Anti-Ribosomal P Protein IgG autoantibodies in systemic lupus erythematosus patients - diagnostic performance and clinical profile

Diana Carmona-Fernandes, MSc\textsuperscript{1}; Maria José Santos, MD PhD\textsuperscript{1,2}; Helena Canhão, MD PhD\textsuperscript{1,3}; João Eurico Fonseca, MD PhD\textsuperscript{1,3}

1- Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal; 2- Rheumatology Department, Hospital Garcia de Orta, Almada, Portugal; 3- Rheumatology and Bone Metabolic Diseases Department, Hospital de Santa Maria, Lisbon, Portugal.

Corresponding author:

João Eurico Fonseca
Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa
Edifício Egas Moniz, Av. Professor Egas Moniz
1649-028 Lisboa
Portugal
Tel: +351-217999545, Fax: +351-217999459
E-mail: jecfonseca@gmail.com
ABSTRACT

Background: We aimed to assess the performance of anti-Rib-P antibodies in systemic lupus erythematosus (SLE) diagnosis and their association with SLE clinical manifestations.

Methods: We have used a fluoroenzyme assay to determine anti-Rib-P levels in SLE patients, diseased controls (rheumatoid arthritis (RA), ankylosing spondylitis, psoriatic arthritis and juvenile idiopathic arthritis) and healthy controls. We also determined anti-Sm and anti-dsDNA levels. Receiver operating characteristic (ROC) curves were performed and the best cut-offs of positivity determined. The relationship between clinical variables and autoantibodies levels was analyzed by regression analysis.

Results: A total of 127 SLE patients, 256 rheumatic disease controls and 100 healthy controls were studied. Anti-Rib-P autoantibodies were positive in 18 (14.2%) SLE patients (mean concentration of 30.6±46.9 U/ml) and in 2 RA patients (0.8% of the disease control group). Twelve (9.4%) SLE patients were positive for anti-Sm (31.1±40.8 U/ml) and 63 (49.6%) tested positive for anti-dsDNA autoantibodies (88.4±88.5 U/ml). Among anti-Rib-P positive samples, 12 were also positive for anti-dsDNA, 2 were positive for the three (anti-Rib-P, anti-Sm and anti-dsDNA) tested autoantibodies, while 4 tested positively only for anti-Rib-P antibodies. The specificity, sensitivity, positive predictive value and negative predictive value of anti-Rib-P for SLE diagnosis was 99.4%, 14.2%, 90% and 76.4%, respectively.

Caucasian ethnicity was associated to lower anti-Rib-P antibody levels. No relation was found between anti-Rib-P levels and neuropsychiatric or other clinical manifestations.
**Conclusions:** Anti-Rib-P autoantibodies are highly specific for SLE diagnosis and their determination might be considered for inclusion in the ACR SLE classification criteria. Caucasian ethnicity was associated to lower anti-Rib-P antibody levels.

**KEYWORDS**

Anti-Rib-P; Systemic Lupus Erythematosus; Antibodies
BACKGROUND

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by multi-organ involvement and by the production of autoantibodies directed against a variety of nuclear and cytoplasmic antigens [1, 2]. Autoantibodies can be detected in patients’ sera years before SLE diagnosis [3]. Some antibodies are relevant for the diagnosis, while others are associated with prognostic features or disease activity status [2, 4].

Antibodies against double stranded DNA (anti-dsDNA) and Sm antigen (anti-Sm) are considered very specific for SLE diagnosis and both are part of the immunologic classification criterion for this disease [5]. Furthermore, high levels of anti-dsDNA are associated with increased SLE disease activity [6].

Among SLE-specific autoantibodies, one subset is directed against ribosomal P (Rib-P) phosphoproteins [2]. The Rib-P antigen consists of three protein components of the 60S ribosomal subunit designated P0 (38 kDa), P1 (19 kDa), and P2 (17 kDa). A pentameric complex of one copy of P0 and two copies each of P1 and P2 interacts with the 28S rRNA molecule to form a GTPase domain that is active during the elongation step of protein translation [7-12]. The major immunoreactive epitope of these ribosomal antigens has been localized to the 22 amino acid carboxy terminal domain, which is present in all three proteins and contains two phosphorylated serine residues proteins [2, 8-14].

Anti-Rib-P antibodies are directed against the three subunits [2, 9, 15] and are able to penetrate certain cells, binding to ribosomal proteins and blocking protein synthesis [15].
Anti-Rib-P antibodies enhance the production of tumor necrosis factor (TNF) and interleukin (IL)-6 by activated monocytes and also up-regulate the expression of TNF and IL-6 messenger RNA in activated monocytes, indicating that human peripheral blood monocytes express the ribosomal P epitope upon activation [15].

Different ethnic backgrounds may influence the frequency with which anti-Rib-P antibodies occur in SLE patients, ranging from 6% to 46% [2, 7, 11, 14-16]. In most ethnic groups anti-Rib-P antibodies have been found in 6-20% of patients, in contrast to Chinese, where 36% SLE patients were reported to be positive [7, 11, 12, 15]. Anti-Rib-P antibodies seem to be highly specific for patients with SLE and might also be a marker for SLE disease activity [12, 14, 15].

The presence of anti-Rib-P antibodies in SLE patients has been associated with younger age at disease onset, multiple organ involvement and overall severe disease course [8], including central nervous system [2, 4, 7, 11, 12, 15], nephritis [2, 7, 12, 15], photosensitivity [2], malar rash [2], and hepatic involvement [2, 7, 12]. Moreover, it has became evident that anti-Rib-P antibodies are more prevalent in juvenile-onset SLE than in adult onset SLE [11, 12]. Bonfa et al. first assessed the association of anti-Rib-P antibodies with psychiatric manifestations in patients with psychosis secondary to SLE [17]. However, this association has not always been confirmed [7, 8].

We hypothesize that anti-Rib-P autoantibodies might be useful for SLE diagnosis. To test that we quantified anti-Rib-P in SLE patients, disease controls (rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), ankylosing spondylitis (AS), and psoriatic arthritis (PsA)) and healthy controls using a new fluoroenzyme assay kit.
METHODS

Patients:

For this study we used serum samples from Biobank - Instituto de Medicina Molecular (IMM), collected between May 2007 and December 2009. Samples were selected from patients with the following diagnosis: SLE fulfilling the revised American College of Rheumatology (ACR) criteria (1997), RA according to the revised American Rheumatism Association (ARA) criteria (1987), JIA according to the second revision of the International League of Associations for Rheumatology (ILAR) classification (2001), AS in agreement with the modified New York criteria (1984) and PsA in accordance to the modified European Spondyloarthropathy Study Group (ESSG) criteria (2006). Healthy volunteers’ samples were used as healthy controls.

In total 127 SLE patients, 256 rheumatic disease controls (100 RA, 99 AS, 34 JIA and 23 PsA) and 100 healthy controls were studied. Information about age, ethnicity and gender was collected. For SLE patients information considering age at disease diagnosis, disease duration, cumulative clinical manifestations (according to ACR classification criteria), the presence of auto-antibodies (anti-dsDNA, anti Sm, anti-cardiolipin, anti-SSA, anti-SSB and anti-RNP), medication (including corticosteroids current dosage and immunosuppressant use), current disease activity (evaluated using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI2K) [18]) and cumulative damage (scored in accordance to the Systemic Lupus International Collaborating Clinics/ACR Damage Index (SLICC) [19]) were obtained at the time of blood sample collection. Clinical manifestations, with particular attention to the occurrence of neuropsychiatric lupus syndromes according to the ACR nomenclature [20], disease activity and damage were assessed semi-annually thereafter.
This study was approved by Centro Hospitalar Lisboa Norte, E.P.E. – Hospital de Santa Maria and by Hospital Garcia de Orta, E.P.E. Ethics Committees and all participants signed a written informed consent prior to any protocol-specific procedure. This study was conducted in accordance with the regulations governing clinical trials such as the Declaration of Helsinki, as amended in Seoul (2008).

Methods:
Anti-Rib-P, anti-Sm and anti-dsDNA antibodies were determined using EliA™ Rib-P, EliA™ Sm and EliA™ dsDNA for quantification of the three antibodies, respectively. This is a fluoroenzyme assay (Phadia®, Sweden - now Thermo Fisher Scientific) for in vitro diagnosis. The tests were performed according to manufacturer’s instructions.

Statistical analysis:
Results are reported as means ± standard deviation for continuous variables or proportions for categorical variables. Anti-Rib-P, anti-Sm anti-dsDNA concentrations are presented in U/ml.
Receiver operating characteristic (ROC) curves were performed for each test comparing SLE patients results with either healthy controls or rheumatic disease controls results. For both ROC curves, for each antibody, a cut-off point was determined as the value of the parameter correspondent to the highest sensitivity without lowering the specificity. The area under the curve (AUC) was also determined. Differences between SLE and control groups were assessed using T-test for continuous variables or $\chi^2$ or Fisher’s exact test for proportions.
The association between clinical variables and these antibodies was investigated for SLE patients using univariate followed by multivariate linear regression analyses. All variables related with the studied outcome in univariate analyses at a \( p \)-value \( \leq 0.20 \) were considered potential predictors and entered in multivariate linear regression models, along with neuropsychiatric manifestations due to its previously association to these antibodies. The selection of covariates was stepwise by backward selection. Statistical calculations were performed using Statistical Package for the Social Sciences (SPSS) Statistics Software, v.15.0 (SPSS Inc., Chicago, USA) and a 2-tailed \( p \)-value <0.05 was selected as significant.

**RESULTS**

Demographic characteristics of all studied subjects are presented in Table 1. SLE patients had a mean age at disease diagnosis of 34.2±14.5 years-old, 8.3±6.5 years of disease duration (range 0.5 to 34 years), a mean SLEDAI2K of 3.3±4.2 and a SLICC damage score of 1.1±2.1 at baseline evaluation. Among SLE patients 79 (62.2%) were under corticosteroids (with mean daily dosage of prednisolone of 12.4 mg), 90 (71%) received antimalarials, 50 (39%) immunossupressants and 1 rituximab.

Receiver operating characteristic (ROC) curves were performed in order to obtain the most adequate cut-off values for the Portuguese population. These curves are of particular relevance for the new anti-Rib-P test kit. They were also made for the other tests for analysis coherence. The curves are presented in Figure 1 the area under the curve (AUC) and correspondent \( p \)-values identified. For both anti-Rib-P and anti-Sm tests the cut-off value after analysis of the ROC curves was established at 4.45 U/ml.
and 3.4 U/ml, respectively. For anti-dsDNA the cut-off was kept as indicated by the manufacturer at 15 U/ml. With the adjustment in the cut-off values, we have found an increased number of SLE patients positive for either anti-Rib-P or anti-Sm, without occurring more false positive results in the control groups than those obtained with the manufacturer cut-off (data not shown).

SLE patients presented anti-Rib-P autoantibodies in a mean concentration of 4.9±20.2 U/ml, significantly greater than the healthy control group (0.07±0.21 U/ml; \(p=0.016\)) and the rheumatic disease control group (0.6±1.8 U/ml; \(p=0.017\)). In 18 (14.2%) samples anti-Rib-P was above the cut-off of positivity (mean concentration of 30.6±46.9 U/ml). Of note, in the disease control group, 2 RA patients (0.8%; 18.9±9.8 U/ml) were positive for anti-Rib-P autoantibodies and none of the healthy controls tested positive for this antibody. In respect to anti-Sm autoantibodies, the whole SLE group presented a mean concentration of 2.8±13.8 U/ml, with 12 positive samples (9.4%; mean concentration of 31.1±40.8 U/ml), significantly greater than the concentration obtained in the healthy control group (0.02±0.11 U/ml; \(p=0.028\)) and in the rheumatic disease control group (0.1±0.3 U/ml; \(p=0.035\)). We only found positive results for anti-Sm autoantibodies in SLE patients. Regarding anti-dsDNA autoantibodies, SLE patients also presented a significantly greater mean concentration (44.6±73.8 U/ml) compared to the healthy control group (3.5±8.1 U/ml; \(p<0.001\)) or the rheumatic disease control group (2.6±4.2 U/ml; \(p<0.001\)). Sixty three (49.6%) SLE samples were positive for anti-dsDNA in a mean concentration of 88.4±88.5 U/ml. In the healthy control group 6 samples (6.0%) and, in the rheumatic disease control group 5 samples (2.0%) were found to be positive for this autoantibody, being one RA, one JIA and three AS samples. These results are summarized in Table 2. The tests
performance was evaluated and the outcome was as follow: anti-rib-P had 14.2% sensitivity, 99.4% specificity, 23.7 positive likelihood ratio (LR⁺) and 0.86 negative likelihood ratio (LR⁻); anti-Sm had 9.4% sensitivity, 100% specificity, LR⁺=∞ and 0.90 LR⁻; anti-dsDNA had 49.6% sensitivity, 96.9% specificity, 16 LR⁺ and 0.52 LR⁻.

Only 2 samples (1.6%) were positive for the three tested autoantibodies, while 12 (9.4%) were positive for both anti-Rib-P and anti-dsDNA and 7 (5.5%) were positive for both anti-Sm and anti-dsDNA. A cross positivity for anti-Rib-P and anti-Sm was not observed (Table 3).

The relationship between clinical variables and anti-Rib-P, anti-Sm and anti-dsDNA autoantibodies was further analyzed in SLE patients (Table 4).

Anti-Rib-P levels were related at a p-value ≤ 0.20 in univariate analysis with age (β=-0.125), Caucasian ethnicity (β=-0.190), erythrocyte sedimentation rate (ESR) (β=0.175), disease activity (SLEDAI2K) (β=0.154), the presence of malar rash (β=0.142), renal disorder (β=0.153), hematologic disorder (β=0.130) and current corticosteroid dosage (β=0.119), and these variables were included in a multivariate model. Caucasian ethnicity (β=-0.190, p=0.034) was the solely factor independently associated with anti-Rib-P levels in SLE patients (Table 4). Anti-Rib-P antibodies were not associated with previous neurologic disorder (seizure or psychosis), neither with the occurrence of neuropsychiatric lupus manifestations within the subsequent three years of follow-up.

The variables potentially associated with anti-Sm levels from the univariate analysis (at a p-value ≤ 0.20) were Caucasian ethnicity (β=-0.060), ESR (β=0.203), C-reactive protein (CRP) (β=0.372), SLEDAI2K (β=0.125), the presence of malar rash (β=-0.138), photosensitivity (β=0.148), serositis (β=0.277), renal disorder (β=0.176), the presence of anti-RNP antibodies (β=0.304), current corticosteroid dosage (β=0.164), and the use
of immunosuppressants (β=0.209). Higher CRP levels (β=0.304, p=0.003), serositis (β=0.321; p=0.002), and previous positivity for anti-RNP antibodies (β=0.297; p=0.003) were found to be independently associated with anti-Sm levels in SLE patients (Table 4).

For anti-dsDNA levels, age (β=-0.237), age at disease onset (β=-0.169), disease duration (β=-0.176), ESR (β=0.187), SLEDAI2K (β=0.413), arthritis (β=-0.150), renal (β=0.287), hematologic (β=0.259) and immunologic disorders (β=0.186), and corticosteroids current dosage (β=0.130) came out as candidate predictors for higher anti-dsDNA levels (at a p-value ≤ 0.20 in univariate analysis). After multivariate analysis shorter disease duration (β=-0.246; p=0.005), SLEDAI2K (β=0.338; p<0.001) and renal disorder (β=0.252; p=0.004) were independently associated with anti-dsDNA levels (Table 4).

DISCUSSION

This work shows that anti-Rib-P protein autoantibodies are very specific for SLE diagnosis.

We have demonstrated that the presence of antibodies against ribosomal P proteins is very specific for SLE patients when compared either with healthy controls or with other rheumatic disease controls. Moreover, the test showed accurate results, both in specificity and sensitivity. However the choice of the most reliable test to determine these autoantibodies requires a comparative study between different tests and the study of a larger and multiethnic population.
Beyond the determination of anti-Rib-P autoantibodies, we have used a detection method based on identical principles to determine anti-Sm and anti-dsDNA autoantibodies in the same population. Both anti-Sm and anti-dsDNA antibodies are also very specific for SLE patients [21-23]. However, anti-dsDNA antibodies were present in low levels in 6% of healthy controls and 2% of rheumatic disease controls samples.

The kit that we have used for the determination of anti-Rib-P protein is an EliA test, a fluorescence enzyme immunoassay (FEIA) designed as a sandwich immunoassay, with a mixture of the three Rib-P antigens (P0, P1 and P2), which has been previously described to have high sensitivity and specificity [7, 11, 24]. Even though, we have looked at the ROC curves in order to check the accuracy of this kit for the Portuguese population. The ROC curves can be used to evaluate the diagnostic performance of a test, adjusting to the study population, and determining the capability of a test to allow distinguishing between the positive group and the control group [25, 26]. We have adjusted the cut-off values for both anti-Rib-P and anti-Sm, respectively, to 4.45 U/ml and 3.4 U/ml. These values corresponded to the lowest concentration that allows the highest possible sensitivity without losing specificity, establishing a cut-off value for SLE patients in comparison to healthy controls and to rheumatic disease controls. For anti-dsDNA determination the cut-off value used in subsequent analyses was the one established by the manufacturer, as this value (15 U/ml) gave the best combination of sensitivity and specificity.

The cut-off confirmation must be done when using a new kit or when testing an existing kit in a different population and the adjusted values might be either higher or
lower than the ones established by the manufacturer, as confirmed by the work of Mahler M. and colleagues [12].

Our results have demonstrated increased levels of all the three autoantibodies in SLE patients, as well as a higher percentage of positive samples for at least one of the autoantibodies in the SLE patients group. Although anti-dsDNA autoantibodies were present in more SLE patients as compared to patients with other diagnosis, the presence of anti-Rib-P or anti-Sm was more specific for SLE diagnosis.

We have reviewed the clinical chart of the non-SLE patients that presented a positive result for either anti-Rib-P or anti-dsDNA antibodies (there are none for anti-Sm). Both non-SLE positive samples for anti-Rib-P were detected in RA patients; one of them presented some lupus-like characteristics (skin rash, leucopenia, and aphthous ulcer) at some point of the disease course and could be classified as an overlap RA/SLE. Interestingly, a similar case was previously reported, referring to a positive anti-Rib-P RA patient that latter on developed renal disease, evolving into a full-blown SLE [11]. None of the healthy controls or the rheumatic disease controls that were positive for anti-dsDNA autoantibodies presented any lupus-like characteristics.

We have found that the only independent association that came up from our multivariate analysis of the results of anti-Rib-P antibodies determination in SLE patients was the ethnicity: lower anti-Rib-P levels were present in individuals of Caucasian ethnicity. To the best of our knowledge no previous reports have established this association. However, given the small number of non-Caucasian patients, these findings need to be replicated in larger SLE populations.

Many studies have reported a relationship between the presence of anti-Rib-P antibodies and some clinical features, namely malar rash, renal involvement and the
occurrence of neuropsychiatric events, particularly psychosis [8, 11, 27, 28]. However, there are also reports that corroborate our findings of an absence of association between the presence of anti-Rib-P antibodies and clinical manifestations or disease activity [7, 13, 16]. In addition, our analysis differed from previous reports because we also took into consideration anti-Rib-P levels.

The positivity for Rib-P was not associated with previous neuropsychiatric manifestations classifiable in the ACR criteria [29, 30] and the presence of these autoantibodies did not reveal a predictive value regarding the occurrence of neuropsychiatric manifestations in the following three years. Thus, these autoantibodies are very specific for SLE but their value for the diagnosis of neuropsychiatric lupus seems to be limited, also because both anti-Rib-P positivity and neuropsychiatric manifestations are relatively rare.

However, given the high specificity, the inclusion of these autoantibodies as part of the SLE classification criteria might be useful. For that further studies encompassing a larger and multiethnic population are needed. Besides the use in established SLE, it will be important to assess the performance of this test in early disease to confirm whether the inclusion of anti-Rib-P testing can improve SLE diagnostic accuracy.

A multivariate analysis was performed for the levels of anti-Sm and anti-dsDNA, revealing some associations with disease features. Serositis and CRP levels were positively associated with higher anti-Sm levels. High CRP levels are usually associated with an ongoing infection in SLE patients, although they have been also associated with serositis, independently of the existence of an infection [31, 32]. This is in line with our results, as we have verified that CRP levels are increased in patients with
serositis (p = 0.047). The association between serositis and anti-Sm antibodies is in contradiction with a previous report from Wang C.L. and co-workers [33].

In the multivariate analysis anti-RNP levels were found to be independently associated with anti-Sm levels. Both anti-Sm and anti-RNP antibodies recognize complexes that contain small nuclear RNA species and the occurrence of anti-Sm antibodies when anti-RNP antibodies are also present has been previously reported [34]. Our observations regarding anti-dsDNA are in line with other classical findings depicting an association with renal involvement, as well as the relation to lower disease duration and higher disease activity [35].

We have also evaluated the crossed positivity for the three studied autoantibodies and verified that 78% of the anti-Rib-P positive patients were also positive for one or for the two other antibodies determined. Previous studies have also demonstrated that the presence of anti-Rib-P antibodies is often associated with anti-dsDNA antibodies, but the simultaneous presence of anti-Rib-P and anti-Sm is not consensual [2, 8, 11, 13]. However, 3.1% of the SLE patients (22% among anti-Rib-P positive patients) were only positive for anti-Rib-P autoantibodies. We have reviewed the clinical records of these four patients and found no particular clinical features in common.

**CONCLUSIONS**

The existence of anti-Rib-P antibodies in anti-DNA and anti-Sm negative patients, might constitute an argument for taking into consideration these autoantibodies for SLE diagnosis, as previously commented by Mahler M. and colleagues [10]. According to what other authors have previously suggested, and considering that these
Classification criteria are constantly subject to confirming and re-evaluation studies, as recently published by Petri M. and colleagues [36], we propose the formal evaluation of anti-Rib-P as candidates to be included in the immunologic disorder component of the ACR criteria for the classification of SLE.
COMPETING INTERESTS

Laboratory expenses were supported by Phadia® (now Thermo Fisher Scientific).
AUTHOR’S CONTRIBUTIONS

DCF was involved in the study design and performed the lab work, data analysis and manuscript writing; MJS, HC and JEF were involved in the study design, data acquisition, interpretation, and analysis and critically revising the article. All authors read and approved the final manuscript.
AKNOWLEDGEMENTS

Technical advice was given by Dr. Teresa Araújo and Dr. Luísa Dupont, both Phadia® (now Thermo Fisher Scientific) collaborators.

We wish to thank Dr. Elsa Vieira-Sousa, Dr. Filipa Mourão and Dr. Ana Maria Rodrigues for helping with data acquisition.
REFERENCES


2. Heinlen LD, Ritterhouse LL, McClain MT, Keith MP, Neas BR, Harley JB, James JA: **Ribosomal P autoantibodies are present before SLE onset and are directed against non-C-terminal peptides.** *J Mol Med* 2010, **88:**719-727.


5. Hochberg MC: **Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus.** *Arthritis Rheum* 1997, **40:**1725.


### Table 1. Demographic characteristics of the studied populations.

<table>
<thead>
<tr>
<th></th>
<th>SLE patients</th>
<th>Healthy controls</th>
<th>Rheumatic disease controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>43.6±14.1</td>
<td>51.7±13.</td>
<td>41.9±10.</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>120 (96.0)</td>
<td>91 (91.9)</td>
<td>99 (99.0)</td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>110 (88.0)</td>
<td>98 (99.0)</td>
<td>90 (90.0)</td>
</tr>
</tbody>
</table>

Values represent mean ± standard deviation for years and proportions for categorical variables.

SLE - Systemic Lupus Erythematosus; RA - Rheumatoid Arthritis; JIA - Juvenile Idiopathic Arthritis; AS - Ankylosing Spondylitis; PsA - Psoriatic Arthritis.
### Table 2. Anti-Rib-P, anti-Sm and anti-dsDNA quantification results.

<table>
<thead>
<tr>
<th></th>
<th>SLE patients</th>
<th>Healthy controls</th>
<th>Rheumatic disease controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-Rib-P (U/ml)</strong></td>
<td>4.9±20.2</td>
<td>0.07±0.21</td>
<td>0.6±1.8</td>
</tr>
<tr>
<td>(p-value)</td>
<td></td>
<td>(p=0.016)</td>
<td>(p=0.017)</td>
</tr>
<tr>
<td><strong>Anti-Rib-P(+)</strong>, n (%)</td>
<td>18 (14.2%)</td>
<td>0 (0%)</td>
<td>2 (0.8%)</td>
</tr>
<tr>
<td>(p-value)</td>
<td></td>
<td>(p&lt;0.001)</td>
<td>(p&lt;0.001)</td>
</tr>
<tr>
<td><strong>Anti-Sm (U/ml)</strong></td>
<td>2.7±13.8</td>
<td>0.02±0.11</td>
<td>0.1±0.3</td>
</tr>
<tr>
<td>(p-value)</td>
<td></td>
<td>(p=0.028)</td>
<td>(p=0.035)</td>
</tr>
<tr>
<td><strong>Anti-Sm(+)</strong>, n (%)</td>
<td>12 (9.4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>(p-value)</td>
<td></td>
<td>(p&lt;0.001)</td>
<td>(p&lt;0.001)</td>
</tr>
<tr>
<td><strong>Anti-dsDNA (U/ml)</strong></td>
<td>44.6±73.8</td>
<td>3.5±8.1</td>
<td>2.6±4.2</td>
</tr>
<tr>
<td>(p-value)</td>
<td></td>
<td>(p&lt;0.001)</td>
<td>(p&lt;0.001)</td>
</tr>
<tr>
<td><strong>Anti-dsDNA(+)</strong>, n (%)</td>
<td>63 (49.6%)</td>
<td>6 (6.0%)</td>
<td>5 (2.0%)</td>
</tr>
<tr>
<td>(p-value)</td>
<td></td>
<td>(p&lt;0.001)</td>
<td>(p&lt;0.001)</td>
</tr>
</tbody>
</table>

Values represent mean ± standard deviation for concentrations in U/ml and proportions of positives. Differences were assessed using T-test for continuous variables or χ² or Fisher’s exact test for proportions.

SLE- Systemic Lupus Erythematosus.
### Table 3. Cross positivity for the three determined autoantibodies in SLE patients (anti-Rib-P, anti-Sm and anti-dsDNA).

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>57</td>
<td>44.9%</td>
</tr>
<tr>
<td>Anti-Rib-P (+) only</td>
<td>4</td>
<td>3.1%</td>
</tr>
<tr>
<td>Anti-Sm (+) only</td>
<td>3</td>
<td>2.4%</td>
</tr>
<tr>
<td>Anti-dsDNA (+) only</td>
<td>42</td>
<td>33.1%</td>
</tr>
<tr>
<td>Anti-Rib-P (+) &amp; Anti-Sm (+)</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Anti-Rib-P (+) &amp; Anti-dsDNA (+)</td>
<td>12</td>
<td>9.4%</td>
</tr>
<tr>
<td>Anti-Sm (+) &amp; Anti-dsDNA (+)</td>
<td>7</td>
<td>5.5%</td>
</tr>
<tr>
<td>The 3 autoantibodies (+)</td>
<td>2</td>
<td>1.6%</td>
</tr>
</tbody>
</table>
**Table 4.** Clinical variables associated with anti-Rib-P, anti-Sm and anti-dsDNA levels in SLE patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Anti-Rib-P</th>
<th>Anti-Sm</th>
<th>Anti-dsDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β coefficient (p-value)</td>
<td>β coefficient (p-value)</td>
<td>β coefficient (p-value)</td>
</tr>
<tr>
<td>Ethnicity (Caucasian)</td>
<td>-0.190 (p=0.034)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.304 (p=0.003)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serositis* (yes)</td>
<td>0.321 (p=0.002)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-RNP positive</td>
<td>0.297 (p=0.003)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td></td>
<td>-0.246 (p=0.005)</td>
<td></td>
</tr>
<tr>
<td>SLEDAI2K</td>
<td></td>
<td></td>
<td>0.338 (p&lt;0.001)</td>
</tr>
<tr>
<td>Renal disorder* (yes)</td>
<td></td>
<td></td>
<td>0.252 (p=0.004)</td>
</tr>
</tbody>
</table>

* according to ACR classification criteria

ESR – erythrocyte sedimentation rate; CRP – C-reactive protein; SLEDAI2K – Systemic lupus erythematosus disease activity index 2000; anti-RNP – anti-ribonucleoprotein.

Multivariate analysis results from multiple linear regression analysis. The total explained variance of the model is (‡) $R^2=0.036$, (§) $R^2=0.325$, and (*) $R^2=0.270$. 
FIGURE LEGENDS

Figure 1. Receiver operating characteristic (ROC) curves for the three antibodies quantified: anti-Rib-P (A), anti-Sm (B) and anti-dsDNA (C). The curves represent the sensitivity and specificity for SLE patients vs healthy controls and for SLE patients vs rheumatic disease controls. For each curve is indicated the area under the curve (AUC) and correspondent p-values. □ points the cut-off established by the manufacturer and * points the new cut-off calculated from the curves (4.45 U/ml for anti-Rib-P and 3.4 U/ml for anti-Sm antibodies; anti-dsDNA does not change).
Systemic Lupus Erythematosus vs Healthy controls

**A** Anti-rib-P antibodies

AUC = 0.800  
$p < 0.001$

Systemic Lupus Erythematosus vs Rheumatic disease controls

**A** Anti-rib-P antibodies

AUC = 0.600  
$p = 0.001$

**B** Anti-Sm antibodies

AUC = 0.857  
$p < 0.001$

**B** Anti-Sm antibodies

AUC = 0.648  
$p < 0.001$

**C** Anti-dsDNA antibodies

AUC = 0.826  
$p < 0.001$

**C** Anti-dsDNA antibodies

AUC = 0.845  
$p < 0.001$