



## Case Report

# Membranous glomerulonephritis in a patient with anti-u1 ribonucleoprotein (RNP) antibody-positive mixed connective tissue disease: A case report

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## ABSTRACT

We report a 33-year-old Japanese man diagnosed with mixed connective tissue disease (MCTD) who developed nephrotic proteinuria. Both speckled antinuclear antibody (ANA) and anti-U1 ribonucleoprotein (RNP) antibody were positive, but anti-double-stranded DNA (dsDNA) antibody and anti-Smith (Sm) antibody were negative, while complement levels were normal. Renal biopsy revealed membranous glomerulonephritis (MGN) with diffuse thickening of the glomerular basement membrane (GBM) plus spike and bubble formation. Immunofluorescence demonstrated granular deposits of IgG and C3 along the GBM. Analysis of IgG subclasses showed predominant deposition of IgG1 and IgG4, unlike typical lupus nephritis in which there is predominant deposition of IgG1, IgG2, IgG3, and C1q. Electron microscopy identified numerous large electron-dense deposits (EDD) of various types in the subepithelial region of the GBM, but there were no EDD localized in the mesangium or subendothelium. Based on these findings, MGN was considered to be closely related to MCTD in this patient.

## 1. Introduction

Mixed connective tissue disease (MCTD) is a connective tissue disorder characterized by a high titer of anti-U1 ribonucleoprotein (RNP) antibody combined with clinical features that are usually seen in systemic lupus erythematosus (SLE) and systemic sclerosis (SSc). While absence of severe renal disease is a hallmark of MCTD, renal involvement occurs to some extent, with membranous glomerulonephropathy (MGN) being the most common renal manifestation and nephrotic proteinuria also being possible [1]. Unlike MCTD, SLE is characterized by a high titer of anti-ds-DNA antibodies and reduced levels of complement components. Renal manifestations of SLE are frequent, and some patients develop MGN-like glomerulonephritis [2–4]. However, the differences between MGN related to MCTD or LN are poorly understood. We encountered an MCTD patient with nephrotic proteinuria,

in whom renal biopsy revealed MGN. Based on the findings in this case, we discuss the differences between MCTD-related and LN-related MGN. (See Table 1.)

## 2. Case report

In January 2013, a 33-year-old Japanese man was admitted to our hospital with edema of the legs, weight gain, and sausage-like digits. At the age of 23 years, Raynaud phenomenon had developed. At age 27, severe back pain occurred due to left renal vein thrombosis. MCTD was diagnosed since he also had Raynaud phenomenon, swollen hands, and a high titer of anti-U1 RNP antibody. Edema of the legs initially occurred in September 2012. The patient had no allergies, drank alcohol socially, and was a nonsmoker. His mother had rheumatoid arthritis.

On admission, the patient was 165.6 cm tall and weighed 54 kg,

**Abbreviations:** Mixed connective tissue disease, (MCTD); Antinuclear antibody, (ANA); Anti-U1 ribonucleoprotein, (RNP) antibody; Anti-double-stranded DNA, (dsDNA) antibody; Anti-Smith, (Sm) antibody; Membranous glomerulonephritis, (MGN); Systemic lupus erythematosus, (SLE); Systemic sclerosis, (SSc); Light microscopy, (LM); Immunofluorescence, (IF); Methylprednisolone, (mPsl); Tacrolimus, (FK); Mycophenolate mofetil, (MMF); Dexamethasone, (DEX); Anti-topoisomerase, 1(Scl-70); Computed tomography, (CT); Glomerular basement membrane, (GBM); Periodic acid methenamine-silver, (PAM); Electron microscopy, (EM); Lupus nephritis, (LN); Mesangial proliferative glomerulonephritis, (MPG)

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**Table 1**  
Laboratory findings on admission.

	Case	Normal range		Case	Normal range
Blood			Anti-centromere antibody	Negative	Negative
White blood cell	10 <sup>4</sup> /μl	6.2	3.4 to 9.2	Anti-RNAP III body	Negative
Red blood cell	10 <sup>6</sup> /μl	4.22	4.00 to 5.66	Anti-SS-A antibody	Negative
Platelet	10 <sup>4</sup> /μl	22	14.1 to 32.7	Anti-PLA2R antibody	Negative
Total protein	g/dl	5.4	6.9 to 8.4	PR3-ANCA	U/ml
Albumin	g/dl	2.9	3.9 to 5.2	MPO-ANCA	U/ml
Urea nitrogen	mg/dl	31	8 to 21	C3	mg/dl
Creatinine	mg/dl	0.7	0.6 to 1.1	C4	mg/dl
Creatinine clearance	ml/min	86.1	> 90	CH50	U/ml
Total cholesterol	mg/dl	275	122 to 240	IgG	mg/dl
CRP	mg/dl	0	0.0–0.3	IgA	mg/dl
ANA	IU/ml	108	< 20	IgM	mg/dl
RF	U/ml	3	0–15	Immuno-electrophoresis	Negative
Anti-U1-RNP antibody	IU/ml	133	< 15	Bence-Jones protein	Negative
Anti-dsDNA antibody	IU/ml	< 10	< 12	Urine	
Anti-Sm antibody		Negative	Negative	Red blood cell	/HPF
Anti-Jo-1 antibody		Negative	Negative	White blood cell	/HPF
Anti-Scl70 antibody		Negative	Negative	Protein	g/day
					3.4
					< 1
					< 1
					< 0.1

with a blood pressure of 129/69 mmHg and heart rate of 110/min. Severe edema was noted at eyelids and lower legs, in addition, swelling and joint pain of both hands (including proximal and distal interphalangeal joints and metacarpophalangeal joints) were definite. Laboratory findings were as follows (Table1): total protein was 5.4 g/dL; albumin, 2.9 g/dL; serum creatinine, 0.7 mg/dL and CRP, 0.0 mg/dL. Immunological tests revealed that speckled type of ANA was 108 index using ELIZA method (normal: < 20). Anti-U1 RNP antibody was also 133 index using ELIZA method(normal: < 15), but anti-dsDNA antibody, and anti-Sm antibody. CH50 was 47 U/mL (normal: > 30 U/mL). Anti-PLA2R antibody was negative. The urinary sediment did not contain erythrocytes or leukocytes. However, 24-h protein excretion was 3.4 g. Computed tomography (CT) showed ground-glass opacities in both the lower lobes of the lungs, but 2-dimensional echocardiography with Doppler flow studies did not reveal any evidence of pulmonary hypertension. Biopsy was performed from the lower pole of the left kidney.

### 3. Renal biopsy

Light microscopy (LM) demonstrated global sclerosis in 1/35 glomeruli. Tubulointerstitial fibrosis associated with atrophy occupied 30 to 40% of the cortical region, but there was minimal sclerotic change of small arteries (Fig. 1a). Examination with periodic acid methenamine-silver (PAM)-Masson staining identified diffuse thickening of the glomerular basement membrane (GBM), with definite spike and bubble formation and deposits between the spikes (Fig. 1b), but there was no mesangial cell proliferation. Immunofluorescence (IF) revealed granular deposits of IgG and C3 along the GBM, but IgA, IgM, C1q, and C4 were negative. Analysis of IgG subclasses showed that IgG1 and IgG4 were predominant (Fig. 1c). Electron microscopy (EM) detected numerous large electron-dense deposits (EDD) in the subepithelial region of the GBM, with perpendicular protrusions (i.e., spikes) arising from the GBM between these deposits. The following types of EDDs were also noted: EDDs extending from the subendothelial into the subepithelial region, hump-like EDDs separate from the lamina densa that bulged toward Bowman's capsule beyond the GBM boundary, and EDDs that extended toward Bowman's capsule from the subepithelial region (Fig. 1d). However, there were no EDD localized in the mesangium or subendothelium. MGN (Stage II and/or III) was diagnosed.

### 4. Diagnosis

This patient was positive for anti-RNP antibody and speckled ANA, but was negative for anti-dsDNA antibody and anti-Sm antibody that

are usually positive in SLE. He was also negative for anti-Scl70 antibody, anti-centromere antibody, and anti-RNAP III antibody (usually positive in systemic sclerosis), as well as being negative for anti-Jo-1 antibody (positive in polymyositis). His clinical manifestations included swelling and pain of joints in the hands, Raynaud phenomenon, and interstitial lung disease. A definite diagnosis of MCTD was made according to the previous criteria [5].

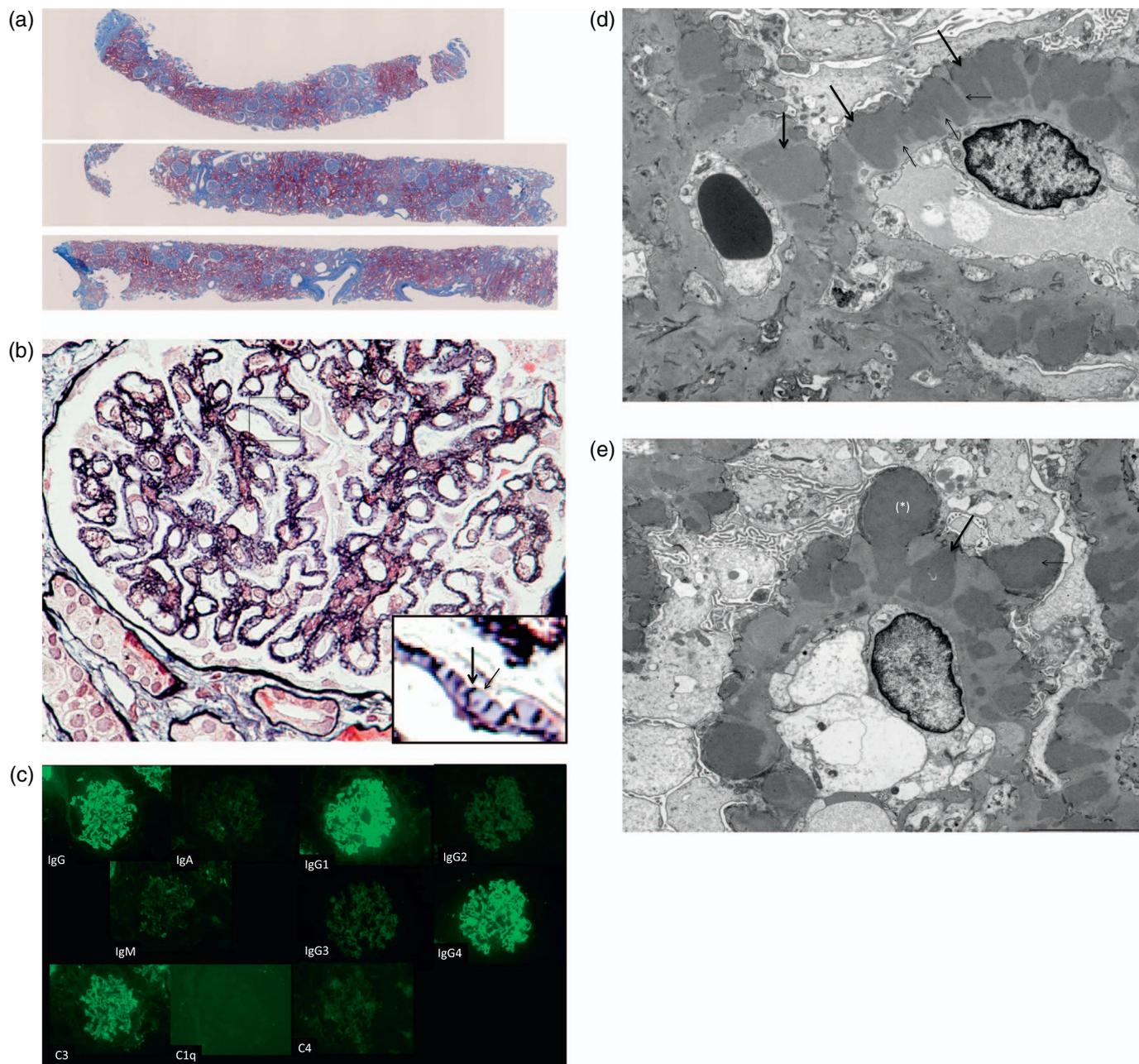
### 5. Clinical course

Treatment was started with methylprednisolone (MPSL) (40 mg/day) and methylprednisolone pulse therapy (1000 mg/day for 3 days) was given concomitantly. However, proteinuria did not subside, so tacrolimus (FK: 1.5 mg/day) and mycophenolate mofetil (MMF: 2000 mg/day) were added, but his proteinuria persisted. MPSL was switched to dexamethasone (DEX: 4 mg/day), and then was changed to prednisolone (PSL: 20 mg/day). In addition, the dose of MMF was decreased to 1500 mg/day and that of FK was decreased to 1 mg/day. Thereafter, proteinuria began to decrease. Pneumonia occurred after eight months of treatment, so MMF and FK were discontinued and PSL was tapered to 5 mg/day. After 13 months, proteinuria increased to 1.23 g/day. Therefore, FK was restarted at 2 mg/day and PSL was increased to 10 mg/day. Thereafter, his proteinuria subsided to 0.3 g/day (Fig. 2).

### 6. Discussion

Various autoantibodies may be detected in patients with SLE, including ANA, anti-dsDNA antibody, anti-Sm antibody, anti-RNP antibody, and anti-SSA (Ro) antibody. Among these autoantibodies, anti-dsDNA antibody is most commonly associated with lupus nephritis (LN), and its titer is usually correlated with disease activity [6]. From 10 to 20% of patients with LN have MGN (classV) [2–4]. It was reported that anti-Sm antibody is also related to renal disease and this association is stronger when anti-dsDNA antibody is positive concomitantly [7], while LN has not been reported in patients who are negative for both anti-dsDNA and anti-Sm antibodies. Our patient was negative for both of these antibodies, which was inconsistent with typical LN.

LN-related MGN was reported to show some different histological features from idiopathic MGN. Concurrent subendothelial or prominent mesangial deposits are detected by EM, as also seen in the proliferative forms of lupus nephritis. Glomerular deposition of all of IgG, IgM, IgA, C3, and C1q (called “full house”) may be seen [8]. In LN, deposition of IgG1, IgG2 and IgG3 is predominant, while predominance of IgG4 is seen in idiopathic MGN [9].



**Fig. 1.** Renal biopsy findings

a: Tubulointerstitial fibrosis and atrophy occupies 30 to 40% of the cortical region. Blue shows connective tissue. (Masson trichrome stain)

b: Spike and bubble formation in the glomerular basement membrane (GBM) is recognized on periodic acid methenamine-silver (PAM)-Masson staining. Large arrow shows a spike (black). Small arrow shows a deposit (blue-white).

c: Immunofluorescence(IF) revealed granular deposits of IgG and C3 along the GBM. Analysis of IgG subclasses showed predominance of IgG1 and IgG4.

d: On electron microscopy (EM), there were numerous large electron-dense deposits (EDD: large arrows) in the subepithelial region of the GBM, with perpendicular protrusions (spikes shown by small arrows) arising from the GBM between the EDD.

e: Various types of EDDs were found in this patient, including deposits that expanded from the subendothelial to subepithelial region (large arrow), hump-like EDDs (small arrow) separate from the lamina densa that bulged toward Bowman's capsule beyond the GBM boundary, and EDDs that extended toward Bowman's capsule from the subepithelial region (\*). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In MCTD patients, renal biopsy displays immune complex glomerulonephritis and the changes are similar to those in LN. Ito studied the renal histology of 6 children with MCTD, reporting that 2 of them had MGN, 3 had MPG, and 4 showed a mixture of MGN and MPG [1]. Ichikawa analyzed the national Japanese renal biopsy database (20,523 patients) and identified 5 patients with MCTD, 3 of whom had MGN [10]. Omokawa reported that MGN shows predominance of IgG2 in patients with MCTD, while lupus-associated MGN is typically positive for IgG1, IgG2, IgG3, C1q, C4 and C3, but they did not report EM

findings [11]. In contrast, our patient predominantly showed deposition of IgG (IgG1 and IgG4).

Its reported that EM revealed subepithelial, intramembranous, and mesangial deposits in patients with MCTD [1], but did not give precise details.

Differences between MGN in MCTD and LN may be summarized as the following; MCTD-related MGN shows the predominant stain of IgG1 and IgG4 on this case, and the predominant stain of IgG2 according to Omokawa' report [11] on IF, and absence of subendothelial and

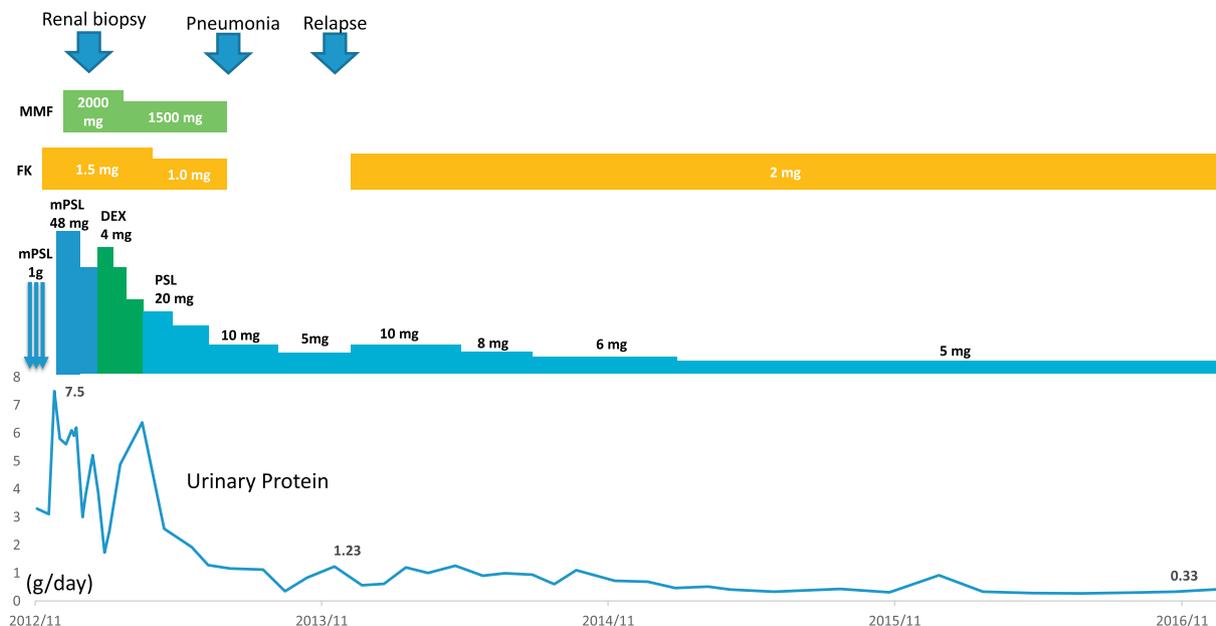


Fig. 2. Clinical course.

mesangial deposits on EM. While, on lupus-associated MGN, IF shows the positive stain of IgG3 and C1q, and IgG3 fix complement component of classical complement pathway such as C1q, C3 and C4, resulting in an influx of inflammatory cells. As well as subepithelial EDDs, concurrent subendothelial or mesangial deposits are present on EM.

In conclusion, we evaluated glomerulonephritis in a patient with MCTD. Renal biopsy showed MGN with diffuse spikes and bubbles of the GBM (stage II and/or III). In addition, analysis of IgG subclasses identified predominant deposition of IgG1 and IgG4, while EM revealed various types of large subepithelial EDD. These histological findings were not consistent with a diagnosis of LN-associated MGN or idiopathic MGN, and the patient's MGN was considered to be related to MCTD.

**Statement of ethics**

The present report adhered to the Declaration of Helsinki, and the patient gave his consent for the case report to be published.

**Conflict of interest and disclosures**

The authors declare no competing financial interests.  
The authors also declare that they have no conflicts of interest.

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