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ORIGINAL RESEARCH

Serum Proteomics Identified the Association of Haptoglobin and Orosomucoid 1 with Disease Activity in Takayasu Arteritis

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Background: Takayasu arteritis (TAK) is a chronic granulomatous vasculitis, and accurate early diagnosis and assessment of disease activity remain significant clinical challenge. Traditional biomarkers such as CRP and ESR have insufficient sensitivity and specificity. High-throughput proteomics provides a robust method for discovering new biomarkers for TAK.

Methods: A comparative serum proteomic analysis of 8 TAK patients and 9 healthy controls (HCs) was conducted using mass spectrometry. Bioinformatic analyses were performed on differentially expressed proteins (DEPs). Candidate biomarkers were prioritized through machine learning and correlation analyses. An independent validation cohort with 51 TAK patients and 31 HCs was used to confirm the biomarkers using ELISA. The correlations were assessed between the new biomarkers and the clinical parameters as well as disease activity scores.

Results: We identified 88 DEPs between TAK patients and HCs. Pathway analysis revealed enrichment in phagosome, platelet activation, and inflammatory response. Among these, Haptoglobin (HP) and Orosomucoid 1 (ORM1) were selected as top candidate biomarkers based on their high feature importance scores and strong correlation with inflammatory markers. ELISA validation confirmed that serum levels of HP and ORM1 were significantly elevated in TAK patients compared with HCs, and further increased in active TAK compared with stable TAK patients. Both biomarkers showed significant positive correlations with clinical inflammatory indicators (ESR, FIB, WBC) and immune cell counts, as well as disease activity scores.

Conclusion: Our integrated proteomic analysis identified HP and ORM1 as 2 novel serum biomarkers significantly associated with disease activity, inflammatory and immune processes in TAK.

Keywords: Takayasu arteritis, serum proteomics, biomarker, haptoglobin, orosomucoid 1

Introduction

Takayasu arteritis (TAK) is a chronic, granulomatous, systemic vasculitis which primarily affects the aorta and its major branches.[1] TAK predominantly affects young women. The incidence rate is significantly higher in Asian populations than in Western populations.[2] The chronic and progressive nature of TAK, as well as its heterogeneity, make it challenging to assess disease activity, which affects clinical treatment decisions. Currently, the assessment of disease activity is primarily based on the serological indicators and imaging techniques.[3,4] However, there are still numerous limitations. Conventional non-specific inflammatory markers, including CRP, ESR, and IL-6, exhibit elevated levels during the active phase of TAK.[5] However, these indicators lack adequate sensitivity and specificity. It is important to note that in approximately 23% of patients with disease activity, these indicators may be within in the normal range. Conversely in patients with inactive disease, up to 44% may still have an elevated ESR, which significantly reduces the reliability of

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judging disease activity. The recent studies have suggested that some novel markers, including PTX-3, MMPs, TIMPs, NO_2^- and NT-proBNP, have the potential to enhance the assessment accuracy.[5,6] Nevertheless, there remains a dearth of large-scale prospective validation. The advanced imaging modalities, such as contrast-enhanced ultrasound (CEUS) and positron emission tomography (PET/CT), have shown to enhance diagnostic sensitivity and specificity for TAK.[7,8] However, their extensive utilisation continues to be constrained by difficulties pertaining to standardisation and accessibility. Consequently, the development of convenient biomarkers for accurate TAK diagnosis and assessment is imperative for optimising the management of TAK.

High-throughput proteomics, a technology founded upon the principles of mass spectrometry (MS), has emerged as a fundamental tool for the large-scale study of protein phenotypes.[9] This technological advancement has been instrumental in the identification of disease-specific biomarkers due to its exceptional sensitivity and resolution capabilities.[10] This technology enables the systematic analysis of the abundance of proteins in plasma or serum, thereby revealing key proteins closely related to disease activity and immunopathological mechanisms.[11] For instance, Luo et al conducted a comparative analysis of serum protein differences between active and inactive TAK, and revealed that 10 proteins, including SAA and C3, exhibited increased expression in active patients, while serum amyloid P demonstrated decreased expression.[12] In a similar vein, Natsuko Tamura et al conducted a comparative analysis of serum protein abundances between TAK patients and controls, utilizing two-dimensional gel electrophoresis combined with mass spectrometry to identify ten proteins with significantly altered abundances, and revealed the presence of Apolipoprotein AI and Conjugated globin among the altered proteins.[13] Huang et al conducted a study in which they analyzed the plasma exosomes of TAK patients by mass spectrometry, which enabled the identification of 357 differentially expressed proteins, suggested that desmosomes might be involved in the destruction of the endothelial barrier.[14] Wen et al identify novel autoantibodies of TAK using HuProt array-based approach.[15] Maughan et al suggest shared pathobiology across the large-vessel vasculitis spectrum involving innate immunity, lymphocyte homeostasis, and tissue remodeling by Plasma proteomic profiling.[15] These findings position serum proteomics as a promising breakthrough for the assessment of disease activity and the discovery of biomarkers. The high-throughput proteomic analysis of TAK serum proteins offers a comprehensive and precise evaluation, which is beneficial for identifying new diagnostic and activity markers for TAK.

The process of validating biomarkers has been hindered by technological limitations. In order to address this discrepancy, a detailed plasma proteomic analysis of TAK patients was performed using ultra-high-resolution mass spectrometry. The objective of this study was to identify differentially expressed proteins in plasma between patients with TAK and healthy control, trying to analyze the biological functions of these proteins and ultimately screen for protein biomarkers of TAK diagnosis and disease activity.

Materials and Methods

Study Population

A total of 59 TAK patients (52 females and 7 males; age range 16–51 years; mean age 34.63±10.25 years) meeting the 2022 ACR/EULAR Classification Criteria[16] was enrolled from the First Affiliated Hospital of Xi'an Jiaotong University between September 2023 and March 2025. The exclusion criteria were as follows: Other rheumatism; acute infections; malignant tumors; unwillingness to participate or incomplete data. Among the 59 TA patients, 8 underwent TAK biomarker screening via proteomic analysis, and 51 participated in TAK biomarker validation using ELISA. A total of 40 gender and age matched healthy controls (35 females and 5 males; age range 17–52 years; mean age 33.62 ± 11.26 years) were concurrently enrolled. Among them, 9 were for biomarker screening and 31 were for biomarker validation. This study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (Ethical Approval Number: XJTU1AF2021LSK-357). The written informed consent was obtained from the participants or a parent of the patient if the patient was under 18 years old. The current study and our previous study[17] were form part of the same research project. The previous study primarily used a retrospective, cross-sectional approach to collect clinical data and explore the relationship between lipids and TAK, while the current study mainly identified new TAK biomarkers through proteomic data analysis. The two

studies used different types of data, and their objectives, clinical research types, and patient populations differed. The demographic data, general data, laboratory test data, and disease activity data (Kerr score, ITAS, ITAS.A score) were obtained from medical records. The TAK patients were divided into two groups according Kerr score, active group (Kerr score ≥ 2) and inactive group (Kerr score < 2). The ITAS score of ≥ 2 or an ITAS-A score of ≥ 5 is conventionally defined as indicating active disease.[18]

Proteomic Analysis

Serum samples (8 TAK patients and 9 HCs) were pretreated using a Low-abundance Protein Enrichment Kit (OMIC SOLUTION, OSFP0002) according to the manufacturer's instructions. Briefly, 100 μL of serum was incubated with pre-washed magnetic beads. After removing high-abundance proteins, the bound low-abundance proteins were subjected to on-bead enzymatic digestion for 2 hours. The peptides were purified with a solid-phase extraction column and then lyophilized into a dry powder for stable storage.

The dried peptide samples were reconstituted in 0.1% formic acid and analyzed using nanoflow liquid chromatography on an UltiMate 3000 RSLCnano system (Thermo Fisher Scientific), fitted with a 25 cm nano-electrospray integrated analytical column (IonOpticks, AUR3-25075C18). Separation was performed at a constant flow rate of 300 $nL \cdot min^{-1}$ using a binary solvent system consisting of solvent A (0.1% FA in water) and solvent B (0.1% FA in 80% acetonitrile). The following gradient profile was applied: 5–20% B over 2 min, 10–40% B over 80 min, 40–55% B over 2 min, 55–90% B over 2 min, and 90–100% B over 5 min. The eluted peptides were ionized via nano-electrospray at a voltage of 2.5 kV and introduced into a high-resolution Orbitrap Exploris 480 mass spectrometer (Thermo Fisher Scientific) for data acquisition. The mass spectrometric data were obtained by data-independent acquisition (DIA) mode. Full-scan MS spectra were collected in the range of m/z 350–1200 at a resolution of 120,000. MS/MS spectra were generated through product ion scan events with a resolution of 30,000. The DIA acquisition was conducted using 42 variable isolation windows covering the entire predefined m/z range. Mass spectrometric data were acquired with full-scan MS spectra recorded in profile mode and fragment ion spectra (MS/MS) acquired in centroid mode.

Raw data from the direct data-independent acquisition (Direct DIA) runs were processed using Spectronaut 18 (Biognosys AG) in library-free mode. The human reference proteome database downloaded from Uniprot (https://www.uniprot.org) was used for Direct-DIA analysis. Database search was conducted under the default BGS Factory Settings provided for Direct-DIA workflows. Trypsin/P was selected as the proteolytic enzyme, permitting up to two missed cleavages. Carbamidomethylation of cysteine was designated as a fixed modification, while oxidation of methionine and N-terminal acetylation were treated as variable modifications. The false discovery rate (FDR) was maintained at 1% for peptide-spectrum matches (PSMs) and peptides, and at 5% for proteins. The initial DEP identification used uncorrected *P*-values for sensitivity, and all findings were subsequently validated in an independent cohort to strengthen the accuracy of biomarkers.

Validation Studies Using ELISA

Serum samples were obtained from 82 subjects (including 51 TAK patients and 31 HCs). Blood was collected into serum separator tubes, and then centrifuged at 2,000 × g for 10 minutes at 4°C. Serum aliquots were subsequently stored at −80°C. Serum levels of haptoglobin (HP) and Orosomucoid 1 (ORM1) were measured using commercial ELISA kits (Quantikine R&D Systems, Minneapolis, MN, USA; Catalogue Nos. DHAPG0 for HP, DAGP00 for ORM1). The ELISA signals were read at 450 nm using a microplate reader. Protein concentrations were determined by generating four-parameter logistic standard curves based on serially diluted standards.

Bioinformatics Analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using the DAVID database (https://david.ncifcrf.gov/). Protein-protein interaction (PPI) networks were constructed using Metascape (https://metascape.org/). Random forest model was trained with the "caret" package (version 7.0.1), and nomograms were visualized using the "rms" package (version 6.7.1). To fit the random forest model, we used 900 trees

(ntree = 900) with mtry set to 3. Internal validation was performed using out-of-bag (OOB) samples, and the OOB error convergence is presented in Supplementary Figure S1. All package-based analyses were performed in R (version 3.8.19).

Statistical Analysis

Data were statistically analyzed by SPSS 23.0 (SPSS, Inc, Chicago, IL). Continuous variables were presented as means ± standard deviations (SD) and categorical variables were presented as counts (percentages). The Shapiro–Wilk test was used to evaluate normality of variance, and Levene's test was applied for homogeneity. According to the situation, the *t*-test or Mann–Whitney *U*-test was used to assess the differences between the two groups. Pearson's correlation coefficient or Spearman correlation coefficient was used to analyze correlations between two variables. The χ^2 test was used to compare the percentages between two group. The diagnostic value of HP or ORM1 was assessed by constructing receiver operating characteristic (ROC) curves. *P* values < 0.05 were considered statistically significant.

Results

Serum Proteome Characterization of TAK Patients

We developed an automated, high-throughput mass spectrometry (MS)-based proteomics workflow employing a data-independent acquisition (DIA) strategy. This workflow enabled proteome profiling of minimal serum volumes, followed by bioinformatics analysis and ELISA validation (Figure 1A). Using this approach, we analyzed the serum proteome of 8 TAK patients and 9 HCs. Principal component analysis (PCA) revealed distinct clustering between the TAK patients and healthy controls, indicating significant differences in their serum proteome (Figure 1B). A total of 573 and 577 proteins were quantified in the serum of TAK patients and HCs, respectively, with 573 TAK-associated proteins shared between both groups (Figure 1C and D). The PCA separation was likely due to differences in protein abundance rather than composition, given the high overlap in proteins. Analysis of serum protein abundance in the two groups revealed that the quantified protein intensities ranged across four orders of magnitude. The top 10 abundant proteins constituted over 37% of the total serum proteins (Figure 1E and F), the antibody affinity method was used to reduce the influence of high-abundance proteins on the detection of low-abundance proteins.

Serum Differential Protein Function and Network Analysis

We identified 88 plasma proteins that were significantly differentially expressed between TAK patients and healthy controls (fold change ≥ 2; $P < 0.05$; Figure 2A). Among these, 49 proteins were up-regulated in TAK patients, including previously validated markers, such as C3, CCL2, CRP, CXCL10, IL-6, MMP2, MMP9, S100A8, and SAA1. Conversely, 39 proteins were down-regulated, some of which were associated with the occurrence of TAK. To identify specific biomarkers of TAK, a bioinformatics analysis was conducted on differentially expressed proteins (DEPs). Pathway analysis of DEPs revealed enrichment in specific pathways, with the most significant KEGG pathways being phagosome function, focal adhesion, and platelet activation (Figure 2B). GO analysis indicated enrichment in biological processes such as platelet degranulation, inflammatory response, regulation of cell motility, and neutrophil degranulation (Figure 2C). Protein-protein interaction (PPI) network analysis revealed high clustering of genes involved in phagocytosis, interleukin signal transduction, and actin cytoskeleton reorganization, processes that were mechanistically linked to TAK vasculitis (Figure 2D).

Screening of Candidate Serum Protein Biomarkers in TAK Patients

To further identify the potential specific protein markers of TAK, a cluster analysis was performed on the DEPs in the serum of TAK patients. This was followed by expression intensity analysis on the top 10 up-regulated proteins (Figure 3A and B). The random forest model was utilized to ascertain the diagnostic importance of the proteins in the classification of TAK (Figure 3C). The subsequent correlation analysis demonstrated significant associations between their expression levels and serum inflammatory markers in patients with TAK (Figure 3D). To identify candidate biomarkers for TAK, two crucial criteria were included: elevated feature importance scores in machine learning (Mean Decrease Accuracy > 3.5) and robust correlations with disease activity indicators (*r* > 0.75). Finally, four potential

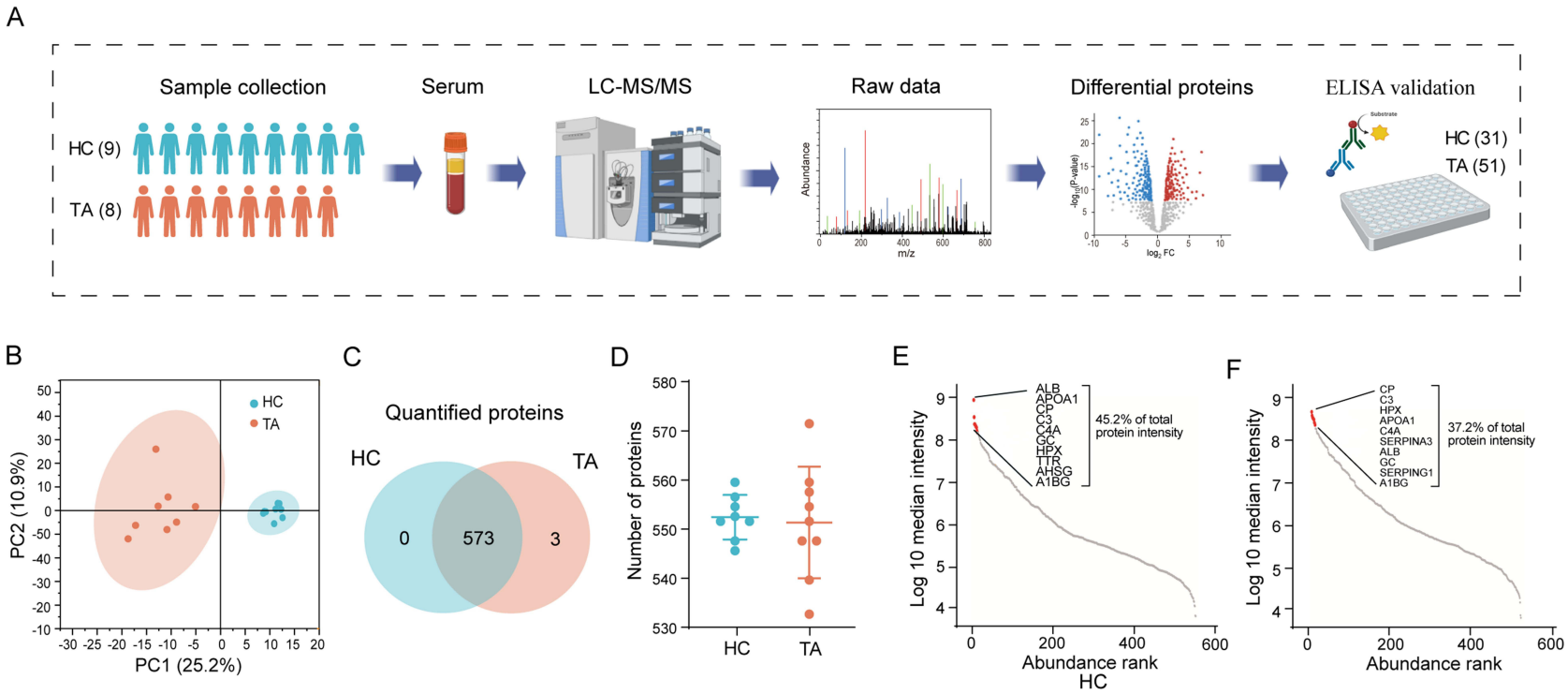

Figure 1 MS-based serum proteomic profiling of TAK patients. (**A**) Schematic workflow of the quantitative proteomics analysis and bioinformatic pipeline applied to serum samples from TAK patients (n = 8) and HCs (n = 9). (**B**) Principal component analysis (PCA) of the discovery cohort serum proteomes showed clear distinction between the TAK and HCs. (**C**) Venn diagram depicted serum proteins quantified in TAK patients and HCs, with 573 proteins common to both cohorts. (**D**) Scatter diagram indicated the number of proteins identified and quantified (1% FDR) per sample in the TAK patients and HCs. (**E** and **F**) Ranked protein abundance profiles for the (**E**) HC and (**F**) TAK cohorts. The top 10 most abundant proteins are labeled, with their relative contribution to the total proteome intensity indicated.

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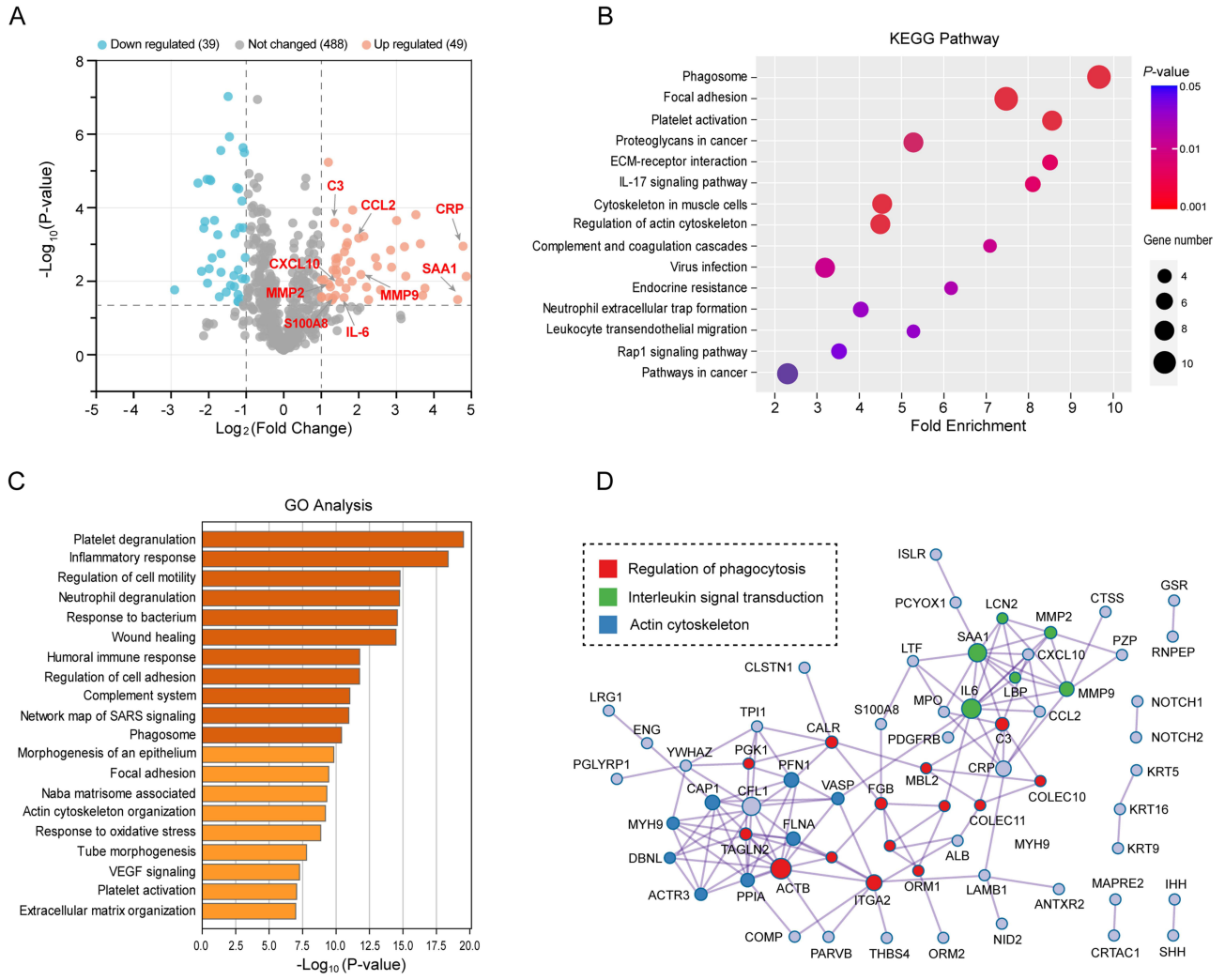

Figure 2 Bioinformatics analysis of differentially expressed plasma proteins (DEPs) in TAK patients. (**A**) Volcano plot of plasma proteome profiles comparing TAK patients and HCs. Red dots: proteins upregulated in TAK patients (fold change ≥2); blue dots: proteins downregulated in TAK patients (fold change ≤-2). (**B**) KEGG pathway enrichment analysis of DEPs. Dot size reflects DEP count per pathway; color scale indicates statistical significance. X-axis indicates enrichment ratio. (**C**) Gene Ontology functional enrichment analysis of DEPs in biological processes. (**D**) Protein-protein interaction network of DEPs highlighting clusters associated with phagocytosis regulation, interleukin signaling, and actin cytoskeleton reorganization.

specific markers of TAK were selected though integrated evaluation, including Haptoglobin (HP), Orosomucoid 1 (ORM1), SAA1 and CRP. The roles of SAA1 and CRP were confirmed to be associated with TAK activity in previous studies.[18] There was no study to assess the value of HP and ORM1 in TAK diagnosis and disease activity. Herein, we selected HP and ORM1 as specific markers of TAK for subsequent study to verify the diagnostic value.

The Association of Haptoglobin and Orosomucoid 1 with Disease Activity in TAK

To validate the candidate TAK biomarkers (HP and ORM1), a total of 51 TAK patients were recruited. The clinical characteristics of the TAK patients in different disease activity were shown in Table 1. Serum samples were collected from the 51 TAK patients and 31 HCs. The levels of HP and ORM1 were tested using ELISA, which were significantly higher in the TAK group compared with the HC group, especially in the active-stage TAK patients (Figure 4A). The Spearman correlation analysis indicated a significant correlation of both biomarkers with ITAS, ITAS.A-ESR, and ITAS.A-CRP scores (Figure 4B).

The nomograms were generated using a logistic regression model. Each variable received a score, which was read from the top scale. By summing these scores and finding their corresponding values on the total score scale, the estimated

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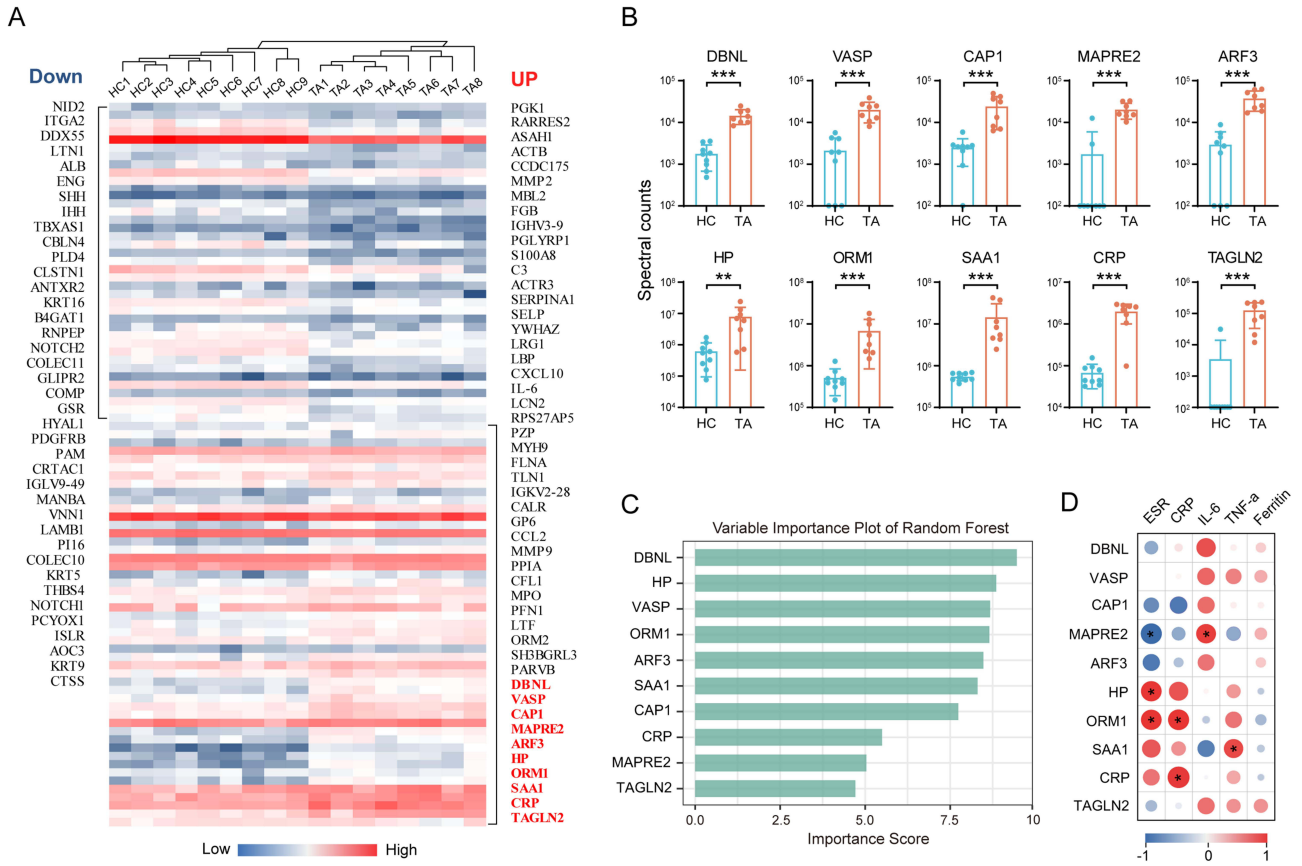

Figure 3 Screening of candidate serum protein biomarkers in TAK patients. (**A**) Hierarchical clustering heatmap of differentially expressed proteins (DEPs) in TAK patients (n = 8) vs. HCs (n = 9). (**B**) MS signal intensities of the top 10 up-regulated proteins in TAK patients. (**C**) Random forest feature importance ranking for TAK diagnosis evaluated by Mean Decrease Accuracy. (**D**) Correlation matrix between the top 10 up-regulated serum proteins and clinical inflammatory markers in TAK patients. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

risk of TAK or disease activity was determined. Higher total scores indicated a higher risk of TAK occurrence or disease activity. Then, ROC curves demonstrated that the superior performance of HP combined with ORM1 for both TAK diagnosis (Figure 4C) and TAK activity (Figure 4D):HP combined with ORM1 (AUC: 0.860, 95% CI: 0.782–0.939 for TAK diagnosis; AUC: 0.756, 95% CI: 0.610–0.901 for TA activity), HP (AUC: 0.844, 95% CI: 0.759–0.928 for TAK diagnosis; AUC: 0.695, 95% CI: 0.548–0.843 for TAK activity), ORM1 (AUC: 0.783, 95% CI: 0.685–0.881 for TAK diagnosis; AUC: 0.740, 95% CI: 0.597–0.883 for TAK activity), CRP (AUC: 0.807, 95% CI: 0.714–0.901 for TAK diagnosis; AUC: 0.536, 95% CI: 0.367–0.706 for TAK activity) and ESR (AUC: 0.809, 95% CI: 0.716–0.903 for TAK diagnosis; AUC: 0.620, 95% CI: 0.466–0.774 for TAK activity).

The Relationship Between HP or ORM1 and Clinical Parameters in TAK Patients

As shown before, the serum HP and ORM1 displayed the superiority for TAK diagnosis and disease activity assessment. We further investigated whether elevated expression levels of both proteins correlated with clinical parameters and immune profiles, which could reveal their roles in TAK pathogenesis (Figure 5A). Significant positive correlations were observed between serum HP levels and erythrocyte sedimentation rate (ESR), fibrinogen (FIB), white blood cell count (WBC), and various immune cell subsets, including natural killer (NK) cells, total lymphocytes, T cells, and $CD4^+$ T cells. Similarly, serum ORM1 levels were positively correlated with ESR, immunoglobulin A (IgA), WBC, NK cells, lymphocytes, T cells and $CD4^+$ T cells (Figure 5B). These findings suggested that both HP and ORM1 were involved in inflammatory and immune regulatory processes in TAK. Further mechanistic study was needed to clarify their specific roles in TAK processes.

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Table 1 The Characteristics of TAK Patients in Different Disease Activity Status

Characteristic	Total (n=51)	Inactive (n=18)	Active (n=33)	*P* value
Age, years	35.67±11.75	36.33±11.76	35.30±11.91	0.768
Female, n (%)	44 (86.27)	17 (94.44)	27 (81.82)	0.398
Disease duration, years	4.51±5.73	4.67±5.39	4.42±5.98	0.467
BMI index (kg/m2)	22.18±2.99	21.96±3.09	22.30±2.98	0.953
Type				
I	12 (23.53)	5 (27.78)	7 (21.21)	0.964
II	7 (13.73)	2 (11.11)	5 (15.15)	
III	2 (3.92)	1 (5.56)	1 (3.03)	
IV	27 (52.94)	9 (50.00)	18 (54.54)	
V	3 (5.88)	1 (5.56)	2 (6.06)	
Symptoms, n (%)				
Fever	5 (9.80)	1 (5.56)	4 (12.12)	0.645
Fatigue	14 (27.45)	6 (33.33)	8 (24.24)	0.525
Dizziness	20 (39.22)	7 (38.89)	13 (39.39)	0.972
Neck pain	8 (15.69)	2 (11.11)	6 (18.18)	0.696
Chest pain	9 (17.65)	1 (5.56)	7 (21.21)	0.233
Abdominal pain	4 (7.84)	1 (5.56)	3 (9.09)	1.000
Signs, n (%)				
Pulseless	21 (41.18)	5 (27.78)	16 (48.48)	0.151
Vasculer bruit	44 (86.27)	15 (83.33)	29 (87.88)	0.686
Vasculer stenosis	50 (98.04)	17 (94.44)	33 (100)	–
Vasculer thickening	35 (68.6)	11 (61.11)	24 (72.72)	0.393
Hypertension	11 (21.57)	4 (22.22)	7 (21.21)	0.042
Hyperlipidemia	5 (9.8)	3 (16.67)	3 (9.1)	0.652
Score				
Kerr scores	2.00±0.89	1	2.55±0.62	–
ITAS scores	3.78±2.94	1.27±0.89	5.15±2.76	< 0.001
ITAS.A-ESR score	4.31±3.11	1.56±1.04	5.82±2.82	< 0.001
ITAS.A-CRP score	4.47±3.01	1.94±1.31	5.85±2.77	< 0.001
Medication history				
Hydroxychloroquine	3 (5.88)	1 (5.56)	2 (6.06)	1.000
Prednisone	40 (78.43)	13 (72.22)	27 (81.81)	0.488
Methotrexate	15 (29.41)	8 (44.44)	7 (21.21)	0.082
Mycophenolate mofetil	4 (7.84)	1 (5.56)	3 (9.09)	1.000
Tofacitinib	24 (47.06)	10 (55.56)	14 (42.42)	0.369

Discussion

In clinical practice, the TAK diagnosis and assessment of disease activity remain complex and challenging.[19,20] Conventional biomarkers, such as CRP and ESR, have been shown to lack sufficient sensitivity and specificity. The advent of proteomic technologies has facilitated the identification of potential biomarkers in TAK.[12–14] However, majority of these candidates have not undergone systematic validation. In this study, a high-throughput DIA-based proteomic approach was applied to profile serum proteins in TAK patients and healthy controls, leading to the identification of 88 DEPs. Functional enrichment analyses revealed that the DEPs were primarily involved in biological processes, such as platelet degranulation, inflammatory response, neutrophil activation, and regulation of cell motility, and were enriched in pathways related to phagosome function, focal adhesion, and platelet activation. These findings were consistent with the established immunopathology of TAK, which encompassed innate and adaptive immune activation, vascular infiltration of inflammatory cells, and endothelial dysfunction.[21,22] The heightened presence of processes, including phagocytosis and interleukin signalling, further substantiates the pertinence of the ascertained proteomic profile in relation to vasculitis. Through integrated bioinformatic analysis and machine learning-based

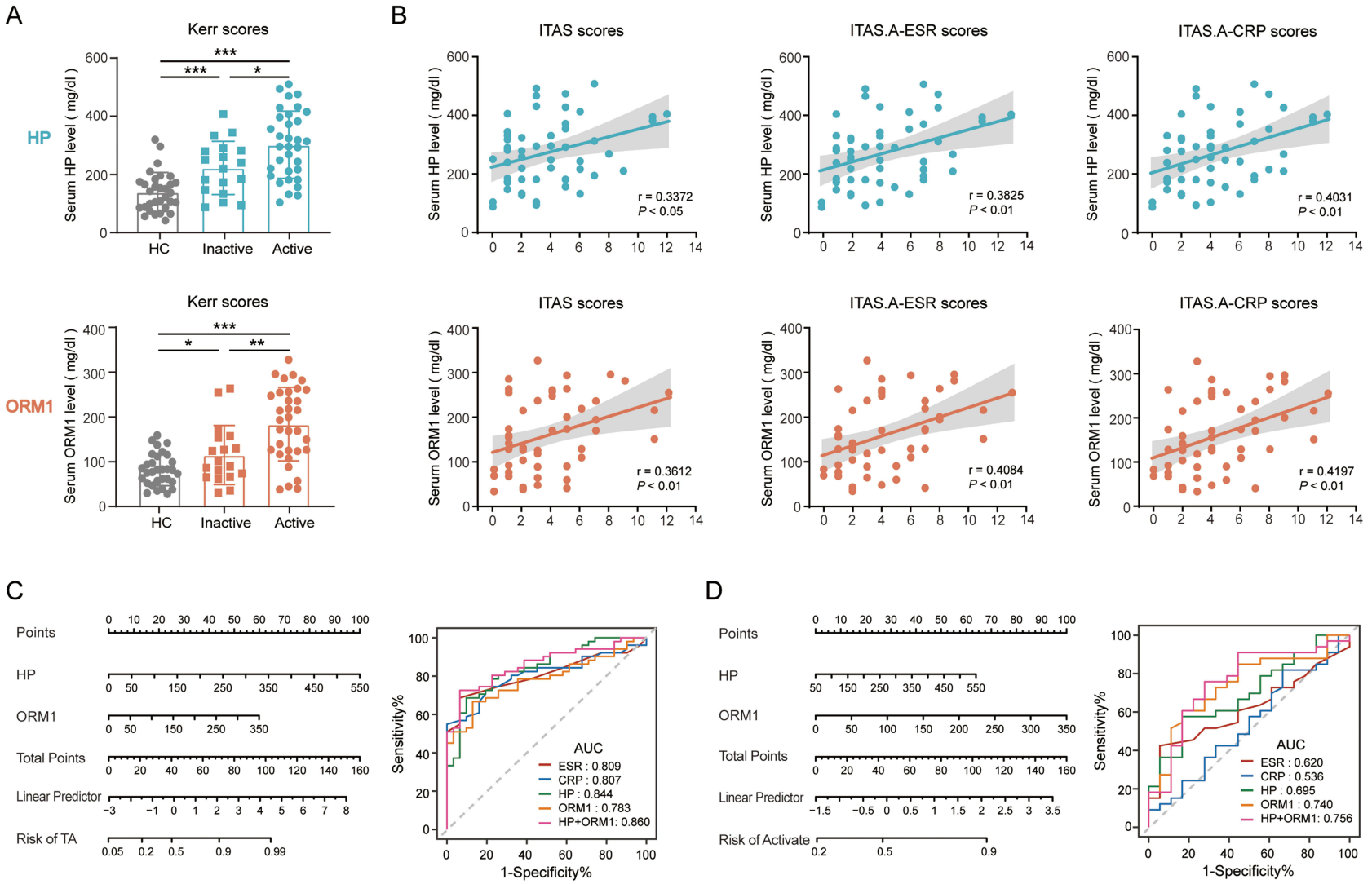

Figure 4 Serum biomarkers validation in TAK patients. (**A** and **B**) The serum levels of HP and ORM1 were measured in TAK patients (n = 51) and HCs (n = 31) using ELISA. Correlation between HP, ORM1 and TAK disease activity score by Spearman correlation analysis. (**C**) The value of TAK occurrence was validated using the nomogram and ROC curve. (**D**) The value of TAK activity was validated using the nomogram and ROC curve. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

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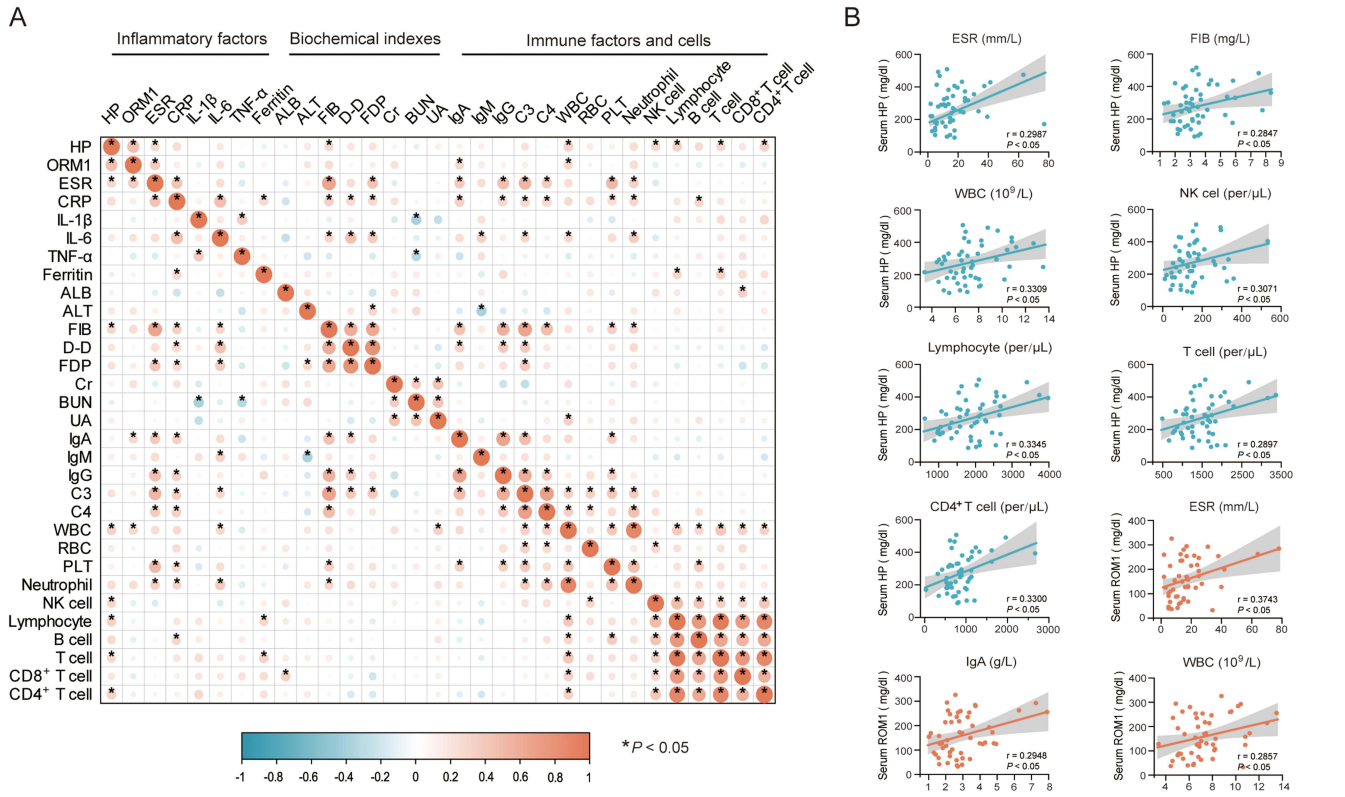

Figure 5 The association of HP or ORM1 with clinical parameters of TAK patients. (**A**) Correlation analysis between HP or ORM1 and clinical parameters in TAK patients, as determined by Spearman's analysis. Red graphs indicated positive correlation, and blue graphs indicated negative correlation. Asterisks denote statistical significance. (**B**) Both serum HP and ORM1 levels showed significant positive correlations with multiple clinical indicators, consistent with the associations displayed in Figure 5A. *$P < 0.05$.

prioritisation, we further identified HP and ORM1 as two novel biomarker candidates strongly associated with TAK diagnosis and disease activity.

HP is a classical acute-phase protein, known to bind free hemoglobin and mitigate oxidative tissue damage, and also modulates immune cell activation.[23] Interestingly, in chronic vasculitic conditions like TAK, HP can exhibit dynamic expression: it is induced during the acute phase of inflammation but is consumed in settings of hemolysis, thus rendering it a context-dependent biomarker.[24,25] Although no study has directly linked HP to TAK, its consumption has been observed in other autoimmune vasculitides accompanied endothelial injury, suggesting that HP may reflect vascular inflammation and hemolytic activity in TAK.

ORM1, another acute-phase reactant, has been less studied in TAK, despite its growing attention of its immunomodulatory roles.[26] We found that ORM1 expression correlated not only with systemic inflammation markers (eg, ESR, IgA), but also with T cell and NK cell counts, indicating a potential role in adaptive immune regulation ORM plays an important regulatory role in the inflammation, immune response, and fibrosis process. In humans, there are two distinct isoforms of ORM, namely ORM1 and ORM2. The existing evidence suggested that ORM1 promoted endothelial activation via NF-κB, enhanced chemokine production, facilitated T cell differentiation towards Th1/Th17 phenotypes, and activate fibrotic signaling pathways (eg, JAK/STAT and TGF-β).[27,28] These mechanisms are consistent with the histopathological features of TA, including vascular infiltration, granulomatous inflammation, and adventitial fibrosis. The presence of ORM1-positive cells in the vascular wall of TAK patients further supports its local role in disease pathogenesis.

In this study, both HP and ORM1 levels exhibited significant up-regulation in TAK patients, particularly during periods of active disease. A robust correlation was observed between these changes and clinical disease activity indices, underscoring their potential as biological markers. Furthermore, the strong correlations of HP and ORM1 with clinical parameters, including ESR, WBC, lymphocyte subsets, and fibrinogen, provided support for them as biomarkers

reflective of inflammatory and immune activity. Of particular significance was the elevation of these markers in active TAK patients, as well as their correlation with standardized disease activity instruments. This underscored their potential as complementary biomarkers to those already in use. However, there were some limitations of this study. Firstly, the sample size of the discovery phase was relatively small, which may have an impact on the generalisability of the proteomic findings. Secondly, although ELISA was utilized to validate HP and ORM1 in a larger cohort, further multi-centre validation with longitudinal sampling was warranted to confirm their clinical utility in monitoring treatment response and relapse. Thirdly, all included patients were receiving treatment at the time of sample collection. Treatments for TAK, such as glucocorticoids and IL-6-targeted agents, are known to significantly influence inflammatory pathways and, consequently, the serum proteomic profile, which may confound the interpretation of DEPs and biomarker performance. Treatment-naïve patients should be enrolled in further studies to obtain a clearer assessment of disease-specific proteomic changes independent of therapeutic effects.

In conclusion, our study provided a comprehensive serum proteomic profile of TAK and highlighted HP and ORM1 as promising biomarker candidates linked to disease activity and immune-inflammatory processes. Future studies should focus on validating these markers in larger prospective cohorts and elucidating their functional contributions to TAK pathogenesis, which may also offer new insights into therapeutic targeting.

Abbreviations

CEUS, contrast-enhanced ultrasound; DEPs, differentially expressed proteins; DIA, data-independent acquisition; ESR, erythrocyte sedimentation rate; FA, formic acid; FIB, fibrinogen; GO, Gene Ontology; HCs, healthy controls; IgA, immunoglobulin A; KEGG, Kyoto Encyclopedia of Genes and Genomes; PCA, Principal component analysis; FDR, false discovery rate; MS, mass spectrometry; NK, natural killer; PET/CT, positron emission tomography; PPI, Protein-protein interaction; PSMs, peptide-spectrum matches; ROC, receiver operating characteristic; SD, standard deviation; TAK, Takayasu arteritis; WBC, white blood cell count.

Data Sharing Statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Integrated Proteome Resources (https://www.iprox.cn/page/PSV023.html;?url=17610132903556JG7) and the accession number is IPX0013749000.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (Ethical Approval Number: XJTU1AF2021LSK-357). The written informed consent was obtained from the participants or a parent of the patient if the patient was under 18 years old.

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Author Contributions

Jing Wang, Xinyi Liu and Xiaohao Wang shared first authorship. YL; Conceptualization, Supervision, Writing – original draft, Investigation, Resources, Writing – review & editing. JW; Conceptualization, Supervision, Methodology, Formal analysis, Data curation, Writing – original, draft,: Investigation, Resources, Writing – review & editing. QL: Conceptualization, Supervision, Writing – review & editing. XL; Methodology, Formal analysis, Data curation, Writing – review & editing. XW; Methodology, Formal analysis, Data curation, Writing – review & editing. FL; Methodology, Formal analysis, Data curation, Writing – review & editing. YH; Methodology, Formal analysis, Data curation, Writing – review & editing. HL; Methodology, Formal analysis, Data curation, Writing – review & editing. LM; Methodology, Formal analysis, Data curation, Writing – review & editing. YH; Methodology, Formal analysis, Data curation, Writing – review & editing. JH; Methodology, Formal analysis, Data curation, Writing – review & editing. All authors gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no other conflicts of interest to declare.

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