Computer-assisted topological analysis of renal allograft inflammation adds to risk evaluation at diagnosis of humoral rejection

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Antibody-mediated rejection is associated with heterogeneous kidney allograft outcomes. Accurate evaluation of risk for graft loss at time of diagnosis is necessary to offer personalized treatment. In contrast with serological and molecular assessment, morpho-histological evaluation of antibody-mediated rejection lesions has not significantly evolved. This relies on Banff classifications designed to be of diagnostic discriminatory power rather than prognostic and face quantitative and qualitative limitations. Here we developed a method of Computer-assisted Analysis of Graft Inflammation (CAGI) to improve the classification of allograft inflammation. Digitization of immunostained biopsy sections, image processing and algorithm-driven analysis allowed quantification of macrophages, T cells, B cells, and granulocytes per unit surface of interstitium, capillaries or glomeruli. CAGI was performed on biopsy specimens of 52 patients with extensively phenotyped antibody-mediated rejection. Macrophage numbers in capillaries and interstitium, but not Banff scores or the amount of other immune cell subsets, correlated with donor-specific antibody (DSA) mean fluorescence intensity and DSA-C3d status. The quantity of macrophages in the interstitium and DSA-C3d status were the only independent predictors for significant allograft loss at the time of antibody-mediated rejection diagnosis (hazard ratio 3.71 and 2.34, respectively). A significant strategy integrating the DSA-C3d assay and the quantification of interstitial macrophages allowed identification of three groups with distinct renal prognosis: DSA-C3d^{-}, DSA-C3d^{+}/macrophages-low and DSAC3d^{+}/macrophages-high. Thus, CAGI brings a missing piece to the antibody-mediated rejection puzzle by identifying morpho-histological processes that bridge *in vitro* parameters of DSA pathogenicity and graft loss. Hence, this approach could be useful in future integrated strategies of risk evaluation.

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KEYWORDS: allograft pathology; antibody-mediated rejection; complement-binding DSA; donor-specific antibodies; inflammation quantification; macrophages

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End-stage renal failure is a major public health problem that is increasing exponentially worldwide. Among all renal replacement therapies, kidney transplantation represents the best option, because it provides patients with the best possible quality of life while prolonging their life expectancy. Nevertheless, late allograft loss remains unavoidable and is responsible for high morbidity and mortality rates and costs.[1]

Late dysfunction of kidney transplants is, in most cases, related to the development of a humoral immune response directed against the graft, a process referred to as "antibody-mediated rejection" (AMR).[2,3] Although AMR is widely recognized as the main cause of late allograft loss, individual outcomes are heterogeneous and difficult to predict.[4,5] Clinicians need reliable prognostic tools to allow evaluation of the risk of graft loss at the time of AMR diagnosis to propose personalized treatments.[6,7]

The pathophysiologic sequence of AMR has been recently clarified by seminal experimental works.[8,9] The sequence is initiated by the binding of circulating donor-specific antibodies (DSAs) directed against mismatched donor human leukocyte antigen molecules expressed by graft endothelium. DSA binding triggers endothelial cell and complement activation, which results in recruitment of innate immune effectors to graft microvasculature. Vascular damage leads to subsequent infiltration of the graft by inflammatory cells,

including macrophages, natural killer cells, granulocytes, T cells, and B cells. These pathophysiologic processes are responsible for progressive tissue destruction and irreversible loss of graft function. Identification of these mechanisms offers interesting opportunities for evaluation of AMR severity at the individual level.

Recent approaches depending on the quantification of these processes have been successfully used for risk stratification at the time of AMR diagnosis. At the serological level, we have recently reported that *in vitro* detection of circulating DSAs able to bind the complement component C3d allowed for prediction of graft loss at the time of diagnosis of AMR.[10] At the molecular level, it has been reported that measuring the level of expression of the genes related to endothelial cell activation or macrophages within a graft biopsy also allows accurate risk prediction in early AMR.[11]

It is currently acknowledged that strategies integrating different methods of risk assessment will provide the greatest precision for personalized evaluation.[7,12,13] Histomorphologic analysis of inflammation present within the graft at the time of AMR likely contains important information for such integrated strategies.[14] Yet, in contrast to serological[10,15] and molecular[16,17] assays, histologic assessment of AMR has not evolved significantly in recent years. Currently, inflammation evaluation depends on traditional pathologic analysis in which lesions are quantified according to criteria defined by the international Banff classification.[6] The Banff classification was designed and refined to be of diagnostic discriminatory power. It was not intended for prognostic evaluation or evaluation of response to treatment. It has quantitative and qualitative limitations: grading of lesions is not continuous with risks of threshold effect, and the nature of inflammatory cells is not considered.

In this study, we evaluated the ability of Banff scores for inflammation to predict the risk for graft loss at the time of AMR in a deeply phenotyped cohort of patients. Because Banff scores failed to predict outcomes, we developed an innovative method of computerized image analysis that improved the quantitative and qualitative characterization of allograft inflammation and allowed identification of patients at higher risk for graft loss. We used this approach to investigate the understudied relationship between *in vitro* criteria of DSA pathogenicity (including mean fluorescence intensity [MFI] level and complement-binding ability) and features of allograft inflammation. Finally, we tested whether a strategy integrating a C3d-binding assay and the thorough topological analysis of inflammation could improve the prognostic stratification of patients at the time of AMR diagnosis.

RESULTS

Description of the study population

Among the 938 kidney transplant recipients who were followed up in our institution over the study period, 69 fulfilled the diagnostic criteria for AMR. Fifty-two of them had enough histologic material left for analysis and were enrolled in the study.

Table 1 shows their baseline characteristics at the time of transplantation and rejection. We could not find any statistical difference between the 52 patients who were enrolled in the study and the 17 patients who were excluded because of lack of available material (Supplementary Table S1).

All transplantations were ABO compatible, and 94% of kidneys were obtained from a deceased donor. AMR occurred, on average, 1439 ± 1475 days after transplantation, and mean estimated glomerular filtration rate (GFR) was

Table 1 | Characteristics of patients included in the study

Variable	Patients with AMR (n = 52)
Characteristics at the time of transplantation	
Recipient	
Gender, male, n (%)	32 (62)
Age, yr	39.4 ± 14.5
Retransplantation, n (%)	18 (35)
Time since dialysis, mo	56.0 ± 63.6
Donor	
Age, yr	38.4 ± 17.1
Deceased, n (%)	49 (94)
Transplantation	
Number of HLA A/B/DR mismatches	3.8 ± 1.4
Combined transplantation,[a] n (%)	7 (13)
Cold ischemic time, minutes	921 ± 379
Delayed graft function, n (%)	16 (31)
Characteristics of AMR	
Clinico-biological characteristics	
Time posttransplantation (d)	1439 ± 1475
Proteinuria, g/d	1.3 ± 2.7
Creatininemia, μmol/L	279 ± 333
Estimated GFR,[b] ml/min/1.73 m^2	36.7 ± 21.1
Subclinical antibody-mediated rejections, n (%)	7 (13)
Histologic characteristics (Banff scores[c])	
Glomerulitis	1.5 ± 1.0
Peritubular capillaritis	1.8 ± 0.7
Microvascular inflammation[d]	3.3 ± 1.2
Transplant glomerulopathy	1.0 ± 1.2
Interstitial inflammation and tubulitis[e]	2.5 ± 2.0
Interstitial fibrosis and tubular atrophy[f]	1.6 ± 0.8
Arteriosclerosis	1.0 ± 1.1
Endarteritis (vasculitis)	0.3 ± 0.5
C4d deposition	1.5 ± 1.0
Immunologic factors	
Number of DSAs	1.8 ± 1.0
Classes of DSAs	
Class I, n (%)	7 (14)
Class II, n (%)	36 (69)
Class I + II, n (%)	9 (17)
MFI of the highest DSA	7630 ± 5724
C3d-binding DSA, ≥ 1, n (%)	31 (60)
Treatments	
Steroids pulses, n (%)	45 (87)
I.v. Igs, n (%)	29 (56)
Rituximab, n (%)	27 (52)
Plasmapheresis, n (%)	25 (48)

Unless noted otherwise results are expressed as mean ± SD.
AMR, antibody-mediated rejection; DSA, donor-specific anti-HLA antibody; MFI, mean fluorescence intensity.
[a]Kidney and pancreas.
[b]Calculated with the Modification of Diet in Renal Disease formula.
[c]Banff scores (0: no significant lesion, 1: mild, 2: moderate, 3: severe).
[d]Sum of the Banff scores for glomerulitis and capillaritis.
[e]Sum of the Banff scores for interstitial inflammation (i) and tubulitis (t).
[f]Grade 1, 2, or 3 of the Banff classification.

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