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IgG glycosylation profiling of systemic lupus erythematosus using lectin microarray

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ABSTRACT

Objectives Research on the specific role of immunoglobulin G (IgG) glycosylation in SLE development and progression is limited, especially regarding changes in IgG glycosylation profiles among different SLE subtypes. In this study, we aimed to characterise the glycosylation profile of serum IgG in patients with SLE.

Methods Lectin microarrays with 56 lectins were used to analyse serum IgG glycosylation in 194 patients with SLE, 100 disease controls (40 primary Sjögren's syndrome (pSS), 60 rheumatoid arthritis (RA)) and 100 healthy controls (HCs). Differences between SLE and control groups, as well as SLE subgroups, were validated by lectin blotting. Altered IgG glycosylation patterns were identified and further confirmed. Receiver operating characteristic (ROC) analysis evaluated the diagnostic value of these glycosylation changes in SLE and its subgroups, including neuropsychiatric SLE (NPSLE), lupus nephritis (LN), pulmonary arterial hypertension, immune thrombocytopaenia and SLE without major organ involvement (WMOI).

Results Compared to DC and HC groups, the IgG glycan level of Galβ3GalNAc (binding Jacalin (11.3%) and Maclura pomifera lectin (14.4%)) was significantly increased, whereas most IgG glycan levels were significantly decreased, including core fucose, high mannose, GlcNAc, GalNAc and Galβ4GlcNAc in the SLE group (all $p<0.05$). The IgG glycan levels were elevated in GalNAc and galactose patterns in the NPSLE group compared to the WMOI group, as well as higher Galβ3GalNAc and galactose patterns in NPSLE and LN compared to HCs. Moreover, ROC curve analysis showed PNA levels might have moderate potential for discriminating SLE from pSS.

Conclusions Patients with SLE show disease-specific alterations in serum IgG glycosylation, and aberrant Galβ3GalNAc, galactose and GalNAc glycosylation may have diagnostic value for SLE and NPSLE. Abnormal IgG glycans may provide new insights into their roles in SLE pathogenesis and progression.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Immunoglobulin (Ig) G glycosylation plays an important role in the development and progression of SLE. However, IgG glycosylation differences across autoimmune diseases and SLE subtypes remain unclear.

WHAT THIS STUDY ADDS

⇒ IgG Galβ3GalNAc levels are significantly elevated in SLE compared with both disease and healthy controls. Neuropsychiatric SLE (NPSLE) exhibits increased GalNAc and galactose glycosylation compared with without major organ involvement, while NPSLE and lupus nephritis show higher Galβ3GalNAc and galactose patterns than healthy controls.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Aberrant Galβ3GalNAc, galactose and GalNAc glycosylation may serve as diagnostic biomarkers for SLE and NPSLE.

INTRODUCTION

SLE is a systemic autoimmune disease that affects multiple organs and systems. Clinical features in individual patients can vary and range from mild joint and skin involvement to severe, life-threatening internal organ disease, with constitutional symptoms, rash, mucosal ulcers, inflammatory polyarthritis, photosensitivity and serositis being the most common clinical features.[1] Prevalence frequencies range from 20 to 240 per 100 000 persons, and reported incidence rates range from 1 to 10 per 100 000 person-years.[2]

Autoantibody production is a hallmark of SLE, and disease activity in SLE usually follows a flare pattern characterised by a relapsing–remitting course. However, many patients have continuously active disease, and only a few have long periods of disease quiescence.[3] Although overall survival rates have improved dramatically over the past few decades, some patients with SLE remain at risk of premature death.[4] Persistent inflammation inevitably results in irreversible major organ damage, which is associated with decreased quality of life and increased mortality.[5 6] The immunopathogenic mechanisms underlying the development of SLE and its related tissue

damage have been extensively explored; however, the exact mechanism underlying the immune dysfunction remains unclear.

Glycosylation is among the most common and diverse post-translational protein modifications,[7] and changes in immunoglobulin G (IgG) glycosylation have been shown to act as master regulators of the inflammatory response. It regulates the binding of IgG to complement proteins or Fc receptors, leading to changes in immunological functions such as complement-dependent cytotoxicity (CDC), antibody-mediated cellular cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) in many immune-mediated diseases.[8 9] However, research on the specific role of IgG glycosylation in SLE development and progression is limited, especially regarding changes in IgG glycosylation profiles among different SLE subtypes.[10]

Lectin has traditionally been used to analyse glycomes because of its specific binding to monosaccharides.[11] The lectin microarray is an emerging technology for glycan analysis[12] that detects target glycoproteins through direct labelling with fluorescent reagents or by overlaying with a fluorescent-labelled lectin antibody. It has the advantages of a simple and rapid procedure, high throughput and high sensitivity without destroying the native structure of glycans,[13 14] which makes it a useful tool for glycosylation discovery. Accordingly, in this study, we aimed to characterise the glycosylation profile of serum IgG in patients with SLE and validate our findings using lectin blotting.

MATERIALS AND METHODS

Participants and samples

In this study, serum samples collected from 194 patients with SLE between 2019 and 2022 were obtained from the Peking Union Medical College Hospital (PUMCH). Among these patients, 46 had lupus nephritis (LN), 30 had pulmonary arterial hypertension (PAH), 45 had neuropsychiatric SLE (NPSLE), 28 had severe immune thrombocytopaenia (ITP) and 45 patients with SLE without major organ involvement (WMOI). All included patients fulfilled the American College of Rheumatology (ACR) 1997 criteria for SLE or the Systemic Lupus International Collaborating Clinics (SLICC) 2012 classification criteria for SLE.

All included patients with LN had definite renal biopsy results, including 30 and 16 patients with stage IV and V LN, respectively. PAH was defined as mean pulmonary artery pressure ≥25 mm Hg at rest, pulmonary artery wedge pressure ≤15 mm Hg and pulmonary vascular resistance >3 Wood units,[15] as assessed by right-sided heart catheterisation. Severe ITP was defined as a platelet count less than 50×10^9/L. Patients with SLE WMOI referred to those with disease duration >5 years and without any internal organ disease, such as LN, NPSLE, ITP or cardiovascular system involvement. Active disease was defined as a Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) >4.

For healthy controls (HCs), 100 samples from individuals undergoing routine health check-ups were recruited at the Medical Examination Center of PUMCH. Those were excluded with abnormal liver or kidney function, lipid profiles, other organ or systemic abnormalities, positive results for common autoantibodies or autoimmune diseases that might affect glycosylation. As for disease control (DC), 100 serum samples were obtained from sex-matched patients with other autoimmune diseases (40 with primary Sjogren's syndrome (pSS) and 60 with rheumatoid arthritis (RA)) in the Rheumatology Department at PUMCH.

Demographic data, clinical manifestations and laboratory results were recorded for each patient with SLE at the time of serum sample collection. Serum samples were collected on admission using red-capped serum vacutainer, allowed to clot at room temperature (20°C–25°C) for 30 min, centrifuged for 5 min at 1000×g, collected the supernatant and stored at −80°C before use.

Lectin microarray analysis

A lectin microarray (BCBIO Biotech, Guangzhou, China) comprising a microchip of 56 lectins was used to analyse the serum samples. Lectin has previously been reported to be effective in biomarker discovery.[16] Briefly, lectin microarrays were taken out from −80°C, warmed up at room temperature for 30 min and incubated with a blocking buffer (3% bovine serum albumin in phosphate-buffered saline (PBS)) at room temperature for 2 hours. After washing three times with PBS with Tween detergent (PBST), 200 µL of 1:1000 diluted serum samples were added and incubated with the microarrays at 4°C overnight. The microarrays were washed three times with PBST and incubated with 5 mL of 1:1000 diluted Cy3-labelled goat anti-human IgG antibody (Jackson ImmunoResearch Labs, Pennsylvania, USA) in the dark at room temperature for 1 hour. Finally, after three PBST washes, the microarrays were rinsed with D.I. water and dried. Microarrays were scanned using a GenePix 4000B Microarray Scanner (Molecular Devices, Sunnyvale, California, USA).

For lectin array assays, the median foreground and background fluorescence intensities for each spot on the arrays were acquired using GenePix Pro V.6.0 (Axon Instruments, California, USA). The signal-to-noise (S/N) ratio (medium intensity of the foreground spot relative to the background) of each lectin spot was calculated. To prevent interarray bias in the lectin microarray, we normalised the S/N data in terms of quality control values between arrays.[17] For the normalisation procedures, each microarray contains a quality control serum sample from the same healthy individual. The data from this control sample are averaged to determine a normalisation coefficient for each microarray. Subsequently, the data from all other serum samples on each microarray are multiplied by their respective normalisation coefficients to obtain the final normalised results.

Lectin blot verification

Lectin blotting was used to validate the results of lectin microarray analysis. SLE serum samples collected from a new smaller cohort containing 24 patients in each SLE subgroup (LN, ITP, NPSLE, PAH and WMOI). HC and DC serum samples were collected from a new set of patients, matched by age and sex with SLE. Referring to the method of Hu and his colleagues,[18] the following rules were used to identify lectins with the most significant binding activities from the lectin microarray result by reducing the false positive rate: (1) fold change (group 1 (S/N) /group 2 (S/N)) ≥1.40 or <0.71; (2) p value <0.05.

Briefly, serum samples were diluted with 1×PBS, mixed with gel electrophoresis loading buffer (CW Biotech, Beijing, China) at a final 1:100 ratio, and boiled for 10 min. Twenty microlitres per sample were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electro-transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, Massachusetts, USA). After washing two times, the membrane was incubated with 10×Carbo Free Blocking Solution (1:10; Vector Laboratories Inc, Newark, California, USA) at room temperature for 2 hours. Next, the membranes were washed twice and incubated with 20 µg/mL of Cy3-labelled (1:1000; GE Healthcare, Chicago, Illinois, USA) lectins overnight in the dark at 4°C. Finally, the washed NC membrane was dried, and the blots were developed using a Typhoon FLA 9500 microarray scanner detection system (GE Healthcare, Chicago, Illinois, USA) using a fluorescence signal.

Statistical analysis

Descriptive data are presented as frequencies for categorical variables. One-way analysis of variance and Mann-Whitney U test were used to analyse differences between the SLE, DC and HC groups, as well as in SLE subgroups. To find the most significant results between groups, the percentage of those higher than mean+2 SD of HCs were compared using the χ^2 test and shown as percentage after each lectin. Both non-parametric test and logistic regression adjusted with age and sex were used to analyse the association between glycan levels and clinical indicators. A receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic value of significant findings. SPSS Statistics for Windows, V.26.0 (SPSS Inc, Chicago, Illinois, USA) was used for statistical analyses, and statistical significance was set at p<0.05. R (V.4.0.2). GraphPad Prism V.8.0 (GraphPad Software Inc, San Diego, California, USA) was used for plot drawing.

RESULTS

Participants characteristics

The baseline information of the 394 study participants included in the lectin microarray analysis is summarised in tables 1 and 2. Among the patients with SLE, 93.8% (182/194) were women, with an average age of 35.1 years old (IQR, 29.0–40.0 years) and a median disease duration of 69.5 months (range, 0–269). The SLE group included 46 patients with LN, 30 with PAH, 45 with NPSLE, 28 with ITP and 45 WMOI. Detailed demographic features, SLEDAI scores and autoimmunity profiles of all the subgroups are presented in table 2.

Lectin microarray results

The results of binding levels of IgG with all 56 lectins bound from the microarray are illustrated in figures 1 and 2. Lectins and their glycan specificities were listed in online supplemental table 1.

Lectin microarray significant results using non-parametric analyse are shown for SLE compared with both DC and HC (figure 1, online supplemental table 2). Significant χ^2 test results are shown as percentage after each lectin (online supplemental table 3).

For patients with SLE compared with HC and DC, most IgG glycan levels were significantly decreased, including core fucose (Fuc, binding PSA), high mannose (Man, binding NPL, GNL (1%), HHL (0%) and VVA), N-Acetylglucosamine (GlcNAc, binding UDA and PWM), N-Acetylgalactosamine (GalNAc, binding Black bean crude) and Galβ4GlcNAc (binding PHA-E). Interestingly, only one IgG glycan level of Galβ3GalNAc (binding Jacalin (11.3%) and MPL (14.4%)) was significantly increased in SLE compared with HC and DC. Other IgG glycan levels were partially elevated, containing sialic acid (Sia, increasingly binding SSA (12.9%) but decreasingly binding SNA, MAL II and SNA-I) and galactose (Gal, increasingly binding GSL I-B4 and PNA but decreasingly binding GHA and RCA I).

In figure 2, selected significant lectin microarray results using non-parametric analyses are shown for NPSLE, LN, PAH and ITP patients versus HC (chart A–E), and WMOI versus other SLE subgroups and HC (chart F–H). Statistical results between subgroups are listed in online supplemental tables 4 and 5.

A similar trend of intergroup results can be found in most SLE subgroups including the WMOI group. Looking into differences of each subgroup compared with HC (with no difference between WMOI vs HC), the glycan levels of lgG galactose (binding GSL I-B4 and PNA (13.3% and 28.3%)), Galβ3GalNAc (binding MPL) were significantly higher in NPSLE and LN vs HC, while IgG core fucose (binding PSA) and Galβ4GlcNAc (binding PHA-E) levels were significantly reduced within NPSLE versus HC. As for the WMOI group, glycan levels of lgG galactose (binding GHA), GalNAc (binding CSA) and Galβ4GlcNAc (binding MAL I) were significantly lower than NPSLE and HC.

Association between changes in IgG glycans with the presence of dsDNAs

Lectin microarray results for all 56 lectins, which revealed the different IgG glycan levels were compared with anti-dsDNA and disease duration in patients with SLE. Logistic regression showed limited results (online supplemental table 6), while the non-parametric test showed

Table 1 Clinical characteristics of study subjects in the lectin microarray cohort

	SLE (n=194) n (%)	DCs (n=100) n (%)		HCs (n=100) n (%)
		pSS (n=40)	RA (n=60)	
Demographic features				
Age, years	35.1 (29, 40)	48.5 (42.5, 56.5)	36.4 (31, 42)	57.2 (47, 63.8)
Female, %	182 (93.8)	37 (92.5)	56 (93.3)	54 (54)
Disease duration	69.5 (0, 269)	—	—	—
Disease assessment				
SLEDAI-2000	4.7 (0, 6)	—	—	—
ESR (mm/hour)	20.6 (6, 28.3)	—	—	—
CRP (mg/L)	3.9 (0.6, 4.5)	—	—	—
C3 (g/L)	0.8 (0.6, 1.0)	—	—	—
C4 (g/L)	0.1 (0.1, 0.2)	—	—	—
IgG (g/L)	14.6 (10.8, 16.7)	—	—	—
Autoimmunity characteristics				
ANA positive	194 (100)	—	—	—
Anti-dsDNA positive	139 (71.6)	—	—	—
Anti-Sm positive	59 (30.4)	—	—	—
Anti-RNP positive	80 (41.5)	—	—	—
Anti-Ro/SSA positive	120 (62.2)	—	—	—
Anti-La/SSB positive	46 (23.8)	—	—	—
Anti-rRNP positive	40 (20.7)	—	—	—
Anti-β2GPI	36 (18.7)	—	—	—
aCL	38 (19.7)	—	—	—
LA	41 (21.2)	—	—	—
Coombs	60 (30.9)	—	—	—

aCL, anticardiolipin; anti-dsDNA antibody, anti-double-strand DNA antibody; anti-La/SSB antibody, anti-Sjögren syndrome B antibody; anti-Ro/SSA antibody, anti-Sjögren syndrome A antibody; anti-rRNP antibody, anti-ribosomal P protein antibody; anti-Sm antibody, anti-Smith antibody; anti-β2GPI antibody, anti-β2glycoprotein antibody I; CRP, C reactive protein; DCs, disease controls; ESR, erythrocyte sedimentation rate; HCs, healthy controls; LA, lupus anticoagulant; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

that patients with positive anti-dsDNA had higher IgG glycan levels of core fucose (binding with LTL and UEA I), sialic acid (binding with SNA), GlcNAc (binding with GSL II) and GalNAc (binding with GSL-IA4) (online supplemental table 7).

Lectin blot analysis for patients with SLE

Lectin blots were performed for selected significant lectins (figure 3). To corroborate the result in lectin microarray, 24 serum samples per group matched for age and sex were collected from a new cohort for SLE, HC, DC and each SLE subgroup. The final results were adjusted according to serum IgG levels.

Similar differences were observed in the lectin blots comparing to the lectin microarray results. A higher binding level of PNA (preferred to Gal) was confirmed in patients with SLE than in HCs, pSS and RA. For patients WMOI, lower binding levels were verified for GHA (preferred to Gal) than in NPSLE, and CSA (preferred to GalNAc) than in NPSLE or PAH.

ROC analysis for discriminating SLE from pSS and RA

We performed ROC curve analysis to assess the ability of PNA to differentiate SLE from pSS and RA (figure 4). Among these, PNA binding levels in the lectin microarray analysis provided better differentiation conditions between SLE and pSS with a sensitivity of 92.8, specificity of 65.0% and area under the curve value of 0.775 (95% CI: 0.676 to 0.874, p<0.001), though it just showed medium potential.

DISCUSSION

Glycosylation is one of the most common and diverse post-translational modification of proteins that enhances the conformational diversity of antibodies, affecting their interactions with receptors. Accumulating evidence has

Table 2 Clinical characteristics of different subgroups in SLE

	NPSLE (n=45) n (%)	LN (n=46) n (%)	PAH (n=30) n (%)	ITP (n=28) n (%)	WMOI (n=45) n (%)
Demographic features					
Age, years	32.8 (21, 41.5)	32.8 (26.5, 37)	38.4 (33.8, 40.8)	35.2 (29, 36.8)	37.6 (30, 42)
Female, %	44 (97.8)	38 (82.6)	30 (100)	25 (89.3)	45 (100)
Disease duration, months	24 (0, 240)	31.5 (0, 223)	89 (40, 254)	25 (0, 206)	89 (60, 269)
Disease assessment					
SLEDAI-2000	9.6 (2, 14.3)	4.7 (0, 6.5)	1.4 (0, 2)	4.7 (1, 6.8)	2.5 (0, 4)
ESR (mm/hour)	25.7 (5.8, 40.3)	16.6 (5, 23)	13.6 (6, 16.5)	33.5 (11, 58)	17.6 (8, 22)
CRP (mg/L)	5.3 (0.6, 5.6)	—	—	1.9 (0.4, 2.8)	—
C3 (g/L)	0.7 (0.5, 1)	0.85 (0.6, 1.1)	0.9 (0.7, 1)	0.9 (0.6, 1.2)	0.9 (0.7, 1)
C4 (g/L)	0.1 (0, 0.1)	0.2 (0.1, 0.2)	0.2 (0.1, 0.2)	0.1 (0.1, 0.2)	0.1 (0.1, 0.2)
IgG (g/L)	13.7 (10.7, 16.6)	12.3 (8.9, 15.7)	16.2 (12.3, 15.5)	14.8 (9.8, 18.2)	16.3 (13.8, 17.8)
Autoimmunity characteristics					
ANA positive	45 (100)	46 (100)	30 (100)	28 (100)	45 (100)
Anti-dsDNA positive	34 (75.6)	36 (78.3)	22 (73.3)	18 (64.3)	29 (64.4)
Anti-Sm positive	9 (20)	20 (43.5)	11 (36.7)	4 (14.3)	15 (33.3)
Anti-RNP positive	16 (36.4)	20 (43.5)	19 (63.3)	7 (25)	18 (40)
Anti-Ro/SSA positive	26 (59.1)	22 (47.8)	23 (76.7)	16 (57.1)	33 (73.3)
Anti-La/SSB positive	15 (34.1)	7 (15.2)	7 (23.3)	5 (17.9)	12 (26.7)
Anti-rRNP positive	10 (22.7)	9 (19.6)	6 (20)	3 (10.7)	12 (26.7)
Anti-β2GPI	12 (27.3)	3 (6.5)	3 (10)	12 (42.9)	6 (13.3)
aCL	12 (27.3)	5 (10.9)	2 (6.7)	12 (42.9)	7 (15.6)
LA	15 (34.1)	7 (15.2)	2 (6.7)	12 (42.9)	5 (11.1)
Coombs	25 (55.6)	9 (19.6)	8 (26.7)	14 (50)	4 (8.9)

aCL, anticardiolipin; anti-dsDNA antibody, anti-double-strand DNA antibody; anti-La/SSB antibody, anti-Sjögren syndrome B antibody; anti-Ro/SSA antibody, anti-Sjögren syndrome A antibody; anti-rRNP antibody, anti-ribosomal P protein antibody; anti-Sm antibody, anti-Smith antibody; anti-β2GPI antibody, anti-β2glycoprotein antibody I; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; ITP, immune thrombocytopaenia; LA, lupus anticoagulant; LN, lupus nephritis; NPSLE, neuropsychiatric SLE; PAH, pulmonary arterial hypertension; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

shown that glycomics hold great promise for the advancement of biomarker research in the context of autoimmune diseases to uncover disease predisposition and development and assist in the identification of specific molecular targets for advanced therapies.[19]

In general, immunoglobulins are central to adaptive immunity, capable of activating complement and binding to IgG-Fc receptors and thereby shape immune responses, including antibody-ADCP, ADCC and CDC.[20 21] Among these are other roles—including securing protein solubility and conformation, as well as intracellular transport and clearance. Interestingly, the presence of certain glycan structures in immunoglobulins is responsible for the regulation of these effector functions and has been associated with proinflammatory antibody activity.[19 22]

Glycan is added to antibody proteins via two types of molecular linkages: asparagine residues (N-glycans) and serine/threonine residues (O-glycans).[23] Human IgG can be divided into 4 subclasses and 36 possible glycoforms, which allow up to 144 functional states, resulting in more complex and precise regulation of antibody effects.[24] Glycosylation of the Fc region is critical for modulating the inflammatory functions of IgG.[25 26] The addition of glycans to antigen-binding fragments through rational engineering enhances the conformational stability of monoclonal antibodies, thereby increasing their half-life.[27] Thus, the identification of differences in the glycosylation of total serum IgG between control individuals and patients would enable accurate diagnosis and guide the rational design of appropriate therapies.

Altered IgG glycans are associated with disease status, disease risk and symptom severity in patients with SLE, thus potentially opening up new avenues for the exploration of personalised treatments based on aberrant IgG glycosylation.[28] Our study found higher levels of Galβ3GalNAc in patients with SLE than in non-lupus patients. Notably, we demonstrated for the first time that SLE and its subgroups are characterised by an abnormal pattern of IgG glycosylation.

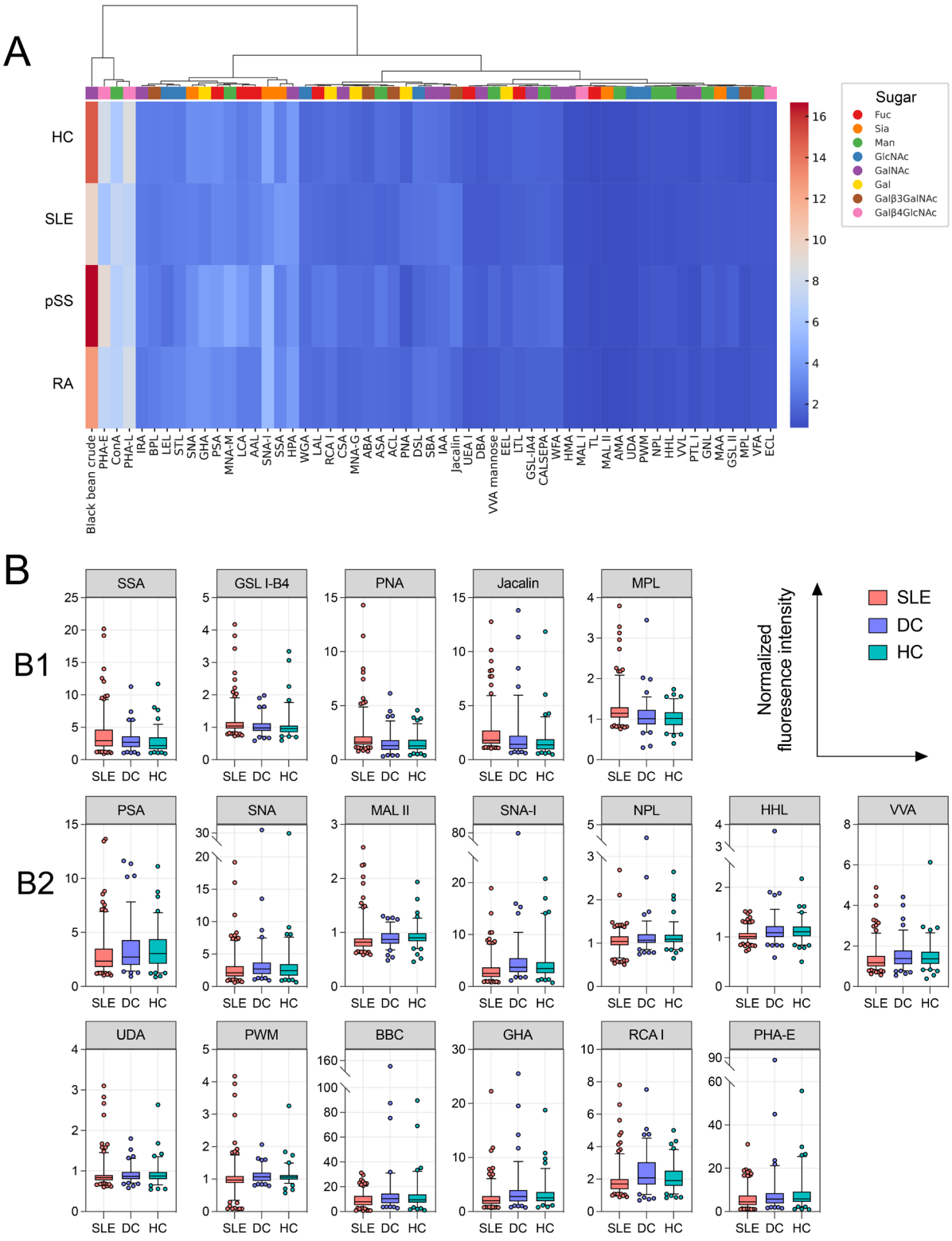

Figure 1 Significant results from lectin microarray for SLE compared with DC (pSS and RA) and HC. Non-parametric test with $p<0.05$ was used to identify significant differences between groups. (A) Heat map showing mean normalised fluorescence intensity of HC, SLE, pSS and RA. (B1) Lectin microarray results for significantly elevated levels in SLE compared with DC and HC. (B2) Lectin microarray results for significantly reduced levels in SLE compared with DC and HC. BBC, Black bean crude (Phaseolus vulgaris sp. Lectin); DC, disease control; GHA, Glechoma hederacea Lectin (ground ivy); GSL I-B4, GSL I-isolectin B4; HC, healthy control; HHL, Hippeastrum hybrid lectin; MAL II, Maackia amurensis lectin II; MPL, Maclura pomifera lectin; NPL, Narcissus pseudonarcissus (daffodil) lectin; PHA-E, Phaseolus vulgaris Erythroagglutinin; PNA, Arachis hypogaea Lectin (Peanut); PSA, Pisum sativum agglutinin; pSS, primary Sjögren's syndrome; PWM, Phytolacca americana Lectin (Pokeweed); RA, rheumatoid arthritis; RCA I, Ricinus communis agglutinin I; SNA, Sambucus nigra lectin; SNA-I, Sambucus nigra (Elderberry Bark); SSA, Salvia sclarea; UDA, Urtica dioica Lectin (Stinging Nettle); VVA, Vicia villosa Lectin (Hairy Vetch, Mannose Specific).

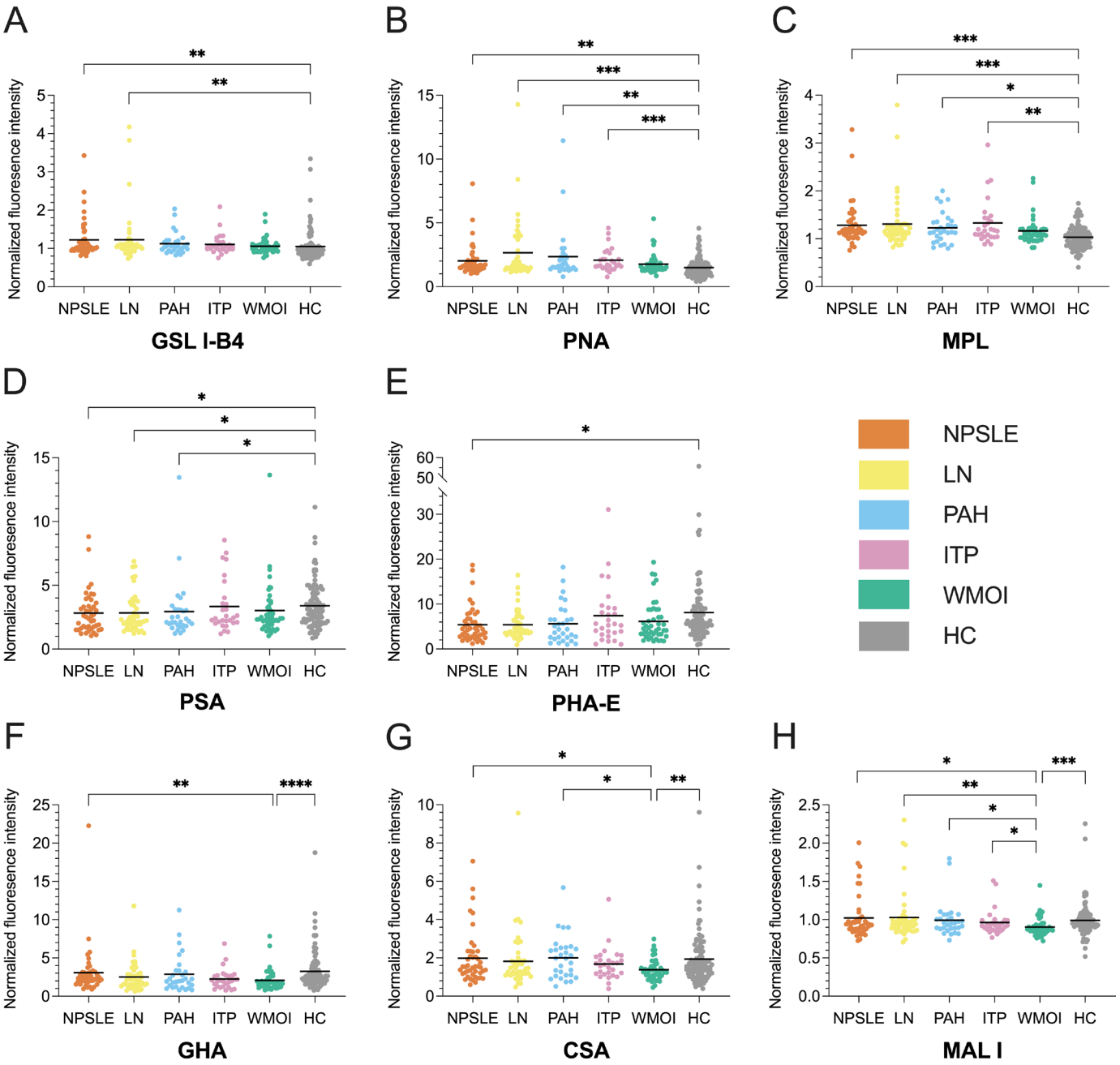

Figure 2 Significant lectin microarray results between SLE subgroups. Microarray results for different binding levels of (A) elevated GSL I-B4 between NPSLE versus HC and LN vs HC; (B) elevated PNA between NPSLE, LN, PAH and ITP versus HC; (C) elevated MPL between NPSLE, LN, PAH and ITP versus HC; (D) decreased PSA between NPSLE, LN and PAH versus HC; (E) decreased PHA-E between NPSLE versus HC. (F) Decreased GHA between WMOI versus NPSLE and HC; (G) elevated CSA between WMOI versus NPSLE, PAH and HC; (H) elevated MAL I between WMOI versus NPSLE, LN, PAH, ITP and HC. CSA, Cytisus sessilifolius Lectin (Portugal Broom); GHA, Glechoma hederacea Lectin (ground ivy); GSL I-B4, GSL I-isolectin B4; HC, healthy control; ITP, immune thrombocytopaenia; LN, lupus nephritis; MAL I, Maackia amurensis lectin I; MPL, Maclura pomifera lectin; NPSLE, neuropsychiatric SLE; PAH, pulmonary arterial hypertension; PHA-E, Phaseolus vulgaris Erythroagglutinin; PNA, Arachis hypogaea Lectin (Peanut); PSA, Pisum sativum agglutinin; WMOI, without major organ involvement. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

In our study, we found that the IgG glycan levels were higher for galactose in the NPSLE versus WMOI group. Unlike our results, reduced galactosylation has been reported in patients with SLE[28–30] and SS.[31] Moreover, an early comparative study to discern disease-specific aberrations in IgG glycosylation in several autoimmune diseases reported that serum IgG was normally galactosylated in patients with SLE and pSS.[32] Other studies reported that increased galactosylation of these antibodies is correlated with increased disease severity.[33–35] Galactosylation decreased over time in patients with and without relapse, which can explain the difference of galactose in NPSLE

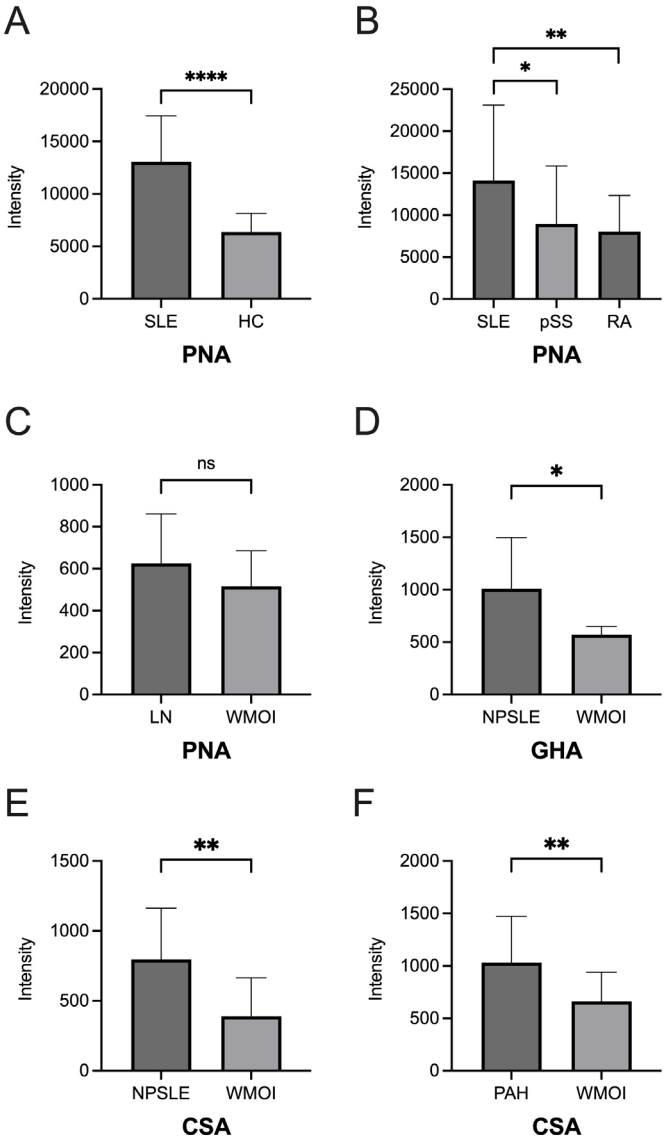

Figure 3 Lectin blot verification of microarray analysis. Lectin blot result for elevated binding levels of (A) PNA between SLE and HCs. (B) NA between SLE and RA. (C) PNA between LN and WMOI. (D) GHA between NPSLE and WMOI. (E) CSA between patients with NPSLE and WMOI patients. (F) CSA between patients with PAH and WMOI patients. CSA, Cytisus sessilifolius Lectin (Portugal Broom); GHA, Glechoma hederacea Lectin (ground ivy); HC, healthy control; LN, lupus nephritis; NPSLE, neuropsychiatric SLE; PAH, pulmonary arterial hypertension; PNA, Arachis hypogaea Lectin (Peanut); pSS, primary Sjogren's syndrome; RA, rheumatoid arthritis; WMOI, without major organ involvement. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

compared with WMOI group, as SLEDAI scores were lower in the WMOI group.[36] The difference in results may be attributed to our different study designs. Previous studies did not mention comparisons between different subgroups of lupus. Our article found that compared with the WMOI group, the NPSLE group had patients who might experience higher IgG glycan levels of galactose.

Fc glycosylation—the presence of the Fc glycan—has been shown to affect antibody effector functions via C1q in the complement system and Fc gamma receptors (FcγR) in immune cells.[37] Elevated IgG-Fc galactosylation stimulates C1q binding and downstream complement activity.[38] These associations have almost exclusively led to the hypothesis that agalactosylated IgGs are proinflammatory, whereas highly galactosylated IgG glycoforms may have an anti-inflammatory effect.

Notably, injection of galactosylated mouse IgG1 immune complexes interfered with the complement-dependent recruitment of neutrophils into the peritoneum and skin, suggesting that galactosylation significantly influences the affinity of IgG1 for FcγRII2b in mice.[39] Various studies have reported contradictory results regarding the effects of galactosylation on antibodies. One suggested that hyper-galactosylation of IgG1 increased the binding to FcγRIIIa, resulting in enhanced NK cell-mediated ADCC.[40] Similarly, Quast *et al* showed that increased galactosylation enhanced C1q binding and CDC in antibody models.[41] However, another study reported no significant variation in the affinity of human IgG isotypes for FcγRIIIa and no increase in ADCC.[38] In these studies, galactosylation varied between patients, with total IgG galactosylation often diverging from antigen-specific IgG galactosylation.

Due to the discrepancies between in vitro and in vivo model results, the potential of galactosylated IgG (IgG-GO) to activate complement remains a topic of debate. Although IgG-GO has been reported to lack C1q-binding properties,[40 42] some researchers suggest that it may activate complement through the lectin pathway.[42] Mannose-binding lectin (MBL) shares structural homology with C1q, and its binding to Man or GlcNAc residues triggers complement activation similar to the classical pathway, leading to C4d generation. Notably, when the galactosylation level of IgG from healthy individuals was enzymatically increased from 20% to 100%, MBL binding was significantly enhanced.[42]

In addition, the exact role of IgG galactosylation and its influence on disease activity remains unclear. Gillian *et al* showed that during remission, total IgG galactosylation is relatively high, whereas during an autoimmune disease flare, total IgG galactosylation is relatively low.[43] As such, the influence of IgG galactosylation on SLE development and activity still requires further investigation. Taken together, these findings suggest that research should not only examine how IgG glycovariants affect Fc-receptor binding but also explore the role of immune complexes rather than focusing solely on monomeric IgG glycosylation variants.

Since Fc receptor polymorphisms are known risk factors for SLE,[44] it is reasonable to assume that variations in Fc receptor affinity for differentially glycosylated IgG contribute to SLE disease pathogenesis.[28] Because only approximately 15% of the total IgG glycome is derived from the Fab portion, the observed differences between patients and controls are likely attributed to variations in Fc glycans. Furthermore, Fab and Fc glycans exhibit distinct compositions, with Fab glycans containing lower

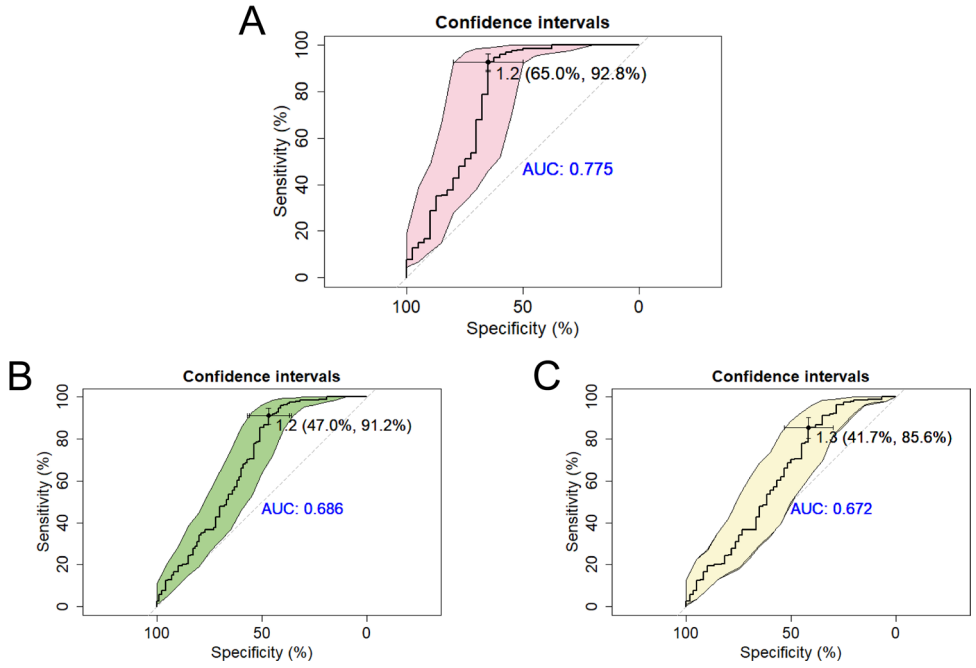

Figure 4 ROC curve analysis of SLE diagnosis. ROC curves and AUC statistics are used to evaluate the capacity of PNA to discriminate SLE from (A) pSS (SLE vs pSS); (B) RA+pSS (SLE vs RA+pSS); (C) RA (SLE vs RA) with excellent specificity and sensitivity. The ROC curve was generated using R software (V.4.0.2, https://cran.r-project.org/bin/windows/base/old/4.0.2/). AUC, area under the curve; PNA, Arachis hypogaea Lectin (Peanut); pSS, primary Sjogren's syndrome; RA, rheumatoid arthritis; ROC, receiver operating characteristic.

core fucosylation and higher levels of galactose, sialic acid and bisecting GlcNAc compared with Fc glycans.[45] Since the method we used cannot distinguish between glycans from the Fab and Fc regions of IgG, the increased galactose levels observed in NPSLE and LN may be partially attributed to the Fab portion.

Given that patients with RA and SLE often present with symptoms overlapping with SS, further IgG glycosylation studies could aid in identifying disease-specific glycosylation traits for SS. This could improve our ability to predict and/or differentiate patients from other rheumatic autoimmune diseases based on altered IgG glycosylation.[10] Eventually, monitoring the glycosylation status, especially the degree of galactosylation, of total or disease-specific antibodies in SLE may be possible. This could help predict or detect an upcoming flare, enabling early intervention after or even before the onset of symptoms.

Limitations

For disease controls, RA and primary pSS are two representative autoimmune diseases sharing characteristics that are more closely aligned with those of SLE, with both having larger patient populations than other autoimmune diseases. And because of the restricted number of clinical samples, DCs apart from RA and pSS were not included, which might limit the generalisability of our findings. In future studies, we aim to incorporate a wider range of autoimmune diseases as controls to enhance the specificity and robustness of our research.

For SLE subgroups, NPSLE, LN and WMOI groups have enough samples for statistical analyses, but PAH and ITP groups were not that sufficient, which may limit the findings of those two subtypes, so we reduced the findings unique to the PAH and ITP groups. In addition, differences in disease severity and duration were observed in the WMOI and PAH groups than the other groups, which were attributed to the characteristics of the enrolled patients. In future studies, these confounding factors, including sample size, should be better taken into account when enrolling patients with PAH and ITP to ensure more accurate attribution.

Further, given that the number of serum samples is limited, the lectin blots did not verify all the results from the microarray. Although lectin microarrays are useful tools for glycosylation studies, they cannot fully clarify the exact glycan structures. And with the development of technology, orthogonal techniques, like mass spectrometry, are crucial to confirm the microarray findings and have a better validation value. Additionally, the association between IgG glycosylation and serum inflammatory markers requires further investigation. To further elucidate the structure of glycans and their roles in SLE pathogenesis, other techniques for glycan analysis—such as affinity chromatography and mass spectrometry—should be applied to investigate the impact of glycosylation on SLE development and progression.

CONCLUSIONS

Patients with SLE showed disease-specific alterations in serum IgG glycosylation. Aberrant Galβ3GalNAc, galactose and GalNAc glycosylation may offer potential

diagnostic value for SLE and NPSLE. These abnormal IgG glycans could also provide new insights into their roles in SLE pathogenesis and progression.

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