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Clinical and serological associations of ribosomal P autoantibodies in systemic lupus erythematosus: prospective evaluation in a large cohort of Italian patients

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Abstract

Objective. To verify the association of ribosomal anti-P antibodies (anti-P), as detected by a sensitive ELISA, with serological findings and clinical manifestations, including neuropsychiatric involvement evaluated according to the American College of Rheumatology (ACR) nomenclature, in a large cohort of patients with systemic lupus erythematosus (SLE).

Methods. Anti-P were evaluated in the serum of 149 consecutive Italian SLE patients by an ELISA using a multiple antigen peptide carrying four copies of a common P0, P1 and P2 epitope. A complete laboratory evaluation and clinical examination were performed in each patient. In addition, all patients underwent an accurate neuropsychiatric and neuropsychological assessment performed by trained specialists according to the 1999 ACR suggestions.

Results. Serum anti-P were detected in 18/149 patients (12.1%). The anti-P prevalence was similar (11.7%) when the analysis was performed in a larger series of sera including 82 additional SLE patients, who were not included in the clinical study. The age of anti-P-positive patients at disease onset was less than 33 yr and, in comparison with the anti-P-negative patients, these patients showed more active disease activity and a higher prevalence of photosensitivity and malar and discoid rash. A strong association between IgG anticardiolipin antibodies and anti-P was also found. However, anti-P were associated with neither neuropsychiatric syndromes nor cognitive impairment.

Conclusion. This study does not seem to confirm the described association of anti-P with SLE neuropsychiatric manifestations. However, it supports the anti-P association with different skin manifestations as well as the presence of anticardiolipin in a subset of patients with SLE characterized by early disease onset.

KEY WORDS: Anti-P antibodies, Systemic lupus erythematosus, Anticardiolipin antibodies, Neuropsychiatric lupus.

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Ribosomal P-protein antibodies (anti-P) mainly react with three phosphoproteins P0, P1 and P2, having molecular masses of 38, 19 and 17 kDa, respectively [1–3]. These autoantibodies have been described almost

exclusively in the sera of subjects with SLE, but show a highly variable prevalence ranging from 6 to 46% [4–7]. It has been shown that different ethnic backgrounds and genetic factors can account, at least in part, for the wide range of anti-P frequencies among SLE patients [7, 8]. Although associations between anti-P antibodies and some clinical SLE manifestations such as hepatitis and nephritis [9–14] have been suggested in some studies, interest in these autoantibodies mainly derived from the finding by Bonfa *et al.* [15, 16] of an association between anti-P and neuropsychiatric manifestations of SLE. However, this association has not always been confirmed [4, 5, 14, 17–26]. The ethnic origin of the patients may partly explain these conflicting results, but several other reasons may account for these discrepancies [6, 27]. Indeed, in some investigations the low number of enrolled patients did not permit reliable evaluation of the data on 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, making difficult the evaluation of the simultaneous presence of anti-P and certain clinical and/or laboratory findings. Furthermore, differences in the methods used for detection of anti-P antibodies may have influenced the results. Data obtained using the two main techniques to detect anti-P antibodies, immunoblotting and enzyme-linked immunosorbent assay (ELISA), usually agree, although ELISA results may vary depending on the nature and purity of the antigen, carrier protein or coupling agent employed [1, 3, 6, 15, 16, 21, 28–32]. In this regard, it has been shown that the use of multiple antigen peptide, rather than simple peptide, improves the sensitivity of the ELISA test, allowing more precise correlations [28].

Another critical point to consider is the definition of central nervous system (CNS) involvement in SLE. It is surprising that the reported prevalence of general SLE neuropsychiatric manifestations or distinct syndromes varies greatly in the different studies, ranging from 10 to 75% [33–38]. Two main explanations have been offered for this variability: the lack of a standard terminology to define and classify neuropsychiatric lupus and the fact that such patients may not have been visited by a trained specialist in psychiatry and neuropsychology [36–38]. This consideration may assume particular relevance in clinical practice for the definition of psychiatric disorders and cognitive impairment in SLE patients with no evidence of organic CNS involvement. Recently, an international and multidisciplinary committee of the American College of Rheumatology (ACR) has proposed a classification of the neuropsychiatric manifestations seen in SLE, with specific recommendations for diagnostic tests, in order to overcome these problems [38].

Conflicting data on the association of anti-P antibodies and clinical manifestations and/or serological findings in SLE patients prompted us to carry out a prospective multicentre study in order to evaluate serum anti-P from a large series of consecutive Italian SLE patients. A sensitive ELISA method was used employing a multiple antigen peptide carrying four copies of a common epitope of P0, P1 and P2, as previously described [28]. Different epidemiological, clinical and laboratory items were simultaneously investigated in each patient and accurate psychiatric and neuropsychological examinations were performed by trained specialists according to the recommendations of the ACR ad hoc Committee on Neuropsychiatric Lupus Nomenclature [38]. Data were then processed by statistical analysis in order to evaluate serological and/or clinical associations.

Patients and methods

Patients

A total of 149 unselected consecutive patients, fulfilling the 1982 ACR criteria [39], modified in 1997 [40], for the classification of SLE, were included in the present study and followed up in five different Italian centres, all with clinical orientation in rheumatology and clinical immunology. Informed consent was obtained from each patient. Analysis of epidemiological, clinical and serological data between the groups of patients enrolled in each of the five clinical centres (51 patients enrolled in Brescia, 46 at the University of Milan, 20 in Perugia, 20 in Milan at the S. Raffaele University, and 12 in Ancona) did not show significant differences (data not shown), so results are presented as pooled data. Serum samples from 82 additional subjects with SLE were also tested only for anti-P in order to verify the prevalence of this autoantibody in a larger group of SLE patients.

The patients comprised 139 women and 10 men, all Caucasians. Mean age was 37.1 yr (range: 15–71 yr) at the time of inclusion in the study and 27.7 yr (8–58 yr) at disease onset, with a mean disease duration of 9.3 yr (1–28 yr) and an average of 10.6 yr (2–20 yr) of school education. At entry, family psychiatric history and current medications were evaluated in all patients; particular attention was paid to corticosteroid treatment and dosage. A blood sample was taken from each patient for serological determinations and all enrolled subjects underwent an accurate physical examination. ACR SLE criteria, met either during the course of the disease or at the time of inclusion in the study, were recorded in a computerized clinical chart. Other different clinical manifestations occurring in the last month before inclusion in the study and haematological results obtained for the same period were registered. In particular, clinical data referred to general complaints, with involvement of muscle, skin, eye, lung, heart, vessels, liver, kidney and peripheral and central nervous system. Haematological determinations evaluated possible anaemia, leucopenia (defined as $<4000/\mu$l white blood cells), thrombocytopenia ($<100\,000/\mu$l), hypocomplementaemia (plasma levels of C3 fraction below 79 mg/dl, as determined by nephelometry) and presence of lupus anticoagulant (as evaluated by Russell viper venom time). Disease activity of SLE was evaluated according to the European Consensus Lupus Activity Measurement (ECLAM) [41].

Each patient subsequently underwent a complete neuropsychiatric assessment performed by the referring trained psychiatrist of each centre in order to evaluate psychiatric syndromes according to the recommendations of the ACR ad hoc Committee on Neuropsychiatric Lupus Nomenclature [38]. In particular, psychiatric disorders were analysed adopting the terminology of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) in order to distinguish primary entities from secondary disease due to underlying medical conditions or drug abuse [42]. In addition, a trained clinical neuropsychologist assessed the cognitive level of each patient by a number of tests evaluating attention (verbal and visual–spatial component), short-term memory (verbal and visual–spatial component), long-term memory (verbal and non-verbal component), and visual–spatial and verbal information processing (see ref. [43] for details).

Serological evaluations

Antinuclear antibodies (ANA) and anti-double-stranded DNA (anti-dsDNA) antibodies were detected by indirect immunofluorescence procedures using HEp-2 cell and *Crithidia luciliae* substrates, respectively. ANA titres $\geqslant$1:160 and anti-dsDNA titres $>$1:20 in at least two consecutive determinations were considered positive. Anti-SSA (Ro), anti-SSB (La), anti-Sm and anti-U1-RNP antibodies were detected by counter-immunoelectrophoresis using human spleen and calf thymus extracts as antigen substrate, as previously described [44]. Reference sera were provided by the Center for Disease Control (Atlanta). Anticardiolipin antibodies (aCL) IgM and IgG titres were assessed by conventional standardized ELISA, as reported by Harris *et al.* [45].

Anti-P antibody evaluation

An ELISA using a multiple antigen peptide, carrying four copies of the C-terminal sequence shared by the three ribosomal P proteins as coating antigen [28], was adopted for anti-P detection in the sera of the 149 patients included in the study and in those of the additional 82 SLE patients who had not been clinically evaluated. Briefly, the multiple antigen peptide was used to coat 96-well microtitre plates at 1 μg/ml in phosphate-buffered saline (PBS). Saturation was obtained with gelatin 1% and samples, diluted in PBS-gelatine 0.5%–Tween 20 0.05%, were incubated for 3 h at room temperature. After washing, anti-IgG antibodies labelled with alkaline phosphatase in the same diluting buffer were incubated for the same period of time. After washing, freshly prepared substrate was delivered and absorbance at 405 nm was read. Each plate contained four normal human sera as negative controls and different dilutions of the same strongly reactive serum as positive controls. Results were calculated as percentages of OD of the same reference serum. Samples (n=231) were all tested in duplicate, subtracting the absorbance due to non-specific binding to the plate of each sample (always very low). Values above 15% (the cut-off was established on 100 normal human sera +3 S.D.) were considered positive. Immunoblot control was performed for a limited number of samples (n=73). Briefly, total ribosomal proteins obtained from rat liver [28] were separated by SDS–PAGE and electrotransferred to a nitrocellulose sheet. After saturation with 5% non-fat milk in PBS, nitrocellulose strips were incubated with sera diluted 1:250 in PBS with 2% casein, washed, incubated with alkaline phosphatase-labelled anti-IgG antibodies, washed again and developed with freshly prepared substrate. Samples were considered to be positive for anti-P antibodies if three bands at 38, 19 and 17 kDa appeared. Serial serum anti-P determinations were performed in randomly selected patients as described in Results.

Statistical analysis

The χ^2-test with Yates' correction and Fisher's exact test, when there was a low frequency in at least one cell, were used to evaluate the balance of prognostic factors between the two groups and to compare differences in the other considered items. Logistic linear models were adopted to evaluate the importance of prognostic factors in explaining the variability of the presence/absence of serum anti-P antibodies. Comparisons between groups were made by Mann-Whitney U-test. All P-values refer to two-tailed tests.

Results

Sera of 231 SLE patients were tested for anti-P by ELISA and 27 of these (11.7%) were positive. A similar prevalence of circulating anti-P was found in the patients included in the study (18/149; 12.1%). They were considered anti-P positive (anti-P+) (Fig. 1), while the sera from the remaining 131 patients did not show anti-P reactivity (anti-P−). Although anti-P titres fluctuated over time, the qualitative distinction of patients into anti-P+ and anti-P− groups was confirmed by: (i) ELISA evaluation of anti-P serum levels in 14 randomly selected patients, i.e. after testing them 6 months later, 8 positive samples remained above and 6 negative samples below the cut-off (Fig. 1); and (ii) immunoblotting results in randomly selected sera, collected from the first blood samples obtained from 73 patients, showed a 100% concordance with ELISA data (data not shown).

Epidemiological data, analysed according to anti-P serum reactivity, did not show statistical differences between the two patient groups according to sex, school education data, age at the time of inclusion and family psychiatric history (data not shown). Although the mean age at disease onset of anti-P+ patients did not differ statistically from that of anti-P− subjects, it is of interest that SLE had developed before the age of 33 yr in all anti-P+ patients (Fig. 2).

The percentage of untreated patients was slightly lower (5.5 *vs* 16.7%) and that of corticosteroid-treated patients slightly higher (94.4 *vs* 71.7%) in the anti-P+ than in the anti-P− group, but neither difference was statistically significant. In fact, overall treatment was

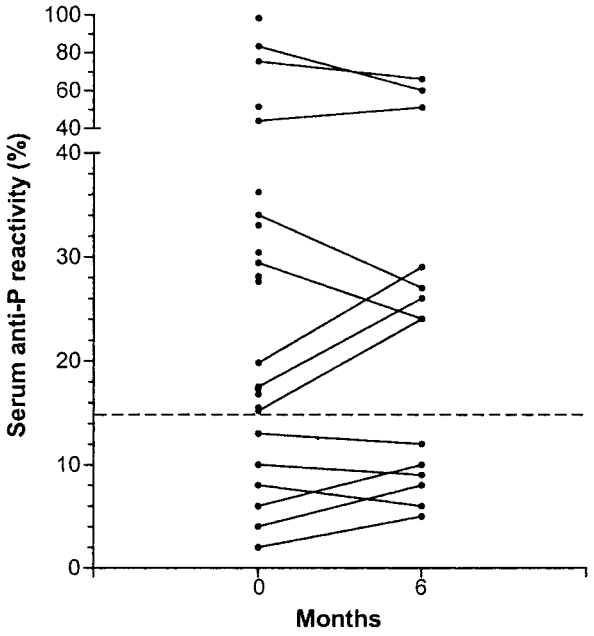

FIG. 1. Serum anti-P reactivity in 14 SLE patients at entry and after a 6-month follow-up (see text for details).

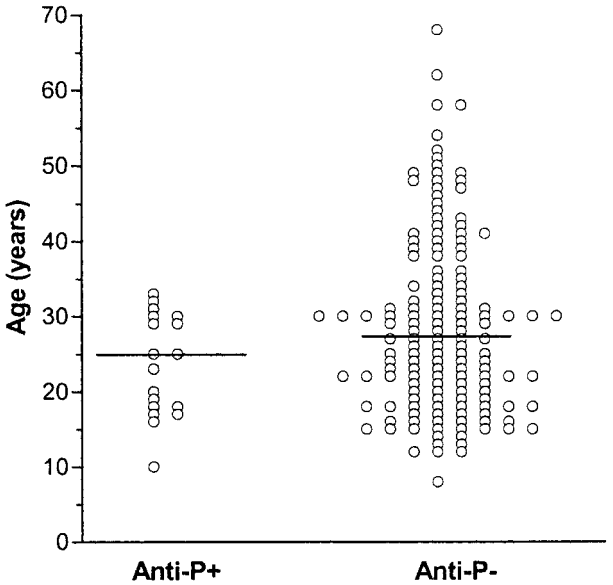

FIG. 2. Age at disease onset of 149 patients with SLE subdivided according to anti-P reactivity.

similar in the two groups of patients, since corticosteroid treatment was similar in terms of patient's age at onset of treatment, years of treatment, mean dosage at entry and corticosteroid dose variation in the last month (data not shown). Treatment regimens were also comparable when cyclophosphamide, antimalarials or other immunosuppressive drugs were considered (data not shown). In addition, serum titres of anti-P were not correlated with the current therapy of each patient (data not shown).

TABLE 1. Disease activity according to the ECLAM score in the patients subdivided according to the presence or absence of serum anti-P antibodies

ECLAM score	Total patient population	Anti-P-negative	Anti-P-positive	Anti-P-negative *vs* anti-P-positive
Complete remission (score 0) (patients %)	39.2	42.3	16.7	0.042
Disease activity (score 1–7) (patients %)	60.8	57.7	83.3	0.042
Score median (range)	1 (0–7)	1 (0–7)	2 (0–4)	0.049

As Table 1 shows, the majority of patients in complete remission were anti-P−, while the presence of anti-P antibodies characterized a group of patients with more active disease as evaluated by ECLAM score, although it was noteworthy that most of the patients included in the study displayed a low degree of disease activity.

At the unifactorial analysis, the comparison of the 11 clinical and laboratory findings included in the ACR SLE diagnostic criteria between the two groups of patients (Table 2) demonstrated a higher prevalence of photosensitivity in the history of anti-P+ patients (61.1 *vs* 36.6%, $P<0.047$). In addition, the anti-P+ subjects had a higher prevalence of malar and discoid rash at the time of anti-P evaluation (61.1 *vs* 31.3%, $P<0.013$ and 16.7 *vs* 2.3%, $P<0.004$, respectively), although when the entire disease history regarding these two skin manifestations was considered, the association with the anti-P was lost. When the data were analysed according to logistic linear models, only malar and discoid rash were found to be important prognostic factors for the presence of anti-P antibodies ($P<0.04$ and $P<0.05$, respectively).

No differences were observed between the anti-P+ and anti-P− patient populations when other clinical findings were analysed (Table 3). However, one patient with lupus hepatitis whom we found in our cohort was anti-P+. Moreover, the analysis of a number of laboratory items, described in Table 4, demonstrated an association between IgG aCL and anti-P reactivity, although there was no strict correlation between the serum levels of these two autoantibodies ($P=0.111$, data not shown). Statistical analysis using logistic linear models confirmed IgG aCL as the only prognostic factor for the presence of anti-P ($P<0.002$). Interestingly, although anticardiolipin antibodies correlated well with cerebrovascular accidents ($P<0.031$) and venous thrombosis ($P<0.025$) (data not shown), no association was found between these vasculopathies and circulating anti-P antibodies (see Table 3).

TABLE 2. SLE ACR classification criteria present in the past or at the time of evaluation in the patients subdivided according to the presence or absence of serum anti-P antibodies

Items	Total patient population (%)	Anti-P-negative (%)	Anti-P-positive (%)	Anti-P-negative *vs* anti-P-positive
Malar rash				
in the past	52.3	49.6	72.2	N.S.
present at entry	34.9	31.3	61.1	0.013
Discoid rash				
in the past	11.4	10.7	16.7	N.S.
present at entry	4.0	2.3	16.7	0.019
Photosensitivity				
in the past	39.6	36.6	61.1	0.047
present at entry	26.2	24.4	38.9	N.S.
Oral ulcers				
in the past	12.1	12.3	5.6	N.S.
present at entry	10.7	10.6	11.1	N.S.
Arthritis				
in the past	74.5	74.8	72.2	N.S.
present at entry	52.3	53.4	44.4	N.S.
Serositis				
in the past	37.6	38.2	33.3	N.S.
present at entry	3.4	3.0	5.5	N.S.
Renal disorders				
in the past	36.9	38.2	27.8	N.S.
present at entry	26.8	28.2	16.7	N.S.
Neurological disorders				
in the past	38.9	39.7	33.3	N.S.
present at entry[a]	–	–	–	–
Haematological disorders				
in the past	67.1	66.4	72.2	N.S.
present at entry	47.6	46.6	55.5	N.S.
Immunological disorders				
in the past	91.9	93.1	83.3	N.S.
present at entry	84.6	84.0	88.9	N.S.
Antinuclear Abs (>1:160)				
in the past	98.0	98.5	94.4	N.S.
present at entry	83.9	84.7	77.8	N.S.

[a]See Table 5.
N.S., not significant.

The overall neuropsychiatric manifestations, including neurological and/or psychiatric disorders and/or cognitive impairment, were not associated with the presence of circulating anti-P (data not shown). Table 5 shows that most patients in the present study had psychiatric manifestations, although only very few of them were linked to SLE according to the specialist. In particular, only 16 (18.6%) of the 86 patients with mood disorder had SLE-dependent depression. Anyway, no significant association was found between anti-P antibodies and specific psychiatric involvement (see Table 6 for details). In particular, it must be noted that only 2 of 16 subjects with SLE-dependent depression were anti-P+. Only one patient had psychosis and another suffered from 'delirium' according to the psychiatric evaluation, but neither displayed anti-P antibodies in the serum.

Finally, Table 6 also shows that anti-P were not associated with cognitive impairment independent of organic CNS involvement.

It is noteworthy that subdividing of anti-P+ patients according to anti-P serum titres (low, medium, high) provided neither different nor additional information about the described clinical and serological associations (data not shown).

Discussion

The overall prevalence of anti-P antibodies found in the present series of SLE patients was around 12%, which is in line with the reported prevalence described in different European populations, ranging from 6% in Bulgarian and Dutch individuals to 18.6% in Greek patients [7, 19, 26]. Although anti-P serum levels vary over time [17, 19, 23], according to our data, anti-P reactivity appears to be a stable feature of an SLE patient subset characterized by disease onset at a young age (less than 33 yr in our series). This interesting finding appears to agree with the documented genetic predisposition to the production of anti-P antibodies [7, 8] and with the higher anti-P prevalence noted in juvenile-onset SLE compared with that of the adult disease [24, 46].

Although the overall SLE activity was mild in our cohort, which was essentially represented by out-patients

TABLE 3. Prevalence of different clinical manifestations, other than SLE criteria, present at the time of evaluation in the patients subdivided according to the presence or absence of serum anti-P antibodies

Items	Total patient population (%)	Anti-P-negative (%)	Anti-P-positive (%)	Anti-P-negative *vs* anti-P-positive
General symptoms (fever, weakness, etc.)	68.5	69.5	61.1	N.S.
Arthralgia/myalgia	50.3	49.6	55.6	N.S.
Myositis	4.0	3.8	5.6	N.S.
Alopecia	24.2	23.7	27.8	N.S.
Generalized rash	17.4	16.8	22.2	N.S.
Cutaneous vasculitis	31.5	31.3	33.3	N.S.
Sicca syndrome	10.7	11.4	5.6	N.S.
Episcleritis/conjunctivitis	4.7	4.6	5.6	N.S.
Lupus pneumonia	0.7	0.76	0	N.S.
Interstitial lung disease	3.4	3.8	0	N.S.
Endocarditis	2.0	2.3	0	N.S.
Myocarditis	0.7	0.76	0	N.S.
Venous thrombosis	10.7	9.9	16.7	N.S.
Arterial thrombosis	5.4	4.6	11.1	N.S.
Raynaud's phenomenon	28.9	29.8	22.2	N.S.
Splenomegaly	8.7	7.6	16.7	N.S.
Aseptic peritonitis	0	0	0	–
Lupus hepatitis	0.7	0	5.6	N.S.
↓ Creatinine clearance (<40 ml/min)	8.1	9.2	0	N.S.
Headache	30.9	32.1	22.2	N.S.
Seizures	3.4	3.8	0	N.S.
Cranial neuropathy	8.7	9.2	5.6	N.S.
Transverse myelitis, ataxia, extrapyramidal disease	1.3	1.5	0	N.S.
Chorea	0.7	0.8	0	N.S.
Peripheral neuropathy	6.7	6.9	5.6	N.S.
Cerebrovascular accidents	10.1	9.9	11.1	N.S.
Other CNS symptoms	26.2	26.7	22.2	N.S.

TABLE 4. Prevalence of various laboratory abnormalities in the patients subdivided according to the presence or absence of serum anti-P antibodies

Items	Total patient population (%)	Anti-P-negative (%)	Anti-P-positive (%)	Anti-P-negative *vs* anti-P-positive
Haemolytic anaemia	12.1	11.4	16.7	N.S.
Non-haemolytic anaemia	38.9	38.2	44.4	N.S.
Leucopenia	32.9	32.8	33.3	N.S.
Thrombocytopenia	12.8	12.2	16.7	N.S.
Lymphopenia	21.5	19.8	33.3	N.S.
Plasma C_3 decrease	29.3	27.9	38.9	N.S.
Anti-dsDNA Abs+	75.7	76.9	66.7	N.S.
Anti-SSA Abs+	31.3	30.9	33.3	N.S.
Anti-SSB Abs+	4.9	5.56	0	N.S.
Anti-Sm Abs+	9.0	9.5	5.5	N.S.
Anti-U_1-RNP Abs+	16.7	15.9	22.2	N.S.
Anticardiolipin IgG+	31.5	27.6	64.3	0.005
Anticardiolipin IgM+	24.8	22.6	42.9	N.S.
Lupus anticoagulant+	17.1	18.4	7.7	N.S.

consecutively seen at the clinic, the anti-P+ patients had a disease activity score, evaluated by the ECLAM scoring system, more elevated than that of anti-P− patients, in agreement with other previously published studies [4, 26, 47]. 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 severe disease. In this regard, some reports showed that anti-P antibodies are associated with renal involvement and correlate with anti-dsDNA serum levels [10–13, 48]. The fact that our study did not confirm these data does not rule out that possible serum peaks of anti-P antibodies may correlate with high anti-dsDNA titres and development of lupus nephritis, as suggested in some studies [12, 13, 48].

We found that anti-P were associated with a previous history of photosensitivity and the presence of malar and discoid rash. The first datum, however, was not

confirmed when statistical analysis was performed according to logistic linear models. It is interesting to note that the few other previously published investigations reporting an association between these autoantibodies and skin rash were all performed on European populations [19, 26, 49], thereby suggesting that the ethnic background may play a role in this association.

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 [9–11]. In our series, we found only one patient with evidence of liver involvement apparently due to the systemic autoimmune disease. He had circulating anti-P antibodies, thus supporting the idea that these autoantibodies may play a pathogenic role in liver damage [50, 51].

The fact that we did not find association of anti-P with either anti-dsDNA, as already mentioned, or other antinuclear antibodies, including anti-Sm, as shown in some previously published reports [10, 13, 52], may be due to methodological differences in antibody detection [31, 53]. Indeed, the techniques adopted in the present report to reveal these specificities essentially give qualitative information and their sensitivity may be not as great as desired, as is well known for the detection of anti-Sm by counterimmunoelectrophoresis [54]. On the contrary, we found a marked association between anti-P and IgG aCL. This association has rarely been investigated in the past. Schneebaum *et al.* [18] found a greater frequency of aCL reactivity in anti-P+ SLE patients, although the difference with the anti-P-group, in the authors' opinion, did not reach statistical significance owing to the low number of patients. Moreover, it is noteworthy that in this study both anti-P and aCL were detected without distinguishing between IgG and IgM isotypes. A Japanese study described five neuropsychiatric SLE patients who had circulating aCL [21]. Four of these were anti-P+, but no conclusions could be drawn from this observation. Three other studies have investigated this topic more recently. In two of these, no association was found: the first one investigated a very heterogeneous multiethnic population in which the prevalence of anti-P was very high [7]; in the other, the anti-P association was investigated with the secondary antiphospholipid antibody (aPL) syndrome and SLE neuropsychiatric involvement rather than with aCL [26]. This report, in particular, showed that no SLE patient with active CNS disease and aCL had circulating anti-P antibodies. Likewise, the third study, investigating anti-P prevalence in a group of Italian patients, different from that analysed in this study, failed to find any association between anti-P and primary or SLE-secondary aPL syndrome [32]. However, anti-P evaluated by immunoblotting were closely associated with aCL. These observations, along with ours, may suggest that the combined presence of serum anti-P and aCL characterizes a subset of SLE patients with a lower risk of thrombotic or other aPL syndrome-related events. This hypothesis seems to be supported by the present data showing that CNS or peripheral thrombotic events, strictly associated with aCL as expected, were not associated with anti-P.

One of the main aims of our study was to verify the possible association of anti-P reactivity with SLE neuropsychiatric manifestations as defined by the recently proposed ACR nomenclature [38]. To our knowledge, this is the first study to investigate anti-P antibodies detected by a sensitive ELISA, in a large series of SLE patients evaluated by trained specialists according

Table 5. Psychiatric manifestations present at the time of evaluation in the patients

Manifestations	Patient number (%)
No psychiatric disorder	27 (18.1)
Psychosis	1 (0.7)
Acute confusional state ('delirium')	1 (0.7)
Total anxiety disorders (SLE-related or not)	20 (13.4)
SLE-related anxiety disorders	2 (1.3)
Total mood disorders (SLE-related or not)	86 (57.7)
with depressive features (non-SLE-related)	68 (45.6)
with depressive features (SLE-related)	16 (10.7)
with manic features	1 (0.7)
with mixed features	1 (0.7)
major depressive-like episode	0 (0)
Drug abuse	14 (9.4)
Total number	149 (100)

Table 6. Psychiatric and neuropsychological manifestions in the patients subdivided according to the presence or absence of serum anti-P antibodies

Items	Total patient population (%)	Anti-P-negative (%)	Anti-P-positive (%)	Anti-P-negative *vs* anti-P-positive
No psychiatric disorder	18.1	17.6	22.2	N.S.
Psychosis/delirium	1.3	1.5	0	N.S.
Anxiety disorders	13.4	14.5	5.6	N.S.
Total mood disorders	57.7	57.2	61.1	N.S.
(SLE-related mood disorders)	10.7	12.2	11.1	N.S.
Drug abuse	9.4	9.2	11.1	N.S.
Cognitive impairment with or without CNS involvement[a]	25.8	26.8	18.7	N.S.
Cognitive impairment without organic CNS involvement[a]	16.4	14.4	12.5	N.S.

[a]Percentages on a total of 128 evaluable patients.

to the above-mentioned criteria. When the overall neuropsychiatric data were analysed according to this classification, no association with anti-P was found. Furthermore, possible associations of anti-P with single neurological, psychiatric and cognitive disorders presented by our patients were also investigated, however without success.

In fact, the lack of association of anti-P 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 [20, 21, 25, 26, 55, 56]. On the contrary, the lack of association with psychiatric disorders contrasts with the findings of a number of previous investigations [5, 7, 15, 16, 18, 22–25]. However, we believe that this datum deserves a more accurate analysis. The first report pointed to an association of anti-P with lupus psychosis [16], an important and well-defined manifestation of SLE, but not so frequent, as its prevalence was estimated in less than 3% of patients [57]. Many of the subsequent studies found that anti-P were associated with broadly defined neuropsychiatric manifestations, including not only psychotic events, but also other manifestations of CNS involvement, such as depression [7, 18]. Some of these studies found a stronger association of anti-P with severe depression than with psychosis [18]. In addition, it has been suggested that, although a single measurement of anti-P would be of limited help in identifying patients with or without psychosis, it would prove to be more helpful in identifying subjects with depression due to SLE [18].

The comparative analysis of neuropsychiatric manifestations in our cohort between anti-P+ and anti-P− patients was not influenced by differences in either family psychiatric history or by treatment, including corticosteroids. A very low frequency of psychotic events was found in the present prospective study, which included only out-patients with mild disease activity. Indeed, we found only 1/149 patient (0.7%) suffering from lupus-related psychosis at the time of inclusion, while another suffered from an acute confusional state. However, both were negative for anti-P. Since the study was planned as prospective with neuropsychiatric evaluation at entry, we did not intentionally consider possible past psychiatric diagnosis, in order to avoid confounding factors of evaluation. On the other hand, as mentioned previously for kidney involvement, we cannot exclude that psychotic events could correlate with anti-P serum peaks.

Finally, in regard to overall neuropsychiatric manifestations, it is interesting to note that more than 80% of our SLE patients had a positive psychiatric diagnosis. However, drug abuse played a role in determining the symptoms in almost 10% of these individuals. In addition, although anxiety or mood disorders were recognized in about 70% of the patients, these manifestations were classified as SLE-related in not more than 12% of them. The fact that anti-P antibodies were not associated with any of the psychiatric syndromes diagnosed in our patients, neither when evaluated together nor when selected according to their relationship to SLE, does not appear to support the suggested link of anti-P with lupus-related depression or other psychiatric syndromes.

References

1. Elkon KB, Parnassa AP, Foster CL. Lupus autoantibodies target ribosomal P proteins. J Exp Med 1985;162:459–71.
2. Francoeur A-M, Peebles CL, Heckman KJ, Lee JC, Tan EM. Identification of ribosomal P protein autoantigens. J Immunol 1985;135:2378–84.
3. Elkon KB, Skelly S, Parnassa A *et al*. Identification and chemical synthesis of a ribosomal protein antigenic determinant in systemic lupus erythematosus. Proc Natl Acad Sci USA 1986;83:7419–23.
4. Sato T, Uchiumi T, Ozawa T *et al*. Autoantibodies against ribosomal proteins found with high frequency in patients with systemic lupus erythematosus with active disease. J Rheumatol 1991;18:1681–4.
5. Nojima I, Minota S, Yamada A, Takaku F, Aotsuka S, Yokohari R. Correlation of antibodies to ribosomal P protein with psychosis in patients with systemic lupus erythematosus. Ann Rheum Dis 1992;51:1053–5.
6. Teh LS, Isenberg DA. Antiribosomal P protein antibodies in systemic lupus erythematosus. A reappraisal. Arthritis Rheum 1994;37:307–15.
7. Arnett FC, Reveille JD, Moutsopoulos HM, Georgescu L, Elkon KB. Ribosomal P autoantibodies in systemic lupus erythematosus. Frequencies in different ethnic groups and clinical and immunogenetic association. Arthritis Rheum 1996;39:1833–9.
8. Teh LS, Doherty DG, Williams BD. HLA-DRB genes and antiribosomal P antibodies in systemic lupus erythematosus. Br J Rheumatol 1994;33:1125–6.
9. Koren E, Schnitz, Reichlin M. Concomitant development of chronic active hepatitis and antibodies to ribosomal P proteins in a patient with systemic lupus erythematosus. Arthritis Rheum 1993;36:1325–8.
10. Hulsey M, Goldstein R, Scully L, Surbeck W, Reichlin M. Anti-ribosomal P antibodies in systemic lupus erythematosus: a case–control study correlating hepatic and renal disease. Clin Immunol Immunopathol 1995;74:252–6.
11. Arnett FC, Reichlin M. Lupus hepatitis: an underrecognized disease feature associated with autoantibodies to ribosomal P. Am J Med 1995;99:465–72.
12. Martin AL, Reichlin M. Fluctuations of antibody to ribosomal P proteins correlate with appearance and remission of nephritis in SLE. Lupus 1996;5:22–9.
13. Chindalore V, Neas B, Reichlin M. The association between anti-ribosomal P antibodies and active nephritis in systemic lupus erythematosus. Clin Immunol Immunopathol 1998;87:292–6.
14. Yoshio T, Masuyama J-I, Minota S *et al*. A close temporal relationship of liver disease to antiribosomal P0 protein antibodies and central nervous system disease in patients with systemic lupus erythematosus. J Rheumatol 1998; 25:681–8.
15. Bonfa E, Elkon KB. Clinical and serological associations of the antiribosomal P protein antibody. Arthritis Rheum 1986;29:981–5.

16. Bonfa E, Golombek SJ, Kaufman LD *et al*. Association between lupus psychosis and anti-ribosomal P-protein antibodies. N Engl J Med 1987;317:265–71.
17. Derksen RHWM, van Dam AP, Gmelig Meyling FHJ, Bijlsma JWJ, Smeenk RJT. A prospective study of antiribosomal P proteins in two cases of familial lupus and recurrent psychosis. Ann Rheum Dis 1990; 49:779–82.
18. Schneebaum AB, Singleton JD, Sterling GW *et al*. Association of psychiatric manifestation with antibodies to ribosomal P proteins in systemic lupus erythematosus. Am J Med 1991;90:54–62.
19. Van Dam A, Nossent H, de Jong J *et al*. Diagnostic value of antibodies against ribosomal phosphoproteins. A cross sectional and longitudinal study. J Rheumatol 1991; 18:1026–34.
20. Teh LS, Hay EM, Amos N *et al*. Anti-P antibodies are associated with psychiatric and focal cerebral disorders in patients with systemic lupus erythematosus. Br J Rheumatol 1993;32:287–90.
21. Yoshio T, Masuyama J-I, Ikeda M *et al*. Quantification of antiribosomal P0 protein antibodies by ELISA with recombinant P0 fusion protein and their association with central nervous system disease in systemic lupus erythematosus. J Rheumatol 1995;22:1681–7.
22. West SG, Emlen W, Wener MH, Kotzin BL. Neuropsychiatric lupus erythematosus: a 10-year prospective study on the value of diagnostic tests. Am J Med 1995; 99:153–62.
23. Watanabe T, Sato T, Uchiumi T, Arakawa M. Neuropsychiatric manifestation in patients with systemic lupus erythematosus: diagnostic and predictive value of longitudinal examination of anti-ribosomal P antibody. Lupus 1996;5:178–83.
24. Press J, Palayew K, Laxer RM *et al*. Antiribosomal P antibodies in pediatric patients with systemic lupus erythematosus and psychosis. Arthritis Rheum 1996; 39:671–6.
25. Isshi K, Hirohata S. Association of antiribosomal P protein antibodies with neuropsychiatric systemic lupus erythematosus. Arthritis Rheum 1996;39:1483–90.
26. Tzioufas AG, Tzortzakis NG, Panou-Pomonis E *et al*. The clinical relevance of antibodies to ribosomal-P common epitope in two targeted systemic lupus erythematosus populations: a large cohort of consecutive patients and patients with active central nervous system disease. Ann Rheum Dis 2000;59:99–104.
27. Iverson GL. Are antibodies to ribosomal P proteins a clinically useful predictor of neuropsychiatric manifestations in patients with systemic lupus erythematosus? Lupus 1996;5:634–5.
28. Caponi L, Pegoraro S, Di Bartolo V, Rovero P, Revoltella R, Bombardieri S. Autoantibodies directed against ribosomal P proteins: use of a multiple antigen peptide as the coating agent in ELISA. J Immunol Methods 1995;179:193–202.
29. Yoshio T, Masuyama J-I, Minota S *et al*. Correlation of serum IgG antibodies to recombinant P0 fusion protein with IgG antibodies to carboxyl-terminal 22 synthetic peptides and carboxyl-terminal 22 amino acid-deleted recombinant P0 fusion protein in patients with systemic lupus erythematosus. Arthritis Rheum 1997; 40:1364–5.
30. Hirohata S, Isshi K, Toyoshima S. The role of carrier protein in the sensitivity of enzyme-linked immunosorbent assay for antiribosomal P protein antibodies: further comment on the article by Yoshio *et al*. Arthritis Rheum 1999;42:593–4.
31. Rayno K, Reichlin M. Evaluation of assays for the detection of autoantibodies to the ribosomal P proteins. Clin Immunol 2000;95:99–103.
32. Ghirardello A, Doria A, Zampieri S, Gerli R, Rapizzi E, Gambari PF. Anti-ribosomal P protein antibodies detected by immunoblotting in patients with connective tissue diseases: their specificity for SLE and association with IgG anticardiolipin antibodies. Ann Rheum Dis 2000;59:975–81.
33. Sibley JT, Olszynski WP, Decoteau WE, Sundaram MB. The incidence and prognosis of central nervous system disease in systemic lupus erythematosus. J Rheumatol 1992;19:47–52.
34. West SG. Neuropsychiatric lupus. Rheum Dis Clin North Am 1994;20:129–58.
35. Mitchell I, Webb M, Hughes R, Maisey M, Cameron JS. Cerebral lupus. Lancet 1994;343:579–82.
36. Rood MJ, Breedveld FC, Huizinga TWJ. The accuracy of diagnosing neuropsychiatric systemic lupus erythematosus in a series of 49 hospitalized patients. Clin Exp Rheumatol 1999;17:55–61.
37. Khoshbin S, Glanz BI, Schur PH. Neuropsychiatric syndromes in systemic lupus erythematosus: a new look. Clin Exp Rheumatol 1999;17:395–8.
38. ACR ad hoc Committee on Neuropsychiatric Lupus Nomenclature. The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. Arthritis Rheum 1999;42:599–608.
39. Tan EM, Cohen AS, Fries JF *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.
40. Hochberg MC. Updating the Americam College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997; 40:1725.
41. Vitali C, Bencivelli W, Isenberg DA *et al*. Disease activity in systemic lupus erythematosus: report of the Consensus Study Group of the European Workshop for Rheumatology Research. II. Identification of the variables indicative of disease activity and their use in the development of an activity score. Clin Exp Rheumatol 1992;10:541–7.
42. American Psychiatric Association. Diagnostic and statistical manual of mental disorders, 4th edn. Washington (DC): American Psychiatric Association, 1994.
43. Sabbadini MG, Manfredi AA, Bozzolo E *et al*. Central nervous system involvement in systemic lupus erythematosus patients without overt neuropsychiatric manifestations. Lupus 1999;8:11–9.
44. Bunn CC, Gharavi AE, Hughes GRV. Antibodies to extractable nuclear antigens in 173 patients with DNA-binding positive SLE: an association between antibodies to ribonucleoprotein and Sm antigens observed by counterimmunoelectrophoresis. J Clin Lab Immunol 1982; 8:13–7.
45. Harris EN. Special report. The second international anti-cardiolipin standardization workshop/the Kingstone Anti-Phospholipid Antibody Study (KAPS) group. Am J Clin Pathol 1990;94:476–84.
46. Reichlin M, Faulkner Broyles T, Hubscher O *et al*. Prevalence of autoantibodies to ribosomal P proteins in juvenile-onset systemic lupus erythematosus compared with the adult disease. Arthritis Rheum 1999; 42:69–75.

47. Elkon KB, Bonfa E, Weissbach H, Brot N. Antiribosomal antibodies in SLE, infection, and following deliberate immunization. Adv Exp Med Biol 1994;347:81–92.
48. Reichlin M, Wolfson-Reichlin M. Evidence for the participation of anti-ribosomal P antibodies in lupus nephritis. Arthritis Rheum 1999;42:2728–9.
49. Caponi L, Pegoraro S, Di Bartolo V, Rovero P, Revoltella R, Bombardieri S. Anti-P protein antibodies in systemic lupus erythematosus: correlations with clinical and serological data. Clin Rheumatol 1995;14:239.
50. Koren E, Koscec M, Reichlin M, Fugate R, Reichlin M. Possible role of autoantibodies to ribosomal P proteins in development of liver disease in patients with systemic lupus erythematosus. Arthritis Rheum 1993;S36:72.
51. Koscec M, Koren E, Wolfson-Reichlin M *et al.* Autoantibodies to ribosomal P proteins penetrate into live hepatocytes and cause cellular dysfunction in culture. J Immunol 1997;159:2033–41.
52. Caponi L, Bombardieri S, Migliorini P. Anti-ribosomal antibodies bind the Sm proteins D and B/B′. Clin Exp Immunol 1998;112:139–43.
53. Caponi L. The cross-reactivity of autoantibodies in connective tissue diseases. Clin Exp Rheumatol 1996; 14:425–31.
54. Navarro E, Palau J. Anti-Sm sera are misdetected by counterimmunoelectrophoresis. A critical analysis of CIE and western blotting on the detection of human antinuclear antibodies. J Autoimmun 1991;4:213–22.
55. Hanly JG, Walsh NM, Fisk JD *et al.* Cognitive impairment and autoantibodies in systemic lupus erythematosus. Br J Rheumatol 1993;32:291–6.
56. Isshi K, Hirohata S. Differential roles of the anti-ribosomal P antibody and anti-neuronal antibody in the pathogenesis of central nervous system involvement in systemic lupus erythematosus. Arthritis Rheum 1998; 41:1819–27.
57. Vitali C, Bencivelli DA, Isenberg DA *et al.* Disease activity in systemic lupus erythematosus: report of the Consensus Study Group of the European Workshop for Rheumatology Research. I. A descriptive analysis of 704 European lupus patients. Clin Exp Rheumatol 1992; 10:527–39.