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BRIEF REPORT

Antiphospholipid antibodies in patients with proliferative and membranous lupus nephritis

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Abstract

The aim of this study was to investigate the role of antiphospholipid antibodies (aPLs) in patients with proliferative and membranous lupus nephritis (LN). Patients hospitalized with LN from July 2014 to December 2017 were selected. Levels of serum IgG, IgA, and IgM anticardiolipin (aCL) antibodies, anti-β2-glycoprotein I (anti-β2-GPI) antibodies, and lupus anticoagulant (LAC) were measured. Other clinical and pathological data were obtained at the time of hospitalization for diagnosis. Overall, 75 patients with proliferative LN and 31 patients with membranous LN were analyzed. Significant differences were found between the two groups in the detection rates of aCL antibodies (45.3% vs 22.7%, $p = 0.029$) and IgG-aCL antibodies (40% vs 12.9%, $p = 0.007$). The aCL antibody–positive proliferative LN patients ($n = 34$) had lower serum complement C3 and C4 levels ($p = 0.010$ and $p = 0.021$, respectively) and higher intensity of complement C1q deposition in kidney tissue than the aCL antibody–negative proliferative LN patients ($n = 41$) ($p = 0.003$). Our work suggests that aCL antibodies, especially IgG-aCL antibodies, may play a role in the damage caused by proliferative LN, and this process may involve the classical pathway of complement activation.

Key Points

- *Classes III + V and IV + V LN, which have both proliferative and membranous LN features, were excluded.*
- *The proliferative LN group had significantly higher detection rates of aCL and IgG-aCL than the membranous LN group.*
- *aCL antibodies, especially IgG-aCL antibodies, may play a role in the damage caused by proliferative LN, and this process may involve the classical pathway of complement activation.*

Keywords Anti-aCL antibodies · Complement activation · Lupus nephritis

Introduction

The term "antiphospholipid antibodies (aPLs)" refers to a heterogeneous family of antibodies including anticardiolipin (aCL) antibodies, lupus anticoagulant (LAC), and anti-beta 2-glycoprotein I (anti-β2-GPI). The presence of aPLs has been closely related to the development of thrombosis and pregnancy complications in antiphospholipid syndrome (APS). APS can occur in patients without an underlying systemic autoimmune disease (primary APS) or with other systemic autoimmune diseases, particularly systemic lupus erythematosus (SLE) [1, 2].

Approximately 30–40% of patients with SLE have aPLs. Except for an increased risk of thrombotic events and pregnancy morbidity, SLE patients with aPLs are also at increased risk of renal involvement or renal insufficiency compared with SLE patients with negative aPLs [3, 4]. However, the role of aPLs in patients with lupus nephritis (LN) has not been fully investigated.

Previous studies have shown a significantly greater prevalence of class V LN in aPL-positive patients than in aPL-negative patients [5, 6]. However, other studies have shown that aPL-positive patients have an increased incidence of proliferative LN and that an association between aPLs and World Health Organization (WHO) classification in LN is lacking [7–9]. This discrepancy is partly due to the existence of classes III + V and IV + V LN, both of which have proliferative and membranous LN features.

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In addition, activation of the complement cascade by aPLs plays an important role in the induction of thrombosis and fetal loss in patients with primary or secondary APS [10, 11]. However, whether there is a relationship between aPLs and complement activation in patients with LN has not been well known. The aim of this retrospective study was to compare the aPL positivity between proliferative and membranous LN and to evaluate the relationship of aPLs and complement activation in proliferative and membranous LN.

Materials and methods

Patients hospitalized in the First Affiliated Hospital of Wenzhou Medical University from July 2014 to December 2017 were selected for this retrospective study. The diagnosis of SLE was established by the SLE criteria revised by the American College of Rheumatology in 1997 [12], and all patients showed clinical manifestations of LN. Renal biopsy was performed at the time of hospitalization for diagnosis in all patients. According to the pathological classification criteria of International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 LN, patients with classes I, II, III + V, IV + V, and VI were excluded [13]. This study was approved by the Wenzhou Medical University Research Ethics Committee. All patients provided verbal consent to participate in the study.

The following data were obtained during the same period before renal biopsy: demographic information; LN duration; SLE disease activity index (SLEDAI); proteinuria; hematuria; serum creatinine; serum albumin; serum complement C3; serum complement C4; serum IgG, IgA, and IgM-aCL; anti-β2-GPI antibodies; and LAC.

IgG, IgA, and IgM-aCLs and anti-β2GPI antibodies were measured with ELISA kits (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). LAC was detected using a LAC Screen/LAC Confirm Kit (Instrumentation Laboratory Company, Lexington, MA) on the IL coagulation system (Instrumentation Laboratory Company), and the dilute Russell's viper venom time was determined according to the manufacturer's instructions.

Renal biopsies were interpreted and reported by one specialized pathologist according to the ISN/RPS 2003 classification of LN [13]. The following data were obtained: classification, activity index and chronicity index, mesangial proliferation, endothelial proliferation, crescent, loop necrosis, micro-thrombosis, and ratio of chronic renal tubular interstitial inflammation. The intensity of glomerular immunofluorescence staining for IgG, IgM, IgA, C3, C4, and C1q was semi-quantitatively scored on a scale of 0 to 3, where 0 = no glomerular staining, 1 = mild glomerular staining, 2 = moderate glomerular staining, and 3 = intense glomerular staining [14].

Variables are presented as the mean and standard deviation or proportion. Chi-square analysis or *t* tests were used to compare differences between two groups. A p value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 19.

Results

Overall, 75 hospitalized patients including 18 with class III LN, 57 with class IV LN, and 31 patients with membranous LN (class V) were analyzed. Demographic and clinical characteristics of the patients with proliferative (class III and class IV) and non-proliferative (class V) LN are summarized in Table 1. Most patients in both groups were female, and the mean age (±SD) of the proliferative and non-proliferative groups was 33.8 ± 13.1 and 35.3 ± 14.1 years, respectively. Patients with proliferative LN had significantly higher serum creatinine levels, higher SLEDAI scores, and more hematuria than patients with membranous LN ($p < 0.001$).

Classification of aPL antibodies of the patients with proliferative and non-proliferative LN is shown in Table 2. Significant differences were found between the proliferative and membranous groups in the detection rates of serum aCL antibodies (45.3% vs 22.7%, respectively, $p = 0.029$) and IgG-aCL antibodies (40% vs 12.9%, respectively, $p = 0.007$). However, the detection rates of anti-β2-GPI antibodies and LAC in the proliferative LN group were not statistically different from those in the membranous LN group ($p > 0.05$ for both).

When we compared the clinical and pathological characteristics of proliferative LN patients with and without aCL antibodies, as shown in Table 3, the aCL antibody–positive patients had lower serum complement C3 and C4 levels ($p = 0.010$ and $p = 0.021$, respectively) and more complement C1q deposition in the kidney than the aCL antibody–negative patients ($p = 0.003$).

Discussion

LN is an immune complex glomerular nephritis that develops as a frequent manifestation of SLE. The pathogenesis of LN involves a variety of pathogenic mechanisms [15]. Given the relatively high prevalence of aPLs in SLE/LN patients, aPLs may play an important role in LN progression [3, 4], but whether aPLs are involved in or alter the course of LN remains unclear.

In a retrospective study, Tsuruta et al. found that aPLs had no prognostic value for long-term renal outcomes in 49 patients with LN, and the frequency of class V LN was low among aPL-positive patients [7]. However, in another study, Moroni et al. evaluated the prevalence of IgG and IgM-aCL

Table 1 Demographic and clinical characteristics of patients with proliferative and membranous LN

Variable	Proliferative LN ($n = 75$)	Membranous LN ($n = 31$)	p value
Sex (male, %)	22 (29.3)	6 (19.4)	0.289
Age (years)	33.8 ± 13.1	35.3 ± 14.1	0.618
LN duration (months)	4.8 (2.0–7.5)	6.2 (2.5–9.2)	0.06
Proteinuria (g/24 h)	3.7 ± 2.3	3.5 ± 2.3	0.674
Hematuria (*n*, %)	60 (80.0)	9 (29.0)	< 0.001*
Albumin (g/L)	25.1 ± 5.4	27.0 ± 8.7	0.261
Serum creatinine (μmol/L)	103.9 ± 38.4	68.2 ± 34.3	< 0.001*
SLEDAI	11.8 ± 3.6	7.5 ± 3.1	< 0.001*
Serum C3 (mg/dL)	39.9 ± 16.4	45.9 ± 13.8	0.057
Serum C4 (mg/dL)	6.6 ± 4.5	7.8 ± 3.4	0.174

LN, lupus nephritis; *SLEDAI*, systemic lupus erythematosus (SLE) disease activity index

Data are shown as the mean ± SD (standard deviation) or percentage

*$p < 0.001$

antibodies in patients with LN and found that the presence of aPLs was an independent predictor of chronic renal function deterioration [6]. Moreover, aPL detection was significantly increased in patients with biopsy-proven membranous LN [6]. Parodis et al. investigated the impact of aPLs on short-term and long-term renal outcomes in patients with LN and showed that neither aPL positivity nor the aPL level was associated with the occurrence of LN in SLE patients [16]. These discrepancies may be due to differences in the characteristics of the study population or the type of aPLs.

In this study, classes III + V and IV + V LN, which have both proliferative and membranous LN features, were excluded. The proliferative LN group had significantly higher detection rates of aCL and IgG-aCL than the membranous LN group, which indicates that aCL antibodies, especially IgG-aCL antibodies, may play an important role in patients with proliferative LN rather than membranous LN.

Buttgereit et al. found that disease activity in SLE was accompanied by significantly increased IgG-aCL but not IgM-aCL, whereas no elevation was found in other diseases including rheumatoid arthritis, reactive arthritis, spondyloarthropathies, and vasculitis [17]. Sarabi et al. investigated the relationship between SLE activity and aPLs and found a positive correlation between IgG-aCL titer and SLEDAI at first visit [18]. These indicated that aCL, especially IgG-aCL, could result in more immune reactivity in patients with SLE. However, LAC or anti-β2-GPI antibodies were more relative to thrombosis than aCL antibodies [14, 19].

We speculated aCL, especially IgG-aCL, may lead to more severe renal pathological injury including such as mesangial proliferation, endothelial proliferation, or cellular crescent. So to further explore the role of aCL antibodies in patients with proliferative LN, we compared the clinical and pathological characteristics in proliferative LN patients with and without aCL antibodies. Our results showed that aCL antibody–positive patients had significantly lower serum C3 and C4 and higher intensity of C1q deposits in the kidney than the aCL antibody–negative patients. Meanwhile, aCL antibody–positive patients had more severe renal injury than the aCL antibody–negative patients, including higher proteinuria, activity index score, and intensity of immunocomplex deposits (IgG, A, M; C3, C4), more hematuria, moderate to severe mesangial proliferation, endothelial proliferation, cellular crescent, loop necrosis, and micro-thrombosis, although there were no significant differences between the two groups. These findings indicate that aCL antibodies might play a role in the progression of proliferative LN, and this process might involve the classical pathway of complement activation.

Table 2 Classification of aPL antibodies in patients with proliferative and non-proliferative LN

aPL assays	Proliferative LN ($n = 75$)	Membranous LN ($n = 31$)	p value
aCL (+) (*n*, %)	34 (45.3)	7 (22.7)	0.029*
IgG-aCL (+) (*n*, %)	30 (40)	4 (12.9)	0.007*
IgM-aCL (+) (*n*, %)	5 (6.7)	2 (6.5)	1.000
IgA-aCL (+) (*n*, %)	7 (9.3)	3 (9.7)	1.000
Anti-β2GPI (+) (*n*, %)	26 (34.7)	9 (29.0)	0.575
LAC (+) (*n*, %)	13 (17.3)	7 (22.6)	0.530

LN, lupus nephritis; *aPL*, antiphospholipid; *aCL*, anticardiolipin; *LAC*, lupus anticoagulant

*$p < 0.05$

Table 3 Clinical and pathological data for patients with proliferative LN with and without aCL antibodies ($n = 75$)

Variables	aCL (+) ($n = 34$)	aCL (−) ($n = 41$)	p value
Proteinuria (g/24 h)	3.9 ± 2.2	3.5 ± 2.4	0.125
Hematuria (n, %)	28 (82.3)	32 (78)	0.643
Serum creatinine (μmol/L)	110.1 ± 38.8	105.5 ± 38.2	0.362
Serum C3 (mg/dL)	34.6 ± 15.5	44.2 ± 16.0	0.010*
Serum C4 (mg/dL)	5.3 ± 3.7	7.7 ± 4.8	0.021*
Activity index score	11.0 ± 2.5	10.2 ± 2.9	0.208
Chronic lesion score	1.1 ± 0.8	1.3 ± 0.6	0.341
Moderate to severe mesangial proliferation (n, %)	13 (38.2)	9 (21.9)	0.123
Endothelial proliferation (n, %)	15 (44.1)	16 (38.0)	0.644
Cellular crescent (n, %)	11 (32.3)	10 (24.3)	0.445
Loops necrosis (n, %)	3 (8.8)	3 (7.3)	0.811
Micro-thrombosis (n, %)	8 (23.5)	7 (17)	0.487
Ratio of chronic renal tubular interstitial inflammation (%)	12.2 ± 8.1	18.3 ± 11.5	0.185
Intensity of deposits			
IgG	2.4 ± 1.2	2.2 ± 0.8	0.390
IgA	1.5 ± 0.4	1.6 ± 0.7	1.000
IgM	2.1 ± 0.8	1.9 ± 0.7	0.385
C3	3.3 ± 0.5	2.8 ± 0.6	0.064
C4	2.5 ± 0.8	2.2 ± 0.9	0.345
C1q	2.7 ± 1.2	1.7 ± 0.9	0.003*

LN, lupus nephritis; *aCL*, anticardiolipin antibody; *SLEDAI*, systemic lupus erythematosus (SLE) disease activity index

Data are shown as mean ± SD (standard deviation) or percentages

*$p < 0.05$

Many previous studies have also found a strong association between aPLs and complement activation. Watanabe et al. assessed the relationship between the complement activation route and clinical manifestations in patients with SLE and suggested that a different complement system mechanism may act in the pathogenesis of APS in patients with SLE [20]. Pierangeli et al. found that the complement system was highly activated and that complement C3 levels were significantly decreased after injecting aPLs into rat models of thrombosis [21]. Garabet et al. demonstrated that aPLs were associated with low complement C3 and C4 levels in patients with SLE [22]. In addition, complement activation was involved in the induction of thrombosis and fetal loss by aPLs in patients with primary or secondary APS [10, 11]. In vitro studies and studies in SLE patients have shown that aPLs promote the deposition of C4 on platelets [23].

However, there are several limitations of our study. First, follow-up data were lacking for most patients, and aPLs were evaluated at only one time point; thus, we could not determine whether aPLs have a role in disease monitoring or prognosis. Second, the study population was limited to SLE patients who underwent renal biopsy and had aPL measurements; therefore, the sample size was small. Third, few measurements of complement activation factors were available; therefore, we could not identify which complement activation pathway plays the most important role. Thus, future studies should be performed with a prospective design and more patients to address the above problems.

In summary, the major findings of this study are that aCL antibodies, especially IgG-aCL antibodies, may play a role in the damage caused by proliferative LN but not membranous LN, and this process may involve the classical pathway of complement activation.

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Compliance with ethical standards

This study was approved by the Wenzhou Medical University Research Ethics Committee. All patients provided verbal consent to participate in the study.

Disclosures None.

References

1. Levine JS, Branch DW, Rauch J (2002) The antiphospholipid syndrome. N Engl J Med 346:752–763
2. Miyakis S, Lockshin MD, Atsumi T et al (2006) International consensus statement on an update of the classification criteria for

definite antiphospholipid syndrome (APS). J Thromb Haemost 4: 295–306
3. Petri M (2000) Epidemiology of the antiphospholipid antibody syndrome. J Autoimmun 15:145–151
4. Taraborelli M, Leuenberger L, Zhang W et al (2014) The effect of clinically significant antiphospholipid antibody positivity on organ damage in systemic lupus erythematosus. Arthritis Rheum 66:8–9
5. Levy Y, Gearge J, Ziporen L et al (1998) Massive proteinuria as a main manifestation of primary antiphospholipid syndrome. Pathobiology 66:49–52
6. Moroni G, Ventura D, Riva P, Panzeri P, Quaglini S, Banfi G, Simonini P, Bader R, Meroni PL, Ponticelli C (2004) Antiphospholipid antibodies are associated with an increased risk for chronic renal insufficiency in patients with lupus nephritis. Am J Kidney Dis 43:28–36
7. Tsuruta Y, Uchida K, Itabashi M et al (2009) Antiphospholipid antibodies and renal outcomes in patients with lupus nephritis. Inter Med 48:1875–1880
8. Fofi C, Cuadrado MJ, Godfrey T, Abbs I, Khamashta MA, Hughes GR (2001) Lack of association between antiphospholipid antibody and WHO classification in lupus nephritis. Clin Exp Rheumatol 19: 75–77
9. Naiker IP, Rughubar KN, Duursma J et al (2000) Anticardiolipin antibodies in South African patients with lupus nephritis: a clinical and renal pathological study. Am J Nephrol 20:351–357
10. Pierangeli SS, Vega-Ostertag M, Liu X et al (2005) Complement activation: a novel pathogenic mechanism in the antiphospholipid syndrome. Ann N Y Acad Sci 1051:413–420
11. Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA (2010) Antiphospholipid syndrome. Lancet 376:1498–1509
12. Hochberg MC (1997) Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 40:1725
13. Weening JJ, D'Agati VD, Schwartz MM et al (2004) The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol 15:241–250
14. Zheng H, Chen Y, Ao W et al (2009) Antiphospholipid antibody profiles in lupus nephritis with glomerular microthrombosis: a prospective study of 124 cases. Arthritis Res Ther 11:R93
15. Tsokos GC (2011) Systemic lupus erythematosus. N Engl J Med 365:2110–2121
16. Parodis I, Arnaud L, Gerhardsson J, Zickert A, Sundelin B, Malmström V, Svenungsson E, Gunnarsson I (2016) Antiphospholipid antibodies in lupus nephritis. PLoS One 11(6): e0158076
17. Buttgereit F, Grünewald T, Schüler-Maué W, Burmester GR, Hiepe F (1997) Value of anticardiolipin antibodies for monitoring disease activity in systemic lupus erythematosus and other rheumatic diseases. Clin Rheumatol 16:562–569
18. Sarabi ZS, Sahebari M, Rezaie AE et al (2018) The relationship between systemic lupus erythematosus activity and persistent positive antiphospholipid antibodies. Curr Rheumatol Rev 14:145–152
19. Shen Y, Chen XW, Sun CY et al (2010) Association between antibeta2 glycoprotein I antibodies and renal glomerular C4d deposition in lupus nephritis patients with glomerular microthrombosis: a prospective study of 155 cases. Lupus 19:1195–1203
20. Watanabe H, Sugimoto M, Asano T, Sato S, Suzuki E, Takahashi A, Katakura K, Kobayashi H, Ohira H (2015) Relationship of complement activation route with clinical manifestations in Japanese patients with systemic lupus erythematosus: a retrospective observational study. Mod Rheumatol 25:205–209
21. Pierangeli SS, Girardi G, Vega Ostertag M et al (2005) Requirement of activation of complement C3 and C5 for antipho8pholipid antibody-mediated thrombophilia. Arthritis Rheum 52:2120–2124
22. Garabet L, Gilboe IM, Mowinckel MC, Jacobsen AF, Mollnes TE, Sandset PM, Jacobsen EM (2016) Antiphospholipid antibodies are associated with low levels of complement C3 and C4 in patients with systemic lupus erythematosus. Scand J Immunol 84:95–99
23. Lood C, Tydén H, Gullstrand B et al (2014) Platelet activation and antiphospholipid antibodies collaborate in the activation of the complement system on platelets in systemic lupus erythematosus. PLoS One 9:e99386

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