

[CASE REPORT]

Membranous Nephropathy with Crescent after Hematopoietic Cell Transplantation

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Abstract:

A 44-year-old man who received allogeneic hematopoietic stem cell transplantation after being diagnosed with acute myeloid leukemia developed nephrosis when the dose of tacrolimus was tapered. A renal biopsy showed the granular deposition of immunoglobulin G in the glomerular basement membrane and subepithelial electron-dense deposits, crescent formation, C4d-positive staining of the peritubular capillary, and subendothelial swelling, suggesting that the main pathological diagnosis was membranous nephropathy and that chronic graft-versus-host disease played a role in the etiology of nephrosis. We herein report a case of membranous nephropathy with various pathological findings. C4d deposition suggests complement activation and the involvement of humoral factors.

Key words: graft-versus-host disease, membranous nephropathy, crescent, C4d positivity on peritubular capillaries

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Introduction

The number of allogeneic hematopoietic cell transplants for leukemia has recently increased. Consequently, the recognition of glomerular diseases associated with nephrotic syndrome as a complication has also increased (1).

According to the pertinent literature, the incidence of nephrotic syndrome in adult recipients ranges from 0.4% to 6% (2). Nephrotic syndrome has many causes; however, the most critical cause of nephrotic syndrome in patients after hematopoietic cell transplantation is graft-versus-host disease (GVHD) (3). Although acute GVHD tends to cause thrombotic microangiopathy (TMA), chronic GVHD tends to cause nephrotic syndrome, especially membranous nephropathy (MN) (2). There have been case series of MN after allogeneic hematopoietic stem cell transplantation that focused

on IgG subclasses and the phospholipase A2 receptor (PLA2R) to distinguish this form of MN from primary MN (4-6). However, the characteristics of MN after hematopoietic stem cell transplantation remain unclear, since several mechanisms are involved in chronic GVHD and the pathological findings may vary among cases.

We herein report a case of MN with unusual renal pathological findings after hematopoietic cell transplantation.

Case Report

A 44-year-old man diagnosed with acute myelogenous leukemia (AML) was treated with idarubicin and cytarabine. He was also administered intensification therapy with the first-course drugs of mitoxantrone and cytarabine and second-course drugs of daunorubicin and cytarabine. Afterward, he underwent unrelated peripheral stem cell transplan-

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Table. Laboratory Data on Admission.

Peripheral blood	AST	25 U/L	Urinalysis	
WBC 10,200 / μ L	ALT	22 U/L	Gravity	≥ 1.050
RBC 5.63×10^6 / μ L	ALP	310 U/L	pH	6.5
Hb 17.5 g/dL	LDH	22 U/L	protein	(4+)
Hct 51.4 %	γ GTP	26 U/L	occult blood	(2+)
Plt 259×10^3 / μ L	GLU	123 mg/dL	glucose	(+/-)
	CRP	0.09 mg/dL	Sediments	
Blood chemistry	Serological tests		RBC	31-50 /HPF
Na 142 mmol/L	anti nuclear Ab	(-)	WBC	1-2 /HPF
K 3.9 mmol/L	CH50	52.1 /mL	hyaline cast	5-10 /LPF
Cl 108 mmol/L	C3	170.4 mg/dL	dysmorphic RBC	(+)
TP 5.2 g/dL	C4	38.1 mg/dL		
Alb 2.0 g/dL	HBs antigen	0.1 COI	Urinary chemistry	
BUN 15 mg/dL	anti HCV Ab	0.1 COI	TP/Cr	23.02 g/gCr
Cr 0.96 mg/dL	IgA	160 mg/dL	NAG	158.5 U/L
UA 7.1 mg/dL	IgG	650 mg/dL	β 2MG	4,600 μ g/L
TC 379 mg/dL	IgM	79 mg/dL	selectivity index.	0.036
TG 322 mg/dL	IgE	269.9 IU/mL		
HDL-C 66 mg/dL	MPO-ANCA	(-)		
LDL-C 250 mg/dL	PR3-ANCA	(-)		

β 2MG: β 2 microglobulin, NAG: N-acetyl- β -D-glucosaminidase

tation, and there was no human leukocyte antigen (HLA) (A, B, C, DR) mismatch with the unrelated donor. A conditioning regimen of methotrexate and total-body irradiation was instituted. GVHD prophylaxis initially consisted of methotrexate but was later changed to tacrolimus monotherapy. Even with reduced-dose tacrolimus, no symptoms related to GVHD were observed, and he was discharged from the hospital four months after transplantation. The tacrolimus dosage was decreased to 0.4 mg/day through 20 months post-transplantation.

Post-transplantation, the serum creatinine (Cr) and serum albumin levels were normal (0.78 mg/dL and 4 g/dL, respectively); however, at 21 months post-transplantation, the patient noticed bilateral leg swelling. New laboratory data showed that the serum Cr level remained normal but had increased to 0.96 mg/dL, and the serum albumin level had decreased to 2 g/dL. The serum total protein level was 5 g/dL, and the serum albumin level was 2 g/dL. The urinary protein/creatinine ratio was 23.02 g/gCr. Due to substantial proteinuria, the patient received a nephrology consultation and was transferred to the nephrology department, where a kidney biopsy was performed at 23 months after transplantation. The urinary protein excretion was 10.5 g/day, and the proteinuria selectivity index was 0.036. The number of red blood cells in urinary sediment was 31-50 per high-power field. The patient's laboratory data upon admission are shown in the table. The only complication the patient experienced was hyperlipidemia. The results of a serological analysis for hepatitis B virus (HBV) and hepatitis C virus (HCV) were negative. Myeloperoxidase anti-neutrophil cytoplasmic antibody (MPO-ANCA), proteinase (PR) 3-ANCA, and antinuclear antibodies were also absent.

The renal biopsy sample contained 39 glomeruli, 2 of

which were globally sclerosed. One glomerulus showed segmental endocapillary hypercellularity and extracapillary cellular proliferation (Fig. 1A and B). The peripheral glomerular capillary walls were not thickened and showed no apparent bubbling or spikes. In the two collapsing glomeruli, the Bowman's capsules were thickened with prominent interstitial fibrosis surrounding them. The proportion of interstitial fibrosis and tubular atrophy area in the cortex was 10%. There was no intimal thickening in the interlobular arteries, but partial nephrotoxicity of calcineurin inhibitors, such as increasing arteriolar hyalinosis and small-vessel narrowing, was observed. Typical TMA morphologies, such as fibrin thrombi within glomeruli and mesangiolysis, were not present.

Immunofluorescent microscopy revealed diffuse, fine, granular deposits of immunoglobulin G (IgG) and C3 along the peripheral capillary walls, suggesting MN (Fig. 2). C4d staining was positive along the peripheral capillary walls and the peritubular capillary wall (Fig. 2). In addition, immunostaining for IgG subclasses and PLA2R showed dominant IgG4 and IgG1 staining and positivity for PLA2R on the glomerular basement membrane [IgG1 (3+), IgG4 (3+) > IgG2 (+), IgG3 (+) and PLA2R (+); Fig. 3].

To define the extracapillary lesion as a crescent or podocyte proliferation, we performed immunostaining of WT-1, podocalyxin, PAX8, and claudin 1. The results for WT-1 and podocalyxin markers for podocytes were negative, but those for PAX8 and claudin 1 markers of the Bowman's capsule epithelial cells were positive (Fig. 4). Taking these findings into consideration, the extracapillary lesion was determined to be a crescent.

Electron microscopy (EM) showed diffuse subepithelial deposits without spike formation or foot process effacement.

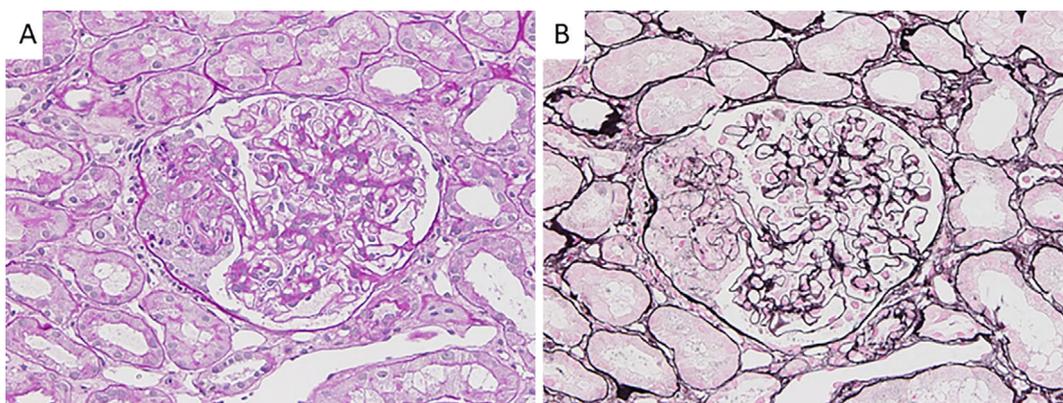


Figure 1. Findings on light microscopy of a kidney biopsy. A: Periodic acid and Schiff staining (magnification, ×200). B: Periodic acid methenamine silver staining (magnification, ×200).

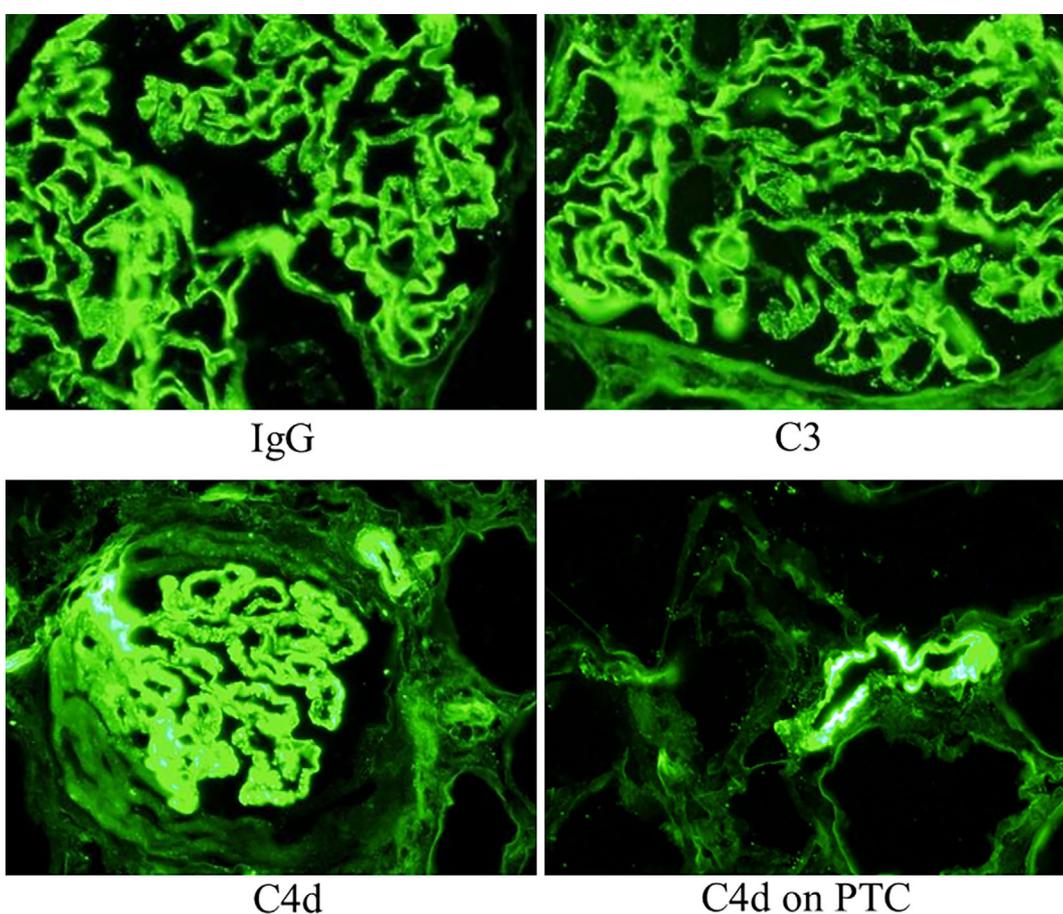


Figure 2. Immunostaining for IgG, C3, and C4d. Glomerular deposition of IgG (2+) and C3 (2+) are observed in the capillary loops. C4d is positive at the capillary loop and peritubular capillaries. PTC: peritubular capillary

These findings were consistent with a diagnosis of MN stage I. In addition to subepithelial deposits, electron micrographs also showed subendothelial widening (Fig. 5).

Complete remission was achieved within 3 months with the intravenous administration of 1 g of methylprednisolone followed by the daily administration of 60 mg of prednisolone and 0.4 mg of tacrolimus. The tacrolimus dosage was not changed after nephrosis. The urinary protein/creatinine

ratio was <0.1 g/gCr, and a urinary sediment examination was negative for hematuria three months after discharge, at which point he transferred to another hospital.

Discussion

This case is a MN after hematopoietic cell transplantation. Our review of the previous literature showed that the inci-

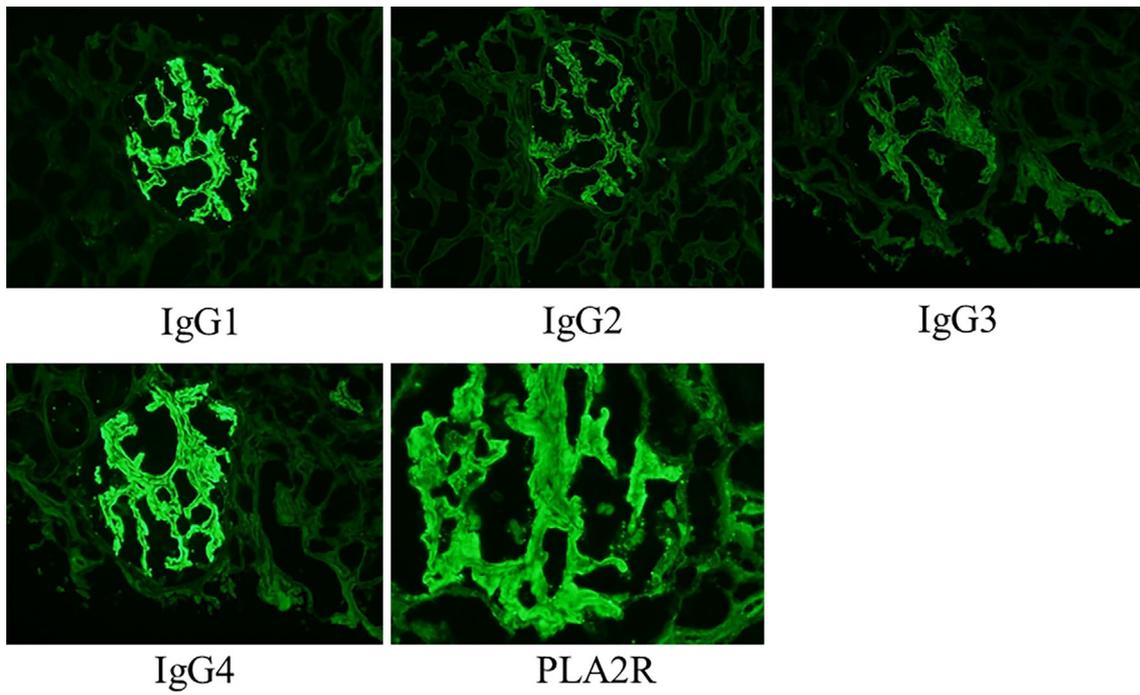


Figure 3. Immunostaining for IgG subclass, and PLA2R. Glomerular deposition of the glomerulus shows predominant IgG4 and IgG1. PLA2R is also positive in the glomerulus. PTC: peritubular capillary, PLA2R: anti-phospholipase-A2 receptor

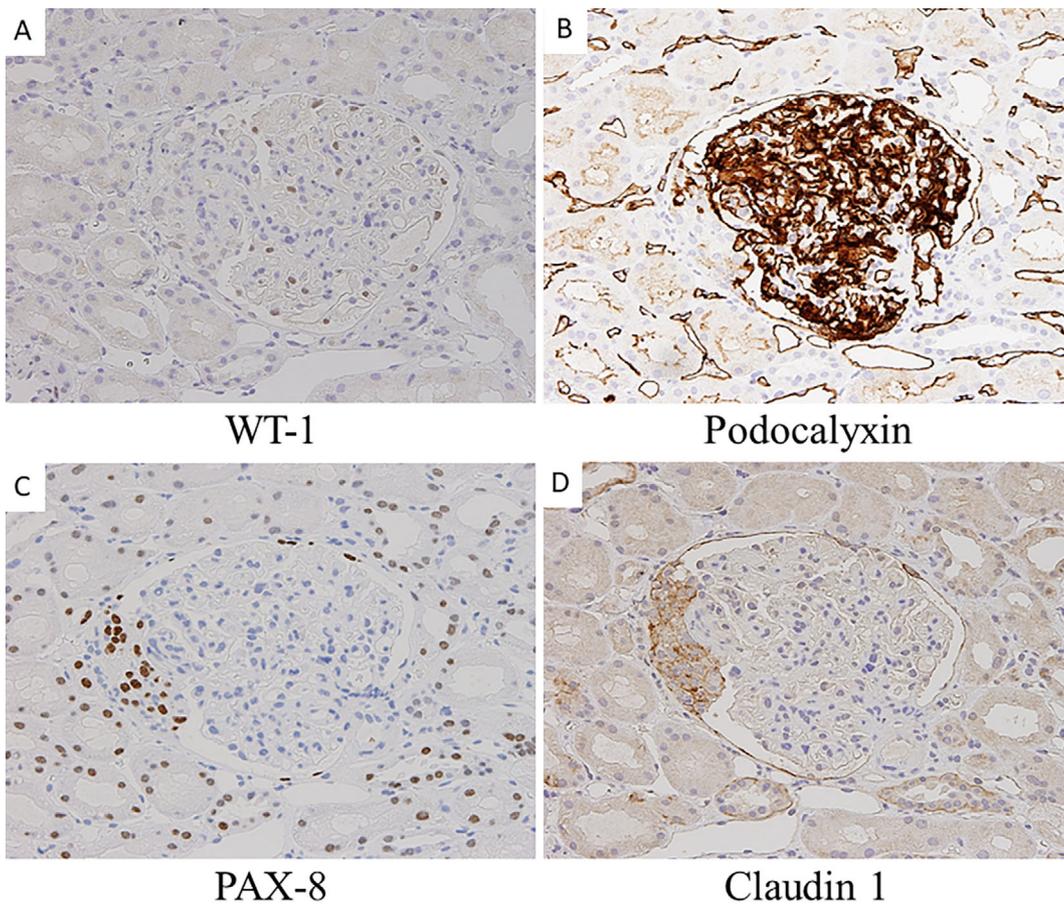


Figure 4. Immunostaining for WT-1, podocalyxin, PAX8, and claudin 1. Stainings for PAX8 and claudin 1 are positive at the extracapillary lesions.

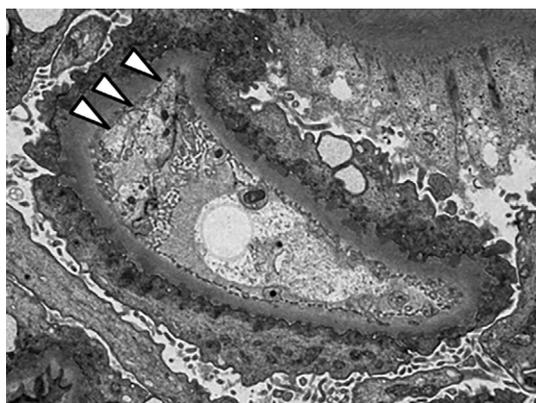


Figure 5. Electron microscopy findings. Subepithelial electron-dense deposits and diffuse foot process effacement are observed. Subendothelial widening is also observed (arrowhead).

dence of MN after hematopoietic cell transplantation with nephrotic syndrome was 61%, followed by minimal change disease at 22%, focal segmental glomerulosclerosis at 7%, and others at 10% (1). Therefore, MN after hematopoietic cell transplantation is not rare, but the findings of crescent, C4d-positive staining in the peritubular capillaries, and subendothelial widening were worth mentioning as the pathological findings in this case.

The prevalence of crescent formation in MN has been reported as nearly 0.4%, and more than half of the cases were secondary causes of MN (7). To confirm the identity of the extracapillary lesion, we performed immunochemical staining. The proteins claudin 1 and PAX8 are expressed solely in parietal epithelial cells (8). In contrast, WT-1 and podocalyxin are expressed exclusively in podocytes (9). Using the antibodies to these four proteins, we investigated the extracapillary lesion and found it to be a crescent, as claudin 1 and PAX8 detection indicated extracapillary lesions, while the expression of WT-1 and podocalyxin was negative. Crescent formation seems to be induced by endocapillary hypercellularity in response to severe injury to the glomerular capillary wall (10). The present case exhibited not thrombosis in the capillaries but subendothelial widening, which is a characteristic pathological finding of TMA. TMA is sometimes observed in chronic GVHD cases (11), and small crescents may occasionally present in TMA cases (12). Since TMA causes subendothelial widening and hampers the cross-talk with podocytes (13), it causes podocyte injury and may produce a crescent, as in this case.

Although the etiology of MN seemed to be GVHD after hematopoietic cell transplantation in this case, IgG4 and IgG1 were predominant among the IgG subclasses, and PLA2R was positive. The IgG subclass and PLA2R expression can help distinguish primary MN from secondary MN; however, the sensitivity and specificity for various IgG subtype staining patterns for primary MN is not high. For example, MN in adults showed a PLA2R expression sensitivity of 75% and specificity of 83% for the detection of primary MN (14). Indeed, there have been several case reports of

MN-associated GVHD in which IgG4 was predominant and PLA2R was positive in the glomeruli (5, 6). Furthermore, corticosteroids have been shown to be more effective for GVHD-associated MN than primary MN, as complete or partial remission was achieved soon after treatment began (5). In the present case, MN occurred more than 100 days after transplantation during the tacrolimus tapering period, and steroid reactivity was good, so the clinical course fit that of MN-associated with GVHD.

In GVHD, no diffuse peritubular C4d staining has been described (14) because GVHD is primarily a T cell-mediated disease in which donor T cells recognize significant histocompatibility complex mismatch. However, B cells also contribute to GVHD through antibody-mediated and antibody-independent mechanisms (15). Mii et al. reported a case of renal TMA associated with chronic GVHD after hematopoietic cell transplantation with C4d deposition on peritubular capillaries (3). They also examined the pathological features of seven renal cases resulting from chronic GVHD using the Banff classification for renal transplant pathology. The pathological characteristics resembled those of antibody-mediated rejection in kidney transplant patients (9). The evidence reported for the cases suggests that humoral factors are also involved because the pathological features partly resemble chronic antibody-mediated rejection after kidney transplantation. According to the Banff classification, subendothelial widening has been regarded as an essential indicator of chronic active antibody-mediated rejection since 2013 (16), and endocapillary hypercellularity is also an important finding in chronic active antibody-mediated rejection. The present case might share the same pathophysiology, and antibody-mediated reactions may be associated with subendothelial widening.

Our case presented with crescent, predominance of IgG4 and IgG1 in the IgG subclass, PLA2R-positive and C4d-positive findings in the peritubular capillaries. Based on the clinical course, we suspect this case was not idiopathic MN but rather MN secondary to chronic GVHD. The crescent formation was induced by endothelial injury associated with intracellular hypercellularity, probably due to T cell-mediated GVHD and humoral immune disorders.

This case report was approved by the Ethics Committee of Nagasaki University Hospital (No. 17121830). Written informed consent to publish this case report and any accompanying images was obtained from the patient.

The authors state that they have no Conflict of Interest (COI).

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References

1. Brukamp K, Doyle AM, Bloom RD, et al. Nephrotic syndrome after hematopoietic cell transplantation: do glomerular lesions repre-

- sent renal graft-versus-host disease? *Clin J Am Soc Nephrol* **1**: 685-694, 2006.
2. Hingorani S. Renal complications of hematopoietic-cell transplantation. *N Engl J Med* **374**: 2256-2267, 2016.
 3. Mii A, Shimizu A, Utsumi K, et al. Renal thrombotic microangiopathy associated with chronic humoral graft versus host disease after hematopoietic stem cell transplantation. *Pathol Int* **61**: 34-41, 2011.
 4. Hiramatsu R, Ubara Y, Imafuku A, et al. Clinicopathological analysis of allogeneic hematopoietic stem cell transplantation-related. *Hum Pathol* **50**: 187-194, 2016.
 5. Huang X, Qin W, Liu Z, et al. Detection of anti-PLA2R autoantibodies and IgG subclasses in post-allogeneic hematopoietic stem cell transplantation membranous nephropathy. *Am J Med Sci* **346**: 32-37, 2013.
 6. Byrne-Dugan CJ, Collins AB, Batal I, et al. Membranous nephropathy as a manifestation of graft-versus-host disease: association with HLA antigen typing, phospholipase A2 receptor, and C4d. *Am J Kidney Dis* **64**: 987-993, 2014.
 7. Rodriguez EF, Nasr SH, Larsen CP, Sethi S, Fidler ME, Cornell LD. Membranous nephropathy with crescents: a series of 19 cases. *Am J Kidney Dis* **64**: 66-73, 2014.
 8. Ohse T, Vaughan MR, Chang AM, et al. De novo expression of podocyte proteins in parietal epithelial cells during experimental glomerular disease. *Am J Physiol Renal Physiol* **298**: 702-711, 2010.
 9. Palmer RE, Kotsianti A, Haber DA, et al. WT1 regulates the expression of the major glomerular podocyte membrane protein Podocalyxin. *Curr Biol* **11**: 1805-1809, 2001.
 10. Charles Jennette J. Rapidly progressive crescentic glomerulonephritis. *Kidney Int* **63**: 1164-1177, 2003.
 11. Mii A, Shimizu A, Fujino T, et al. Renal thrombotic microangiopathy associated with chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Pathol Int* **61**: 518-527, 2011.
 12. Jennette JC, Silva FG, D'Agati VD, et al. Heptinstall's pathology of the kidney. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, PA, 2015.
 13. Dimke H, Maezawa Y, Quaggin SE. Crosstalk in glomerular injury and repair. *Curr Opin Nephrol Hypertens* **6**: 356-372, 2015.
 14. Larsen CP, Messias NC, Walker PD, et al. Determination of primary versus secondary membranous glomerulopathy utilizing phospholipase A2 receptor staining in renal biopsies. *Mod Pathol* **26**: 709-715, 2013.
 15. Jacobson CA, Ritz J. B-cell-directed therapy for chronic graft-versus-host disease. *Haematologica* **95**: 1811-1813, 2010.
 16. Haas M, Sis B, Colvin RB, et al. Banff 2013 meeting report: inclusion of C4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am J Transplant* **14**: 272-283, 2014.

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