigated further in a larger cohort.

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Table 1: Initial characteristics of the overall population (n=41):

	CDD KTs without DGF (n=6)	DGF CDD KTs (n=9)	LD KTs (n=26)	p-value
Recipient: donor and transplantation characteristics				
<i>Recipient</i>				
Age (years)	56 [50.25; 58.75]	53 [49; 55]	36.5 [30; 54.25]	0.05
Gender (male)	6 (100)	9 (100)	18 (60.23)	0.06
BMI	26.86 [24.3; 28.02]	24.84 [22.74; 26.23]	23.08 [21.23; 26.12]	0.32
Initial nephropathy				0.56
Uropathy	1 (16.66)	0	4 (15.38)	
Polycystic kidney disease	1 (16.66)	4 (44.44)	6 (23.08)	
Glomerulopathy	2 (33.33)	2 (22.22)	7 (26.92)	
Metabolic	2 (33.33)	1 (11.11)	2 (7.69)	
Other	0	2 (22.22)	7 (26.92)	
HLA sensitization	0	0	5 (19.23)	0.29
<i>Donor</i>				
Donor age (years)	47 [40.25; 48.5]	43 [38; 46]	50 [39.5; 55.0]	0.17
Serum creatinine at graft removal (μmol/L)	94.5 [82.25; 139.75]	118 [87; 128]	74.5 [64.5; 79.75]	<0.01
<i>Transplantation</i>				
Cold ischemia time (min)	660.5 [566.2; 918.2]	797 [617; 824]	80 [75.25; 95.5]	<0.01

Number of HLA mismatches	5 [4; 7.5]	5 [4; 6]	3.5 [2; 5]	0.08
Number of HLA-DQ mismatches	1 [1; 1.75]	1 [1; 1]	1 [0; 1]	0.06
30-min resistive index	0.14 [0.12; 0.16]	0.25 [0.11; 0.28]		0.19
Biomarker characteristics				
uNAG day 1 (IU/mmol)	0.68 [0.42; 0.77]	1.89 [1.42; 5.64] ^a	0.52 [0.38; 0.94] ^a	<0.01
uNGAL day 1 (µg/mmol)	59.87 [52.13; 108.27]	298.9 [248; 442.75] ^b	46.68 [15.33; 100.95] ^b	0.01
sLDH day-1 (IU/L)	310.5 [289.2; 416.5]	954 [633.8; 1487.2] ^b	220 [185; 245] ^b	<0.01
Serum creatinine day 1 (µmol/L)	506 [470; 541.2]	679 [620; 1130]	358.5 [243.5; 430.2]	<0.01
[TIMP2].[IGFBP7] ((ng/mL)²/1000)	0.17 [0.07; 0.29]	0.72 [0.65; 2.39] ^c	0.07 [0.05; 0.13] ^a	0.01
Outcome				
3-month persistent dysfunction	1 (16.66)	3 (33.33)	1 (3.85)	0.04
Biopsy results				0.44
Normal	3 (50.0)	4 (44.44)	11 (42.3)	
Reject	2 (33.33)	0 (0)	4 (15.38)	
Other	0 (0)	4 (44.44)	8 (30.77)	
No biopsy available	1 (16.66)	1 (11.11)	3 (11.54)	
BK virus at 3-month				0.30

Decoy cells	2 (33.33)	2 (22.22)	4 (15.38)	
Serum PCR	0	1 (11.11)	0	
<i>De-novo</i> DSA at 3-month	0	0	1 (3.85)	1

Quantitative variables are presented as medians (interquartile ranges) and qualitative variable as frequencies (corresponding percentages).

Abbreviations: CDD, donor after cardiac death; KT, kidney transplantation; DGF, delayed graft function; LD, living donor; BMI, body-mass Index; HLA, Human leukocyte antigen; uNAG, urinary N acetyl β -D glucosaminidase; uNGAL, urinary neutrophil gelatinase-associated lipocalin; sLDH, serum lactate dehydrogenase; [TIMP2].[IGFBP7], [tissue inhibitor of metalloproteinases 2].[insulin-like growth factor-binding protein 7]; PCR, polymerase chain reaction; DSA, donor-specific antibody

^a 2 missing values

^b 3 missing values

^c 4 missing values

Figures

Figure 1: Flow chart

Abbreviations: CDD, donor after cardiac death; KT, kidney transplantation; LD, living donor; DGF, delayed graft function

Figure 2: Urinary NGAL, NAG, [TIMP2].[IGFBP7] and serum LDH on day 1 post-transplantation according to donor type

Abbreviations: CDD, donor after cardiac death; DGF, delayed graft function; LD, living donor; NAG, N acetyl β -D glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; [TIMP2].[IGFBP7], [tissue inhibitor of metalloproteinases 2].[insulin-like growth factor-binding protein 7]; LDH, lactate dehydrogenase

Figure 3: ROC curves of urinary NGAL, NAG, [TIMP2].[IGFBP7] and serum LDH at day 1, and prediction of DGF after CDD kidney transplantation

Abbreviations: ROC, receiver operating characteristics; NAG, N acetyl β -D glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; [TIMP2].[IGFBP7], [tissue inhibitor of metalloproteinases 2].[insulin-like growth factor-binding protein 7]; LDH, lactate dehydrogenase; DGF, delayed graft function; CDD, donor after cardiac death.

Figure 4: Urinary NGAL, NAG, [TIMP2].[IGFBP7] and serum LDH evolution from day 1 to day 3 post transplantation in CDD KT patients

Patients with DGF are indicated in light-grey; dark-grey indicates absence of DGF

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; NAG, N acetyl β -D glucosaminidase; CCAM, cell-cycle arrest markers; LDH, lactate dehydrogenase; CDD, donor after cardiac death; KT, kidney transplantation

Figure 5: Urinary NGAL kinetics over the first 3 months post-transplantation, presented as the NGAL median value for LD and CDD KT patients at each time point.

Abbreviations: NGAL, neutrophil gelatinase-associated lipocalin; CDD, donor after cardiac death; LD, living donor; KT, kidney transplantation