Clinical and diagnostic value of ribosomal P autoantibodies in systemic lupus erythematosus

Samy Haddouk1, Sameh Marzouk2, Moez Jallouli2, Hajer Fourati1, Makram Frigui2, Youssef B. H. Hmida1, Faten Koubaa1, Wassim Sellami3, Sofiene Baklouti4, Jamil Hachicha5, Zouheir Bahloul2 and Hatem Masmoudi1

Objective. To analyse prospectively the diagnostic sensitivity and specificity as well as the clinical relevance of ribosomal P (anti-P) autoantibodies in a large cohort of SLE patients.

Methods. The anti-P autoantibodies were evaluated in the serum of 200 Tunisian SLE patients at disease onset and 130 various control subjects by a sensitive immunodot assay. A complete laboratory evaluation and clinical examination were performed in each SLE patient. During the follow-up, the patients were regularly monitored for clinical parameters. Global SLE activity was measured by the ECLAM.

Results. The sensitivity and specificity of anti-P testing for SLE were 23.5 and 98.4%, respectively. The anti-P-positive samples 14/47 (29.8%), 27/47 (57.4%) and 5/47 (10.6%) were negative for anti-dsDNA, anti-Sm or both antibodies, respectively. The anti-P-positive patients showed more active disease activity and a much higher prevalence of arthritis. An association between IgG aCLs and anti-P antibodies was also found. However, anti-P antibodies were not associated with neuropsychiatric manifestations or lupus nephritis.

Conclusion. This study does not seem to confirm the described association of anti-P antibodies with neuropsychiatric manifestations of SLE. However, it supports the anti-P antibody association with arthritis and disease activity as well as the presence of aCL. Based on our study and other related studies, we propose that, akin to anti-Sm and anti-dsDNA, anti-P antibodies detected by one agreed method may be considered for inclusion as a criterion for the classification of SLE.

KEY WORDS: Anti-ribosomal P antibodies, Systemic lupus erythematosus, Sensitivity, Specificity, Clinical associations.

Introduction

Although antibodies to ribosomal P (anti-P) antibodies have only been recognized since 1985 [1], many recent research developments have served to highlight the special significance of these autoantibodies. These autoantibodies occur very specifically in the disease SLE [2]. The molecular target of these autoantibodies are three highly conserved ribosomal phosphoproteins of molecular mass 38 (P0), 19 (P1) and 17 (P2) kDa, respectively. A major immunodominant epitope shared by these three proteins is the carboxy terminal 22 amino acids [3].

The reported prevalence of anti-P antibodies in SLE population ranges from 6 to 46%; it is higher in Asian patients and at a relatively lower prevalence in black and Caucasian patients [4–7]. Although the association between anti-P antibodies and some clinical SLE manifestation such as hepatitis and nephritis has been suggested in some studies [8, 9], interest in these autoantibodies mainly derives from the finding by Bonfa et al. [2, 10] of an association between anti-P antibodies and NPSLE. Some groups have disputed this relationship [11–13] while others have confirmed it [4, 14–17].

The ethnic origin of the patients may partly explain these conflicting results, but several other reasons may account for these discrepancies [18, 19]. Indeed, in some investigations the low number of enrolled patients did not permit reliable evaluation of the data of the few anti-P-positive subjects. On the other hand, the majority of the studies carried out on a large number of patients were retrospective, thus making the evaluation of the simultaneous presence of anti-P antibodies and certain clinical and/or laboratory findings difficult. Furthermore, differences in the methods used for detection of anti-P antibodies may have influenced the results.

The aim of our study is to evaluate the sensitivity and specificity of anti-P antibodies in a large cohort of Tunisian SLE patients at disease onset, before any drug regimen, and to assess a correlation between anti-P antibodies, clinical parameters and SLE disease activity.

Patients and methods

Patients

In this prospective study, 200 consecutive unselected SLE patients were recruited in several clinical units (Medicine, Nephrology and Rheumatology) at the University Hospital in the city of Sfax, Tunisia. Twenty-seven were men and 173 were women. The median age at the time of diagnosis was 30.5 ± 11.7 (range 11–67) years. All patients fulfilled the revised criteria of the ACR for classification of SLE [20]. Drug-induced SLE was excluded.

At time of diagnosis, a complete laboratory evaluation and clinical examination of each patient were performed. During the follow-up, the patients were regularly monitored (every 3 months) for clinical evaluation. Cumulative clinical manifestations up to the time of assessment were included. The mean duration of the follow-up was 5.11 ± 2.9 years (range 6 months to 11 years).

Clinical features were defined according to the ACR criteria [20]. Active neuropsychiatric lupus was diagnosed according to ACR guidelines [21]. Disease activity of SLE was evaluated according to the ECLAM score [22], only at time of diagnosis.

Blood samples were drawn from SLE patients at the time of diagnosis. Sera from 20 healthy subjects (HSS) and from 110 various control diseases including RA (n = 50), SSc (n = 30) and SS (n = 30) were used as controls.

All sera were kept at −20°C until analysis. The study was conducted after approval by the local ethics committee (the ethics committee of the Habib Bourguiba Hospital of Sfax, Tunisia). Informed consent was obtained from all patients.
Assay for anti-P antibodies

Anti-P antibodies were evaluated by a specific immunodot assay (Alphadia, Waver, Belgium), in which the corresponding antigen is a purified ribosomal P protein from a native source. The test is based on the principle of qualitative enzyme immunoassay. Similar test has been previously used and validated [23, 24].

The test strip is composed of a membrane fixed on plastic support. During test procedure, the strips are incubated with diluted patients’ sera. After washing, the strips were incubated with ALP-labelled protein A, and subsequently with BCIP/NBT substrate for colour development. A sample is positive for anti-P antibody if the colour intensity of the corresponding antigen dot is higher than the intensity of the cut-off dot, otherwise it is considered as negative.

After anti-P antibodies were assayed, the SLE patients were divided into two groups: group with anti-P antibodies and group without anti-P antibodies.

Serological evaluations for SLE patients

ANA and anti-dsDNA antibodies were detected by IIF procedures using HEp-2 cell (Alphadia) and Crithidia luciliae (Binding Site, Birmingham, UK) substrates, respectively. ANA titres \( \geq 1 : 160 \) and anti-dsDNA titres \( > 1 : 20 \) were considered positive.

Anti-Sm, anti-Sm/RNP, anti-SSA/Ro and anti-SSB/La were analysed by the Euroline ANA-profile 1 (Euroimmun, Lübeck, Germany) as described previously [25].

Each patient was also assayed for IgG aCLs by ELISA (Binding Site).

Statistics

Data were analysed using SPSS (Chicago, IL, USA). The Student’s t-test was used for comparison of ECLAM score indices, expressed as mean ± s.d. Clinical and serological parameters were analysed using conventional chi-square test \( (\chi^2) \) or Fisher’s exact test. We computed odds ratios (ORs) and their 95% CI.

\( P \)-values <0.05 were considered statistically significant.

Results

When sera from SLE patients \( (n = 200) \) and various controls \( (n = 130) \) were assayed for anti-P antibodies, a clear discrimination between SLE patients and various controls was found (Fig. 1). The sensitivity and the specificity of anti-P antibodies for the diagnosis of SLE were 23.5 and 98.4%, respectively. Statistical evaluation by chi-square analysis and Fisher’s exact test showed a statistically relevant difference between SLE patients and various controls \( (P < 0.0001; \chi^2 = 28.34; \text{OR} = 19.66; 95\% \text{ CI} = 4.56, 119.37) \).

ANA, anti-dsDNA, anti-Sm, anti-Sm/RNP, anti-SSA/Ro and anti-SSB/La were found in 98.5, 63.5, 33.5, 32, 56.5 and 22% of SLE patients, respectively.

Remarkably, 14/47 (29.8%), 27/47 (57.4%) and 5/47 (10.6%) anti-P-positive samples were negative for anti-dsDNA, anti-Sm or both antibodies, respectively.

Epidemiological data, analysed according to anti-P serum reactivity, did not show statistical differences between the two patient groups according to sex and age at the time of diagnosis. Mean age at disease onset of anti-P+ and anti-P− patients did not differ statistically. However, it is of interest to note that anti-P antibodies were more frequently found in patients younger than 18 years old (30.7 vs 22.4% for adult patients) even though this difference was not statistically significant.

At the unifactorial analysis, the comparison of the clinical findings included in the ACR SLE diagnostic criteria between the two groups of patients demonstrated a much higher prevalence of arthritis in the history of anti-P+ patients (83 vs 42.5%; \( P < 0.0001; \chi^2 = 23.62; \text{OR} = 6.60; 95\% \text{ CI} = 2.73, 16.48) \). None of the other characteristic clinical manifestations of SLE differed in their rate of occurrence between patients with and without anti-P antibodies. No differences were observed between the anti-P+ and anti-P− patients populations when other clinical features were analysed. However, one patient with lupus hepatitis whom we found in our cohort was anti-P+ (Table 1).

As Table 1 shows, we found a weak correlation of anti-P antibodies with disease activity that is expressed by an ECLAM score significantly higher in patients with anti-P antibodies \( (6.97 \pm 1.98 \text{ vs } 6.14 \pm 2.20; P = 0.021) \).

Table 2 lists the prevalence of other autoantibodies in the SLE patients with or without anti-P antibodies. As seen, the prevalence of anti-dsDNA, anti-Sm and anti-Ro/SSA was greater in the anti-P-positive patients, but statistical tests did not find a significant difference. However, the prevalence of IgG antibodies to cardio-lipin was significantly associated with anti-P antibodies \( (P = 0.006; \chi^2 = 7.53; \text{OR} = 2.50; 95\% \text{ CI} = 1.21, 5.17) \).

Discussion

Although most studies have found that anti-P antibodies are highly specific for SLE, they have yet to achieve the clinical impact that anti-Sm or anti-dsDNA antibodies have [26–28]. This might be explained by the limited reliability of IIF assays for the detection of anti-P antibodies or by the absence of an international reference serum [27].

Our study, with a specificity of 98.4%, confirms that anti-P antibodies are highly specific for patients with SLE. In a recent international multicenter study of 947 SLE patients and 1113 controls, the high disease specificity (99.3%) of anti-P antibodies was clearly demonstrated [27]. Of interest, in one patient initially diagnosed with RA, anti-P antibodies predicted the later clinical conversion into definite SLE [26, 27]. We cannot exclude this hypothesis in the two RA patients with anti-P antibodies isolated in our study.

Additionally, in the same study, 52/143 (36.3%) of anti-P-positive samples have been reported as anti-dsDNA negative. This observation is in keeping with the findings of the present study in which a significant portion of anti-P-positive samples show no anti-dsDNA reactivity. In patients who have anti-P antibodies but no antibodies to dsDNA or Sm, confirmatory serology may be missing and this in turn could result in an unfortunate delay of diagnosis and treatment of such patients.

Using a sensitive assay at disease onset, we found that the frequency of anti-P antibodies was 23.5% in 200 unselected consecutive patients with SLE, which is in line with the reported prevalence of 20% in a study of randomly selected Caucasian SLE patients [13]. Some studies have shown that different ethnic
Anti-P antibodies in SLE

Table 1. The prevalence of clinical features in the SLE patients with or without anti-P antibodies

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Group with anti-P antibodies, n = 47</th>
<th>Group without anti-P antibodies, n = 153</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at SLE onset, mean (range)</td>
<td>30.6 (14–61)</td>
<td>30.5 (11–67)</td>
<td>0.95</td>
</tr>
<tr>
<td>General symptoms, n (%)</td>
<td>25 (53.2)</td>
<td>82 (53.6)</td>
<td>0.96</td>
</tr>
<tr>
<td>Malar rash, n (%)</td>
<td>27 (57.4)</td>
<td>83 (54.2)</td>
<td>0.70</td>
</tr>
<tr>
<td>Photosensitivity, n (%)</td>
<td>26 (55.3)</td>
<td>73 (47.7)</td>
<td>0.36</td>
</tr>
<tr>
<td>Alopecia, n (%)</td>
<td>13 (27.7)</td>
<td>39 (25.5)</td>
<td>0.76</td>
</tr>
<tr>
<td>Oral and nasopharyngeal ulcers, n (%)</td>
<td>6 (12.8)</td>
<td>14 (9.1)</td>
<td>0.65</td>
</tr>
<tr>
<td>Endocarditis, n (%)</td>
<td>3 (6.4)</td>
<td>7 (4.6)</td>
<td>0.91</td>
</tr>
<tr>
<td>Myocarditis, n (%)</td>
<td>2 (4.2)</td>
<td>4 (2.6)</td>
<td>0.93</td>
</tr>
<tr>
<td>RP, n (%)</td>
<td>5 (10.6)</td>
<td>19 (12.4)</td>
<td>0.74</td>
</tr>
<tr>
<td>Arthritis, n (%)</td>
<td>39 (83)</td>
<td>65 (42.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lupus nephritis, n (%)</td>
<td>28 (53.5)</td>
<td>97 (63.3)</td>
<td>0.31</td>
</tr>
<tr>
<td>Neurological involvement, n (%)</td>
<td>18 (38.3)</td>
<td>47 (30.7)</td>
<td>0.33</td>
</tr>
<tr>
<td>Central</td>
<td>8 (17)</td>
<td>19 (12.4)</td>
<td>0.42</td>
</tr>
<tr>
<td>Peripheral</td>
<td>3 (6.4)</td>
<td>10 (6.5)</td>
<td>0.76</td>
</tr>
<tr>
<td>Psychiatric, n (%)</td>
<td>7 (14.9)</td>
<td>18 (11.5)</td>
<td>0.57</td>
</tr>
<tr>
<td>Psychosis</td>
<td>1 (2.1)</td>
<td>2 (1.3)</td>
<td>0.55</td>
</tr>
<tr>
<td>Anxiety disorders</td>
<td>1 (2.1)</td>
<td>16 (10.4)</td>
<td>0.40</td>
</tr>
<tr>
<td>Pericarditis, n (%)</td>
<td>9 (19.1)</td>
<td>30 (18.6)</td>
<td>0.94</td>
</tr>
<tr>
<td>Pleural effusion, n (%)</td>
<td>9 (19.1)</td>
<td>21 (13.7)</td>
<td>0.36</td>
</tr>
<tr>
<td>Haematological disorders*, n (%)</td>
<td>38 (80.1)</td>
<td>130 (85)</td>
<td>0.50</td>
</tr>
<tr>
<td>Liver involvement, n (%)</td>
<td>1 (2.1)</td>
<td>0 (0)</td>
<td>0.23</td>
</tr>
<tr>
<td>Sicca syndrome, n (%)</td>
<td>5 (10.6)</td>
<td>14 (9.1)</td>
<td>0.98</td>
</tr>
<tr>
<td>ECLAM score, mean ± s.d.</td>
<td>6.97 ± 1.98</td>
<td>6.15 ± 2.20</td>
<td>0.024</td>
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</tbody>
</table>

*Anaemia and/or leucopenia and/or lymphopenia and/or thrombocytopenia.

Table 2. The prevalence of autoantibodies in the SLE patients with or without anti-P antibodies

<table>
<thead>
<tr>
<th>Autoantibodies</th>
<th>Group with anti-P antibodies, n = 47</th>
<th>Group without anti-P antibodies, n = 153</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-dsDNA, n (%)</td>
<td>33 (70.2)</td>
<td>94 (61.4)</td>
<td>0.27</td>
</tr>
<tr>
<td>Anti-Sm, n (%)</td>
<td>20 (42.6)</td>
<td>47 (30.7)</td>
<td>0.13</td>
</tr>
<tr>
<td>Anti-Sm/RNP, n (%)</td>
<td>18 (38.3)</td>
<td>46 (30.1)</td>
<td>0.28</td>
</tr>
<tr>
<td>Anti-SSA/Ro, n (%)</td>
<td>30 (63.3)</td>
<td>84 (54.2)</td>
<td>0.24</td>
</tr>
<tr>
<td>Anti-SSB/La, n (%)</td>
<td>9 (19.2)</td>
<td>35 (22.9)</td>
<td>0.58</td>
</tr>
<tr>
<td>IgG aCL, n (%)</td>
<td>29 (61.7)</td>
<td>60 (39.2)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

backgrounds may influence the frequency with which anti-P occurs in SLE patients, ranging from 6 to 46% [18]. In most of the ethnic groups (Whites, Blacks and Hispanics), anti-P antibodies have been found in 6–20%, in contrast to the Chinese and Japanese groups where 36 and 46% were reported to be positive, respectively [27, 29, 30]. In a group of Chinese SLE patients from Malaysia who have high prevalence of anti-P antibodies, an increased frequency of an HLA-DRB gene allele DR16X was found [31]. This allele has only been found in the South East Asian population, which could explain the high prevalence of anti-P antibodies in SLE patients from this region. These observations lead to the conclusion that prevalence results are dependent in part on the ethnic origin of the study patients.

Age differences may also be a factor, since anti-P antibodies have been found more frequently with younger onset SLE [32]. In our study, although mean age was not lower in the group with anti-P antibodies, we found that young patients (under the age of 18 years) had greater frequency of anti-P antibodies.

The variation in the observed frequency may also depend on the test system used to detect the autoantibodies. Results can vary across techniques. Nowadays, immunoenzymetric methods (ELISA, immunoblot, immunodot, etc.) have the highest sensitivity and specificity [33]. We used an immunodot technique in which the antigen is a purified native protein comprising the epitope shared by the three ribosomal phosphoproteins P0, P1 and P2.

In our study, disease activity score, evaluated by the ECLAM scoring system, is slightly more elevated in anti-P+ patients than in anti-P– patients; this result is in agreement with other previously published studies [4, 15, 34, 35]. These observations may support the concept that the presence of circulating anti-P antibodies characterizes a subset of SLE patients with a persistently more active disease, but they do not clarify whether these autoantibodies are in fact associated with more active disease.

In this regard, some reports showed that anti-P antibodies are associated with lupus nephritis [36, 37]. The fact that our prospective study did not confirm these data, as other studies [34, 35, 38], does not rule out that the possible serum peaks of anti-P antibodies may correlate with high anti-dsDNA titres and development of lupus nephritis, as suggested in some studies [39, 40].

We were unable to demonstrate any association between the presence of anti-P antibodies in the sera of SLE patients and NPSLE as described by others [9–11, 16, 17]. In fact, the lack of association of anti-P antibodies with neurological disorders, cognitive impairment or both is in agreement with the majority of the studies published up until now, since positive associations with cognitive dysfunctions or organic CNS involvement without psychiatric disease have been, respectively, never or only sporadically reported [15–17, 41, 42]. On the contrary, the lack of association with psychiatric disorders contrasts with the findings of a number of previous investigations. However, most of the recent reports conclude that anti-P antibody testing has negligible diagnostic utility for NPSLE overall or for particular neuropsychiatric presentations of SLE [30, 34, 38, 43] and we believe that these data deserve a more accurate analysis.

We found a strong association between anti-P antibodies and arthritis. Our findings are consistent with those of a multicentre study conducted in France [23] to investigate the clinical significance of anti-P antibodies in general and anti-P antibodies in particular. This study found a significant association between anti-P antibodies and arthritis but no correlation with other specific clinical manifestations of SLE. This original association points out the necessity of further research and encourages the design of studies that address the issue of whether anti-P antibodies participate in the development of arthritis.

Another intriguing clinical association with anti-P antibodies which has been described in the past is that of lupus hepatitis, a rare clinical manifestation of SLE [8, 44]. In our series, we found only one patient with evidence of liver involvement apparently due to the systemic autoimmune disease. He had
categorizing Anti-P antibodies, thus supporting the idea that these autoantibodies may play a pathogenic role in liver damage [45, 46].

We found a greater frequency of anti-dsDNA, anti-Sm and anti-SSA reactivities in anti-P- SLE patients although the difference with the anti-P group did not reach statistical significance while association with anti-CL was statistically significant. Coexistence and cross-reactivity of anti-dsDNA or anti-Sm and anti-P antibodies have been previously reported [47, 48]. The fact that we did not find a significant association of anti-P antibodies with either anti-dsDNA or anti-Sm, as shown in some previously published studies [27, 35, 40], may be due to methodological differences in antibody detection.

Association between anti-P antibodies and anti-SSA or anti-CL has previously been described [2, 34]. Whether these associations are also due to cross-reactivity remain speculative and require further research. Our findings are also similar to Ghirardello et al. [28] and Schneebaum et al. [49]. These authors found an association between anti-P and aCL antibodies, but could not establish a correlation with anti-dsDNA or with Sm antibodies. In conclusion, we found that anti-P reactivity in SLE patients is correlated significantly with arthritis, disease activity and IgG cardioliopin antibodies. With regard to the sensitivity >20% and the high predictive value of anti-P antibodies for SLE patients, we propose that the serological ACR criteria for the classification of SLE should be reconsidered and revised to include anti-P antibodies, detected by one agreed method, as a criterion.

Rheumatology key messages

- Anti-P antibodies are highly specific biomarkers for SLE.
- Anti-P antibodies are associated with arthritis, disease activity and aCL.
- Anti-P antibodies may be considered for inclusion as a criterion for SLE classification.

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References