Arthritis Research & Therapy

RESEARCH

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Profiling of IgG N-glycosylation for axial spondyloarthritis and other rheumatic diseases

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Abstract

Background Axial spondyloarthritis (axSpA) is an inflammatory rheumatic disease with challenges in diagnosis and disease activity assessment. While alterations in immunoglobulin G (IgG) N-glycosylation have been observed in varied rheumatic diseases, those in axSpA remains unclear. This study aims to explore the role of IgG N-glycan profiles in diagnosis and disease activity of axSpA.

Methods A clinical case-control study was conducted involving patients with axSpA ($n=138$), systemic lupus erythematosus ($n=102$), rheumatoid arthritis ($n=106$), osteoarthritis ($n=33$), gout ($n=41$) and healthy controls ($n=117$). Ultra-performance liquid chromatography was employed to analyze the composition of the serum IgG N-glycome. Associations between IgG N-glycans and axSpA were investigated and compared to healthy controls and other four rheumatic diseases. The relationship among IgG N-glycosylation, disease activity, and inflammatory cytokines of axSpA patients were analyzed. The receiver operating characteristic (ROC) curve analysis was applied to evaluate the diagnostic/classification performance of IgG N-glycans to distinguish axSpA and its disease activity.

Results In patients with axSpA, the abundances of IgG galactosylation and sialylation were significantly lower than healthy controls, while the abundance of fucosylation was higher than the other four studied rheumatic diseases. Additionally, two asialylated IgG N-glycans (FA2 and FA2 [3]G1) were associated with axSpA, with adjusted odds ratios (AORs) of 5.62 (95% CI: 3.41–9.24) and 0.33 (95% CI: 0.22–0.50), respectively. Notably, decreased FA2 [3] G1 emerged as a characteristic IgG N-glycan associated with all five studied rheumatic diseases, while decreased FA2BG2S2 was a unique IgG N-glycan differentiating axSpA from the other four rheumatic diseases. Furthermore, FA2 displayed positive association with disease activity indicators (ASDAS-CRP, SPARCC-SIJ and SPARCC-spine) in axSpA. IgG N-glycans, particularly FA2 [3]G1, FA2BG2S2 and FA2, demonstrated canonical correlation with inflammatory cytokines, including interleukin-23 and tumor necrosis factor α, in axSpA ($r=0.519$, $P=0.017$).

Conclusions Specific IgG N-glycans hold potential as novel biomarkers to enhance diagnosis and disease activity assessment in axSpA management.

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Keywords Axial spondyloarthritis, Rheumatic diseases, Immunoglobulin G, Glycosylation, Biomarker

Background

Rheumatic diseases encompass a broad spectrum of conditions, including inflammatory joint diseases, autoimmune diseases, degenerative joint diseases and metabolic bone diseases [1], and among them axial spondyloarthritis (axSpA), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), osteoarthritis (OA), and gout are common rheumatic diseases [2]. More specifically, axSpA is a "mixed-pattern" disease, ranked in between inflammation and autoimmune disease [3, 4]. Although axSpA and other rheumatic diseases share some similar symptoms, such as swelling and pain in the joints, they still exhibit many different clinical manifestations, therapeutic approaches and prognoses [5, 6].

Currently, there is no single test to diagnose axSpA [7]. In addition, serum biomarkers for axSpA have generally demonstrated both low sensitivity and specificity, typically below 60% [8]. Furthermore, the insidious progression of axSpA often leads to a delay in diagnosis, averaging up to 5.7 years, which prevents patients from receiving timely treatment and consequently impacting their physical function and the quality of life [9, 10]. Moreover, although the Ankylosing Spondylitis Disease Activity Score (ASDAS) is a commonly used tool for evaluating disease activity in axSpA, this method has a high level of subjectivity as it relies on patient self-reporting [11]. The Spondyloarthritis Research Consortium of Canada (SPARCC) scoring system, which assesses active inflammation in the sacroiliac joint (SPARCC-SIJ) and spine (SPARCC-spine) through magnetic resonance imaging (MRI), is also used to evaluate disease activity. However, due to its high cost and complex interpretation, it is not routinely recommended in clinical practice [5, 12–14]. Therefore, it is essential to improve the diagnostic approach and disease activity assessment for axSpA.

Although the exact pathogenesis of axSpA remains unclear, the interleukin-23 (IL-23)/Th17 (T helper 17) axis and tumor necrosis factor α (TNFα) are known to play crucial roles in driving the disease by contributing to inflammation and inducing bone erosion [15, 16]. Immunoglobulin G (IgG) N-glycosylation, a co- and post-translational modification process, plays a crucial role in regulating the balance between pro- and anti-inflammatory responses [17, 18]. Recent mechanistic and human case-control studies have suggested the involvement of IgG N-glycans as functional effectors in several inflammatory and autoimmune diseases, such as SLE, RA and inflammatory bowel disease (IBD) [19–21]. For example, the absence of galactose on IgG can activate the complement system via the lectin pathway, exacerbating the pro-inflammatory response [22]. Conversely, elevated abundances of IgG sialylation are associated with an anti-inflammatory effect by inhibiting antibody-dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) [23]. Furthermore, alterations in IgG N-glycosylation are promising as potential biomarkers for disease prediction, owing to their discriminative capacity [20, 24]. A recent study has found that the abundance of IgG agalactosylation increased shortly before the onset of RA, peaked during active disease stages and decreased upon inflammation resolution, suggesting a potential relationship between IgG N-glycosylation and disease activity [25].

Moreover, recent research has identified that inflammatory cytokines in the immune microenvironment can modulate IgG N-glycosylation by regulating the action of glycosyltransferases [26]. For instance, IL-23 may exert regulatory control over T helper 17 (Th17) cells to secrete cytokines and inhibit the expression of β-galactoside α-2,6-sialyltransferase 1 (St6Gal1), in turn leading to a reduction in IgG sialylation in mice with arthritis [27]. Additionally, pro-inflammatory cytokines such as TNFα and IL-6 have been shown to upregulate sialylation by stimulating α-2,3-sialyltransferase 4 (St3Gal4) in cancer cells [18]. However, the connection between inflammatory cytokines and IgG N-glycosylation in patients with axSpA remains unclear.

In the current study, we investigated the association of IgG N-glycosylation with axSpA and other rheumatic diseases, and identified potential glycomic biosignatures for distinguishing axSpA from other rheumatic diseases and healthy controls. Additionally, we identified the relationship between IgG N-glycosylation and disease activity and inflammatory factors in axSpA patients, thereby providing deeper insights into the role of IgG N-glycosylation in diagnosis and disease activity assessment in axSpA.

Methods

Study participants

A clinical case-control study was conducted in the First Affiliated Hospital of Shantou University Medical College in China from 2021 to 2022. The case group consisted of 138 axSpA patients who fulfilled the 2009 Assessment of SpondyloArthritis International Society (ASAS) classification criteria for axSpA [28]. These patients were recruited from the Clinical characteristics and Outcomes in the Chinese Axial Spondyloarthritis (COCAS; registration no. ChiCTR2100049357), as previously described [29]. Furthermore, for the control group, the study included patients with SLE ($n = 102$) meeting the 2019 American College of Rheumatology/European League

Against Rheumatism (ACR/EULAR) classification criteria [30], patients with RA ($n = 106$) meeting the 2010 ACR/EULAR classification criteria [31], patients with OA ($n = 33$) meeting the 1986 ACR classification criteria [32], patients with gout ($n = 41$) meeting the 2015 ACR/EULAR classification criteria [33], as well as healthy controls ($n = 117$).

The participants met the following inclusion criteria: (1) aged 18 years or older; (2) Chinese Han ethnicity; and (3) patients with either axSpA, SLE, RA, OA or gout. Individuals were excluded based on the following criteria: (1) severe infectious diseases; (2) other rheumatic diseases; (3) chronic renal and/or liver diseases, cancer and mental illness; and (4) pregnancy or lactation.

Data collection and disease activity assessment

The data including age, gender, IgG, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), disease duration (in years from symptom onset to the date of the first clinic visit) and age at disease onset (age when the patient recalled disease-related manifestations) and human leucocyte antigen B27 (HLA-B27) were obtained from electronic medical records.

Disease activity was assessed using the ASDAS along with CRP (ASDAS-CRP), SPARCC-SIJ and SPARCC-spine. Additionally, according to the classification criteria recommended by ASAS, high disease activity (HDA) of axSpA was defined as ASDAS-CRP ≥ 2.1, and low and inactive disease activity (LIDA) as ASDAS-CRP < 2.1 [5].

Analysis of blood samples

For IgG N-glycan analysis, serum IgG isolation and IgG Fc N-linked glycan release were conducted following previously established protocols [34, 35]. In brief, a 100 μL serum sample was applied to a 96-well protein G monolithic plate (BIA Separations, Slovenia) for IgG isolation. The isolated IgG samples were denatured with 1 mL of 0.1 $mol{\cdot}L^{-1}$ formic acid, and then promptly neutralized with 1 $mol{\cdot}L^{-1}$ ammonium bicarbonate. To release N-glycans, 4 μL of peptide-N-glycosidase F (PNGase F) enzyme was added to the IgG samples, followed by an 18-hour incubation period in a 37 °C water bath. The released N-glycans were then tagged with 2-aminobenzamide (2-AB), and transferred to an oven at 65 °C for 3 h. Following this, the labeled glycans were analyzed via an ultra-performance liquid chromatography (UPLC) instrument (Walters Corporation, USA), enabling the detection of 24 directly measured IgG N-glycans (Table S1). Additionally, 54 derived glycan traits were calculated from the ratios of 24 directly measured N-glycans, as previously described (Table S2). Among them, 10 derived glycan traits were calculated to represent the abundances of four glycosylation features: galactosylation, sialylation, bisecting acetylglucosamine (GlcNAc) and fucosylation (Table S3) [36].

Plasma samples were used to measure inflammatory cytokines (IL-6, IL-17, IL-21, IL-22, IL-23, and TNFα) using ultrasensitive sandwich enzyme-linked immunosorbent assay (ELISA) kits (Cusabio, USA) according to the manufacturer's instructions. Briefly, plasma samples were added to 96-well plates, followed by sequential incubation with biotin-antibody, HRP-avidin, and TMB substrate, with washing steps between each stage. The reaction was stopped with a stop solution, and optical density was measured at 450 nm using a microplate reader. Cytokine concentrations were calculated using standard curves generated from serial dilutions of the provided cytokine standards.

Statistical analysis

The normality of variables was tested using the Kolmogorov-Smirnov method. Continuous data, presented as median [P_{25}, P_{75}] due to a skewed distribution, were analyzed using the Kruskal-Wallis H-test with Benjamini-Hochberg false discovery rate (FDR) correction. Categorical data was presented as frequency (n) and proportion (%), and the differences between groups were analyzed using the χ^2 test.

To control the experimental batch variation in the measurements, batch correction and normalization techniques were applied to the IgG N-glycan data. Multivariate linear regression was used to analyze the association between IgG N-glycans and axSpA adjusting for patient characteristics and other clinical features.

The Least Absolute Shrinkage and Selection Operator (LASSO) algorithm and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) were used to screen IgG N-glycans that were associated with the respective rheumatic diseases, to prevent overfitting and to control the model's complexity. Shared IgG N-glycans between the LASSO and OPLS-DA methods were incorporated into multivariate logistic regression analysis to evaluate the associations between IgG N-glycans and axSpA and other rheumatic diseases. The discriminative ability of the models was assessed by receiver operating characteristic (ROC) curve analysis. The discriminant models were trained using logistic regression classifiers with five-fold cross-validation to minimize overfitting. Spearman's rank correlation and partial correlation analysis were used to analyze the correlation between IgG N-glycans and inflammatory factors and clinical features. Canonical correlation analysis (CCA) was used to explore the overall correlation between the IgG N-glycans (x) and inflammatory factors (y). Identified variables with a statistically significant impact on the canonical variables were judged by canonical loadings. In general, an absolute value of

canonical loading greater than 0.30 was used to define significant loading [37].

R software version 4.1.1 (https://www.r-project.org) and SPSS software version 29.0 (IBM, USA) were used for statistical analyses, whereby $P<0.05$ was considered statistically significant.

Results

Clinical characteristics of the study participants

Descriptive information on patients with axSpA, SLE, RA, OA, or gout, and healthy controls are presented in Table 1. In patients with axSpA, the percentage of males was significantly higher than in those with SLE, RA, or OA, but lower than in those with gout. There was no significant difference in the percentage of males between axSpA patients and healthy controls. The median age of axSpA patients was 27 years, which was significantly lower than that of patients with OA (62 years), RA (52.5 years), gout (40 years), SLE (37 years), and healthy controls (34 years). Additionally, the levels of inflammatory cytokines (IL-6, IL-17, IL-21, IL-22, IL-23, and TNFα) were significantly increased in patients with axSpA compared to those of healthy controls ($P<0.001$), but showed no significant difference when compared to those of other four rheumatic diseases.

The comparison of IgG N-glycan profiles among axSpA, other rheumatic diseases and healthy controls

Quantitative measures of 24 directly measured IgG N-glycans and 54 derived glycan traits between patients with axSpA, SLE, RA, OA, gout, and healthy controls are different (Tables S4 and S5). Among these IgG N-glycans, 10 derived glycan traits were presented the abundances of four glycosylation features in Fig. 1 and Table S6.

Compared to that of healthy controls (52.85%), the abundances of total galactosylation gradually decreased in patients with axSpA (48.26%), OA (47.22%), SLE (47.17%), gout (44.18%) and RA (43.04%) ($P<0.05$). In contrast, the abundances of agalactosylation (G0) were significantly increased in patients with RA (33.31%), SLE (29.04%), gout (28.82%), axSpA (28.52%), or OA (28.40%) compared to that of healthy controls (22.56%) ($P<0.05$).

Additionally, when compared to that of healthy controls (23.21%), the abundances of total sialylation decreased in patients with axSpA (21.19%), OA (21.19%), SLE (22.35%), RA (22.92%), or gout (22.94%). However, only the decrease in axSpA patients showed statistical significance ($P<0.05$).

Moreover, the abundances of IgG fucosylation were decreased in patients with axSpA (95.65%), SLE (94.86%), RA (94.67%), OA (89.45%), or gout (88.73%) when compared to that of healthy controls (95.81%). However, this decrease was not statistically significant in axSpA patients. Contrastingly, compared to that of healthy controls (14.91%), the abundances of bisecting GlcNAc were significantly increased in patients with RA (18.40%), SLE (19.05%), OA (20.40%), or gout (20.54%) ($P<0.05$). Notably, in patients with axSpA, the abundance of fucosylation was significantly decreased, while the abundance of bisecting GlcNAc was increased when compared to other four rheumatic diseases ($P<0.05$).

The associations of IgG N-glycans with axSpA and other rheumatic diseases

The shared IgG N-glycans between the different health condition groups based on the LASSO and OPLS-DA analysis are presented in Figure S1. After the multivariate logistic regression analysis adjusted for gender and age, increased abundance of FA2 and decreased abundance of FA2 [3]G1 were found to be significantly associated with axSpA, with adjusted odds ratios (AORs) of 5.62 (95% CI: 3.41–9.24) and 0.33 (95% CI: 0.22–0.50), respectively. Similarly, four IgG N-glycans (reduced abundance of FA2 [3]G1, A2BG2 and FA2G2S2, as well as an increased abundance of FA2BG2S2) demonstrated significant associations with SLE, while four IgG N-glycans (increased abundance of M5 and FA2BG2S2, and decreased abundance of FA2 [3]G1 and FA2G2) were significantly associated with RA. Additionally, only decreased abundance of FA2 [3]G1 exhibited a significant association with OA, whereas decreased abundance of FA2 [3]G1 and FA2G2 were significantly associated with gout (Fig. 2a).

In addition, five IgG N-glycans (increased abundance of A2BG2, FA2G1S1 and FA2G2S2, as well as reduced abundance of A2G2S1 and FA2BG2S2) exhibited significant differences between axSpA and SLE. Four IgG N-glycans (increased abundance of FA2 [3]G1 and FA2G2, and reduced abundance of FA2BG2 and FA2BG2S2) demonstrated significant differences between axSpA and RA. Additionally, two IgG N-glycans (decreased abundance of A2G2 and FA2BG2S2) exhibited significant differences between axSpA and OA, while only decreased abundance of FA2BG2S2 showed significant difference between axSpA and gout. Moreover, compared to the patients with other rheumatic diseases (including SLE, RA, OA and gout), three IgG N-glycans (increased abundance of FA2 [3]G1 and FA2G2S2, and decreased abundance of FA2BG2S2) showed significant differences in axSpA (Fig. 2b). The detailed results of multivariate logistic regression analyses are shown in Table S7-S16. Based on these results, the IgG N-glycan-based models exhibited outstanding performance in distinguishing patients with axSpA from healthy controls, SLE, RA, OA and gout, with the area under the curves (AUCs) of 0.869, 0.882, 0.889, 0.920 and 0.927, respectively (Figure S2). After conducting a five-fold cross-validation, the average AUCs of all the glycan-based models were higher than 0.80 (Table S17). These results indicate

Table 1 Demographic and clinical characteristics of the study participants

Variables	axSpA (*n* = 138)	SLE (*n* = 102)	RA (*n* = 106)	OA (*n* = 33)	Gout (*n* = 41)	HC (*n* = 117)	*P*
Male/Female (*n*, %)	100 (72.46) / 38 (27.54)	10 (9.80) / 92 (91.20) [a, b]	17 (16.04) / 89 (83.96) [a, b]	5 (15.16) / 28 (84.84) [a, b]	40 (97.56) / 1 (2.44) [a, b]	70 (59.83) / 47 (40.17)	< 0.001
Age (years)	27.00 [22.00, 33.00] [a]	37.00 [27.00, 47.25] [b]	52.50 [46.00, 59.25] [a, b]	62.00 [54.50, 72.50] [a, b]	40.00 [32.00, 49.00] [b]	34.00 [28.50, 42.00]	< 0.001
Disease duration (years)	4.00 [1.50, 8.00]	6.00 [1.00, 11.25]	7.00 [2.00, 11.25] [b]	10.00 [3.00, 16.00] [b]	6.00 [3.00, 10.00]	-	0.006
Age at disease onset (years)	22.00 [17.00, 27.75]	28.00 [21.00, 40.00] [b]	45.00 [34.00, 51.00] [b]	51.50 [46.50, 59.50] [b]	29.50 [25.75, 38.50] [b]	-	< 0.001
IgG (g/L)	13.65 [11.60, 15.28]	15.25 [11.53, 18.60] [b]	13.20 [11.10, 17.20]	12.80 [11.30, 15.00]	10.96 [9.05, 12.80] [b]	-	< 0.001
CRP (mg/L)	9.78 [2.80, 26.28]	5.44 [2.48, 12.80] [b]	12.95 [4.91, 43.85] [b]	5.72 [3.11, 17.30]	17.40 [7.39, 82.40] [b]	-	< 0.001
ESR (mm/h)	15.00 [7.00, 42.75]	31.00 [14.25, 50.00] [b]	35.00 [22.00, 70.00] [b]	26.50 [19.00, 52.00] [b]	37.00 [20.00, 63.00] [b]	-	< 0.001
HLA-B27 positive (*n*, %)	116 (84.06)	-	-	-	-	-	NA
ASDAS-CRP	2.30 [1.77, 3.25]	-	-	-	-	-	NA
SPARCC-SIJ	13.00 [4.00, 15.00]	-	-	-	-	-	NA
SPARCC-spine	25.00 [10.00, 29.00]	-	-	-	-	-	NA
IL-6 (pg/mL)	181.57 [102.60, 267.24] [a]	190.20 [125.35, 256.85] [a]	180.89 [113.44, 252.57] [a]	186.00 [117.20, 255.32] [a]	178.36 [116.09, 243.77] [a]	34.84 [29.34, 39.32]	< 0.001
IL-17 (pg/mL)	463.28 [187.94, 708.87] [a]	489.70 [263.60, 755.76] [a]	516.50 [242.08, 751.46] [a]	448.06 [184.50, 637.42] [a]	411.79 [124.86, 730.11] [a]	214.04 [198.11, 231.52]	< 0.001
IL-21 (pg/mL)	32.92 [21.17, 43.77] [a]	35.82 [28.35, 45.03] [a]	35.52 [25.01, 45.47] [a]	38.29 [21.41, 47.33] [a]	35.37 [23.82, 42.06] [a]	14.06 [13.68, 14.69]	< 0.001
IL-22 (pg/mL)	377.93 [214.47, 484.51] [a]	357.08 [264.11, 447.99] [a]	355.20 [203.89, 503.99] [a]	337.42 [189.86, 501.29] [a]	372.25 [263.48, 533.85] [a]	119.63 [105.36, 126.01]	< 0.001
IL-23 (pg/mL)	591.95 [357.64, 834.85] [a]	619.84 [462.83, 805.72] [a]	636.49 [409.08, 839.70] [a]	630.26 [377.25, 834.85] [a]	670.49 [435.97, 922.22] [a]	351.48 [317.74, 390.02]	< 0.001
TNFα (pg/mL)	64.94 [50.73, 78.49] [a]	62.17 [51.36, 77.35] [a]	59.41 [44.29, 76.33] [a]	63.11 [41.85, 84.89] [a]	57.87 [38.96, 80.98] [a]	31.78 [29.60, 33.66]	< 0.001

[a] Statistically significant compared to healthy controls

[b] Statistically significant compared to axSpA

Data are shown as medians [P_{25}, P_{75}]. $P < 0.05$ was considered statistically significant. ASDAS, ankylosing spondylitis disease activity score; axSpA, axial spondyloarthritis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HC, healthy controls; HLA-B27, human leucocyte antigen B27; IgG, immunoglobulin G; IL, interleukin; NA, not applicable; OA, osteoarthritis; P_{25}, the 25th percentile; P_{75}, the 75th percentile; RA, rheumatoid arthritis; SIJ, sacroiliac joint; SLE, systemic lupus erythematosus; SPARCC, spondyloarthritis research consortium of Canada; TNFα, tumor necrosis factor α

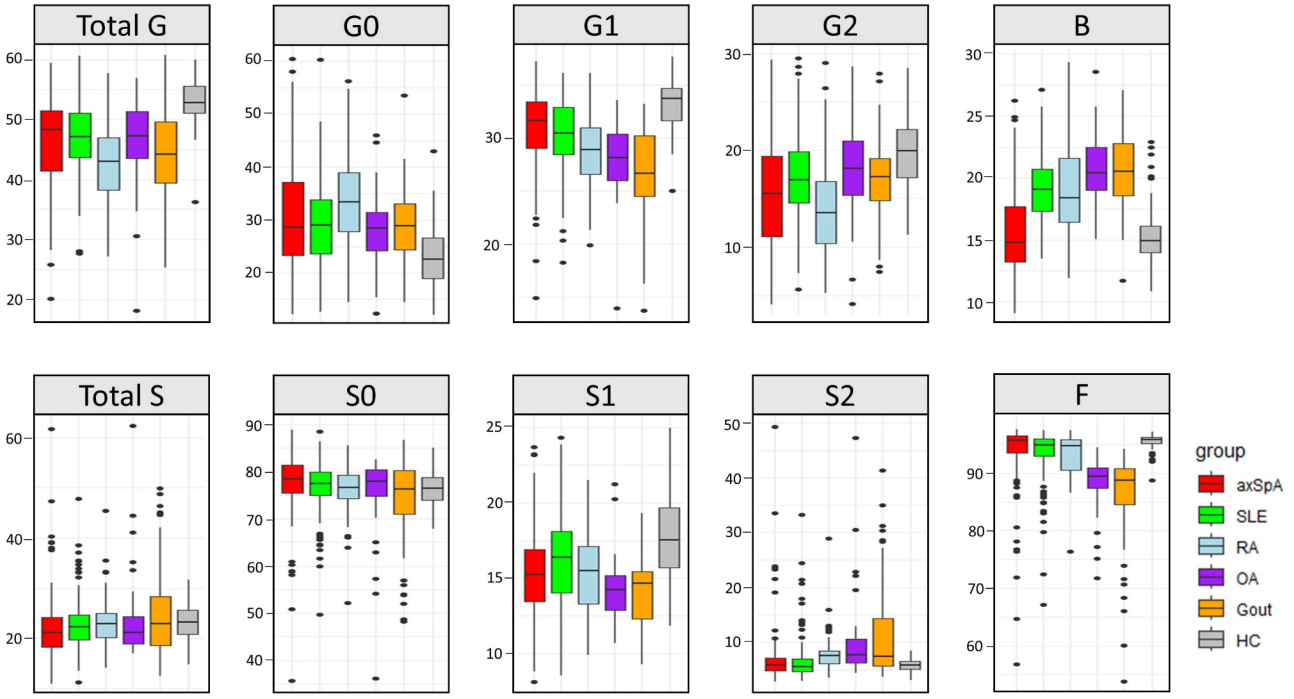

Fig. 1 Comparison of the abundances of derived glycan traits between patients with axSpA, SLE, RA, OA, or gout, and healthy controls. Each box represents the 25th to 75th percentiles (interquartile range). The lines inside the boxes represent the medians. axSpA, axial spondyloarthritis; B, bisecting GlcNAc; F, fucosylation; G, glycosylation; G0/G1/G2, a-/mono-/di-galactosylation; HC, healthy controls; OA, osteoarthritis; RA, rheumatoid arthritis; S, sialylation; S0/S1/S2, a-/mono-/di-sialylation; SLE, systemic lupus erythematosus

that the classification ability of the IgG N-glycan-based models is relatively reliable.

Furthermore, the shared and unique characteristics of IgG N-glycans between the different health conditions based on the association analysis are presented in Venn diagrams (Fig. 2c and d). Only one IgG N-glycan (FA2 [3] G1) was found to be associated with all rheumatic disease groups, indicating that decreased FA2 [3]G1 was a shared IgG N-glycan trait among all the rheumatic diseases (Fig. 2c). Additionally, decreased FA2BG2S2 was found as a unique glycan in patients with axSpA compared to those with other four rheumatic diseases (Fig. 2d).

The relationship between IgG N-glycans and disease activity in axSpA

Firstly, 10 derived IgG N-glycans traits were utilized to analyze the correlation with disease activity in axSpA (Figure S3). The results demonstrated that ASDAS-CRP and SPARCC-spine were negatively correlated with total galactosyaltion and total sialylation, while these were positively correlated with IgG G0 and asialylation (S0). Notably, SPARCC-SIJ exhibited negatively correlation with IgG digalactosyaltion (G2) and disialylation (S2), and positive correlation with IgG S0.

Additionally, to determine which directly measured IgG N-glycans were associated with disease activity in axSpA, univariate correlation analysis (Figure S4) and multivariate linear regression analysis were conducted. The results revealed that FA2 was positively associated with ASDAS-CRP (B = 0.32), SPARCC-SIJ (B = 0.24) and SPARCC-spine (B = 0.23) (Tables S18-S20).

Moreover, to examine the association between IgG N-glycans and disease activity status in axSpA, 138 patients with axSpA were divided into an HDA group ($n = 81$) and a LIDA group ($n = 57$). A comparison of IgG N-glycan profiles between HDA and LIDA groups is shown in Table S21. Adjusting for gender, age, IgG, disease duration, age at disease onset and HLA-B27, multivariate logistic regression demonstrated that an increased abundance of FA2 was significantly associated with HDA in axSpA patients (AOR = 1.10, 95% CI: 1.02–1.18) (Table S22). Moreover, FA2 (AUC = 0.734, 95% CI: 0.652–0.805) exhibited relatively high performance in distinguishing patients with high disease activity from low and inactive disease activity compared to CRP (AUC = 0.727, 95% CI: 0.644–0.799) and ESR (AUC = 0.696, 95% CI: 0.615–0.774) (Fig. 3a).

Furthermore, compared to CRP and ESR, FA2 exhibited a stronger correlation with ASDAS-CRP ($r = 0.47$, $P < 0.01$), SPARCC-spine ($r = 0.52$, $P < 0.01$) and SPARCC-SIJ ($r = 0.26$, $P < 0.01$) (Fig. 3b and d). After adjusting for CRP and ESR, partial correlation analysis showed that FA2 was positively correlated with ASDAS-CRP ($r = 0.44$,

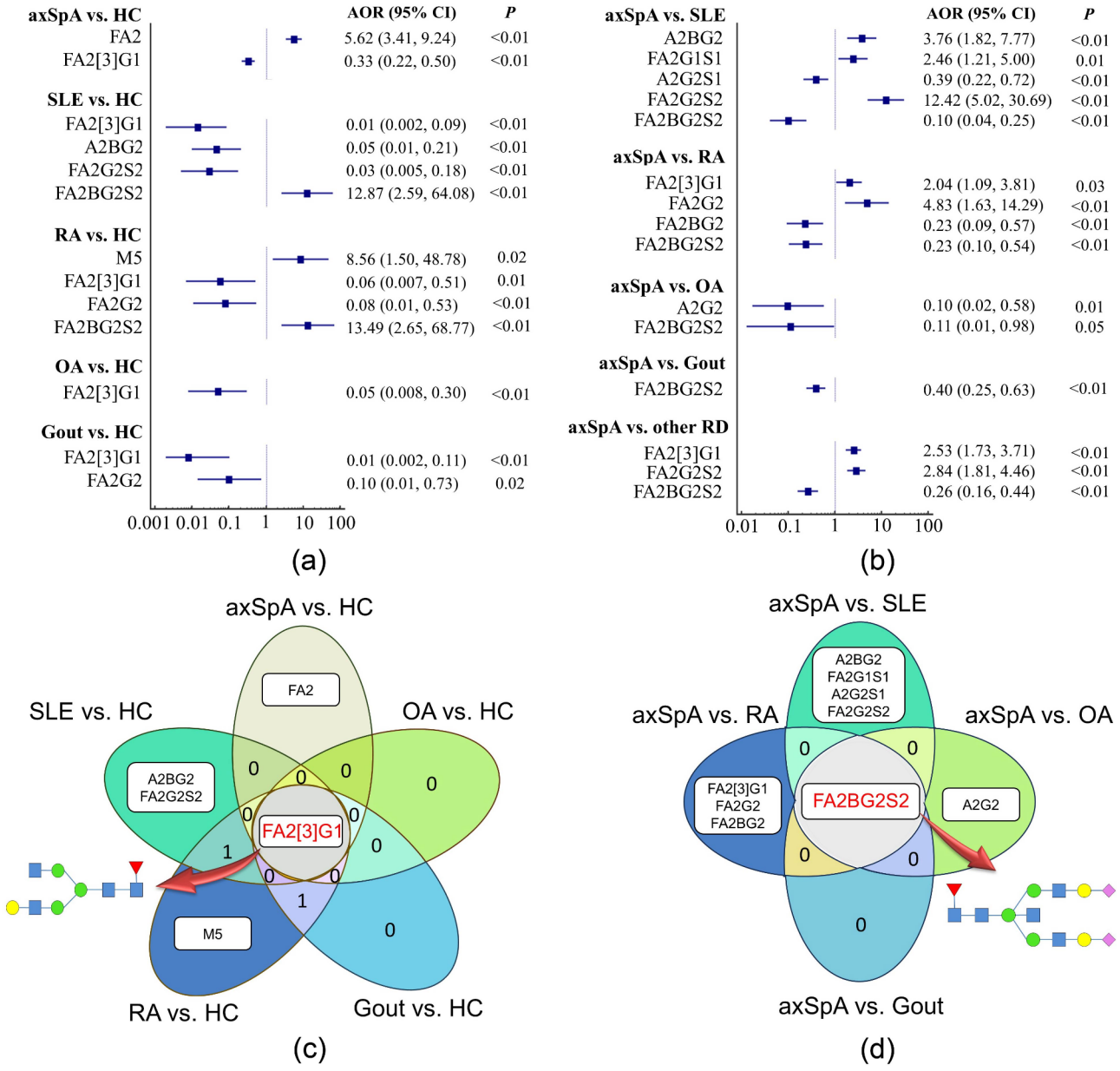

Fig. 2 The associations between IgG N-glycans and axSpA and other rheumatic diseases. Forest plot displaying the AOR and 95% CI for the associations of IgG N-glycans with axSpA, SLE, RA, OA, or gout versus HC adjusted for gender and age, respectively (**a**); with axSpA versus SLE, RA, OA, gout or other rheumatic diseases (including SLE, RA, OA and gout) adjusted for gender and age, respectively (**b**). Venn diagram showing the numbers of shared and unique IgG N-glycans in the five pairwise comparison groups (**c**) and four pairwise comparison groups (d) based on the result of logistic regression analysis. Glycan structure: A1/A2, mono-/di-antennary; B, bisecting GlcNAc; F, fucosylation; G1/G2, mono-/di-galactosylation; M5, five mannose residues; S1/S2, mono-/di-sialylation; [3]G1, galactose on the antenna of the *α*1–3 linked mannose; [6]G1, galactose on the antenna of the *α*1–6 linked mannose. AOR, adjusted odds ratio; axSpA, axial spondyloarthritis; CI, confidence interval; HC, healthy controls; OA, osteoarthritis; RA, rheumatoid arthritis; RD, rheumatic disease; SLE, systemic lupus erythematosus

$P < 0.01$), SPARCC-spine ($r = 0.45$, $P < 0.01$) and SPARCC-SIJ ($r = 0.15$, $P = 0.08$).

The correlation between IgG N-glycans and inflammatory factors in axSpA

Spearman's rank correlation was conducted to evaluate the correlation between each of the 24 directly measured IgG N-glycans and each of 8 inflammatory factors (CRP, ESR, IL-6, IL-17, IL-21, IL-22, IL-23 and TNF*α*) in axSpA (Figure S5). Following this analysis, the IgG N-glycans significantly correlated with inflammatory factors were screened for canonical correlation analysis (CCA). The CCA results demonstrated that IgG N-glycans were significantly correlated with inflammatory factors in

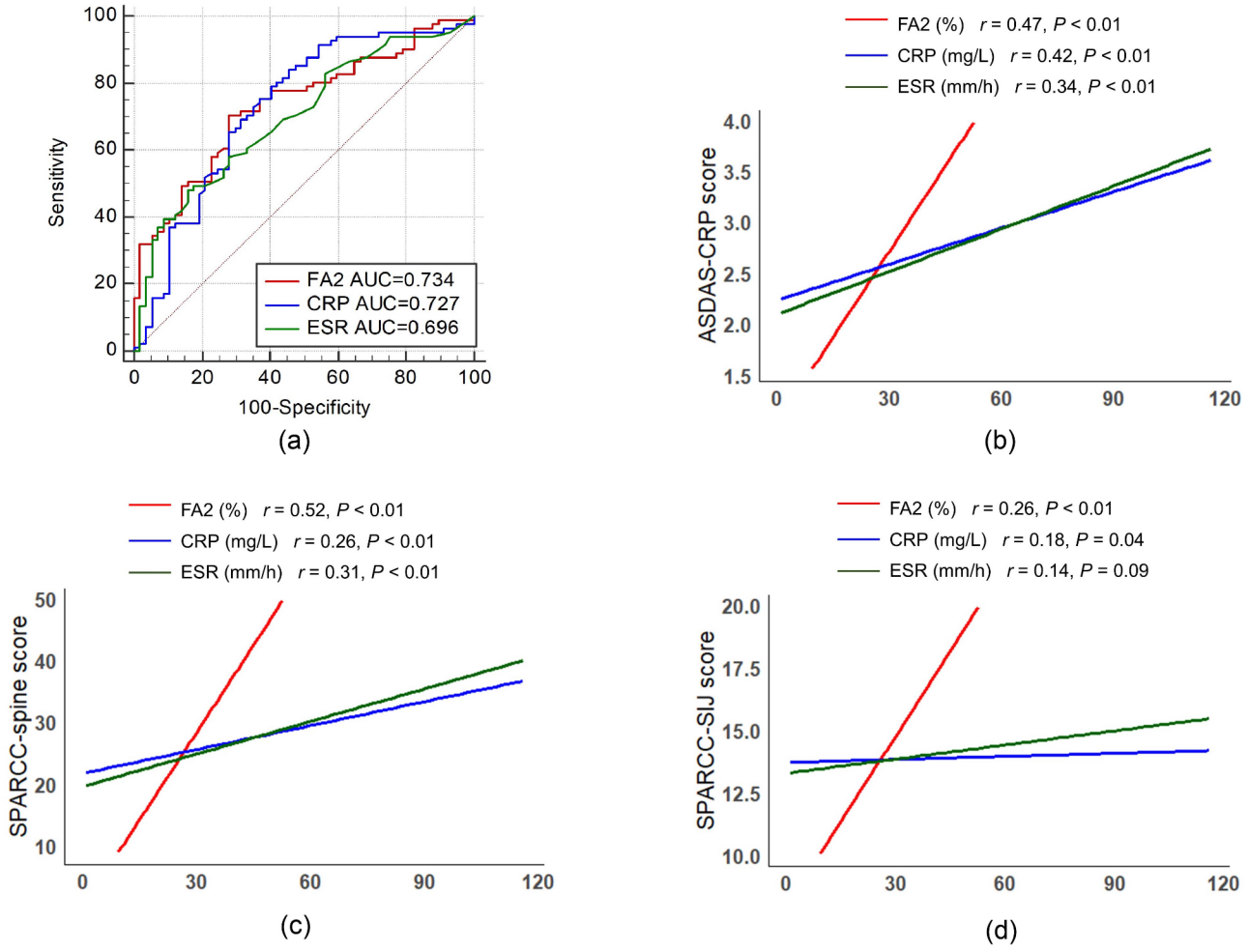

Fig. 3 Comparison of FA2 with CRP and ESR in relation to disease activity in axSpA. (**a**) The discriminative performance of FA2 and CRP, ESR between HDA and LIDA groups in axSpA. The correlation between FA2, CRP, ESR and ASDAS-CRP (**b**), SPARCC-spine (**c**), and SPARCC-SIJ (**d**) in axSpA. ASDAS, ankylosing spondylitis disease activity score; axSpA, axial spondyloarthritis; AUC, area under the ROC curve; A2, di-antennary; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; F, fucosylation; LIDA, low and inactive disease activity; HDA, high disease activity; ROC, receiver operating characteristic; SIJ, sacroiliac joint; SPARCC, spondyloarthritis research consortium of Canada

the first canonical set, with a canonical correlation of 0.519 ($F=1.479$, $P=0.017$). Nine IgG N-glycans (especially FA2BG2S2, FA2 and FA2 [3]G1) exhibited significant correlation with three inflammatory factors (IL-23, TNFα and ESR) in patients with axSpA, due to the absolute value of canonical loading greater than 0.30 (Fig. 4). These findings indicate that there is a notable association between IgG N-glycan patterns and inflammatory factors related to axSpA.

Discussion

This study comprehensively analyzed the association of IgG N-glycan profiles with axSpA, SLE, RA, OA, and gout patients. In our study, decreased FA2 [3]G1 was found as a shared IgG N-glycan trait among all five types of rheumatic diseases, whereby decreased FA2BG2S2 was a unique marker in patients with axSpA compared to those with other four rheumatic diseases. Additionally, FA2 demonstrated a robust discriminatory capacity in distinguishing axSpA patients with varying disease activity status. Furthermore, IgG N-glycans (especially FA2BG2S2, FA2 and FA2 [3]G1) exhibited strong canonical correlations with inflammatory factors (IL-23, TNFα and ESR) in patients with axSpA.

Since the initial discovery of decreased IgG galactosylation in RA, similar alterations in IgG glycosylation have been reported in various inflammatory and autoimmune conditions, such as IBD, OA, RA and SLE [19–21]. Reduced IgG galactosylation has been widely recognized as a hallmark of diseases associated with inflammatory activity [38]. This is consistent with our current study, i.e., a decrease in IgG galactosylation was observed in patients with axSpA, SLE, RA, OA, or gout. IgG Fc glycans that lack terminal galactose residues are thought to promote inflammation by activating the complement system through the lectin pathway, facilitated by binding to

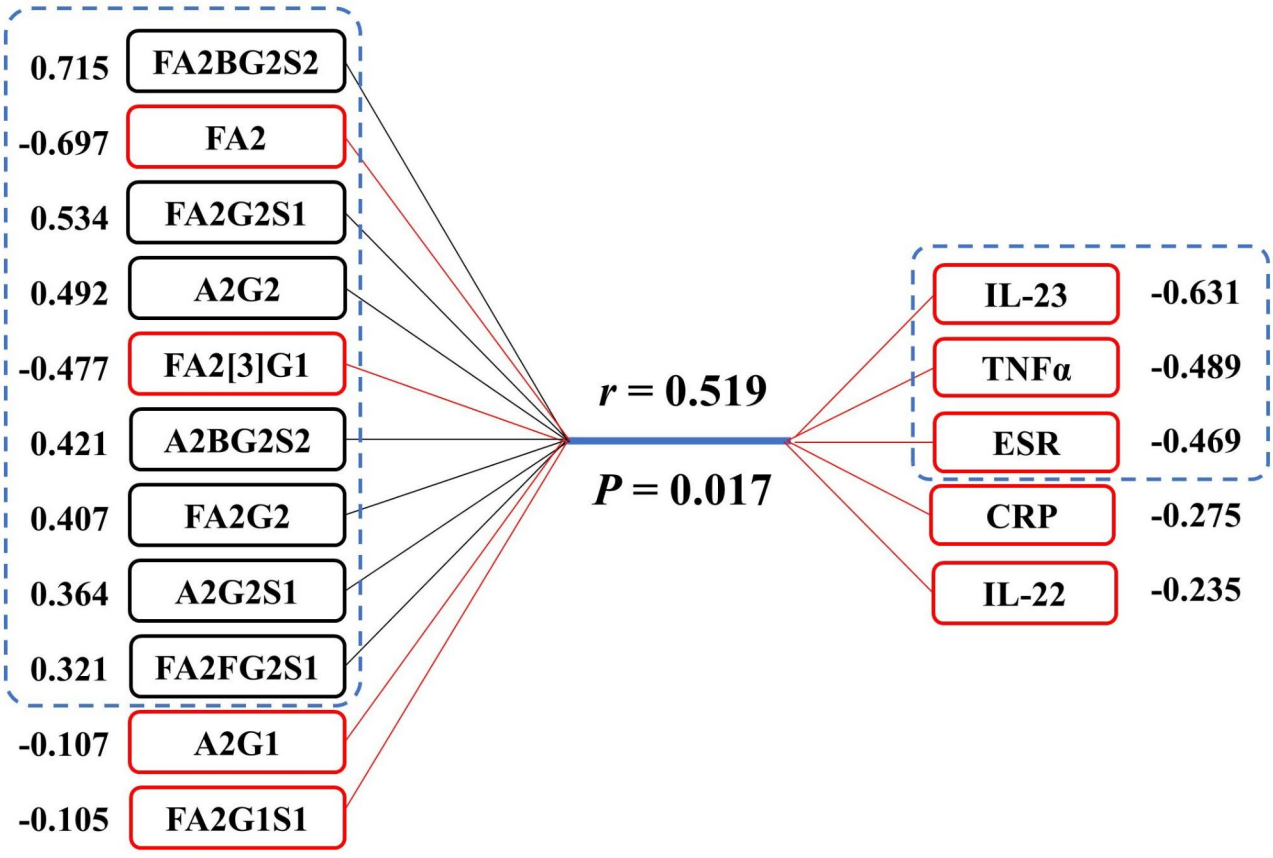

Fig. 4 Canonical structures of IgG N-glycans and inflammatory factors in the first canonical set of canonical correlation analysis in axSpA. The positive relationships are represented in black boxes, while negative relationships are shown in red boxes. The absolute value of canonical loading greater than 0.30 was used to define significant loading. Glycan structure: A2, di-antennary; B, bisecting GlcNAc; F, fucosylation; G0/G1/G2, a-/mono-/di-galactosylation; S0/S1/S2, a-/mono-/di-sialylation; [3]G1, galactose on the antenna of the *α*1–3 linked mannose. axSpA, axial spondyloarthritis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL, interleukin; TNF*α*, tumor necrosis factor *α*

mannose-binding lectin (MBL) [18]. More specifically, we found that a mono-galactosylated IgG N-glycan (FA2 [3]G1) was associated with the occurrence of all five types of rheumatic diseases investigated. This finding aligns with previous studies, showing that FA2 [3]G1 not only demonstrated a significant decrease in patients with SLE and hyperuricemia but also exhibited outstanding performance in diagnosing these conditions [21, 39, 40]. Taken together, this finding suggests that a low FA2 [3]G1 signature at diagnosis may be associated with rheumatic diseases and could potentially aid in the diagnostic workup.

In the current study, the abundances of IgG fucosylation were decreased in patients with axSpA, SLE, RA, OA and gout when compared to that of healthy controls. Whilst this decrease of IgG fucosylation did not reach statistical significance in patients with axSpA, this trend is consistent with previous studies on related autoimmune diseases, such as SLE and ulcerative colitis [19–21]. When compared to patients with axSpA, IgG fucosylation was found to be significantly decreased in patients with SLE and RA. Recent studies have revealed that fucosylation significantly influences the functional activity and structure of IgG [41, 42]. The absence of fucose residues can increase fragment crystallizable receptor (Fc*γ*RIIIa) affinity, thereby dramatically enhancing ADCC against self-antigens [23]. This process leads to the destruction of tissues, contributing to the development of autoimmune diseases, such as SLE and RA. Additionally, a previous study has demonstrated that the reduction of IgG fucosylation can enhance pro-inflammation by activating ADCC [41]. We observed a significant decrease in the abundance of IgG fucosylation in patients with gout compared to those with axSpA. This finding aligns with the observation that patients with gout exhibit more severe inflammation, as evidenced by notably higher CRP levels compared to patients with axSpA. Notably, a decrease in FA2BG2S2, a fucosylated IgG N-glycan, was uniquely observed in patients with axSpA compared to those with other rheumatic diseases. However, the heterogeneity of rheumatic diseases often necessitates additional diagnostic markers specific to each disease. For example, SLE diagnosis commonly relies on anti-nuclear

antibody (ANA) and anti-dsDNA, RA is associated with rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibody (anti-CCP), OA is diagnosed through imaging or synovial fluid analysis, and gout diagnosis typically involves serum uric acid measurement. Taken together, these findings suggest that the IgG N-glycans could serve as biomarkers to differentiate axSpA from other rheumatic diseases, potentially reducing the reliance on disease-specific markers and diagnostic procedures. This could, in turn, alleviate the diagnostic burden and associated costs for patients.

Similar to the roles of IgG galactosylation and fucosylation, IgG sialylation also plays a crucial role in the regulation of inflammatory responses, with its reduction being linked to the pathogenesis of various inflammatory diseases [21, 43]. In the current study, a reduction of IgG sialylation was observed in patients with axSpA, SLE, RA, OA, or gout. Although the decrease in IgG sialylation did not reach statistical significance in patients with SLE, RA, OA, or gout, this trend is consistent with the previous data [21, 41]. The absence of terminal sialic acid on the IgG N-glycan is known to exert a pro-inflammatory effect via modulating the FcγRIIIa affinity and activating ADCC [41]. Conversely, increased sialylation of IgG promotes its binding affinity to FcγRIIa, mediating antibody-dependent cellular phagocytosis (ADCP) and ultimately attenuating inflammatory response [44]. Additionally, our findings also show that IL-23 levels were negatively correlated with sialylated IgG N-glycan (i.e., FA2BG2S2) and positively correlated with asialylated IgG N-glycans (i.e., FA2 and FA2 [3]G1) in patients with axSpA. Previous studies have identified that IL-23/Th17 signaling pathway, which plays a pivotal role in the development of axSpA [15], has been implicated in the modulation of IgG sialylation [27]. Specifically, IL-23 stimulates Th17 cells to secrete inflammatory cytokines such as IL-21 and IL-22, which downregulate the expression of the glycosyltransferase (St6Gal1) on B cells, thereby reducing the abundance of IgG sialylation in mice with arthritis [27]. Furthermore, our data demonstrate a negative correlation between IgG sialylation and inflammation in the SIJ and spine. Prior research has demonstrated that IgG asialylation promotes osteoclastogenesis and bone loss [45], whereas increased sialylation alleviates inflammation and reduces osteoclast activity, offering protection against collagen-induced arthritis in mice [46]. In patients with RA, reduced IgG sialylation is significantly correlated with a decrease in bone volume and trabecular number [45]. Given the inflammation characteristic of axSpA in the SIJ and spine, structural changes such as bone erosion and bone loss may result from osteoclast-mediated bone resorption [16]. In addition, we also identified a negative correlation between TNFα and IgG sialylation (i.e., FA2BG2S2) in axSpA patients. TNF has been shown to inhibit St6Gal1 activity, reducing sialylation in synovial fibroblasts and potentially enhancing pro-inflammatory effects in RA [47]. Although there are no cellular or animal studies directly examining the effects of TNFα on IgG glycosylation, based on the results mentioned above, it is reasonable to hypothesize that TNFα may similarly affect St6Gal1 activity in B cells, leading to a reduction in IgG sialylation. Taken together, these findings suggest that the IL-23/Th17 axis and TNFα may contribute to the pro-inflammatory state in axSpA by modulating IgG sialylation, thereby contributing to osteoclastogenesis and subsequent bone erosion.

Notably, alterations in IgG N-glycosylation may parallel the disease activity of axSpA. In this study, both IgG galactosylation and sialylation were found to be negatively correlated with disease activity (i.e., ASDAS-CRP and SPARCC-spine) in axSpA, which aligns with previous findings in RA, SLE and IBD [20, 27, 48]. These correlations are supported by evidence from mouse models of autoimmune diseases, where increased IgG galactosylation and sialylation enhance binding affinity to FcγRIIb, thereby reducing inflammation and disease activity [49, 50]. Additionally, a notable finding in our study was that a specific glycan (FA2) showed not only a stronger correlation with disease activity indicators (ASDSA-CRP, SPARCC-spine and SPARCC-SIJ) than CRP, but also exhibited high performance in the ability to distinguish axSpA patients with varying disease activity status. Furthermore, among patients with high disease activity (26.13%), the abundance of FA2 was significantly elevated compared to those with low disease activity (20.97%) and healthy controls (18.29%) (Tables S4 and S21). These results suggest that the abundance of FA2 may dramatically increase in high disease activity status and decrease in low disease activity status of axSpA. While MRI is an effective way to evaluate disease activity in the SIJ and spine, its widespread clinical use is limited by high cost [12]. Moreover, although CRP is a classical inflammatory marker, it exhibited only a weak correlation with spine inflammation on MRI and no correlation with SIJ inflammation [51]. Thus, our findings suggest that FA2 may serve as an additional indicator of disease activity and a dynamic biomarker involved in the pathogenesis of axSpA.

Our study contributes to understanding the role of IgG N-glycosylation in the diagnosis and disease activity assessment of patients with axSpA. Nevertheless, the interpretation of the study findings should consider the study's limitations. Firstly, the current research is a case-control study; therefore, the causal relationship between IgG N-glycosylation and axSpA, as well as other rheumatic diseases, cannot be established. Thus, the underlying mechanisms linking IgG N-glycosylation and axSpA warrant further investigation. Secondly, although we

used LASSO, OPLS-DA, and logistic regression analysis to minimize overfitting in our IgG N-glycan-based diagnostic model, its validity is still limited by the small sample size. Future studies should include larger sample sizes and expand the investigation of IgG N-glycan profiles in other rheumatic diseases, such as Sjögren syndrome and fibromyalgia (FM), which are key differential diagnoses for axSpA, particularly in cases with enthesopathic involvement. This will help determine whether decreased FA2BG2S2 is a unique marker for axSpA, aiding physicians in differentiating it from FM and Sjögren syndrome without specific autoantibodies. Additionally, including patients with mechanical back pain will further assess the specificity of glycan profiles, further validating their clinical utility in distinguishing between inflammatory and non-inflammatory back pain.

Conclusions

This study has demonstrated that decreases in IgG galactosylation and sialylation are associated with axSpA. More specifically, it has identified that decreased FA2[3]G1 is a shared IgG N-glycan trait among all five types of rheumatic diseases investigated, while decreased FA2BG2S2 is a unique IgG N-glycan that differentiates axSpA from the other four rheumatic diseases. Furthermore, FA2 demonstrated a robust discriminatory capacity in distinguishing axSpA patients with varying disease activity statuses. These findings suggest that individual variations in IgG N-glycosylation might contribute to axSpA via their effects on pro-inflammatory pathways, potentially modulated by the IL-23/Th17 axis and TNFα. The specific IgG N-glycans offer possibilities for developing new diagnostic markers to improve the diagnosis and disease activity assessment for axSpA management.

Abbreviations

2AB	2-aminobenzamide
ACR	American College of Rheumatology
ADCC	Antibody-dependent cell cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
ANA	Anti-nuclear antibody
AOR	Adjusted odds ratio
ASAS	Assessment of SpondyloArthritis International Society
ASDAS	Ankylosing spondylitis disease activity score
AUC	Area under the curve
axSpA	Axial spondyloarthritis
B	Bisecting GlcNAc
CCA	Canonical correlation analysis
CCP	Cyclic citrullinated peptide
CDC	Complement-dependent cytotoxicity
CI	Confidence interval
COCAS	Clinical characteristics and outcomes in the Chinese axial spondyloarthritis
CRP	C-reactive protein
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
F	Fucosylation
FcγRIIa	Fragment crystallizable γ receptor IIa
FcγRIIb	Fragment crystallizable γ receptor IIb
FcγRIIIa	Fragment crystallizable γ receptor IIIa
FDR	False discovery rate
FM	Fibromyalgia
G0	Agalactosylation
G1	Monogalactosylation
G2	Digalactosylation
GlcNAc	Acetylglucosamine
HC	Healthy controls
HDA	High disease activity
IBD	Inflammatory bowel disease
IgG	Immunoglobulin G
IL-17	Interleukin-17
IL-21	Interleukin-21
IL-22	Interleukin-22
IL-23	Interleukin-23
IL-6	Interleukin-6
LASSO	Least absolute shrinkage and selection operator
LIDA	Low and inactive disease activity
MRI	Magnetic resonance imaging
OA	Osteoarthritis
OPLS-DA	Orthogonal partial least squares discriminant analysis
PNGase F	Peptide-N-glycosidase F
RA	Rheumatoid arthritis
RF	Rheumatoid factor
ROC	Receiver operating characteristic
S0	Asialylation
S1	Monosialylation
S2	Disialylation
SIJ	Sacroiliac joint
SLE	Systemic lupus erythematosus
SPARCC	Spondyloarthritis Research Consortium of Canada
St3Gal4	α-2,3sialyltransferase 4
St6Gal1	βgalactoside α-2,6sialyltransferase 1
Th17	T helper 17
TNFα	Tumor necrosis factor α
UPLC	Ultra-performance liquid chromatography

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-025-03505-y.

Supplementary Material 1

Acknowledgements

We would like to thank the patients who participated in the study. We also extend our gratitude to the physicians Danmin Wang, Jianhua Peng, Yao Gong, Hongjin Liang, Zhaopeng Chen, and Zhengyu Xiao for their insightful advice and expert guidance on the clinical aspects of our research.

Author contributions

LL, WW, MS and XX conceived and designed the study. XX, ZC, ZH and YH were involved in the acquisition of data. XX, ZC, LB, HH and CZ contributed to the analysis and interpretation of data. LL and XX are the guarantors and provided supervision throughout the study. XX wrote the manuscript, and all authors revised the manuscript and approved the final version.

Funding

This study was supported by grants from the Guangdong Basic and Applied Basic Research Foundation (2024A1515012910), the Special Funds for science and technology of Guangdong Province ([2021]-88), the Science and Technology Special Fund Project of Guangdong Province (200113095894089), and the Science and Technology Planning Projects of Shantou City ([2022]-88, [2024]-124). MS is supported by the Western Australian Future Health Research and Innovation Fund (WANMA/Ideas2023-24/10). The funding sources had no involvement in the study design, collection, analysis and interpretation of data, writing of the manuscript, and the decision to submit the manuscript for publication.

Data availability
Data are available on reasonable request. All data relevant to the study are presented in the article or uploaded as supplementary information.

Declarations

Ethics approval and consent to participate
This study involves human participants and was approved by the Ethics Committee of the First Affiliated Hospital of Shantou University Medical College (No. B-2021-134) and the Research Ethics Committee at Edith Cowan University (No. 2023-04559-XU). All participants provided written informed consent.

Consent for publication
All authors agreed to the publication of this study.

Conflict of interest
The authors declare that they have no conflicts of interest.

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Received: 11 November 2024 / Accepted: 11 February 2025
Published online: 22 February 2025

References

1. Vanhoof J, Declerck K, Geusens P. Prevalence of rheumatic diseases in a rheumatological outpatient practice. Ann Rheum Dis. 2002;61(5):453–5. https://doi.org/10.1136/ard.61.5.453
2. Branco JC, Rodrigues AM, Gouveia N, Eusebio M, Ramiro S, Machado PM, et al. Prevalence of rheumatic and musculoskeletal diseases and their impact on health-related quality of life, physical function and mental health in Portugal: results from EpiReumaPt- a national health survey. RMD Open. 2016;2(1):e000166. https://doi.org/10.1136/rmdopen-2015-000166
3. Generali E, Bose T, Selmi C, Voncken JW, Damoiseaux J. Nature versus nurture in the spectrum of rheumatic diseases: classification of spondyloarthritis as autoimmune or autoinflammatory. Autoimmun Rev. 2018;17(9):935–41. https://doi.org/10.1016/j.autrev.2018.04.002
4. McGonagle D, McDermott MF. A proposed classification of the immunological diseases. PLoS Med. 2006;3(8):e297. https://doi.org/10.1371/journal.pmed.0030297
5. Ramiro S, Nikiphorou E, Sepriano A, Ortolan A, Webers C, Baraliakos X, et al. ASAS-EULAR recommendations for the management of axial spondyloarthritis: 2022 update. Ann Rheum Dis. 2023;82(1):19–34. https://doi.org/10.1136/ard-2022-223296
6. Szekanecz Z, McInnes IB, Schett G, Szamosi S, Benko S, Szucs G. Autoinflammation and autoimmunity across rheumatic and musculoskeletal diseases. Nat Rev Rheumatol. 2021;17(10):585–95. https://doi.org/10.1038/s41584-021-00652-9
7. Taurog JD, Chhabra A, Colbert RA. Ankylosing spondylitis and Axial Spondyloarthritis. N Engl J Med. 2016;374(26):2563–74. https://doi.org/10.1056/NEJMra1406182
8. Lorenzin M, Ometto F, Ortolan A, Felicetti M, Favero M, Doria A, et al. An update on serum biomarkers to assess axial spondyloarthritis and to guide treatment decision. Ther Adv Musculoskelet Dis. 2020;12:1759720X20934277. https://doi.org/10.1177/1759720X20934277
9. Redeker I, Callhoff J, Hoffmann F, Haibel H, Sieper J, Zink A, et al. Determinants of diagnostic delay in axial spondyloarthritis: an analysis based on linked claims and patient-reported survey data. Rheumatology (Oxford). 2019;58(9):1634–8. https://doi.org/10.1093/rheumatology/kez090
10. Garrido-Cumbrera M, Delgado-Dominguez CJ, Galvez-Ruiz D, Mur CB, Navarro-Compan V, Atlas G, Working. The Effect of Axial Spondyloarthritis on Mental Health: results from the Atlas. J Rheumatol. 2019;46(10):1284–9. https://doi.org/10.3899/jrheum.180868
11. Tsang HHL, Chung HY. The discriminative values of the bath ankylosing Spondylitis Disease Activity Index, Ankylosing Spondylitis Disease activity score, C-Reactive protein, and Erythrocyte Sedimentation Rate in Spondyloarthritis-Related Axial Arthritis. J Clin Rheumatol. 2017;23(5):267–72. https://doi.org/10.1097/RHU.0000000000000522
12. Maksymowych WP. The role of imaging in the diagnosis and management of axial spondyloarthritis. Nat Rev Rheumatol. 2019;15(11):657–72. https://doi.org/10.1038/s41584-019-0309-4
13. Maksymowych WP, Inman RD, Salonen D, Dhillon SS, Williams M, Stone M, et al. Spondyloarthritis Research Consortium of Canada magnetic resonance imaging index for assessment of sacroiliac joint inflammation in ankylosing spondylitis. Arthritis Rheum. 2005;53(5):703–9. https://doi.org/10.1002/art.21445
14. Maksymowych WP, Inman RD, Salonen D, Dhillon SS, Krishnananthan R, Stone M, et al. Spondyloarthritis Research Consortium of Canada magnetic resonance imaging index for assessment of spinal inflammation in ankylosing spondylitis. Arthritis Rheum. 2005;53(4):502–9. https://doi.org/10.1002/art.21337
15. Tsukazaki H, Kaito T. The role of the IL-23/IL-17 pathway in the pathogenesis of Spondyloarthritis. Int J Mol Sci. 2020;21(17). https://doi.org/10.3390/ijms21176401
16. Nakamura A, Towheed T. Pathogenesis, assessment, and management of bone loss in axial spondyloarthritis. Semin Arthritis Rheum. 2024;64:152345. https://doi.org/10.1016/j.semarthrit.2023.152345
17. Wang W. Glycomedicine: the current state of the art. Engineering. 2023;26(7):12–5. https://doi.org/10.1016/j.eng.2022.03.009
18. Radovani B, Gudelj I. N-Glycosylation and inflammation; the Not-So-Sweet relation. Front Immunol. 2022;13:893365. https://doi.org/10.3389/fimmu.2022.893365
19. Sun DH, Hu FL, Gao HY, Song ZJ, Xie WC, Wang P, et al. Distribution of abnormal IgG glycosylation patterns from rheumatoid arthritis and osteoarthritis patients by MALDI-TOF-MSn. Analyst. 2019;144(6):2042–51. https://doi.org/10.1039/c8an02014k
20. Simurina M, de Haan N, Vuckovic F, Kennedy NA, Stambuk J, Falck D, et al. Glycosylation of Immunoglobulin G Associates with Clinical features of Inflammatory Bowel diseases. Gastroenterology. 2018;154(5):1320–e3310. https://doi.org/10.1053/j.gastro.2018.01.002
21. Vuckovic F, Kristic J, Gudelj I, Teruel M, Keser T, Pezer M, et al. Association of systemic lupus erythematosus with decreased immunosuppressive potential of the IgG glycome. Arthritis Rheumatol. 2015;67(11):2978–89. https://doi.org/10.1002/art.39273
22. Pincetic A, Bournazos S, DiLillo DJ, Maamary J, Wang TT, Dahan R, et al. Type I and type II fc receptors regulate innate and adaptive immunity. Nat Immunol. 2014;15(8):707–16. https://doi.org/10.1038/ni.2939
23. Russell A, Adua E, Ugrina I, Laws S, Wang W. Unravelling immunoglobulin G fc N-Glycosylation: a dynamic marker potentiating predictive, preventive and Personalised Medicine. Int J Mol Sci. 2018;19(2):390. https://doi.org/10.3390/ijms19020390
24. Pan H, Wu Z, Zhang H, Zhang J, Liu Y, Li Z, et al. Identification and validation of IgG N-glycosylation biomarkers of esophageal carcinoma. Front Immunol. 2023;14:981861. https://doi.org/10.3389/fimmu.2023.981861
25. Seeling M, Bruckner C, Nimmerjahn F. Differential antibody glycosylation in autoimmunity: sweet biomarker or modulator of disease activity? Nat Rev Rheumatol. 2017;13(10):621–30. https://doi.org/10.1038/nrrheum.2017.146
26. Cao Y, Song Z, Guo Z, Zhao X, Gong Y, Zhao K, et al. Cytokines in the Immune Microenvironment change the glycosylation of IgG by regulating Intracellular glycosyltransferases. Front Immunol. 2021;12:724379. https://doi.org/10.3389/fimmu.2021.724379
27. Pfeifle R, Rothe T, Ipseiz N, Scherer HU, Culemann S, Harre U, et al. Regulation of autoantibody activity by the IL-23-TH17 axis determines the onset of autoimmune disease. Nat Immunol. 2017;18(1):104–13. https://doi.org/10.1038/ni.3579

28. Rudwaleit M, van der Heijde D, Landewe R, Listing J, Akkoc N, Brandt J, et al. The development of Assessment of SpondyloArthritis international society classification criteria for axial spondyloarthritis (part II): validation and final selection. Ann Rheum Dis. 2009;68(6):777–83. https://doi.org/10.1136/ard.2009.108233
29. Huang SX, Li HG, Liang HJ, Wang DM, Peng JH, Shen FC, et al. Comparison of clinical characteristics between adult-onset and juvenile-onset non-radiographic axial spondyloarthritis in Chinese patients: results from the COCAS cohort. Eur J Med Res. 2023;28(1):388. https://doi.org/10.1186/s40001-023-01387-x
30. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, et al. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for systemic Lupus Erythematosus. Arthritis Rheumatol. 2019;71(9):1400–12. https://doi.org/10.1002/art.40930
31. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569–81. https://doi.org/10.1002/art.27584. 3rd.
32. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum. 1986;29(8):1039–49. https://doi.org/10.1002/art.1780290816
33. Neogi T, Jansen TL, Dalbeth N, Fransen J, Schumacher HR, Berendsen D et al. 2015 *Gout classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative.* Ann Rheum Dis. 2015;74(10):1789-98. https://doi.org/10.1136/annrheumdis-2015-208237
34. Liu. X, Xu Y, Li J, Zhang X, Zhang Q, Li, et al. Immunoglobulin G N-Glycan analysis by Ultra-performance Liquid Chromatography. J Vis Exp. 2020;155https://doi.org/10.3791/60104
35. Lu JP, Knezevic A, Wang YX, Rudan I, Campbell H, Zou ZK, et al. Screening novel biomarkers for metabolic syndrome by profiling human plasma N-glycans in Chinese Han and Croatian populations. J Proteome Res. 2011;10(11):4959–69. https://doi.org/10.1021/pr2004067
36. Radovani B, Vuckovic F, Maggioni AP, Ferrannini E, Lauc G, Gudelj I. IgG N-Glycosylation is altered in coronary artery disease. Biomolecules. 2023;13(2):375. https://doi.org/10.3390/biom13020375
37. Liu D, Zhao Z, Wang A, Ge S, Wang H, Zhang X, et al. Ischemic stroke is associated with the pro-inflammatory potential of N-glycosylated immunoglobulin G. J Neuroinflammation. 2018;15(1):123. https://doi.org/10.1186/s12974-018-1161-1
38. Gudelj I, Lauc G, Pezer M. Immunoglobulin G glycosylation in aging and diseases. Cell Immunol. 2018;333:65–79. https://doi.org/10.1016/j.cellimm.2018.07.009
39. Hou H, Xu X, Sun F, Zhang X, Dong H, Wang L, et al. Hyperuricemia is Associated with Immunoglobulin G N-Glycosylation: A Community-based study of glycan biomarkers. Omics. 2019;23(12):660–7. https://doi.org/10.1089/omi.2019.0004
40. Pan H, Wang J, Liang Y, Wang C, Tian R, Ye H, et al. Serum IgG Glycan Hallm Systemic Lupus Erythematosus Eng. 2023;26:89–98. https://doi.org/10.1016/j.eng.2023.01.006
41. Zhou X, Motta F, Selmi C, Ridgway WM, Gershwin ME, Zhang W. Antibody glycosylation in autoimmune diseases. Autoimmun Rev. 2021;20(5):102804. https://doi.org/10.1016/j.autrev.2021.102804
42. Wada R, Matsui M, Kawasaki N. Influence of N-glycosylation on effector functions and thermal stability of glycoengineered IgG1 monoclonal antibody with homogeneous glycoforms. MAbs. 2019;11(2):350–72. https://doi.org/10.1080/19420862.2018.1551044
43. Kissel T, Toes REM, Huizinga TWJ, Wuhrer M. Glycobiology of rheumatic diseases. Nat Rev Rheumatol. 2023;19(1):28–43. https://doi.org/10.1038/s41584-022-00867-4
44. Kuhns S, Shu J, Xiang C, Guzman R, Zhang Q, Bretzlaff W, et al. Differential influence on antibody dependent cellular phagocytosis by different glycoforms on therapeutic monoclonal antibodies. J Biotechnol. 2020;317:5–15. https://doi.org/10.1016/j.jbiotec.2020.04.017
45. Harre U, Lang SC, Pfeifle R, Rombouts Y, Fruhbeisser S, Amara K, et al. Glycosylation of immunoglobulin G determines osteoclast differentiation and bone loss. Nat Commun. 2015;6:6651. https://doi.org/10.1038/ncomms7651
46. Du N, Song L, Li Y, Wang T, Fang Q, Ou J, et al. Phytoestrogens protect joints in collagen induced arthritis by increasing IgG glycosylation and reducing osteoclast activation. Int Immunopharmacol. 2020;83:106387. https://doi.org/10.1016/j.intimp.2020.106387
47. Wang Y, Khan A, Antonopoulos A, Bouche L, Buckley CD, Filer A, et al. Loss of alpha2-6 sialylation promotes the transformation of synovial fibroblasts into a pro-inflammatory phenotype in arthritis. Nat Commun. 2021;12(1):2343. https://doi.org/10.1038/s41467-021-22365-z
48. Rombouts Y, Ewing E, van de Stadt LA, Selman MH, Trouw LA, Deelder AM, et al. Anti-citrullinated protein antibodies acquire a pro-inflammatory fc glycosylation phenotype prior to the onset of rheumatoid arthritis. Ann Rheum Dis. 2015;74(1):234–41. https://doi.org/10.1136/annrheumdis-2013-203565
49. Schwab I, Mihai S, Seeling M, Kasperkiewicz M, Ludwig RJ, Nimmerjahn F. Broad requirement for terminal sialic acid residues and FcgammaRIIB for the preventive and therapeutic activity of intravenous immunoglobulins in vivo. Eur J Immunol. 2014;44(5):1444–53. https://doi.org/10.1002/eji.201344230
50. Yamada K, Ito K, Furukawa J, Nakata J, Alvarez M, Verbeek JS, et al. Galactosylation of IgG1 modulates FcgammaRIIB-mediated inhibition of murine autoimmune hemolytic anemia. J Autoimmun. 2013;47:104–10. https://doi.org/10.1016/j.jaut.2013.09.001
51. Tian H, Li T, Wang Y, Lu H, Lin L, Wu X, et al. The correlations between C-reactive protein and MRI-detected inflammation in patients with axial spondyloarthritis: a systematic review and meta-analysis. Clin Rheumatol. 2023;42(9):2397–407. https://doi.org/10.1007/s10067-023-06658-w

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