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Original article

Glomerular fibrin thrombi in ABO and crossmatch compatible renal allograft biopsies

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ABSTRACT

Glomerular fibrin thrombi may be an early indication of antibody-mediated rejection in renal allograft biopsies. However, fibrin thrombi have a broad differential; thus, we sought to evaluate the etiology and implications of glomerular fibrin thrombi in allograft biopsies of blood group and cytotoxic crossmatch compatible renal allografts.

Biopsies were identified from the pathology files of Oregon Health & Science University. Detailed histopathologic findings were retrospectively correlated with clinical data, treatment, and outcome.

Sixteen early posttransplant biopsies had glomerular fibrin thrombi, including three surveillance biopsies. Six of 16 biopsies had no other histopathologic findings; 5/16 had glomerulitis and peritubular capillaritis; 4/16 had concomitant cellular vascular rejection; one had parenchymal infarction. C4d staining was positive in 4/16 cases. Most patients were treated with IVIg and plasmapheresis, others with rapamycin, thymoglobulin, or rituximab. At an average follow-up of 62 months, 8 patients with functioning grafts had a mean serum creatinine of 1.4 mg/dL (122 μmol/L).

Antibody-mediated rejection is an important consideration in blood group compatible allograft biopsies with glomerular fibrin thrombi, even with C4d-negative biopsies. However, multidisciplinary evaluation is necessary, given other etiologies, including drug toxicity, hemolytic-uremia syndrome, and large vessel thrombosis. Despite aggressive treatment, both short and long-term graft survival may be compromised.

Introduction

The histologic discovery of fibrin thrombi in renal allograft biopsies is a rare but very concerning finding. There are a number of widely varying etiologies of graft microthrombosis, including surgical-anastomotic complications, hyperacute or antibody-mediated rejection, thrombotic microangiopathy due to calcineurin inhibitors or recurrent hemolytic uremia syndrome, anti-phospholipid antibody, sepsis, malignant hypertension donor parameters (disseminated intravascular coagulation, head trauma), and viral infection [2,19,25,26,35,46]. However, with evolution in transplantation practice, the prevalence and implications of these conditions have changed over time. Before the era of consistent HLA-typing and pre-transplant cross-matching, hyperacute rejection was the predominant cause of fibrin thrombi, with devastating consequences. In the early era of calcineurin inhibitors, glomerular microthrombi were often associated with calcineurin inhibitor toxicity (CNIT) [4]. In the current era of closely monitored drug levels, antibody-mediated (humoral) rejection has re-emerged as a prime concern in the setting of microthrombi in allograft biopsy, despite HLA cross-matching. Detection of C4d deposition in peritubular capillaries in renal allograft biopsies is an extremely useful ancillary diagnostic tool for recognition of antibody-mediated rejection (ABMR) [4,5,32,40]. However, C4d is not entirely sensitive for ABMR [1,4,27]. In 2004, Fidler et al. published a detailed study of protocol biopsies ABO blood group-incompatible renal allografts [9]. They noted that fibrin thrombi in glomerular capillaries may be the first manifestation of antibody-mediated rejection, preceding C4d positivity by several days [9]. ABO-incompatible renal allograft recipients are heavily pretreated with desensitization protocols, and ABO-incompatible allografts appear to have unique mechanisms of accommodation [4]. Thus, it is not entirely clear whether these findings directly translate to ABO-compatible and crossmatch compatible renal allografts. We sought to study the etiology and implications of glomerular fibrin thrombi in initial posttransplant biopsies from a modern single-institution series of crossmatch-compatible, ABO-compatible renal allografts.

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Table 1
Criteria for histocompatibility test interpretation.

Interpretation	Threshold
Flow crossmatch before 2/2006	
Positive T-lymphocyte	≥10 Channel shift (2 SD[a])
Positive B-lymphocyte	≥25 Channel shift (2 SD[a])
Flow crossmatch since 2/2006 (pronase treatment protocol)	
Positive T-lymphocyte	≥30 Channel shift (2 SD[a])
Positive B-lymphocyte	≥30 Channel shift (2 SD[a])
Flow PRA	
Positive Class I	Discrete positive peak(s) > 3% of negative control cut-off
Positive Class II	Discrete positive peak(s) > 6% of negative control cut-off
Negative Class I, II	Fluorescent intensity cut off equal to negative control cut-off
Luminex single antigen bead	
Positive Class I	>1000 Normalized mean fluorescent intensity
Positive Class II	>1000 Normalized mean fluorescent intensity
Donor specific antibody	>1000 Normalized mean fluorescent intensity
Luminex MICA[b]	
Positive	>2.1 Normalized background ratio
Equivocal	1.8–2.1 Normalized background ratio
Negative	<1.8 Normalized background ratio

[a] SD: standard deviation.
[b] MICA: Major histocompatiblity complex Class I related chain A antibody.

Materials and methods

Case selection

This study was approved by the Institutional Review Board at Oregon Health & Science University (OHSU). The computerized pathology files of OHSU (2000–2008) were searched for renal allograft biopsies containing thrombi; only initial allograft biopsies were included in the study. Sixteen cases were identified.

Cross-matching and immunosuppression

Donor–recipient cross-matching was performed using standard methods (Table 1). Briefly, NIH standard, antiglobulin, and B-cell cytotoxic crossmatches were performed [8,30]. Flow crossmatches (T- and B-cells) along with Flow Panel Reactive Antibody (PRA, classes I and II) were also performed [10,17,31]. Flow antibody identification using single HLA antigen beads (FlowID) was performed for recipients who had a positive flow PRA (Table 1) [10,23]. Post-transplant monitoring for the presence of donor-specific antibodies was performed using FlowPRA and FlowID. Major histocompatibility complex Class I-related chain A antibody (MICA) testing was performed for four patients, with the LabScreen MICA Single Antigen Bead Kit from One Lambda Inc. (Canoga Park, CA) (Table 1) [23,50].

All patients, except those receiving HLA identical kidney transplants, received induction immunosuppression. The choice of induction agent was determined by an immunologic scoring system with highly sensitized patients with PRA > 50% or positive flow crossmatch receiving anti-thymocyte globulin antibody (thymoglobulin 3 mg/kg in the operating room, and then 1 mg/kg for 3 doses on postoperative days 1–3), and others receiving an IL-2 receptor blocker (daclizumab or basiliximab) [12]. Standard maintenance immunosuppression consisted of triple therapy (see Table 2), beginning on postoperative day (POD) zero: tacrolimus or cyclosporine; mycophenolate mofetil (MMF); and prednisone. However, in patients receiving thymoglobulin induction, calcineurin inhibitors were delayed until POD four. Allograft biopsies were performed for clinical causes, such as creatinine increase or plateau, delayed graft function, or oliguria. Surveillance biopsies were routinely performed at 3 and 12 months beginning September 2001. Based on local observations and recent literature [9], one week surveillance biopsies were performed for rare, highly sensitized patients.

For treatment of antibody-mediated rejection, standard plasmapheresis protocol entailed plasmapheresis (1.5 plasma volume exchange) followed by 500 mg/kg IVIg, for 3–4 days. Standard thymoglobulin protocol for treatment of acute cellular rejection was 1.5 mg/kg for 7 doses.

Histopathologic assessment and C4d staining

Formalin-fixed, renal biopsy tissue was routinely processed and paraffin-embedded. Two to three micrometer sections were stained with hematoxylin and eosin, periodic-acid-schiff (PAS), trichrome, and Jones silver; 8–16 levels were examined from each biopsy by a renal pathologist (MLT). Rejection and histologic features (interstitial inflammation (i), tubulitis (t), vasculitis (v), glomerulitis (g), peritubular capillaritis (ptc), arteriolar hyaline as per alternative quantitative scoring (aah), mesangial changes (mm)) were scored according to the Banff 2007 criteria [11,32,34,40] with the following modifications: glomerulitis was defined as 5 or more leukocytes per glomerular cross section; a trace category was added as 3–4 cells per glomerulus in few glomeruli (10–25%). Cells in direct association with fibrin thrombi were not scored. Interstitial hemorrhage and arteriolar/arterial fibrin was scored as: negative(−) = none; 1+ = involvement of 1–2 vessels/foci; 2+ = involvement of 3–4 vessels/foci; 3+ = greater than 4 (extensive). The presence or absence of fragmented red blood cells and tubular vacuolization was noted, as were other histopathologic findings.

C4d indirect immunofluorescent staining was performed on frozen biopsy tissue initially held in Michel's transport media, with the 10–11 monoclonal antibody (1:50, Biogenesis/Serotec, Raleigh, NC) [3]. Where frozen tissue was not available (Cases #2, #7, #8), C4d staining was performed retrospectively on formalin-fixed, paraffin-embedded tissue using the Biomedica polyclonal antibody (distributed by ALPCO, Salem, New Hampshire) at a dilution of 1:40 using cc1 mild pretreatment conditions, an amplification step, and Biotin-free Envision detection on Ventana XT instruments (Ventana, Tucson, Arizona) [43]. Extent of peritubular capillary staining (0—negative; <10%—minimal; 10–50%—focal; >50%—diffuse) and intensity of staining (0–3+) was recorded per Banff 2007, and classified based on respective staining method [40]. Cases #1 and #11 were part of a prior study of C4d staining [43].

Clinical correlation and follow-up data

Renal transplant charts and subsequent biopsy slides were reviewed; chronic changes were assessed according to Banff criteria [32,40].

Results

Patients and biopsies

From a single institution series of 1156 renal transplants, we identified 16 initial renal biopsies containing glomerular fibrin thrombi. All biopsies were derived from recipients of ABO-compatible, cytotoxic-crossmatch compatible renal allografts. Details of patient and transplantation characteristics are listed in Table 2. Biopsies were performed at a mean of POD 7 (range 0–22 days), mainly for elevation of serum creatinine; 3 were planned