Significance of anti-ribonucleoprotein (RNP) antibody and anti-Smith (anti-Sm) antibody among patients with SLE or MCTD

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Abstract

Background

The significance of anti-ribonucleoprotein (RNP) antibody and anti-Smith (anti-Sm) antibody remains unclear in patients with systemic lupus erythematosus (SLE) or mixed connective tissue disease (MCTD).

Methods

Thirty patients were retrospectively enrolled in this study. They were all positive for antinuclear antibody (ANA) and anti-RNP antibody, were diagnosed with SLE(n = 25) or MCTD(n = 5), and underwent renal biopsy at our hospital between January 1990 and December 2014. These 30 patients were classified into 4 groups based on their anti-dsDNA and anti-Sm status. Renal histology was classified into 3 patterns, including pure subepithelial membranous nephropathy (MN), mesangial and/or subendothelial lesions (MES), and MN combined with MES.

Results

Comparison between groups A [dsDNA(-)and SM(-)] and B□dsDNA(-)and SM(+)□ revealed that group A was with pure MN(n = 5) + MN + MES(n = 4) + MES(n = 1) and normocomplementemia, while group B was with MES + MN(n = 2) + MES(n = 5) and hypocomplementemia. Comparison between groups C□ dsDNA(+)and SM(-)□ and D□dsDNA(+)and SM(+)□showed that group C was with pure MES and normocomplementemia, while group D was with MN + MES(n = 4) + pure MES(n = 3) and hypocomplementemia. Among 10 patients in group A, all 5 patients with MCTD were categorized as pure MN, while the 5 patients with SLE were MN + MES or pure MES.

Conclusion

Among patients showing positivity for anti-RNP antibody alone, those with MCTD seem likely to develop pure MN, while those with SLE tend to have MES as well as MN.

Introduction

Human spliceosomal U1 small nuclear ribonucleoprotein (snRNP), consisting of U1 snRNA and ten proteins, recognizes the 5'-splice site within precursor-mRNAs and initiates assembly of the spliceosome for intron excision (1). Anti-RNP antibodies react with proteins (70Kd, A, and C) that are associated with U1 RNA and form U1 snRNP. While, anti-smooth muscle (anti-Sm) antibodies are directed against 7 proteins (B/B', D1, D2, D3, E, F, and G) that constitute the common core of the U1, U2, U4, and U5 snRNP particles (2).

Renal involvement is one of the major complications of mixed connective tissue disease (MCTD), and a variety of renal pathologies have been described in 10–50% of MCTD patients (3). In patients with MCTD, renal biopsy usually reveals membranous glomerulonephritis or mesangial glomerulonephritis, although focal or diffuse proliferative glomerulonephritis can sometimes be detected (3, 4).

Pulmonary arterial hypertension is the leading cause of death in these patients, and that studies of inception cohorts with MCTD have described a cumulative incidence of renal disease of only 6% at 10 years (5, 6). Besides the renal biopsy results described in MCTD, autopsy studies in these patients have shown intimal thickening of renal arteries resembling scleroderma kidney findings (7).

In this study, we clarify the significance of anti-ribonucleoprotein (RNP) antibody and anti-Smith (anti-Sm) antibody in patients with systemic lupus erythematosus (SLE) or mixed connective tissue disease (MCTD).

Methods

We retrospectively reviewed patients who were positive for anti-RNP antibody, were diagnosed as having SLE or MCTD, and underwent renal biopsy at our nephrology center between January 1990 and December 2014. All patients fulfilled the 1997 American College of Rheumatology (ACR) criteria for classification of SLE (8, 9) or the 2004 Japanese Ministry of Health, Labor and Welfare criteria for classification of MCTD, which are the revised Kasukawa criteria (7). The criteria require and all of the following three major conditions to be fulfilled: (i) at least one of three findings (Raynaud's phenomenon, swollen hands, or pulmonary hypertension), (ii) anti-RNP antibody positivity, and (iii) mixed findings of at least two of connective tissue diseases (SLE, systemic sclerosis, or polymyositis). Clinical characteristics, laboratory data, and histological findings were obtained from the medical records of the patients. All clinical data were measured at the time of renal biopsy. Anti-dsDNA antibody was defined as negative when the titer was ≤ 15 IU/ml. Proteinuria was measured by 24-hour urine collection.

Renal biopsy samples were processed by using standard techniques for light microscopy (LM), immunofluorescence microscopy (IF), and electron microscopy (EM) according to the published methods (10). Three pathologists at our hospital assessed the renal biopsy findings. Renal disease was divided into three patterns: (1) membranous nephropathy (MN) was defined as nephropathy with subepithelial deposits represented by spikes and a bubble-like appearance (Fig. 1a), (2) mesangial and/or subendothelial lesions (MES) were defined as nephropathy with mesangial and/or subendothelial deposits (Fig. 1b), and (3) MN combined with MES was defined as (1) + (2).

For statistical analysis, the chi-square test or Fisher's exact test was employed to compare categorical data between patients with and without the relevant antibodies, while the Mann-Whitney test or Student t-test was used for comparison of continuous variables. The Kruskal-Wallis test was employed to compare nonparametric data among multiple groups. Probability (*P*) values of less than 0.05 were considered significant. All analyses were performed by using Microsoft Office Excel 2016 (Microsoft, WA, USA) and

Statcel4 for Windows software (OMS, Saitama, Japan). This retrospective study was approved by the institutional ethics committee of our hospital.

Results

The 30 patients who were positive for anti-RNP antibody included 25 patients with SLE and 5 with MCTD. Their mean age (SE) was 38.6 (2.5) years, and 26 of the 30 patients (87%) were women. Thirteen patients (43%) were positive for anti-dsDNA antibody and 14 patients (47%) were positive for anti-Sm antibody. In 17 patients (57%), urinary occult blood (U-OB) was positive. The median daily urine protein excretion (range) was 1.1 (0.08–14.6) g and median serum creatinine (range) was 0.8 (0.4–3.7) mg/dL.

The 30 patients were classified into 4 groups based on the combination of anti-dsDNA and anti-Sm positivity/negativity: group A (n = 10) was anti-dsDNA(-) and anti-Sm (-), group B (n = 7) was anti-dsDNA (-) and anti-Sm (+), group C (n = 6) was anti-dsDNA (+) and anti-Sm (-), and group D (n = 7) was anti-dsDNA (+) and anti-Sm (+). Age, sex, U-OB positivity, proteinuria, and serum creatinine showed no significant differences among these 4 groups. However, the levels of CH_{50}, C3, and C4 showed a significant difference between groups A and B or between groups C and D ($P < 0.01$, $P < 0.01$, and $P < 0.01$, respectively). Groups B and D showed significant hypocomplementemia (Fig. 2).

In group A (n = 10), 5 patients had pure MN, 4 patients had MN + MES, and 1 patient showed pure MES. In group B (n = 7), there were 2 patients with MN + MES and 5 patients with pure MES. All 6 patients in group C (n = 6) showed pure MES, while group D (n = 7) included 4 patients with MN + MES and 3 patients showing pure MES.

Comparison between groups A and B showed that anti-Sm antibody (-) status was closely related with normocomplementemia and MN in patients who were anti-RNP antibody (+) and anti-ds-DNA antibody (-), while anti-Sm antibody (+) status was closely related with hypocomplementemia and MES.

Comparison between groups C and D showed that anti-Sm antibody (+) status was closely related to hypocomplementemia, but did not have any significant influence on renal histology.

Among 10 patients in group A, all 5 patients with MCTD were categorized as pure MN, while the 5 patients with SLE were categorized as MN + MES or pure MES. In groups B, C, and D, all 20 patients were classified as having SLE.

Discussion

In SLE, various autoantibodies can be positive, including ANA, anti-dsDNA antibody, anti-Sm antibody, anti-RNP antibody, anti-Ro/SS-A antibody, and anti-La/SS-B antibody. Among them, anti-ds DNA antibody is most commonly associated with lupus nephritis (LN), and the antibody titer is usually correlated with disease activity (11). Anti-Sm antibody has also been reported to show a close relation with renal disease, and this association is stronger when anti-Sm antibody is positive together with anti-dsDNA antibody

(12). Anti-RNP antibody is detected in 25–47% of SLE patients, with high anti-RNP antibody titers being diagnostic of MCTD. Both anti-Sm and anti-RNP antibodies are more important for diagnosis of SLE than for monitoring disease progression, although anti-RNP antibody shows a higher prevalence in patients with Raynaud's phenomenon and is associated with milder renal involvement, while anti-Sm antibody reflects the severity and of renal involvement and renal disease activity (2). Kitridou et al. performed renal biopsy in 10 MCTD patients with renal disease, and reported that six patients (60%) had membranous nephropathy, two (20%) had mesangial glomerulonephritis, one (10%) had local sclerosing glomerulonephritis, and one (10%) had membranoproliferative nephritis (3). In addition, Bennet et al. performed renal biopsy in four of 20 patients with MCTD, and found that three patients had membranous glomerulonephritis (13) .

In SLE, overproduction of type I interferons (IFNs) and autoantibodies targeting nucleic acids has been reported (14). Kirou et al. suggested that activation of the IFN pathway defines a subgroup of SLE patients whose have more severe disease, including more severe renal disease, and increased disease activity, which is reflected by activation of complement and the presence of autoantibodies targeting RNA binding proteins (RBP), i.e., antibodies specific for Ro, U1 RNP, and Sm (15). Lövgren et al. reported that the induction of IFN production by SLE serum was associated with the presence of anti-RBP antibodies with or without anti-DNA antibodies, but not with anti-DNA antibodies alone (16) .

Two distinct mechanisms for the formation of immune deposits in glomerulonephritis have been suggested, which are passive trapping of immune complexes associated with mesangial or subendothelial deposits, or in situ formation of immune complexes at subepithelial, subendothelial, and mesangial sites (17). The latter mechanism might be considered in MCTD patients with concomitant membranous nephropathy because they predominantly have subepithelial deposits. However, the precise immunological processes underlying membranous nephropathy in MCTD patients are still unclear.

In conclusion, the present study indicated that 5 patients out of 10 patients with anti-RNP antibody positivity only showed MN and normocomplementemia. On the other hand, in patients who are positive for both anti-Sm antibody and anti-RNP antibody, the main renal disease is MES with hypocomplementemia. Among patients showing positivity for anti-RNP antibody alone, those with MCTD seem likely to develop pure MN, while those with SLE tend to have MES as well as MN.

The Limitations Of This Study

This retrospective study is made on only 30 patients, but these patients are even rarer than the two rare diseases (SLE and MTCD). The true evaluation is a future problem. This study cannot assess the association of anti-RNP with anything, as all patients had anti-RNP, therefore there is no comparison group without anti-RNP.

Abbreviations

systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), antinuclear antibody (ANA), anti-ribonucleoprotein antibody (anti-RNP antibody), anti-smooth muscle antibody (anti-Sm antibody), anti-double-stranded DNA antibody (anti-dsDNA antibody), estimated glomerular filtration rate (eGFR), high-power field (HPF), light microscopy (LM), immunofluorescence (IF), electron microscopy (EM), International Society of Nephrology/Renal Pathology Society classification (ISN/RPS), periodic acid-methenamine-silver (PAM)

Declarations

Compliance with ethical standards

The authors obtained approval from the institutional review board of Toranomon Hospital (1487), and informed consent was waived by the institutional review board.

Conflict of interest

The authors have declared that no conflict of interest exists.

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Figures

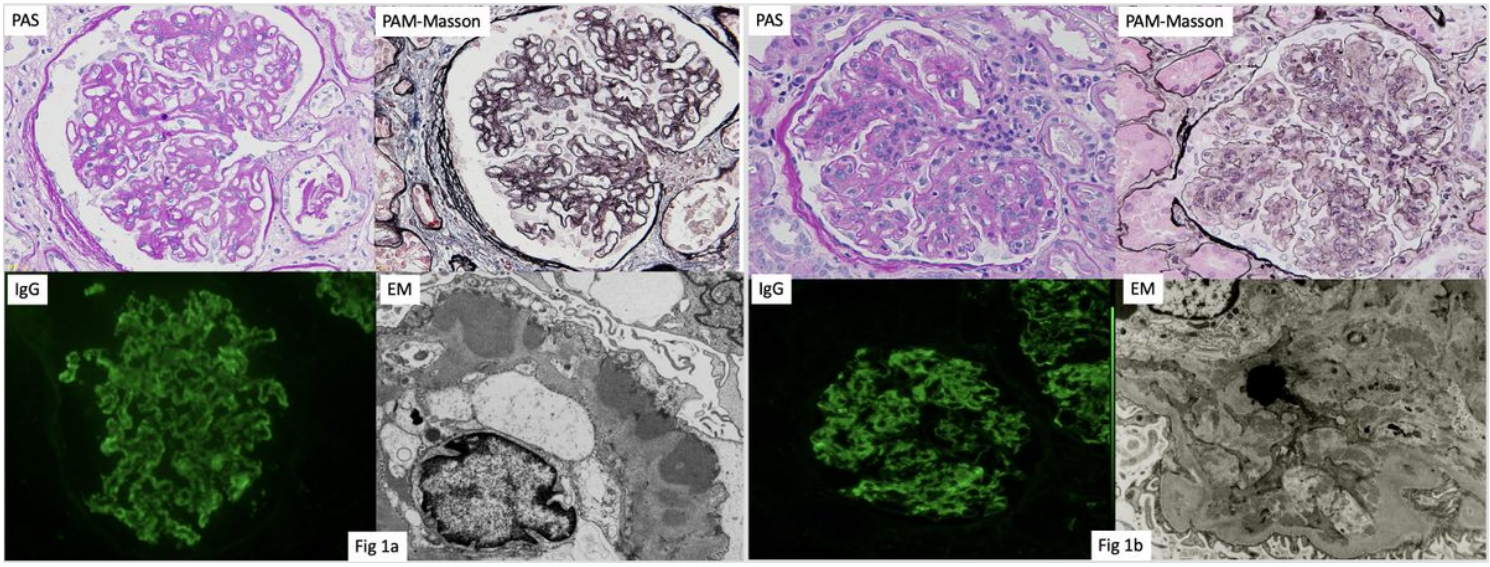
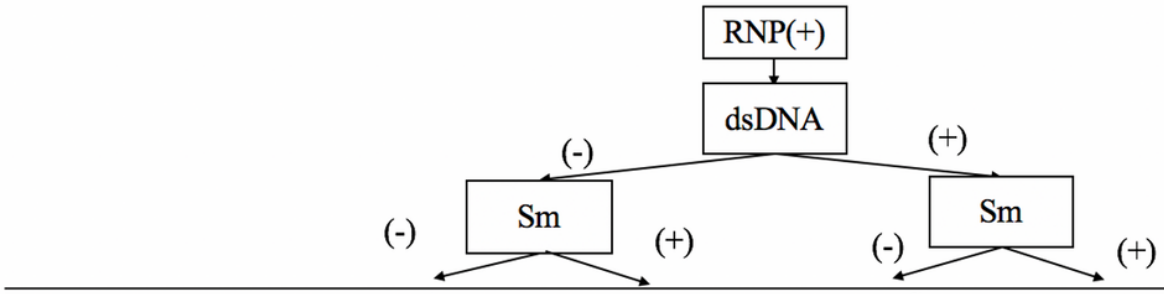


Figure 1

Representative renal biopsy findings. 1-a: A 33-year-old man who was anti-RNP antibody (+), anti-Sm antibody (-), and anti-dsDNA antibody (-). PAS staining shows diffuse thickening of the glomerular basement membrane (GBM), while PAM-Masson staining reveals spike and bubbles of the GBM. In addition, IF shows granular staining for IgG and EM displays subepithelial electron-dense deposits. The diagnosis was Class V (ISN/RPS classification). 1-b: A 48-year-old woman who was anti-RNP antibody (+), anti-Sm antibody (+), and anti-dsDNA antibody (-). PAS and PAM-Masson staining show mesangial proliferation and endocapillary glomerulonephritis. IF reveals mesangial staining for IgG, while EM shows mesangial and subendothelial electron dense deposits. The diagnosis was Class 111 or IV (ISN/RPS classification).



	A (n=10)	B (n=7)	*P*	C (n=6)	D (n=7)	*P*
Age, years (mean±S.E)	42.7 ± 3.5	40.9 ± 6.3	0.788	33.7 ± 3.2	34.9 ± 6.4	0.878
Female, n (%)	8 (80%)	7 (100%)	0.331	5 (83%)	6 (86%)	0.731
U-OB positivity, n (%)	4 (40%)	5 (71%)	0.218	3 (50%)	5 (71%)	0.413
Proteinuria, g/day, median (range)	3.0 (0.14 - 6.5)	0.5 (0.08 - 3.61)	NS	2.1 (0.18 - 14.6)	1.3 (0.18 - 6.5)	NS
Serum Cr, mg/dl, median (range)	0.8 (0.4 - 3.7)	0.8 (0.5 - 1.7)	NS	0.6 (0.5 - 1.3)	0.8 (0.7 - 1.1)	NS
CH50, U/ml, median (range)	38.5 (12 - 52)	19.0 (6 - 37)	< 0.01	30.5 (11 - 50)	8.0 (2 - 19)	< 0.05
C3, mg/dL, median (range)	71.5 (48 - 109)	50.0 (22 - 76)	< 0.05	70.0 (23 - 82)	25.0 (18 - 49)	< 0.05
C4, mg/dL, median (range)	24.5 (3 - 31)	9.0 (2 - 13)	< 0.05	11.5 (8 - 27)	5.0 (3 - 13)	< 0.1
Light microscopy findings						
Pure MN	5 (MCTD)	0		0	0	
MN+MES	4 (SLE)	2 (SLE)		0	4 (SLE)	
Pure MES	1(SLE)	5 (SLE)		6 (SLE)	3 (SLE)	

Figure 2

Comparison among A,B,C and D.