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The *prognostic* significance of glomerular infiltrating leukocytes during acute renal allograft rejection

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ABSTRACT

Transplant glomerulitis, observed in T cell-mediated and antibody-mediated rejection, is histologically characterized by intracapillary mononuclear cell infiltration. However, the prognostic value of counting various glomerular inflammatory cells during rejection has not been elucidated, which is a key step for the introduction of novel biomarkers in the clinics. We immunophenotyped glomerulitis during episodes of acute rejection in order to investigate their predictive value for transplant outcomes. To do so, we included 57 transplant biopsies of 57 renal transplant recipients with biopsy-proven acute rejection with a median follow-up of 4.2 years. We determined average glomerular cell counts for T cells, B cells, Tregs, IL-17^{+} cells, neutrophils and macrophages. Logistic and Cox regression models were used to investigate the association of glomerular inflammatory cells with response to therapy and graft failure on a population level. We used novel time-dependent ROC curve analyses to investigate the value of glomerular inflammatory cell infiltrates for the prediction of transplant outcomes, applicable to the individual patient. We identified three cell types that were responsible for glomerulitis during rejection: macrophages, T cells and neutrophils. By quantification of glomerular macrophages, an emerging cell type associated with antibody-mediated rejection, we were able to predict the progression towards death-censored graft failure within the first 500 days after the initial episode of rejection. With the use of novel time-dependent ROC analyses, we propose dynamic sensitivities, specificities, and positive and negative predictive values with their corresponding cut-off values for the average amount of glomerular macrophages, depending on what time after rejection death-censored graft failure needs prediction.

1. Introduction

According to the Banff classification, histologically transplant glomerulitis is characterized by intracapillary glomerular mononuclear cell infiltration in the kidney allograft. In the first Banff classification of renal allograft pathology, tubulitis and intimal arteritis were regarded as the principal lesions indicative of acute rejection. However, this classification did not include glomerulitis as a defining feature [1]. Nowadays, glomerulitis is recognized as a form of microcirculatory inflammation in the context of allograft injury related to antibody-mediated rejection (ABMR) [2,3], however this feature has also been observed in T cell-mediated rejection (TCMR). The Banff classification has included semi-quantitative lesion grading, which makes it simple and quick to apply [1]. However, the reproducibility of lesion scoring might be a limiting factor and true quantification of leukocytes might be more suitable and accurate [4,5]. Methodological aspects of histological grading of glomerulitis are the subject of ongoing debate. No clear cut-off value exists to render a diagnosis of glomerulitis in the context of ABMR. Batal et al. addressed this issue in a series of 111 index renal allograft biopsies and compared three different histological methods to grade glomerulitis (defined by the presence of >5 leukocytes/glomerulus) and their correlations with clinical parameters [6]. They concluded that grading glomerulitis based on the percentage of affected glomeruli (as recommended by the Banff) was superior to grading based on the most inflamed glomerulus, or the presence of capillary loop occlusion

Abbreviations: ABMR, antibody-mediated rejection; TCMR, T cell-mediated rejection; C4d, complement factor 4d; HLA, human leukocyte antigen; ROC, receiver operating characteristics; AUC, area under the curve; DSA, donor-specific antibodies; MPNS, methylprednisolone; ATG, anti-thymocyte globulin; CDC, complement dependent cytotoxicity; PRA, panel reactive antibodies; IF/TA, interstitial fibrosis and tubular atrophy; OR, odds ratio; HR, hazard ratio; IQR, interquartile range; IFN, interferon; DBD, deceased after brain death; DCD, deceased after cardiac death; eGFR, estimated glomerular filtration rate.

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by inflammation. As far as glomerulitis is concerned, the Banff definition is based on identification of mononuclear cells in particular and granulocytes are not included, which was the case in the initial study of Batal [6]. Both cell types might be difficult to discriminate on conventional histological stainings and exclusion of the granulocytes might underestimate the pathogenic role of these cells. A limited number of studies is available concerning the immunohistochemical phenotyping of glomerular leukocytes and their relationship with clinical parameters, response to anti-rejection therapy and renal allograft outcome. Emerging data from several studies show that even though associated with indices of ABMR, transplant glomerulitis associates with death-censored graft loss, independent of C4d or donor-specific antibody (DSA) status [6–9]. Therefore, there is a need to better define glomerulitis immunophenotypically and to assess the prognostic value of the cell types for the prediction of graft outcomes.

2. Objective

We thoroughly phenotyped glomerular inflammation during episodes of acute rejection. The principle aim of our present study was to investigate the predictive value of the various types of transplant glomerulitis in the context of acute renal allograft rejection for the response to therapy and the development of graft failure, since this is currently lacking. In order to do so, we used novel time-dependent receiver operating characteristics analyses.

3. Materials and methods

3.1. Renal allograft recipients

In the current study we collected renal transplant patients with a diagnosis of acute rejection (TCMR or mixed TCMR/ABMR rejection) on their index biopsy from the database of the Academic Medical Center. Patients were included when after diagnostic work-up, paraffin embedded biopsy material was still available (excluded patients were in this case considered missing completely at random). An initial group of 28 renal biopsies was analyzed by immunohistochemistry for the presence of T cells, B cells, macrophages, FoxP3^{+} cells, IL-17^{+} cells and neutrophils in the glomeruli. From this preliminary assessment, we concluded that B cells, plasma cells, and FoxP3^{+} cells were hardly present in glomeruli during acute rejection (Fig. 1). IL-17 was only positive in neutrophils and we therefore omitted further sub-analysis based on this immunohistochemical staining as well. We completed the group with another 29 renal biopsies with acute rejection and in this cohort, immunostains for T cells, macrophages and neutrophils were performed. We excluded acute rejection biopsies with the coexistence of biopsy-proven polyomavirus nephropathy (N = 3). Complete data

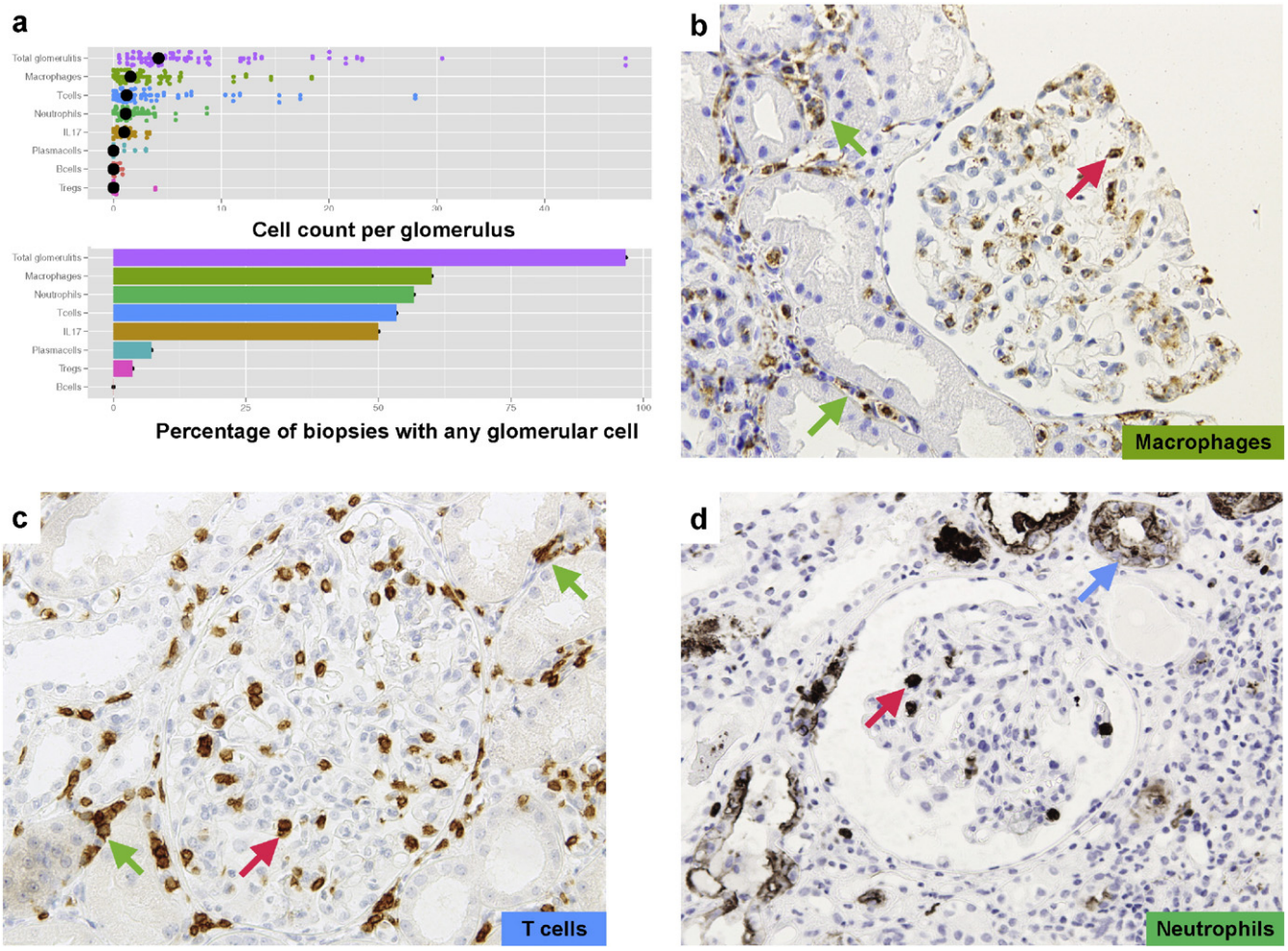

Fig. 1. Distribution of glomerular cell counts. (a) Upper panel: averaged glomerular cell count per biopsy during an episode of acute rejection. The black dot represents the group median glomerular cell count. Lower panel: the percentage of patients with any glomerulitis in their biopsy. (b) CD68^{+} macrophage, (c) CD3^{+} T cells and (d) CD15^{+} neutrophils. The red arrows indicate the existence of glomerular cells, the green arrow shows concomitant peritubular capillaritis (virtually absent for neutrophils) and the blue arrow indicates tubular CD15 staining.