Clinicopathologic Relevance of Vascular Changes Associated with Transplant Glomerulopathy Secondary to Chronic Antibody-mediated Rejection in the Renal Allograft

Summary of PhD thesis

Deján Dobi, MD

Szeged, 2018

Department of Pathology, Faculty of Medicine,
University of Szeged

Thesis supervisor: Béla Iványi, MD, DSc

Doctoral School of Clinical Medicine

This PhD thesis is based on the following publications:

I. Dobi D, Bodó Z, Kemény É, et al. Morphologic features and clinical impact of arteritis concurrent with transplant glomerulopathy. *Pathol Oncol Res.* 2015; 22: 15–25. (Original Research Article) **IF: 1,736**

II. Dobi D, Tatapudi V, Rajalingam R, et al. Quantitative changes of kidney microvasculature in transplant glomerulopathy *Virchows Arch*. 2015; 467:S34. (Abstract)

III. Dobi D, Bodó Z, Kemény É, et al. Peritubular capillary basement membrane multilayering in early and advanced transplant glomerulopathy: quantitative parameters and diagnostic aspects. *Virchows Arch.* 2016; 469: 563-573. (Original Research Article) **IF: 2,848**

Introduction

Chronic antibody mediated rejection (ABMR) persists in being the most significant barrier that hinders long-term renal transplant survival [1, 2]. Its clinical presentation is characterized by slowly declining graft function and proteinuria that eventually lead to end-stage renal disease [3]. The gold standard for the diagnosis of rejection is renal biopsy with conventional histologic evaluation. Diagnostic lesions of T cell-, and antibody-mediated rejection -that usually manifest focally in the biopsy sample- are classified by the biannually revised Banff criteria [4].

CABMR triggers progressive injury of the graft vasculature via donor specific alloantibodies (DSAs). DSAs sublytically injure and activate the endothelial lining of the graft vasculature. The subsequent repair mechanisms result in the formation of subendothelial basement membranes (BMs) observed as double-contoured glomerular capillary loops (transplant glomerulopathy - TxG) on tissue sections stained with periodic acid-Schiff or methenamine silver that highlight

the glomerular BM. The BM multiplication around the peritubular capillaries (peritubular capillary basement membrane multilayering - PTCBMML) can be best detected by electron microscopy [5]. The endothelial damage also induces intimal fibrosis in the arteries that one can examine by special stains (elastic, trichrome).

Based on the Banff 2015 classification [6] the diagnosis of chronic active ABMR requires the following criteria: 1) DSA-induced chronic tissue damage (TxG, or severe PTCBMML, or arterial intimal fibrosis), 2) evidence of current/recent alloantibody interaction with the vascular endothelium [C4d deposition around the peritubular capillaries (PTCs), or at least moderate microvascular inflammation, or increased expression of ABMR-related transcripts], and 3) serologic evidence of DSAs.

While grafts with TxG have worse survival compared to those without, several co-factors can modify outcomes for patients with the condition: increased urine protein to creatinine ratio, class II DSAs, and C4d deposition around the PTCs [7] are amongst the determinants of graft loss once TxG is established.

However, there are certain morphological lesions the clinical relevance of which is less studied. Little is known about the prognostic implications of arteritis in TxG, especially if it is associated with intimal fibrosis. In addition, the utility of ultrastructural metrics in the risk stratification of patients with TxG has not yet been examined systematically.

Electron microscopy (EM) can also contribute to the early diagnosis of chronic ABMR. The current diagnostic criteria for chronic active ABMR lists two ultrastructural lesions [6]: early, very mild glomerular double contouring that can only be identified by EM and severe PTCBMML that requires the presence of at least one PTC profile with seven BM layers and two PTCs with at least five layers.

Based on our previous studies, less severe PTCBMML can also be indicative of chronic ABMR if non-alloimmune causes (thrombotic microangiopathy and tubulointerstitial nephritis) can be excluded [8-10]. However, it is not known whether these milder forms of PTCBMML represent an early or an advanced stage of chronic ABMR, and their utility in routine diagnosis has not yet been clarified.

Aims

To better understand the clinicopathologic relevance of macro-, and microvascular changes associated with TxG, we set the following research goals:

1) To analyze the prognostic relevance, morphological characteristics, and the immunophentoype of fibrosing intimal arteritis concurrent to TxG and to investigate its relationship to other morphological alterations associated with chronic ABMR.
2) To describe the microvascular lesions related to TxG at the ultrastructural level by using morphometry tools and to explore the utility of the ultrastructural metrics in graft survival prognostication.
3) To study whether the current Banff threshold or previously proposed cut-off values for DSA-induced PTCBMML corresponds best to an early stage of chronic ABMR, and to establish a PTC_{CIRC} value typical of early and fully developed chronic ABMR.

Material and Methods

General aspects

Three, retrospective, case-control studies were designed and carried out at the Departments of Pathology, University of Szeged, and University of California, San Francisco to adress the above-listed questions. Renal biopsy samples were processed according to standardized protocols for light microscopic, immunofluorescent and electron microscopic analysis. Samples were assessed histologically based on the 2015 Banff classification [6] for the presence and severity of acute and chronic lesions in the arteries, arterioles, glomeruli, PTCs, interstitium, and tubuli. DSAs were measured by the Luminex technology.

For statistical analysis, Independent samples t-test, Mann-Whitney U test, Kruskal-Wallis test, and chi-square test were used where applicable. The graft survivals were tested by medians of Kaplan-Meier curves. Graft failure was defined as the restarting of dialysis.

Morphologic Features and Clinical Impact of Arteritis Concurrent with Transplant Glomerulopathy

In the first study, 59 patients with TxG were enrolled. To explore the clinicopathologic relevance of arteritis, patients were split into two groups based on the presence of the lesion. The clinical and morphological characteristics and graft survival of the study cohorts were compared. In addition, the evaluation of chronic arterial lesion (cv) was modified relative to the Banff classification. We defined three categories: fibrosing intimal arteritis, in which leukocytes could be detected in the fibrotic intima (cv_{mo}), intimal fibrosis without intimal inflammatory infiltrate (cv_{IF}), and intimal fibroelastosis (cv_{IFE}) with multiple elastic laminae due to aging or hypertension. The severity of luminal narrowing was compared across these three categories. Furthermore, hierarchical cluster analysis (HCA) was used to explore the class relationships among the newly defined lesions and those part of the Banff classification. CD8/TIA-1, CD68 and CD3 antibodies were used to characterize the intimal infiltrate by immunohistochemistry.

Quantitative Changes of the Renal Microvasculature in Transplant Glomerulopathy

Forty-seven patients with TxG were enrolled into the second study along with 11 patients with normal management biopsies as controls to explore the severity of ultrastructural changes typical of glomerular double contouring. In addition to the routine diagnostic workup, a detailed electron microscopic analysis of the glomeruli and the PTCs was performed that included the measurement of mean harmonic BM thickness, endothelial thickness and endothelial fenestrae (EF) frequency in both microvascular compartments, and counting of filtration slits (FSs). The mean number of PTCBM layers (PTC_{CIRC}) was also recorded. Ultrastructural morphometric data were correlated with renal function, and serological parameters. HCA based on glomerular EF frequency, glomerular endothelial thickness, mean harmonic glomerular BM membrane thickness (δ_{GBM}), and FS frequency was used to establish TxG subgroups, of which the clinical, serological, and

morphological parameters, as well as their graft survival rates were compared.

Peritubular Capillary Basement Membrane Multi-layering in Early and Advanced Transplant Glomerulopathy: Quantitative Parameters and Diagnostic Aspects

In the third study, the ultrastructural quantitative aspects of PTCBMML were examined in 57 kidney transplant biopsies with TxG. DSA-induced PTCBMML was defined by three different thresholds: permissive (1 PTC with 5 BM layers), intermediate (3 PTCs with 5 layers, or 1 PTC with seven layers), and strict (the current Banff criterion for PTCBMML). PTC_{circ} was also registered in each case. The clinical, serological, and morphological characteristics including the quantitative PTC features of patients with mild TxG vs. moderate-to-severe TxG were compared. The capacity of the different PTCBMML thresholds to discriminate between these two grades of TxG severity were expressed using the sensitivity and the specificity of the thresholds for the moderate-to-severe TxG lesion.

Results

Morphologic Features and Clinical Impact of Arteritis Concurrent with Transplant Glomerulopathy

Fifteen out of the 16 cases with arteritis fulfilled the morphological diagnostic criteria for chronic active ABMR, and 11 cases with arteritis showed morphological evidence of concurrent, ongoing T-cell-mediated alloimmune response. Interstitial inflammation in the scarred and non-scarred cortex, total cortical inflammation, and arterial luminal narrowing were significantly more severe in biopsies with arteritis. By HCA, cv_{mo} was grouped with mostly acute lesions, cv_{if} was associated with chronic vascular alterations, and cv_{ife} remained separate. Immunohistochemistry revealed T-lymphocyte predominance over macrophages in the intimal infiltrates in 14 out of 16 cases, and cytotoxic T-lymphocytes were identified among intimal mononuclears in 10 cases. Patients with arteritis demonstrated a significantly shorter renal survival [7.5 months (95 % CI, 2 to 13) *vs* 29 months (95 % CI, 17 to 41), p=0.001].

Quantitative Changes of the Renal Microvasculature in Transplant Glomerulopathy

There was a significant ($p < 0.0005$) difference between the TxG and the protocol group for EF frequency [720 unit/mm (640) *vs* 2160 unit/mm (120)] GBM thickness [890 nm (520) *vs* 319 nm (90)], and FS frequency [1110 unit/mm (410) *vs* 1490 unit/mm (280)]. We observed ultrastructural changes of similar magnitude in the PTCs. Significant correlation was found between eGFR and mean harmonic endothelial thickness in the PTCs ($r_S = -0.713$, $p < 0.001$) and between urine protein/creatinine ratio and δ_{GBM} ($r_S = 0.626$, $p < 0.001$). HCA based on selected ultrastructural parameters separated the TxG patients into two subgroups. Cumulative survival proportion for the subgroup with less severe glomerular changes did not dip below 0.5 by the end of the follow-up period, unlike that of the patients with more severe glomerular alterations who had a median graft survival rate of 25 months (95 % CI, 4 to 46).($p < 0.0005$). GBM thickness $\geq$ 925 nm could differentiate the two clusters with 100 % sensitivity and 95 % specificity.

Peritubular Capillary Basement Membrane Multi-layering in Early and Advanced Transplant Glomerulopathy: Quantitative Parameters and Diagnostic Aspects

The clinical data revealed that mild TxG corresponded to early chronic ABMR, while moderate-to-severe TxG represented the advanced stage of the disease. The permissive threshold displayed the lowest specificity (73 %) and the highest sensitivity (83 %) for moderate-to-severe TxG, and its corresponding PTC_{circ} value was 3 layers. Sensitivity and specificity of the "intermediate" cut-off were 87 %, and 64 %. In contrast, the strict threshold -adopted by the Banff 2013 classification- displayed a specificity and sensitivity of 93 % and 52 %, and the corresponding PTC_{circ} was 4 layers. In mild TxG, 26 % of the cases met the permissive cut-off and 6 % the strict cut-off. Mild TxG was associated with a lower PTC_{circ} (2.6 layers *vs* 4.5 layers in moderate-to-severe TxG; $p < 0.0001$). Patients who met the permissive threshold for DSA-induced PTCBMML had a lower median graft survival rate [19 months (95 % CI, 15 to 23)] than those who did not [40 months (95 % CI, 19 to 61), $p = 0.020$].

Discussion

First, we examined the clinicopathologic relevance of arteritis associated with TxG and found a relatively frequent coincidence of the two lesions in an unselected biopsy series (16/59). In all but one sample, arteritis was accompanied by intimal fibrosis, a constellation of morphological changes that we defined as fibrosing intimal arteritis (cv_{mo}). Our statistical test (HCA) clustered cv_{mo} mainly with inflammatory lesions, confirming the notion that it is an active process. In contrast, intimal fibrosis without leukocytes (cv_{IF}), along with TxG and PTCBMML formed another group, emphasizing the chronic nature of these injuries. Intimal fibroelastosis (cv_{IFE}) did not have statistical association with other morphological lesions of the Banff classification, reflecting the fact that this lesion is due to ageing and/or hypertension. In addition, specimens with cv_{mo} showed a significantly more severe luminal narrowing of the arteries and displayed higher inflammatory load in the cortex relative to patients with cv_{IF} or cv_{IFE}. These morphological characteristics probably contributed to the lower survival

rate that we registered in patients with arteritis. Based on the immunhistochemical studies, the intimal inflammatory infiltrate was a mixture of cytotxic T-lymphocytes, and macrophages. This result suggests -in agreement with the literature- that the patomechanism of the lesion probably involves both arms of the alloimmune response.

In our subsequent study we focused on the microvascular alterations and showed that the renal microvasculature undergoes major remodeling compared to the normal, baseline parameters by the time the histological diagnosis of TxG is established. In addition, glomerular BM thickening positively correlates with urine protein/creatinine ratio, and a cut-off of 925 nm of δ_{GBM} identifies a TxG subgroup with worse renal survival. This cut-off can capture those patients who, despite having a light microscopically apparent lesion, could still expect a significantly less steep survival curve.

Lastly, we studied PTCBMML associated with TxG. Based on our findings, the current (“strict”) threshold for DSA-induced PTCBMML cannot facilitate the early detection of chronic ABMR since it is mainly present in cases where glomerular double contours are

already obvious by light microscopy and further ultrastructural examination for diagnostic purposes is unnecessary. In addition, this threshold also fails to demonstrate any prognostic value in patients with TxG. Based on these findings, we suggest using the permissive criterion/PTCcirc ≥ 3.0 to diagnose DSA-mediated PTCBMML, because it represents the earliest, prognostically relevant morphologic manifestation of chronicity due to ABMR in an appropriate clinico-pathologic context (e.g. DSAs/C4d positivity/at least moderate microvascular inflammation).

In summary, chronic ABMR remains a major challenge that needs to be overcome to prolong long-term renal graft survival. Our findings prove that the detailed examination of the morphological changes in the renal vasculature can contribute to these ongoing efforts by identifying prognostic markers and refining diagnostic criteria that help to define a patient population that can benefit the most from the current therapeutic options.

References

1. Sellares J, De Freitas D, Mengel M, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and non-adherence. *Am J Transplant*. 2012; 12: 388-399.

2. Sis B, Campbell PM, Mueller T, et al. Transplant glomerulopathy, late antibody-mediated rejection and the ABCD tetrad in kidney allograft biopsies for cause. *Am J Transplant.* 2007; 7: 1743-1752.

3. Husain S, Sis B. Advances in the Understanding of Transplant Glomerulopathy. *Am J Kidney Dis.* 2013; 62: 352-363.

4. Haas M, Loupy A, Lefaucheur C, et al. The Banff 2017 kidney meeting report: Revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant.* 2018; 18: 293-307.

5. Ivanyi B, Kemeny E, Rago P, et al. Peritubular capillary basement membrane changes in chronic renal allograft rejection: Comparison of light microscopic and ultrastructural observations. *Virchows Arch.* 2011; 459: 321-330.

6. Loupy A, Haas M, Solez K, et al. The Banff 2015 kidney meeting report: Current challenges in rejection classification and prospects for adopting molecular pathology. *Am J Transplant*. 2017; 17: 28-41.

7. Lesage J, Noël R, Lapointe I, et al. Donor-specific antibodies, C4d and their relationship with the prognosis of transplant glomerulopathy. *Transplantation.* 2014; 99: 69–76.

8. Iványi B, Fahmy H, Brown H, et al. Peritubular capillaries in chronic renal allograft rejection: a quantitative ultrastructural study. *Hum Pathol.* 2000; 31: 1129-1138.

9. Ivanyi B, Kemeny E, Szederkenyi E, et al. The value of electron microscopy in the diagnosis of chronic renal allograft rejection. *Modern Pathol*. 2001; 14: 1200-1208.

10. Ivanyi, B. Transplant capillaropathy and transplant glomerulopathy: ultrastructural markers of chronic renal allograft rejection. *Nephrol Dial Transplant.* 2003; 18: 655-660.