American Journal of Transplantation 2014; 14: 908–915
Wiley Periodicals Inc.

doi: 10.1111/ajt.12639

Sterile Leukocyturia Is Associated With Interstitial Fibrosis and Tubular Atrophy in Kidney Allograft Protocol Biopsies

S. Coelho[1], F. Ortíz[2], R. Gelpi[3], P. Koskinen[2], N. Porta[4], O. Bestard[3], E. Melilli[3], O. Taco[3], J. Torras[3], E. Honkanen[2], J. M. Grinyó[3] and J. M. Cruzado[3,*]

[1]*Department of Nephrology, Hospital Fernando da Fonseca, Lisbon, Portugal*
[2]*Department of Nephrology, Helsinki University Central Hospital, Helsinki, Finland*
[3]*Department of Nephrology, Hospital Universitari de Bellvitge, University of Barcelona, IDIBELL, L'Hospitalet de Llobregat, Spain*
[4]*Central Unit of Clinical Research and Clinical Trials (UCICEC), IDIBELL, L'Hospitalet de Llobregat, Spain*
[*]*Corresponding author: Josep M. Cruzado, jmcruzado@bellvitgehospital.cat*

Kidney allograft interstitial fibrosis and tubular atrophy (IF/TA) is associated with a poorer renal function and outcome. In the current clinical practice, an early diagnosis can only be provided by invasive tests. We aimed to investigate the association of sterile leukocyturia with Banff criteria histological findings in kidney allograft protocol biopsies. We studied 348 allograft biopsies from two different European countries performed at 8.5 + 3.5 months after transplantation. In these cases, the presence of sterile leukocyturia (Leuc+, n = 70) or no leukocyturia (Leuc−, n = 278) was analyzed and related to Banff elementary lesions. Only IF/TA was significantly different between Leuc+ and Leuc− groups. IF/TA was present in 85.7% of Leuc+ and 27.7% of Leuc− patients ($p < 0.001$). IF/TA patients had higher serum creatinine and presence of proteinuria ($p < 0.05$). Independent predictors of IF/TA were donor age, donor male sex, serum creatinine and Leuc+ (hazard ratio 18.2; 95% confidence interval, 8.1–40.7). The positive predictive value of leukocyturia for predicting IF/TA was 85.7% whereas the negative predictive value was 72.3%. These studies suggest that leukocyturia is a noninvasive and low-cost test to identify IF/TA. An early diagnosis may allow timely interventional measures directed to minimize its impact and improve graft outcome.

Keywords: Interstitial fibrosis, leukocyturia, renal transplant, tubular atrophy

Abbreviations: AR, acute rejection; CCL2, chemokine (C-C motif) ligand 2; CI, confidence interval; CIT, cold ischemia time; CNI, calcineurin inhibitor; DD, deceased donor; DGF, delayed graft function; ESRD, end-stage renal disease; HR, hazard ratio; IF/TA, interstitial fibrosis and tubular atrophy; LD, living donor; Leuc+, sterile leukocyturia; Leuc−, no leukocyturia; MM, mismatch; NPV, negative predictive value; PPV, positive predictive value; RNA, ribonucleic acid; SD, standard deviation

Received 17 September 2013, revised 13 December 2013 and accepted for publication 29 December 2013

Introduction

Interstitial fibrosis and tubular atrophy (IF/TA) constitute one of the most important histopathological entities associated with long-term renal allograft failure in protocol biopsies taken early after renal transplantation (1,2). The Banff grade of IF/TA correlates with lower estimated glomerular filtration rate and higher proteinuria (3,4), and its predictive value on outcome has been shown to be independent from clinical and analytical parameters such as serum creatinine, acute rejection or proteinuria (2). The term ''IF/TA without evidence of any specific etiology'' was introduced in the revised Banff 2005 classification, to replace the previous ''chronic allograft nephropathy,'' because this name was thought to diminish attempts to determine the underlying cause of the histologic lesions (5,6). In fact, IF/TA seems to be the consequence of several processes involving both alloantigen-dependent and alloantigen-independent factors (3). Its progression has been associated with preexisting donor damage, degree of sensitization, cold ischemia time (CIT), clinical and subclinical acute rejection, cyclosporine exposure and renal calcifications (7). More recent studies have advocated that graft implantation injury may cause an early ''silent inflammation'' that leads to the development of IF/TA (8).

An early diagnosis of IF/TA is important given its negative impact on renal graft prognosis and its unpredictable development. Surveillance biopsies can diagnose IF/TA at a preclinical stage, while graft function still remains normal (9). Therefore, it has been advocated that they can be used to predict the risk of subsequent graft function deterioration, allowing early intervention designed to decelerate or abrogate development of fibrosis and

ultimately improve graft survival (10). However, protocol biopsies have several limitations associated with morbidity, cost and potential for sampling error, and not all kidney transplantation units have the human and logistical resources to implement such a program (11). Noninvasive diagnostic tests of IF/TA have been searched. Urinary markers such as the protein CCL2 or the messenger RNA levels of kidney injury molecule-1 and vimentin showed promising results (12–14). Several recent manuscripts have been published reporting on the diagnosis of acute rejection through messenger RNA amplification in the urine of some lymphocyte-derived messages (15,16). However, none of these expensive studies controls the presence of leukocytes in the samples analyzed.

We aimed to investigate whether a routine urinalysis could predict subclinical histopathological changes in protocol biopsies with important impact in prognosis. Specifically, we hypothesized that sterile leukocyturia could predict histological findings of acute and/or chronic inflammatory damage classified by Banff criteria.

Materials and Methods

Patient population and study design

Between January 2006 and July 2010, a total of 820 patients received a kidney transplant at Bellvitge University Hospital and Helsinki University Hospital. A kidney allograft biopsy was taken by protocol, after patient's written consent, in 422 patients at a median time of 8 months after transplantation, as previously described (17). We retrospectively reviewed all kidney transplant patients with a protocol biopsy who had a urinalysis determination between 1 month before and 1 month after the protocol biopsy.

Definition of clinical variables

Leukocyturia was determined by flow cytometry (Sysmex UF-1000i; Sysmex Europe GmbH, Norderstedt, Germany) in a morning fresh urine sample. The measurement range is 1–5000/μL. Leukocyturia was considered present when urinary leucocytes were $>10/\mu$L. This technique does not provide any information about the type of leukocyte present in the urine sample. Moreover, the following variables were evaluated at the time of surgery: age, sex, cause of end-stage renal disease (ESRD), dialysis time, *peak/last* panel reactivity antibodies, number of HLA mismatches, donor age and sex, CIT, and immunosuppressive treatment. Regarding immunosuppression protocols, basiliximab was used in the case of kidneys from extended criteria donors and thymoglobulin in high-immunological-risk recipients. As a calcineurin inhibitor (CNI), cyclosporine was mainly used in Helsinki while tacrolimus was preferred in Barcelona. Steroids and mycophenolate mofetil were given to all patients. After surgery, the following variables were recorded: time of biopsy, delayed graft function (DGF), acute rejection, proteinuria and serum creatinine at the time of biopsy, graft loss or death.

Kidney allograft biopsies

Protocol biopsies were performed under ultrasound guidance with an automated biopsy gun and processed for light and immunofluorescence microscopy. Renal lesions were graded and diagnosed according to the 2007 Banff update of Banff 1997 diagnostic criteria (5) by local pathologists who were unaware of leukocyturia assessment. In this classification (6), biopsies have fibrosis if they had a Banff chronic interstitial score $\geq$1. According to this classification, IF/TA is graded mild (grade I) when IF/TA involve $<$25% of cortical area, moderate (grade II) when they affect 26–50% of cortical area and severe (grade III) when $>$50% of cortical area is involved.

Statistics

All data are presented as frequencies for categorical variables or mean and standard deviation for normally distributed continuous variables. Groups were compared using the chi-squared test for categorical variables, t-test for normally distributed data and nonparametric Mann–Whitney U-test for nonnormally distributed variables. To identify independent predictors of IF/TA, univariate and multivariate logistic regression analyses were performed. From the probability of IF/TA predicted from these models, sensitivity and specificity were computed to assess the predictive performance of leukocyturia in this study. A cutoff of 0.6 in the predicted probability (corresponding to the observed prevalence of IF/TA) was considered to classify patients. Cumulative percent graft survival was estimated by Kaplan–Meier analysis. All statistics were performed with SPSS version 20.0 (IBM SPSS Statistics for Windows, Version 20.0; Armonk, NY). The statistical significance level was defined as p-value $<$ 0.05.

Results

Baseline patient characteristics

We evaluated 422 protocol kidney allograft biopsies performed in 422 recipients (Figure S1). Seventy-four were excluded: 7 by inadequate biopsy sample, 59 because urinalysis was unavailable and 8 because there was a concomitant positive urine culture. Therefore, a total of 348 biopsies were suitable for the analysis. Among the 348 patients with histological evaluation, 70 patients (20.1%) had sterile leukocyturia (Leuc+) at the biopsy time and 278 (79.9%) had no leukocyturia (Leuc−). Both groups had similar demographic characteristics with no differences in the etiology of ESRD (Table 1). They also had similar transplant characteristics, namely with respect to donor demographics, immunological compatibility, immunosuppressive therapy and short-term clinical outcome. Induction therapy was given to one-third of the patients (80% basiliximab and 20% thymoglobulin, equally in both groups). CNI was given to nearly 90% of the patients (60% tacrolimus and 40% cyclosporine, without differences in Leuc+ and Leuc−). Ten percent of the patients were on CNI-free protocols in both groups, either on sirolimus/everolimus or on belatacept. No patient had diagnosis of BK virus nephropathy. The source of kidneys from deceased donors was always from brain death donors.

Pathological findings and laboratory determinations according to leukocyturia

Regarding histology (Table 2), IF/TA was present in 85.7% of Leuc+ and 27.7% of Leuc− patients ($p < 0.001$). When all parameters of the Banff classification were analyzed separately, only IF/TA were significantly different between Leuc+ and Leuc− groups. Of note, the presence of tubular and/or interstitial inflammation was not associated with leukocyturia. Moreover, the addition of tubulointerstitial inflammation to IF/TA did not increase the presence of leukocyturia further. When the severity of IF/TA was

Table 1: Demographic and clinical data

	All patients (N = 348)	With leukocyturia (N = 70)	Without leukocyturia (N = 278)	p-Value
Cause of ESRD (%)				
Diabetes	60 (17.2)	11 (18.3)	49 (17.6)	–
Glomerular	97 (27.9)	25 (35.7)	72 (25.9)	–
Interstitial	30 (8.6)	2 (2.9)	28 (10.1)	–
Polycystic kidney disease	64 (18.4)	15 (21.4)	49 (17.6)	–
Vascular	22 (6.3)	7 (10)	14 (5.4)	–
Other	75 (21.5)	10 (14.3)	65 (23.4)	0.09*
Recipient sex (male/female)	230/118	11/13	69/26	0.29
Recipient age (mean, SD)	49.19 (SD 12.8)	49.82 (SD 13.5)	49.04 (SD 12.6)	0.65
Donor sex (male/female)	208/140	40/30	168/110	0.62
Donor age (mean, SD)	48.3 (SD 14.2)	50.03 (SD 13.5)	47.87 (SD 14.4)	0.26
Retransplantation (%)	31 (8.9)	5 (7.2)	26 (9.4)	0.72
DD/LD	334/14	66/4	268/10	0.42
Previous acute rejection (%)	60 (17.2)	17 (24.3)	43 (15.5)	0.08
Dialysis time (mean, SD)	27.32 (SD 24.2)	25.71 (SD 23.0)	27.7 (SD 24.5)	0.53
Induction therapy (%)	120 (34.6)	24 (34.3)	96 (34.7)	0.95
CNI (%)	312 (89.7)	62 (88.6)	250 (89.9)	0.74
HLA-A MM (SD)	1.37 (0.6)	1.54 (0.6)	1.33 (0.6)	0.11
HLA-B MM (SD)	1.35 (0.5)	1.38 (0.6)	1.35 (0.5)	0.83
HLA-DR MM (SD)	0.73 (0.6)	0.66 (0.6)	0.75 (0.6)	0.24
Cold ischemia time (SD)	18.85 (5.9)	18.5 (7.2)	18.9 (5.6)	0.54
DGF (%)	116 (33.3)	25 (35.7)	91 (32.7)	0.64

CNI, calcineurin inhibitor; DD, deceased donor; DGF, delayed graft function; ESRD, end-stage renal disease; LD, living donor; MM, mismatch; SD, standard deviation.
*p-Value by chi-squared test to the overall relationship.

analyzed, we found IF/TA grade I in 84% and grade II in 16% of the cases without any difference between Leuc+ and Leuc− groups. Moreover, there was no relationship between the degree of IF/TA and the number of leukocytes in urine. Although there was a trend for a higher rate of clinical acute rejections in the Leuc+ group (Table 1), there was no statistically significant difference in the proportion of acute rejection or borderline changes in both groups (Table 2). On the other hand, the Leuc+ group had a higher serum creatinine at the time of biopsy ($p = 0.019$). There were also a higher proportion of the Leuc+ patients with proteinuria (Table 2).

Table 2: Main differences in histological and laboratory findings between patients with and without leukocyturia

	All patients (N = 348)	With leukocyturia (N = 70)	Without leukocyturia (N = 278)	p-Value
Time of biopsy (days, SD)	263.9 (105.8)	276.6 (121.3)	260.7 (101.5)	0.261
Subclinical AR (%)	29 (8.3)	6 (8.6)	23 (8.3)	0.881
Borderline changes (%)	118 (33.9)	23 (32.9)	95 (34.2)	0.835
IF/TA (%)	137 (39.4)	60 (85.7)	77 (27.7)	**<0.001**
g (%)	42 (12.3)	35 (12.8)	7 (10.3)	0.577
i (%)	126 (36.5)	24 (34.8)	102 (37.0)	0.737
t (%)	75 (21.8)	14 (20.6)	61 (22.1)	0.787
v (%)	6 (1.7)	2 (2.9)	4 (1.5)	0.413
aah (%)	60 (17.5)	16 (23.5)	44 (16.0)	0.143
cg (%)	4 (1.2)	1 (1.5)	3 (1.1)	0.794
ci (%)	154 (44.6)	57 (82.6)	97 (35.1)	**<0.001**
ct (%)	159 (46.1)	52 (75.4)	107 (38.8)	**<0.001**
mm (%)	28 (8.6)	7 (10.6)	21 (8.0)	0.507
Proteinuria, >0.15 g/day (%)	119 (36.2)	34 (50.7)	85 (32.4)	**0.005**
Proteinuria, >0.5 g/day (%)	21 (6.4)	8 (11.9)	13 (5.0)	**0.037**
sCreatinine (μmol/L; SD)	120.3 (35.9)	129.3 (39.1)	118.0 (34.8)	**0.019**

For g, i, t, v, aah, cg, ci, ct, mm, % means the percentage of cases with the value of the corresponding variable above 0.
aah, arteriolar hyaline thickening; AR, acute rejection; cg, allograft glomerulopathy; ci, interstitial fibrosis; ct, tubular atrophy; g, glomerular inflammation; i, interstitial inflammation; IF/TA, interstitial fibrosis and tubular atrophy; mm, mesangial matrix increase; t, tubulitis; v, intimal arteritis.
Statistically significant p-values in bold.

Table 3: Variables associated with IF/TA in kidney allograft protocol biopsies

	All patients (N = 348)	With IF/TA (N = 137)	Without IF/TA (N = 211)	p-Value
Recipient sex (male/female)	230/118	96/41	134/77	0.206
Recipient age (mean, SD)	49.1 (12.8)	50.3 (13.1)	48.5 (12.5)	0.210
Donor sex (male/female)	208/140	92/45	116/95	**0.024**
Donor age (mean, SD)	48.3 (14.2)	51.1 (14.1)	46.5 (14.0)	**0.003**
Retransplantation (%)	31 (9.0)	13 (9.5)	18 (8.6)	0.931
DD/LD	334/14	132/5	202/9	0.775
Previous acute rejection (%)	60 (17.2)	33 (24.1)	27 (12.8)	**0.006**
Dialysis time (mean, SD)	27.3 (24.2)	26.3 (25.1)	28.0 (23.6)	0.537
CNI (%)	312 (89.7)	118 (86.1)	194 (91.9)	0.082
HLA-A MM (SD)	1.37 (0.6)	1.45 (0.6)	1.28 (0.6)	0.125
HLA-B MM (SD)	1.35 (0.5)	1.37 (0.5)	1.33 (0.6)	0.723
HLA-DR MM (SD)	0.73 (0.6)	0.78 (0.6)	0.70 (0.6)	0.185
Cold ischemia time (SD)	18.8 (5.9)	18.8 (6.0)	18.9 (5.9)	0.819
DGF (%)	116 (33.3)	48 (35.0)	68 (32.2)	0.587
Leukocyturia (%)	70 (20.1)	60 (43.8)	10 (4.7)	<**0.001**
Proteinuria, >0.15 g/day (%)	119 (36.2)	63 (48.8)	56 (28.0)	<**0.005**
Proteinuria, >0.5 g/day (%)	21 (6.4)	12 (9.3)	9 (4.5)	0.082
sCreatinine (μmol/L; SD)	120.2 (35.9)	130.2 (38.2)	113.8 (32.9)	<**0.005**

CNI, calcineurin inhibitor; DD, deceased donor; DGF, delayed graft function; IF/TA, interstitial fibrosis and tubular atrophy; LD, living donor; MM, mismatch; SD, standard deviation.
Statistically significant p-values in bold.

Variables associated with IF/TA in kidney allograft protocol biopsies

The overall IF/TA prevalence in our study population was 39.4%. These patients more frequently received kidneys from older and/or male donors (Table 3). The antecedent of clinical acute rejection was also associated with IF/TA. Regarding laboratory determinations, IF/TA patients had higher serum creatinine and presence of proteinuria and leukocyturia (Table 3). On the other hand, the significant variables associated with IF/TA in the univariate analysis were included in a multivariate logistic regression model, together with other clinical variables that may have an impact on the association between leukocyturia and IF/TA (e.g. DGF). Donor age, donor male sex, serum creatinine and particularly Leuc+ (hazard ratio [HR] 18.2, 95% confidence interval [CI], 8.1–40.7) were predictors of IF/TA. Therefore, leukocyturia was associated with a higher risk to observe IF/TA in the kidney allograft protocol biopsy (Table 4). The specificity of leukocyturia for predicting IF/TA was 95.3% whereas the sensitivity was 44%. Also, its positive predictive value (PPV) was 85.7% and negative predictive value (NPV) was 72.3%.

Leukocyturia and graft survival

The mean follow-up (from transplantation until graft loss or censored at July 12, 2012) was 5.1 ± 2.1 years, rather short to observe major differences. Nevertheless, there is a trend to poor graft survival in patients showing IF/TA and inflammation in the protocol biopsy (Figure 1A). Leukocyturia had no effect on patient and graft survival ($p = 0.98$, log-rank test). Moreover, the addition of leukocyturia to pathological findings was not associated with any significant change in graft survival (Figure 1B).

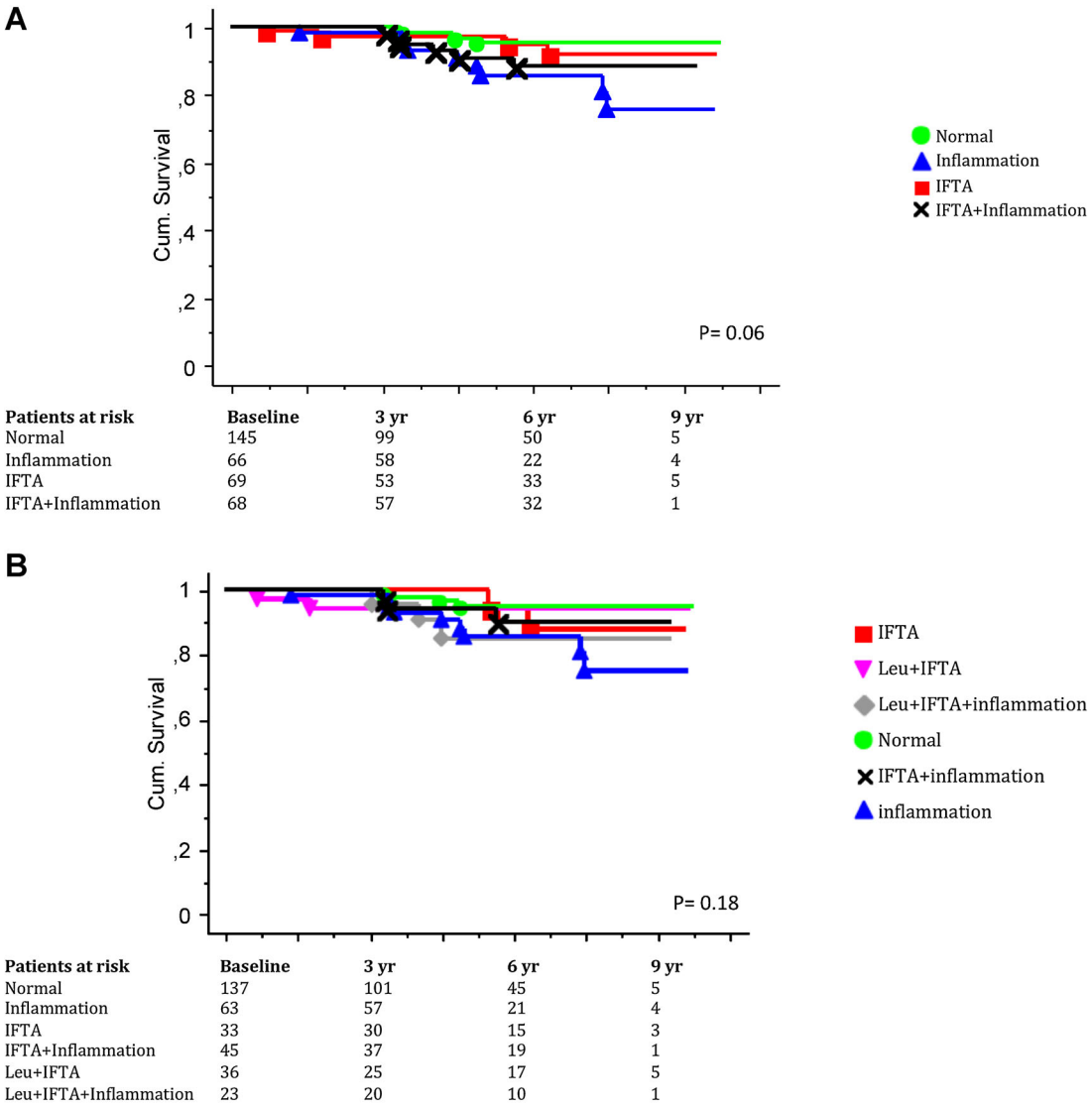
Discussion

In our study, we analyzed the relationship between a classical urine analysis and the Banff classification and

Table 4: Logistic regression analysis for IF/TA predictors

		Univariate			Multivariate		
Covariates		HR	95% CI	p-Value	HR	95% CI	p-Value
Donor sex	Male vs. female (ref)	1.76	(1.07, 2,62)	0.024	2.68	(1.48, 4.86)	0.001
Donor age (year)		1.024	(1.008, 1.041)	0.003	1.027	(1.004, 1.049)	0.018
Acute rejection	Presence vs. absence (ref)	2.16	(1.23, 3.80)	0.007	2.01	(0.98, 4.12)	0.056
DGF	Presence vs. absence (ref)	1.13	(0.72, 1.79)	0.58	0.73	(0.40, 1.35)	0.321
Proteinuria	Presence vs. absence (ref)	2.46	(1.55, 3.90)	0.0001	1.67	(0.953, 2.914)	0.073
Leukocyturia	Presence vs. absence (ref)	15.7	(7.63, 32.2)	<0.0001	18.2	(8.13, 40.7)	<0.0001
1 year sCreatinine (μmol/L)		1.013	(1.007, 1.020)	<0.0001	1.010	(1.001, 1.019)	0.032

Proteinuria is >0.15 g/day.
CI, confidence interval; DGF, delayed graft function; HR, hazard ratio; IF/TA, interstitial fibrosis and tubular atrophy.



Patients at risk	Baseline	3 yr	6 yr	9 yr
Normal	145	99	50	5
Inflammation	66	58	22	4
IFTA	69	53	33	5
IFTA+Inflammation	68	57	32	1

Patients at risk	Baseline	3 yr	6 yr	9 yr
Normal	137	101	45	5
Inflammation	63	57	21	4
IFTA	33	30	15	3
IFTA+Inflammation	45	37	19	1
Leu+IFTA	36	25	17	5
Leu+IFTA+Inflammation	23	20	10	1

Figure 1: Death-censored graft survival according histological findings. (A) In the Kaplan–Meier analysis, the association of IF/TA with inflammation shows a trend toward poor graft survival, although the mean follow-up is relatively short (5.1 ± 2.1 years) to observe high number of events. (B) The presence or absence of leukocyturia was not associated with any significant change in graft survival. p-Values were obtained by log-rank test. There were eight patients with leukocyturia and normal biopsy and three cases with leukocyturia and inflammation. These groups are not included in the Kaplan–Meier analysis due to the small number and the absence of events. IF/TA, interstitial fibrosis and tubular atrophy.

provide evidence that leukocyturia can be useful in predicting IF/TA in early protocol biopsies.

Sterile leukocyturia, defined as the presence of leukocytes in the urine without bacterial growth on conventional immersion culture media, has been associated with interstitial nephritis, nephrolithiasis, uroepithelial tumors and infection with atypical organisms (18–20). Interestingly, Baquero and Santiago-Delpin (21) have also described an association of sterile leukocyturia with high serum creatinine and worse allograft outcome in kidney transplant patients, although these authors did not report any histological assessment. Although we did not observe any effect of leukocyturia on graft survival, probably because of the relatively short follow-up, our results were in agreement with Baquero and Santiago-Delpin (21) because leukocyturia was associated with high serum creatinine and presence of proteinuria.

Kidney interstitial fibrosis is defined by the accumulation of collagen and related molecules in the renal interstitium. Tubular atrophy is usually associated with interstitial

fibrosis, although it probably has different mechanisms, being defined as loss of tubular specialized transport and metabolic capacity (22). IF/TA is thought to result from the orchestration of multiple cell types: tubular cells, renal vasculature cells and inflammatory cells, including lymphocytes, monocyte/macrophages, mast cells and dendritic cells (22). The renal tubular and endothelium cells are thought to undergo transitional changes into a mesenchymal phenotype, responsible for the production of fibrosis and extracellular matrix deposition. Inflammatory cells seem to mediate the epithelial to mesenchymal transition (22) and this may thus explain why we found leukocyturia to be associated with IF/TA.

Recently, the Mayo Clinic group reported data of almost 600 patients with 1- and 5-year protocol biopsies (23). During the first posttransplant year, 53% had fibrosis, but only 23% of patients with mild fibrosis progressed to more severe forms at 5 years. Similarly, in our study, we described 39.4% prevalence of IF/TA at 6–10 months posttransplantation. There are several well-known risk factors for IF/TA (3). As an example, in a series of 688 protocol biopsies performed in 258 patients, Schwarz et al (24) reported that the risk factors for IF/TA at 26 weeks after transplantation were GFR at 3 months, previous acute rejection and nephrocalcinosis in a previous biopsy. In our study, IF/TA was associated with donor age and sex and acute rejection, in accordance to previous studies. A higher donor age and male sex probably represent baseline damage already present in the allograft at the time of implantation. However, we could not confirm this notion due to the lack of preimplantation kidney biopsy assessment in our study. An acute rejection episode before the protocol biopsy suggests an alloimmune injury that healed into IF/TA. Interestingly, a third of patients with IF/TA also had tubulitis and more than 40% had interstitial inflammation, which represents ongoing acute damage on chronic tubular and interstitial lesions. We found IF/TA with inflammation was associated with a poorer outcome in our study, as previously described (25). Recently, the current apparently less severe and less progressive nature of early IF/TA in the absence of inflammation led some authors to compare it to the process of wound healing that evolves into mild and stable lesions (26). However, although these chronic lesions probably have a lower impact on kidney allograft and patient outcome than previously thought and possibly lower than other factors like acute rejection, this does not mean that they have no impact at all. In fact, in our study, patients with IF/TA had higher serum creatinine and proteinuria at the time of protocol biopsy than patients without IF/TA. A larger number of cases and a higher follow-up might have shown a difference in graft and patient survival.

Early identification of IF/TA is important in order to implement strategies that can slow its progression and ideally reverse its development. Several studies have shown that progression of IF/TA is related to immunosuppression (27,28) and Gelens et al (4) even suggested that it should be guided according to the grade of IF/TA at preimplantation biopsy. Although there is still not enough evidence, there are some surrogate markers that point to a beneficial effect of angiotensin-converting enzyme inhibitor/angiotensin receptor blocker in the treatment of IF/TA (29). Along these lines, it is important to develop biomarkers of kidney allograft lesions. For instance, Suthanthiran et al (15) reported a urinary test that accurately diagnoses acute rejection of kidney transplants by just measuring three genetic molecules in a urine sample. Nowadays, the use of omics-technologies in further refining the diagnostic criteria and predict outcomes is being actively explored in both experimental and clinical settings (30,31). The study of the genomic profile (genomics) and proteins (proteomics) revealed that the genes and proteins more commonly expressed in patients with IF/TA were those related to immune response, inflammation and matrix deposition (32–34). Transcriptomics (the study of gene transcripts) associated areas of IF/TA with distinct secondary inflammation patterns: lymphoid aggregates, B cell and plasma cell transcripts (35), mast cell transcripts (36), and FoxP3 mRNA (37). Although these results are promising, they are complex, not accessible in daily practice and time-consuming. Although many of these studies are seeking leukocyte products, surprisingly none of them control the mere presence of leukocytes in the samples analyzed. Our study suggests that it is feasible to overcome the need for a biopsy in patients with leukocyturia (PPV of 85.7%), though it should not be considered as a fair screening test since its sensitivity is 44%. Leukocyturia could be a highly specific test for IF/TA, since the estimated probability of a positive test of leukocyturia among patients without IF/TA is low ($100 - \text{specificity} = 5\%$). However, the NPV of 72.3% suggests that it may not be possible to avoid the biopsy to diagnose IF/TA in patients without leukocyturia. The predictive performance of leukocyturia should be validated in an independent data set. On the other hand, it would also be interesting to explore a study looking at the measurement variability between institutions and comparing different techniques (flow cytometry, urinary dipstick and standard urinary sediment).

Our study has some limitations associated with its retrospective design. Nevertheless, we analyzed a considerable number of patients from two different European countries, which strengthens our conclusions and minimizes the impact of possible confounders. In addition, although leukocyturia was useful for IF/TA detection, it was useless for identifying those patients with IF/TA who were at risk of graft failure. Urinary infections with atypical organisms have not been systematically excluded as a cause of sterile leukocyturia.

In conclusion, in the current-omics era, our results suggest that classical urinalysis still provides valuable clinical information for the follow-up of kidney allograft recipients,

although prospective studies on the assessment of the type of leukocytes in urine are necessary to validate our retrospective results. Sterile leukocyturia might be an accessible, low-cost marker of IF/TA.

Acknowledgments

This research was supported by The Red de Investigación Renal (REDinREN, ISCIII RD12/0021/0003), ISCIII grant PI12/01427 and Helsinki University Central Hospital research funds (EVO TYH2010206).

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

References

1. Servais A, Meas-Yedid V, Noel LH, et al. Interstitial fibrosis evolution on early sequential screening renal allograft biopsies using quantitative image analysis. Am J Transplant 2011; 11: 1456–1463.
2. Serón D, Moreso F, Bover J, Condom E, Gil-Vernet S. Early protocol renal allograft biopsies and graft outcome. Kidney Int 1997; 51: 310–316.
3. Nankivell BJ, Borrows RJ, Fung CL-S, O'Connell PJ. The natural history of chronic allograft nephropathy. N Engl J Med 2003; 349: 2326–2333.
4. Gelens MA, Steegh FM, van Hooff JP, et al. Immunosuppressive regimen and interstitial fibrosis and tubules atrophy at 12 months postrenal transplant. Clin J Am Soc Nephrol 2012; 7: 1010–1017.
5. Solez K, Colvin RB, Racusen LC, John S, John S. Banff'05 meeting report: Differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). Am J Transplant 2007; 7: 518–526.
6. Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. Kidney Int 1999; 55: 713–723.
7. Serón D, Moreso F. Protocol biopsies in renal transplantation: Prognostic value of structural monitoring. Kidney Int 2007; 72: 690–697.
8. Brouard S, Renaudin K, Soulillou JP. Revisiting the natural history of IF/TA in renal transplantation. Am J Transplant 2011; 11: 647–649.
9. Yilmaz S, Tomlanovich S, Mathew T, et al. Protocol core needle biopsy and histologic Chronic Allograft Damage Index (CADI) as surrogate end point for long-term graft survival in multicenter studies. J Am Soc Nephrol 2003; 14: 773–779.
10. Henderson LK, Nankivell BJ, Chapman JR. Surveillance protocol kidney transplant biopsies: Their evolving role in clinical practice. Am J Transplant 2011; 11: 1570–1575.
11. Rush D. Can protocol biopsy better inform our choices in renal transplantation? Transplant Proc 2009; 41: S6–S8.
12. Anglicheau D, Muthukumar T, Hummel A, Ding R, Sharma VK. Discovery and validation of a molecular signature for the noninvasive diagnosis of human renal allograft fibrosis. Transplantation 2012; 93: 1136–1146.
13. Nogare AL, Dalpiaz T, Veronese FJ, Gonçalves LF, Manfro RC. Noninvasive analyses of kidney injury molecule-1 messenger RNA in kidney transplant recipients with graft dysfunction. Transplant Proc 2012; 44: 2297–2299.
14. Ho J, Rush DN, Gibson IW, et al. Early urinary CCL2 is associated with the later development of interstitial fibrosis and tubular atrophy in renal allografts. Transplantation 2010; 90: 394–400.
15. Suthanthiran M, Schwartz JE, Ding R, et al. Urinary-cell mRNA profile and acute cellular rejection in kidney allografts. N Engl J Med 2013; 369: 20–31.
16. Hricik DE, Nickerson P, Formica RN, et al. Multicenter validation of urinary CXCL9 as a risk-stratifying biomarker for kidney transplant injury. Am J Transplant 2013; 13: 2634–2644.
17. Ortiz F, Gelpi R, Koskinen P, et al. IgA nephropathy recurs early in the graft when assessed by protocol biopsy. Nephrol Dial Transplant 2012; 27: 2553–2558.
18. Michel DM, Kelly CJ. Acute interstitial nephritis. J Am Soc Nephrol 1998; 9: 506–515.
19. Gómez E, Aguado S, Baltar J, Alvarez R, Laures A, Alvarez-Grande J. Sterile leukocyturia as a manifestation of urinary tuberculosis in renal transplant patients. Nephrol Dial Transplant 1993; 13: 1610–1611.
20. van Rossum AM, Dieleman JP, Fraaij PL, et al. Persistent sterile leukocyturia is associated with impaired renal function in human immunodeficiency virus type 1-infected children treated with indinavir. Pediatrics 2002; 110: 1–7.
21. Baquero A, Santiago-Delpin EA. The prognostic significance of sterile leukocyturia after kidney transplantation. Dial Transpl 1984; 13: 758–762.
22. Farris AB, Colvin RB. Renal interstitial fibrosis: Mechanisms and evaluation. Curr Opin Nephrol Hypertens 2012; 21: 289–300.
23. Stegall MD, Park WD, Larson TS, Gloor JM, Cornell LD, Sethi S. The histology of solitary renal allografts at 1 and 5 years after transplantation. Am J Transplant 2011; 11: 698–707.
24. Schwarz A, Mengel M, Gwinner W, Radermacher J. Risk factors for chronic allograft nephropathy after renal transplantation: A protocol biopsy study. Kidney Int 2005; 67: 341–348.
25. Park WD, Griffin MD, Cornell LD, Cosio FG, Stegall MD. Fibrosis with inflammation at one year predicts transplant functional decline. J Am Soc Nephrol 2010; 21: 1987–1997.
26. Mengel M, Chang J, Kayser D, et al. The molecular phenotype of 6-week protocol biopsies from human renal allografts: Reflections of prior injury but not future course. Am J Transplant 2011; 11: 708–718.
27. Mota A, Arias M, Taskinen EI, et al. Rapamune maintenance regimen trial: Sirolimus-based therapy following early cyclosporine withdrawal provides significantly improved renal histology and function at 3 years. Am J Transplant 2004; 4: 953–961.
28. Moreso F, Serón D, Carrera M, et al. Baseline immunosuppression is associated with histological findings in early protocol biopsies. Transplantation 2004; 78: 1064–1068.
29. Cruzado JM, Rico J, Grinyo JM. The renin angiotensin system blockade in kidney transplantation: Pros and cons. Transpl Int 2008; 21: 304–313.
30. Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: Updates and future directions. Am J Transplant 2008; 8: 753–760.
31. Naesens M, Sarwal MM. Molecular diagnostics in transplantation. Nat Rev Nephrol 2010; 6: 614–628.
32. Henger A, Kretzler M, Doran P, Bonrouhi M, Schmid H, Kiss E. Gene expression fingerprints in human tubulointerstitial inflammation and fibrosis as prognostic markers of disease progression. Kidney Int 2004; 65: 904–917.

33. Maluf DG, Mas VR, Archer KJ, Yanek K, Gibney EM, King AL. Molecular pathways involved in loss of kidney graft function with tubular atrophy and interstitial fibrosis. Mol Med 2008; 14: 276–285.
34. Kurian SM, Heilman R, Mondala TS, Nakorchevsky A, Hewel JA, Campbell D. Biomarkers for early and late stage chronic allograft nephropathy by proteogenomic profiling of peripheral blood. PLoS ONE 2009; 4: e6212.
35. Einecke G, Reeve J, Mengel M. Expression of B cell and immunoglobulin transcripts is a feature of inflammation in late allografts. Am J Transplant 2008; 8: 1434–1443.
36. Mengel M, Reeve J, Bunnag S. Molecular correlates of scarring in kidney transplants: The emergence of mast cell transcripts. Am J Transplant 2009; 9: 169–178.
37. Bunnag S, Allanach K, Jhangri GS. FOXP3 expression in human kidney transplant biopsies is associated with rejection and time post transplant but not with favorable outcomes. Am J Transplant 2008; 8: 1423–1433.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Flow chart depicting study selection process.