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Prediction of Long-term Renal Allograft Outcome By Early Urinary CXCL10 Chemokine Levels

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Background. Predictive biomarkers for long-term renal allograft outcome could help to individualize follow-up strategies and therapeutic interventions. **Methods.** We investigated the predictive value of urinary CXC chemokine ligand 10 (CXCL10) measured at different timepoints (ie, at 3 and 6 months, and mean of 3 and 6 months coined CXCL10-burden) for long-term allograft outcomes in 154 patients. The primary outcome was a composite graft endpoint of death-censored allograft loss and/or biopsy-proven rejection and/or decline of estimated glomerular filtration rate greater than 20% occurring beyond 6 months after transplantation. **Results.** After a median follow-up of 6.6 years (interquartile range, 5.7-7.5 years) the endpoint was reached in 43/154 patients (28%). In a multivariable Cox-regression model independent predictors were 6-month CXCL10 levels, the CXCL10-burden, HLA-mismatches, donor age and delayed graft function while previous (sub)clinical rejection, estimated glomerular filtration rate and proteinuria at 6 months, as well as 3-month CXCL10 levels were not. Time-dependent receiver operating characteristic analysis revealed an area under the curve of 0.68 (6-month CXCL10) and 0.67 (CXCL10-burden). Grouped by optimal cutoff, low 6-month CXCL10 (<0.70 ng/mmol) was associated with a 95% endpoint-free 5-year survival compared to 78% with high 6-month CXCL10 (P = 0.0007). Only 2 of 62 patients (3%) with low 6-month CXCL10 levels (<0.70 ng/mmol) experienced late rejection or graft loss due to rejection compared to 15 of 92 patients (16%) with high 6-month CXCL10 levels (P = 0.008). Similar results were obtained when patients were grouped according to CXCL10-burden (cutoff, 1.06 ng/mmol). **Conclusions.** Six-month urinary CXCL10 is an independent predictor for long-term graft outcome and thus might be a supplementary tool to tailor surveillance strategies and therapy.

(*Transplantation* 2015;1: e31; doi: 10.1097/TXD.0000000000000537. Published online 24 September 2015.)

Using current immunosuppression, the frequency and severity of renal allograft rejection have changed dramatically. Indeed, the frequency of clinical rejection within the first year after transplantation dropped to 10% to 20%[1,2] and even subclinical rejection fulfilling the current Banff classification only ranges between 10% and 20%.[3-6] These observations contrast with several studies showing that alloimmune-mediated injury is still the leading cause for allograft loss.[7-10] This suggests that with current immunosuppression the alloimmune response is a low-grade inflammation

Received 11 June 2015.

Accepted 17 June 2015.

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The authors declare no conflicts of interest.

This study was supported by the Else Kröner-Fresenius-Stiftung (grant 2012_A255). P.M.H. is supported by the Astellas foundation for biomedical research (grant CH-02-RG-248). J.H. is supported by the Canadian Institutes of Health Research (grant 287559) and an MMSF Dr. F.W. Du Val Clinical Research Professorship salary award. P.N. is supported by the Flynn Family Chair in Renal Transplantation.

P.H.M., J.H., D.R., P.N., and S.S. participated in designed research/study. P.H.M., J.H., A.G., and S.S. performed research/study. P.H.M., P.A., H.H., and S.S. collected data. P.H.M., J.H., M.T.K., and S.S. analyzed the data. All of the authors wrote the article.

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Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantjournal.com).

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000000537

process that is not easy to diagnose and is not currently classified as rejection in the Banff schema.

In support of this concept earlier studies demonstrated that inflammation below the Banff threshold for borderline changes is associated with declining allograft function over 5 years and is an independent predictor of graft loss.[11-13] Furthermore, there is accumulating evidence that persisting allograft rejection/inflammation can culminate in late antibody-mediated rejection (AMR), which is the most frequent histological phenotype observed in lost allografts.[9,10,14,15] Therefore, adjunctive diagnostic tools to screen for those low-grade inflammatory processes are urgently needed. Not surprisingly, there are many efforts to develop—mostly noninvasive—biomarkers for this purpose.[16,17]

Urinary CXC chemokine ligand 9 (CXCL9) and CXCL10 are among these biomarkers; both CXC-receptor 3 chemokines showed the potential to detect (sub)clinical rejection in several studies[6,18-24] and can be regarded as largely equivalent.[21,23,24] A very important observation in the multicenter CTOT-01 study was that patients with high urinary CXCL9 levels at 6 months after transplantation had a higher risk to subsequently develop rejection or declining allograft function.[24] Unfortunately, the CTOT-01 study had a limited follow-up time of 2 years and could not provide a urinary CXCL9 cutoff for its prognostic use. In addition, it is currently unknown at which time biomarker assessment provides the most prognostic information.

Thus, the aims of this study were to investigate whether (i) early measured urinary CXCL10 predicts long-term outcomes and (ii) to calculate detailed prognostic characteristics of urinary CXCL10 levels measured at different timepoints (ie, at 3 months, at 6 months, and using the arithmetic mean of measurements at 3 and 6 months coined urinary CXCL10-burden).

MATERIALS AND METHODS

Patient Population

The study protocol was approved by the ethics committee of the University of Basel and all participating patients gave written informed consent. The patient flow diagram of the study is detailed in Figure 1. Briefly, all patients consecutively transplanted at the University Hospital of Basel between October 2005 and March 2009 were considered for inclusion (n = 228). Two hundred and eight of 228 patients (91%) were finally included as they had both a functioning graft at 6 months after transplantation and at least 1 pair of surveillance biopsy/urine sample obtained at 3 or 6 months after transplantation. One hundred fifty-four of 208 patients (74%) had 2 pairs of surveillance biopsy/urine sample obtained at 3 and 6 months after transplantation, 54 of 208 patients (26%) had 1 pair of surveillance biopsy/urine obtained at 3 or 6 months after transplantation. Only 1 of 208 patients was lost to follow-up beyond 6 months after transplantation.

Investigated Outcomes

Outcomes were prospectively determined as of March 31, 2014. We assessed 2 composite endpoints occurring beyond 6 months after transplantation defined as follows:

– (i) Graft outcome endpoint: death-censored graft loss and/or biopsy-proven clinical rejection and/or greater than 20% decline of estimated glomerular filtration rate (eGFR) between 6 months and last follow-up.
– (ii) Clinical composite endpoint: patient death and/or graft outcome endpoint as described above.

Immunosuppressive Regimens

Initial immunosuppression was selected based on the presence/absence of donor-specific HLA antibodies (HLA-DSA; all defined by single-antigen flow beads), AB0 blood group compatibility and HLA matching as described elsewhere[25] and is listed in Table 1. Patients with HLA-DSA or AB0 incompatibility were maintained on a triple immunosuppression consisting of tacrolimus, mycophenolate mofetil (MMF), and prednisone. In all other patients, we reduced immunosuppression to a dual therapy consisting of tacrolimus and MMF or mycophenolate sodium, if surveillance biopsies at 3 and 6 months were free of rejection.

Evaluation of Allograft Biopsies and Treatment of Rejection

Clinically indicated allograft biopsies were performed when serum creatinine increased by more than 20% from baseline. Surveillance biopsies were scheduled at 3 and 6 months after transplantation. All obtained allograft biopsies (2 cores obtained with a 16-gauge needle) were evaluated by light microscopy, immunofluorescence (C4d, HLA-DR), and immunohistochemistry (SV40 large T-antigen) and were scored according to the Banff classification.[26,27] Rejection episodes were treated according to the histological phenotype and severity. Briefly, clinical and subclinical T cell–mediated rejection including borderline rejection were mostly treated with steroids and increased maintenance immunosuppression. Clinical AMR was mostly treated with plasmapheresis, steroids +/– intravenous immunoglobulins. Subclinical AMR was mostly treated with steroids +/– intravenous immunoglobulins +/– rituximab.

Screening for and Management of Polyomavirus BK Viremia

Screening for active polyomavirus BK (BKV) infection was done according to a standard protocol consisting of urine cytology for decoy cells. Patients with positive decoy cells were tested for plasma BKV viremia by quantitative real-time PCR at the following visit.[28] In patients with sustained BKV viremia (ie, ≥1000 copies/mL) immunosuppression was reduced stepwise as reported previously in detail.[29]

Urine Processing and Standard Urine Analyses

Midstream urine samples were collected immediately before performing an allograft biopsy. Total urine protein, urinary α1-microglobulin (α1m), and urine creatinine were measured as part of clinical routine. An aliquot of midstream urine samples was centrifuged at 1750*g* for 10 minutes and supernatants stored at –80°C without any additives for future analysis.

Measurement of Urinary CXCL10

Urinary CXCL10 measurements were performed retrospectively on stored urine samples using a sandwich enzyme-linked immunosorbent assay as described previously.[6] To correct for different urine dilutions, excretion of urinary CXCL10 is given in relation to urine creatinine (ie, ng protein/mmol creatinine). Urinary CXCL10 results were

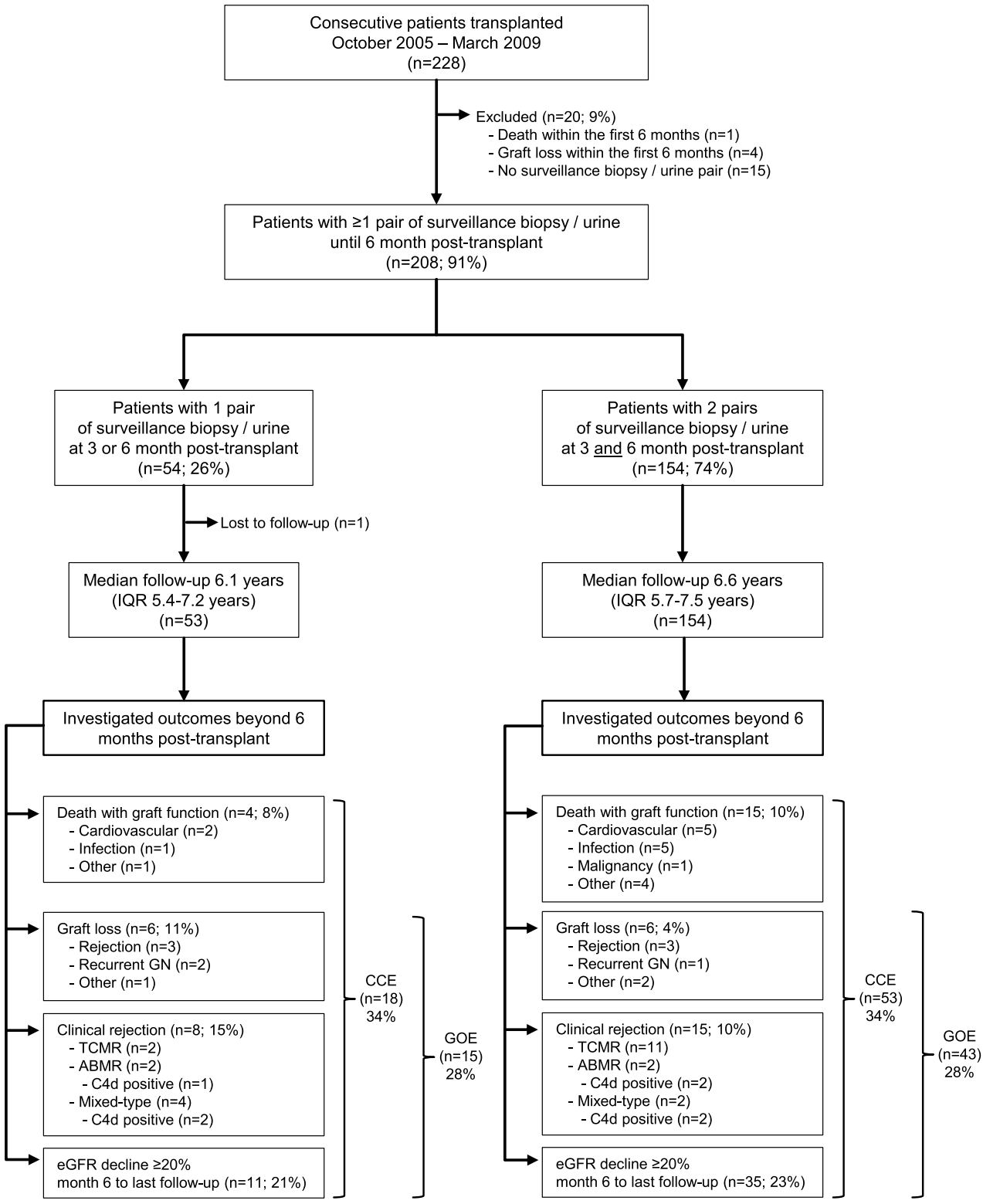

FIGURE 1. Patient flow diagram demonstrating exclusions, follow-up time and outcomes. Patients were stratified by the number of surveillance biopsy/urine pairs (ie, 1 pair obtained at 3 or 6 months posttransplant vs 2 pairs obtained at 3 and 6 months after transplantation).

TABLE 1.

Comparison of baseline and follow-up characteristics of patients with 1 or 2 surveillance biopsy/urine pairs

	1 surveillance biopsy/urine pair (n = 53)	2 surveillance biopsy/urine pairs (n = 154)	*p*
Recipient characteristics			
Age, median (IQR), y	57 (42-64)	54 (44-62)	0.48
Female, n (%)	12 (23)	48 (31)	0.29
Primary disease, n (%)			
ADPKD	8 (15)	31 (20)	0.43
Diabetic	7 (13)	17 (11)	
Vascular	4 (8)	14 (9)	
Glomerulopathy	15 (28)	56 (37)	
Other	19 (36)	36 (23)	
Immunological risk			
Normal risk, n (%)	36 (68)	122 (79)	0.20
Pretransplant HLA-DSA, n (%)	14 (26)	24 (16)	
MFI, median (IQR)	2241 (1294-5943)	2542 (1570-7434)	
Class I, n	4	12	
Class II, n	6	7	
Class I + II, n	4	5	
ABOi, n (%)	3 (6)	8 (5)	
HLA-A-B-DR mismatches			
n with 0/1/2/3/4/5/6	4/4/5/9/9/16/6	4/6/18/41/38/35/12	0.26
Known sensitizing events[a]			
First/second/third kidney transplant, %	70/26/4	85/13/2	0.05
Blood transfusions, n (%)	19 (36)	64 (42)	0.76
Pregnancies, n (%)	9 (17)	35 (23)	0.86
Induction therapy			
Basiliximab, n (%)	34 (64)	122 (79)	0.02
ATG +/– Ivlg, n (%)	15 (28)	30 (20)	
None, n (%)	4 (8)	2 (1)	
Maintenance immunosuppression			
Tac-MMF-P, n (%)	30 (56)	82 (53)	0.74
Tac-MPS-mTOR, n (%)	20 (38)	66 (43)	
Other	3 (6)	6 (4)	
Donor characteristics			
Age, median (IQR)	50 (39-62)	53 (44-63)	0.47
Female, n (%)	31 (58)	74 (48)	0.21
Deceased donor, n (%)	25 (47)	84 (55)	0.43
DGF, n (%)	18 (34)	33 (21)	0.10
Acute rejection within first 6 mo[a]			
Any clinical +/– subclinical rejection, n (%)	17 (32)	72 (47)	0.08
Clinical rejection, n (%)	10 (19)	14 (9)	0.08
TCMR borderline, n	2	4	
TCMR ≥ Banff Ia, n	4	5	
AMR (including category "suspicious"), n	3	5	
Mixed type, n	—	—	
Subclinical rejection, n (%)	8 (15)	63 (41)	0.0007
TCMR borderline, n	2	38	
TCMR ≥ Banff Ia, n	2	16	
AMR (including category "suspicious"), n	2	6	
Mixed-type, n	2	3	
Allograft function at 6 mo			
Creatinine: median (IQR), μmol/L	137 (109-180)	133 (108-161)	0.31
eGFR: median (IQR), mL/min	44 (36-57)	46 (37-55)	0.44
Prot:Creat ratio: median (IQR), mg/mmol	14 (8-26)	12 (9-20)	0.42
BKV viremia within first 6 mo	10 (19)	17 (11)	0.16
CXCL10 chemokine levels[b]			
CXCL10:Creat ratio: median (IQR), ng/mmol	1.3 (0.6-4.2)	1.1 (0.5-3.1)	0.37

[a] A patient can contribute to more than 1 group.

[b] For patients with 2 surveillance biopsy/urine pairs the arithmetic mean of urinary CXCL10 concentrations was calculated (=CXCL10-burden). eGFR (mL/min) calculated by the MDRD formula (mL/min per 1.73 m^2 of body surface).

ABOi, ABO incompatible; ADPKD, autosomal dominant polycystic kidney disease; ATG, antithymocyte globulin; Tac, tacrolimus; TCMR, T cell–mediated rejection; MFI, mean fluorescence intensity; MPS, mycophenolate sodium; mTOR, mechanistic target of rapamycin.

not available to clinicians and thus, did not influence therapeutic or further diagnostic interventions.

Statistical Analysis

We used JMP Pro software version 11.0 (SAS Institute Inc., Cary, NC) for statistical analyses. For categorical data, Fisher exact test or Pearson χ^2 test was used and data presented as counts and percentages. Parametric continuous data were analyzed by Student *t* tests. Nonparametric continuous data were summarized as median (interquartile range) unless stated otherwise and analyzed by the Wilcoxon rank-sum or Kruskal-Wallis rank sum tests as appropriate. The predictive value of urinary CXCL10 was investigated as measurement (i) at 3 months, (ii) at 6 months, and (iii) arithmetic mean of measurements at 3 and 6 months (=urinary CXCL10-burden). Multivariable Cox proportional hazards regression analysis was performed to assess independent predictors for the defined composite endpoints as described above. Within the multivariable model, variables with either a *P* value less than 0.10 in the univariable analyses or known risk factors for worse graft outcome (ie, pretransplant DSA, second/third transplant, deceased donor status, delayed graft function [DGF], proteinuria at 6 months and early rejection episodes occurring within the first 6 months after transplantation) were selected as explanatory variables for the multivariable models. Time-dependent receiver operating characteristic analyses (R statistical software version 3.2.0; www.r-project.org) were used to define the best cutoff of urinary CXCL10 levels and to calculate prognostic characteristics. According to these cutoff values, patients were grouped as "high CXCL10" and "low CXCL10" and compared with a time-to-endpoint analysis (Kaplan-Meier method using the log-rank test). A *P* value less than 0.05 was considered to indicate statistical significance.

RESULTS

Patient Characteristics

Two hundred seven patients were evaluated in the study. As detailed in the patient flow diagram (Figure 1), 154 of 207 patients (74%) had 2 pairs of surveillance biopsy/urine sample obtained at 3 and 6 months after transplantation, whereas the remaining 53 of 207 patients (26%) had only 1 pair of surveillance biopsy/urine obtained at 3 or 6 months after transplantation. Baseline and follow-up data at 6 months after transplantation of these 2 groups are summarized in Table 1. There were no differences between the 2 groups with the exception of the induction therapy and the frequency of subclinical rejection episodes. The latter is most likely related to the fact that the group with two pairs of surveillance biopsy/urine had more surveillance biopsies than the group with only 1 pair of surveillance biopsy/urine. Notably, urinary CXCL10 chemokine levels did not differ between the 2 groups ($P = 0.37$).

Long-Term Outcomes

After a median follow-up time of 6.5 years (interquartile range [IQR], 5.7-7.5 years), the graft outcome endpoint was reached in 15 of 53 patients (28%) with 1 pair of surveillance biopsy/urine and 43 of 154 patients (28%) with 2 pairs of surveillance biopsy/urine (*P* = ns). The frequency of patients reaching the clinical composite endpoint was similar as well (34% vs 34%, *P* = ns). The median time until occurrence of the graft outcome endpoint was 5.9 years (IQR, 5.0-6.5 years) and did not differ between the 2 groups ($P = 0.62$). The median time until occurrence of the clinical composite endpoint was 5.1 years (IQR, 3.5-6.1 years) and was not different between the 2 groups as well ($P = 0.99$). The details of the individual endpoints are summarized in Figure 1.

Predictors of Long-Term Outcomes

As baseline characteristics and long-term outcomes were not different between patients with 1 and 2 pairs of surveillance biopsy/urine, we used the group with 2 pairs for the following analyses. This allowed us to accurately investigate the predictive value of CXCL10 levels measured at 3 months, at 6 months as well as CXCL10 levels calculated as the arithmetic mean of 3 and 6 months values (=urinary CXCL10-burden).

First, we investigated predictors for the graft outcome endpoint in an univariable analysis using the urinary CXCL10-burden. The urinary CXCL10-burden significantly differed between patients reaching the graft outcome endpoint compared to those who were event-free (median [IQR] urinary CXCL10/creatinine ratio 2.0 ng/mmol [0.7-4.4 ng/mmol] vs 0.9 ng/mmol [0.4-2.5 ng/mmol]; $P = 0.0007$) (Table 2). Other parameters with *P* value less than 0.10 in the univariable analysis were total HLA-mismatches, donor age, BKV viremia within the first 6 months after transplantation, and urine protein/creatinine ratio at 6 months. Surprisingly, pretransplant HLA-DSA, eGFR at 6 months after transplantation, and allograft rejection within the first 6 months after transplantation were not predictive for the graft outcome endpoint. We used 5 different definitions of rejection in the univariate analysis, but none was significantly associated with the graft outcome endpoint (Table 2). All 5 variables with *P* value less than 0.10 as well as known risk factors for worse graft outcome (pretransplant DSA, second/third transplant, deceased donor status, DGF, proteinuria, and early rejection episodes) were entered into a multivariable Cox proportional hazard analysis. Taking the whole model into account, total HLA mismatches (hazards ratio [HR], 1.36; 95% confidence interval [95% CI], 1.03-1.82; $P = 0.03$), donor age (HR, 1.02; 95% CI, 1.00-1.05; $P = 0.02$), DGF (HR, 0.38; 95% CI, 0.13-0.95; $P = 0.04$) and the urinary CXCL10-burden (HR, 1.12; 95% CI, 1.04-1.20; $P = 0.007$) emerged as independent predictors (Table 2). Similar results were obtained when using 6-month urinary CXCL10 levels (HR, 1.08; 95% CI, 1.02-1.13; $P = 0.01$), whereas 3-month urinary CXCL10 levels were not a significant independent predictor ($P = 0.10$).

Next, we performed the same analysis investigating the clinical composite endpoint. HLA mismatches (HR, 1.39; 95% CI, 1.09-1.80; $P = 0.008$), donor age (HR, 1.03; 95% CI, 1.01-1.05; $P = 0.0008$), and DGF (HR, 0.35; 95% CI, 0.14-0.78; $P = 0.01$) were independent baseline predictors and the urinary CXCL10-burden was the only significant independent 6-month predictor for the clinical composite endpoint (HR, 1.10; 95% CI, 1.02-1.17; $P = 0.02$) (Table 3). Similar results were obtained when using 6-month urinary CXCL10 levels (HR, 1.07; 95% CI, 1.02-1.12; $P = 0.01$), whereas 3-month urinary CXCL10 levels were again not a significant independent predictor ($P = 0.45$).

In addition, urinary CXCL10 level kinetics between the 2 time points (ie, delta urinary CXCL10 between the 2 measurements at 3 and 6 months) were evaluated to predict both

TABLE 2.

Associations of clinical and laboratory variables with graft outcome endpoint (n = 154 patients)

	Graft outcome endpoint (n = 43)	No graft outcome endpoint (n = 111)	Univariable, *p*	Multivariable cox proportional analysis hazard ratio (95% CI); *p*
Baseline predictors				
Recipient age: median (IQR), y	55 (44-61)	54 (43-62)	0.78	
Primary disease, n (%)				
ADPKD	11 (26)	20 (18)	0.43	
Diabetic	5 (12)	12 (11)		
Vascular	2 (4)	12 (11)		
Glomerulopathy	12 (28)	44 (39)		
Other	13 (30)	23 (21)		
Pretransplant HLA-DSA, n (%)	9 (21)	15 (14)	0.24	0.70 (0.25-1.73); 0.45
Total HLA-A-B-DR mismatches, mean ± std.	4.0 ± 1.2	3.5 ± 1.5	0.03	1.36 (1.03-1.82); 0.03
First/second/third kidney transplant, %	79/19/2	87/11/2	0.38	2.03 (0.75-4.94); 0.16
Donor age: median (IQR), y	55 (44-65)	51 (43-63)	0.08	1.02 (1.00-1.05); 0.02
Deceased donor, n (%)	26 (60)	58 (52)	0.18	1.65 (0.78-3.48); 0.19
DGF, n (%)	7 (16)	26 (23)	0.27	0.38 (0.13-0.95); 0.04
6-mo predictors				
Rejection within the first 6 mo, defined[a]				
Any clinical rejection (including borderline), n (%)	2 (5)	12 (11)	0.44	0.30 (0.04-1.27); 0.11
Any sub(clinical) rejection (including borderline), n (%)	22 (51)	50 (45)	0.23	1.45 (0.74-2.86); 0.27
Persisting early rejection, n (%)[b]	7 (16)	14 (13)	0.51	0.98 (0.33-2.44); 0.97
Phenotype				
(Sub)clinical TCMR ≥ Ia, n (%)	5 (11)	16 (14)	0.83	1.30 (0.42-3.35); 0.62
(Sub)clinical AMR (including category "suspicious"), n (%)	4 (9)	10 (9)	0.55	0.56 (0.12-2.18); 0.41
AMR with pretransplant HLA-DSA, n (%)	3 (7)	6 (5)	0.61	0.34 (0.06-1.62); 0.18
BKV viremia within first 6 mo, n (%)	8 (17)	9 (8)	0.06	0.78 (0.25-2.30); 0.65
eGFR at 6 mo: median (IQR), mL/min	47 (36-56)	46 (37-54)	0.36	
Prot:creat ratio at 6 mo: median (IQR), mg/mmol[c]	13 (9-24)	12 (8-18)	0.07	1.00 (1.00-1.01); 0.09
CXCL10 chemokine burden: median (IQR), ng/mmol[d]	2.0 (0.7-4.4)	0.9 (0.4-2.5)	0.0007	1.12 (1.04-1.20); 0.007

eGFR (mL/min) calculated by the MDRD formula (mL/min per 1.73 m^2 of body surface).

[a] Each definition of rejection was added individually to the model including all other variables.

[b] Persisting rejection was defined as rejection observed in two biopsies (clinical or surveillance biopsy), which were at least 8 weeks apart.

[c] Three patients with primary focal segmental glomerulosclerosis were excluded from analysis.

[d] The arithmetic mean of urinary CXCL10 concentrations obtain at 3 and 6 months posttransplant was used for analysis = urinary CXCL10-burden.

long-term endpoints. However, delta urinary CXCL10 was neither a univariable predictor of the graft outcome endpoint ($P = 0.81$) nor the clinical composite endpoint ($P = 0.45$) and thus was not included in the multivariable Cox models.

Finally, using the entire patient population (n = 207), multivariable Cox proportional hazard analysis for both endpoints revealed the same independent predictors as for the described subgroup of patients with 2 pairs of surveillance biopsy/urine sample (n = 154) (data not shown).

Prognostic Characteristics of Urinary CXCL10

We used a time-dependent receiver operating characteristic analysis to further investigate the prognostic characteristics of the 6-month urinary CXCL10 levels and the urinary CXCL10-burden for prediction of both endpoints. The area under the curve were between 0.66 and 0.68 with sensitivities of 72% to 81%, specificities of 48% to 59%, positive predictive values of 38% to 49%, and negative predictive values of 81% to 87%. The optimal cutoff for the 6-month urinary CXCL10 levels was 0.70 ng/mmol, whereas it was 1.06 ng/mmol for the urinary CXCL10-burden (Table 4). According to these cutoff values, patients were classified as "high urinary CXCL10" and "low urinary CXCL10." Baseline and follow-up data at 6 months of patients with low and high 6-month urinary CXCL10 levels, as well as low and high urinary CXCL10-burden are summarized in **Tables S1 and S2** (**SDC**, http://links.lww.com/TXD/A11), respectively. Patients with high urinary 6-month CXCL10 levels had a higher frequency of second and deceased donor transplantations (both $P = 0.01$), a higher rate of BKV viremia ($P = 0.0001$) and higher proteinuria ($P = 0.04$) compared to patients with low 6-month urinary CXCL10 levels (**Table S1, SDC**, http://links.lww.com/TXD/A11). Patients with high urinary CXCL10-burden had more rejection episodes ($P = 0.02$; driven by more subclinical rejections), a higher rate of BKV viremia ($P < 0.0001$), and higher proteinuria ($P = 0.005$) compared to patients with low urinary CXCL10-burden (**Table S2, SDC**, http://links.lww.com/TXD/A11).

A low 6-month urinary CXCL10 level was associated with a 95% 5-year freedom from reaching the graft outcome endpoint compared to only 78% with a high 6-month urinary CXCL10 level ($P = 0.0007$). Similar results were observed comparing low and high urinary CXCL10-burden (95% vs 76%; $P = 0.001$) (Table 4 and Figure 2). Indeed, the time-to-event analysis showed no difference between the low 6-month CXCL10 level and the low CXCL10-burden groups ($P = 0.85$) as well as between the high 6-month CXCL10 level and the high CXCL10-burden groups ($P = 0.59$) (Figure 2A).

TABLE 3.

Associations of clinical and laboratory variables with clinical composite endpoint (n = 154 patients)

	Clinical composite endpoint (n = 53)	No clinical composite endpoint (n = 101)	Univariable, *p*	Multivariable cox proportional analysis, hazard ratio (95% CI); *p*
Baseline predictors				
Recipient age: median (IQR), y	55 (44-62)	53 (43-62)	0.35	
Primary disease, n (%)				
ADPKD	13 (25)	18 (18)	0.76	
Diabetic	8 (15)	9 (9)		
Vascular	4 (7)	10 (10)		
Glomerulopathy	15 (28)	41 (40)		
Other	13 (25)	23 (23)		
Pretransplant HLA-DSA, n (%)	10 (19)	14 (14)	0.39	0.68 (0.27-1.56); 0.38
Total HLA-A-B-DR mismatches, mean ± std.	4.1 ± 1.2	3.4 ± 1.4	0.009	1.39 (1.09-1.80); 0.008
First/second/third kidney transplant, %	81/17/2	87/11/2	0.54	1.87 (0.76-4.20); 0.17
Donor age: median (IQR), y	57 (45-66)	50 (43-62)	0.02	1.03 (1.01-1.05); 0.0008
Deceased donor, n (%)	31 (58)	53 (52)	0.25	1.56 (0.82-2.98); 0.18
DGF, n (%)	8 (15)	25 (25)	0.12	0.35 (0.14-0.78); 0.01
6-mo Predictors				
Rejection within the first 6 mo, defined[a]				
Any clinical rejection (including borderline), n (%)	4 (7)	10 (10)	0.41	0.94 (0.25-2.66); 0.91
Any sub(clinical) rejection (including borderline), n (%)	27 (51)	45 (45)	0.82	1.57 (0.87-2.87); 0.13
Persisting rejection, n (%)[b]	9 (17)	12 (12)	0.18	1.38 (0.33-1.77); 0.45
Phenotype				
(Sub)clinical TCMR ≥ Ia, n (%)	6 (11)	15 (15)	0.35	0.90 (0.38-2.49); 0.82
(Sub)clinical AMR (including category "suspicious"), n (%)	5 (9)	9 (9)	0.45	1.06 (0.33-3.91); 0.93
AMR with pretransplant HLA-DSA, n (%)	4 (7)	5 (5)	0.36	0.20 (0.02-1.66); 0.15
BKV viremia within first 6 mo, n (%)	10 (19)	7 (7)	0.02	0.79 (0.17-3.30); 0.75
eGFR at 6 mo: median (IQR), mL/min	47 (36-56)	46 (38-54)	0.54	
Prot:creat ratio at 6 mo, median (IQR), mg/mmol[c]	13 (9-22)	11 (8-17)	0.15	1.00 (0.98-1.01); 0.22
CXCL10 chemokine burden: median (IQR), ng/mmol[d]	1.8 (0.9-4.2)	0.8 (0.4-2.0)	0.002	1.10 (1.02-1.17); 0.02

eGFR (mL/min) calculated by the MDRD formula (ml/min/1.73 m^2 of body surface).

[a] Each definition of rejection was added individually to the model including all other variables.

[b] Persisting rejection was defined as rejection observed in two biopsies (clinical or surveillance biopsy), which were at least 8 weeks apart.

[c] Three patients with primary focal segmental glomerulosclerosis were excluded from analysis.

[d] The arithmetic mean of urinary CXCL10 concentrations obtain at 3 and 6 months posttransplant was used for analysis = urinary CXCL10-burden.

The same observation was made regarding the clinical composite endpoint (Table 4 and Figure 2B).

Individual Longitudinal Urinary CXCL10 Levels and Graft Outcome

Individual urinary CXCL10 levels measured at 3 and 6 months after transplantation of the 154 patients with 2 pairs of surveillance biopsies/urines were ordered by the 6-month CXCL10 values and correlated with the graft outcomes (Figure 3). Only 8 of 62 patients (13%) with a urinary 6-month CXCL10 value less than 0.70 ng/mmol reached the graft outcome endpoint compared to 35 of 92 patients (38%) with a urinary 6-month CXCL10 value greater than 0.70 ng/mmol ($P < 0.0001$). Furthermore, only 2 of 62 patients (3%) with low 6-month CXCL10 levels experienced late rejection or graft loss due to rejection compared with 15 of 92 patients (16%) with high 6-month CXCL10 levels ($P = 0.008$). Interestingly, patients with low 6-month CXCL10 levels often had stable and low 3-month CXCL10 levels, whereas patients with high 6-month CXCL10 levels demonstrated much more variation in a generally higher range.

Patients with HLA-DSA had slightly but significantly higher 6-month CXCL10 levels compared with HLA-DSA-negative patients (median, 1.8 ng/mmol [IQR, 0.8-6.3 ng/mmol] vs median, 0.8 ng/mmol [IQR, 0.4-2.1 ng/mmol]; $P = 0.02$), whereas urinary 3-month levels were similar ($P = 0.34$). Further, HLA-DSA–positive patients showed a trend to have a higher urinary CXCL10-burden compared to HLA-DSA–negative patients (median, 1.9 ng/mmol [IQR, 0.7-8.0 ng/mmol] vs median, 1.0 ng/mmol [IQR, 0.5-2.8 ng/mmol]; $P = 0.04$).

DISCUSSION

The main observation in this study was that the 6-month urinary CXCL10 levels and the urinary CXCL10-burden are both independent predictors for long-term renal allograft outcome with equivalent prognostic value. This is in line with the data from the multicenter CTOT-01 study, which demonstrated that elevated levels of urinary CXCL9 chemokine at 6 months are associated with a subsequent decline in eGFR or allograft rejection until 24 months after transplantation.[24] The novelty of our study is the calculation of a defined urinary CXCL10 cutoff derived from an unselected consecutive patient population having 2 surveillance biopsies at 3 and 6 months and a median follow-up time of 6.6 years. These results extend the work of the multicenter CTOT-01 trial and support the utility of a urinary chemokine-based risk stratification for renal transplantation.

TABLE 4.

Prognostic characteristics of urinary CXCL10

	Graft outcome endpoint (43/154 patients [28%] reached endpoint)		Clinical composite endpoint (53/154 patients [34%] reached endpoint)	
	Urinary CXCL10 measurement		Urinary CXCL10 measurement	
	At 6 mo	Mean of 3 mo + 6 mo	At 6 mo	Mean of 3 mo + 6 mo
AUC	0.68	0.67	0.66	0.67
P level	0.003	0.0008	0.003	0.002
Cutoff CXCL10/creat ratio, ng/mmol	0.70	1.06	0.70	1.06
Sensitivity	81%	72%	79%	74%
Specificity	48%	56%	50%	59%
PPV	38%	39%	45%	49%
NPV	87%	84%	82%	81%
Five-year freedom from reaching the endpoint	95% vs 78% ($P = 0.0007$)	95% vs 76% ($P = 0.001$)	90% vs 71% ($P = 0.0005$)	92% vs 66% ($P < 0.0001$)

AUC, area under the curve

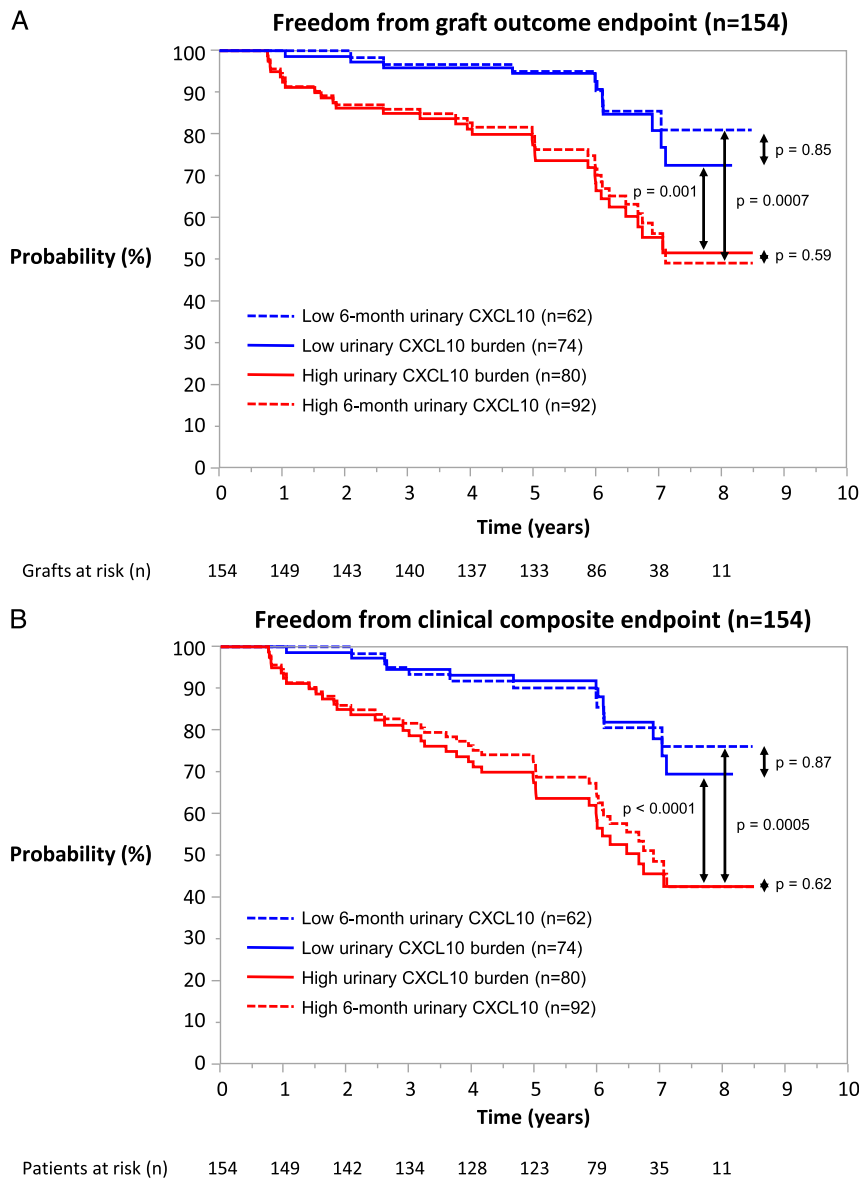

FIGURE 2. Outcomes of 154 patients stratified by early urinary CXCL10 levels. A, Freedom from graft outcome endpoint (ie, death-censored graft loss, clinical rejection beyond 6 months posttransplant, >20% decline of eGFR). B, Freedom from clinical composite endpoint (ie, death, graft loss, clinical rejection beyond 6 months posttransplant, >20% decline of eGFR).

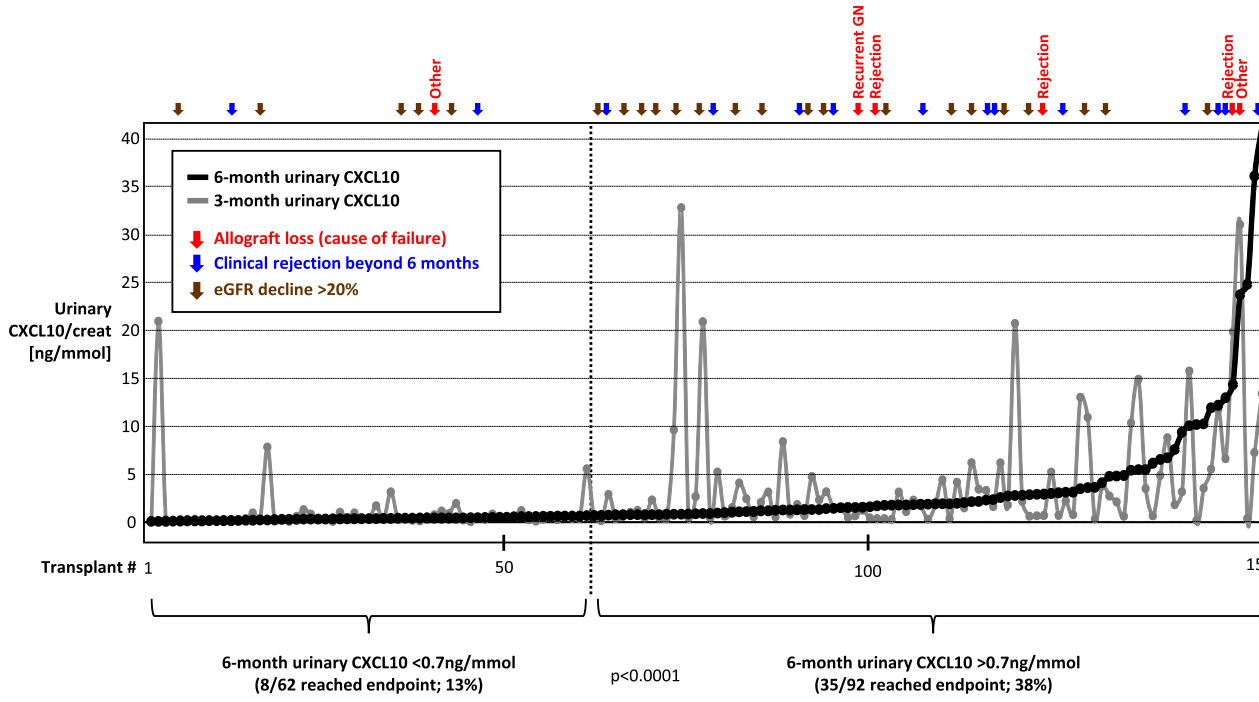

FIGURE 3. Individual longitudinal urinary CXCL10 levels and graft outcome. Urinary CXCL10 chemokine levels measured at 3 and 6 months after transplantation of the 154 patients with 2 pairs of surveillance biopsies/urines were ordered by the 6-month CXCL10 values and correlated with graft outcomes (coloured arrows). The cause of graft failures are indicated above the corresponding arrow. GN, glomerulonephritis.

A surprising finding in this study was the missing association of clinical and/or subclinical rejection within the first 6 months after transplantation and long-term allograft outcome. Although we used five different definitions of rejection, none was predictive. Several explanations exist for this finding. First, it could be due to an intervention bias as we treated all rejection episodes—even subclinical borderline rejection—with augmented immunosuppression likely preventing progression to significant organ damage. This assumption is supported by a recently published study by Loupy et al[15] who found that patients with treated subclinical T cell–mediated rejection had similar long-term prognosis that those individuals without any rejection. Second, standard histology can assess the number of cells in different renal compartments (eg, tubulitis), but it provides very limited information regarding the cell activity, which might be more critical.[30] Third, rejection might have been missed due to sampling error.

Another intriguing observation was that pretransplant HLA-DSA were not predictive for long-term graft outcome. One explanation for that could be that all patients with pretransplant HLA-DSA received induction therapy with antithymocyte globulin +/– IvIg and were maintained on a triple immunosuppression consisting of tacrolimus, MMF, and prednisone.[31] Furthermore, the group with HLA-DSA (ie, 24/154 [16%]) was rather small, and we might miss a significant impact because of insufficient statistical power. In addition, patients with HLA-DSA had rather low-level antibodies with median mean fluorescence intensity around 2500.

Two of 4 independent predictors for long-term allograft outcome in our study were immunological parameters (ie, HLA mismatches and urinary CXCL10 levels), supporting the established concept that inflammation, mainly induced by alloimmune responses, is a key factor for inferior allograft survival.[11,32] However, the lacking association of (sub)clinical rejection defined by the current Banff criteria with long-term allograft outcome suggests that treatment of (sub)clinical rejection was successful and/or that more subtle phenotypes of rejection might be involved. Several studies highlighted that even mild interstitial fibrosis/tubular atrophy plus inflammation (IF/TA plus i), where the degree of inflammation does not meet the diagnostic criteria for Banff borderline rejection, is strongly associated with functional decline and death-censored graft loss.[11-13,33,34] Remarkably, IF/TA plus i is also associated with higher number of HLA-mismatches,[11,33] as well as a rejection-like gene expression signature,[12,34] suggesting that IF/TA plus i may indeed indicate an ongoing, low-grade rejection process, which is not recognized in the current Banff classification. Thus, we postulate that urinary CXCL10 levels might reflect presence/absence of such low-grade rejection phenotypes.

A rational use of a biomarker-guided posttransplant surveillance strategy relies on a well-defined biomarker cutoff for clinical decision-making. Our study demonstrates that the urinary CXCL10 cutoff is time-dependent, likely because the intensity of inflammation becomes in general less severe later posttransplant. Therefore, the reported cutoff is only valid for measurements at 6 months after transplantation.

We acknowledge that the prognostic characteristics of 6-month urinary CXCL10 for prediction of long-term allograft outcome are only moderate. However, 6-month urinary CXCL10 outperformed important traditional risk factors of graft outcome like proteinuria, eGFR, allograft rejection, and pretransplant HLA-DSA which were all not predictive in our study. As the negative predictive value (NPV) of a 6-month urinary CXCL10 value less than 0.70 ng/mmol is 87%, the best use of this biomarker is to identify patients at very low risk for adverse outcome. By contrast, a 6-month

urinary CXCL10 value greater than 0.70 ng/mmol has a very low positive predictive value (PPV) of only 38%. This is consistent with the CTOT-01 study, which also revealed high NPV, but low PPV.[24] We were hoping that the urinary CXCL10-burden—reflecting the cumulative inflammation/rejection burden over time—would provide better specificity and PPV compared with a single point measurement. Unfortunately, this was not the case.

Ultimately, only a prospective trial can determine if a urinary chemokine-based monitoring and/or intervention strategy improves long-term renal allograft outcomes. A possible monitoring strategy for patients with elevated urinary chemokine levels could be to schedule additional surveillance biopsies (ie, at 12 and 24 months after transplantation) and/or a more dense screening for de novo HLA antibodies. On the other hand, if a patient has low levels of urinary chemokines, surveillance biopsies can be omitted with continuing noninvasive monitoring. This would be the benefit of a high NPV. Reasonable therapeutic interventions might be to maintain patients with elevated urinary chemokine levels at a higher maintenance immunosuppression (eg, triple immunosuppression including low dose steroids) and not subject them to drug minimization or avoidance protocols. Furthermore, patients at high risk of long-term allograft outcomes could be targeted for novel interventional trials, such as evaluating the efficacy of increased immunosuppression for IF/TA and inflammation.

This study has some limitations. There are no surveillance biopsies at 1 or 2 years after transplantation to investigate whether urinary CXCL10 levels are still predictive beyond histology obtained at later timepoints. In addition, we did not collect serial sera beyond 6 months after transplantation to assess the occurrence of de novo HLA-DSA, which is a risk factor for subsequent rejection and allograft loss. As the frequency of de novo HLA-DSA at 6 months after transplantation is very low (ie, <3%),[10] we regard it as unlikely that HLA-DSA at 6 months after transplantation would be a relevant confounder in our study. Notably, the aim of the study was to investigate whether a biomarker obtained at early specific timepoints (ie, 3 and/or 6 months) allows the prediction of long-term outcomes. Finally, nonadherence has emerged as a prominent risk factor for late rejection and allograft loss.[10] Again, nonadherence is mostly a problem beyond the first year after transplantation, and as such an assessment of the adherence status at 6 months after transplantation is unlikely to change the predictive value of urinary CXCL10 values.

In conclusion, 6-month urinary CXCL10 is an independent predictor for long-term graft outcome and might thus be a supplementary tool to tailor surveillance strategies and therapeutic interventions in renal transplant patients.

ACKNOWLEDGMENTS

The authors thank the staff of the University Hospital Basel Renal Transplant Unit and the histocompatibility laboratory for collection and processing of urine samples.

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