Molecular diagnostics identifies risks for graft dysfunction despite borderline histologic changes

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The significance of borderline changes in kidney allograft biopsies is widely debated. To help resolve this, we studied differences in intrarenal gene expression patterns between early clinical and 3-month protocol biopsies, all of which had borderline histologic changes. The gene expression profiles in training set of patients by microarray analysis and data were validated in a larger cohort using RT-qPCR. There was greater expression of immunity- and inflammation-related genes in the early clinical biopsies compared to the 3-month protocol biopsies with borderline changes. In early clinically manifested borderline changes, graft deterioration within 24 months due to chronic rejection was associated with increased activation of immune, defense, and inflammatory processes. Regression modeling identified higher donor age and expression of macrophage receptor *CLEC5A* as risk factors for progression. In the 3-month protocol biopsies with borderline changes, graft dysfunction was associated with increased expression of fibrinogen complex transcripts. The discrimination power of fibrinogen was confirmed by cross-validation on two independent cohorts. Thus, our study highlights variations in gene expression between clinical and subclinical borderline changes despite similar histological findings. The data also support a recommendation for frequent patient monitoring, especially in those with borderline changes who received grafts from older donors.

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In renal allograft pathology, borderline changes (BL) are defined as interstitial inflammation with tubulitis where the severity of either or both of these is insufficient to meet Banff criteria for a diagnosis of acute cell-mediated rejection, type 1a. The incidence of BL histopathology in renal grafts is approximately 20%.[1–4] Treatment in response to BL findings is still debated, as some studies suggest that BL infiltrates are benign and occur in patients with stable graft function.[5,6] while others suggest that persistence of BL infiltrates may negatively influence graft outcome.[7] Although most patients with untreated BL infiltrates will not progress into rejection,[4] many transplant centers treat BL rejection with additional steroids or augmentation of maintenance immunosuppression. Studies suggest that only approximately 30% of patients with BL infiltrates do not respond to anti-rejection therapy;[3] therefore, BL histological findings need not be associated exclusively with rejection processes.

The development of microarray technologies coupled with bioinformatics tools has allowed the analysis of whole transcriptomes and the molecular pathology of transplantation.[8–11] There have been only 3 microarray studies dealing with BL biopsies as separate category.[9,12,13] The only conclusive microarray study of BL biopsies compared the molecular phenotypes of BL to T cell mediated rejection (TCMR) and non-rejection samples, and found that 67% of BL biopsies had non-rejection patterns.[13] Moreover, published studies have analyzed transcripts from biopsies with BL regardless of the time after transplantation. This fact could explain the molecular heterogeneity and absence of rejection patterns in most, frequently late, biopsies. Similarly, immune-related transcripts identified in case biopsies may differ from biopsies performed per protocol. In this study, we focused on transcriptome description in early clinical and 3-month protocol biopsies to determine the molecular and clinical predictors of kidney allograft dysfunction.

Table 1 | Patient´s characteristics of training set

	Early clinical biopsies			Protocol biopsies (3-months)		
	Non-progressors (*n* = 6)	Progressors (*n* = 7)	*P*	Non-progressors (*n* = 8)	Progressors (*n* = 7)	*P*
Donor's characteristics						
Age (mean, SD)	53 (5.6)	57.4 (10.6)	0.380	51.1 (14.4)	60.1 (6.8)	0.223
Gender (female, %)	33.3%	14.3%	1	37.5%	42.9%	0.622
Deceased/living donors (*n*)	6/0	7/0	1	6/2	6/1	1
ECD, *n* (%)	4 (66.7%)	6 (85.7%)	0.559	5 (62.5%)	6 (85.7%)	0.765
Hypertension, *n* (%)	2 (33.3%)	5 (71.4%)	0.280	5 (62.5%)	4 (57.1%)	1
Recipient's characteristics						
Age (mean, SD)	46.3 (13.2)	59.3 (8.7)	0.057	53.9 (10.9)	49.9 (14.2)	0.546
Gender (female, %)	33.3%	42.8%	1	25%	14.3%	0.554
HLA mismatch (mean, SD)	4.2 (1.3)	4.4 (1.1)	0.820	4.4 (1.3)	3.4 (0.5)	0.156
PRA (mean, SD)	11(22.4)	9.7 (10.4)	0.240	3.5 (6.9)	16.3 (23.3)	0.142
Retransplantation, *n*	1	1	1	1	1	1
Cold ischemia (hod, mean, SD)	19.3 (5.8)	19.5 (3.9)	0.946	14.8 (8.7)	16.3 (8.1)	0.355
Clinical characteristics						
Induction therapy						
AntiCD25 antibodies, *n* (%)	0	0		5 (62.5%)	1 (14.3%)	
Thymoglobulin, *n* (%)	0	1 (14.3%)		0	3 (42.9%)	
Without induction, *n* (%)	6 (100%)	6 (85.7%)		3 (37.5%)	3 (42.9%)	
Maintenance immunosuppressive regimens at 3 months						
MMF, tacrolimus and steroids	6 (100%)	5 (71.4%)		6 (75%)	4 (57.1%)	
MMF, cyclosporine and steroids	0	0		2 (25%)	2 (28.6%)	
Others	0	2 (28.6%)	0.592	0	1 (14.3%)	0.467
Tacrolimus (μg/l) at 24 months, mean (SD)	7.3 (1.9)	5.8 (1.15)	0.181	6.04 (1.6)	5.9 (2.4)	0.684
DGF, *n* (%)	2 (33.3%)	4 (57.1%)	0.858	2 (25%)	0 (0%)	0.858
DSA I class, *n* (%)	1 (25%)[a]	1 (20%)[a]		1 (20%)[b]	1 (25%)[b]	
DSA II class, *n* (%)	0[a]	0[a]		0[b]	0[b]	
MICA, *n* (positive patients / measured patients)	0[a]	0[a]		0[b]	0[b]	
Serum creatinine (μmol/l)						
At biopsy, mean (SD)	406.3 (245.6)	327.6 (144.5)	0.668	127.1 (34.8)	139.7 (32)	0.477
At 3 months, mean (SD)	146.7 (26.1)	155.3 (64.3)	0.766	127.1 (34.8)	139.7 (32)	0.477
At 24 months, mean (SD)	145.5 (28.6)	241.1 (103)	0.041	126.4 (30.5)	216.1 (50.9)	0.001
Proteinuria (g/24 hod)						
At 3 months, mean (SD)	0.89 (1.2)	0.44(0.16)	0.340	0.37 (0.29)	0.4 (0.23)	0.518
At 24 months, mean (SD)	0.29 (0.23)	0.37 (0.32)	0.650	0.49 (0.47)	5.28 (5.9)	0.009
Banff scores in measured biopsy(grade 0/1/2/3) (*n*)						
Interstitial fibrosis (ci)	6/0/0/0	2/5/0/0	0.021	4/3/1/0	2/4/0/1	0.431
Total inflammation (ti)	2/4/0/0	1/6/0/0	0.416	4/2/2/0	2/3/1/1	0.543
Peritubular capillaritis score (ptc-s)	5/1/0/0	3/3/1/0	0.296	7/1/0/0	5/1/1/0	0.529
Peritubular capillaritis quality (ptc-q)	5/1/0/0	3/1/3/0	0.179	6/2/0/0	4/2/1/0	0.512
Peritubular capillaritis excess (ptc-e)	5/1/0/0	3/3/1/0	0.296	7/1/0/0	5/1/1/0	0.529
Reasons for dysfunction						
Acute T-cell mediated rejection (*n*, %)	0	1(14.3%)		0	0	
Acute/active antibody-mediated rejection (*n*, %)	0	0		0	2 (28.6%)	
Chronic/active T cell-or antibody-mediated rejection (*n*, %)	0	4 (57.1%)		0	1 (14.3%)	

Abbreviations: DGF, delayed graft function; DSA, donor specific antibodies; ECD, expanded criteria donor; HLA, human leukocyte antigens; MMF, mycophenolate mofetil; PRA, panel reactive antibodies.
[a]Data not available for 2 patients.
[b]Data not available for 3 patients.

RESULTS

Differences in gene expression between early clinical and 3-month protocol renal allograft biopsies with BL changes

Gene expression patterns change over time after transplantation and may vary widely, even among tissues with similar morphological changes. Therefore, we analyzed transcriptomes in tissues with BL diagnosed from clinically indicated biopsy performed soon after surgery (median 7 postoperative days, $n = 13$), and from tissues collected at 3-month protocol biopsy ($n = 15$) when kidney graft function was stable.

Training set. Using microarray-based gene expression profiling in a training set of patients ($n = 28$) (Table 1), we identified 200 upregulated and 118 downregulated genes in early clinical biopsies compared to protocol biopsies (Supplementary Table S1). According to information from the DAVID database (http://david.abcc.ncifcrf.gov), that