De Novo Membranous Nephropathy Associated With Antibody-Mediated Rejection in Kidney Transplant Recipients

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ABSTRACT

Background. Membranous nephropathy (MN) is a rare autoimmune disease that can develop a persistent nephrotic syndrome and end-stage kidney disease, with a recurrence rate of 30% to 40% after kidney transplant.

Methods. Retrospective case series of membranous nephropathy observed in a cohort of kidney transplant recipients with donor-specific anti−human leukocyte antigen antibodies and biopsy-proven antibody-mediated rejection (AMR).

Results. We report 4 cases of membranous nephropathy associated with AMR. MN was diagnosed 10 to 92 months posttransplant, associated with de novo donor-specific antibodies, specific to class I in 2 cases, and class II in another 2. All cases presented typical morphology of membranous nephropathy, with subepithelial deposits with spikes at electron microscopy. Immunostaining for immunoglobulin G4 was negative in all cases, and podocyte-expressed M-type phospholipase A2 receptor was detected in glomerular basement membrane of 3 cases. Biopsy specimens from patients with longer follow-up showed more intense microvascular inflammation and chronic injury markers, possibly because of subclinical immunologic injury. AMR therapy included immunoglobulin 2g/kg in 3 patients, isolated or associated with plasmapheresis. One patient was not treated because of an active disseminated infection. Two patients remain with functioning grafts and under antiproteinuric therapy. Two grafts were lost, 1 because of chronic failure and the other because of death secondary to infection. Despite treatment, donor-specific antibodies remain detectable in a 6-month follow-up.

Conclusions. De novo MN is a rare manifestation associated with AMR in kidney transplant recipients. The occurrence of podocyte-expressed M-type phospholipase A2 receptor in de novo MN suggests antibody-mediated activation, despite the use of maintenance immunosuppression.

Membranous nephropathy (MN) is a rare autoimmune disease and the most common cause of idiopathic nephrotic syndrome in White adults without diabetes [1]. About 80% of patients have are primary membranous nephropathy (PMN), and 20% have secondary, which is associated with other systemic diseases [2]. The histologic MN diagnosis consists of immune complex deposits of immunoglobulins (IgG, mainly IgG4) and complement factors (C3 and C4) along the glomerular basement membrane [1], as well as electron-dense subepithelial deposits with spikes at electron microscopy. Most cases of PMN present circulating IgG4 autoantibody to the podocyte-expressed M-type phospholipase A2 receptor (anti-PLA2R), thrombospondin type

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1 domains containing 7A (3%-5%), or other unidentified mechanisms (10%) [2].

MN recurrence rate in kidney transplant ranges from 10% to more than 50%, depending on the indication of graft biopsy results and their frequency, the sample size, and the immunosuppressive therapy [3]. Unlike PMN, the recurrent posttransplant MN usually is a progressive disease that requires treatment at the earliest stage [3]. De novo posttransplant MN might be related to immunologic phenomena induced by transplant, such as T cell−mediated rejection or antibody-mediated rejection (AMR) [4]. We reported a series of cases of biopsy-proven AMR that presented histologic findings compatible with MN. We also reviewed the literature about de novo MN associated with AMR.

MATERIALS AND METHODS

Patients and Clinical Data

Secondary analysis of a single-center retrospective cohort of kidney transplant recipients with donor-specific anti-HLA antibodies and biopsy-proven AMR. In a previous study [5], we aimed to analyze the clinical and histologic effects of the AMR treatment with intravenous immunoglobulins (IVIGs) and plasmapheresis. Inclusion criteria were recipients older than18 years at transplant who received therapy for biopsy-proven AMR. Diagnosis of AMR included the presence of microvascular injury in the graft biopsy specimen and detection of donor-specific antibody (DSA) in peripheral blood. Exclusion criteria were pediatric kidney transplant recipients and incomplete data for AMR diagnosis.

For this secondary analysis, we included 4 patients with the diagnosis of MN associated with AMR, characterized by the presence of subepithelial deposits with spikes in the glomerular basement membrane at electron microscopy. Data collection included demographic data: age at kidney transplant, sex, etiology of the chronic kidney disease (CKD), pretransplant blood transfusion, previous transplant, pretransplant pregnancies, HLA-A, -B, and -DR mismatches, pretransplant panel-reactive antibodies, presence of preformed DSA, donor source, age and sex of donors, expanded criteria donors, kidney function at donation, and Kidney Profile Donor Index (KDPI). The collected transplant data included initial immunosuppressive therapy, length of cold ischemia, and occurrence of delayed graft function (DGF). AMR related data included time posttransplant, DSA class, mean fluorescence intensity (MFI) in anti-HLA antibody screening by solid-phase assay, graft function, and proteinuria AMR.

The study protocol was approved by the University of Campinas Ethics Committee (CAAE: 51485415.6.0000.5404). Written informed consent was obtained from the patients or their next of kin to publish this case report and any accompanying images.

Histologic, Immunohistochemistry, and Electron Microscopy Analysis

The graft morphology was analyzed according to the Banff 2013 classification, revised in 2015 [6]. The paraffin-embedded biopsy slices were stained with monoclonal anti-C4d antibody, as previously described [7]. Transmission electron microscopy analyzed the presence of immunocomplex deposits, podocyte effacement, and glomerular basement membrane duplication.

The kidney biopsy specimens were screened for the expression of PLA2R by immunohistochemistry on the paraffin-embedded graft section. Four microbiopsies were deparaffinized using 3 immersions in xylol (Labsynth, Diadema, Brazil) for 5 minutes each, and the tissues were rehydrated in sequential ethanol gradient (100%/95%/80%). Antigen was retrieved by incubation with Tris-EDTA buffer (pH 9.0) for 40 minutes at 95°C. After 2 wash cycles of 5 minutes with Tris-buffered saline (TBS) containing 0.05% Tween 20 (Labsynth) (pH 7.4), endogenous peroxidase was blocked by incubation with 3% H_2O_2 solution for 15 minutes. Protein blockade used protein blocking solution from Immunohistoprobe 2-step polymer kit (Advanced Biosystems, Redwood City, Calif, United States), following the manufacturer's recommendations. Primary antibody (mouse monoclonal anti-PLA2R, Sigma Aldrich St Louis, MO, United States) was diluted at 1:250 in 1% bovine serum albumin inTBS and incubated for 18 hours at 4°C. After this period, 3 wash cycles with TBS containing 0.05% Tween 20T were performed. Samples were incubated with the secondary anti-mouse antibody, followed by horseradish peroxidase polymer, both from Immunohistoprobe 2 step polymer kit (Advanced Biosystems), according to manufacturer's instructions. The 3,3'-Diaminobenzidine staining kit (Dako North America, Calif, United States) was used to reveal immunostaining following the kit instructions, and slides were counterstained with hematoxylin solution (Dinâmica Química, Indaiatuba, Brazil). Biopsy specimens stained with only secondary HPR-polymer conjugated antibody were used to check the background of nonspecific staining. Slides were analyzed by light microscopy (400 × magnification) using Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan), and images were captured and processed in real time using the NIS-Elements AR program (Nikon).

Literature Review

Two researchers performed a literature review in the databases PubMed, Scopus, and Web of Science using the terms "membranous nephropathy," "antibody-mediated rejection," "DSA," and "anti-HLA antibodies" between March 2019 and December 2020. We selected case reports, systematic reviews, clinical trials, multicenter studies, and meta-analyses with MN cases associated with AMR.

RESULTS

Four kidney transplant recipients who presented MN associated with acute AMR were included in this study. The clinical and histologic characteristics are shown in Table 1. The biopsy specimen features are presented in Fig 1.

Patient 1 was a male 25-year-old with CKD of unknown etiology and negative serology for HIV, hepatitis B and C, syphilis, and Chagas disease. He received a kidney from a standard deceased donor with KDPI of 2%, serum creatinine 88.4 μmol/L at donation, and preimplantation kidney donor biopsy results without abnormalities. HLA antigen compatibility showed 1 mismatch in each HLA-A, -B, -DR, and DQ loci. Induction therapy included basiliximab and steroids, and the maintenance regimen was tacrolimus, with adjustment aiming at a trough level of 5 to 8 ng/mL, sodium mycophenolate, and steroids. Hospital discharge occurred at day 6 posttransplant, with a serum creatinine of 219.3 μmol/L and progressive renal function improvement reaching a serum creatinine of 109.6 μmol/L, without proteinuria, and normal urinalysis. Twenty-four months posttransplant, the patient presented peripheral edema, serum creatinine of 107.8 μmol/L, urine protein-to-creatinine ratio (UPC) of 6.19,

Table 1. Clinical and Histologic Characteristics of the Cases of Membranous Nephropathy Associated With Antibody-Mediated Rejection

Case	1	2	3	4
Transplant recipients				
Age, y	25	44	44	28
Sex	Male	Female	Female	Female
Etiology of CKD	Unknown	FSGS	HUS	FSGS
Transfusions pretransplant	No	Yes	Yes	Yes
Previous transplant	No	No	No	No
Pretransplant pregnancies	-	Yes	Yes	Yes
HLA-A, -B, -DR mismatches	3	4	6	1
Pretransplant class I PRA, %	0	0	27	78
Pretransplant class II PRA, %	0	0	0	0
Preformed DSA	No	No	No	No
Donors				
Type of donors	Deceased	Deceased	Deceased	Deceased
Age, y	19	35	27	17
Sex	Male	Male	Male	Male
Expanded criteria donors	No	No	Yes*	No
Serum creatinine, μmol/L	68.9	61.9	406.6	97.2
KDPI, %	2	20	89	5
Transplant				
Initial immunosuppression	IL2RAb/TAC/MS	ATG/CYA/MS	ATG/TAC/MS	ATG/CYA/MS
Cold ischemia, h	14.3	31.0	17.4	22.7
DGF	No	Yes	Yes	No
Antibody-mediated rejection				
Time posttransplant, mo	24	92	10	36
DSA class	II	II	I	I
DSA MFI_{SUM}	44,115	23,794	945	13,356
Immunodominant DSA MFI_{MAX}	22,745	23,794	945	13,356
Current immunosuppression	TAC/MS	CYA/MS	CYA/MS	CYA/MS
SCr, μmol/L	106.1	170.6	167.9	159.1
eGFR (CKD-EPI), mL/min	83.5	30.9	30.8	37.6
UPC, g/g	6.19	11.8	2.3	12.1
Graft morphology				
Light microscopy - Banff score				
i, 0-3	0	1	0	1
t, 0-3	1	1	0	1
v, 0-3	0	0	0	0
g, 0-3	0	2	0	2
ptc, 0-3	0	2	0	2
MVI g + ptc, 0-6	0	4	0	4
C4d, 0-3	3	2	1	3
ci, 0-3	0	2	1	0
ct, 0-3	0	2	2	1
Electron microscopy				
IC deposits	Subepithelial	Subepithelial	Subepithelial	Subepithelial
Podocyte effacement	No	Diffuse	No	Diffuse
GBM duplication	No	Yes	No	Yes
IgG4 deposition	No	Mild	No	No
PLA2R	NA	Yes	Yes	Yes
Immunofluorescence positivity	IgG/C3 κ/λ/C1q	IgG/C3/λ	IgG/C3 κ/λ	IgG/IgM/IgA C3/κ/λ

ATG, antithymocyte globulin; ci, interstitial fibrosis; CKD, chronic kidney disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; ct, tubular atrophy; CYA, cyclosporine; DGF, delayed graft function; DSA, donor-specific anti−human leukocyte antigen antibody; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; g, glomerulitis; GBM, glomerular basement membrane; HLA, human leukocyte antigen; HUS, hemolytic uremic syndrome; i, inflammation; IC, immune complex; Ig, immunoglobulin; IL2RAb, interleukin 2 receptor antibody; KDPI, Kidney Donor Profile Index; MFI, mean fluorescence intensity; MS, mycophenolate sodium; MVI, microvascular inflammation; NA, not available; PLA2R, M-type phospholipase A2 receptor; PRA, panel-reactive antibody; ptc, peritubular capillaritis; SCr, serum creatinine; TAC, tacrolimus; t, tubulitis; UPC, urine protein-to-creatinine ratio; v, intimal arteritis; GBM, glomerular basement membrane.

* Donor with acute kidney injury.

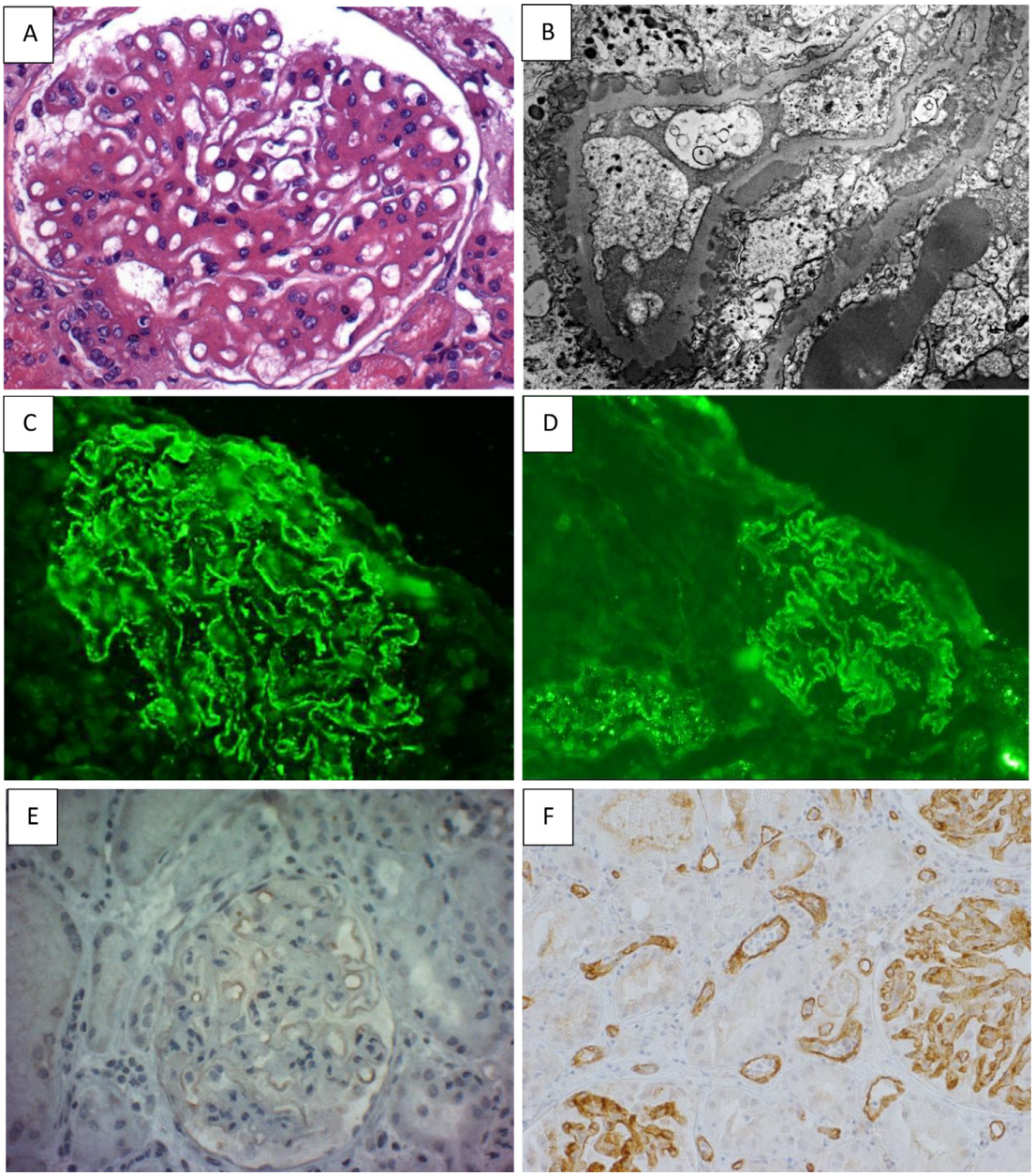

Fig 1. Morphology of membranous nephropathy associated with antibody-mediated rejection. **(A)** Glomerular basement membrane thickening, (light microscopy: hematoxylin and eosin, × 400); **(B)** Diffuse thickening of the glomerular basement membrane, subepithelial electron-dense deposits, and spike formation (electron microscopy); **(C)** Immunoglobulin G deposition along the capillary wall (immunofluorescence assay, × 400); **(D)** C3 deposition along the capillary wall (immunofluorescence assay, × 400); **(E)** M-type phospholipase A2 receptor glomerular staining (immunostaining assay, × 400); **(F)** C4d staining (immunostaining, × 400).

dyslipidemia, serum albumin of 2.3 g/dL, and negative HIV, hepatitis B, and hepatitis C serologies. Chest radiography and abdominal ultrasonography findings were normal. He reported discontinuation of tacrolimus inadvertently for 1 week. He presented de novo DSA anti-DQA3 (21,373 MFI) and anti-DQ8 (MFI 22,742 MFI). The graft biopsy specimen, analyzed according to the Banff score, showed thickened glomerular basement membrane, mild mesangial expansion, and mild tubulitis (t1). There was no glomerulitis (g0), peritubular capillaritis (ptc0), or glomerular basal membrane double contours (cg0). Immunofluorescence was positive for IgG, C3, kappa, and lambda in a granular capillary pattern, and C1q mesangial

granular and negative for IgM, and IgA. There was no IgG4 expression in the glomerular capillary in the immunohistochemistry. C4d immunostaining was positive and diffuse along peritubular capillaries. PLA2R was not analyzed because of insufficient kidney tissue for staining. Electron microscopy analysis revealed subepithelial deposits with spikes, without double contouring or multilayering of the glomerular basement membrane. Histopathologic findings were compatible with the diagnosis of MN associated with AMR. Rejection therapy included tacrolimus reintroduction, a 3-day pulse therapy of intravenous 500 mg methylprednisolone, and human intravenous immunoglobulin 2g/kg. Likewise, adjuvant therapy included loop diuretic, angiotensin-converting enzyme blockade, and statins. Kidney function remained stable during the first 3 months of treatment, with a reduction of UPC to 3.4 g/g, recovery of serum albumin to 3.5 g/dL, and complete remission of peripheral edema. One year post treatment, the class II DSAs remained detected (anti-DQA3 24,429 MFI and anti-DQ8 22,247 MFI), with persistent proteinuria (UPC 3.1 g/g) and a stable renal function (serum creatinine of 123.8 μmol/L).

Patient 2 was a female 44-year-old with CKD secondary to focal segmental glomerulosclerosis (FSGS), negative serology for HIV, hepatitis B and C, syphilis, and Chagas disease, without preformed anti-HLA antibodies. She received a kidney from a standard deceased donor with KDPI of 20% and normal preimplantation biopsy results. There were 2 mismatches in each HLA-A and -B loci. The induction of immunosuppression consisted of thymoglobulin 7 mg/kg and steroids, and maintenance therapy included cyclosporine, sodium mycophenolate, and steroids. She presented DGF and chronic graft dysfunction during the follow-up (serum creatinine 159.1 μmol/L), without proteinuria. Eight years posttransplant, the patient developed proteinuria (UPC 11.8 g/g) and de novo DSA anti-DQ7 (23,794 MFI). Graft biopsy results showed moderate glomerulitis (g2), thickened glomerular basement membrane, mild inflammation (i2), mild tubulitis (t1), moderate peritubular capillaritis (ptc2), mild interstitial fibrosis (ci1), and mild tubular atrophy (ct1). Immunofluorescence showed C3 and lambda granular deposits in glomerular capillaries and negative for IgG, IgM, IgA, C1q, and kappa. Immunohistochemistry (IHC) showed mild IgG4 expression in the glomerular capillary, focal C4d staining of peritubular capillaries, and PLA2R expression in the glomeruli. Electron microscopy revealed subepithelial deposits with spikes, compatible with the diagnosis of MN associated with AMR, with double contouring of the glomerular basement membrane and diffuse podocyte effacement. Therapy included a 3-day pulse therapy of intravenous 500 mg methylprednisolone, IVIG 2g/kg, and angiotensin-converting enzyme blockade. Nine months after treatment, graft biopsy results showed the persistence of glomerulitis and peritubular capillaritis, as well as reduction of tubulitis. Twenty months after AMR treatment, DSA remained detectable (anti-DQ7 24,419 MFI), with chronic graft dysfunction (serum creatinine 194.5 μmol/L), and proteinuria (UPC 4.8 g/g).

Patient 3 was a female 44-year-old with CKD secondary to hemolytic uremic syndrome, who underwent hemodialysis for 6 years, with negative serology for HIV, hepatitis B and C, syphilis, and Chagas disease. She reported 2 blood transfusions, with pretransplant panel-reactive antibody (PRA) 26% class I and 0 class II pretransplant, without DSA. She received a kidney from a 27-year-old standard deceased donor with KDPI of 23%, acute kidney injury at donation (serum creatinine 406.6 μmol/L), and normal preimplantation biopsy results. The HLA antigen compatibility showed 2 mismatches in each HLA-A, -B, -DR, and DQ loci. Induction immunosuppressive was thymoglobulin 6 mg/kg and steroids, and maintenance regimen included sodium mycophenolate and steroids. There was no need for hemodialysis, with hospital discharge at day 14 posttransplant, with a serum creatinine of 116.7 μmol/L. Calcineurin inhibitor was introduced day 21 posttransplant, with tacrolimus adjustment aimed at a trough level of 5 to 8 ng/mL. Because of to the donor's tuberculosis diagnosis, confirmed after transplant, the recipient received isoniazid for 6 months. Three months after transplant, the patient presented graft dysfunction (serum creatinine raised from 132.6 μmol/L to 212.2 μmol/L), proteinuria (UPC 0.81 g/g), and presence of decoy cells in routine urine cytology screening. Tacrolimus dose was progressively reduced, and switched to sirolimus. Ten months after transplant, proteinuria increased to 2.3 g/g and de novo DSA anti-A2 (945 MFI) was detected. Graft biopsy results showed thickened glomerular basement membrane without glomerulitis (g0), peritubular capillaritis (ptc0), tubulitis (t0), tubular cells viral inclusions, or vascular or interstitial abnormalities. Immunofluorescence was positive for IgG, C3, kappa, and lambda in a granular capillary pattern. There was no IgG4 expression in the glomerular capillary in the IHC, C4d staining in peritubular capillaries was minimal, and PLA2R expression was present in glomeruli. Electron microscopy revealed subepithelial deposits with spikes, without double contouring or multilayering of the glomerular basement membrane, compatible with the diagnosis of MN associated with AMR. Therapy included calcineurin inhibitor reintroduction, and DSA was undetectable after 3 months. The patient persisted with graft dysfunction (serum creatinine 196.2 μmol/L) and proteinuria (UPC 1.0 g/g). Thirty months after transplant, the patient returned to dialysis because of infectious complications.

Patient 4 was a female 28-year-old with CKD secondary to FSGS, who underwent hemodialysis for 5 years, pretransplant PRA 78% class I and 0 class II, and negative serology for HIV, hepatitis B and C, syphilis, and Chagas disease. She received a kidney from a 17-year-old standard donor KDPI of 5%, serum creatinine of 97.2 μmol/L at donation, and normal preimplantation biopsy results. There was 1 mismatch in each HLA-A and -DR loci, without DSA. Induction therapy included thymoglobulin 4.5 mg/kg and steroids, and the maintenance regimen consisted of cyclosporine, sodium mycophenolate, and steroids. She presented DGF and chronic graft dysfunction (serum creatinine 159.1 μmol/L) during follow-up, without proteinuria. Three years after transplant, the patient developed proteinuria (UPC 12.1 g/g) and de novo DSA anti-A1 (13,356 MFI). The graft biopsy results showed moderate glomerulitis (ptc2), thickened glomerular basement, moderate peritubular capillaritis (ptc2), mild tubulitis (t1), and tubular atrophy (ct1). Immunofluorescence revealed capillary granular deposits of IgG, IgA, IgM, C3, kappa, and lambda, without C1q. There was no

glomerular IgG4 expression in the IHC, diffuse C4d staining was present in peritubular capillaries, and PLA2R expression was noticed in glomeruli. Electron microscopy showed subepithelial deposits with spikes, double contouring of the glomerular basement membrane, and diffuse podocyte effacement, compatible with the diagnosis of MN associated with AMR. Therapy included a switch from cyclosporine by tacrolimus, 3 plasmapheresis sessions, and IVIG 2g/kg. After treatment, the DSA persisted as detectable (anti-A1 7760 MFI), with the progression of graft dysfunction and graft failure after 7.3 months.

DISCUSSION

After kidney transplant, membranous nephropathy can occur either as a recurrence of the disease or de novo glomerular disease [8]. In the present series, none of the patients had MN as the etiology of CKD. In case 1, the etiology of CKD was unknown, and although it is not possible to rule out MN, clinical data, such as young age at diagnosis and absence of nephrotic syndrome, differs from the natural history of MN, most often affecting elderly patients, with nephrotic syndrome and a slower progression to end-stage CKD [9]. Favoring de novo MN, the onset of posttransplant symptoms coincided with the interruption of calcineurin inhibitor and de novo DSA detection in the peripheral blood. The C4d deposits on graft verified by immunostaining consist of additional AMR criteria, despite the absence of glomerulitis or peritubular capillaritis in the biopsy specimen.

Patients 2 and 4, whose primary renal disease was FSGS, developed proteinuria and AMR later after transplant. The morphology of MN was associated with more intense microvascular inflammation and markers of chronic graft lesion, suggesting the occurrence of continuous subclinical immunologic injury. The patient 2 de novo DSA could be a consequence of poor adherence to immunosuppressive treatment. Patient 4 presented an increased risk of developing AMR because of previous HLA sensitization, with a pretransplant class I PRA of 78%, without DSA. The patient 3 posttransplant polyomavirus infection was associated with the need to reduce immunosuppressive therapy, which could trigger the development of de novo DSA.

Several studies in kidney transplant recipients reported de novo MN associated with hepatitis C infection, but few studies showed de novo MN associated with AMR (Table 2). Patel et al [8] reported 1 case of de novo MN-associated AMR, with detection of class I and II DSA and treated with intravenous immunoglobulin and rituximab, with reduction of proteinuria and intensity of DSA fluorescence after 9 months. Honda et al [4], in a series of 17 patients with de novo MN, detected 4 who presented de novo DSA at the time of MN diagnosis, the majority with class II DSA. El Kossi et al [10] reported a case of de novo MN associated with DSA directed against HLA-DQ7 at 50th month posttransplant, treated with angiotensin receptor blocker and switched from azathioprine to mycophenolate mofetil, with resolution of proteinuria and improvement of kidney function. However, the DSA remained detectable, with a reduction of MFI.

MN is an antibody-mediated glomerular disease that binds antibodies to podocyte antigens and favors deposition of immune complexes [11]. The anti-PLA2R antibodies are present in most primary MN in native kidneys, and positive anti-PLA2R MN has also been reported in patients with sarcoidosis, autoimmune liver disease, hepatitis B virus infection, and class V lupus nephritis [11]. In normal kidney tissue, PLA2R is found in the cytoplasm of podocytes. In patients with primary MN, PLA2R is concentrated in subepithelial deposits, while cytoplasmic expression decreases [12]. In general, the glomerular search for the antigen is more sensitive than the serum measurement of the antibody [13,14]. The PLA2R antigen can still be detected in glomerular deposits, even in the absence of circulating antibody [13].

Posttransplant MN can result from 2 types of mechanisms: recurrence of primary MN or de novo MN [11]. Unlike the cases of primary MN recurrence, where anti-PLA2R and IgG4 are usually detected, de novo MN might represent a form of immune response triggered by exposure of hidden antigens, probably different from those antigens observed in idiopathic

Table 2. Reported Cases of Posttransplant De Novo Membranous Nephropathy Associated With Antibody-Mediated Rejection

Study	MN Cases	HCV	DSA Class	Treatment and Effectiveness
de Sousa et al [5]	4	No	I and II	CNI, MYF, steroids, IVIG, and ACEi: effective
				CNI, MYF, steroids, IVIG, and ACEi: effective
				CNI, MYF, steroids, and ACEi*
				CNI, MYF, steroids, IVIG, PP, and ACEi: not effective
Patel et al [8]	1	No	I and II	IVIG and rituximab: effective
Honda et al [4]	5	NA	I and II	PP, CNI, MP, MMF, and ARB: effective
				CNI and MP: not effective
				CNI, MMF, and MP: not effective
				CNI, MP, and DSG: not effective
				CNI, MP, DSG, PP, and Pulse: not effective
El Kossi et al [10]	1	No	II	Switch azathioprine to mycophenolate: effective

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockade; CNI, calcineurin inhibitor; DSA, donor-specific anti–human leukocyte antigen antibodies; DSG, deoxyspergualin; HCV, hepatitis C virus; IVIG, intravenous immunoglobulin; PP, plasmapheresis; MMF, mycophenolate mofetil; MN, membranous nephropathy; MP, methylprednisolone; MYF, sodium mycophenolate; NA, not available; Pulse, intravenous steroid pulse therapy.

* Death with functioning graft due to infectious disease.

MN, and IgG1 staining seems to be dominant [15]. The donor's graft endothelium expresses histocompatibility complex molecules that target antibody production and complement activation, resembling C4d positivity in peritubular capillaries [16]. Endothelial cell activation is associated with circulating DSAs, mainly class II, and accompanies vascular injury in AMR [17]. In the reported cases, we observed de novo circulating anti-DQ antibodies and C4d deposits in the graft. Probably, these antigens elicited immune response, which can lead to damage of podocytes and release of cytoplasmic- or membrane-associated podocytes proteins [15], with a production of antibodies and immunocomplex and its deposition in the subepithelial area. In our series, none of the cases presented IgG4 as the dominant or codominant IgG subclass in the glomerular deposits, in agreement with other studies [18,19]. However, most of our patients showed PLA2R expression, different from the other authors' reports [10,13,20]. We hypothesized that besides the antibody reactivity to HLA class II antigens expressed on podocytes, the anti-HLA antibodies might have cross-linked with non-HLA antigens on the podocyte surface, such as PLA2R. Another possible explanation is that the AMR could stimulate anti-PLA2R production and cause tissue damage, even in the absence of anti-HLA class II antibodies.

AMR treatment aims to remove circulating antibodies, block their effects, and reduce their production [21]. Despite the lack of strong evidence from reliable randomized clinical trials in AMR therapy, plasmapheresis is widely used, combined with IVIG and antirejection drugs [22,23]. The goal of plasmapheresis is the removal of alloantibodies from the circulation, mainly DSA. Additional therapies include IVIG (2g/kg), anti-CD20 antibody, and lymphocyte-depleting antibody, with or without steroids [23,24]. IVIG has strong immunomodulatory effects, regulating Fc antibody receptors' expression and function, interfering with the activation of complement and the cytokine network, providing anti-idiotypic antibodies and exerting effector functions on T and B cells [25]. Anti-CD20 antibodies can suppress antibody production, including DSAs [23]. The treatment with high-dose steroids and lymphocyte-depleting antibodies can increase sepsis and cancer [26]. Its use must be carefully indicated, considering the severity of rejection severity and chronic graft dysfunction intensity.

In our center, once AMR is diagnosed, we add a calcineurin inhibitor to the maintenance immunosuppressive therapy in cases when it is not already in use, associated with the antiproliferative drug, preferably mycophenolate, and steroids. The treatment usually includes 2 g/kg IVIG and 5 alternate day plasma exchanges using 5% human serum albumin. In the reported cases, the treatment consisted of calcineurin inhibitor reintroduction in cases where it had been withdrawn or switching from cyclosporine to tacrolimus because of its higher immunosuppressive potential. A 3-day intravenous steroid pulse was administered in case of associated acute cellular rejection, with interstitial inflammation. Three patients received IVIG 2 g/kg because of its infectious events, except the third case. Because of the low clearance of anti-DQ antibodies in other AMR cases treated with plasma exchange in our center, we decided not to perform plasmapheresis in patients 1 and 2. Additional therapies were antiproteinuric and statins. We observed 1 death due to infectious events and 1 graft failure in our series. The other 2 patients remain with functioning grafts and receiving antiproteinuric drugs. DSA remains detectable in these cases, with routine solid-phase DSA test control every 6 months.

CONCLUSIONS

De novo MN is a rare manifestation associated with AMR in kidney transplant recipients, which could be suspected in the presence of proteinuria and de novo DSA. Treatment of AMR associated with antiproteinuric drugs can reduce proteinuria and prolongs graft survival. The occurrence of PLA2R in de novo MN suggests antibody-mediated activation, despite the use of maintenance immunosuppression.

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