Article type: Original article

Title: Phosphatidylserine-Dependent Anti-prothrombin Antibodies as a Key Predictor for Systemic Lupus Erythematosus in Patients with Primary Antiphospholipid Syndrome: A retrospective longitudinal cohort study

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Running title: aPS/PT in PAPS and SLE

Conflict of interest

T.Atsumi reports personal fees from Chugai, during the conduct of the study; grants and personal fees from Astellas, grants and personal fees from Takeda, grants and personal fees from Mitsubishi Tanabe, grants and personal fees from Chugai, grants and personal

fees from Pfizer, grants from Daiichi Sankyo, grants from Otsuka, personal fees from Eisai, personal fees from AbbVie, outside the submitted work. M.Kato has received research grants from AbbVie, Actelion, and GlaxoSmithKline and speaking fees from Eli Lilly. M. Kono reports grants and/or speaking fees from AbbVie Inc., Asahi-Kasei Co., Astellas Pharma Inc., AstraZeneca plc., AYUMI Pharmaceutical Co., Ltd., Bristol-Myers Squibb Co., Chugai Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Eisai Co. Ltd., Eli Lilly Japan K.K., Gilead Sciences K.K., GlaxoSmithKline K.K., Janssen Pharmaceutical K.K., Kowa Co. Ltd., KYOCERA Co., Ltd., LOTTE CO., LTD., Nippon Boehringer Ingelheim Co., Ltd., NIPPON SHINYAKU CO., LTD., Mitsubishi Tanabe Pharma Co., MOCHIDA PHARMACEUTICAL CO.,LTD., Pfizer Inc., Taiju Life Social Welfare Foundation, Taisho Pharmaceutical, Takeda Pharmaceutical Co., Ltd., Terumo Co., Ltd., UCB Japan Co. Ltd. and YAMAZAKI BAKING CO.,LTD., outside the submitted work. Y.Fujieda has received research grants from MEDICAL & BIOLOGICAL LABORATORIES CO., LTD., and speaking fees from AbbVie Inc., Asahi-Kasei Co., Astellas Pharma Inc., AstraZeneca plc., Bristol-Myers Squibb Co., Chugai Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd.,Eisai Co. Ltd., Eli Lilly Japan K.K., Janssen Phar- maceutical K.K., Kyowa Kirin Co., Ltd., Novartis Pharma K.K., and Nippon Boehringer Ingelheim Co., Ltd., Mit- subishi Tanabe Pharma Co., Ono Pharmaceutical Co. Ltd., Otsuka Pharmaceutical Co., Ltd., Pfizer Inc., Takeda Phar- maceutical Co., Ltd., Taisho Pharmaceutical Co., Ltd., Teijin Pharma Ltd., and UCB Japan Co. Ltd. R. Hisada has received speaking fees from AbbVie Inc., Asahi-Kasei Co.; Astellas Pharma Inc., AYUMI Pharmaceutical Co., Bristol-Myers Squibb Co., Chugai Pharmaceutical Co., Ltd.; Daiichi Sankyo Co., Ltd., Eisai Co. Ltd., Janssen Phar- maceutical K.K., Nippon Boehringer Ingelheim Co., Mit- subishi Tanabe Pharma Co., Otsuka Pharmaceutical Co., Ltd.; Pfizer Inc. The other authors state that they have no conflict of interest.

Acknowledgements: This work was partly supported by the project of junior scientist promotion in Hokkaido University.

Keywords

Primary antiphospholipid syndrome (PAPS), Systemic lupus erythematosus (SLE), phosphatidylserine-dependent anti-prothrombin antibody (aPS/PT), antiphospholipid antibodies, longitudinal study

Abstract

[Objectives] Primary antiphospholipid syndrome (PAPS) is an autoimmune disorder characterized by thrombosis and pregnancy morbidity. Although PAPS is distinguished from systemic lupus erythematosus (SLE), the two conditions share clinical features and susceptibility genes. Progression from PAPS to SLE is well-recognized. However, risk factors for this transition are poorly understood. We aimed to identify predictors of progression to SLE from patients with PAPS.

[Methods] A longitudinal single-center study was conducted at Hokkaido University Hospital from 1990-2021. Baseline characteristics including clinical features, laboratory data, aPL profiles were compared between patients who progressed to SLE (SLE group) and those who did not (non-SLE group).

[Results] Among 64 patients diagnosed with PAPS at the baseline, nine (13.8%) progressed to SLE over a mean follow-up of 9 years (incidence rate, 1.61 per 100 person-years). At the diagnosis of PAPS, the SLE group had a higher prevalence of anti-phosphatidylserine/prothrombin antibodies (aPS/PT) and anti-dsDNA antibodies compared to the non-SLE group. Other clinical findings, autoantibody profiles, and serum complement levels were similar between the two groups. Multivariate Cox analysis showed that IgG aPS/PT was significantly associated with SLE development (Hazard ratio: 10.3, 95%CI: 1.13-92.6, p=0.04).

[Conclusion] IgG aPS/PT may be a predictive factor for new-onset SLE in patients with PAPS, suggesting its utility in guiding risk stratification and monitoring strategies for these patients.

Introduction

Antiphospholipid syndrome (APS) is an acquired autoimmune thrombophilia characterized by arterial thrombosis, venous thrombosis and recurrent pregnancy loss[1]. The diagnosis of APS requires the persistent detection of antiphospholipid antibodies (aPL)[2,3] which bind to the complex of phospholipids and plasma proteins such as beta-2-glycoprotein I (β2GPI), prothrombin (PT)[4]. The syndrome manifests either as primary APS (PAPS) in the absence of other connective tissue diseases. Systemic lupus erythematosus (SLE) characterized by a variety of autoantibodies and the activation of the complement system[5], is the most common systemic autoimmune disease [6,7]. The overlap in clinical manifestations, pathogenesis, genetic factors, and immunological profiles between APS and SLE has led to a debate about whether they represent a single autoimmune disorder with a spectrum of phenotypes or distinct diseases sharing similar characteristics with different underlying mechanisms [8,9].

Notably, aPL are frequently detected in SLE patients and some SLE patients have been observed to eventually develop overt APS [6,10-12]. SLE patients with APS are more prone to thrombocytopenia compared to primary APS[6,13]. Moreover, several studies have proposed the potential for PAPS patients to develop SLE [14-18]. The progression from PAPS to SLE is thought to involve a complex interplay of genetic, immunologic and environmental factors. Genetic analyses have implied that patients with PAPS and SLE share lupus susceptibility genes[19-21]. The profile of T and B cell subsets in PAPS are similar to those in SLE [22], leading to the breakdown of self-tolerance and autoantibody production. However, no specific risk factors contributing to the development of SLE in PAPS patients have been identified. Currently, the management of PAPS patients mainly focuses on the prevention and treatment of thrombotic events

and pregnancy morbidity[23]. The identification of specific risk factors for the progression to SLE in PAPS patients could enable closer monitoring, early detection and timely intervention, potentially improving patient outcomes. Therefore, the aim of this study was to identify specific risk factors that contribute to the development of SLE in patients with PAPS.

Methods

Study population

This retrospective, longitudinal and observational study of patients with APS, was conducted in a single center at Hokkaido University Hospital in Sapporo, and in accordance with ethical principles of the Declaration of Helsinki and Good Clinical Practice guidelines approved by Hokkaido University Hospital ethics committee (approval number: 012-0338). The cohort comprised consecutive patients who were diagnosed as APS between April 1990 and March 2021 in our Rheumatology department. The attending physicians and authors verified the diagnosis of APS according to the Sydney-revised Sapporo criteria [6] for definite APS. We included PAPS patients with more than 2 years follow-up. Patients' clinical records were retrospectively reviewed including the development of SLE.

Endpoint

The endpoint was set as event free survival period and event was defined as the development of SLE. SLE was classified according to the criteria of 1997 American College of Rheumatology (ACR) [24]. The observation period of each patient was defined to begin at the time when they were diagnosed with APS, and the end at the time of an event or at the end of the observation period.

Antiphospholipid antibody (aPL) tests

Blood samples for aPL testing were collected at the diagnosis of APS in our hospital.

For the detection of lupus anticoagulant (LA), clotting tests of activated partial thromboplastin time (aPTT) and dilute Russell's viper venom time (dRVVT) were performed using a semi-automated hemostasis analyzer (STart 4; Diagnostica Stago). The guidelines recommended by the Subcommittee on LA /aPL of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis (SSC-ISTH) were followed[25].

IgG or IgM anticardiolipin antibodies (aCL) were measured according to a standard enzyme-linked immunosorbent assay (ELISA) [26]. IgG or IgM anti-β2GPI antibodies (aβ2GPI) were determined by ELISA as previously reported [27] or commercial chemiluminescence assays (CLIA) of aβ2GPI (QUANTA Flash® Werfen, Barcelona, Spain). IgG or IgM phosphatidylserine-dependent anti-prothrombin antibody(aPS/PT) were assayed by home-made ELISA as described previously [28].

Data collection and laboratory tests

Medical records were retrospectively reviewed and clinical/laboratory data extracted. Sex, age, clinical manifestations, and history of treatment including glucocorticoid were recorded at the diagnosis of APS. Clinical manifestations including venous thrombosis, arterial thrombosis, obstetric complications associated with APS, neurological disorder defined as neuropsychiatric criteria of the ACR classification criteria for SLE [29], were recorded at the diagnosis of APS. Laboratory tests included thrombocytopenia (< 100,000/ μ L), leucopenia (<4,000/mm^3), lymphopenia (<1,500 /mm^3), and low complement (C3, C4 and CH50), direct Coombs test, and anti-DNA antibody (aDNA). aDNA was measured by radioimmunoassay (RIA, normal value <6 IU/ml).

Statistical analysis

Categorical variables were described as counts and percentages. Fisher's exact test was used for qualitative data analyses. Continuous variables were expressed as the mean ± standard deviation or the median and quartiles and were assessed using the Student's t-

test or the Mann-Whitney U test to identify differences between the SLE group and non-SLE group at baseline. Kaplan-Meier analysis and multivariate analysis using Cox proportional hazards model were applied to identify the risk factors of development of SLE in PAPS. In all statistical analyses, $p < 0.05$ was taken to were considered statistically significant. All statistical analyses were performed using JMP® Pro 16.2.0 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Patients' Characteristics

The demographic, clinical, and laboratory data of all patients are summarized in **Table1**. A total of 64 PAPS patients were included, comprising 54 females (84%) with a median age of onset of 39 years (interquartile range of 29-52 years) and follow-up for a median duration of 9 years (interquartile range of 4.3-15.8 years). The primary clinical manifestations were venous and arterial thrombosis in sixteen (25.0%) and thirty-nine (60.9%) patients, respectively, and obstetric complications in twenty-two out of fifty-four females (40.7%).

Development of SLE in patients with primary APS

During the observation period, 9 (13.8%) out of 64 PAPS patients were newly diagnosed with SLE (incidence rate 1.61 per 100 patient-years) (**Figure 1**). In these patients, LA was detected in 9 (100%) patients, aCL IgG in 6 (66.7%), aCL IgM in 2 (22.2%), aβ2GPI IgG in 6 (66.7%), aβ2GPI IgM in 2 (22.2%), aPS/PT IgG in 8 (88.9%) and aPS/PT IgM in 3 (33.3%) (**Table 1**). Patients in the SLE group were significantly younger than those in the non-SLE group (median 29 years vs median 41 years, p=0.016). The SLE group had higher prevalence of aPS/PT (88.9% vs 34.6%, p=0.003) and aDNA (66.7% vs 9.1%, p=0.0004) at baseline than the non-SLE

group. Other clinical findings, autoantibody profiles, and serum complement levels were similar between the SLE and non-SLE groups.

Risk factors for developing SLE

Kaplan-Meier analysis showed a high association between SLE development and positivity of aPS/PT IgG ($p = 0.006$) and aDNA ($p < 0.0001$). Among 27 patients with aPS/PT IgG positive, 8 cases (29.6 %; 3.2 /100 person-year) of SLE development were found, which was significantly more frequent compared to one case out of 37 patients with aPS/PT IgG negative (2.7%; 0.3 /100 person-year) (Log-rank P value= 0.006) (**Figure 2A**). Similarly, 5 cases of SLE development were observed among 11 patients with aDNA positive (45.5%; 6.1 /100 person-year), and 4 cases among 53 patients with aDNA negative (7.5%; 0.8 /100 person-year) (**Figure 2B**).

Univariate analysis showed a significant association between IgG aPS/PT and aDNA antibodies with SLE development. Multivariable analysis using Cox proportional hazards model, after adjustment for age, showed a significantly higher association of IgG aPS/PT with SLE development (hazard ratio 10.3, 95%CI 1.13-92.6, p=0.04) (**Table 2**).

Discussion

In our longitudinal observation study, we followed up 64 patients with PAPS, for a median period of 9 years, and 9 (13.8%) were newly diagnosed with SLE, reflecting an incidence rate of 1.61 per 100 patient-years. Notably, a significant association between aPS/PT IgG and SLE development were found by multivariate analysis.

Previous studies estimated that between 5-10% of PAPS patients develop SLE during a period of 5-10 years [14-18], consistent with the SLE development rate observed in our study. In terms of incidence rate, only one previous study from Taiwan's National Health Insurance Research Database (2003-2013) showed an incidence rate of 289.79 per 10,000 person-months[18], with 10.1% (126/1245) of APS patients developing

SLE. In comparison, our study (1990-2021) found a similar prevalence of SLE development at 13.8% (9/64); however, the incidence rate in our study was considerably lower at 13.27 per 10,000 person-months. Several factors may contribute to the discrepancy in incidence rates between the Taiwanese study and our study. Firstly, the Taiwanese study used a national health insurance database, which may capture a broader or different subset of SLE cases due to International Classification of Diseases (ICD) coding compared to the clinical criteria used in our study. Secondly, genetic and environmental factors specific to each population may have influenced the observed differences. Finally, the longer observation period in our study (1990-2021) compared to the Taiwanese study (2003-2013) might have diluted the incidence rate, as SLE development may be more frequent in the earlier years following PAPS diagnosis.

Our study identified aPS/PT IgG as a key predictor of SLE development in PAPS patients, alongside known risk factors such as a positive Coombs test[15], and the presence of antinuclear antibodies, anti-dsDNA [16]. The aPS/PT are known as non-criteria aPL that recognize the complex of phosphatidylserine (PS) and prothrombin (PT) [30]. The aPS/PT is strongly associated with thrombosis and pregnancy morbidity in patients with APS [31]. In a systematic review, the prevalence of aPS/PT IgG was 34.1% (187/548) for PAPS and 54.5% (218/404) for secondary APS[32]. Conversely, in SLE, aPL profile prevalence was 11-30% for LA[33], 17- 40% for aCL IgG or IgM [33], 32-37% for aβ2GPI IgG or IgM [34,35]. In terms of aPS/PT in SLE, positive rates differed across studies. Bertolaccini et al. discovered aPS/PT in 66 (31%) of 212 SLE patients; 16% for IgG and 6% for IgM [36]. Sciascia et al. reported that aPS/PT in 35 (46.7%) of 75 SLE patients; 44% for IgG and 30.7% for IgM[37]. Elbagir et al. reported aPS/PT in 50 (15%) of 341 SLE patients [38]. These findings suggested that the prevalence of aPS/PT in SLE is comparable to other aPL. Notably, no other specific aPL tests have been identified to be associated with SLE development in PAPS patients. Our study is the first to identify specific aPL, aPS/PT IgG, as a predictor of SLE development in PAPS patients. The aPS/PT showed a strong association with the

presence of LA and are expected to serve as a surrogate for LA as well as an indicator for risk stratification of thrombosis in patients with APS [31]. The reason why LA was not detected as a predictive factor for the development of SLE might be attributed to the inclusion of not only aPS/PT but also aβ2GPI, or other aPL that possess LA activity.

A longitudinal study clarified the progressive accumulation of autoantibodies prior to the diagnosis of SLE[39]. Of them, aDNA antibodies can be detected in individuals without SLE, and in some cases, these antibodies may precede the clinical diagnosis of SLE by several years[40]. In our study, among the 11 patients testing positive for aDNA, 8 also positive for aPS/PT. Of the 9 patients who developed SLE, 6 were positive for aDNA and 5 of them were positive for aPS/PT and all 3 patients who were negative for aDNA tested positive for aPS/PT IgG (data not shown). Hence, PAPS patients with positive for aPS/PT IgG might already have features of SLE, supposing that aPS/PT were one of lupus-associated antibodies, at the onset of PAPS, indicating a potential development of SLE in the future. Our results suggest that aPS/PT IgG may serve as a predictive marker for the occurrence of SLE, raising the potential need for its regular monitoring of aPS/PT IgG levels.

There are some limitations to this study that should be considered. Firstly, the study was conducted retrospectively at a single center, which may limit the statistical power to detect significant associations and the generalizability of the findings to other populations. Secondly, no patients were treated with Hydroxychloroquine for APS in this study due to lack of the availability in Japan. This could have potentially skewed our results. Furthermore, the mechanisms underlying the association between aPS/PT and SLE remain unclear. Establishing a causal relationship between the presence of aPS/PT positivity and the development of SLE in PAPS is challenging. Further basic research and larger, multicenter studies are needed to validate our findings.

In conclusion, aPS/PT IgG may predict new-onset SLE in PAPS patients. These results could help identify PAPS patients at higher risk of developing SLE, enabling closer monitoring and timely intervention.

Acknowledgment

This work was partly supported by the project of junior scientist promotion in Hokkaido University.

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Figure Legends

Figure 1. Incidence of SLE development in patients of PAPS. During the observation period, 9 (13.8%) out of 64 PAPS patients were newly diagnosed with SLE (incidence rate 1.61 per 100 patient-years).
SLE: systemic lupus erythematosus, PAPS: primary antiphospholipid syndrome.

Figure 2. Incidence of SLE development in patients of PAPS. (**A**) Comparison between patients with aPS/PT IgG positive and aPS/PT negative. Incidence of SLE development was 29.6 % (3.2 /100 person-year) in aPS/PT IgG positive and 2.7 % (0.3 /100 person-year) in aPS/PT negative (aPS/PT IgG positive vs aPS/PT negative, Log-rank p value= 0.006). (**B**) Comparison among patients with aDNA positive and aDNA negative. Incidence of SLE development was 45.5% (6.1 /100 person-year) in aDNA positive, and 7.5 % (0.8 /100 person-year) in aDNA negative (aDNA positive vs aDNA negative, Log-rank p value < 0.0001).
SLE: systemic lupus erythematosus, PAPS: primary antiphospholipid syndrome, aPS/PT: Phosphatidylserine-dependent anti-prothrombin antibodies, aDNA: anti DNA antibodies

Table 1. The demographic, clinical, and laboratory

		PAPS (N=64)	Non-SLE group (N=55)	SLE-group (N=9)	P-value
Variables					
Age at diagnosis (years)	Median (IQR)	39 (29 - 52)	41 (31 - 56)	29 (23 - 40)	0.016*
Female	N (%)	54 (84.3)	45(81.8)	9 (100)	0.33
Observation period (years)	Median (IQR)	9 (4.3 - 15.8)	8 (4 - 14)	16 (5 - 17.5)	0.25
LA	N (%)	41 (64.1)	33 (60.0)	8 (88.9)	0.14
aCL IgG	N (%)	40 (62.5)	34 (61.8)	6 (66.7)	1.00
aCL IgM	N (%)	9 (14.1)	7 (12.7)	2 (22.2)	0.60
aβ2GPI IgG	N (%)	26 (40.6)	20 (36.4)	6 (66.7)	0.14
aβ2GPI IgM	N (%)	14 (21.9)	12 (21.8)	2 (22.2)	1.00
aPS/PT IgG	N (%)	27 (42.2)	19 (34.6)	8 (88.9)	0.003*
aPS/PT IgM	N (%)	17 (26.5)	14 (25.5)	3 (33.3)	0.69
Arterial thrombosis	N (%)	39 (60.9)	34 (61.8)	5 (55.6)	0.73
Venous thrombosis	N (%)	16 (25.0)	12 (21.8)	4 (44.4)	0.21
Obstetric complications (N=54**)	N (%)	22 (22/54**, 40.7)	19 (19/45**, 42.2)	3 (3/9** ,33.3)	0.901
Neurological disorder	N (%)	11 (17.2)	8 (14.6)	3 (33.3)	0.18
Thrombocytopenia	N (%)	9 (14.1)	9 (16.4)	0 (0)	0.34
Leukopenia/lymphopenia	N (%)	49 (76.6)	40 (72.7)	9 (100)	0.10
Anti-DNA antibody	N (%)	11 (17.1)	5 (9.1)	6 (66.7)	0.0004*
Anti-nuclear antibody (> 1:80)	N (%)	35 (54.6)	30 (54.6)	5 (55.6)	1.00

Low complement	N (%)	33 (51.6)	27 (49.1)	6 (66.7)	0.48
Direct Coombs test (N=60***)	N (%)	9 (9/60***, 15.0)	6 (6/51***, 11.8)	3 (3/9*** ,33.3)	0.12
Glucocorticoid treatment	N (%)	14 (21.9)	13 (23.7)	1 (11.1)	0.67

IQR: interquartile range, N(%) :number (percentage) LA: lupus anticoagulant, aCL: anticardiolipin antibody, aβ2GPI: anti-β2GPI antibody, aPS/PT: phosphatidylserine dependent anti-prothrombin antibody, P values were by the Mann–Whitney U test or the chi-square test (Non-SLE group vs SLE group), * <0.05

** The denominators for obstetric complications are different from the total population. The percentages are calculated based on the respective subgroup populations (54 for obstetric complication in PAPS, 45 for non-SLE group and 9 for SLE group).

*** The denominators for direct coombs test are different from the total population. The percentages are calculated based on the respective subgroup populations (60 for direct coombs test in PAPS, 51 for non-SLE group and 9 for SLE group).

Table2. Characteristics of the patients at the diagnosis of systemic lupus erythematosus (SLE)

	Sex	Age*	Years**	Clinical manifestations***	Thrombocytopenia	Leukopenia Lymphopenia	Low complement	Anti-DNA antibody
1	F	47	2	nephritis, arthritis	-	-	+	+
2	F	31	7	nephritis, arthritis	-	-	+	+
3	F	39	13	arthritis	+	-	-	+
4	F	30	1	nephritis, rash, arthritis	-	-	-	-
5	F	51	8	rash, arthritis	-	+	+	-
6	F	20	2	nephritis	+	-	+	+
7	F	31	10	epilepsy	-	+	+	-
8	F	32	1	nephritis	-	-	+	+
9	F	44	8	neurological disorder	-	+	+	+

*Age: age at the diagnosis of systemic lupus erythematosus (SLE)

**Years: years from primary antiphospholipid syndrome (PAPS) onset to SLE onset

***Clinical manifestation: clinical manifestations at the diagnosis of SLE

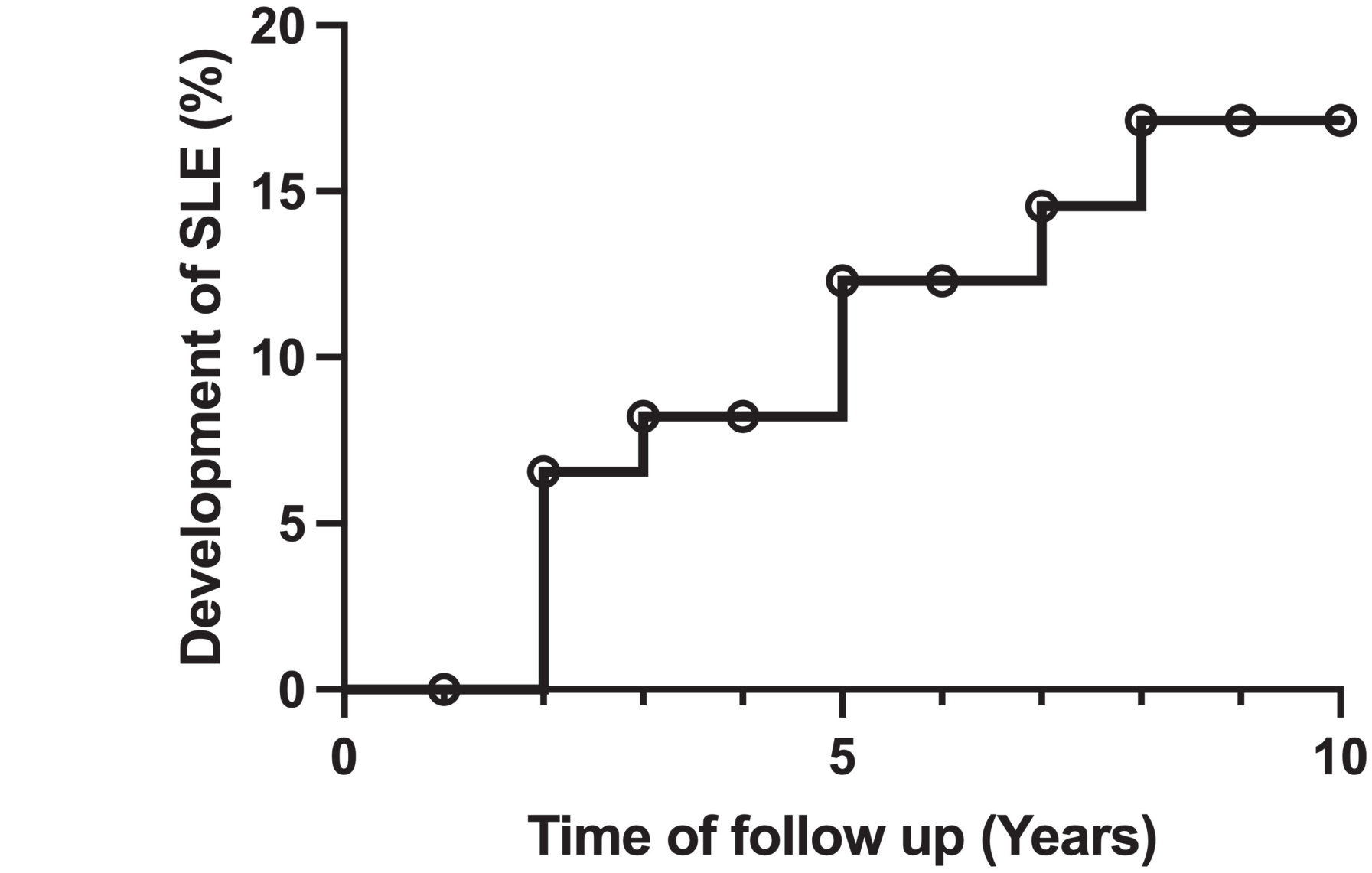
Table 3. Risk factors for developing systemic lupus erythematosus (SLE)

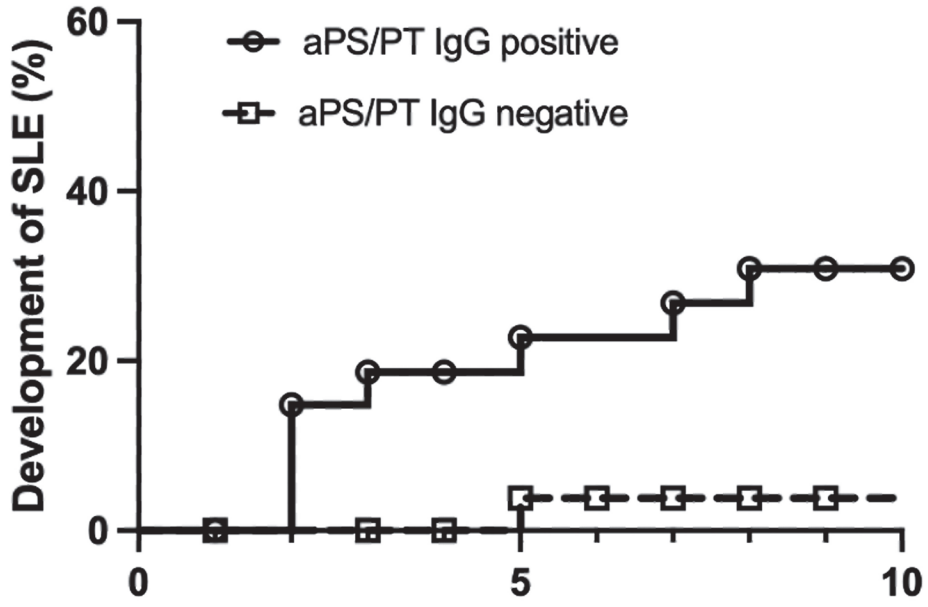
	Univariate analysis			Multivariate analysis		
Variables	HR	(95% CI)	*P* value	HR	(95% CI)	*P* value
Age at diagnosis < 40 years	0.33	(0.07 – 1.61)	0.17	0.39	(0.07 – 2.09)	0.27
LA	4.72	(0.59 – 37.8)	0.14			
aCL IgG	0.92	(0.23 – 3.73)	0.91			
aCL IgM	1.82	(0.38 – 8.77)	0.46			
aβ2GPI IgG	2.70	(0.67 – 10.8)	0.16			
aβ2GPI IgM	0.96	(0.20 – 4.65)	0.96			
aPS/PT IgG	10.6	(1.32 – 84.5)	0.03	10.3	(1.13 – 92.6)	0.04*
aPS/PT IgM	1.16	(0.29 – 4.68)	0.83			
Venous thrombosis	2.45	(0.66 – 9.15)	0.18			
Arterial thrombosis	0.74	(0.20 – 2.75)	0.65			
Obstetric complications	0.98	(0.25 – 3.92)	0.98			
Neurological disorder	2.59	(0.65 – 10.4)	0.20			
Thrombocytopenia	0.00	(0 – ∞)	1.00			
Leukopenia/lymphopenia	8×10^8	(0 – ∞)	1.00			
Anti-DNA antibody	9.27	(2.32 – 37.1)	0.002	4.75	(1.12 – 20.1)	0.03*
Anti-nuclear antibody (> 1:80)	1.01	(0.27 - 3.76)	0.99	0.59	(0.14 – 2.47)	0.47
Low complement	1.72	(0.43 - 6.92)	0.44			
Direct Coombs test	2.92	(0.49 – 17.5)	0.24			

Glucocorticoid treatment	0.47	(0.06 – 3.71)	0.47

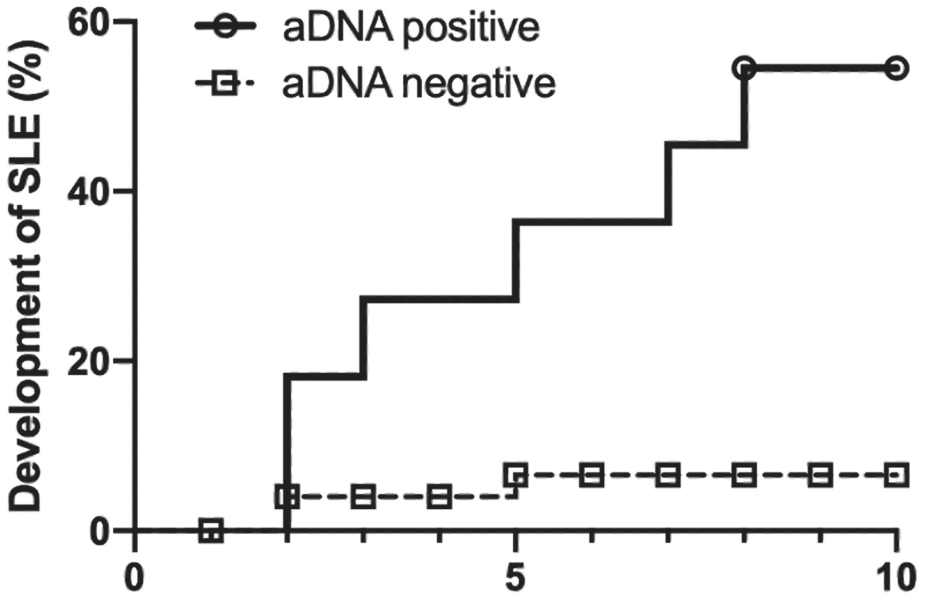
LA: lupus anticoagulant, aCL: anti-cardiolipin antibody, aβ2GPI: anti-β2GPI antibody, aPS/PT: phosphatidylserine dependent anti-prothrombin antibody,

HR : Hazard ratio, CI：confidence interval, P values were by Cox proportional hazards model, * <0.05


Development of SLE (%)
20
15
10
5
0
0
5
10
Time of follow up (Years)
Numbers at risk 64 64 61 56 52 45 42 39 33 26 23



Numbers at risk											
aPS/PT IgG positive	27	27	26	21	20	19		18	17	14	13
aPS/PT IgG negative	37	37		35	32	26	24	21	16	13	



Numbers at risk											
aDNA positive	11		11	9		8		7	6		4
aDNA negative	53	53	50	47	44	37	35	32	27	23	20