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Rheumatologie und Klinische Immunologie
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DISSERTATION

Diagnostic value and clinical laboratory associations of antibodies against recombinant ribosomal P0, P1, P2 proteins and their native heterocomplex in a Caucasian cohort with systemic lupus erythematosus

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Charite Universitätsmedizin Berlin

von Barkhudarova Fidan
aus Baku (Aserbaidschan).

Referee: 1. Prof. Dr. med. F. Hiepe

2. Prof. Dr. med. G. Keyßer

3. Priv.-Doz. Dr. med. K. Conrad

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Summary

Objective: Aim of this study were to assess a) the diagnostic value of autoantibodies against recombinant ribosomal P0, P1, P2 proteins and their native heterocomplex in SLE, b) their prognostic value, c) clinicolaboratory associations.

Patients and methods:

Serum samples were obtained from patients with systemic lupus erythematosus (SLE; n=163), rheumatoid arthritis (RA; n=90), systemic sclerosis (SSc; n=66), primary Sjögren's syndrome (pSS; n=54), and healthy donors (n=100). Disease activity of SLE patients was characterized using the activity index SLEDAI-2000. Serum autoantibodies to recombinant ribosomal P0, P1, P2 proteins and their native heterocomplex were measured by ELISA. Test results were correlated to ACR criteria, SLEDAI-2000, laboratory data and medications of all SLE patients.

Results: Sensitivities of 22.0% for anti-RibP_R0, 14.9% for anti-RibP_R2, 14.3% for anti-RibP_NH and 10.7% for anti-RibP_R1 autoantibodies were obtained at a specificity of 99%. Anti-RibP_R0 has the best diagnostic value among all anti-Rib autoantibodies. 10% of anti-Sm and anti-dsDNA negative sera were positive for anti-RibP_R0 at a specificity of 100%. Anti-RibP_R0 positive patients had significantly lower lymphocyte counts, and anti-RibP_R1 positive patients had higher γ-glutamyltransferase (GGT) levels than their negative counterparts. No specific damage occurred in anti-RibP positive lupus patients compared to a group of age-, gender- and nephritis-matched anti-RibP negative SLE patients within 3 years.

Conclusions: The measurement of autoantibodies against ribosomal P proteins improves the diagnosis of SLE and should therefore be considered in upcoming criteria for the diagnosis or classification of SLE. Lymphocytopenia is associated with high titers of anti-RibP_R0, and elevated GGT levels with high titers of anti-RibP_R1. Anti-RibP autoantibodies have not shown any evidence for a damage prediction in SLE.

Inhaltsverzeichnis

1 Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease, which is characterized by multiorgan involvement and by the production of autoantibodies directed mainly against nuclear proteins and nucleic acids [1, 2]. However, antibodies against ribonucleoproteins, such as anti-ribosomal P proteins or anti-Sm (Smith), have been reported to be specific for SLE as well [2, 3]. In contrast to anti-Sm and anti-dsDNA antibodies, anti-ribosomal P protein antibodies are not included in the current American College of Rheumatology (ACR) classification criteria for SLE [4, 5]. Notably, antibodies to phospholipids are included in the ACR criteria, although they are less specific for the disease [4, 5].

The human native Rib-P antigen consists of one copy of P0-anchor (M_{P0}=38 kDa) and two copies of P1/P2 heterodimers (M_{P1}=19 kDa¸ M_{P2}=17 kDa), forming a pentameric complex that is located within the 60S ribosomal subunit and is involved in the elongation step of protein translation [3]. The constituents of that pentamer have a common immunodominant epitope at the carboxyl terminus [6], which can cross-react with anti-ribosomal P0, P1, P2 antibodies. The ribosome-free forms of all 3 P proteins were reported to exist in the cytoplasm as well [6, 7]. Interestingly, the P0-like protein is also detectable in the plasma membranes of hepatocytes, lymphocytes and other cells [8-11].

The prevalence of anti-ribosomal antibodies varies widely depending on the patient's ethnicity, disease activity and antigens used in detection systems [12-14]. Anti-ribosomal P protein antibodies have been associated with a number of clinical presentations including short disease duration [15], rash [16, 17], lymphocytopenia [18] and lupus hepatitis [11, 19-23]. Ohira et al. [22] showed that patients with lupus hepatitis have significantly higher and more frequent levels of aRibPR0 than patients with autoimmune hepatitis. There are also contradictory reports with juvenile onset SLE [24-27], neuropsychiatric SLE [3, 28, 29], lupus nephritis class V [3, 27, 30], high disease activity [15, 16, 26, 31] and low levels of complement component 3 or 4 [16, 17, 22, 32].

A comparative investigation of the clinical laboratory associations (including diagnostic and prognostic value) of antibodies against recombinant ribosomal P0, P1, P2 protein has never been conducted. Thus, the goal of this study was to evaluate the diagnostic value of anti-RibP_NH, anti-RibP_R0, anti-RibP_R1 and anti-RibP_R2 for SLE and to analyse their associations with disease features and prognosis.

2 Patients und Methods

2.1 Study participants

Overall 479 serum samples were collected from the following groups:

a) patients with SLE (n=163), who met the ACR 1982 revised criteria for the classification of SLE [4],

b) patients with systemic sclerosis (SSc, n=66), who fulfilled ACR criteria of scleroderma 1980 [33],

c) patients with primary Sjogren's syndrome (pSS, n=54), meeting the preliminary EULAR criteria of Vitali et al. [34],

d) patients with rheumatoid arthritis (RA, n=90), who fulfilled the ACR 1987 revised criteria for the classification of rheumatoid arthritis [35],

e) healthy donors (HD, n=100).

SLE activity was calculated in 101 patients using the systemic lupus erythematosus disease activity index 2000 (SLEDAI-2000) [36-38]: 6 of them had no activity (SLEDAI=0), 35 were mildly active (0<SLEDAI≤5), 41 had moderate disease activity (5<SLEDAI≤10), 14 were highly active (10<SLEDAI≤20), and 5 had very high activity (SLEDAI>20). If the age at diagnosis was 18 years or younger according to the Pediatric Rheumatology International Trials Organization [39], the onset was categorized as a juvenile. Twenty-four (14.7%) patients with juvenile onset and 139 (85.3%) patients with adult onset SLE were studied. Disease damage was assessed with the standard protocol of SLICC, Systemic Lupus International Collaborative Clinics [40, 41], and WDS, weighted damage score [40]. All patients were recruited from the out- and in-patient facilities of the Departments of Rheumatology and Clinical Immunology, Charité University Hospital, Berlin, Germany. The Ethics Committee of the Medical Faculty of Charité approved the study, and written informed consent was obtained from all subjects. Sera from healthy donors were enlisted in cooperation with the University of Lübeck, Germany. Written informed consent was obtained from all healthy subjects.

2.2 Measurement of antibodies

Microtiter plates (Nunc, Roskilde, Denmark) were coated with 1 µg/ml full-length recombinant ribosomal protein P0, P1 or P2 expressed in insect cells (DIARECT, Freiburg, Germany). Sera diluted 1:201 in PBS-0.1% (w/v) casein were added and allowed to react for 30 minutes, followed by three washing cycles with PBS-0.05% (v/v) Tween 20. For detection

of bound antibodies the plates were incubated with anti-human IgG conjugated with peroxidase (EUROIMMUN, Lübeck, Germany) for 30 minutes, washed three times, and allowed to react with tetramethylbenzidine (EUROIMMUN) for 15 minutes. After addition of acidic stopping solution (EUROIMMUN), the optical density (OD) was read at 450 nm using an automated spectrophotometer (Spectra Mini, Tecan, Crailsheim, Germany). All steps were performed at room temperature. A highly positive index patient serum was used to generate a standard curve consisting of three calibrators (2, 20 and 200 relative units (RU)/mL). RU/mL was calculated for all samples using this 3-point standard curve. The analytical reproducibility of all anti-RibP assays was evaluated by repeated testing of 2 serum samples (10 determinations each) in the same run, giving intra-assay coefficients of variation (CV) of 2.4% (anti-RibP_R0), 2.1% (anti-RibP_R1) and 2.7% (anti-RibP_R2), respectively. Relationships between sensitivity and specificity at different cut-off values were examined for all assays by ROC curve analyses, allowing also the determination of test characteristics at pre-defined specificities.

The Anti-RibP_NH ELISA (IgG, CV 2.6%), Anti-Sm ELISA, Anti-dsDNA RIA (Farr assay) and Anti-dsDNA ELISA are commercially available assays from EUROIMMIUN and were performed following the manufacturer's instructions.

2.3 Statistical analysis

Data were analysed using the statistical software GraphPad Prism 5 (GraphPad Software, La Lolla, USA). By means of receiver-operating characteristics (ROC) analysis, the diagnostic significance of anti-ribosomal protein N, P0, P1, P2 antibodies was assessed and areas under curves (AUC) were created. To determine associations, Mann-Whitney test (for comparing medians between groups; MWT), Fisher's exact test (FET) and Spearman rank test (SRT) were used. Two-tailed t-tests were used throughout. Differences with p-value <0.05 were considered significant.

3 Results

3.1 Reactivity and diagnostic significance of anti-ribosomal protein N, P0, P1, P2 antibodies

In sera from 163 SLE patients, 210 with other rheumatic autoimmune diseases and 100 healthy controls, antibodies against recombinant ribosomal P_R0, P_R1, P_R2 proteins and against their native heterocomplex (Figure 1), Sm and dsDNA (ELISA and Farr assay) were measured in order to define and compare the sensitivity and specificity in ROC curve analysis (Table 1).

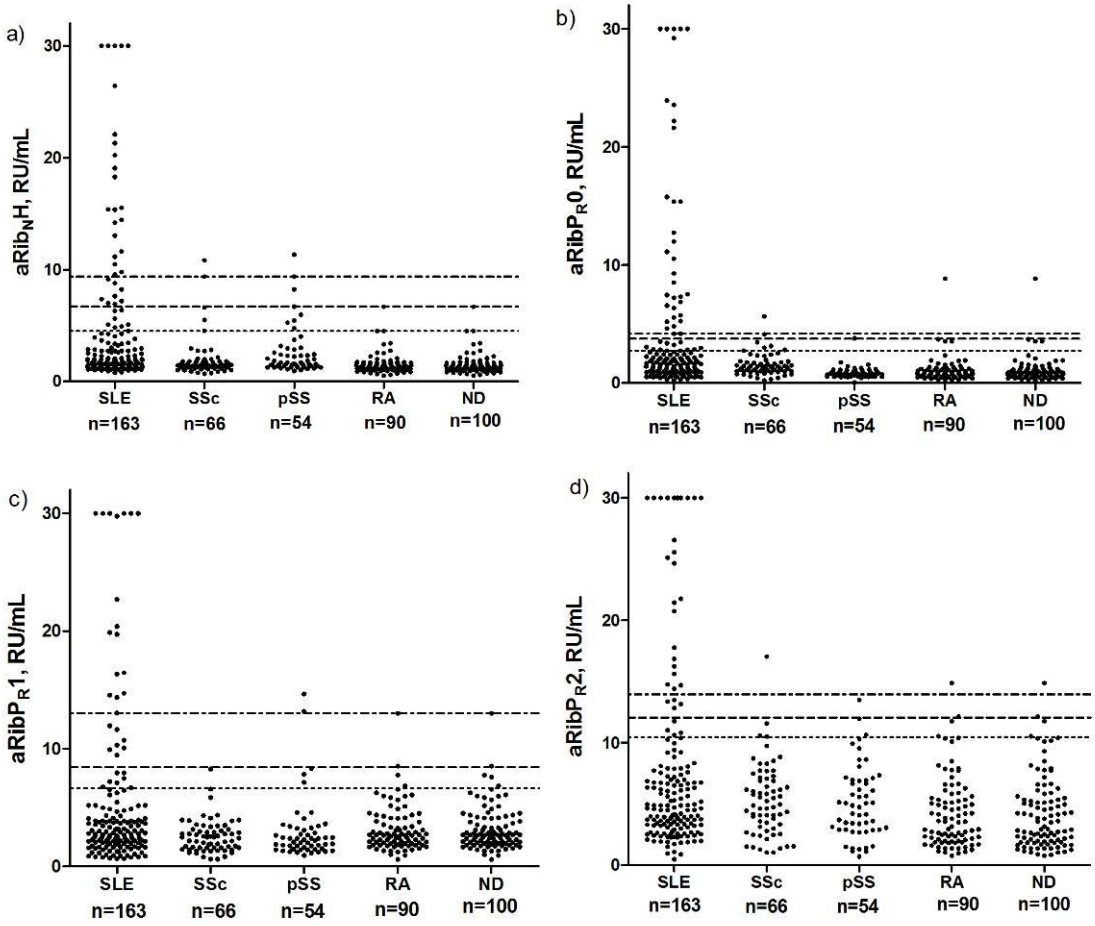

Figure 1: **Anti-ribosomal P protein antibodies P_NH, P_R0, P_R1, P_R2 in SLE, other rheumatic diseases and healthy donors.** Dotted lines represent the threshold obtained through ROC-test by specificity 95% (dotted line), 98% (broken line), 99% (dotted and broken line): for aRibP$_N$H (Fig.1a) , aRibP$_R$0 (Fig. 1b), aRibP$_R$1 (Fig. 1c) , aRibP$_R$2 (Fig. 1d). Values > 30 RU/mL were set to 30 RU/mL for the clearer arrangement of the figures.

For anti-RibP_NH, a sensitivity of 5.5% and specificity of 100% were calculated using the manufacturer's cutoff (20 RU/mL). At a specificity of 98%, among 210 patients with other rheumatic diseases (SSc, pSS, RA), only 5 (2.4%), 4 (1.9%), 4 (1.9%), 4 (1.9%) had elevated anti-RibP_NH, anti-RibP_R0, anti-RibP_R1 and anti-RibP_R2 titers, respectively. At the same specificity, among 100 healthy donors, only 0 (0%), 1 (1.0%), 2 (2.0%), 2 (2.0%) had high titers of anti-RibP_NH, anti-RibP_R0, anti-RibP_R1 and anti-RibP_R2. Anti-RibP_R0 had the highest performance with regard to criteria like area under curve (AUC) and maximum sum of sensitivity and specificity, followed by anti-RibP_NH (Table 1), in comparison with other anti-ribosomal P protein antibodies. All parameters of anti-RibP_R0 were inferior to those of the Anti-dsDNA ELISA or the Farr assay, but almost equal to those of the Anti-Sm ELISA (see Table 1).

Table 1: Test values of anti-ribosomal P_NH, P_R0, P_R1, P_R2 antibodies calculated in ROC analysis. The highest values of sensitivity, AUC and the lowest values of cut-offs (in parentheses) of anti-RibP autoantibodies are shown in bold.

	aRibP$_N$H	**aRibP$_R$0**	**aRibP$_R$1**	**aRibP$_R$2**	**anti-Sm**	**a-dsDNA-RIA**	**a-dsDNA-ELISA**
Area under curve	0.7014	**0.7368**	0.5811	0.6220	0.6791	0.8463	0.8621
95% confidence interval	0.65-0.75	0.69-0.79	0.52-0.64	0.57-0.67	0.62-0.74	0.80-0.89	0.82-0.90
P value	<0.0001	<0.0001	0.0021	<0.0001	<0.0001	<0.0001	<0.0001
Sensitivity at specificity of 95% (cutoff)	24.4 % (4.5)	**29.2 % (2.7)**	20.4 % (6.6)	20.2 % (10.5)	38.7% (2.0)	61.4% (5.4)	53.9% (73.9)
Sensitivity at specificity of 98% (cutoff)	19.1 % (6.7)	**22.0 % (3.7)**	16.1 % (8.4)	17.9 % (12.1)	33.7% (2.4)	56.4% (6.5)	42.9% (105.8)
Sensitivity at specificity of 99% (cutoff)	14.3 % (9.4)	**22.0 % (4.2)**	10.7 % (13.0)	14.9 % (13.9)	19.6% (4.8)	55.8% (6.8)	37.4% (151.0)
Sensitivity at specificity of 100% (cutoff)	**11.9 (11.5)**	11.3 % (9.1)	8.9 % (14.7)	11.3 % (17.4)	12.3% (7.9)	49.1% (9.0)	31.3% (169.7)
Max sum of specificity+ sensitivity	133.2 %	**140.7 %**	118.2 %	117.9 %	138.9%	161.8%	160.8%

3.2 Patients negative for anti-RibP_NH, but positive for anti-RibP_RP0, 1, 2

Though the native heterocomplex of ribosomal P consists of 3 subunits P0, P1 and P2, there were considerable differences in the cut-offs and in sensitivities for the detection of anti-RibP_NH, anti-RibP_R0, anti-RibP_R1 and anti-RibP_R2 with outstanding results for anti-RibP_R0 (Figure 2). Furthermore, we investigated if there were patients negative for anti-RibP_NH, but positive in anti-RibP_R0, anti-RibP_R1 or anti-RibP_R2. Sera meeting these criteria would suggest that there are some epitopes of ribosomal P proteins that are present in free subunits P0, P1

and P2 and are not accessible to autoantibodies directed against the native heterocomplex due to the spatial conformation of the latter.

At 99% specificity, among 141 anti-RibP_NH-negative patients there were 19 (13.5%) positive for anti-RibP_R0, 6 (4.3%) for anti-RibP_R1 and 11 (7.8%) for anti-RibP_R2. Some of those sera were exclusively positive for one of the recombinant RibPs and showed an increased titer up to the 2-fold of the corresponding cut off (Figure 2B). **Fold change indices** of positive anti-Rib P_R0, P_R1, P_R2 antibodies in anti-RibP_NH-negative SLE patients show how high the levels of antibodies were and were calculated through dividing the anti-Rib P_R0, P_R1, P_R2 levels by their cut-offs, respectively.

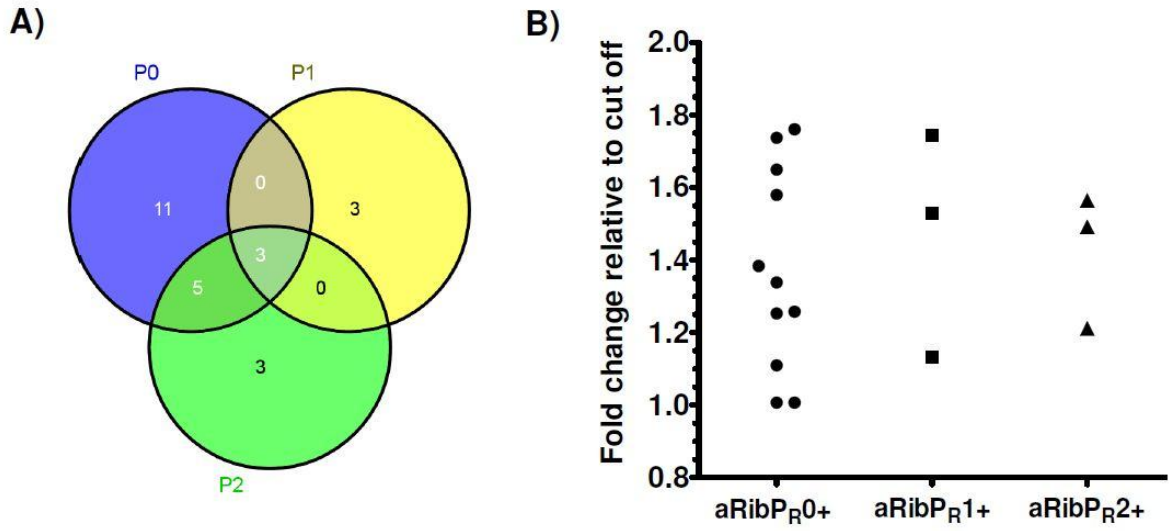

Figure 2: Frequencies of anti-RibPR0, anti-RibPR1 and anti-RibPR2 in anti-RibPNH-negative lupus patients. A) Venn diagram of anti-ribosomal P_R0, P_R1, P_R2 antibody frequencies in anti-RibP_NH-negative SLE patients by antibody specificity 99%. **B)** Fold change indices of anti-Rib P_R0, P_R1, P_R2 antibodies positive in anti-RibP_NH-negative SLE patients at an antibody specificity of 99%.

3.3 Diagnostic value of anti-ribosomal P protein antibodies in SLE

To estimate the supplementary diagnostic value of anti-ribosomal P protein antibodies in SLE, we looked for patients that were negative for antibodies against dsDNA and Sm, but were positive for anti-ribosomal P protein antibodies at a specificity of 100% (Figure 3). This analysis was carried out twice using the results of the Anti-dsDNA ELISA (Figure 3A) or those of the Farr assay (Figure 3B). 63 (38.7%) patients were regularly diagnosed by the presence of anti-dsDNA-ELISA or anti-Sm antibodies, whereas 11 individuals could only be

diagnosed by detection of anti-RibP antibodies. In the case with the Farr assay (Anti-dsDNA-RIA), the results were 89 (54.6%) and 5 (3.1%) correspondingly.

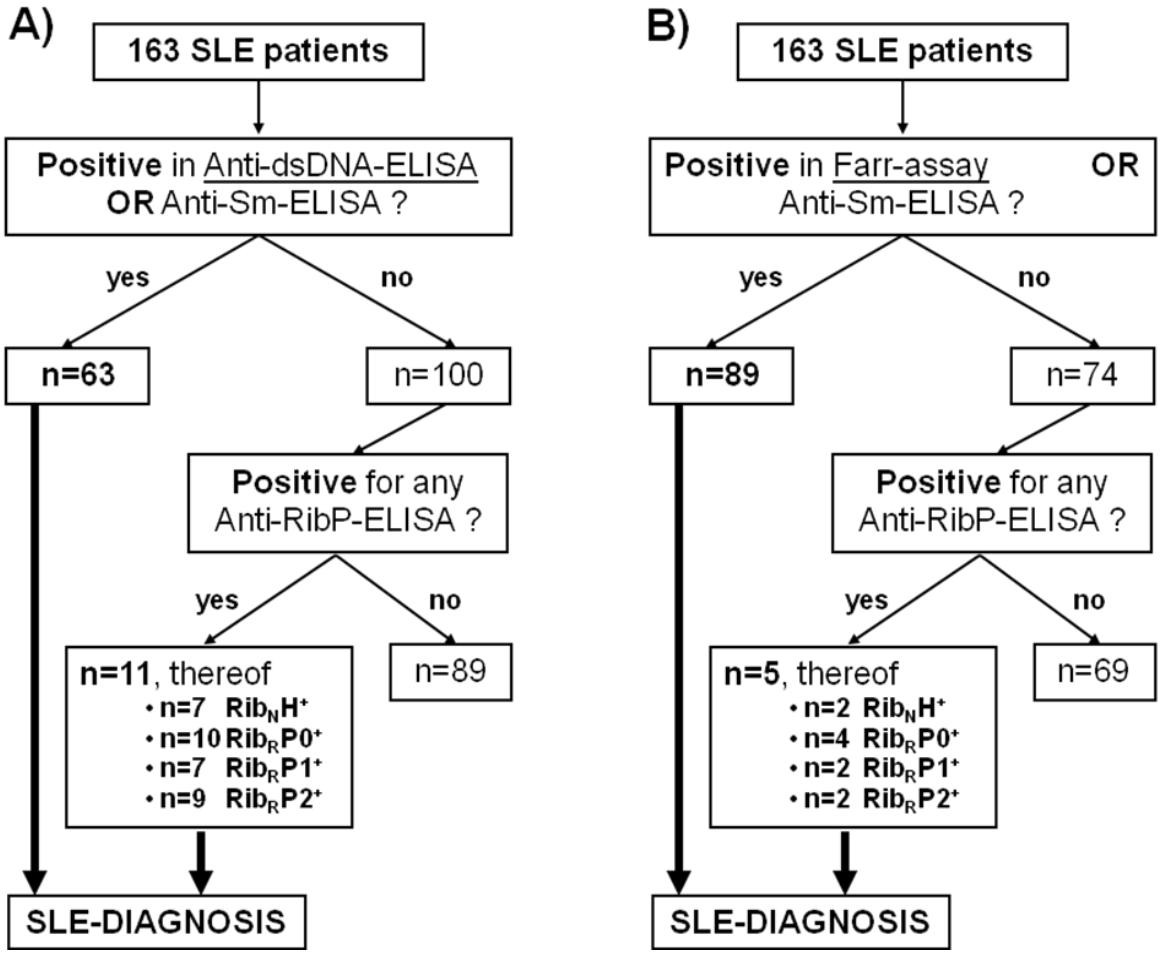

Figure 3: Diagnostic contribution of anti-ribosomal P protein antibodies in SLE revelation. Analysis using Anti-dsDNA-ELISA is shown in Figure 2A and using Anti-dsDNA-RIA (Farr assay) is shown in Figure 2B.

3.4 Comparison of disease features in anti-RibP positive vs. aRibP negative SLE patients

To detect the special features of SLE patients with high anti-RibP antibodies, we compared medical records (ACR-criteria, SLEDAI-2000, drugs, laboratory parameters including autoantibodies, liver enzymes and etc.) of anti-RibP negative lupus patients with those of positive counterparts. Table 2 demonstrates significant clinico-laboratory associations. All results and detailed demographic information about the study cohort are shown in Table 2 of the original publication [42] (see Appendix). Anti-RibP_NH positive patients fulfilled significantly more ACR criteria, had more frequently photosensitivity and decreased

complement component 3 levels. Anti-RibP$_R$0 positive patients had a significantly lower number of lymphocytes, and higher GGT levels were found in anti-RibP$_R$1 positive patients. Prevalence of high anti-Sm, anti-dsDNA and anti-U1RNP antibodies was higher in all aRibP positive patients.

Table 2. Comparison of frequencies: demographical and clinical data in anti-RibP-positive and negative SLE patients (reduced to significant results)

Features	All pts, n=163	aRibP$_N$H			aRibP$_R$0			aRibP$_R$1			aRibP$_R$2		
		pos. n=30	neg. n=133	p value	pos. n=34	neg. n=129	p value	pos. n=24	neg. n=139	p value	pos. n=28	neg. n=135	p value
ACR-Criteria	n=163	n=30	n=133		n=34	n=129		n=24	n=139		n=28	n=135	
No. of ACR criteria[1], median	6.00	**7.00**	**6.00**	**0.031**	7.00	6.00	0.059	6.50	6.00	0.236	7.00	6.00	0.076
Photosensitivity [2],%	46.6	**63.3**	**42.9**	**0.046**	58.8	43.4	0.125	62.5	43.9	0.121	53.6	45.2	0.533
SLEDAI	n=101	n=17	n=84		n=22	n=79		n=17	n=84		n=17	n=84	
Arthritis[2],%	33.7	23.5	35.7	0.408	22.7	36.7	0.309	**11.8**	**38.1**	**0.048**	**11.8**	**38.1**	**0.048**
LABORATORY													
Lymphocytes[1], median	0.87	0.67	0.87	0.164	**0.63**	**0.92**	**0.036**	0.91	0.86	0.957	0.70	0.93	0.076
GGT*[1], median	23.0	26.0	21.6	0.278	24.0	21.1	0.423	**29.0**	**21.0**	**0.047**	29.0	21.1	0.108
low C3 [2], %	47.6	**65.5**	**43.2**	**0.038**	58.1	44.8	0.227	54.2	46.3	0.511	61.5	44.6	0.134
AUTOANTIBODIES													
↑ anti-Sm[2], %	33.7	**63.3**	**27.1**	**0.0004**	**70.6**	**24.2**	**$<10^{-4}$**	**66.7**	**28.1**	**0.0007**	**60.7**	**28.1**	**0.002**
↑anti-dsDNA in ELISA[2], %	42.3	**70.0**	**36.8**	**0.0018**	**67.8**	**36.4**	**0.002**	**75.0**	**37.4**	**0.0007**	**60.7**	**39.3**	**0.058**
↑anti-dsDNA in RIA [2], %	56.4	**76.7**	**51.9**	**0.015**	**79.4**	**50.4**	**0.003**	**83.3**	**51.8**	**0.004**	**78.6**	**51.9**	**0.012**
↑anti-U1[2] in anamnesis, %	28.9	**59.1**	**21.7**	**0.001**	**50.0**	**23.3**	**0.021**	**62.5**	**23.5**	**0.003**	**54.5**	**22.7**	**0.007**
↑anti-Nucleosomes, %	50.9	60.0	48.9	0.315	64.7	47.3	0.084	62.5	48.9	0.271	**70.4**	**47.1**	**0.035**
↑anti-La, %	12.3	20.0	10.5	0.213	8.82	13.2	0.769	**0.00**	**14.4**	**0.046**	3.57	14.1	0.203

Table 2: [1]- the p values in MWT; [2]- the p values in FET. The significant findings are marked in bold. *GGT values for men and from other laboratories have been standardized on cut-offs of GGT for women in Charité Central Laboratory.

3.5 Comparison of disease damage in anti-RibP positive vs. anti-RibP negative SLE patients

Damage burdens at time of blood sampling and three years later were completely assessable in 41 out of all 58 patients that were positive for any of the four anti-ribosomal P protein ELISA. Among these 41 patients, 22, 27, 18 and 23 individuals were positive for anti-RibP$_N$H, anti-RibP$_R$0, anti-RibP$_R$1 and anti-RibP$_R$2, respectively. As a control, 41 age-, gender- and nephritis-matched anti-RibP negative patients were used. Changes in damage scores (ΔSLICC, ΔWDS) were calculated and compared. SLICC and WDS correlated significantly with disease duration (for SLICC p=0.018, r=0.259; for WDS p=0.021, r=0.255) and age of patients (for SLICC p<0.0001, r=0.443; for WDS p<0.0001, r=0.426), but not with the rest of clinic-laboratory parameters. Neither total disease damage nor damage to separate organ systems in anti-RibP positive patients was significantly higher than in their negative

counterparts within these three years. Thus, we found no predictive role for anti-RibP autoantibodies at the year 3.

4 Discussion

Herein, we present the first comparative study of clinic-laboratory associations, the diagnostic and prognostic potentials of anti-ribosomal P protein autoantibodies in a large SLE cohort comprising 163 SLE patients, where not only antibodies against native ribosomal P-heterocomplex, but also against its recombinant constituents P0, P1, P2 were investigated. We found that anti-RibP_R0 antibodies have the best diagnostic value of all anti-RibP autoantibodies, and additional measuring of anti-RibP_R0 offered most diagnostic benefit in SLE patients negative for anti-dsDNA and anti-Sm antibodies. Moreover, anti-RibP_R0 positive lupus patients tend to have significantly lower lymphocyte counts than their negative counterpart. Finally, anti-RibP antibodies showed no association with disease damage over a 3-year period.

Our findings regarding the frequency and high specificity of anti-RibP antibodies for SLE are in line with data described before [3, 43]. We further found sensitivities of $P_R0>P_NH>P_R2>P_R1$ at specificities of 98-99% and $P_NH>P_R0=P_R2>P_R1$ at a specificity of 100% in a cohort of 163 lupus patients. Mahler et al. found in a cohort of 50 SLE patients other sensitivities at a specificity of 100%: $P_R2=P_R1=P_R0=18\%$ [13]. The cause of those divergent observations might have been the use of different detection systems and patient cohorts.

We further showed that negative anti-RibPNH does not automatically imply negativity of antibodies against its subunits. The higher anti-RibP_R0 prevalence could be explained through the presence of ribosomal P0-like protein in the cell membranes of many cells which could contribute to an increased immunogenicity [8-11] and, as a consequence, to freely accessible epitopes that are not within the spatial conformation of the native heterocomplex. This observation agree somewhat with previous reports that anti-ribosomal P antibodies can target non-C-terminal epitopes [44].

Among the great variety of autoantibodies that are described in SLE, anti-dsDNA and anti-Sm antibodies are highly specific and mostly used for the verification of diagnosis. Though less specific antiphospholipid antibodies are included in the ACR criteria as well [4]. However, anti-RibP antibodies are also discussed as a diagnostic criterion. Herein, we raised the question if anti-RibP antibodies contain an auxiliary diagnostic value to the immunological criteria of ACR. Among sera negative in the anti-Sm and anti-dsDNA at a specificity of 100%, 10% were positive for anti-RibP_R0 in the case of anti-dsDNA ELISA and 5.4% in the

case of the Farr assay. Laboratories using less sensitive assays seem to benefit more from testing for anti-RibP antibodies in suspected cases of SLE. Hence, measurement of anti-ribosomal P protein antibodies would improve the classification and diagnosis of SLE, especially in cases with borderline or negative anti-dsDNA, anti-Sm antibodies and/or with ACR criteria less than 4.

The most remarkable association of anti-RibPs with clinical parameters was that between positive anti-RibP_R0 antibodies and significantly lower lymphocytes. Of note, a P0-like protein was demonstrated to be present on the surface the plasma membranes of different cells including lymphocytes [11]. Further, the anti-RibP antibodies are able to bind and penetrate T cell lines [45, 46] and especially anti-RibP_R0 can induce apoptosis on Jurkat T cells [47]. Hence, clinicians should keep in mind high anti-RibP antibodies as a differential diagnostic cause of lymphocytopenia along with viral status, drugs, hematologic malignancies, etc. Thus, anti-RibP_R0 should be born in mind as a differential diagnosis for lymphocytopenia in SLE together with the viral status, drug side effects, hematologic malignancies, etc.

In our investigation, we were unable to confirm an association between anti-RibP positivity and lupus nephritis, short disease duration, high disease activity or juvenile onset. These findings might be influenced by the Caucasian ethnicity of the study cohort, number of patients with active disease and different test systems. The number of patients with neuropsychiatric lupus was insignificant in our study.

This is the first study confirming a statistically significant association between GGT and anti-RibP_R1 in a large cohort of Caucasian lupus patients. In a study of 61 Japanese patients [22], no significant association was found between anti-RibP_R0 and liver enzymes AST, ALT - but the GGT was not assessed. The involvement of anti-RibP antibodies in liver pathology of SLE was previously reported in cell cultures [9, 11, 47] and in case reports [19-21]. But the focus in previous studies was on ribosomal P0 protein as autoantigen because of the membrane-bound P0-isoform [8-11]. Interestingly, it was reported that the penetration of anti-RibP_R0 can result in inhibition of the apolipoprotein B synthesis evoking a threefold increase in cellular cholesterol with lipid droplet accumulation and global protein synthesis [9, 46]. The liver enzyme GGT is a sensitive marker for cholestatic damage. The same mechanism could function in the case of anti-RibP_R1. However, it is difficult to differentiate liver involvement from other causes of cholestatic damage (such as nutrition, drugs and other autoimmune hepatitis forms) in SLE patients. Longitudinal analysis of anti-RibP antibodies with liver function tests might unravel this association best.

In this work, we were the first to examine the prognostic role of anti-RibP antibodies and could show that anti-RibP antibodies are not a prognostic parameter in SLE in a three-year-period. To date, no prognostic laboratory long term parameter is known. Except age and disease duration, there was no correlation with clinical-laboratory parameters. However, prospective investigations with larger patient cohorts and longer observation period are needed.

In a nutshell, anti-ribosomal P protein antibodies are highly specific for SLE, can be positive in patients with negative anti-dsDNA and anti-Sm antibodies and, therefore, have to be discussed to be included in upcoming classification and diagnosis criteria for SLE. High anti-$RibP_R0$ titers can be associated with low lymphocyte count, and high anti-$RibP_R1$ with an elevated GGT level. Over a three-year-period, a prognostic value of anti-ribosomal P protein antibodies was not found in this study, but should be explored in larger cohorts in future.

5 APPENDIX

5.1 References

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5.2 List of Abbreviations

ACR	American College of Rheumatology
anti-dsDNA	anti-double-stranded DNA antibody
anti-Sm	anti-Smith antibody
aRibPs	anti-ribosomal P protein antibodies
$aRibP_NH$	antibodies against native ribosomal P heterocomplex
$aRibP_R0$	antibodies against recombinant ribosomal P0 protein
$aRibP_R1$	antibodies against recombinant ribosomal P1 protein
$aRibP_R2$	antibodies against recombinant ribosomal P2 protein
ALT	Alanine aminotransferase
AST	aspartat aminotransferase
AUC	area under curve
CRP	C-reactive protein
ELISA	Enzyme-linked immunosorbent assay
ESR	erythrocyte sedimentation rate
FET	Fisher's exact test
GGT	γ-glutamyl transpeptidase
HD	Healthy donors
La/SS-B	anti- Sjögren's syndrome antigen B
MWT	Mann-Whitney test
pSS	Primary Sjögren syndrome
RA	Rheumatoid arthritis
RIA	Radioimmunoassay
ROC	receiver-operating characteristics analysis
Ro/SS-A	anti- Sjögren's syndrome antigen A
SLE	systemic lupus erythematosus
SLEDAI-2000	systemic lupus erythematosus disease activity index 2000
SLICC	Systemic Lupus International Collaborative Clinics
SRT	Spearman rank test
SSc	systemic sclerosis
U1-RNP	U1-ribonucleoprotein
WDS	weighted damage score

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5.4 Curriculum vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

5.5 Declaration in lieu of oath

Hereby I, Barkhudarova Fidan, declare that I have written this thesis with the topic "Diagnostic value and clinical laboratory associations of antibodies against recombinant ribosomal P0, P1, P2 proteins and their native heterocomplex in a Caucasian cohort with SLE" by my own. Furthermore, I confirm that no other sources have been used than those specified in the thesis itself.

Berlin, the 22.02.2012

Barkhudarova Fidan
Doctoral candidate

Erklärung

Ich, Barkhudarova Fidan, erkläre, dass ich die vorgelegte Dissertation mit dem Thema: “Diagnostic value and clinical laboratory associations of antibodies against recombinant ribosomal P0, P1, P2 proteins and their native heterocomplex in a Caucasian cohort with SLE” selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.

Berlin, 22.02.2012

5.6 List of publications

1. Barkhudarova F, Dähnrich C, Rosemann A, Schneider U, Stöcker W, Burmester GR, Egerer K, Schlumberger W, Hiepe F, Biesen R. **Diagnostic value and clinical laboratory associations of antibodies against recombinant ribosomal P0, P1, P2 proteins and their native heterocomplex in a Caucasian cohort with SLE.** Arthritis Res Ther. 2011 Feb 10;13(1):R20. Impact factor Arthritis Res Ther. 2009 = 4.27
2. Biesen R, Dahnrich C, Rosemann A, Barkhudarova F, Rose T, Jakob O, Bruns A, Backhaus M, Stocker W, Burmester GR, Schlumberger W, Egerer K, Hiepe F. **Anti-dsDNA-NcX ELISA: dsDNA-loaded nucleosomes improve diagnosis and monitoring of disease activity in systemic lupus erythematosus.** Arthritis Res Ther. 2011 Feb 17;13(1):R26. Impact factor Arthritis Res Ther. 2009 = 4.27
3. Biesen R, Demir C, Barkhudarova F, Grün JR, Steinbrich-Zöllner M, Backhaus M, Häupl T, Rudwaleit M, Riemekasten G, Radbruch A, Hiepe F, Burmester GR, Grützkau A. **Sialic acid-binding Ig-like lectin 1 expression in inflammatory and resident monocytes is a potential biomarker for monitoring disease activity and success of therapy in systemic lupus erythematosus.** Arthritis Rheum. 2008 Apr 58(4):1136-45. Impact factor Arthr. Rheum. 2008 = 6.787

5.7 Declaration about contribution to publications

The doctoral candidate Fidan Barkhudarova has contributed to the following publications:

Publication 1: Barkhudarova et al., Arthritis Res Ther. 2011 Feb 10;13(1):R20.
Input (ca. 60 %): collecting of patients' data, clinical assessment of SLE patients, statistical analysis of clinico-laboratory data, drafting of publication in the present form.

Publication 2: Biesen R et al., Arthritis Res Ther. 2011 Feb 17;13(1):R26.
Input (ca. 30 %): collecting of patients' data, interpretation of statistical analysis, discussion.

Publication 3: Biesen R. et al., Arthritis Rheum. 2008 Apr 58(4):1136-45.
Input (ca. 20 %): collecting of patients' data, clinical assessment of SLE patients, statistical analysis, discussion.

Prof. Dr. Falk Hiepe	Fidan Barkudarova
Doctoral thesis supervisor	Doctoral candidate