

Beta2-glycoprotein I Expression in Lupus Nephritis Patients with Antiphospholipid-associated Nephropathy

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ABSTRACT. Objective. Antiphospholipid-associated nephropathy (aPLN) is a severe condition in patients with lupus nephritis (LN). aPLN should be distinguished from other reasons for renal ischemia. The most important cofactor of antiphospholipid antibodies (aPL), β 2-glycoprotein I (β 2GPI), was shown *in vitro* to bind endothelial cells and to induce a procoagulant phenotype. The objectives of this study were to investigate whether β 2GPI expression was involved in patients with LN with aPLN and to determine its specificity.

Methods. We retrospectively investigated β 2GPI expression in 231 renal biopsy specimens of patients with LN. Data from biopsy reports and clinical information were collected. Immunohistochemical staining for β 2GPI expression was performed.

Results. Histological aPLN was detected in 88 patients with LN (38.1%). The LN with aPLN consisted of 43 patients (18.6%). Expression of β 2GPI was detected in endothelial cells in 14 (32.6%) in renal arteries or arterioles, 11 (25.6%) in glomerular or peritubular capillaries, and a total of 15 (34.9%) of the 43 patients with LN with aPLN. It was mainly expressed in the endothelial cells in patients with LN with aPLN ($p < 0.05$). The specificity of β 2GPI expression in patients with LN with aPLN was 97.5%.

Conclusion. Expression of β 2GPI may be involved in the formation of aPLN in patients with LN. This expression in endothelial cells in kidney tissue may be considered a useful marker for aPLN. (J Rheumatol First Release September 15 2016; doi:10.3899/jrheum.151395)

Key Indexing Terms:

B2-GLYCOPROTEIN I
NEPHRITIS

ANTIPHOSPHOLIPID SYNDROME
SYSTEMIC LUPUS ERYTHEMATOSUS

Lupus nephritis (LN), the most common secondary glomerular disease, develops in up to 60% of patients with systemic lupus erythematosus (SLE) during the course of the disease¹. Lesions of renal small-artery vasculopathy and chronic renal ischemia in patients with LN are common^{2,3,4}. Antiphospholipid antibodies (aPL) correlate with these lesions, including thrombotic microangiopathy (TMA); fibrous intimal hyperplasia (FIH) involving organized thrombi, fibrous and/or fibrocellular occlusions of arteries and arterioles; focal cortical atrophy (FCA); and tubular

thyroidization. These renal lesions in aPL-associated nephropathy (aPLN) may worsen the prognosis of LN⁵. The recognition of the lesions may have therapeutic significance, including antithrombotic and/or vasoprotective therapy. However, these types of histopathologic vascular damage may be nonspecific, and should be distinguished from vasculitis, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, malignant hypertension (HTN), and other reasons for renal ischemia^{5,6}. Otherwise, the definition of a histopathologic lesion of aPLN was not identical in articles. In the most recent consensus criteria of the antiphospholipid syndrome (APS), FIH should involve organized thrombi⁶. However, some authors defined FIH only as with or without recanalization, and that organized thrombi are not essential⁷.

The mechanism of aPL-associated renal lesions is largely unknown. β 2-glycoprotein I (β 2GPI; apolipoprotein H), a plasma protein known to bind to anionic phospholipids, is the most important cofactor of aPL, such as anticardiolipin antibodies (aCL), lupus anticoagulant (LAC), and anti- β 2GPI antibodies⁸. The β 2GPI-aPL complex has a pivotal role to promote thrombus formation⁹. It has been shown *in vitro* that β 2GPI binds nonstimulated endothelial cells, which then enables anti- β 2GPI antibodies to bind the cells and to induce

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a procoagulant phenotype¹⁰. The β 2GPI expression was present in the trophoblast surfaces of placentae obtained from 4 patients with primary APS by indirect immunofluorescence¹¹. Therefore, we investigated whether β 2GPI expression was involved in patients with LN with aPLN and determined its specificity in aPLN. A retrospective study of 231 patients with LN was performed to address this issue.

MATERIALS AND METHODS

Patients. We studied 231 patients with LN who attended the Division of Nephropathy of Peking Union Medical College Hospital. All these patients fulfilled the 1997 revised American College of Rheumatology classification criteria for the diagnosis of SLE. Included in addition were 4 patients with benign HTN, 10 with malignant HTN, 2 with systemic sclerosis (SSc), 3 with thrombotic thrombocytopenic purpura/hemolytic uremic syndrome, 10 with antineutrophil cytoplasmic antibodies-associated vasculitis (AAV), 2 with immunoglobulin (Ig) A nephropathy, and 2 with minimal change disease.

Clinical evidence was obtained and pathologic findings of renal biopsy specimen confirmed the diagnoses according to the appropriate classification criteria. Plasma samples were collected within 3 days before renal biopsy. The following demographic, clinical, and serologic data of patients with SLE were collected at the time of renal biopsy: sex, age, weight, duration of the disease, history of pregnancy and symptomatic thrombosis, prevalence of systemic HTN, proteinuria, levels of serum albumin, and serum creatinine. Estimated glomerular filtration rate (eGFR) was calculated according to the abbreviated Modification of Diet in Renal Disease Study equation. The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was

scored for each patient with SLE at the time of serum collection. The levels of aPL (LAC, aCL, anti- β 2GPI antibody) were measured in all patients 2x or more, at least 12 weeks apart. An overview of the clinical and laboratory data is given in Table 1.

All patients had given informed written consent to participate in the study and the study protocol was reviewed and approved by the regional ethics committee at Peking Union Medical College Hospital in Beijing, China.

Histology of renal biopsy samples. All patients underwent ultrasound-guided renal needle biopsy. The renal tissues obtained by biopsy were fixed in 10% neutral buffered formalin, dehydrated gradually, and embedded in paraffin. Paraffin-embedded tissue sections were stained with H&E, periodic acid-Schiff, Masson trichrome, and periodic acid–silver methenamine. Small portions of fresh renal tissue were snap-frozen and 4-mm cryostat-cut sections were incubated with fluorescein isothiocyanate-conjugated rabbit antisera against human IgG, IgA, IgM, C1q, complement factor 3 (C3), and C4 (Dako), and the direct immunofluorescence of these sections was examined. The biopsy specimens of the patients with SLE were classified using the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification of LN¹². The classification data are given in Table 1.

Particularly, the presence or absence of histopathologic lesions with aPLN was determined in each specimen. The lesions were TMA involving both arterioles and glomerular capillaries, FIH with or without recanalization, fibrous or fibrocellular occlusions of arteries and arterioles, FCA, and tubular thyroidization. The definition of aPLN was the coexistence of aPL (laboratory criteria for APS)⁶ along with the histopathologic detection of above lesions. Histological data with aPLN are given in Table 2.

Immunohistochemical staining of renal biopsy samples. Immunohistochemical staining for β 2GPI was performed on paraffin-embedded tissue by

Table 1. Demographic and clinical characteristics and laboratory findings of the patients with LN. Values are mean \pm SD unless otherwise specified.

Variables	aPL+ with Histological aPLN, Group 1, n = 43	aPL- with Histological aPLN, Group 2, n = 45	aPL- without Histological aPLN, Group 3, n = 119	aPL+ without Histological aPLN, Group 4, n = 24	Overall Comparison, p	Group 1 vs Group 2, p	Group 1 vs Group 3, p	Group 1 vs Group 4, p
Age, yrs	32 \pm 11	34 \pm 10	26 \pm 10	24 \pm 11	< 0.001	0.312	0.008	0.004
Male:female ratio	13:30	5:40	17:102	3:21	0.057	0.027	0.021	0.105
SLE-biopsy time interval, mos*	85.1 \pm 47.4	62.4 \pm 45.7	43.0 \pm 27.6	48.0 \pm 38.4	0.256	0.814	0.243	0.700
APS, n (%)	6 (14.0)	0	0	3 (12.5)	0.002	0.011	< 0.001	1.000
Systolic BP, mmHg	142 \pm 26	135 \pm 24	126 \pm 22	134 \pm 26	0.002	0.210	0.001	0.231
Mean BP, mmHg	106 \pm 20	102 \pm 16	96 \pm 17	101 \pm 21	0.006	0.408	0.002	0.277
Hypertension, n (%)	26 (60.5)	22 (48.9)	45 (37.8)	10 (41.7)	0.701	0.276	0.010	0.139
Scr at biopsy, μ mol/l	123 \pm 108	150 \pm 139	91 \pm 41	112 \pm 141	0.001	0.707	0.003	0.027
eGFR at biopsy, ml/min \cdot 1.73m ²	67.6 \pm 29.5	65.4 \pm 39.6	87.7 \pm 23.9	89.4 \pm 35.0	< 0.001	0.524	< 0.001	0.020
24 h proteinuria, g/day	5.20 \pm 4.03	6.91 \pm 6.34	4.21 \pm 3.80	5.09 \pm 4.42	0.025	0.226	0.145	0.873
24 h proteinuria \geq 3 g/day, n (%)	25 (58.1)	30 (66.7)	59 (49.6)	13 (54.2)	0.260	0.490	0.288	0.672
SLEDAI	14 \pm 6	13 \pm 6	13 \pm 5	13 \pm 5	0.912	0.558	0.488	0.689
ISN/RPS classification, n								
II	3	2	20	5	0.069	0.673	0.113	0.124
III	13	5	16	6	0.038	0.026	0.014	0.649
IV	16	26	51	6	0.052	0.053	0.519	0.308
V	3	7	23	5	0.276	0.316	0.059	0.124
III + V	1	3	3	1	0.595	0.617	1.000	1.000
IV + V	7	2	6	1	0.067	0.086	0.043	0.242

* Time interval between diagnosis of SLE and renal biopsy. Significant values at < 0.05 are in bold face. LN: lupus nephritis; aPL: antiphospholipid antibodies; aPLN: aPL-associated nephropathy; SLE: systemic lupus erythematosus; APS: antiphospholipid syndrome; BP: blood pressure (1 mmHg = 0.133 Kpa); Scr: serum creatinine; eGFR: estimated glomerular filtration rate; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; ISN/RPS: International Society of Nephrology/Renal Pathology Society.

Table 2. Histological findings association in patients with LN with aPLN in the analyzed subgroups. Values are n unless otherwise specified.

Variables	aPL+ with Histological aPLN, Group 1, n = 43	aPL- with Histological aPLN, Group 2, n = 45	aPL- without Histological aPLN, Group 3, n = 119	aPL+ without Histological aPLN, Group 4, n = 24	Group 1 vs Group 2, p
Thrombotic microangiopathy	12	6	0	0	0.896
Chronic lesions	35	39	0	0	0.699
Fibrous intimal hyperplasia	35	37	0	0	0.852
Arterial fibrous or fibrocellular occlusion	6	9	0	0	0.451
Tubular thyroidization	8	9	0	0	0.868
Focal cortical atrophy	2	2	0	0	1.000

LN: lupus nephritis; aPL: antiphospholipid antibodies; aPLN: aPL-associated nephropathy.

using a rabbit polyclonal anti-human β 2GPI antibody from Sigma-Aldrich Inc. The paraffin-embedded tissue, 2- μ m sections, was deparaffinized and rehydrated through a series of washes with xylene and graded alcohols. Antigen retrieval was performed by flooding the slides with 10-mM citrate buffer (pH 6.0) and heating in a 750 W microwave at 100°C for 10 min. Endogenous peroxidase was blocked by treatment with 3% H₂O₂ for 10 min. Then nonspecific binding was blocked with 10% normal goat serum in phosphate buffered saline (PBS) for 20 min. Primary rabbit polyclonal anti-human β 2GPI antibody was applied to the slides at a dilution of 1:50 and the slides were subsequently incubated overnight at 4°C. The slides were then incubated with a secondary goat anti-rabbit IgG antibody (HPA001654, Sigma) for 20 min at 37°C. The slides were stained by DAB immunohistochemical staining for 3–5 min. These sections were then washed with PBS (pH 7.4) between each step (3 \times for 5 min each time). Finally, the sections were counterstained with hematoxylin, air-dried, cleared in xylene, and coverslipped. Typically, β 2GPI were stained in several parts of the cortex and the medulla of the kidney, including tubular epithelium and tubular lumen (Figure 1A–F). Sometimes Bowman capsules were also stained (not shown). Typical β 2GPI expression in endothelial cells in the kidney in patients with LN with aPLN is shown in Figure 1B–D.

Statistical analysis. For comparison purposes, the whole series was divided into 4 groups according to the aPL status and the existence of histological aPLN: (1) LN with aPL and histological aPLN, (2) LN with histological aPLN without aPL, (3) LN without aPL or histological aPLN, and (4) LN with aPL without histological aPLN. SPSS version 13.0 software (SPSS Inc.) was used to perform all statistical analyses. Categorical variables were compared using Fisher's exact test or chi-square test. Differences between the median values of defined patient groups were compared using the nonparametric Mann–Whitney U test. A p value of < 0.05 was considered statistically significant.

RESULTS

Demographic and clinical characteristics and laboratory findings of patients with LN. In our study, we examined 231 patients with LN (193 women and 38 men) with a mean age (\pm SD) of 29 \pm 11 years. The LN with aPL and histological aPLN group consisted of 43 patients (18.6%; 30 women and 13 men), the LN with histological aPLN without aPL group consisted of 45 patients (19.5%; 40 women and 5 men), the LN without aPL or histological aPLN group consisted of 119 patients (102 women and 17 men), and the LN with aPL without histological aPLN group consisted of 24 patients (21 women and 3 men).

The mean ages (\pm SD) of the patients in the 4 groups were 32 \pm 11 years, 34 \pm 10 years, 26 \pm 10 years, and 24 \pm 11 years, respectively. The patients in the LN with aPL and histological aPLN group were significantly older than the patients in the

LN without aPL or histological aPLN group and in the LN with aPL without histological aPLN group ($p < 0.01$ for both). No significant difference was observed between the LN with aPL and histological aPLN group and the LN with histological aPLN without aPL group in terms of age ($p > 0.05$). The male to female ratio in the LN with aPL and histological aPLN group was significantly higher than that in the LN with histological aPLN without aPL group and the LN without aPL or histological aPLN group ($p < 0.05$ for both).

The patients with APS in the LN with aPL and histological aPLN group were significantly more than the patients in the LN with histological aPLN without aPL group and in the LN without aPL or histological aPLN group ($p = 0.011$ and $p < 0.001$, respectively). Systolic blood pressure (BP), mean arterial pressure, and the frequency of systemic HTN were all greater in the LN with aPL and histological aPLN group than in the LN without aPL or histological aPLN group ($p = 0.001$, $p < 0.05$, $p < 0.05$, respectively). BP and the frequency of systemic HTN did not differ between the LN with aPL and histological aPLN group and the LN with histological aPLN without aPL group or the LN with aPL without histological aPLN group ($p > 0.05$ for all). Serum creatinine levels at biopsy were greater in the LN with aPL and histological aPLN group than in the LN without aPL or histological aPLN group and in the LN with aPL without histological aPLN group ($p < 0.01$, $p < 0.05$, respectively). At biopsy, eGFR were appropriately lower in the LN with aPL and histological aPLN group than in the LN without aPL and histological aPLN group and in the LN with aPL without histological aPLN group ($p < 0.001$ and $p < 0.05$, respectively). However, serum creatinine levels and eGFR at biopsy did not differ between the LN with aPL and histological aPLN group and the LN with histological aPLN without aPL group. SLE biopsy time interval, the antecedent history of vascular thrombosis and pregnancy morbidity (data not shown), 24-h proteinuria, the frequency of heavy proteinuria, or SLEDAI did not differ between the LN with aPL and histological aPLN group and the other groups ($p > 0.05$ for all).

The distribution of the ISN/RPS classification of the 231 patients was as follows: 30 were class II, 40 were class III, 99 were class IV, 38 were class V, 8 were class III + V, and 16 were class IV + V. The frequency of class III LN in the

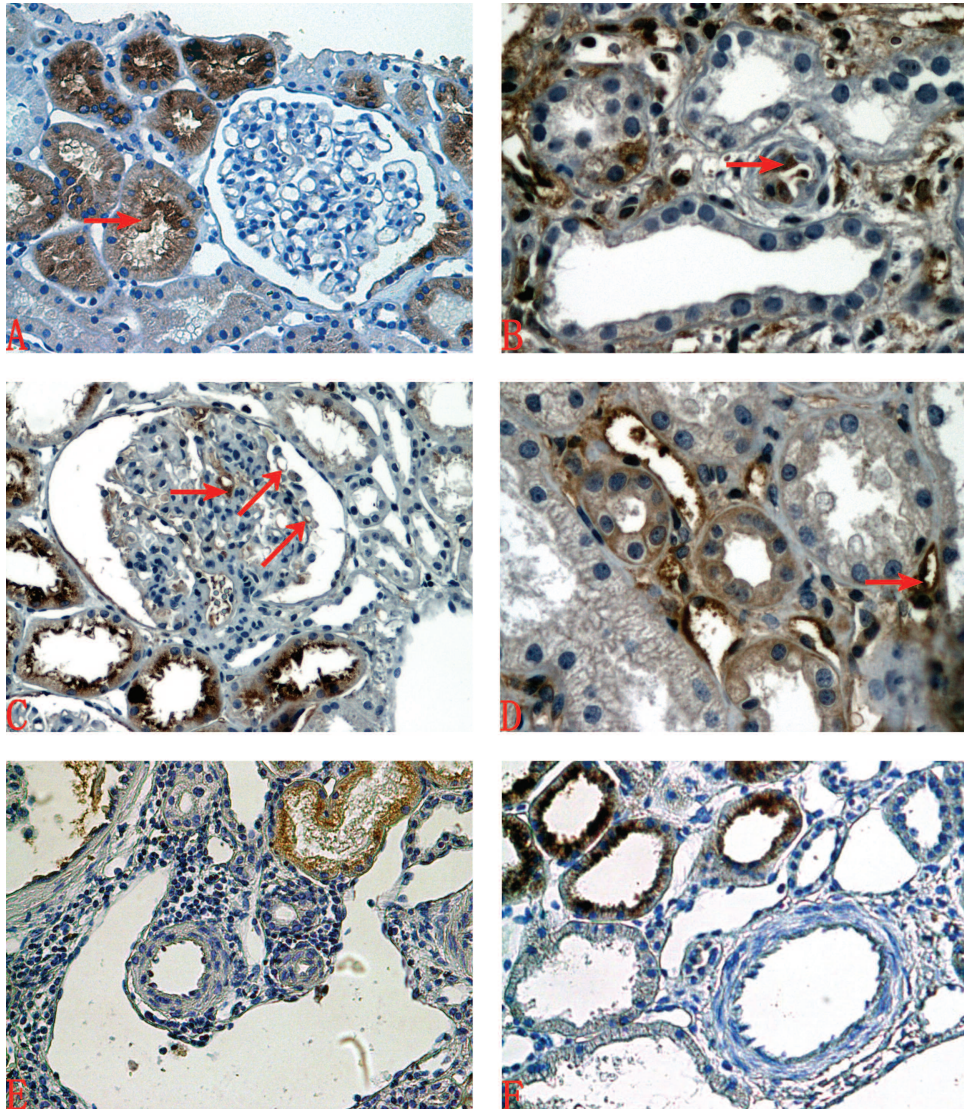


Figure 1. β 2GPI expression on the endothelial cells in the kidney by immunohistochemistry. (A) β 2GPI expression (red arrow) on tubular epithelial cells in a patient with minimal disease change. (B) β 2GPI expression (red arrow) on the endothelial cells in a kidney arteriole showing fibrocellular occlusions in a patient with LN with positive aPL. (C) β 2GPI expression (horizontal red arrow) on the glomerular capillary endothelial cells in a patient with LN with positive aPL. Mild β 2GPI expression on endothelial cells was also shown (oblique arrows). (D) Intense β 2GPI expression (red arrow) on peritubular capillary endothelial cells in a patient with LN with positive aPL and histological aPLN. (E) β 2GPI expression was shown only on tubular epithelial cells, not on endothelial cells in fibrocellular occlusions of arterioles in a patient with LN without aPL. (F) β 2GPI expression was shown only on tubular epithelial cells, not on endothelial cells in a patient with IgA nephropathy. Original magnification $\times 200$ except for Figure 1D, which had original magnification $\times 400$. β 2GPI: β 2 glycoprotein-I; LN: lupus nephritis; aPL: antiphospholipid antibodies; aPLN: aPL-associated nephropathy; IgA: immunoglobulin A.

LN with aPL and histological aPLN group (30.2%) was higher than in the LN with aPL without histological aPLN group (11.1%) and in the LN without aPL or histological aPLN group (13.4%; $p < 0.05$ for both).

Relationships between β 2GPI expression and the presence of aPLN. Histological aPLN was detected in 88 patients (38.1%) with LN. In the LN with aPL and histological aPLN group, TMA was found in 12 patients (27.9%). In 35 patients

(81.4%), we found chronic vascular damage including FCA, FIH, fibrous and/or fibrocellular occlusions of arteries and arterioles, or tubular thyroidization. Two patients had FIH involving organized thrombi. Twelve patients (27.9%) had both TMA and chronic lesions. TMA lesions or chronic vascular damage did not differ between the LN with aPL and histological aPLN group and the LN with histological aPLN without aPL group (Table 2).

We detected β 2GPI expression in endothelial cells in 14 subjects (32.6%) in renal arteries or arterioles, 11 (25.6%) in glomerular or peritubular capillaries, and a total of 15 (34.9%) of the 43 patients of the LN with aPL and histological aPLN group; 1 (2.2%) in renal arteriole of the 45 patients of the LN with histological aPLN without aPL group; 1 (0.8%) in glomerular capillaries of the 119 patients of the LN without aPL or histological aPLN group; 2 (8.3%) in arteries or arterioles of the 24 patients of the LN with aPL without histological aPLN group (Figure 2); and 1 (3.0%) in renal arteriole of the 33 patients with glomerular diseases including benign HTN, malignant HTN, SSc, thrombotic thrombocytopenic purpura/hemolytic uremic syndrome, AAV, and minimal change disease.

Expression of β 2GPI in renal arteries or arterioles or capillaries in the LN with aPL and histological aPLN group was significantly greater than in the other 3 groups ($p < 0.01$, $p < 0.001$, and $p < 0.01$, respectively).

According to our definition of aPLN, the specificity of

β 2GPI expression in kidney tissue for aPLN diagnosis in patients with LN was 97.5%. However, the sensitivity of β 2GPI expression for aPLN in patients with LN in our study was 34.8%.

DISCUSSION

In our study, we observed that β 2GPI was mainly expressed in the endothelial cells of interlobular artery or arteriole, or glomerular or peritubular capillaries in the kidney, with the presence of aPLN in patients with LN. Perhaps β 2GPI expression is crucial for the development of aPLN in patients with LN.

The most recent diagnosis criteria for aPLN, based on clinical and histopathologic features, may be nonspecific. Especially in patients with SLE⁶, aPLN should be distinguished from renal small-artery vasculopathy or chronic renal ischemia. However, these diseases themselves may be caused by aPL. Malignant HTN may be a feature of aPL-related renal lesions¹³. Occurrence of thrombotic thrombocytopenic

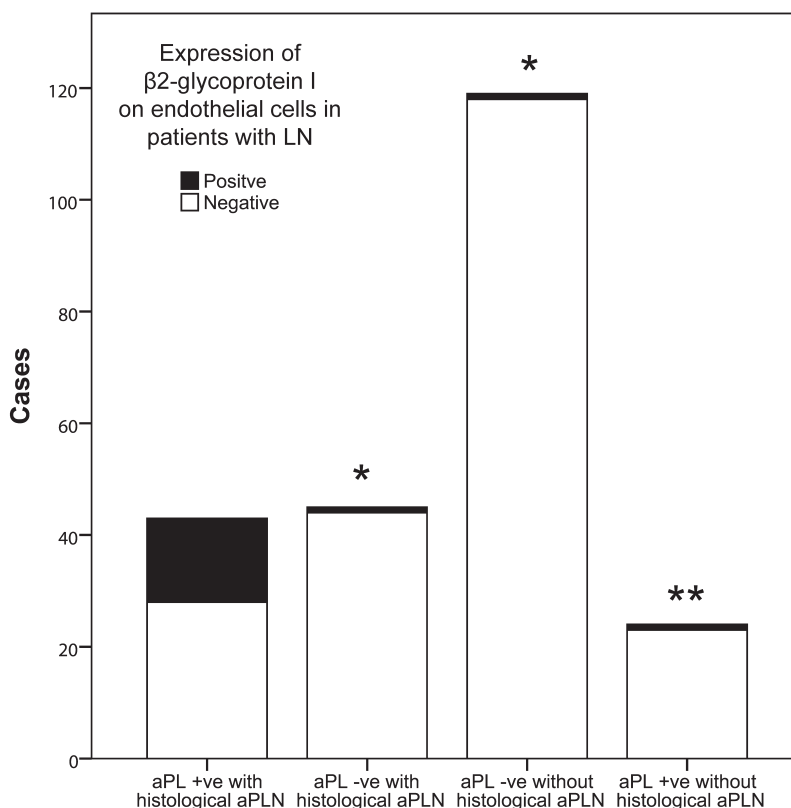


Figure 2. β 2GPI expression profile on renal vessels in patients with LN. A total of 15 patients (34.9%) of the LN with aPL and histological aPLN group showed β 2GPI expression on endothelial cells of renal arteries or arterioles, or glomerular capillaries. Only 4 patients including other groups showed β 2GPI expression. One of 33 patients with other glomerular diseases (including benign HTN, malignant HTN, systemic sclerosis, thrombotic thrombocytopenic purpura/hemolytic uremic syndrome, antineutrophil cytoplasmic antibodies-associated vasculitis, and minimal change disease) showed mild β 2GPI expression in renal arteriole. * < 0.001 and ** < 0.01 , compared with the LN with aPL and histological aPLN group. β 2GPI: β 2 glycoprotein-I; LN: lupus nephritis; aPL: antiphospholipid antibodies; aPLN: aPL-associated nephropathy; HTN: hypertension.

purpura in patients with SLE who have aPL was also reported^{14,15,16}. A few studies have been published regarding markers such as arteriolar C4d deposition for TMA¹⁷. However, a specific marker for aPLN is absent. There are few studies focusing on β 2GPI expression in patients with LN with aPLN. To our knowledge, our study is the first to evaluate β 2GPI expression in renal biopsy specimens of patients with LN who have aPLN. We found that β 2GPI was mainly expressed in patients with LN with the presence of aPLN. The specificity of β 2GPI expression in patients with LN who have aPLN is 97.5%. Therefore, our study suggests that β 2GPI expression in renal biopsy specimens may be an element to be included in the criteria of aPLN.

However, the sensitivity of β 2GPI expression in patients with LN who have aPLN was low: only 34.8%. Moreover, in the positive kidney tissue of β 2GPI expression, only a few endothelial cells were stained. There are several possible reasons. First, aPL in this disorder are directed antigenic targets other than β 2GPI. A few targets have been identified in patients with APS, including prothrombin, tissue plasminogen activator¹⁸, plasmin¹⁹, annexin A2²⁰, and thrombin²¹. Combined detection with multiple antigens may increase the sensitivity. The possible involvement of complement activation in APS pathogenesis is also suggested. Shen, *et al* reported anti- β 2GPI antibodies may be involved in TMA formation, and this process might involve complement activation²². Second, the expression of β 2GPI may be very mild and cannot be detected by current immunohistochemical study. Actually, mild β 2GPI expression in our study was common (Figure 1C).

Overall, the prevalence of aPLN in patients with LN varied from 10.4%–34%. The prevalence of aPLN in our cohort of patients with LN was similar to other series^{2,5,23,24,25}. However, the prevalence of histological aPLN in patients with LN without aPL in our cohort was higher². In fact, most histological aPLN lesions may not be specific and therefore less reproducible because of the retrospective design of the studies and the fact that the pathologists analyzed and identified aPLN lesions⁶. Of note, unspecific arterial changes were more common and we defined all FIH as 1 histological aPLN lesion. Indeed, β 2GPI expression was detected in some patients in our cohort with FIH and cellular myofibroblastic proliferation in the intima with luminal narrowing of small arteries, but without organized thrombi. Therefore, FIH with cellular proliferation in the intima may be a candidate lesion of aPLN.

Our results demonstrate that β 2GPI was mainly expressed in the endothelial cells in patients with LN with aPLN. The specificity of β 2GPI expression in patients with LN with aPLN was 97.5%. The formation of aPLN in patients with LN may involve β 2GPI expression. The β 2GPI expression in endothelial cells in kidney tissue may be considered a useful marker for aPLN.

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