学　位　論　文

Predictive and Monitoring Markers in Systemic Lupus Erythematosus

（全身性エリテマトーデスにおける疾患予測・活動性評価のためのマーカー研究）

2025 年 6 月

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List of Publications and Presentations

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1. Jiang W, Fujieda Y, Fujita Y, Ogata Y, Hisada R, Kono M, Amengual O, Kato M & Atsumi T. Phosphatidylserine-Dependent Anti-prothrombin Antibodies as a Key Predictor for Systemic Lupus Erythematosus in Patients with Primary Antiphospholipid Syndrome: A retrospective longitudinal cohort study. Modern Rheumatology.

List of Presentations

1. Jiang W & Fujieda Y. Clinical significance of urine Presepsin levels in patients with lupus nephritis. 23rd Asia-Pacific League of Associations for Rheumatology (APLAR) Congress. August 28-31, 2021. Kyoto, Japan.
2. Jiang W, Fujieda Y, Hisada R, Kono M, Kato M, Amengual O & Atsumi T. Phosphatidylserine-dependent antiprothrombin antibodies are a risk factor for developing systemic lupus erythematosus in patients with primary antiphospholipid syndrome. 24rd Asia-Pacific League of Associations for Rheumatology (APLAR) Congress. Dec 6-9, 2022. Hong Kong.

1. Summary

Systemic lupus erythematosus (SLE) is an autoimmune disease that presents with diverse clinical symptoms, requiring early detection and careful monitoring for diagnosis and treatment. SLE can affect multiple organs including joints, skin, kidneys, and the nervous system, potentially causing significant deterioration in patients' quality of life. Despite recent advances in understanding the underlying pathophysiology, the field lacks robust biomarkers for disease monitoring. Here we focused on IgG anti-phosphatidylserine/prothrombin antibodies (aPS/PT) as novel biomarkers and clarified their clinical significance.

[Background and Objectives] Antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of antiphospholipid antibodies (aPL) in the blood, leading to arterial thrombosis, venous thrombosis, and pregnancy complications. Autoimmune diseases are complex disorders characterized by immune system dysfunction, where the body's defense mechanisms incorrectly target its own tissues. Among these conditions, APS and systemic lupus erythematosus (SLE) represent significant challenges in both diagnosis and management. Primary APS (PAPS) is defined as APS without coexisting other autoimmune disorders. Although PAPS is distinct from 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 in patients with PAPS.

[Methods] A longitudinal single-center study was conducted at Hokkaido University Hospital from 1990 to 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).

[Discussion] In this longitudinal investigation of primary antiphospholipid syndrome (PAPS), we demonstrate that IgG anti-phosphatidylserine/prothrombin antibodies (aPS/PT IgG) serve as a novel predictor for systemic lupus erythematosus (SLE) development. While PAPS-to-SLE progression rates aligned with established literature, the identification of aPS/PT IgG as a predictive marker represents a significant advance in understanding disease trajectory. Although previously recognized for its association with thrombotic manifestations, aPS/PT IgG's capacity to forecast SLE development was previously unrecognized. These findings reveal aPS/PT IgG potentially enabling targeted surveillance and timely therapeutic intervention in high-risk populations.

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

2. List of Abbreviation

APS	Antiphospholipid syndrome
aPL	Antiphospholipid antibodies
aPS/PT	Anti-phosphatidylserine/prothrombin antibodies
ACR	American College of Rheumatology/
APOE	Apolipoprotein E
aβ2GPI	Anti-Beta2 Glycoprotein I
aPTT	activated partial thromboplastin time
aDNA	anti-DNA antibody
anti-dsDNA	Anti-double stranded DNA
CLEIA	Chemiluminescent Enzyme Immunoassay
Cre	Cre
C5a	Complement Component 5a
C3a	complement component 3a
CH50	homolytic complement activity
IQR	interquartile range
ICD	International Classification of Diseases
dRVVT	dilute Russell's viper venom time
ELISA	The enzyme-linked immunosorbent assay
EULAR	European League Against Rheumatism
EUROAPS	Endoplasmic reticulum
FE-PSEP	European Registry on Obstetric Antiphospholipid Syndrome
LDL	Low-Density Lipoprotein

LRP8	LDL Receptor Related Protein 8
LA	lupus anticoagulant
LN	lupus nephritis
PSEP	Presepsin
P-PSEP	plasma levels of PSEP
PAPS	primary APS
RIA	radioimmunoassay
SLE	Systemic lupus erythematosus
SSC-ISTH	Standardisation Committee of the International Society on Thrombosis and Haemostasis
U-PSEP	urinary levels of PSEP

3. Introduction

3.1 Systemic lupus erythematosus.

Systemic lupus erythematosus (SLE) represents one of the most complex autoimmune disorders in rheumatology (Bertsias GK et al, 2012). This chronic condition is characterized by its heterogeneous clinical manifestations. The hallmark of SLE pathophysiology is the production of autoantibodies against nuclear and cytoplasmic antigens, coupled with dysregulation of multiple immunopathogenic pathways (Tsokos GC, 2011). This immune system dysfunction can affect any organ system, presenting clinicians with diverse manifestations ranging from mild mucocutaneous involvement to severe central nervous system complications. Such heterogeneity not only poses significant challenges in diagnosis and classification but also necessitates individualized therapeutic approaches, considering disease activity, severity, and organ involvement. (Truedsson L et al, 2007)

The impact of SLE extends well beyond immediate disease manifestations, encompassing increased cardiovascular risk, pregnancy-related complications, and substantial effects on patients' quality of life. As our understanding of SLE continues to evolve, new therapeutic paradigms emerge, including biological agents and novel immunomodulatory approaches, offering hope for improved disease management.

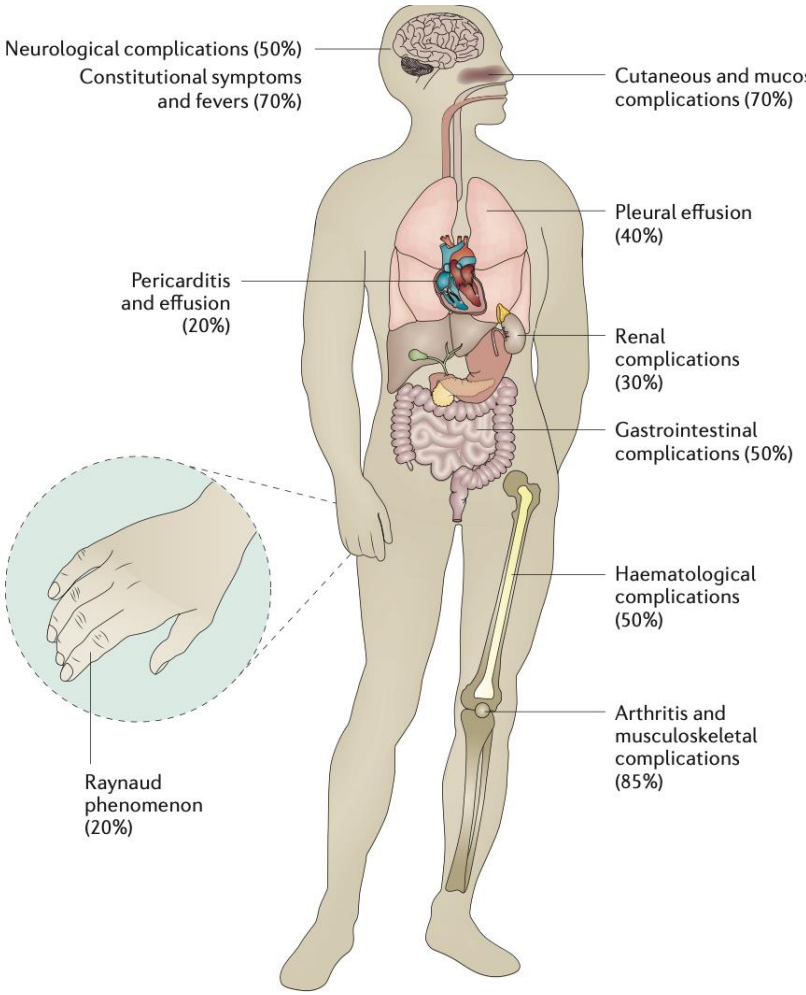

Figure1. Clinical heterogeneity of SLE. (Truedsson L et al, 2007)
The multifaceted nature of systemic lupuserythematosus (SLE) is shown by the number of different organ systems that can be affected. In addition, each organ-specific complication can manifest in different ways. For example, cardiac complications can be the consequence of myocarditis, pericarditis, pericardial effusion, pulmonary hypertension and Libman–Sacks endocarditis. Gastrointestinal involvement varies from oral ulcers to full-blown lupus enteritis, pancreatitis, hepatitis and ascites. Neurological involvement is complex with symptoms such as headache, seizures and thrombotic features including stroke. The average frequency of the most common complications is indicated in parentheses.

3.2 Lupus nephritis

Lupus nephritis (LN) represents one of the most severe organ manifestations of systemic lupus erythematosus, affecting approximately 50-60% of patients and serving as the primary cause of morbidity and mortality. The condition typically develops within the first five years of SLE diagnosis, though it can also present as the initial manifestation of lupus. This renal involvement significantly impacts patient outcomes, with 5-20% of LN patients progressing to end-stage renal disease within a decade despite current therapeutic interventions (Houssiau, FA et al, 2010).

The pathophysiology of LN is characterized by a complex interplay of immunological processes, including the production of pathogenic autoantibodies (particularly anti-double-stranded DNA and anti-C1q antibodies), immune complex deposition in the glomeruli, and complement activation (Tsokos GC, 2011). Recent evidence has illuminated the crucial role of long-lived plasma cells in the kidney and dysregulated microRNA expression, offering new perspectives on disease mechanisms and potential therapeutic targets (Sanz I and Lee FE, 2010). The resulting inflammation and tissue damage manifest through various pathological patterns, classified into six distinct classes based on renal biopsy findings, with class III and IV representing the most common and severe forms requiring aggressive therapeutic intervention (Weening JJ et al, 2004). Current treatment protocols, including mycophenolate mofetil, cyclophosphamide, and corticosteroids, achieve suboptimal remission rates, highlighting a critical unmet need in LN management. The emergence of biological therapies, such as belimumab and rituximab, represents promising additions to the therapeutic arsenal, though their optimal positioning in treatment algorithms remains under investigation. Furthermore, the management of LN is complicated by treatment-related complications, including infections, osteoporosis, and cardiovascular disease, necessitating careful monitoring and individualized therapeutic approaches.

3.3 Antiphospholipid syndrome

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by the presence of antiphospholipid antibodies (aPL) in the blood, leading to arterial thrombosis, venous thrombosis, and pregnancy complications.

aPL is a collective term for autoantibodies against phospholipids or phospholipid-binding proteins, as well as lupus anticoagulant (LA), an immunoglobulin that inhibits phospholipid-dependent coagulation reactions (Atsumi T et al, 2011). APS is recognized as a common condition causing acquired thrombotic tendencies and various pregnancy complications, and it holds an important clinical position.

The Sapporo criteria from 1998 have been used for diagnosis (Wilson et al, 1999). However, minor revisions were made in 2006, and these revised criteria are currently in use (Figure 2: Sydney revision of the Sapporo criteria) (Miyakis et al, 2006).

The Sydney revision of the Sapporo criteria (Wilson WA et al, 1999) has been widely used as an international classification criterion for APS. However, in 2023, a new classification criterion by ACR/EULAR was announced (Barbhaiya M et al, 2023) (Figure 3: ACR/EULAR criteria). APS is not a single disease but includes various conditions such as: (1) APS with multiple positive antiphospholipid antibodies causing thrombosis and pregnancy complications, (2) APS accompanied by thrombotic risks like atherosclerosis, and (3) secondary APS represented by SLE (Khamashta et al, 2004). The new criteria are designed to classify a limited population assuming clinical trials. Therefore, patient profiles that were classified as APS under the previous criteria but cannot be classified under the new criteria have been presented as an agenda requiring future consideration.

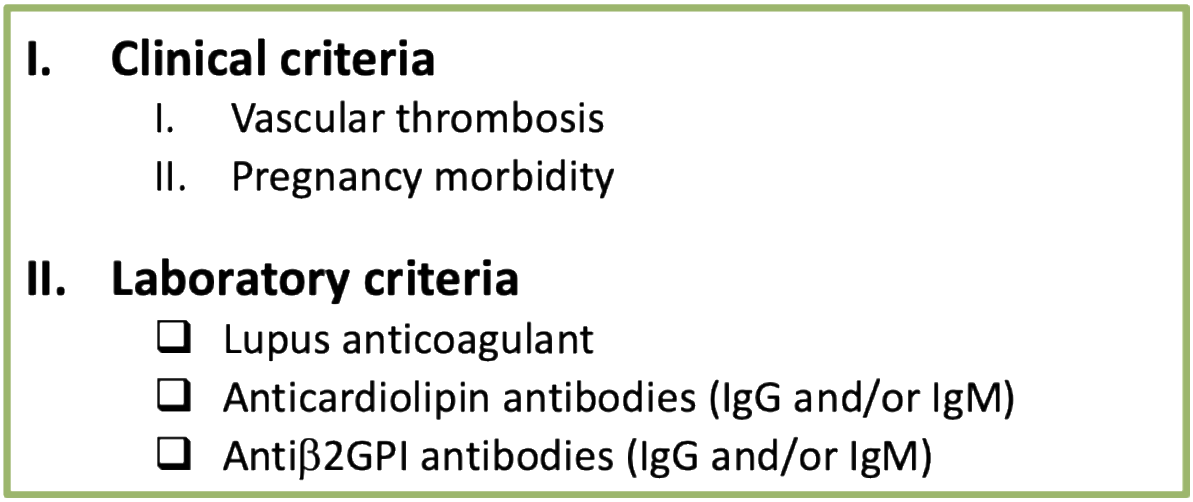

Figure 2. Sydney revised Sapporo Criteria. (Miyakis Set, 2006)

For a definitive diagnosis of antiphospholipid syndrome, at least one clinical criterion and one laboratory criterion must be met.

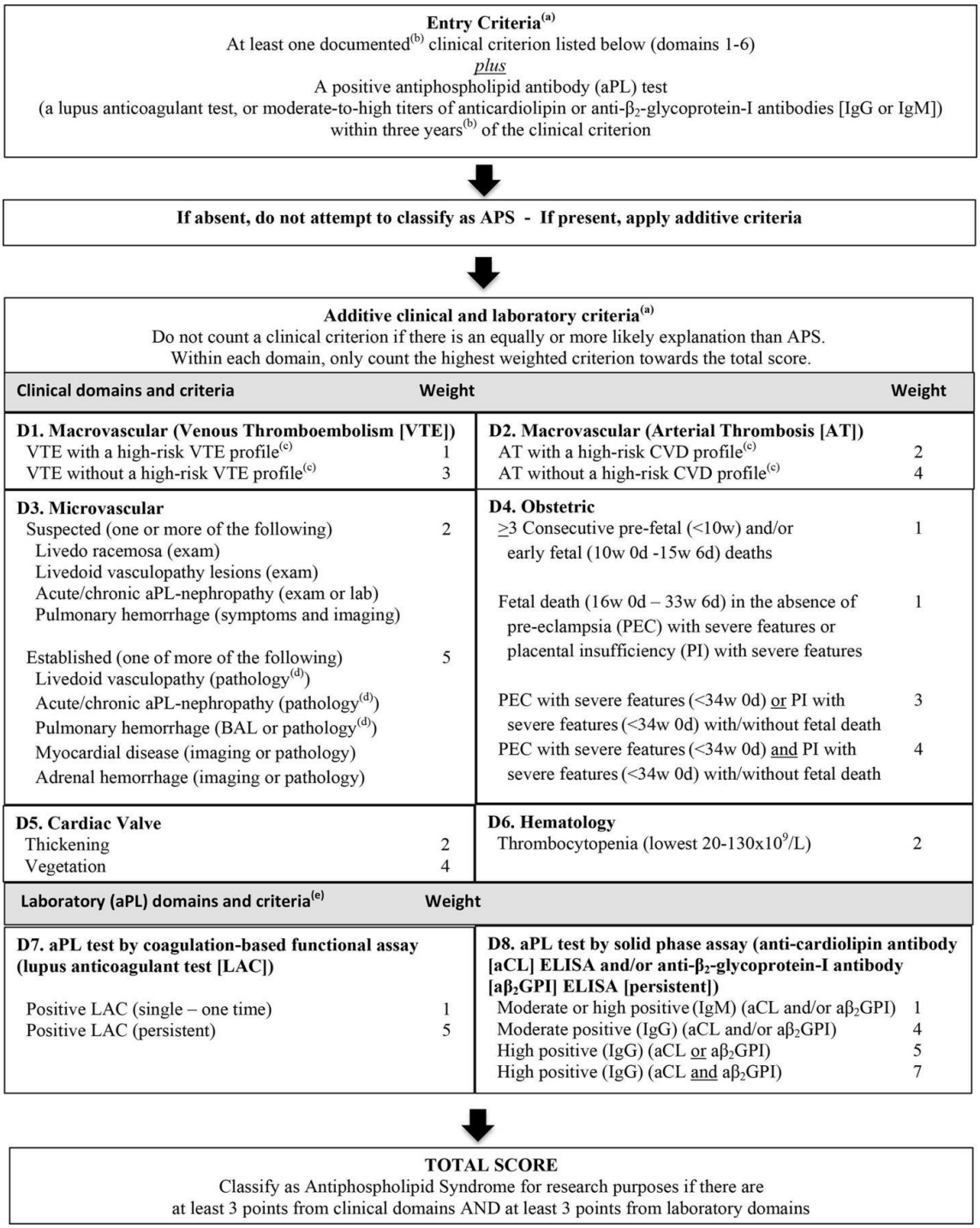

Figure 3. 2023 ACR/EULAR antiphospholipid syndrome classification criteria. (Barbhaiya M et al, 2023)

A total score of 6 or more points is required for a classification of RA. The criteria emphasize the importance of joint involvement, serological markers, and the duration of symptoms, facilitating an early and accurate diagnosis, which is crucial for effective treatment and management of the disease.

3.4 Antiphospholipid antibodies

Pathogenic aPLs include anti-cardiolipin antibody (aCL), anti-β2 glycoprotein I (β2GPI) antibody (aβ2GPI), and lupus anticoagulant (LA). Prothrombin can also be a target of aPL, and the corresponding autoantibody known as phosphatidylserine-dependent anti-prothrombin antibody (phosphatidylserine/prothrombin complex: aPS/PT) has been identified. (Figure 4)

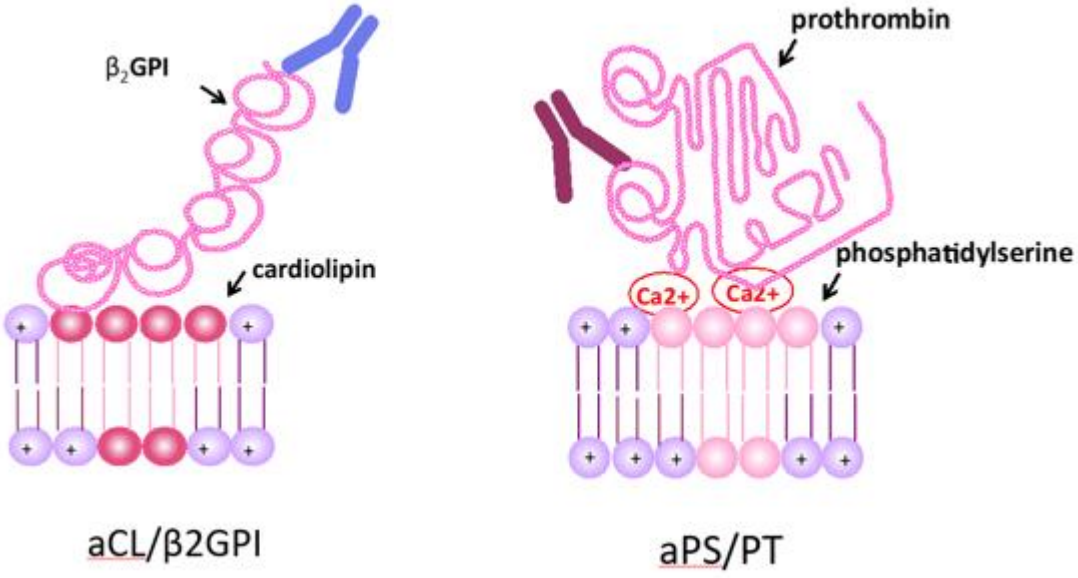

Figure 4. The structure of anti cardiolipin and β2 GP1 antibodies, anti phosphatidylserine and prothrombin.

This figure shows antiphospholipid antibodies, which are actually thought to be pathogenic. The main antigens that antiphospholipid antibodies respond to are said to be β2-glycoprotein I, which binds to cardiolipin (CL), and prothrombin, which binds to phosphatidylserine (PS).

3.5 Mechanism of thrombosis formation by antiphospholipid antibodies

The pathophysiology of thrombosis caused by antiphospholipid antibodies involves the activation of prothrombotic cells such as vascular endothelial cells, monocytes, and platelets (Miyakis S et al, 2006), as well as neutrophil extracellular traps (NETs) (Yu Z et al, 2020), and the activation of the complement pathway (Shruti C et al, 2019). Thrombus formation requires two steps: the "first hit," which is the presence of antiphospholipid antibodies, and the "second hit," which is the trigger for thrombosis. Additionally, thrombotic risk factors inherent to individual patients also contribute to thrombus formation. (Figure 5)

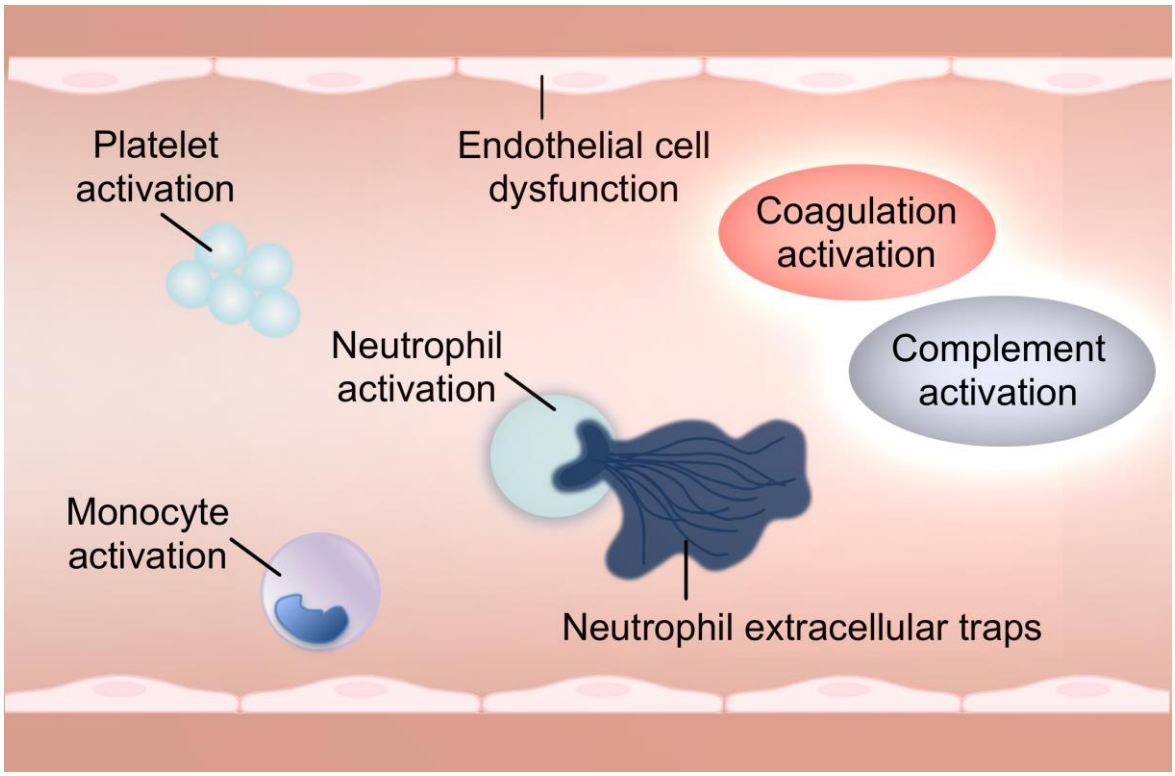

Figure 5. Antiphospholipid antibodies can lead to thrombosis through the activation of several cells.（Fujieda Y et al, 2021）

The pathophysiology of thrombosis associated with antiphospholipid antibodies involves activating prothrombotic cells like vascular endothelial cells, monocytes, and platelets, as well as neutrophil extracellular traps (NETs) and the complement pathway.

3.6 Relationship between SLE and APS

Antiphospholipid syndrome (APS) is an acquired autoimmune thrombophilia characterized by arterial thrombosis, venous thrombosis and recurrent pregnancy loss (Hughes GR et al, 1983). The diagnosis of APS requires the persistent detection of antiphospholipid antibodies (aPL) (Miyakis S et al, 2006) which bind to the complex of phospholipids and plasma proteins such as beta-2-glycoprotein I (β2GPI), prothrombin (PT) (Amengual O et al, 2018). The syndrome manifests either as primary APS (PAPS) in the absence of other connective tissue diseases or as secondary APS, associated with various connective tissue diseases. Systemic lupus erythematosus (SLE) characterized by a variety of autoantibodies and the activation of the complement system (Tsokos GC et al, 2020), is the most common systemic autoimmune disease associated with APS (Cervera R et al, 2015; Fujieda Y et al, 2012). 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 (Agmon-Levin N et al, 2014; Tincani A et al, 2009). Notably, aPL are frequently detected in SLE patients and some SLE patients have been observed to eventually develop overt APS (Cervera R et al, 2015; Giannakopoulos B et al, 2013; Cervera R et al, 2002; Tarr T et al, 2007). SLE patients with APS are more prone to thrombocytopenia compared to primary APS (Cervera R et al, 2015; Unlu O et al, 2019). Moreover, several studies have proposed the potential for PAPS patients to develop SLE (Mujic F et al, 1995; Gomez-Puerta JA et al, 2005; Freire PV et al, 2014; Taraborelli M et al, 2017; Chen HH et al, 2021). 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. The profile of T and B cell subsets in PAPS is similar to those in SLE (Lorena Á et al, 2018), 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 (Tektonidou MG et al, 2019). 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.

4. Method

4.1 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 the 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 with 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 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.

4.2 Endpoint

The endpoint was set as the 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) (Hochberg MC et al, 1997). 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.

4.3 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 (Pengo V et al, 2009).

IgG or IgM anticardiolipin antibodies (aCL) were measured according to a standard enzyme-linked immunosorbent assay (ELISA) (Harris EN et al, 1987). IgG or IgM anti-b2GPI antibodies (a b2GPI) were determined by ELISA as previously reported (Amengual O et al, 1996) or commercial chemiluminescence assays (CLIA) of ab2GPI (QUANTA Flash® Werfen, Barcelona, Spain). IgG or IgM phosphatidylserine-dependent anti-prothrombin antibody(aPS/PT) was assayed by home-made ELISA as described previously (Atsumi T et al, 2000).

4.4 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 (Liang MH et al, 1999), were recorded at the diagnosis of APS. Laboratory tests included thrombocytopenia (< 100,000/μL), leucopenia (<4,000/mm3), lymphopenia (<1,500 /mm3), 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).

4.5 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 the 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 considered statistically significant. All statistical analyses were performed using JMP® Pro 16.2.0 (SAS Institute Inc., Cary, North Carolina, USA).

5. Results

5.1 Patients' Characteristics

The demographic, clinical, and laboratory data of all patients are summarized in Table 1. 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 thrombosis in sixteen (25.0%) and arterial thrombosis in thirty-nine (60.9%) patients, respectively, and obstetric complications in twenty-two out of fifty-four females (40.7%).

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).

5.2 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 person-years) (Figure 6, Table 2). Among those 9 patients, all of them developed typical lupus clinical features when they were diagnosed as SLE. In these patients, LA was detected in 9 (100%) patients, aCL IgG in 6 (66.7%), aCL IgM in 2 (22.2%), ab2GPI IgG in 6 (66.7%), ab2GPI 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.

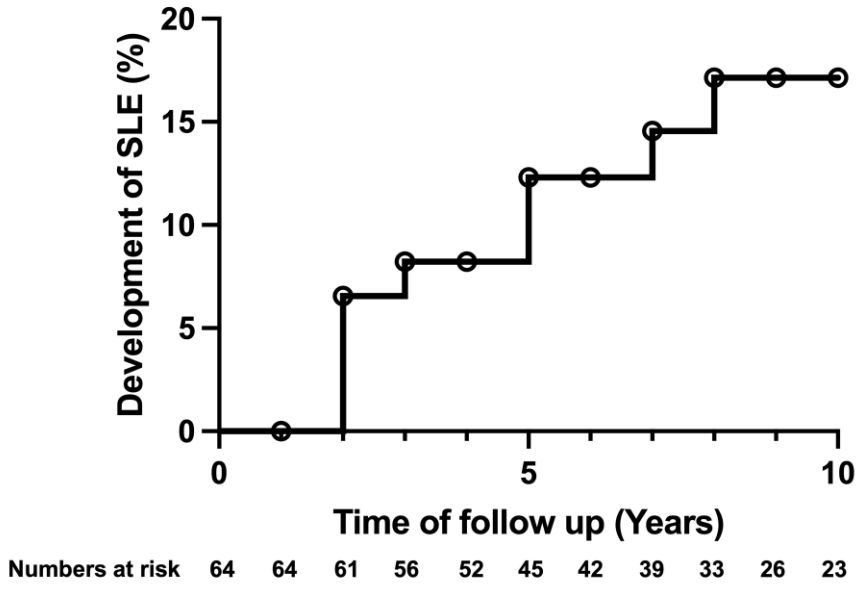

Figure 6. Incidence of SLE development in patients of PAPS.
During the observation period, 9 (13.8%) out of 64 PAPS patients were newly diagnosed with SLE (incidence rate 1.61 per 100 person-years).
SLE: systemic lupus erythematosus, PAPS: primary antiphospholipid syndrome.

Table 2. Characteristics of the patients at the diagnosis of 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 SLE
**Years: years from PAPS onset to SLE onset
***Clinical manifestation: clinical manifestations at the diagnosis of SLE

5.3 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 per 100 person-years) 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 per 100 person-years) (Log-rank P value = 0.006) (Figure 7A). Similarly, 5 cases of SLE development were observed among 11 patients with aDNA positive (45.5%; 6.1 per 100 person-years), and 4 cases among 53 patients with aDNA negative (7.5%; 0.8 per 100 person-years) (Figure 7B).

Univariate analysis showed a significant association between IgG aPS/PT and aDNA antibodies with SLE development. Multivariable analysis using the 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 3)

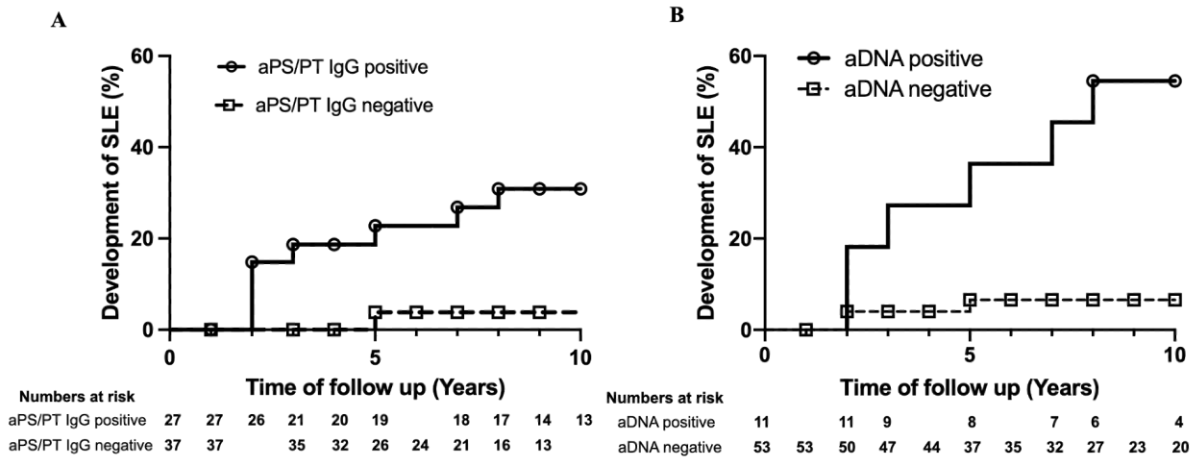

Figure 7. Risk factors for developing SLE in patients of PAPS.

(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 3. Risk factors for developing 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

6. 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 was found by multivariate analysis.

Previous studies estimated that between 5-10% of PAPS patients develop SLE during a period of 5-10 years (Mujic F et al, 1995; Gomez-Puerta JA et al, 2005; Freire PV et al, 2014; Taraborelli M et al, 2017), 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 (Chen HH et al, 2021), 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 (Gomez-Puerta JA et al, 2005), and the presence of antinuclear antibodies, anti-dsDNA (Freire PV et al, 2014). The aPS/PT

is known as non-criteria aPL that recognize the complex of phosphatidylserine (PS) and prothrombin (PT) (Amengual O et al, 2017). The aPS/PT is strongly associated with thrombosis and pregnancy morbidity in patients with APS (Zhang Y et al, 2023). 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 (Zhu R et al, 2022). Conversely, in SLE, aPL profile prevalence was 11-30% for LA (Unlu O et al, 2016), 17- 40% for aCL IgG or IgM, 32-37% for aβ2GPI IgG or IgM (Bruce IN et al, 2000; Petri M et al, 2010). 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, 6% for IgM and 9% for both isotypes of aPS/PT (Bertolaccini ML et al, 2005). Sciascia et al. reported that aPS/PT in 35 (46.7%) of 75 SLE patients: 44% for IgG and 30.7% for IgM (Sciascia S et al, 2014). Elbagir et al. reported aPS/PT in 50 (15%) of 341 SLE patients (Elbagir S et al, 2023). 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 .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 (Arbuckle MR et al, 2003). 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. aDNA antibodies are expressed in a variety of immune diseases (Aringer M et al, 2022). They are also expressed in some healthy people (Samantha

S W et al, 2016). For APS, aDNA antibodies have low sensitivity and specificity. Therefore, aDNA antibodies are not included in the diagnostic criteria for APS. (Miyakis S, 2006). 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 the 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.

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.

7. Conclusion

This study yielded the following new insights.

- We demonstrated a significant association between the prevalence of IgG aPS/PT and aDNA in PAPS patients who subsequently developed SLE.

Future Implications and Research Directions

Our findings suggest that the presence of aPS/PT IgG and aDNA antibodies may serve as a predictive biomarker for SLE development in PAPS patients. This discovery has important clinical implications for risk stratification and early intervention strategies.

However, several critical areas require further investigation:

1.Validate the predictive value of these autoantibody combinations.

2. Establish optimal monitoring intervals.

3.Determine appropriate intervention thresholds.

In conclusion, our study has demonstrated the clinical significance of marker in SLE for disease progression prediction. This finding represent a significant advancement toward precision medicine in rheumatology, and we anticipate their contribution to improved patient care and outcomes.

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9. Disclosure of Conflict of Interest

There is no conflict of interest to declare.

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11. Supplementary：Urin-Presepsin Levels in Patients with Lupus Nephritis

11.1 Summary

Title： Urin-Presepsin Levels in Patients with Lupus Nephritis

[Background and Objectives] Presepsin (PSEP) is a 13kDa glycoprotein containing amino acid sequence 1-64 of CD14, discovered in Japan in 2002 as a marker that shows elevated levels in sepsis patients. It is a CD14 fragment product released when monocytes and neutrophils containing elastase phagocytose bacteria and antigens under inflammatory stress. It is currently measured at the general clinical level as a diagnostic and treatment marker for sepsis. Additionally, elevated plasma PSEP (P-PSEP) levels have been reported in hemophagocytic syndrome, where abnormal monocyte activation is the main pathological feature. Lupus nephritis (LN) remains one of the most important manifestations of SLE, significantly impacting patient outcomes and quality of life. Despite advances in therapeutic approaches, the assessment of disease activity and prediction of prognosis continue to rely heavily on renal biopsy, which serves as the gold standard for diagnosis, evaluation of disease activity, and treatment decision-making. Additionally, since kidney biopsies are invasive for patients, it is difficult to regularly check their disease progression or treatment response. Therefore, the development of reliable urinary biomarkers is required as a critical advancement in LN management. We previously showed that the P-PSEP in patients with SLE are elevated in association with disease activity of SLE. However, no data is available on urinary levels of PSEP (U-PSEP) in LN. The aim of this study is to clarify whether U-PSEP is high in LN as a potential urinary biomarker.

[Methods] We conducted cross-sectional study, including LN, SLE without LN, and other autoimmune diseases admitted to our department from August 2013 to September 2020. Urinary and P-PSEP levels were measured by CLEIA system. U-PSEP levels were compared between LN and non-LN. In addition, sub analysis was performed using each PSEP data compared among the following three groups [1. Active LN, 2. Inactive LN, 3. SLE without LN]. Active LN is defined as clinical and laboratory manifestations that meet ACR criteria (persistent proteinuria > 0.5g/gCre or active urinary sediment).

[Results] A total of 82 patients were enrolled in this study， including 36 patients with SLE [11 active LN patients, 8 inactive LN， 17 without LN] and 46 patients with other autoimmune diseases. There was no statistically significant difference in U-PSEP levels in between the SLE group and other immune disease group (average (SD): 461.5 (1312.9) VS 584.39 (1221.17), P = 0.3285). In sub analysis of SLE groups, U-PSEP levels of SLE with LN is higher than SLE without LN (161±1761.3 vs 56±289.5, $p < 0.0001$). The analysis revealed a statistically significant difference between the SLE with active LN group and SLE with inactive LN group (average (SD):1280(2209). VS 48(52.9), $P < 0.001$), SLE with active LN group and SLE without LN (average (SD):1280(2209). VS 127(14), $P < 0.001$).

[Discussion] Our results suggest that while U-PSEP levels show some association with lupus nephritis, several limitations need to be carefully considered. Although we observed differences in U-PSEP levels between active and inactive LN groups, U-PSEP elevation is not specific to LN, as similar elevations can be seen in various inflammatory conditions affecting the kidney. The presence of CD14-positive cells in urine, while mechanistically plausible, may reflect general inflammation rather than LN-specific pathology. Additionally, factors such as infection, medication use, and other comorbidities could potentially confound U-PSEP measurements, making interpretation challenging in clinical practice.

[Conclusion] We could detect U-PSEP in patients with active LN. Additional markers are needed for showing the specificity and further experiment should be needed.

11.2 Background

11.2.1 Systemic lupus erythematosus (SLE)

SLE is an autoimmune disease of unknown etiology that primarily affects young to middle-aged women. It is characterized by the production of various autoantibodies, including antinuclear antibodies and anti-ds-DNA antibodies, and causes organ damage through immune complex deposition in tissues throughout the body (Lisnevskaia et al, 2014). The development and pathogenesis of SLE involve B lymphocyte hyperreactivity, CD4-positive T lymphocyte activation, immune system dysfunction, environmental factors, and genetic background. Recently, abnormal clearance of apoptotic cells such as monocytes and dendritic cells, and the resulting type I interferons and inflammatory cytokines have been reported to be involved in clinical and serological abnormalities in SLE (Grossmayer et al, 2005; Herrmann et al, 1998). The importance of monocyte activation/functional abnormalities and their pathological significance is increasingly being recognized in SLE (Taylor et al, 2000).
Lupus nephritis (LN) remains a severe manifestation of SLE, significantly impacting patient outcomes and quality of life. Despite advances in therapeutic approaches, the assessment of disease activity and prediction of prognosis continue to rely heavily on renal biopsy, which serves as the gold standard for diagnosis, evaluation of disease activity, and treatment decision-making. However, renal biopsy has several inherent challenges: it is an invasive procedure with associated risks, and there are limitations on how frequently biopsies can be performed. These factors make it difficult to effectively monitor both disease progression and treatment response over time. Therefore, urinary biomarkers have emerged as promising candidates for monitoring lupus nephritis (LN), as they enable non-invasive sampling, allow repeated measurements, and directly reflect kidney pathology. Recent research has identified several urinary biomarkers, including soluble CD163 (sCD163), which correlates with disease

activity, and interleukin-16 (IL-16), which shows high diagnostic specificity for proliferative LN. Additionally, CD14+ cells have demonstrated significant elevation in Class IV LN, potentially aiding in distinguishing between different proliferative LN subtypes. The development of urinary biomarkers represents a critical advancement in LN management, potentially reducing patient burden while enabling more precise diagnosis, treatment monitoring, and outcome prediction.

11.2.2 Presepsin (P-SEP)

Presepsin (PSEP) is a 13kDa glycoprotein containing amino acid sequence 1-64 of CD14, discovered in Japan in 2002 as a marker that shows elevated levels in sepsis patients. It is a CD14 fragment product released when monocytes and neutrophils containing elastase phagocytose bacteria and antigens under inflammatory stress (Mussap et al, 2011; Tsujimoto et al, 2016; Zou et al, 2014). The name presepsin was changed from its original designation as sCD14 sub-type in 2011, derived from the fact that blood concentrations increase before the onset of sepsis (Shirakawa et al, 2011). P-PSEP is less affected by conventional inflammatory markers in conditions of high cytokine levels without infection, such as severe trauma and burns, and has been reported to increase in early infection. It is currently measured at the general clinical level as a diagnostic and treatment marker for sepsis (Endo et al, 2014). Additionally, elevated P-PSEP levels have been reported in hemophagocytic syndrome, where abnormal monocyte activation is the main pathological feature (Nanno et al, 2016). (Figure S1)

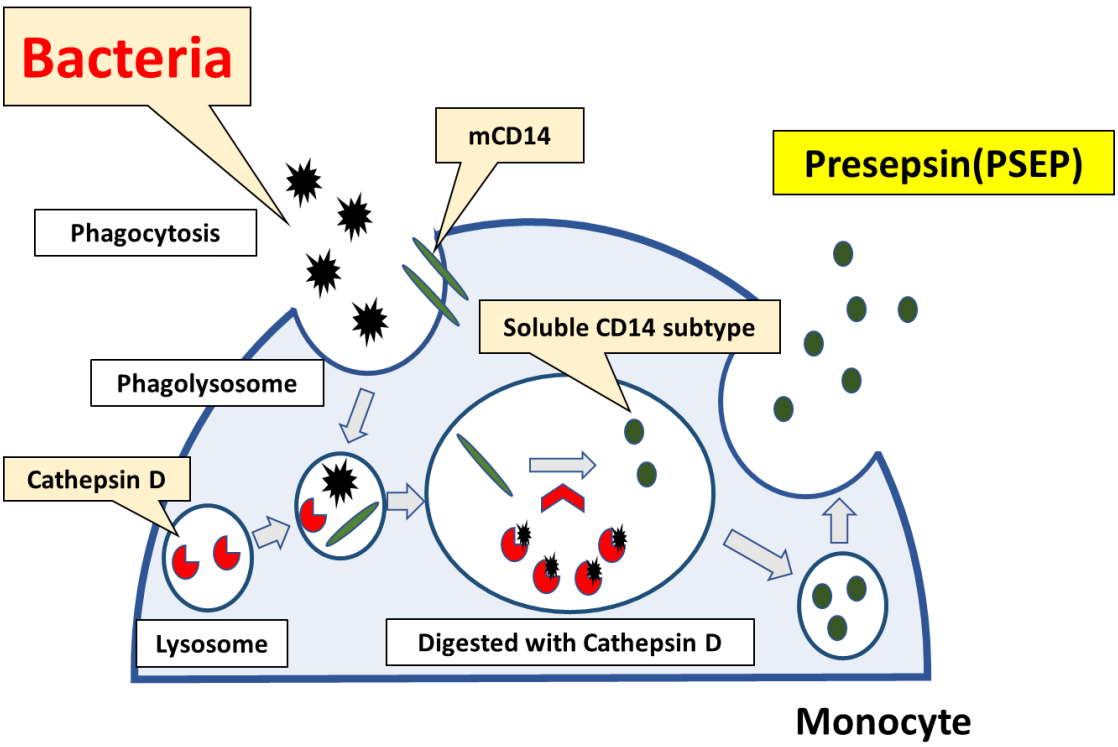

Figure S1. Mechanism of presepsin Production.

During the acute phase response to infection, monocytes engage in phagocytosis of pathogenic organisms, simultaneously internalising membrane-bound CD14 (mCD14). The internalised CD14 undergoes proteolytic processing within the lysosomal compartment by aspartic proteases, primarily cathepsin D. This enzymatic cleavage generates multiple fragments, of which the 13kDa soluble fraction—termed soluble CD14 subtype (sCD14-ST) or presepsin—is subsequently released into the systemic circulation. This molecular pathway represents a crucial mechanism for generating this emerging biomarker of systemic infection.

11.2.3 Rationale and Hypothesis

In 2015, they reported that patients with active lupus nephritis showed CD14-positive cells in urine and the number of CD14-positive cells in urine was associated with the severity of lupus nephritis (Kopetschke et al, 2015). Additionally, other previous studies have supported the evidence of the presence of CD14-positive cells in the urine of patients with lupus nephritis, and the correlation between urinary CD14-positive cell counts and disease severity (Abeer Abdelati et al, 2020). CD14-positive cells were observed in significantly higher numbers in class IV than class III LN (Abeer AA et al, 2021). Moreover, urinary CD14-positive cell counts showed strong correlation with disease activity and severity, with increased detection during acute exacerbations and tendency to disappear during remission (María M et al, 2021). Given that PSEP is a soluble CD14 subtype released from monocytes and macrophages upon activation, its presence in urine suggests heightened monocyte/macrophage activity within the kidneys. If U-PSEP levels are elevated in active LN patients, the observed elevation reflects increased local production and release of PSEP from inflamed renal tissue. Therefore, we hypothesized that Urine PSEP (U-PSEP) may have the potential to be a disease activity marker in patients with Lupus nephritis. The ability to non-invasively assess renal involvement through U-PSEP measurements could have significant clinical implications, potentially aiding in early detection and management of renal flares in SLE patients.

11.3 Method

11.3.1 Patients' Characteristics

We conducted cross-sectional study, including LN, SLE without LN, and other autoimmune diseases admitted to our department from August 2013 to September 2020. The diagnosis of each autoimmune disease was made by at least two specialists based on the criteria shown below, SLE: American College of Rheumatology (ACR) 1997 revised classification criteria (Hochberg, 1997), Vasculitis syndrome (ANCA (antineutrophil cytoplasmic antibody)-associated vasculitis, polyarteritis nodosa): Watts classification (Watts et al, 2007), ACR 1990 revised classification criteria (Arend et al, 1990; Hunder et al, 1990). Systemic sclerosis (6), 2013 ACR/EULAR Classification Criteria (Hoogen et al, 2013).Membranous Nephropathy(1), Evidence-Based Clinical Practice Guidelines for Nephrotic Syndrome (Shinichi N et al, 2014). Polymyositis/Dermatomyositis (4), clinical features laboratory tests. Polymyalgia rheumatica (3), International Classification of Disease (ICD)-10 code assignment. TAFRO syndrome (1), Proposed diagnostic criteria, disease severity classification and treatment strategy for TAFRO syndrome (Yasufumi Masaki et al, 2016). Spondyloarthritis (3), The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part I): classification of paper patients by Expert Opinion Including Uncertainty Appraisal (Rudwaleit M et al, 2009). Sjogren syndrome (2), classification of paper patients by Revised Japanese criteria for Sjögren's syndrome (1999): availability and validity (Takashi Fujibayashi et al, 2004). IgG4 related diseases (1), The 2020 revised comprehensive diagnostic (RCD) criteria for IgG4-RD (Hisanori Umehara et al, 2020). Giant cell arteritis (3), 2022 American College of Rheumatology/EULAR Classification Criteria for Giant Cell Arteritis (Cristina Ponte et al, 2022), Behcet Disease (1), Clinical and Genetic Aspects of Behçet's Disease in Japan

(Yohei Kirino et al, 2019). Adult onset Still’s disease (2), Preliminary criteria for classification of adult Still's disease (Yamaguchi M et al, 1992)

11.3.2 Definition of lupus nephritis

The study included SLE patients diagnosed according to the 1997 ACR criteria with eGFR ≥ 45mL/min/1.73m² (G3a). Active lupus nephritis (LN) was defined as having a medical history of suspected LN or being under treatment for LN, along with pathological urinary cast and urinary protein > 0.15 g/gCre. Non-LN was defined as having no medical history of suspected LN or treatment for LN, and no pathological urinary cast and urinary protein > 0.15 g/gCre.

11.3.3 Measurement of plasma and urinary presepsin levels

P-PSEP and U-PSEP levels were measured using the PATHFAST® automated immunoanalyzer (Mitsubishi Chemical Analytec Co. Ltd., Tokyo, Japan) based on the chemiluminescent enzyme immunoassay (CLIA) method (Behnes et al, 2014). The detection range for P-PSEP using PATHFAST® was from a lower limit of 20 pg/ml to an upper limit of 20,000 pg/ml, and the normal P-PSEP levels in healthy individuals were established as 73-209 pg/ml (mean ± SD: 132.5 ± 50.6 pg/ml) (Okamura and Yokoi, 2011).

U-PSEP levels were compared between LN and non-LN. In addition, sub analysis was performed using each PSEP data compared among the following three groups [1. Active LN, 2. Inactive LN, 3. SLE without LN and 4. disease controls]. Active LN is defined as clinical and laboratory manifestations that meet ACR criteria (persistent proteinuria > 0.5g/gCre or active urinary sediment).

Fe-PSEP (the fraction excretion of PSEP) represents the percentage of filtered PSEP exerted in the urine and was calculated using the formula:

Fe-PSEP (%)= (U-PSEP × S-creatine) / (S-PSEP × U-creatine) × 100

11.3.4 Statistical Analysis

Continuous variables that could not be assumed to follow a normal distribution were expressed as median [range], while categorical variables were expressed as absolute numbers or percentages. For comparison of continuous variables between groups, the Wilcoxon test or Kruskal-Walli’s test was used. Agreement between two groups was evaluated using Cohen's kappa statistic, and correlations between two groups were assessed using Spearman's rank-order correlation coefficient. Statistical significance was set at $p < 0.05$ for all analyses. Statistical analyses were performed using JMP Pro software (ver. 17.0; SAS Institute Inc., Cary, NC, USA) and GraphPad Prism software (ver. 8; GraphPad Software Inc., San Diego, CA, USA).

11.4 Results

11.4.1 Urine-Presepsin and SLE

We analyzed a total of 82 samples to compare the U-PSEP levels between patients with SLE and disease controls, including Rheumatoid Arthritis (RA), Systemic Sclerosis (SSc), Dermatomyositis(DM), Antineutrophil Cytoplasmic Antibody-associated Vasculitis(AAV),and others (Table S1 and S2).There was no statistically significant difference in U-PSEP levels in between the SLE group and disease controls (Average (SD): 461.5 (1312.9) VS 584.39 (1221.17), P = 0.3285; Figure S2(A)). Subsequently, we categorized the SLE patients (n = 36) into three subgroups: SLE with active lupus nephritis LN(n = 11), SLE with inactive LN (n = 8), and SLE without LN (n = 17). The analysis revealed a statistically significant difference between the SLE with active LN group and SLE with inactive LN group (Average (SD):1280(2209). VS 48(52.9), P < 0.001; Figure 9(B)), SLE with active LN group and SLE without LN (Average (SD):1280(2209). VS 127(14), P < 0.001; Figure S2(B)).

Table S1. Characteristics of the patients with systemic lupus erythematosus (SLE)

		SLE Group(n=36)			
Variable		SLE(n=36)	Active LN(n=11)	Inactive LN(n=8)	Witout LN(n=17)
Age	Median(IQR)	46(31-52)	44.5(33-49)	47(41-52)	47(31-56)
Female(%)	n(%)	35(97.3%)	10(90.9%)	8(100%)	17(100%)
eGFR	Median(IQR)	81.3(57.4-95.7)	83.54(52.2-93.6)	77.06(71.7-82.7)	87.9(38.3-98.5)
Urine protein positive	n(%)	21(58.4%)	11(100%)	5(62.5%)	5(29.4%)
Urine Total protein/Cre	Median(IQR)	0(0-0.66)	1.41(0.57-2.22)	0.17(0.16-0.53)	0(0-0)
CRP	Median(IQR)	0.258（0.045-0.788）	0.3(0.06-0.9)	0(0-0)	0.06(0.04-0.45)
ANA positive	n(%)	7(19.5%)	6(54.5%)	0(0%)	1(5.89%)
C3	Median(IQR)	66(57.8-92)	41.5(29-60.8)	84(74.8-92.3)	70(65-102.5)
C4	Median(IQR)	12(6.5-20)	6.5(3.3-10.8)	15.5(9.3-22.5)	14(12-21.5)
CH50	Median(IQR)	44.55（31.5-52）	24.3(15.7-33.1)	50.6(46.1-56.2)	46.2(35.7-56)
Plasma-Presepsin	Median(IQR)	136(73.5-257.7)	251(159-343)	86.95(72.9-150.8)	81.2(61.8-168)
Urine-Presepsin	Median(IQR)	67.85(45.9-375.3)	606(303-880)	33.3(15.5-61.8)	56(24.7-79.2)
FE-PSEP	Median(IQR)	0.59(0.22-1.78)	1.35(0.59-4.3)	0.43(0.2-0.7)	0.35(0.13-1.5)

Table S2. Characteristics of the control group

		Other immune diseases(n=46)					
Variable		Total(n=46)	AAV(n=4)	EGPA(n=3)	RA(n=9)	DM(n=3)	Particular(n=27)
Age	Median(IQR)	62(45-72)	69.5(63-71)	51(50-56)	67(54-78)	44(44-47)	62(44-70)
Female(%)	n(%)	25(54.34%)	0(0%)	2(66.7%)	7(77.8%)	1(33.4%)	15(55.6%)
eGFR	Median(IQR)	71.85(54.7-94.15)	55.34(51.3-75.8)	56.42(55.62-79.14)	56.74(47.23-85.99)	60.15(51.18-71.55)	81.2(64.15-99.79)
Urine protein positive	n(%)	27(58.7%)	4(100%)	1(33.4%)	7(77.8%)	3(100%)	12(44.5%)
Urine Total protein/Cre	Median(IQR)	0.094(0-0.38)	0.38(0.25-1.72)	0(0-0.3)	0.048(0-0.106)	0.079(0.039-0.118)	0(0-0.361)
CRP	Median(IQR)	0.33(0.06-2.63)	4.2(2.19-8.21)	-	0.33(0.24-4.56)	0.12(0.075-0.39)	0.345(0.058-3.15)
ANA positive	n(%)	23(50%)	1(25%)	1(33.4%)	6(66.7%)	1(33.4%)	14(51.85%)
C3	Median(IQR)	118(106-133.8)	119(115.5-176.8)	-	120(116.25-129)	-	117(96-129)
C4	Median(IQR)	26(19.5-30.5)	29(25-51.5)	-	27(18.5-28.75)	-	25(20.25-30.25)
CH50	Median(IQR)	59.5(53.25-69.75)	71.6(63.7-90.2)	-	57.45(55.2-65.4)	-	59.7(58.6-67.98)
Plasma-Presepsin	Median(IQR)	150(69.5-273.8)	290.5(181.8-501.5)	60.8(59.1-65.6)	129(69.1-1026)	188(1168.5-227.5)	134(83.35-273.5)
Urine-Presepsin	Median(IQR)	125(32.4-381.5)	621(443.5-976.5)	45(38.75-83)	119(37.6-347)	212(199-708.5)	97(25-282)
FE-PSEP	Median(IQR)	1.22(0.34-2.22)	1.51(1.36-3.09)	1.49(1.09-3.44)	1.05(0.38-2.12)	3(2.496-3.51)	0.81(0.21-2.15)

Other autoimmune diseases (n=46) include: Antineutrophil Cytoplasmic Antibody-associated Vasculitis(AAV)(4),
Particular(n=27) include: Systemic sclerosis (6), Membranous Nephropathy(1), Polymyositis/Dermatomyositis (4), Polymyalgia rheumatica (3), TAFRO syndrome(1), Spondyloarthritis (3), Sjogren syndrome (2), IgG4 related diseases (1),
Giant cell arteritis (3), Behcet Disease (1), Adult onset Still's disease (2)

11.4.2 Urine-Presepsin and infection

During the analysis, we found that even if the LN was not in the active stage, some patients would have elevated U-PSEP. We found that these patients might be in an infected state by comparing the clinical diagnosis of the patients. However, due to limited data, we only collected clinical diagnosis information on whether 43 patients were infected. The results showed that infection may cause an increase in U-PSEP, but since only 3 patients could be confirmed to be in the infection period, it was impossible to accurately analyze whether U-PSEP was also related to infection. (Figure S2(C)).

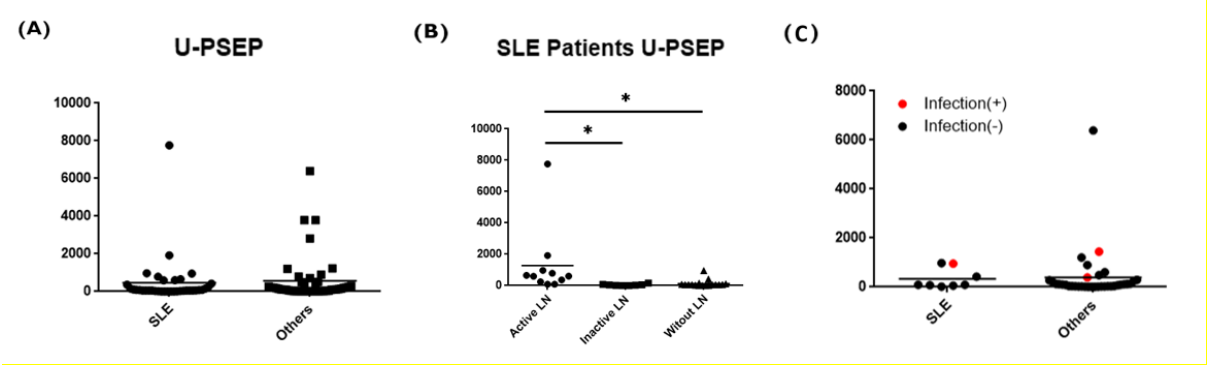

Figure S2

(A) Comparison of Urine-PSEP values between SLE and other immune diseases. SLE patients (n=36) and other immune disease patients (n=36) were analyzed by Wilcoxon test and concluded that there was no statistical difference between the two groups (p value=0.3285). (B) SLE with active lupus nephritis LN (n = 11), SLE with inactive LN (n = 8), and SLE without LN (n = 17). Pairwise Wilcoxon test analysis of Urine-PSEP was performed among the three groups. There was a statistical difference between the SLE with active LN group and the SLE with inactive LN group ($p < 0.0001$). There was also a statistical difference between the SLE with active LN group and the SLE without LN group ($p < 0.0001$). (C)Comparison of Urine-PSEP values between SLE and other immune diseases. SLE patients (n=8) and other immune disease patients (n=35) The red dots represent patients in the infectious period, and the black dots represent other patients.

11.4.3 The fraction excretion of Presepsin

The assessment of lupus nephritis activity has been limited by urinary biomarker measurements being affected by renal function changes and proteinuria. We developed the Fractional Excretion of PSEP (Fe-PSEP) metric by simultaneously measuring blood and urinary PSEP, applying sodium excretion rate calculations with creatinine ratio correction. This novel approach enables more precise evaluation of PSEP dynamics at the renal parenchymal level, leading to improved assessment of lupus nephritis activity.

Regarding the Fe-PSEP comparison among Active LN, Inactive LN, and Without LN groups, there were lack of significant differences (Average (SD): 4.81 (9.69). VS 0.67 (0.79). VS 0.58 (0.68) $P < 0.01$; Table S3) .

Table S3. The fraction excretion of Presepsin

SLE Group(n=36)	FE-SEP	Disease controls(n=46)	FE-SEP
SLE(n=36)	0.59(0.22-1.78)	**Total(n=46)**	1.22(0.34-2.22)
Active LN(n=11)	1.35(0.59-4.3)	**RA(n=9)**	1.05(0.38-2.12)
Inactive LN(n=8)	0.43(0.2-0.7)	**SSc(n=6)**	0.88(0.4-3.31)
Witout LN(n=17)	0.35(0.13-1.5)	**DM(n=5)**	1.378(0.61-2.5)
		AAV(n=4)	1.51(1.36-3.09)
		Others(n=22)	1.17(0.24-2.17)

Kruskal-Wallis one-way analysis among active LN, inactive LN, and SLE without LN.

11.5 Discussion

We observed a significant difference in U-PSEP levels between patients with active LN and those with inactive LN. However, U-PSEP levels associated with urin-protein, suggesting it is hard to show its potential utility as a biomarker for LN.

A meta-analysis of urinary biomarker studies in LN identified three primary limitations. First, significant heterogeneity exists in urine collection and processing methods across studies. Second, most studies compare active LN with healthy controls rather than with inactive LN or other glomerulonephritides. Third, validation cohorts are typically small and lack ethnic diversity (Birmingham et al, 2017).

Several urinary proteins have shown promise in recent large-scale studies. Monocyte chemoattractant protein-1 (MCP-1) and neutrophil gelatinase-associated lipocalin (NGAL) demonstrated correlation with disease activity in a prospective cohort of 635 LN patients (Brunner et al, 2016). Additionally, urinary TWEAK levels showed significant association with LN activity in a multicenter study of 173 patients (Schwartz et al., 2015). Recent advances in proteomics have enabled more comprehensive analysis of the urinary proteome in LN. The Renal Activity Index for Lupus (RAIL) combines multiple urinary biomarkers and has shown improved diagnostic accuracy compared to single markers in a validation cohort of 325 patients (Petri et al, 2021).

Our study has several important limitations. Most notably, we did not perform concurrent renal biopsies at the time of U-PSEP measurement. This limitation prevents us from establishing direct correlations between U-PSEP levels and specific histopathological features of lupus nephritis, such as the class of nephritis, degree of inflammatory infiltrates, or extent of tissue damage. The absence of histological data also makes it difficult to determine whether elevated U-PSEP levels reflect active inflammation, chronic damage, or both.

Furthermore, we were unable to elucidate the precise mechanism by which PSEP appears in the urine and its relationship to disease pathogenesis. Several possibilities exist: PSEP might be released by infiltrating monocytes/macrophages in the kidney, it could represent filtration of P-PSEP, or it might be produced by resident renal cells under inflammatory conditions. Understanding these mechanisms would require additional studies combining histological analysis, molecular techniques, and longitudinal monitoring of U-PSEP levels.

Key priorities include standardization of urine collection and processing protocols, establishment of larger validation cohorts, and development of composite biomarker panels.

11.6 Conclusion

While urinary biomarkers show promise for non-invasive monitoring of LN, current evidence does not support their use as a replacement for renal biopsy. Future progress requires larger, well-designed validation studies with standardized methodologies.

11.7 References

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