Early Prediction of Graft Outcomes After Kidney Transplantation From Donors After Circulatory Death: Biomarkers and Transplantation Characteristics

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ABSTRACT

Background. This study aimed to identify transplantation characteristics and biomarkers that predict outcomes for kidney transplant (KT) patients from donors after circulatory death (DCDs).

Methods. Consecutive patients receiving a KT from a DCD in our center between 2014 and 2016 were included; the reference population was recipients with a living donor KT. The urinary tubular injury biomarker-to-creatinine ratio and serum lactate dehydrogenase (LDH) were measured at post-transplant days 1 and 3. The primary outcome was the occurrence of delayed graft function (DGF). Descriptive and receiver operating characteristic analyses were performed.

Results. Forty-one patients were included in the analysis: 15 (36.59%) DCD KTs (9 of which suffered from DGF) and 26 (63.41%) living donor KTs. For the primary endpoint, neutrophil gelatinase-associated lipocalin, N-acetyl-beta-D-glucosaminidase, urinary tubular injury biomarker-to-creatinine ratio, and LDH areas under the curve were 1 and 0.96 (95% confidence interval: 0.84-1.0), 1 and 0.92 (95% confidence interval: 0.73-1.0), respectively. Among the transplant characteristics, only the 30-minute resistive index on the perfusion machine was significantly higher in DCD KTs with DGF vs those without DGF (0.26 mm Hg/mL/min [0.20; 0.32] vs 0.14 mm Hg/mL/min [0.12; 0.16], $P = .05$). Median 3-month creatinine clearance among DGF DCD KTs was 49 mL/min/1.73 m^2 [IQR: 42; 65] and 65 mL/min/1.73 m^2 [IQR: 62; 66] among DCD KTs without DGF ($P = .22$).

Conclusion. In the DCD KT population, clinical and biological markers were identified that provided predictive tools for DGF. Thus, systematic measurement of these biomarkers, particularly LDH, could improve the management of kidney graft recipients' immunosuppressive therapy.

A shortage of kidney allografts has resulted in extending the pool of potential donors, for example living donor (LD) kidney transplantation (KT) to nonrelated donors and organs from donors after circulatory death (DCDs), which has been made possible by the development of hypothermic-perfusion machines that improve organ preservation. KT using DCDs has shown similar results to those from donors after brain death (DBDs) in terms of patient and graft survival rates [1] and is associated with enhanced survival compared with patients who remain on dialysis while

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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-transplant [4] and is a risk factor in 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-transplant objective. The use of baseline characteristics alone (donor age, serum creatinine level at graft removal, cold ischemia time) has limited accuracy to predict early and late allograft outcomes. Unfortunately, serum creatinine clearance cannot be considered as a perfect marker for kidney injury.

An increase in serum creatinine level 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 level is always delayed with regard to the injury occurrence; therefore, it can be used to diagnose rather than predict DGF [10].

Early biomarkers are needed to improve prediction of 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 LDs and DBD KTs [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 KT [13–15]. Finally, serum lactate dehydrogenase (sLDH), a nonspecific marker for cell injury, has been little studied following KT, 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. 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 HLA-incompatible KTs. Six patients with LD KT had major graft dysfunction in the early post-transplant period (ie, within less than 3 months post-transplant) secondary to complications; thus, they could no longer be considered as reference KT patients and their data were excluded (of the 6: 1 died, 2 had graft artery stenosis, 1 had acute kidney injury, 1 had graft rejection on day 11 post-transplant, and 1 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 participant signed an informed consent form before enrollment. This study was an ancillary study to another that aimed to identify the urinary proteomic profile of the accommodation phenomenon in the presence of donor-specific antibodies among KT recipients (Grenoble Institutional Review Board number 6705).

DCD KT

All patients, except 1, received a Maastricht category II graft; the exception was Maastricht category III [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

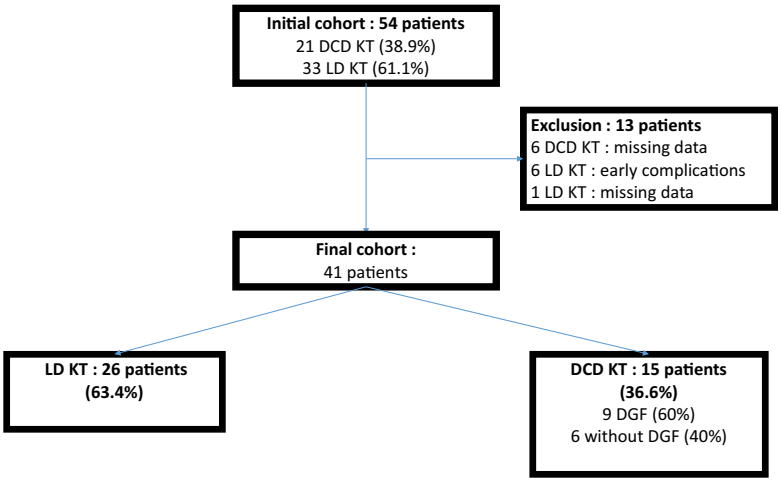

Fig 1. Flow chart. DCD, donor after cardiac death; DGF, delayed graft function; KT, kidney transplantation; LD, living donor.

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				
Recipient				
Age, y	56 [50.25; 58.75]	53 [49; 55]	36.5 [30; 54.25]	.05
Sex, (male)	6 (100)	9 (100)	18 (60.23)	.06
BMI	26.86 [24.3; 28.02]	24.84 [22.74; 26.23]	23.08 [21.23; 26.12]	.32
Initial nephropathy				.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)	.29
Donor				
Donor age, y	47 [40.25; 48.5]	43 [38; 46]	50 [39.5; 55.0]	.17
Serum creatinine at graft removal, μmol/L	94.5 [82.25; 139.75]	118 [87; 128]	74.5 [64.5; 79.75]	< .01
Transplantation				
Cold ischemia time, min	660.5 [566.2; 918.2]	797 [617; 824]	80 [75.25; 95.5]	< .01
Number of HLA mismatches	5 [4; 7.5]	5 [4; 6]	3.5 [2; 5]	.08
Number of HLA-DQ mismatches	1 [1; 1.75]	1 [1; 1]	1 [0; 1]	.06
30-min resistive index	0.14 [0.12; 0.16]	0.25 [0.11; 0.28]		.19
Biomarker Characteristics				
uNAG day 1, IU/mmol	0.68 [0.42; 0.77]	1.89 [1.42; 5.64]*	0.52 [0.38; 0.94]*	< .01
uNGAL day 1, μg/mmol	59.87 [52.13; 108.27]	298.9 [248; 442.75]†	46.68 [15.33; 100.95]†	.01
sLDH day 1, IU/L	310.5 [289.2; 416.5]	954 [633.8; 1487.2]†	220 [185; 245]†	< .01
Serum creatinine day 1, μmol/L	506 [470; 541.2]	679 [620; 1130]	358.5 [243.5; 430.2]	< .01
[TIMP2]·[IGFBP7], $(ng/mL)^2/1000$	0.17 [0.07;0.29]	0.72 [0.65; 2.39]‡	0.07 [0.05; 0.13]*	.01
Outcome				
3-month persistent dysfunction	1 (16.66)	3 (33.33)	1 (3.85)	.04
Biopsy specimen results				.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 specimen available	1 (16.66)	1 (11.11)	3 (11.54)	

Table 1. (continued)

	CDD KTs Without DGF ($n = 6$)	DGF CDD KTs ($n = 9$)	LD KTs ($n = 26$)	*P Value*
BK virus at 3 month				.30
Decoy cells	2 (33.33)	2 (22.22)	4 (15.38)	
Serum PCR	0	1 (11.11)	0	
De novo 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: BMI, body mass index; CDD, donor after cardiac death; DGF, delayed graft function; DSA, donor-specific antibody; KT, kidney transplantation; LD, living donor; PCR, polymerase chain reaction; sLDH, serum lactate dehydrogenase; [TIMP2]·[IGFBP7], product of tissue inhibitor of metalloproteinases 2 and insulin-like growth factor-binding protein 7 concentrations; uNAG, urinary N-acetyl-β-D-glucosaminidase; uNGAL, urinary neutrophil gelatinase-associated lipocalin.
*2 missing values.
†3 missing values.
‡4 missing values.

(LifePort Kidney Transporter 1.1, Organ Recovery Systems, Itasca, IL, United States), and kidneys with end-perfusion resistive indices greater than 0.3 mm Hg/mL/min were discarded [18]. No routine biopsy was performed on the graft before transplant.

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 to 12 μg/L, and then 5 to 8 μg/L was targeted.

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

Data Collection

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

Serum creatinine, sLDH, urinary creatinine, urinary NGAL (uNGAL), and urinary NAG (uNAG) levels were measured at days 1 and 3, and uNGAL and serum creatinine levels were assessed at day 7, 6 weeks, and 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 minutes at 2000*g*, then decanted, aliquoted, frozen, and stored at -20°C. uNGAL was measured using an enzyme-linked immunosorbent assay (Human NGAL ELISA kit 036CE, Bioporto Diagnostics, Hellerup, Denmark), uNAG was measured using a colorimetric test (10875406001 kit, Roche Life Science, Penzberg, Germany), and the product of TIMP2 and IGFBP7 concentrations ([TIMP2][IGFBP7]) was measured using a sandwich immunoassay technique (NephroCheck, Astute Medical, San Diego, CA, United States). uNAG and uNGAL are presented as a ratio, that is, they were divided by the urinary creatinine value.

Outcomes

The primary outcome was the occurrence of DGF, defined as the requirement of at least 1 dialysis session within the first week post-transplant.

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

Missing Data

Patients that did not have complete uNAG, uNGAL, creatinine, or sLDH values at day 1 or 3 were excluded from the analysis (ie, 6 DCD KTs and 1 LD KT). It was decided to conserve the data from patients when [TIMP2]·[IGFBP7] measurement was absent because this dosage was added afterward. 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 the Fisher exact test (because of the small population size).

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

Receiver operating characteristic (ROC) analysis was performed to assess uNAG, uNGAL, [TIMP2]·[IGFBP7], and sLDH on day 1 and their respective predictive performances of the primary outcome. No logistic regression for multivariate analyses was performed because of the small population size. A P value of .05 was considered to be statistically significant. All analyses were conducted using R software, version 3.3.1.

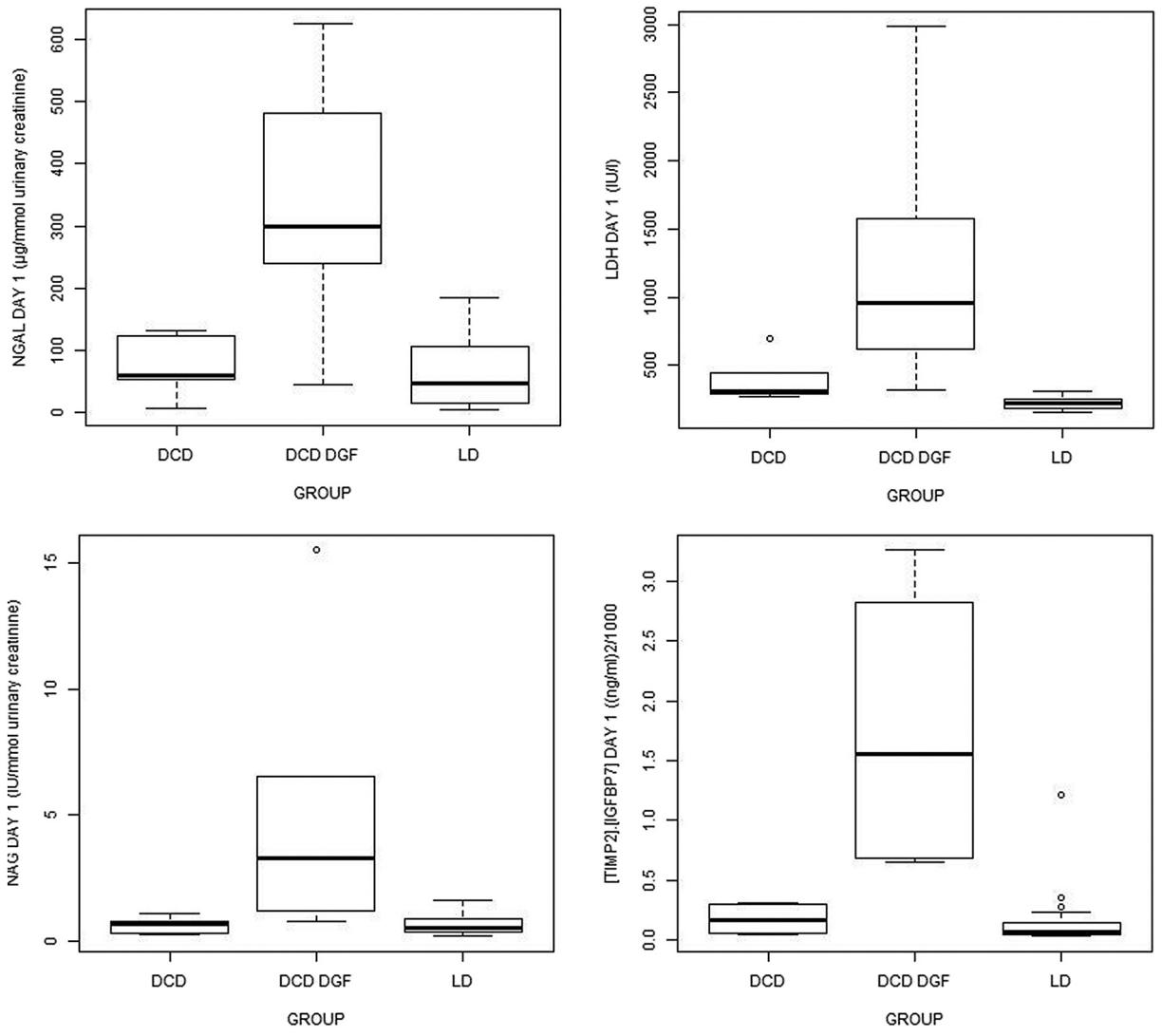

Fig 2. Urinary NGAL, NAG, [TIMP2].[IGFBP7], and serum LDH on day 1 post-transplant according to donor type. DCD, donor after cardiac death; DGF, delayed graft function; LD, living donor; LDH, lactate dehydrogenase; NAG, N-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; [TIMP2].[IGFBP7], [tissue inhibitor of metalloproteinases 2].[insulin-like growth factor-binding protein 7].

RESULTS

Patients' Characteristics

Of the 54 patients screened, 41 were included in the analysis: 15 (36.6%) DCD KTs and 26 (63.4%) LD KTs (Fig 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 years) and 36.5 years (range: 30.0-54.25 years) for DCD KT and LD KT patients, respectively ($P = .02$). Concerning the donors' characteristics, DCDs were slightly younger without reaching statistical difference (46.0 years [range: 38.0-48.0 years] vs 50.0 years [range: 39.5-55.0 years]) ($P = .07$) and had higher creatinine values at graft removal (111 µmol/L [range: 83.5-134.5 µmol/L] vs 74.5 µmol/L [range: 64.5-79.75 µmol/L], $P = < .01$).

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: DGF

A total of 34 patients with complete data were included in the analysis, including 12 with DCD KT, 6 of whom had DGF, with a median of 16 days between transplantation procedure and the last dialysis session.

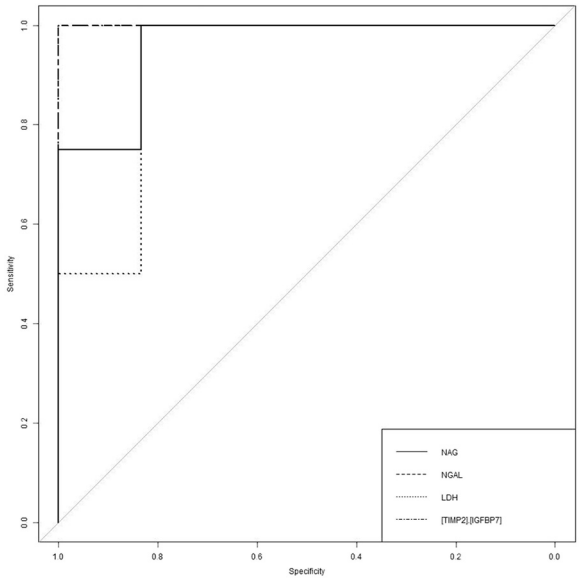

Fig 3. ROC curves of urinary NGAL, NAG, [TIMP2].[IGFBP7], and serum LDH at day 1, and prediction of DGF after DCD kidney transplant. DCD, donor after cardiac death; DGF, delayed graft function; LDH, lactate dehydrogenase; NAG, N-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; ROC, receiver operating characteristics; [TIMP2].[IGFBP7], [tissue inhibitor of metalloproteinases 2].[insulin-like growth factor-binding protein 7].

Among the biomarkers measured at day 1, there was a statistically significant difference between LD and DCD KTs for sLDH, serum creatinine, uNAG, and uNGAL levels (sLDH: 221 IU/L [IQR: 184.5; 247] vs 531 IU/L [IQR: 310.2; 828], $P < .01$; serum creatinine: 358.5 μmol/L [IQR: 243.5; 430.2] vs 562 μmol/L [IQR: 513.5; 710.5], $P < .01$; uNAG: 0.52 IU/mmol [IQR: 0.38; 0.9] vs 0.96 [IQR: 0.69; 2.6], $P = .02$; uNGAL: 45.62 μg/mmol [IQR: 15.14; 103.24] vs 126.58 μg/mmol [IQR: 52.38; 285.36], $P = .01$), and between DCD KTs with DGF vs DCD KTs without DGF (sLDH: 954 IU/L [IQR: 633.8; 1487.2] vs 310.5 IU/L [IQR: 289.2; 416.5], $P = .04$; serum creatinine: 742 μmol/L [IQR: 634.8; 1048.8] vs 506 μmol/L [IQR: 470; 541.2], $P < .01$; uNAG: 3.30 IU/mmol [IQR: 1.37; 6.10] vs 0.68 IU/mmol [IQR: 0.42; 0.77], $P < .01$; uNGAL: 298.9 μg/mmol [IQR: 248; 442.75] vs 59.87 μg/mmol [IQR: 52.13; 108.27], $P = .04$) (Fig 2).

Similarly, there was a statistically significant difference between DCD KTs without DGF compared with LD KTs for sLDH and serum creatinine levels (sLDH: 310.5 IU/L [IQR: 289.2; 416.5] vs 221 IU/L [IQR: 184.5; 247], $P < .01$; serum creatinine: 506 μmol/L [IQR: 470; 541.2] vs 385.5 μmol/L [IQR: 243.5; 430.2], $P = .01$), whereas there was no difference for uNAG and uNGAL (uNAG: 0.68 IU/mmol [IQR: 0.42; 0.77] vs 0.52 IU/mmol [IQR: 0.38; 0.90], $P = .76$; uNGAL: 59.87 μg/mmol [IQR: 52.13; 108.27] vs 45.62 μg/mmol [IQR: 15.14; 103.24], $P = .53$).

For patients with data available for [TIMP2]·[IGFBP7] (n = 32, including 4 DCD KT patients with DGF), there was a statistically significant difference between LD KTs and DCD KTs (0.07 $[ng/mL]^2$/1000 [IQR: 0.05; 0.14] vs 0.31 $[ng/mL]^2$/1000 [IQR: 0.13; 0.7], $P = .01$) and between DCD KTs with DGF and DCD KTs without DGF (1.56 $[ng/mL]^2$/1000 [IQR: 0.70; 2.61] vs 0.17 $[ng/mL]^2$/1000 [IQR: 0.07; 0.29], $P = .01$). In contrast, the difference between DCD KTs without DGF and LD KTs was not statistically significant (0.17 $[ng/mL]^2$/1000 [IQR: 0.07: 0.29] vs 0.07 $[ng/mL]^2$/1000 [IQR: 0.05; 0.14], $P = .23$). When performing ROC analysis on the 10 DCD KTs, 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% confidence interval: 0.73-1.0) and was 0.96 (95% confidence interval: 0.84-1) for uNAG (Fig 3). A comparison between AUCs was not performed because of the small population size.

Of the transplantation characteristics (n = 12), only 30-minute resistive index was significantly higher for DCD KTs with DGF compared with DCD KTs without DGF (0.26 mm Hg/mL/min [IQR: 0.20; 0.32] vs 0.14 mm Hg/mL/min IQR: [0.12; 0.16], $P = .05$). It is notable that the resistive index, when the kidney was placed on the perfusion machine, did not differ between groups (0.43 mm Hg/mL/min [0.34; 0.51] vs 0.34 mm Hg/mL/min [IQR: 0.31; 0.35], $P = .38$). 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, 3 suffered from persistent graft dysfunction at 3 months, without dialysis dependency. Median creatinine clearance among DGF DCD KT was 49 mL/min/1.73 m^2 [IQR: 42; 65] and 65 mL/min/1.73 m^2 [IQR: 62; 66] among DCD KTs without DGF ($P = .22$). Day 3 uNAG, uNGAL, and [TIMP2]·[IGFBP7] were not associated with the 3-month graft outcomes ($P = .27$, .6, 0.15, respectively), whereas sLDH was associated ($P = .02$). When considering the median day 3 sLDH value (400 IU/L), median creatinine clearance was 54.5 mL/min/1.73 m^2 [IQR: 41; 68.75] in patients with higher sLDH values vs 61 mL/min/1.73 m^2 [IQR: 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 (Fig 4). Similarly, 30-minute resistive index did not differ between the 2 groups ($P = .08$). For the 12 patients with complete day 1 values (ie, 2 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, 2 DCD KTs (15.38%) and 4 LD KTs

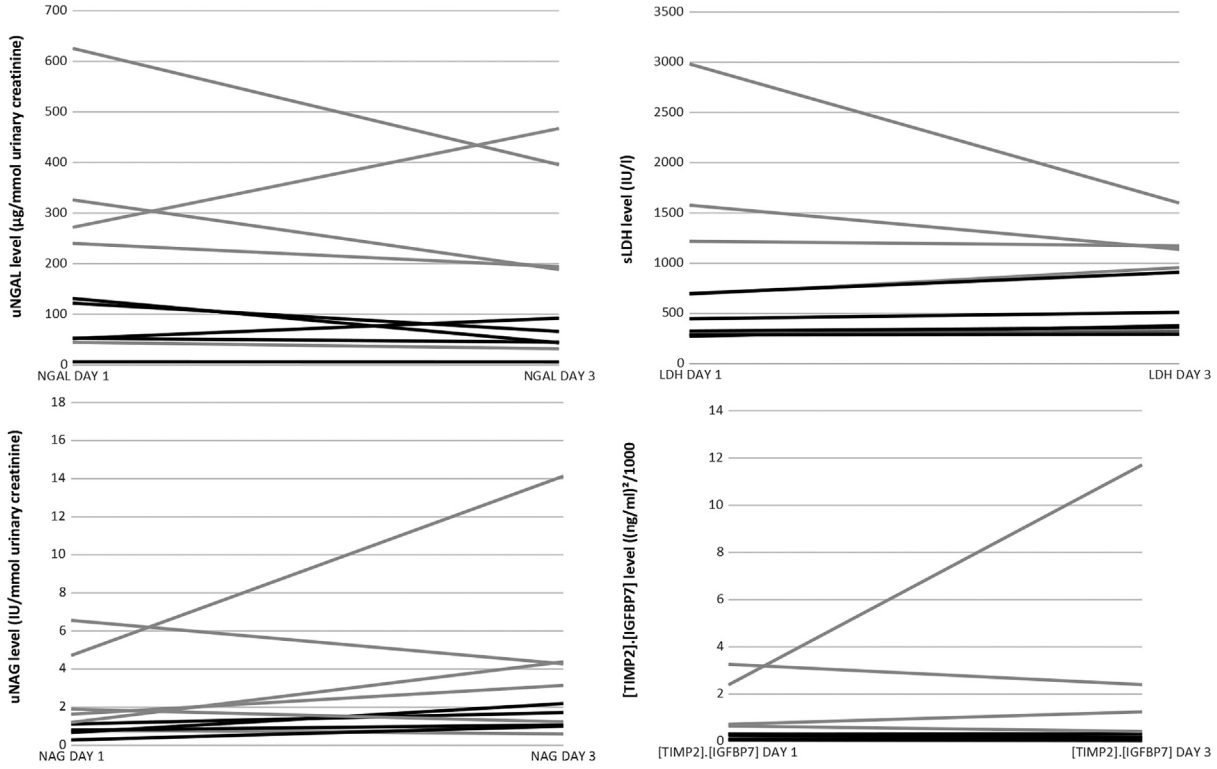

Fig 4. Urinary NGAL, NAG, [TIMP2].[IGFBP7], and serum LDH evolution from day 1 to day 3 post-transplant in DCD KT patients. Patients with DGF are indicated in light gray; dark gray indicates absence of DGF. CCAM, cell cycle arrest markers; DCD, donor after cardiac death; DGF, delayed graft function; KT, kidney transplantation; LDH, lactate dehydrogenase; 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].

(17.39%) experienced subclinical graft rejection ($P = 1.00$). Of the 31 patients with complete day 1 values, 5 had a subclinical graft rejection. Neither the overall population nor the patient subgroups (ie, 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-transplant are presented in Fig 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-transplant. 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 level, in association with urine output, despite its obvious limitations. Increased serum creatinine level is only evident 24 to 48 hours 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 clearance cannot be considered as a "gold standard" [23]. In the setting of KT, diagnosis of DGF requires a kinetic approach rather than a unique measurement, which leads, in most cases, to a 48-hour delay.

To predict DGF, uNGAL values on day 1 were analyzed. In previous studies, uNGAL values appeared to reach a maximum at 24 to 48 hours after KT [24,25], therefore, the timing used to measure it in this study seems appropriate. Moreover, we considered that values measured at 48 to 72 hours postsurgery were of little benefit because they were mostly performed after the first dialysis session, thus, providing no further information other than serum

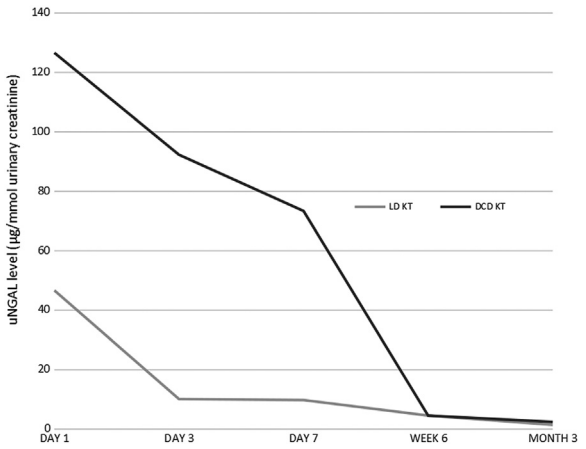

Fig 5. Urinary NGAL kinetics over the first 3 months post-transplant, presented as the NGAL median value for LD and DCD KT patients at each time point. DCD, donor after cardiac death; KT, kidney transplantation; LD, living donor; NGAL, neutrophil gelatinase-associated lipocalin.

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 level may reflect lesion severity. [TIMP2]·[IGFBP7] is associated with cell cycle arrest of tubular cells in gap 1 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 KT 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 everyday 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 are 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 nonfunction [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 clearance, and 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 preconditioning, 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 with 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 with 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 1 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 antiapoptotic 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-transplant period. The delay between transplant 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 that 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 (uNGAL, uNAG, [TIMP2]·[IGFBP7], and sLDH) markers could provide a predictive score for DGF, and they could thus identify patients at risk and allow modification of their management accordingly. In particular, owing 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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