Significance of Antibodies Against the Native Ribosomal P Protein Complex and Recombinant P0, P1, and P2 Proteins in the Diagnosis of Chinese Patients with Systemic Lupus Erythematosus

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Background: This study aimed to evaluate autoantibodies against the native ribosomal P complex (anti-Rib-P_C) and recombinant ribosomal P proteins (anti-Rib-P0, anti-Rib-P1, anti-Rib-P2) for their prevalence, diagnostic relevance and clinical associations in a Chinese cohort with systemic lupus erythematosus (SLE). **Methods:** Anti-Rib-P, anti-dsDNA and anti-Smith antigen (Sm) antibodies were analyzed in sera from 198 patients with SLE, 33 with rheumatoid arthritis, 61 with Sjögren's syndrome and 70 healthy individuals by means of ELISA. **Results:** Antibody prevalences were 29.8% (anti-Rib-P_C), 33.3% (anti-Rib-P0), 42.9% (anti-Rib-P1) and 34.3% (anti-Rib-P2), at a specificity of 99%. Among SLE patients lacking anti-dsDNA and anti-Sm, 27.8% showed positive for at least one of the investigated anti-Rib-P types. The sero-

logical hit rate provided by anti-dsDNA/anti-Sm detection (72.7%) was increased upon parallel testing for anti-Rib-P_C (77.3%) or anti-Rib-P0/P1/P2 (80.3%). Anti-Rib-P positivity was associated with disease activity, neuropsychiatric events, lupus nephritis, skin rash, lymphocytopenia, increased erythrocyte sedimentation rates, decreased complement C3/C4 and elevated IgA/IgG levels. **Conclusion:** Based on these results, antibodies against ribosomal P proteins are important complementary parameters to anti-dsDNA and anti-Sm, and should be considered for inclusion in the classification criteria for SLE. The diagnostic value of anti-Rib-P0/P1/P2 is diagnostically superior to that of anti-Rib-P_C. *J. Clin. Lab. Anal.* 27:87–95, 2013. © 2013 Wiley Periodicals, Inc.

Key words: antiribosomal P protein antibody; autoimmunity; ELISA; Rib-P; SLE

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by the presence of a variety of autoantibodies. Antiribosomal P protein antibodies (anti-Rib-P) are regarded as specific for SLE, with a prevalence of 10–40% in SLE patients in contrast to their rarity in healthy subjects and other autoimmune diseases (1). Unlike antibodies against double-stranded DNA (dsDNA) or Smith antigen (Sm), anti-Rib-P is not among the SLE classification criteria of the American College of Rheumatology (ACR) (2, 3). The wide range of anti-Rib-P prevalence rates is mainly due to methodological factors (antigen, detection method) (4) and different eth-

nic backgrounds. For Asian patients, higher prevalences have been reported compared to Afro-Americans and Caucasians (5–10). Reports about clinical associations of

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anti-Rib-P antibodies are in part controversial, including disease activity (10–15), neuropsychiatric symptoms (6,16–23), lupus nephritis (12,24–26), hepatic involvement (26–28), skin manifestations (11), juvenile SLE (12, 29), and others (1,30).

The antibodies recognize three specific ribosomal phosphoproteins designated P0 (38 kDa), P1 (19 kDa), and P2 (17 kDa). In the 60S subunit of ribosomes, these proteins are organized in a pentameric complex containing one P0 monomer and two P1/P2 dimers. This complex, together with the 28S rRNA, constitutes the major part of a GTPase domain, which is active during the elongation step of protein translation (31). The C-terminal 22 amino acids (C22) are shared by all three Rib-P proteins and contain the immunodominant epitope (32, 33). Besides, non-C-terminal epitopes have been described (34, 35).

Several studies have addressed the diagnostic value of anti-Rib-P. However, a comprehensive analysis on the clinical associations of antibodies against the ribosomal P complex (anti-Rib-P_C) and its three subunits (anti-Rib-P0, anti-Rib-P1, and anti-Rib-P2) has so far been reported only once for a Caucasian SLE cohort (36). Following up on this, the present study was designed to evaluate the diagnostic performance of these antibodies as well as their correlations with clinical manifestations and laboratory parameters in a large cohort of Chinese SLE patients.

MATERIALS AND METHODS

Patient Characteristics

Serum samples were collected from 198 SLE patients (181 women, 17 men; mean age 37 years, range from 14 to 78 years) attending the Department of Rheumatology and Immunology, People's Hospital of Peking University Medical School, China. The mean disease duration was 6.3 ± 5.1 years. The patients satisfied the revised and updated ACR criteria for SLE (2, 3). A similar number of controls ($n = 164$) was chosen from the same hospital. The control group comprised two subgroups, one was the disease control group ($n = 94$) consisting of 33 patients with rheumatoid arthritis (RA) and 61 patients with Sjögren's syndrome (SS), all of which fulfilled the diagnostic criteria for RA and SS (37, 38). The second control subgroup was composed of 70 healthy individuals.

At the time of diagnosis, clinical examination and a routine laboratory evaluation of each patient were performed. Meanwhile, patient sera were obtained by centrifugation after blood coagulation. Sera were kept at -80°C and thawed only once for antibody measurements.

Clinical features of SLE patients were defined and recorded according to the ACR criteria (2, 3), including lupus nephritis, vasculitides, skin rash, photosensitivity,

oral ulcers, arthralgia, Raynaud's phenomenon, alopecia, myalgia, and fever. Presence, type, and severity of neuropsychiatric SLE (NPSLE) manifestations were determined according to the 1999 ACR nomenclature and case definition system for NPSLE (39). Disease activity was quantified at the time of diagnosis using the SLE disease activity index (SLEDAI) score (40). Additionally, routine examination of blood, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum levels of complement C3, and C4, IgA, IgG, IgM, white blood cell count, lymphocytopenia and thrombocytopenia was performed for all study subjects. The study was conformed to the standards set by the Declaration of Helsinki.

Detection of Autoantibodies by ELISA

Full-length recombinant Rib-P0, Rib-P1, and Rib-P2 were expressed from human cDNA in Sf9 cells using the baculovirus system. Microtiter plates were coated with the recombinant proteins (1 $\mu\text{g}/\text{ml}$ in PBS) overnight at 4°C , washed with PBS-0.05% (v/v) Tween-20 and blocked for two h with PBS-0.1% (w/v) casein. After washing, sera diluted 1:200 in PBS containing 0.1% (w/v) casein were incubated for 30 min at room temperature. Bound antibodies were detected using antihuman IgG peroxidase conjugate and stained with tetramethylbenzidine (EUROIMMUN, Lübeck, Germany). The optical density was read at 450 nm using an automated spectrophotometer (Spectra Mini, Tecan, Crailsheim, Germany). Receiver operating characteristics (ROC) curve analysis was used for analyzing the assays' diagnostic accuracy, selection of cut-off values and determination of test characteristics at predefined specificities.

The anti-Rib-P ELISA based on native ribosomal P protein complex (Rib-P_C), anti-dsDNA-NcX ELISA, and anti-Sm ELISA are commercial assays (EUROIMMUN) and were performed according to the manufacturer's instructions using cut-off values optimized by ROC curve analysis (anti-Rib-P_C) or as recommended by the manufacturer (anti-dsDNA, anti-Sm).

Statistical Analysis

Results were evaluated using SPSS 13.0 (SPSS Inc., Chicago, IL), SigmaPlot 10.0 (SSI, San Jose, CA), and GraphPad QuickCalcs (GraphPad Software Inc., La Jolla, CA). The significance of differences between two groups was assessed by Student's *t*-test for continuous variables and chi-square test for categorical variables, as appropriate. The correlation coefficients (*r*) between the anti-Rib-P titer and SLEDAI scores were calculated by Spearman's rank correlation. *P* values below 0.05 were considered statistically significant.

TABLE 1. Overall Test Characteristics of Anti-Rib-P ELISA in ROC Curve Analysis^a

Test variables	Anti-Rib-P _C	Anti-Rib-P0	Anti-Rib-P1	Anti-Rib-P2
Area under the curve (AUC)	0.759	0.844	0.864	0.655
95% confidence interval	0.713–0.804	0.805–0.884	0.827–0.901	0.598–0.711
Maximal sum of sensitivity and specificity (Cut-off ^b)	144.9% (5.66)	153.8% (0.04)	159.2% (0.05)	134.2% (0.18)
Sensitivity at 99% specificity (Cut-off ^b)	29.8% (27.85)	33.3% (0.14)	42.9% (0.12)	34.3% (0.19)

^aROC curve analysis is based on antibody determinations in sera from 198 patients with systemic lupus erythematosus, 94 disease controls (33 patients with rheumatoid arthritis, 61 patients with Sjögren's syndrome), and 70 healthy controls.

^bCut-off values are presented in relative units/ml for anti-Rib-P_C, and in optical densities (OD₄₅₀) for anti-Rib-P0, anti-Rib-P1, and anti-Rib-P2.

RESULTS

Overall Diagnostic Performance of Antiribosomal P Protein ELISA

To analyze and compare the diagnostic performance of the four ELISA systems based on native Rib-P_C or recombinant Rib-P0, Rib-P1, and Rib-P2, ROC curve analysis was performed by antibody measurements in sera from 198 SLE patients and 164 controls. According to the area under the curve values and maximum sum of sensitivity and specificity, Rib-P1 showed the highest efficiency in distinguishing between antibody-positive and -negative specimens, followed by Rib-P0, Rib-P_C, and Rib-P2 (Table 1, Fig. 1). At a predefined specificity of 99%, the

assays' sensitivities were 42.9% (Rib-P1), 34.3% (Rib-P2), 33.3% (Rib-P0), and 29.8% (Rib-P_C), respectively (Table 1). Among the controls, only one RA patient had a positive result of anti-Rib-P_C, and one SS patient showed weakly positive reactivity against Rib-P0, Rib-P1, and Rib-P2 (Fig. 2).

Diagnostic Benefit of Antiribosomal P Protein Antibody Determination

Antibodies against dsDNA and Sm were found in 129/198 (65.2%) and 65/198 (32.8%) of SLE patients, respectively. A total of 144/198 patients were positive for either or both of these antibodies, resulting in combined hit rate of 72.7% (Fig. 3).

Among all SLE patients, 54/198 (27.3%) were lacking both anti-dsDNA and anti-Sm antibodies. Detection of anti-Rib-P reactivity in these patients at high specificity levels would substantiate the diagnostic benefit of anti-Rib-P testing in suspected cases of SLE. Using cut-off values ensuring a specificity of 99%, 15/54 (27.8%) anti-dsDNA/anti-Sm negative patients were reactive against at least one of the four investigated Rib-P antigens. In particular, antibodies against Rib-P_C, Rib-P0, Rib-P1, and Rib-P2 were found in 9 (16.7%), 13 (24.1%), 13 (24.1%), and 14 (25.9%) of the 54 cases, respectively (Table 2).

The parallel determination of anti-dsDNA, anti-Sm, and anti-Rib-P_C resulted in a total hit rate of 77.3%, whereas additional testing of reactivity against Rib-P0, Rib-P1, and Rib-P2 further increased the hit rate up to 80.3% (Fig. 3).

Frequency of Reactivity against the Native Ribosomal P Complex and Recombinant Ribosomal P Subtype Proteins

Out of all studied SLE patients, 139/198 (70.2%) were anti-Rib-P_C negative. Among these anti-Rib-P_C negative individuals, 37 (26.6%) had antibodies against at least one of recombinant antigens (Rib-P0, Rib-P1, and/or

Fig. 1. Comparative receiver operating characteristics (ROC) curve analysis of anti-Rib-P ELISA. ROC curves were analyzed and compared using the SigmaPlot 10.0 software. Analysis results are summarized in Table 1. There were significant differences in the area under the curve (AUC) values between any of the anti-Rib-P ELISA ($P < 0.0001$), except between anti-Rib-P0 and anti-Rib-P1 ($P = 0.257$).

Fig. 2. ELISA determination of antiribosomal P protein antibodies in sera from patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome (SS), and from healthy blood donors (HBD). ELISA were based on (A) native ribosomal P protein complex (Rib-P_C) or on the recombinant ribosomal P subtype proteins (B) Rib-P₀, (C) Rib-P₁, and (D) Rib-P₂. Data points represent relative units (RU)/ml or optical densities (OD) measured at 450 nm. Dashed lines indicate cut-off values at 99% specificity according to ROC curve analysis (see Table 1). The prevalence of antibodies is indicated in percent for each cohort. Numbers in brackets represent positive and total cases, respectively.

Rib-P₂), with anti-Rib-P₁ occurring most frequently (23.0%) (Table 2, Fig. 4A). Reversely, only 6/198 (3.0%) patients were found anti-Rib-P_C positive, but not reactive against Rib-P₀, Rib-P₁, or Rib-P₂.

Moreover, among the 15 SLE patients which were anti-dsDNA/anti-Sm negative and anti-Rib-P positive, nine showed positive results in all four anti-Rib-P assays. In the remaining six cases, serum reactivity was exclusively directed against the recombinant Rib-P proteins, but not against the native Rib-P_C (Fig. 4B).

Correlation Between Antiribosomal P Protein Antibodies and Clinical Manifestations in SLE

In order to reveal clinical associations of autoantibodies against Rib-P_C, Rib-P₀, Rib-P₁, and Rib-P₂, the frequency of several clinical features in antibody-positive

SLE patients was compared to that in antibody-negative patients.

As shown in Table 3, the prevalence of neuropsychiatric events (NPSLE) was significantly higher in patients with than without antiribosomal reactivity, with statistical differences being more evident for anti-Rib-P_C, anti-Rib-P₀, and anti-Rib-P₂ antibodies ($P < 0.01$) compared to anti-Rib-P₁ ($P < 0.05$). Among 198 SLE patients, 26 (13.1%) patients were found with NPSLE manifestations. Of 19 different clinical manifestations of NPSLE, only cerebrovascular disease, headache, seizure disorders, cognitive dysfunction, mood disorder, psychosis, neuropathy, and polyneuropathy were observed. However, we found no significant correlation of neural symptoms with anti-Rib-P_C, P₀, P₁, or P₂, respectively ($P > 0.05$).

A statistical association was also observed between the presence of lupus nephritis and anti-Rib-P_C ($P < 0.05$).

Fig. 3. Hit rates for diagnosing systemic lupus erythematosus (SLE). Sera from 198 lupus patients were analyzed for antibodies against the indicated antigens by means of ELISA. Positivity was based on cut-off values recommended by the manufacturer (anti-dsDNA, anti-Sm) or cut-off values ensuring a specificity of 99% according to ROC curve analysis (anti-Rib-P; see Table 1). Bars indicating the hit rate for a group of antibodies represent all patients positive for at least one of the indicated parameters. Numbers in brackets represent positive and total cases, respectively.

In addition, the frequency of skin rashes was higher in patients with anti-Rib-P0 or anti-Rib-P1 positivity ($P < 0.05$). Regarding the involvement of vasculitides, photosensitivity, oral ulcers, arthralgia, Raynaud's phenomenon, alopecia, myalgia, and fever, there were no significant differences between the antibody-positive and -negative groups.

Furthermore, we found that the titers of anti-Rib-PC and subtype antibodies were strongly clustered with the clinical disease activity as measured by the SLEDAI ($P < 0.01$). Anti-Rib-PC antibodies correlated slightly better with the SLEDAI scores ($r = 0.393$) than anti-Rib-P0 ($r = 0.379$), anti-Rib-P1 ($r = 0.364$), and anti-Rib-P2 ($r = 0.370$).

Fig. 4. Antiribosomal P protein reactivity in lupus patients. (A) Prevalence of anti-Rib-P0, anti-Rib-P1, and anti-Rib-P2 antibodies in 139 anti-Rib-PC negative lupus patients. (B) Prevalence of anti-Rib-PC, anti-Rib-P0, anti-Rib-P1, and anti-Rib-P2 in 54 lupus patients negative for anti-dsDNA and anti-Sm.

Correlation Between Antiribosomal P Protein Antibodies and other Laboratory Parameters in SLE

ESR elevation was found to be associated with anti-Rib-P0 ($P < 0.05$) and anti-Rib-P1 ($P < 0.01$) positivity. The frequency of low serum complement C3 correlated with the presence of anti-Rib-P1 ($P < 0.05$). Moreover, the prevalence of low serum complement C4 and of elevated IgG was significantly higher in all anti-Rib-P-positive groups compared to the negative groups ($P < 0.01$, $P < 0.05$). Clustering of IgA elevation was high in patients with anti-Rib-P2 ($P < 0.05$), whereas lymphocytopenia was associated with reactivity against Rib-P0 ($P < 0.05$). Regarding other serological parameters, the prevalence of positive anti-dsDNA titers correlated significantly with anti-Rib-P1 positivity ($P < 0.01$), whereas positive anti-Sm titers were more frequent in all anti-Rib-P-positive groups ($P < 0.01$, $P < 0.05$). The prevalence of elevated CRP levels, increased IgM, leukopenia, and thrombocytopenia was similar in the antibody-positive and -negative groups; statistical testing confirmed the lack of significant differences (Table 3).

TABLE 2. Prevalence of Anti-Rib-P Antibodies Detected by ELISA in Lupus Patients Lacking Specific Autoantibodies

Antibodies	<i>n</i>	Anti-Rib-PC positive (%)	Anti-Rib-P0 positive (%)	Anti-Rib-P1 positive (%)	Anti-Rib-P2 positive (%)	Anti-Rib-PC/P0/P1/P2 positive (%) ^a	Anti-Rib-P0/P1/P2 positive (%) ^a
Anti-dsDNA negative	69	17 (24.6)	19 (27.5)	21 (30.4)	20 (29.0)	25 (36.2)	23 (33.3)
Anti-Sm negative	133	24 (18.0)	36 (27.1)	44 (33.1)	38 (28.6)	50 (37.6)	49 (36.8)
Anti-dsDNA/anti-Sm double-negative ^b	54	9 (16.7)	13 (24.1)	13 (24.1)	14 (25.9)	15 (27.8)	15 (27.8)
Anti-Rib-PC negative ^b	139	-	16 (11.5)	32 (23.0)	18 (12.9)	-	37 (26.6)

^aSera positive for at least one of the indicated anti-Rib-P types.

^bRefer to Figure 4 for detailed results.

TABLE 3. Prevalence of Clinical Manifestations, Laboratory Parameters and Autoantibodies in 198 Lupus Patients With and Without Anti-Rib-P Antibodies

	Anti-Rib-P _C			Anti-Rib-P ₀			Anti-Rib-P ₁			Anti-Rib-P ₂		
	Negative <i>n</i> = 139	Positive <i>n</i> = 59	<i>P</i> value ^a	Negative <i>n</i> = 132	Positive <i>n</i> = 66	<i>P</i> value ^a	Negative <i>n</i> = 113	Positive <i>n</i> = 85	<i>P</i> value ^a	Negative <i>n</i> = 130	Positive <i>n</i> = 68	<i>P</i> value ^a
Clinical features												
NPSLE (%)	8.6	23.7	0.004	7.6	24.2	0.001	8.0	20.0	0.013	7.7	23.5	0.002
Lupus nephritis (%)	47.5	64.4	0.029	47.7	62.1	0.056	47.8	58.8	0.124	47.7	61.8	0.060
Vasculitides (%)	5.0	6.8	0.624	6.1	4.5	0.661	5.3	5.9	0.862	6.2	4.4	0.611
Skin rash (%)	42.4	57.6	0.050	41.7	57.6	0.027	39.8	56.5	0.020	42.3	55.9	0.069
Photosensitivity (%)	18.0	15.3	0.641	18.9	13.6	0.351	19.5	14.1	0.323	20.0	11.8	0.145
Oral ulcers (%)	15.8	15.3	0.919	18.9	9.1	0.072	18.6	11.8	0.267	17.7	11.8	0.276
Arthralgia (%)	32.4	37.3	0.504	32.6	36.4	0.595	32.7	35.3	0.707	32.3	36.8	0.529
Raynaud's phenomenon (%)	14.4	18.6	0.451	16.7	13.6	0.580	16.8	14.1	0.605	16.9	13.2	0.498
Alopecia (%)	28.8	37.3	0.238	28.8	36.4	0.279	27.4	36.5	0.175	28.5	36.8	0.232
Myalgia (%)	6.5	6.8	0.937	8.3	3.0	0.156	7.1	5.9	0.736	6.9	5.9	0.779
Fever (%)	25.2	28.8	0.595	28.0	22.7	0.424	26.5	25.9	0.916	27.7	23.5	0.527
SLEDAI, mean	9.0	14.9	<0.0001	9.3	13.8	<0.0001	9.1	13.1	<0.0001	9.2	13.9	<0.0001
Laboratory parameters												
ESR↑ (%)	65.5	78.0	0.082	63.6	80.3	0.017	60.2	81.2	0.002	64.6	77.9	0.054
CRP↑ (%)	33.8	27.1	0.355	31.8	31.8	1.000	32.7	30.6	0.747	31.5	32.4	0.907
C3↓ (%)	79.9	89.8	0.089	80.3	87.9	0.183	77.9	89.4	0.033	80.8	86.8	0.288
C4↓ (%)	64.7	84.7	0.005	65.9	80.3	0.036	61.1	83.5	0.001	65.4	80.9	0.023
IgA↑ (%)	18.0	22.0	0.508	16.7	24.2	0.202	15.9	23.5	0.179	14.6	27.9	0.024
IgG↑ (%)	36.0	57.6	0.005	27.3	57.6	<0.0001	36.3	50.6	0.044	35.4	55.9	0.006
IgM↑ (%)	5.0	5.1	0.989	3.8	7.6	0.251	4.4	5.9	0.643	5.4	4.4	0.767
Leukopenia (%)	28.1	40.7	0.081	29.5	36.4	0.332	28.3	36.5	0.223	29.2	36.8	0.280
Lymphocytopenia (%)	48.9	54.2	0.494	45.5	60.6	0.044	49.6	51.8	0.759	46.9	57.4	0.163
Thrombocytopenia (%)	19.4	23.7	0.494	17.4	27.3	0.107	17.7	24.7	0.228	17.7	26.5	0.148
Autoantibodies												
Anti-dsDNA positive (%)	62.6	71.2	0.246	62.1	71.2	0.206	57.5	75.3	0.009	62.3	70.6	0.246
Anti-Sm positive (%)	21.6	59.3	<0.0001	26.5	45.5	0.008	21.2	48.2	<0.0001	26.9	44.1	0.014

^a*P* values were calculated using chi-square test, except for SLEDAI, which was examined by *t*-test. Bold *P* values indicate statistically significant differences.

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NPSLE, neuropsychiatric systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index.

DISCUSSION

It has long been known that some SLE patients' sera contain anti-Rib-P antibodies (1, 8, 41), but the correlation between anti-Rib-P subtype antibodies and SLE has not been well examined. The current study addressed this issue by evaluating the diagnostic efficiency as well as the clinical and laboratory associations of antibodies against Rib-P_C, Rib-P₀, Rib-P₁, and Rib-P₂. Our analyses were performed in a cohort of Chinese SLE patients, thus contrasting with similar studies based on Caucasian cohorts (4, 36).

The study revealed antibody prevalences in the range of 29.8% (anti-Rib-P_C) to 42.9% (anti-Rib-P₁), at a specificity level of 99%. These values are in accordance with previous studies reporting anti-Rib-P positivity in 28–42% of Asian SLE patients, but slightly higher than shown for European, African, or American populations (6–35%)

(5–10). Interestingly, Barkhudarova et al. found prevalence rates of 14.3% (anti-Rib-P_C), 22.0% (anti-Rib-P₀), 10.7% (anti-Rib-P₁), and 14.9% (anti-Rib-P₂) among 163 German SLE patients at 99% specificity, employing the same setup as in the present study (36). Thus, ethnicity-based differences between patient cohorts are likely to account for the divergence of prevalence data. In addition, the multitude of anti-Rib-P detection systems and absence of standardization have to be considered (4, 8). Among others, gel immunoprecipitation, indirect immunofluorescence, radioimmunoassay, immunoblot, and ELISA have been used for anti-Rib-P detection, the latter two being the most popular formats today. These assays are based on native Rib-P, recombinant proteins, or synthetic peptides. Epitope-mapping studies revealed that the C-terminus, which is shared by the three Rib-P subtypes, contains a major epitope recognized by the antibodies (32, 33, 42). However, assays using C-terminal antigen (e.g., C22) often

revealed lower sensitivities than assays using full-length protein (8,43).

The diagnostic benefit of an additional serological marker for SLE would be reflected by the potential to identify SLE patients negative for anti-dsDNA and anti-Sm. We found 27.8% of anti-dsDNA and anti-Sm negative patients positive for at least one of the investigated anti-Rib-P types at a specificity of 99%. The hit rate provided by anti-dsDNA and anti-Sm (72.7%) was increased by 4.6% upon additional testing for anti-Rib-P_C, and by 7.6% upon additional testing for anti-Rib-P0/P1/P2. Together with similar findings by other studies (36,43), this substantiates the relevance of anti-Rib-P serology in suspected cases of SLE.

Our results indicate that ELISA coated with any of the three recombinant Rib-P proteins have a higher diagnostic efficiency than ELISA based on native Rib-P_C. This applies to the whole SLE cohort including the subgroup of anti-dsDNA/anti-Sm negative patients. In particular, the anti-Rib-P1 assay showed superior performance in ROC curve analysis, suggesting that Rib-P1 may be more suitable for diagnosing SLE than Rib-P_C, Rib-P0, and Rib-P2. The observation that anti-Rib-P2 was slightly more frequent than anti-Rib-P0 and anti-Rib-P1 among the anti-dsDNA/anti-Sm negative patients should be of minor importance because of the low case number. However, Barkhudarova et al. highlighted Rib-P0 as the optimal antigen, presumably reflecting demographic differences of their Caucasian cohort (36). Other studies recommended to use a mixture containing all three (recombinant) Rib-P proteins in the molar ratio as they occur in their native pentameric complex P0(P1/P2)₂ since this was found to be diagnostically superior to purified native antigen, Rib-P0, and synthetic C22 (8,43). In view of all these results, the determination of reactivity against the three recombinant Rib-P subtype proteins seems to be preferable with regard to utmost sensitivity.

Moreover, the finding that 26.6% of 139 anti-Rib-P_C negative patients showed positive for at least one of the anti-Rib-P subtype antibodies, does not only indicate that anti-Rib-P0, anti-Rib-P1, and anti-Rib-P2 are valuable in diagnosing SLE. It also suggests that some epitopes could be specific to antigenic sites of individual subtype proteins, but might not be accessible in the native pentameric complex. Besides, several sera showed exclusive reactivity against only one of these proteins, suggesting that some epitopes are located outside the common C-terminal sequence. This corresponds to previous reports on the presence of more N-terminally located immunoreactive sequences (34,35,43). Only six of 198 SLE patients showed anti-Rib-P_C positivity while antibodies against the Rib-P subtypes were not detectable, presumably due to the presence of conformational determinants.

Among the many clinical manifestations of SLE, central nervous system involvement is of prognostic importance, leading to neuropsychiatric syndromes including anxiety, cognitive dysfunction, myelopathy, psychosis, seizure disorders, and others. Several autoantibodies may contribute to the pathogenesis of these complications, but the ACR committee for NPSLE judged most of them as investigational except testing for antiphospholipid antibodies and, in limited circumstances, anti-Rib-P (39). Bonfa et al. first assessed the association between anti-Rib-P and neuropsychiatric manifestations in 20 patients with psychosis secondary to SLE (41). Despite substantial subsequent investigations, this issue is still controversial (6,16–23). In a recent review, 12 of 17 studies confirmed an association between elevated titers of anti-Rib-P and psychiatric symptoms (23). Our study revealed that all four investigated antibody types were correlated significantly with NPSLE, with syndromes of the central nervous system occurring more frequently than of the peripheral nervous system. The neural manifestation disappeared during our treatment of lupus, indicating that neuropsychiatric features are due to the lupus process. Presumably, the discrepant findings obtained in various studies may be associated with the low number of patients being investigated, demographics of the patient cohort and/or different technical features of the test systems. A large cohort, follow-up and multicenter study might be interesting.

Remarkably, our anti-Rib-P_C positive patients had a significantly higher prevalence of lupus nephritis compared to anti-Rib-P_C negative patients (it remains unclear why this association does not include reactivity against Rib-P0, Rib-P1, and Rib-P2). This finding confirms previous studies showing presence of anti-Rib-P in the majority of nephritic SLE patients with titers decreasing during disease remission, and suggesting an immunopathogenic role of the antibodies in lupus nephritis (12,24–26). Thus, SLE patients at higher risk of kidney involvement may be identified by positive anti-Rib-P titers.

Another finding of this study was that the frequency of reduced lymphocyte levels was significantly elevated in anti-Rib-P0 positive SLE patients. This association has been reported before (36) and is in line with studies classifying anti-Rib-P as subtypes of antilymphocyte antibodies leading to penetration, apoptosis, and depletion of T cells (44–46).

Moreover, our data gave evidence for an association of anti-Rib-P with disease activity. Noteworthy, a recent multicenter evaluation reported a relationship between anti-Rib-P (C22) and SLEDAI scores only for a subgroup of participants and concluded that this serological parameter is likely not appropriate for measuring global disease activity (14). Considering that there are other reports substantiating or refuting this association (10–13,15,36), further prospective large-scale studies may clarify this issue.

Summarizing, anti-Rib-P antibodies are highly specific SLE markers which enable the identification of several anti-dsDNA and anti-Sm negative patients, thus demanding consideration as an additional classification criterion for SLE. This study investigated the prevalence of antibodies to Rib-P_C, Rib-P0, Rib-P1, and Rib-P2 in Chinese SLE patients for the first time, and revealed best diagnostic accuracy in diagnosing SLE for the anti-Rib-P1 assay. The highest hit rates for SLE were obtained by parallel testing of anti-dsDNA, anti-Sm, and anti-Rib-P0/P1/P2. There was evidence for anti-Rib-P positivity being associated with lupus activity, neuropsychiatric, kidney and skin manifestations, lymphocytopenia, increased ESR rates, C3/C4 reduction, and IgA/IgG elevation.

Conflicts of Interest:

YS, XJW, XSC, DHW, XFC, SS, and CD are employees of EUROIMMUN AG, Lübeck, Germany. WST and WSC are board members of EUROIMMUN AG. EUROIMMUN AG develops, produces, and distributes reagents and equipments for medical laboratory diagnostics.

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