Heavy Proteinuria as a Manifestation of Acute Allograft Rejection Presenting Early after Kidney Transplantation: A Retrospective, Single-Center Case Series

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Abstract: The differential diagnosis of heavy proteinuria presenting early after kidney transplantation has generally included de novo or recurrent glomerulonephritis (GN), whereas proteinuria is thought to be an unusual presentation of acute allograft rejection. We retrospectively analyzed the characteristics of 7 patients who presented with early (median 9 days post-transplant) heavy proteinuria with or without renal failure, in association with the development of new donor anti-HLA antibody. End Stage Renal Disease (ESRD) was due to primary GN in three patients. Mean proteinuria at presentation was 7.46 ± 2.44 gm/24 hours. Donor specific anti-HLA antibody was associated with each episode. Diffuse peritubular C4d staining was noted in three cases. Response to therapy with intravenous immunoglobulin G (IVIg) was good, with mean creatinine of 1.48 ± 0.13 mg% at last follow-up of 2-78 months with resolution of proteinuria, and no graft loss. Based on this series, we recommend screening for proteinuria post transplant in all allograft recipients, not only to detect de-novo or recurrent GN in the allograft, but also to detect antibody mediated rejection.

Keywords: IVIg, acute rejection, proteinuria, alloantibody, post-transplant FSGS, glomerulonephritis.

INTRODUCTION

Case reports and series of antibody mediated rejection (AMR) presenting with heavy proteinuria are documented, although this is still considered an unusual presentation of AMR [1-3]. We describe a retrospective case series of 7 renal transplant patients who presented with heavy proteinuria, with and without acute renal failure, in the early post-transplant period. The biopsy findings were variable but all included foot process effacement. Clinically and histopathologically, many of the features of presentation were similar to that seen in post-transplant FSGS. However, all of these patients had developed donor specific anti-HLA antibody. Intravenous immunglobulin (IVIg) was administered to these patients as specific therapy for antibody-mediated rejection. All seven responded to IVIg therapy by clinical measures and/or by biopsy.

METHODS

A retrospective review of data from chart, pathology and computer records was performed on seven patients transplanted at Walter Reed Army Medical Center (WRAMC) between 1994 and 2001. After collection of all necessary data, identifiers were removed, and analysis done. The patients described were those that presented after renal transplant with the onset of heavy proteinuria (with or without acute renal failure) and a newly associated donor-specific antibody, with foot process fusion on transplant renal biopsy. These were considered to have acute antibody-mediated rejection. Proteinuria was identified by repeatedly positive urine dipstick, and was quantified by either a spot urine protein to creatinine ratio or a 24-hour urine collection by standard methods. All descriptive numeric data are presented as the mean ± SEM or as the median (range).

The methodology by which donor-specific antibody was detected is as follows. For Panel Reactive Antibody (PRA) screening and identification, the standard complement-dependent cytotoxicity (CDC) assay was used. From 1999 onward, ELISA Class I screening was performed using solid-phase cell based technology; ELISA class II screening was established in 2001 and used the same methods as for ELISA Class I screening. Donor Specific Antibody (DSA) was measure by Complement dependent cytotoxicity with cell trays.

Renal transplant biopsies were done with the standard technique. Each biopsy sample was sent for light microsco-

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py, EM and immunoflourescence with albumin, fibrinogen, C3/C4, IgA, IgG, and IgM.

Immunohistochemical Staining for C4d

Only 2 patients (#6 and 7) had C4d staining done at the time of kidney biopsy. Patient #7 had C4d staining performed on fresh tissue. Patient #6's specimen was performed using a formalin-fixed paraffin section. In all other cases, staining was done using archived paraffin sections.

On fresh and formalin-fixed, paraffin-embedded tissues, standard immunohistochemical staining was performed with antibodies to human complement split product C4d (Biomedica Gruppe, Vienna, Austria) on the Discovery XT (Ventana Medical Systems, Tucson, Ariz). Briefly, 5-µm-thick sections were treated with Cell Conditioning 2 (CC2) solution (Ventana Medical Systems) for antigen retrieval and then washed in phosphate-buffered saline. Endogenous peroxidases were blocked with hydrogen peroxide and Vector Blocking Kit (Vector Laboratories, Burlingame, Calif) was added to block endogenous avidin-biotin. Non-specific background staining was blocked with Super Block (Super Stain System (HRP), ID Labs, London, Ontario, Canada). The sections were incubated with the rabbit polyclonal anti-human C4d antibody at the dilution of 1:50. After incubation for 30 minutes at room temperature, the sections were incubated with biotinylated goat-anti-rabbit IgG (iView DAB Detection Kit, Ventana Medical Systems), followed by conjugated streptavidin horseradish peroxidase (iView DAB Detection Kit, Ventana Medical Systems), and reacted with diaminobenzidine tetrahydrochloride (iView DAB Detection Kit, Ventana Medical Systems) as the chromogen. Appropriate positive and negative controls were run in parallel with the test tissues. Sections were considered positive when crisp, brown staining was identified in peritubular capillaries and glomerular loops.

RESULTS

Between 1994 and 2001 there were 166 renal transplants done at WRAMC. The acute rejection rate in the first year post transplant was 17.5% (29 patients).

Patient demographic features are shown in Table **1**. There were 6 females and one male. The average age was 47 ± 4 years. One patient was Caucasian, one Asian, and the rest were African American. Three (# 1, 5, and 7) had received a second transplant. Only one patient was known to have FSGS as the etiology of ESRD.

The initial immunosuppressive regimens were as follows:

Patient 1: Antibody induction with rabbit thymoglobulin. Maintenance therapy with rapamycin, tacrolimus, and prednisone.

Patient 2: No antibody induction. Maintenance therapy with cyclosporine, MMF, and prednisone.

Patient 3: No antibody induction. Maintenance therapy with tacrolimus, MMF, and prednisone.

Patient 4: Antibody induction with anti-lymphocyte globulin. Maintenance therapy with cyclosporine, azathioprine, and prednisone.

Patient 5: No antibody induction. Maintenance therapy with tacrolimus, MMF, and prednisone.

Patient 6: Antibody induction with rabbit thymoglobulin. Maintenance therapy with rapamycin, tacrolimus, and prednisone.

Table 1. Patient Demographics and Clinical Characteristics. AA= African American; Asi=Asian; Cau=Caucasian

Patient ID	Age (years)	Sex	Race	Type of Donor	Transplant Number	Cause of ESRD	Time post transplant at presentation	Proteinuria at presentation	Nadir Cr(mg/dl)	Peak Cr(mg/dl)	Proteinuria post treatment	Last f/u Cr(mg/dl)	IVIg Dose
1	53	F	Cau	Deceased Donor	2	Reflux Disease	38 days	5.7 gm/d	1.6	1.6	312 mg	1.4	2gm/Kg over 2d
2	39	F	AA	Living Related	1	Unknown	14 days	100mg% dipstick	1.0	5.5	Urine dip negative	2.1	2gm/Kg over 2d
3	33	F	AA	Deceased Donor	1	Reflux Disease	10 days	17 gm/d	0.8	3.9	Urine dip negative	1.1	2gm/Kg over 2d
4	46	F	Asi	Deceased Donor	1	IgA Nephropathy	9 days	100mg% dipstick	1.5	5.8	Urine dip negative	1.5	0.5 gm/Kg/d x 5d
5	53	F	AA	Deceased Donor	2	Membranous Glomerulonephritis	7 days	6.7 gm/d	1.5	3.1	Urine dip negative	1.8	0.5gm/Kg/d x 5d
6	66	M	AA	Deceased Donor	1	Primary FSGS	4 days	3.9 gm/d	2.7	5.6	379mg	1.3	2 gm/Kg over 2d
7	39	F	AA	Deceased Donor	2	SLE	9 days	4.0 gm/d	1.1	2.2	Urine dip negative	1.2	2 gm/Kg over 1d

Patient 7: Antibody induction with rabbit thymoglobulin. Maintenance therapy with mycophenolate mofetil, tacrolimus, and prednisone.

Acute rejection episodes occurred 4-38 days (median of 9 days) after transplant. Kidney biopsy was performed in all patients. 6/7 patients had deterioration of kidney function concurrent with the onset of heavy proteinuria. In patient #1, heavy proteinuria alone was the indication for biopsy. Clinical characteristics at the time of biopsy are shown in Table **1**.

Proteinuria was not quantified in 2 patients, but was identified repeatedly by dipstick. In the 5 patients in which it was quantified, all were nephrotic, with mean proteinuria of 7.46 ± 2.44 gm/day. All but one patient (#1) experienced a decline in renal function at the time of acute rejection, with at least a doubling of serum creatinine concentration. The mean peak creatinine was 3.96 ± 0.38 mg%.

Prior to transplant patients 1, 6, and 7 were anuric. Patients 2, 3 and 4 had 30 mg% or less proteinuria on dipstick urinalysis immediately post transplant and prior to presentation with heavy proteinuria. There was incomplete information for patient # 5.

Donor specific antibody was newly detected in all cases with new positive cross-matches with donor cells reported in 3 cases. 6/7 cases were associated with a previous antigen stimulation (history of pre-transplant transfusion, pregnancy, or previous transplant) and the development of new donor specific antibody may have represented an amnestic response to a previous antigen stimulation. Patient #7 had known specific antibodies to a previous donor kidney, but the antigens were not shared with the second donor kidney. A new anti-DR7 antibody was demonstrated to have arisen, specific to the second donor. Results are shown in Table **2**.

Biopsies from all the 7 patients had foot process fusion. 3/7 patients had positive C4d staining. Only patients 6 and 7 had C4d staining done at the time of biopsy; both had positive peritubular C4d staining. The remaining 5 patients had C4d staining done on archived paraffin sections. 2 of the 5 had insufficient tissue available for staining, and only 1 of the 3 with sufficient tissue had positive peritubular C4d staining. 2/7 patients had evidence of thrombotic microangiopathy, and both had positive C4d staining, but no evidence of acute cellular rejection. 5/7 patients had evidence of acute cellular rejection. Patient #5 had subepithelial and intramembranous electron dense deposits, in addition to diffuse foot process fusion and evidence of acute cellular rejection. This patient had a history of membranous glomerulonephritis as the cause of ESRD. Tissue was not adequate for Banff classification in 3/7 patients, however, 1

Table 2. Patient and Donor HLA and Donor Specific Antibody Characteristics

Pt ID	HLA Pt	HLA Donor	New Donor Specific Antibody Characteristics	Possible Associated Source
1	A3,- B7,44 DR 11,16	A3,11 B35,- DR1,4	Anti-A11	History of pre-transplant transfusion, history of previous transplant
2	A28,- B8,70 DR3,12	A28,30 B40,70 DR12,13	Anti-B7/12 Creg -Incl B40 & A30	History of pre-transplant transfusion, history of pregnancy
3	A2,3 B14,62 DR15,4	A1,2 B44,62 DR7,13	Anti-Bw4 (Associated with B44)	None known
4	A33,- B35,56 DR6,10	A2,- B46,51 DR 8,12	Anti-A2	History of pre-transplant transfusion
5	A33,36 B35,53 DR11,13	A1,3 B8,35 DR15,7	Anti-B8	History of pre-transplant transfusion
6	A26,34 B35,65 DR15,13	A28,74 B58,70 DR13,9	Anti-A2 Creg -Incl B58 IgG by ELISA	History of pre-transplant transfusion, history of previous transplant
7	A34,- B44,35 DR15,11	A34,66 TX#2 B44,70 DR7,11	Prev Ab:A1,2,9,28,36 New: Anti-DR7	History of previous transplant

Creg: Cross reactive group

of these had C4d positive staining, and the other 2 had evidence of acute cellular rejection.

The antibody-mediated rejection episodes were treated with solumedrol and intravenous immunoglobulin G (IVIg). Patients 1, 2, 3, 6 and 7 received 2 gm/kg IVIg over 1-2 days. Patients 4 and 5 received 0.5 mg/kg/day for 5 days. Patient 3 also received OKT3 and ATG at the time of the rejection episode. Patient 6 received one treatment of plasmapheresis (with 1 plasma volume exchanged with albumin saline) prior to the IVIg treatment.

Renal function improved and proteinuria resolved to < 1 gm/day in all patients after therapy. The mean serum creatinine at last follow-up was 1.48 ± 0.13 mg% with a median follow-up of 3 years (2-78 months). This response corresponded with a decline in the PRA in all patients. Foot process fusion was shown to have diminished in the 1 patient who was re-biopsied for another indication.

DISCUSSION

The Banff criteria for AMR require that at least 3 of 4 criteria be present: 1) Graft dysfunction, 2) Histologic evidence of tissue injury, 3) Positive staining for C4d, and 4) Demonstration of donor specific antibody. In the present series, we describe 7 patients who presented early after kidney transplantation with heavy proteinuria, DSA, and diffuse foot process effacement on allograft biopsy. All of these patients met the criteria for AMR cited above. All had evidence of allograft tissue injury on biopsy other than diffuse foot process fusion.

All patients responded to treatment with steroids and IVIg with resolution of acute renal failure (if present) and proteinuria, with an associated decline in panel reactive antibody. Patient 3 also received OKT3 and ATG for acute cellular rejection, and patient 6 received one plasma volume exchange via plasmapheresis, based on the early presumed diagnosis of recurrent FSGS, which was not consistent with biopsy findings or C4d staining. The excellent response of all patients argues against a misdiagnosis of post-transplant GN, which in most series does not respond well to IVIg [4]. If DSA had not been tested for in these patients, they would have been likely treated as either de-novo or recurrent GN/FSGS, based on their presentation with proteinuria, acute renal failure, and foot process fusion on biopsy (with or without evidence of cellular rejection).

C4d staining was not available to us at the time of presentation for the first 5 patients. The lack of positive staining in 2/3 patients with sufficient archived tissue could be due to storage and handling as well as the passage of time, and thus a false negative test, although one study has reported that there are no significant differences between the three currently available methods for detecting C4d [5]. However, all patients did have increased titers of DSA, which is the most consistent standard for comparison of C4d staining [6-8]. In chronic allograft nephropathy, podocyte effacement can be variably present in this condition, but not commonly described.

The importance and relative rarity of persistent, nephrotic range proteinuria post transplant has now been documented in recipients of both living donor [9] and deceased donor

[10] kidneys. Thus, the detection of heavy/nephrotic range proteinuria should not be attributed to native kidney disease. In fact, persistent post-transplant proteinuria has been reported in 22-30% of renal transplant patients and is associated with decreased graft survival [11,12]. The most alarming finding from this study was that the biopsy proven cases of chronic rejection and transplant glomerulopathy appeared significantly earlier after transplantation than the cases of GN, and that cases of allograft rejection could have been missed by reliance on serum creatinine alone.

In the pathology community, diffuse foot process efface-ment in allograft biopsies is sometimes taken as diagnostic of GN, and sometimes thought to actually argue against allograft rejection. However, our series demonstrates that this finding may be present in cases of antibody mediated allograft rejection. Although de-novo/recurrent GN and acute rejection/AMR are not mutually exclusive conditions, one would argue against implicating more disease processes than necessary. Only one patient (#6) in our series had a primary disease (FSGS) described to commonly occur with associated acute renal failure post transplant. Although foot process fusion was clearly present on his allograft biopsy, the presence of concurrent thrombotic microangiopathy, positive C4d staining, and DSA, make it much more likely that his primary cause of proteinuria and acute renal failure was AMR rather than FSGS. Most of our patients were African American, including this patient, which is associated with a lower risk of recurrent FSGS [13,14]. Patient (#5) who had a history of membranous GN did indeed have evidence of subepithelial and intramembranous deposits on biopsy, consistent with recurrence. However, she also had a new positive DSA (as did all the patients in the series) and responded to IVIg with resolution of proteinuria and acute renal failure with decline in DSA which was consistent with the presence of AMR.

The era of this study was prior to the broader use of plasmapheresis for AMR, and we used IVIg alone. At present there is no consensus that plasmapheresis is superior to high dose IVIg alone, at least in patients with a negative CDC prior to transplant [15].

Our report reinforces the recommendations by the American society of Transplantation that a DSA determi-nation should be obtained and C4d staining be done in all allograft biopsies performed for deterioration of allograft function [6]. In addition, we would advocate the frequent screening of all recipients for worsening of proteinuria after transplant, especially in programs that do not perform protocol biopsies.

CONFLICT OF INTEREST

The opinions are solely those of the authors and do not represent an endorsement by the Department of Defense. There is no financial conflict of interest.

DISCLAIMER

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This study was approved by the Walter Reed Army Medical Centre Institutional Review Board in compliance with all Federal regulations governing the protection of human subjects.

We certify that all individuals who qualify as authors have been listed; each has participated in the conception and design of this work, the analysis of data (when applicable), the writing of the document, and the approval of the submission of this version; that the document represents valid work; that if we used information derived from another source, we obtained all necessary approvals to use it and made appropriate acknowledgements in the document; and that each takes public responsibility for it.

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