Significance of antibodies to ribosomal P proteins in lupus nephritis patients and their relation to disease activity: clinical and laboratory study

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Background

Lupus nephritis (LN) is a prominent feature in systemic lupus erythematosus (SLE), present in 15–30% of patients with lupus at the time of the initial diagnosis and in 30–50% during the disease progression.

Objectives

The aim of this study was to determine the significance of anti-ribosomal P protein (anti-P) antibodies and LN and their relation to disease activity and other SLE manifestations.

Patients and methods

Fifty active LN patients were subjected to full clinical examination (assessment of SLE disease activity using the systemic lupus erythematosus disease activity index and assessment of SLE disease severity using Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index), routine laboratory investigations, anti-dsDNA antibodies, and anti-P antibodies.

Results

Comparison between the data of LN in both groups (anti-P positive/anti-dsDNA negative and anti-P negative) shows that there was a statistically significant difference in age (P=0.042), hypertension (P=0.00), and psychiatric manifestations (P=0.004). On comparison of both groups as regards vasculitis, there was a borderline statistical significance (P=0.050). Comparison of both groups as regards creatinine level or biopsy class V showed a statistically significant difference with a higher percentage in the anti-P positive/anti-dsDNA negative group (P=0.024 and 0.040, respectively).

Conclusion

Anti-P antibody-positive patients have younger ages, lower creatinine level, lower incidence of hypertension, usually class V in renal biopsy, but more susceptible to have psychiatric manifestations compared with anti-P antibody negative patients.

Introduction

Systemic lupus erythematosus (SLE) is a syndrome of multifactorial etiology, characterized by widespread inflammation. Actually every organ and/or system of the body may be involved, although the skin and the joints are the most frequently affected. The course of the disease is typically one of remissions and exacerbation [1].

Kidney involvement is a great predictor of poor outcome in SLE, with 5–10% progression to end-stage renal disease despite immunosuppressive therapy [2].

Lupus nephritis (LN) affects from one-third to one-half of lupus patients, accounting for significant morbidity and mortality. Kidney disease in lupus typically appears within the first 5 years after diagnosis [3].

There is a shadow of renal injury that can be assessed in part on clinical grounds, and more definitely by means of biopsy [4], which assesses the pathological pattern, activity, and damage indices [5].

Other clinical manifestation such as creatinine clearance, proteinuria, urine sediments, and complement level are not sensitive or specific enough for detecting ongoing

disease activity in lupus kidney and early relapse of nephritis [6].

A biomarker refers to a biologic, biochemical, or molecular event that can be measured qualitatively and quantitatively using laboratory methods and their levels must correlate with disease pathogenesis or activity in different organ systems [6].

Anti-dsDNA is considered as the best available biomarker for LN as it correlates well with renal activity, worse prognosis, and histology severity [2]. However, this antibody is not a public finding in patients with LN, and therefore we need to search for other specific markers for long-term outcome.

Antibodies to ribosomal P proteins (anti-P) are very specific for SLE diagnosis. The presence of antibodies against anti-P was observed to be very specific for patients with SLE compared with either Healthy Controls (HCs) or with controls who had other rheumatic diseases. Moreover, the test had high levels of specificity and sensitivity [7].

Biochemical and structural studies have shed lights on the structural features of the anti-P autoimmune target, which is composed of ribosomal proteins P0, P1, and P2. The C-terminal tails of these three proteins share a common sequence, which could serve as the anti-P epitope. These three proteins form a pentameric complex, $P0(P1–P2)_2$, in which the five C-terminal tails could move freely over a wide area, and the N-terminal domain of P0 binds to the 28 s rRNA. This unique structural property of the $P0(P1–P2)_2$ complex might provide immunogenic stimulus for the anti-P production [8].

Anti-P antibody is a possible manifestation for lupus renal disease [9,10] as their level changes in parallel with renal flares [11] and with disease activity [12,13].

A retrospective study with 4 years of follow-up concluded that the isolated presence of anti-P antibodies during nephritis flares is a good marker to predict a better long-term renal outcome in lupus patients compared with patients with isolated anti-dsDNA antibodies or absence of both antibodies. Serum creatinine at biopsy is a significant risk factor for end-stage renal failure, but anti-P was more accurate to know a better prognosis [14].

In some studies, anti-P antibodies correlated with disease activity. These observations may support the concept that the presence of circulating anti-P antibodies characterizes a group of SLE patients with a persistently more active disease, but they do not differentiate whether these autoantibodies are associated with more active disease [15].

Aim
The aim of this study was to determine the significance of anti-P antibodies and LN and their relation to disease activity and other SLE manifestations.

Patients and methods
This study was conducted on 50 LN patients during renal flare (42 female and eight male) who were selected from the Rheumatology and Rehabilitation Department of Beni Suef University Hospital and diagnosed according to Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE [16] from December 2013 to October 2015. All patients gave informed consent, and this study was approved by the local ethics committee.

The LN group was defined as those having a renal systemic lupus erythematosus disease activity index (SLEDAI) of greater than or equal to 4 (at least two abnormal results for renal parameters on at least two occasions).

Inclusion criteria
One serum sample at the time of renal flare and biopsy.

Exclusion criteria
Concomitant presence of anti-P and anti-dsDNA, renal injury due to diabetes (those with essential hypertension having hypertension before the onset of lupus) or medications.

Methods
All patients were subjected to full clinical examinations and routine laboratory investigations, assessment of SLE disease activity using the SLEDAI [17], and assessment of SLE disease severity using SLICC/American College of Rheumatology Damage Index [18].

Laboratory investigations
Laboratory investigations included the following.

Complete blood count, erythrocyte sedimentation rate, C reactive protein, 24 h urinary proteins, serum urea and creatinine, complete urine analysis, liver function test, lipid profile (cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein). Quantitative determination of serum complement levels (C3, C4) [19], antinuclear antibodies using

the immunoflorescence technique [20], and anti-dsDNA antibodies [21] was carried out.

Anti-ribosomal P protein antibodies
Summary and explanation of the test.

Antibodies to ribosomal proteins (anti-P) are of special clinical relevance in the differential diagnosis of SLE. Anti-P antibodies are directed against a common epitope of three phosphoproteins (P0, P1, and P2), which are major compounds of the 60 s subunit of ribosomal RNP complexes. In cases of systemic lupus that are associated with depression and/or psychotic symptoms, anti-P antibodies have been reported with an incidence of 40–90%. Determination of antibodies directed against ribosomal P proteins is a valuable diagnostic method for the differential diagnosis of SLE, especially in cases in which classic SLE typical antibodies are not found [22].

Principle of the test.

Highly purified ribosomal P protein (anti-P) is added to microwells. Antibodies against this antigen, if present in diluted serum or plasma, are combined with the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase-conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color.

The addition of an acid stops the reaction from forming a yellow end product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of color is directly proportional to the concentration of IgG antibodies found in the original sample.

Interpretation of results.

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the anti-P test:

Normal: <10 U/ml.
Elevated: 10^3 U/ml.

Sampling.

All participants were subjected to collection of 6 ml of venous blood and 24 h urine.

Renal biopsy.

Renal biopsy was performed for patients with persistent hypertension, rising creatinine levels, persistent hematuria, proteinuria, and casts. It was classified according to the classification of LN using the International Society of Nephrology/Renal Pathology Society [23].

Statistical analysis
All data were tabulated and statistically analyzed. The data were coded and entered using the statistical package SPSS, version 15; SPSS Inc. The data were summarized using descriptive statistics: mean, SD, minimal and maximum values for quantitative variables, and using number and percentage for qualitative values. Statistical differences between groups were tested using the χ^2-test for qualitative variables, independent sample t test for quantitative normally distributed variables, and the nonparametric Mann–Whitney test was used for quantitative variables that are not normally distributed. P values less than or equal to 0.05 were considered statistically significant.

Results
The total number of patients was 50 patients. The patients were classified according to anti-dsDNA and anti-P antibody, which was considered positive if its level was greater than or equal to 10 μg/dl. There were seven anti-dsDNA positive and anti-P positive patients and were excluded from our study. The anti-P positive/anti-dsDNA negative group (group 1) included seven patients and the anti-P negative group (group 2) comprised 36 patients; 18 patients of them were anti-P negative/anti-dsDNA positive.

The number of patients included in our study was 43. There were seven (16.2%) male and 36 (83.7%) female patients. Their ages ranged from 18 to 52 years with a mean of 27.46±7.17 years.

The disease duration ranged from 2 to 144 months with a mean of 39.92±36.56 months. The renal duration ranged from 2 to 144 months with a mean of 33.84±34.05.

Comparison between the demographic data of lupus nephritis in groups 1 and 2 (anti-P positive/anti-dsDNA negative and anti-P negative)
It showed that there was a statistically significant difference between age ($P=0.042$) in the anti-P positive/anti-dsDNA negative group compared with the anti-P negative group. Other parameters did not show any statistically significant difference (Table 1).

Comparison between the demographic data of lupus nephritis patients [anti-P positive/anti-dsDNA negative (seven patients) and anti-P negative/anti-dsDNA positive (18 patients)]

On comparing the anti-P positive/anti-dsDNA negative group compared with the anti-P negative/anti-dsDNA positive) group, it showed that there was a statistically significant difference in age range 18–26 (22.57±3.25) years and 19–43 (28.94±7.42) years, respectively (*P*=0.007) and age at onset ranged from 18 to 24 (20.71±2.42) years and 18 to 41 (25.44±5.91) years, respectively (*P*=0.054). Other parameters did not show a statistically significant difference (Fig. 1).

Comparison between systemic lupus erythematosus disease activity index, Systemic Lupus International Collaborating Clinics scores, and clinical data of lupus nephritis patients in groups 1 and 2 (anti-P positive/anti-dsDNA negative and anti-P negative)

As for group 1 (anti-P positive/anti-dsDNA negative) and group 2 (anti-P negative), comparison between them as regards hypertension showed a statistically significant difference (anti-P positive/anti-dsDNA negative) (*P*=0.001). There was a statistically significant difference in anti-P positive/anti-dsDNA negative compared with anti-P negative as regards psychiatric manifestations (*P*=0.004). Moreover, there was a borderline statistical significance between the anti-P positive/anti-dsDNA negative group and the anti-P negative group as regards vasculitis (*P*=0.050), whereas other clinical parameters did not show any statistically significant difference (Table 2).

Comparison between the systemic lupus erythematosus disease activity index and Systemic Lupus International Collaborating Clinics scores and clinical data of the anti-P positive/anti-dsDNA negative and anti-P negative/anti-dsDNA positive groups
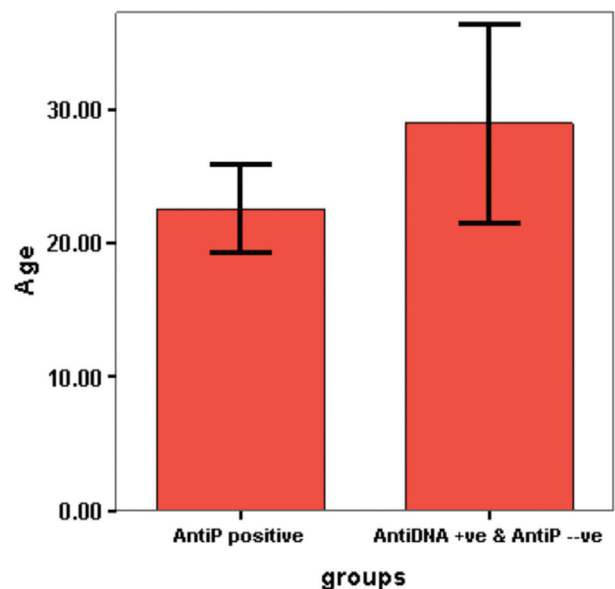
As for the anti-P positive/anti-dsDNA negative group and the anti-P negative/anti-dsDNA positive group, comparison between them as regards hypertension showed a statistically significant difference in anti-P positive/anti-dsDNA negative subgroup (*P*<0.001). Diastolic blood pressure also showed a statistically significant difference (*P*<0.057) (Fig. 2). Moreover, there was a statistically significant difference as regards psychiatric manifestations (*P*<0.007), whereas other clinical parameters did not show a statistically significant difference (Fig. 3).

Comparison between the laboratory parameters of lupus nephritis patients (anti-P positive/anti-dsDNA negative and anti-P negative)

The comparison showed that there was a statistically significant difference in creatinine (*P*=0.024) in the anti-P positive/anti-dsDNA negative group compared with the anti-P negative group, whereas other laboratory parameters did not show any statistically significant difference (Table 3).

Figure 1

Comparison of age range between lupus nephritis patients (the anti-P positive/anti-dsDNA negative and anti-P negative/anti-dsDNA positive groups). Anti-P positive in the diagrams means anti-P positive/anti-dsDNA negative

Table 1 Comparison between the demographic data of lupus nephritis patients (anti-P positive/anti-dsDNA negative and anti-P negative) (*N*=43)

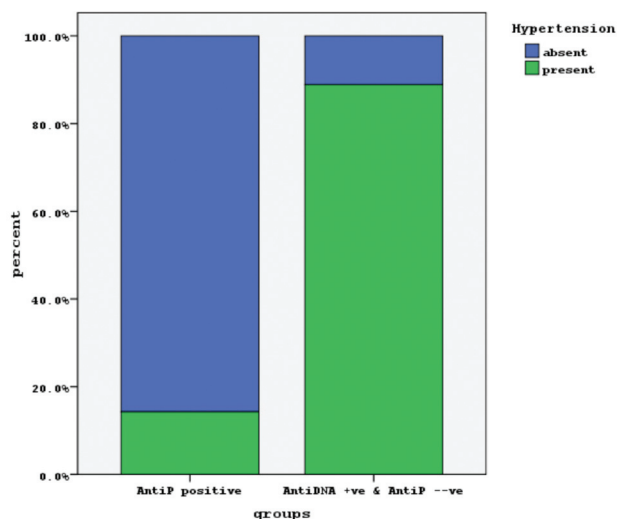| Variables	Anti-P positive/anti-dsDNA (*N*=7)		Anti-P negative (*N*=36)		*P* value
	Range	Mean±SD	Range	Mean±SD	
Age	18–26	22.57±3.25	19–52	28.9±7.8	0.042[*]
Age at onset	18–24	20.71±2.42	17–49	25.3±7.2	0.105
Disease duration (months)	4–48	22.0±16.12	2–144	43.0±40.3	0.263
Duration of renal affection (months)	4–48	18.57±15.86	2–144	35.8±37.6	0.292
Sex [*n* (%)]					
Female		7 (100)		29 (80.6)	0.577
Male		0 (0)		7 (19.6)	

[*]Statistically significant.

Comparison between the laboratory parameters of [anti-P positive/anti-dsDNA negative (seven patients) and anti-P negative/anti-dsDNA positive (18 patients)]
This comparison showed that there was a statistically significant difference in serum creatinine level (*P*=0.001)
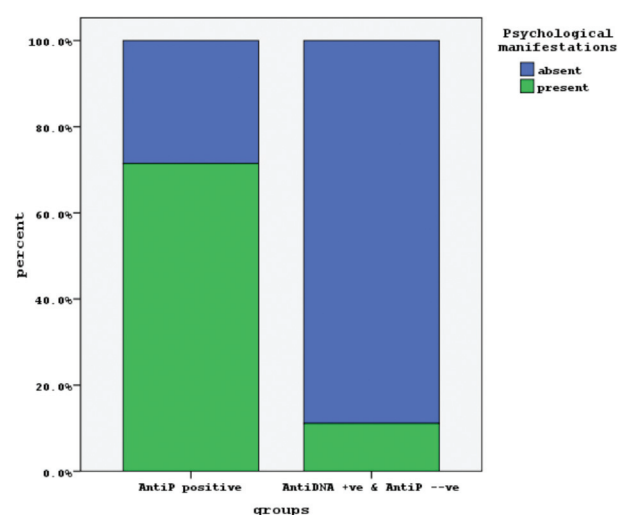
in the anti-P positive/anti-dsDNA negative [range: 0.7–1.40 (0.76±0.41) mg/dl] compared with anti-P negative/anti-dsDNA positive [range: 1.0–5.0 (2.03± 1.28) mg/dl], whereas other laboratory parameters did not show a statistically significant difference (Table 4).

Figure 2

Comparison of the percentage of patients with hypertension between the anti-P positive/anti-dsDNA negative and anti-P negative/anti-dsDNA positive groups. Anti-P positive in the diagrams means the anti-P positive/anti-dsDNA negative group

Figure 3

Comparison of the percentage of patients with psychiatric manifestations between the anti-P positive/anti-dsDNA negative and anti-P negative/anti-dsDNA positive groups. Anti-P positive in the diagrams means the anti-P positive/anti-dsDNA negative group

Table 2 Comparison between the systemic lupus erythematosus disease activity index, Systemic Lupus International Collaborating Clinics scores and clinical data of lupus nephritis patients (anti-P positive/anti-dsDNA negative and anti-P negative) (*N*=43)

Variables	Anti-P positive/anti-dsDNA negative (*N*=7)		Anti-P negative (*N*=36)		*P* value
	Range	Mean±SD	Range	Mean±SD	
SLEDAI	5–38	19.4±10.6	10–30	17.6±5.2	0.640
SLICC	0.0–4.0	1.28–1.49	0.0–2	0.75±0.73	0.508
Systolic blood pressure	110–150	118.5±14.6	110–260	166.9±28.0	0.765
Diastolic blood pressure	70–90	75.7±7.8	70–130	101.1±12.8	0.302
n (%)					
Hypertension	1 (14.3)		31 (86.1)		0.00[*]
General manifestations	4 (57.1)		15 (41.7)		0.680
Serositis	6 (85.7)		17 (47.2)		0.100
Malar rash	4 (57.1)		26 (72.2)		0.655
Oral ulcers	3 (42.9)		22 (61.1)		0.427
Photosensitivity	3 (28.6)		21 (58.3)		0.222
Alopecia	5 (71.4)		15 (41.7)		0.222
Arthralgia	1 (14.3)		18 (50.0)		0.112
Arthritis	3 (42.9)		15 (41.7)		1.00
Myalgia	1 (14.3)		5 (11.1)		1.00
Myositis	1 (14.3)		2 (5.6)		0.421
Hepatitis	2 (28.6)		7 (19.4)		0.624
Psychiatric manifestations	5 (71.4)		5 (13.9)		0.004[*]
Vasculitis	4 (57.1)		6 (16.7)		0.050
Thrombosis	1 (14.3)		3 (8.3)		0.523
Anemia	1 (14.3)		8 (22.2)		1.00
Thrombocytopenia	2 (28.6)		11 (30.6)		1.00
Leukopenia	5 (71.4)		12 (33.3)		0.093

SLEDAI, systemic lupus erythematosus disease activity index; SLICC, Systemic Lupus International Collaborating Clinics. [*]Statistically significant.

Comparison between biopsy classes of lupus nephritis patients (anti-P positive/anti-dsDNA negative and anti-P negative)

Renal biopsy class V showed a statistically significant difference in the anti-P positive/anti-dsDNA negative group (*P*<0.040) compared with the anti-P negative group, whereas there was no statistically significant difference in other classes (Table 5).

Comparison between biopsy classes of lupus nephritis patients (anti-P positive/anti-dsDNA negative and anti-P negative/anti-dsDNA positive)

As for the anti-P positive/anti-dsDNA negative group and the anti-P negative/anti-dsDNA positive group, comparison between them as regards biopsy class V showed a statistically significant difference with a higher percentage in the anti-P positive/anti-dsDNA negative group (*P*<0.017), whereas the percentage in other classes did not show a statistically significant difference (Fig. 4).

Discussion

On comparison between the demographic data of the anti-P positive/anti-dsDNA negative and anti-P negative groups, a significant statistical difference was found as regards age (22.57±3.25 vs. 28.9±7.8, *P*=0.042), respectively. This finding was in agreement with López-Longo *et al.* [24], who suggested that anti-

Table 3 Comparison between the laboratory parameters of lupus nephritis patients (anti-P positive/anti-dsDNA negative and anti-P negative) (*N*=43)

Variables	Anti-P positive/anti-dsDNA negative (*N*=7)		Anti-P negative (*N*=36)		*P* value
	Range	Mean±SD	Range	Mean±SD	
ESR	50–110	74.85±24.73	37–150	87.2±26.03	0.323
Hemoglobin (g/dl)	6.4–12.1	9.08±2.06	5.8–13.0	9.6±1.8	0.480
White blood cells (10^3/µl)	2.10–16	7.18±5.09	2.9–15.0	6.9±2.6	0.735
Lymphocytes%	7–33	18.0±8.16	8–41	18.4±7.6	0.987
Platelets (10^3/µl)	15–506	286.4±146	55–640	228.2±126.6	0.236
ALT (mg/dl)	14–64	29.0±22.9	8–60	21.6±11.3	0.711
AST (mg/dl)	10–87	36.7±31.7	13–102	26.0±17.4	0.687
Serum albumin (g)	1.3–3.5	2.27±0.70	2.0–3.6	2.6±0.42	0.063
Complement 3 (mg/dl)	27.3–110	62.5±28.95	19–147	59.3±24.5	0.834
Complement 4 (mg/dl)	0.3–1.03	1.14±0.1008	2–40	15.0±9.7	0.097
Serum creatinine (mg/dl)	0.7–1.40	0.76±0.41	0.4–5.0	1.5±1.1	0.024[*]
Blood urea	19–202	75.8±68.5	13–200	55.2±46.2	0.687
Triglycerides (mg/dl)	88–350	182.7±94.6	66–540	158.0±111.9	0.277
Cholesterol (mg/dl)	139–364	228.2±90.6	110–568	191.8±84.3	0.307
24 h urinary proteins (mg/dl)	0.7–6.9	3.90±2.3	0.6–6.5	2.6±1.5	0.146

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ESR, erythrocyte sedimentation rate. [*]Statistically significant.

Table 4 Comparison between laboratory parameters of (anti-P positive/anti-dsDNA negative and anti-P negative/anti-dsDNA positive) lupus nephritis patients (*N*=25)

Variables	Anti-P positive/anti-dsDNA negative (*N*=7)		Anti-P negative/anti-dsDNA positive (*N*=18)		*P* value
	Range	Mean±SD	Range	Mean±SD	
ESR	50–110	74.85±24.73	37–150	86.55±32.32	0.423
Hemoglobin (g/dl)	6.4–12.1	9.08±2.06	5.8–13.0	9.48±2.03	0.662
White blood cells (10^3/µl)	2.10–16	7.18±5.09	2.9–15.0	6.61±3.05	0.929
Lymphocytes%	7–33	18.0±8.16	8–30	16.6±5.45	0.745
Platelets (10^3/µl)	15–506	286.4±146.8	98–430	198.3±83.8	0.110
ALT (mg/dl)	14–64	29.0±22.9	8–60	26.0±14.1	0.976
AST (mg/dl)	10–87	36.7±31.7	13–102	31.3±23.4	0.790
Serum albumin (g)	1.3–3.5	2.27±0.70	2.0–3.6	2.68±0.39	0.073
Complement 3 (mg/dl)	27.3–110	62.5±28.95	19–147	53.2±29.8	0.357
Complement 4 (mg/dl)	0.3–1.03	1.14±0.1008	0.60–1.5	0.38±0.39	0.07
Serum creatinine (mg/dl)	0.7–1.40	0.76±0.41	1.0–5.0	2.03±1.28	0.001[*]
Blood urea	19–202	75.8±68.5	18–167	64.8±45.8	0.976
Triglyceride	88–350	182.7±94.6	66–540	160.7±126.4	0.220
Cholesterol	139–364	228.2±90.6	110–410	193.7±62.9	0.534
24 h urinary proteins (g/dl)	0.7–6.9	3.90±2.31	0.8–6.5	3.1±1.7	0.458

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ESR, erythrocyte sedimentation rate. [*]Statistically significant.

P antibodies are more prevalent in younger age group [25].

We also compared the SLEDAI and SLICC scores and clinical manifestations in groups 1 and 2 (anti-P positive/anti-dsDNA negative and anti-P negative).

The SLEDAI score was 19.4±10.6 versus 17.6±5.2 (*P*=0.640), respectively. In our study we selected patients during renal activity.

The SLICC score was 1.28–1.49 versus 0.75±0.73 (*P*=0.508), respectively. Anti-P antibodies seem to

Table 5 Comparison between renal biopsy classes of lupus nephritis patients (anti-P positive/anti-dsDNA negative and anti-P negative) (N=43)

Variables	Anti-P positive/anti-dsDNA negative (*N*=7) [*n* (%)]	Anti-P negative (*N*=36) [*n* (%)]	*P* value
Class I	0 (0.0)	0 (0.0)	–
Class II	0 (0.0)	0 (0.0)	–
Class III	0 (0.0)	5 (14)	0.686
Class IV	1 (14.3)	14 (38.9)	0.391
Class V	5 (71.4)	10 (27.8)	0.040[*]
Classes II and III	0 (0.0)	1 (2.8)	–
Classes II and V	1 (14.3)	1 (2.8)	–
Class III and V	0 (0.0)	1 (2.8)	–
Classes IV and V	0 (0.0)	4 (11.2)	0.830

[*]Statistically significant.

Figure 4

Comparison of the percentage of patients with class V between the anti-P positive/anti-dsDNA negative and anti-P negative/anti-dsDNA positive groups. Anti-P positive in the diagrams means the anti-P positive/anti-dsDNA negative group. Anti-P negative in the diagrams means the anti-P negative/anti-dsDNA positive group

be related to illness activity level. However, it is not clear whether these antibodies are associated with more illness severity [26]. Moreover, there was no evidence for a prognostic value of anti-P for damage [27].

The percentage of hypertensive patients in groups 1 and 2 (anti-P positive/anti-dsDNA negative and anti-P negative) was 14.3 and 86.1% (*P*=0.00), respectively, showing a significant statistical difference. As regards this finding, it was mostly due to the high percentage of class V in this group.

There was a significant statistical difference in the percentage of psychiatric manifestations (anti-P positive/anti-dsDNA negative and anti-P negative) (71.4 vs. 13.9%, *P*=0.004, respectively). The association between anti-P antibodies and psychosis using SLE was first reported by Bonfa *et al.* [28]. They had found that 18 of 20 (90%) patients with psychosis using SLE had anti-P antibodies. These observations were reproduced by Schneebaum *et al.* [29] but denied in the studies of Teh and Isenberg [30] and Iverson [31]. Shi *et al.* [32] concluded that anti-P antibody is potentially related to neuropsychiatric SLE.

These differences have been attributed to methodological differences and in reporting and analyzing results. A number of subsequent studies had supported the association between anti-P antibodies and neuropsychiatric manifestations by lupus [33–35]. For example, Tzioufas*et al.* [26] had found that 11 of 28 (39.3%) patients with SLE and neurological affection (psychiatric 71% and epilepsy 75%) had anti-P antibodies.

The percentage of our patients with cutaneous vasculitis (palpable purpura and livedo reticularis), most of them diagnosed clinically, in both groups was 57.1 versus 16.7% (*P*=0.050), respectively. In a study conducted on 140 patients who had a history of vasculitis, Shinjo and Bonfá [36] found that SLE cutaneous vasculitis without the associated anti-phospholipid antibodies or Sjögren syndrome characterizes a subgroup of patients with more ribosomal P and anti-P protein antibodies.
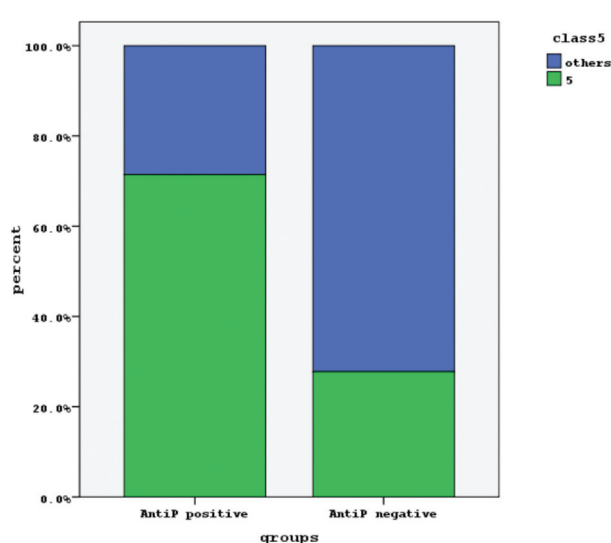
We also compared the laboratory data between groups 1 and 2 (anti-P positive/anti-dsDNA negative and anti-P negative) and a significant statistical difference was found as regards serum creatinine (0.76±0.41 vs. 1.5±1.1, *P*<0.024, respectively). As regards this finding we had an agreement with do Nascimento *et al.* [22], who conducted a study on 81 patients and observed that renal function was

preserved in six of seven anti-P positive patients with class V LN. Moreover, Andrade de Macedo *et al.* [14], who conducted a study on 60 patients found that anti-P positive/anti-dsDNA negative patients had a significantly lower creatinine level compared with anti-P negative patients.

Meanwhile, no statistical difference was found as regards other laboratory renal parameters such as proteinuria in the anti-P positive/anti-dsDNA negative and anti-P negative groups (3.90±2.31 vs. 2.6±1.5, *P*=0.146, respectively). Our result was different from that of do Nascimento *et al.* [22], who reported that anti-P positive patients had a higher proteinuria level compared with anti-P antibody-negative patients due to the higher frequency of class V LN in anti-P positive patients. Moreover, no significant statistical findings were found as regards alanine aminotransferase (*P*=0.711) and aspartate aminotransferase (*P*=0.687), but a meta-analysis by Shi *et al.* [32] concluded that anti-P antibody is potentially associated with hepatic damage. However, a study found an association of anti-P with anemia [37]. A study found that high titers of aRibPR0 can be associated with lymphocytopenia and no significant association was found between aRibPR0 and liver enzymes, alanine aminotransferase or aspartate aminotransferase [27].

On comparison between biopsy classes between the anti-P positive/anti-dsDNA negative and the anti-P negative group, a significant statistical difference was found as regards class V (71.4 vs. 27.8%, *P*=0.040, respectively). This finding was in agreement with Andrade de Macedo *et al.* [14], who reported that anti-P antibodies were a potential serologic marker for lupus membranous glomerulonephritis, because the frequency of anti-P antibodies in patients with class V LN was significantly higher than the frequency between patients with other classes of renal disease (72 vs. 28%, *P*=0.005). In contrast, Bertolaccini *et al.* [38] observed that anti-P antibodies did not discriminate membranous types of nephritis. The most possible explanation for this apparent discrepancy was the small sample size, particularly with regard to the representation of class V nephritis. In fact, in the study by Bertolaccini *et al.* [38], only 18 patients had class V glomerulonephritis, compared with 35 patients in our study. Supporting the possibility that the study by Bertolaccini *et al.* [38] did not have the statistical power to detect this difference was the fact that those investigators also had observed a higher frequency of class V nephritis in anti-P antibody-positive patients (41%) compared with

anti-P antibody-negative patients (20%), although the difference was not statistically significant (*P*=0.08). This trend is reinforced by their finding of higher titers of anti-P antibodies in patients with class V nephritis than in those with other classes as a whole (*P*=0.02). Moreover, the unexpected absence in the same population of an association of proliferative LN with anti-dsDNA antibodies (*P*=0.3), a well-established serologic marker, emphasizes the relevance of adequate sample sizing to know this difference [22]. Other less likely explanations are genetic differences, because the anti-P antibody response in lupus was strongly affected by certain class II major histocompatibility complex alleles and may be was correlated with a special clinical manifestation [39]. Indeed, the prevalence of this antibody may vary between different populations [23]. A high heterogeneity was found due to ethnicity and publication bias. Moreover, the other important point was the distinct methodology used to detect anti-P antibodies. In this regard, all of our patients were anti-P antibody positive using enzyme-linked immunosorbent assay (>3 SD) and Western blotting, whereas in the study of Bertolaccini *et al.* [38], anti-P antibodies were known by multiplexed bead technology. Although this latter methodology seems to be a useful diagnostic tool for antibody screening, additional investigations are still needed to define its performance for individual autoantibody profiles [40].

Bonfa *et al.*'s [28] group had found in a study with 60 consecutive patients with biopsy-proven LN that the overall renal survival was significantly higher in the anti-P positive/anti-dsDNA group compared with the anti-P group, supposing that anti-P might be a replacing marker for better renal survival [14].

It is concluded that, anti-P could be used to predict the severity of kidney affection. Anti-P can be used to distinguish neuropsychiatric events attributed to SLE and non-SLE causes. Long term and frequent follow-up for this group of patients is recommended to detect any possibility of progression of psychiatric manifestations early.

Moreover, it is recommended that further studies should be conducted on the anti-P antibodies to support its utility in the LN patients.

Conflicts of interest
There are no conflicts of interest.

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