Plasma proteomic screening and validation of novel biomarkers in Takayasu's arteritis

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Abstract

Objective

To screen and validate differential proteins as novel biomarkers in active Takayasu's arteritis (TAK).

Methods

Plasma samples from 40 active, 40 inactive patients, and 40 healthy controls were collected. Protein profiles of plasma were mapped by two-dimensional gel electrophoresis. Differential protein spots were detected and identified by image analysis and mass spectrometry. Plasma concentrations of proteins were measured to validate candidate biomarkers. The area under the receiver operating characteristic (ROC) curve (AUC) of circulating plasma concentrations of candidate biomarkers were calculated to assess diagnostic value.

Results

With a total of 1507 matched gel spots, there were 170 differential expression spots between active and inactive TAK, including 139 up-regulated and 31 downregulated. Only 11 proteins could be identified by mass spectrometry. Serum amyloid A(SAA), fibrinogen, complement C4a, complement C3c, complement C4b binding protein(C4bp), recombination acting gene protein 1(RAG1), alpha-1-acid glycoprotein, alpha-1-microglobulin, complement C7, complement factor H related protein-1 were up-regulated in active patients, while serum amyloid P was down-regulated. Active patients had higher circulating levels of RAG1($P<0.001$), C4bp ($p=0.012$) and SAA($p<0.001$), compared to inactive patients, while inactive patients had higher levels than controls (RAG1, $p=0.011$; C4bp, $p=0.012$; SAA, $p=0.005$). The composite AUC with SAA, RAG1, and C4bp was 0.94 (95%CI 0.86-0.98) for discriminating activity, larger than 0.71(95% CI 0.60–0.80) for ESR ($p=0.0004$) or 0.75(95%CI 0.64–0.84) for CRP ($p=0.0014$), respectively.

Conclusion

Some acute-phase and immunology-related proteins may serve as novel biomarkers of TAK. Further study of these proteins may be helpful to elucidate the pathologic mechanism.

Key words

Takayasu's arteritis, biomarkers, two-dimensional difference gel electrophoresis, mass spectrometry

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Competing interests: none declared.

Introduction

Takayasu's arteritis (TAK) is a non-specific arteritis with unknown aetiology, mainly involving the aorta and its branches. Assessment of disease activity is essential for the management of TAK. Severe vascular comprise may require surgical or endovascular treatment. Surgical and endovascular procedures are generally performed during the remission period or after controlling disease activity because restenosis is common after bypass or angioplasty in patients with active TAK (1-4). Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level are widely used to assess the disease activity, but are generic markers for inflammation. Surgical biopsy revealed active inflammation in 44% of patients thought to be in the inactive phase (4). There are no reliable biomarkers that accurately reflect the disease activity in TAK (5).

The plasma proteome is highly complex and a rich source of potential diagnostic and prognostic biomarkers. Through proteomic techniques, more and more acute-phase proteins have been discovered to be differentially altered (6). This study used proteomics to conduct a comprehensive survey of differential plasma proteins to screen candidate biomarkers. Our pilot study detected some differential expression of plasma protein profiles between patients with active and inactive Takayasu arteritis (7). Some acute-phase proteins have been discovered which might serve as biomarkers (8). In this study, we made a further analysis of differentially expressed proteins in a larger size sample to validate the novel biomarkers of disease activity of TAK.

Patients and methods

Ethics

This study was carried out following the Helsinki declaration and approved by the ethics committee of the Beijing Shijitan Hospital and Beijing Anzhen Hospital, affiliated with Capital Medical University. All subjects signed written informed consent before enrolment.

Study subjects

Forty patients with active TAK were enrolled at Beijing Anzhen Hospital and Beijing Shijitan Hospital. Forty inactive TAK patients with matched age, sex, and disease classification acted as the inactive group. The diagnosis of TAK was consistent with Sharma's modified criteria (9). Enrolment excluded patients with a history of other autoimmune or genetic diseases, surgery, or endovascular treatment for TAK, and infarction events (*e.g.* myocardial infarction, major stroke, visceral infarction). All patients underwent angiography included digital subtraction angiography, computerised tomography angiography, or magnetic resonance angiography. The disease activity was assessed according to the National Institutes of Health criteria for active disease, as suggested by Kerr *et al.* (10). TAK was classified according to the 1996 angiographic classification of the International TAK Conference in Tokyo (11).

A control group of 40 healthy volunteers, age- and sex-matched, were recruited, and underwent physical examination with ultrasound duplex examination of the peripheral artery to rule out vascular diseases. Inclusive criteria of healthy controls included normal function of liver, kidney, lung, heart; no history of vascular diseases, autoimmune and connective diseases; no acute or chronic infection or inflammation state; and no recent surgery or endovascular treatment for at least three months.

Plasma proteome profiling

Ten millilitres of blood was drawn from the antecubital vein into BD vacutainers (Beckton Dickinson and Company) containing ethylene diamine tetra-acetic acid. Samples were immediately centrifuged at 1509(×g) for 15 minutes at 4°C. High-abundant proteins (human albumin, immunoglobin G, antitrypsin, immunoglobin A, transferrin, haptoglobin) were removed from plasma supernatant by the Agilent multiple affinity removal spin cartridge (Agilent Tech, Santa Clara, USA) according to the instruction manual. Plasma supernatant was collected and stored in a refrigerator at -80°C.

Sample protein concentrations were determined by the Bradford method. Samples with 120 μg of proteins were diluted to 350 μL with rehydration solu-

tion including1.25μL immobilised pH gradient (IPG) buffer and 7μL 65mM DTT. Samples were applied onto an 18cm ph3-10 non-linear IPG strip (Amersham Pharmacia Biotech, Sweden) after centrifugation at 23500(×g) for one minute. Strips were focused on the IPG Phor system (Amersham Pharmacia Biotech, Sweden). The voltage started at 50V and gradually increased to 8000V overnight to reach 80000VHr. After focusing, strips were equilibrated on a shaker for 15 minutes in a solution containing 1.5mM Tris (pH8.8), 6M urea, 20% glycerol, 0.1% sodium dodecyl sulfate (SDS), and 1% DTT. A second equilibration step was carried out for 15 minutes in the same solution, except replacing DTT with 259 mM iodoacetamide. The second dimension (SDS-PAGE) electrophoresis was performed on ProteinIICell unit (Bio-Rad, Berkeley, USA) using 13% SDS-polyacrylamide gel without stacking gel at a constant current of 20 mA/gel for 40min and 30mA/gel until the bromophenol blue dye marker reached the bottom of the gel.

Silver (0.25% silver nitrate, 37% formaldehyde) and Coomassie Brilliant Blue R-250 (0.25% Brilliant Blue, 10% v/v ethanol, 10% v/v acetic acid) staining were used for analytical (120 μg of sample protein) and preparative (1500 μg of sample protein) gels, respectively. Image Scanner densitometer (Amersham Pharmacia Biotech, Uppsala, Sweden) captured the 2-DE images. Image Master 2D platinum 5.01 software (Bio-Rad, Berkeley, USA) was used to detect, quantify, and align spots. Spot intensity volumes were normalised for every gel (spot volume/Σ spot volumes × 100) to correct for subtle variation in protein loading and gel staining between compared gels.

The image of the preparative gel was matched with the analytical gels. Spots of interest were manually excised from gels and washed twice with 50% acetonitrile (ACN) and 100 mM ammonium bicarbonate buffer until the gel pieces were opaque. The gel pieces were dried by centrifugal vacuum concentration. The particles were soaked with 1% (w/v) trypsin in 25 mM NH_4HCO_3 and incubated at 37°C for at least 12h. Subsequently, peptides were extracted twice from particles with 50% ACN/5% trifluoroacetic acid (TFA), combined, dried, and resuspended in 0.1% TFA. The peptides were finally eluted with 50% ACN/0.1% formic acid from particles for mass spectrometric analysis.

MALDI-TOF protein analysis

Matrix solution (α-cyano-4-hydroxycinnamic acid in 50% ACN and 0.1% TFA) and sample solutions were mixed and applied onto the target plate. Mass spectra were acquired by a Bruker Reflex III MALDI-TOF-MS (Bruker-Franzen, Bremen, Germany) in positive ion mode at an accelerating voltage of 20kv. The obtained peptide mass finger printings were analysed to search proteins in the Swiss-Prot, NCBInr, and MSDB databases by the MASCOT search engine (http://www.matrixscience.com/). The following search parameters were selected: trypsin digest (one missed cleavage allowed); species: *Homo sapiens*; mass value: monoisotopic; peptide mass tolerance: ±0.3 Da; oxidation of methionine and carbamidomethyl modification of cysteine.

Circulating concentration measurement

Circulating plasma concentrations of candidate proteins were measured using the commercially available enzyme-linked immunosorbent assay (ELISA) kits (Uscnlife Sciences & Technology Co. Ltd, Wuhan, China).

Table I. Demographic and clinic characteristic of subjects.

Variables	Active TAK	Inactive TAK	Healthy control	*p*
Number of patients	40	40	40	
Female n (%)	38 (95%)	38 (95%)	38 (95%)	1.00 [a]
Age (years)*	24.50 (21.00-26.00)	24.00 (21.0-26.0)	24.00 (20.00-26.75)	0.910 [b]
ESR (mm/H)*	23.00 (7.00-32.00)	7.00 (5.25-9.00)	6.50 (4.00-9.00)	0.015 [c]
CRP (mg/L)*	7.50 (4.00-12.00)	5.00 (2.00-6.00)	5.00 (2.00-5.75)	<0.001 [d]
Angiographic				1.00 [e]
Classification				
Type I, n (%)	12 (30%)	12 (30%)		
Type II, n (%)	2 (5%)	2 (5%)		
Type III, n (%)	3 (7.5%)	3 (7.5%)		
Type IV, n (%)	3 (7.5%)	3 (7.5%)		
Type V, n (%)	20 (50%)	20 (50%)		

*Median (p_{25}-p_{75}). [a]p=1.00, active TAK group *vs*. inactive TAK group *vs*. healthy control. [b]p=0.91, active TAK group *vs*. inactive TAK group *vs*. healthy control. [c]p=0.006, active group *vs*. inactive group; p=1.00, inactive group *vs*. control. [d]p<0.001, active *vs*. inactive; p=1.00, inactive *vs*. control. [e]p=1.00, active TAK group *vs*. inactive TAK group.

Statistical analysis

Data were expressed as means ± standard deviation if they followed a normal distribution assessed by Kolmogorov-Smirnov test, or medians and interquartile range (PM25, PM75) for abnormal distribution. Comparisons of differences among the three groups was determined by Student-Newman-Keuls or Kruskal-Wallis H test. The *p*-value of comparison between two groups was adjusted by Bonferroni correction for multiple comparisons. Statistical analyses were conducted with SPSS software v. 26.0 (SPSS Inc, Chicago, IL, USA). ROC curve was analysed by Medcalc software v. 19.04 (MedCalc Software Ltd, Ostend, Belgium).

Results

The demographic and clinical characteristic of patients are shown in Table I. Most patients were female with multiple lesions. There were no significant differences of age or sex among three groups, also no difference in angiographic type between the active and inactive group, while ESR and CRP were higher in the active group than inactive group. Figure 1 illustrates two-dimensional map of plasma protein profiling from the active, inactive patient, and health control. Among those maps, image analysis matched a total of 1507 spots in silver-stained gels. Between the active and inactive group, the quantitative and statistical analysis detected 170 differential spots, of which 139 were up-regulated and 31 down-regulated.

Table II. Identified protein spots.

Spot no.	Protein name	Accession no.	Molecular weight (Da)	Isoelectric point	Sequence coverage	MASCOT score
1939	Amyloid related serum protein SAA	gil225986	8298	5.76	61%	82
1649	Amyloid related serum protein SAA	gil225986	18083	5.54	75%	97
1306	Chain A, Serum amyloid p component	gil576259	37057	7.23	36%	102
1477	Unidentified		29654	5.73		
1469	Chain A, Serum amyloid p component	gil576259	29870	5.56	28%	70
1499	Unidentified		28268	5.60		
1013	Unidentified		50545	5.57		
1032	Unidentified		49371	5.63		
959	Chain B, fibrinogen	gil2781208	52797	5.90	47%	99
838	Chain B, Fibrinogen	gil2781208	57643	5.80	36%	71
872	Unidentified		57014	5.92		
626	C4b-binding protein alpha chain precursor	gil4502503	72542	5.80	32%	114
474	complement C7, partial	gil899271	10513	5.98	24%	94
879	fibrinogen gamma-prime chain	gil182440	55980	5.45	32%	57
1429	Unidentified		31509	5.15		
1004	Alpha-1-acid glycoprotein 2	OMHU2	46055	3.77	35%	68
792	Fibrinogen alpha chain isoform alpha precursor	gil11761629	60969	6.92	32%	136
796	Fibrinogen alpha chain isoform alpha precursor	gil11761629	60747	7.09	30%	138
876	fibrinogen beta chain isoform 1 preproprotein	gil70906435	55504	7.46	49%	205
1043	Fibrinogen alpha chain isoform alpha precursor	gil11761629	48891	7.00	30%	102
1021	Unidentified		49855	7.39		
1204	Unidentified		40880	6.81		
1232	Fibrinogen alpha chain	FGHUA	39974	7.93	18%	67
1336	Fibrinogen alpha chain	FIBA_HUMAN	35928	7.55	15%	72
1216	Fibrinogen alpha chain isoform alpha precursor	gil11761629	40273	7.55	21%	82
1266	Fibrinogen alpha chain isoform alpha precursor	gil11761629	38797	8.00	23%	96
1363	Complement C4, A fragment	Q5JNX0_HUMAN	34871	6.77	33%	73
1384	Unidentified		33989	6.20		
1385	Recombination activating gene 1 protein	gil23337081	33917	6.27	18%	71
1238	Fibrin alpha C term fragment	gil223057	39889	6.47	52%	66
1242	Complement factor H related protein 1	FHR1_HUMAN	39550	6.37	27%	60
1311	Unidentified		36860	6.29		
1164	Complement factor H-related protein 1	gil543981	43120	5.88	36%	70
926	Unidentified		8743	8.96		
687	Unidentified		17138	6.81		
1170	fibrinogen alpha chain isoform alpha precursor	gil11761629	42890	10.5	13%	64
1380	Fibrinogen alpha chain isoform alpha precursor	gil11761629	33917	10.4	18%	88
726	Unidentified		15106	6.03		
635	Unidentified		19528	6.68		
645	Chain C, Complement Component C3c	gil78101271	18454	6.68		127
689	Unidentified		16891	6.69		
1427	Alpha1 microglobulin	gil223373	32292	5.04	41%	94
964	Unidentified		52732	8.00		
974	Fibrinogen alpha chain preproprotein, isoform alpha	gil13591823	52029	8.01	19%	72

However, only 44 spots were revealed by Coomassie blue staining in the preparative gels. MALDI-TOF-MS successfully identified 28 fragments from 11 proteins. Table II summarises details of these identified protein fragments with molecular weight (MW), isoelectric point (PI), sequence coverage, identification scores from MASCOT. Spot intensity of fragments of serum amyloid A (SAA), fibrinogen (FIB), complement C7 (C7), complement C4a (C4a), complement C3c (C3c), complement C4b binding protein (C4bp), complement factor H-related protein 1 (CFHRP1), alpha-1-acid glycoprotein 2 (AAG), recombination activating

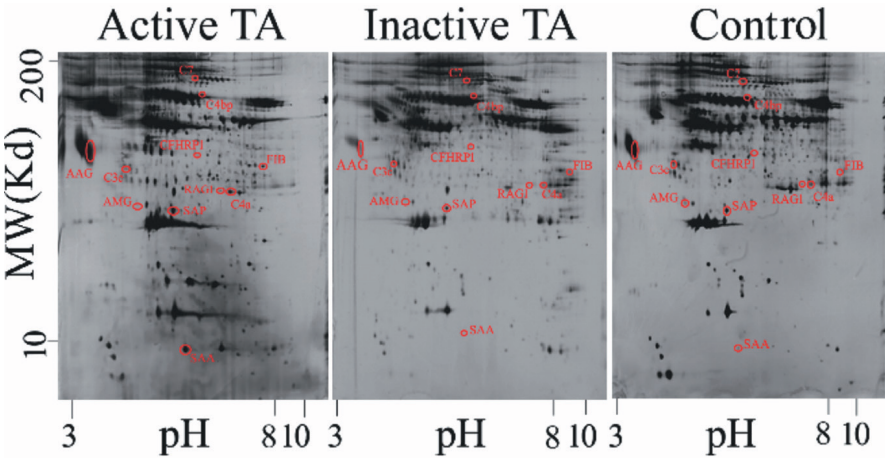

Fig. 1. Two-dimensional gel electrophoresis maps of plasma profiles of active, inactive Takayasu arteritis and healthy controls.

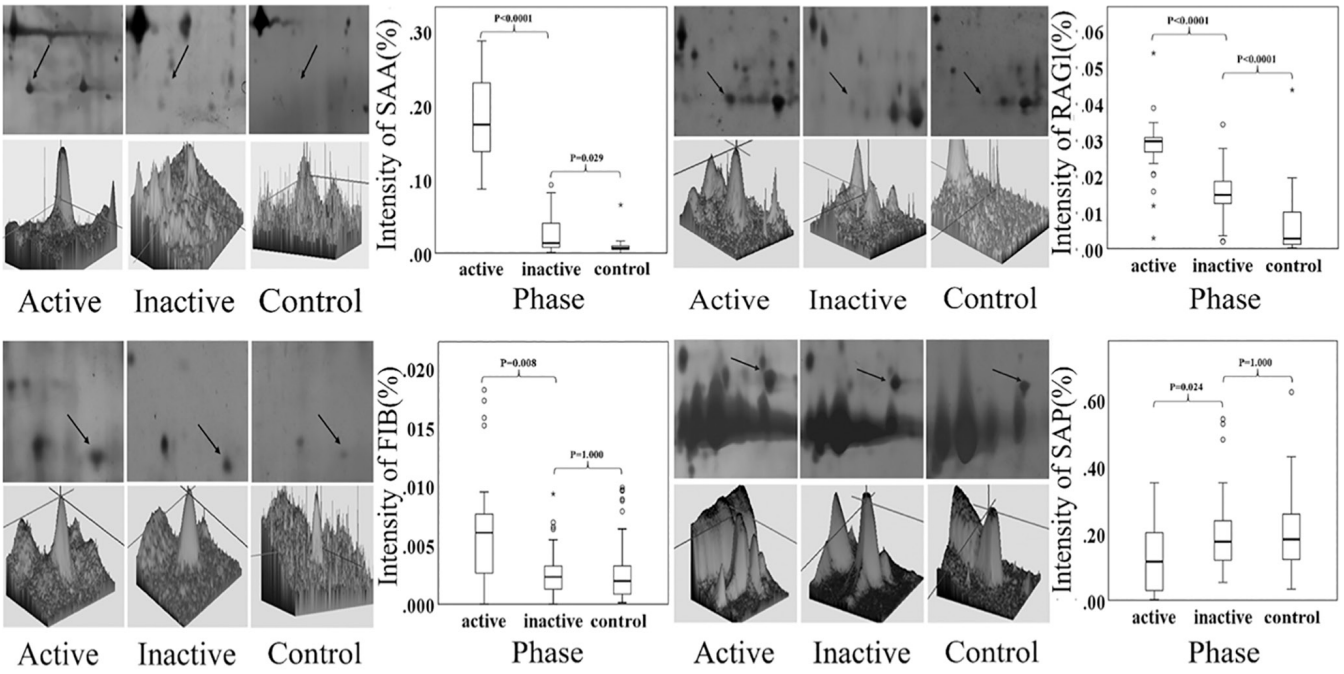

Fig. 2. Two-dimension and three-dimension view and spot intensity of serum amyloid A(SAA), recombination activating gene-1(RAG1) protein, fibrinogen (FIB), serum amyloid P component (SAP).

Table III. Circulation plasma concentrations of candidate biomarkers.

Protein	Active TA*	Inactive TA*	Healthy controls*	*p*
SAA (mg/L)	100.16 (90.53-123.41)	75.78 (65.09-95.37)	50.06 (44.60-69.10)	<0.001[a]
C4bp (mg/L)	294.06 (278.93-338.40)	260.50 (221.01-303.39)	222.41 (204.98-249.95)	0.012[b]
FIB (g/L)	3.65 (3.01-4.30)	3.43 (2.73-4.03)	3.18 (2.49-3.62)	0.095
C7 (mg/L)	103.54 (96.82-109.64)	99.60 (95.24-105.90)	95.12 (92.18-106.72)	0.080
AAG (mg/L)	863.54 (742.93-950.70)	781.96 (752.50-863.97)	772.78 (724.32-849.05)	0.073
SAP (mg/L)	36.13 (25.72-54.30)	40.55 (25.21-56.17)	32.59 (25.89-47.70)	0.459
RAG1 (mg/L)	95.99 (87.26-104.08)	80.25 (63.06-88.17)	59.93 (45.64-76.13)	<0.001[c]
C3c (mg/L)	808.30 (757.55-860.51)	795.91 (745.20-832.62)	769.88 (717.80-852.56)	0.173
C4a (mg/L)	1.92 (1.63-2.22)	1.70 (1.57-1.91)	1.66 (1.55-1.87)	0.060
AMG (mg/L)	30.23 (27.42-32.30)	28.10 (23.64-34.35)	26.04 (22.83-31.94)	0.077
CFHRP1 (mg/L)	144.37 (107.07-167.51)	134.73 (110.28-155.59)	125.23 (95.83-157.78)	0.174

SAA: serum amyloid A; C7: complement 7; C4bp: C4b binding proteins; FIB: fibrinogen; AAG: alpha-1-acid glycoprotein 2; SAP: serum amyloid p component; RAG: recombination activating gene protein 1; C3c: complement C3, C chain; C4a: complement C4, A fragment; AMG: alpha-1-microglobulin; CFHRP1: complement factor H related protein 1.
*Median(p_{25}-p_{75}).[a]p<0.0001, active *vs*. inactive TA; p=0.005, inactive TA *vs*. controls. [b]p=0.012, active *vs*. inactive TA; p=0.012, inactive TA *vs*. control. [c]p<0.0001, active *vs*. inactive TA; p=0.011, inactive *vs*. control.

gene protein1 (RAG1), and alpha-1-microglobulin (AMG) increased in active patients compared to inactive patients, while serum amyloid P (SAP) decreased. Furthermore, the spot intensity of serum amyloid A, RAG1, and C4bp increased in the inactive group compared with healthy controls. Figures 2 and 3 illustrated the comparison of the intensity of differential protein spots.

Circulating concentration of differential proteins are shown in Table III. Circulating levels of SAA, C4bp, and RAG1 significantly increased in active patients compared with those in inactive patients. All three were also significantly higher in inactive patients compered to healthy conrols.

Figure 4 illustrates the ROC curves of plasma concentrations of SAA, RAG1, C4bp, ESR, and CRP. AUC of SAA, RAG1, C4bp, ESR, and CRP was 0.833 (95%CI, 0.73–0.91), 0.82 (95%CI 0.72–0.90) and 0.69 (95%CI 0.58–0.79), 0.71 (95%0.60–0.80) and 0.75 (95%CI, 0.64–0.84) respectively. There was no difference in AUC among them. The composite AUC of SAA, C4bp, and RAG1 was 0.94 (95%CI 0.86-0.98), which was larger than that of ESR (p=0.0004) or CRP (Pp0.0014) individually, as seen in Figure 5.

Discussion

Many studies have tried to search for novel biomarkers of TAK in serum or plasma, demonstrating some kinds of cytokines, chemokines, inflammatory factors, or proteases that might be useful biomarkers, such as interleukin (IL)-6, IL-8, interferon-γ, tissue inhibitor of metalloproteinases-1, matrix

metalloproteinase-9, tumour necrosis factor-α, or YKL-40 (12-17). Serum IL-6 level was higher in TAK patients than healthy controls, and longitudinally decreased when patients achieved remission (13, 17, 18). However, tumour necrosis factor-α and interferon-γ were not significantly different between the active and stable group in another study (18). Recent studies demonstrated that the pentraxin-3 level was associated with disease activity and more accurate than ESR and CRP for distinguishing active from the inactive disease (19-21). Those findings require further investigation to confirm the sensitivity and specificity of these candidate biomarkers.

In this study, the discovered plasma proteins with differential abundance on two-dimensional gel electrophoresis can be categorised by function into acute-phase proteins (APP) or immune-related proteins. The majority of these proteins, such as SAA, AAG, SAP, AMG, FIB, are APPs. RAG1, C3c, C7, C4a, C4BP, CFHRP-1 are immunity-related proteins. Some proteins, like C4b binding protein and CFHRP-1, are both acute-phase and immunity-related proteins. These proteins respectively reflected reactions of systemic inflammation, adaptive immunity, coagulation, tissue damage or repair in TAK. Further studies of the role of these proteins in the TAK process may help to elucidate the pathologic mechanism of this disease.

Our finding with SAA validates Nair's study which even found that SAA in TAK patients decreased during follow-up in treatment responders in a longitudinal observation (22). Koga also reported elevated levels of circulating SAA and low-grade abnormal uptake of [18F]-FDG remained detectable when the patient was in remission and had normalised CRP and ESR (23). SAA is synthesised primarily in the liver, reflecting systemic inflammatory reaction. Some chronic rheumatic diseases susceptible to amyloidoses, such as Behçet's disease and giant cell arteritis, have increased SAA (24, 25) . If the SAA level reflected not only systemic inflammation but also revealed a local pathologic change in TAK, it could explain that SAA might be a more sensitive biomarker and its elevated level in inactive patients. It is worth exploring the association between the level of SAA and local histopathology or angiographic change.

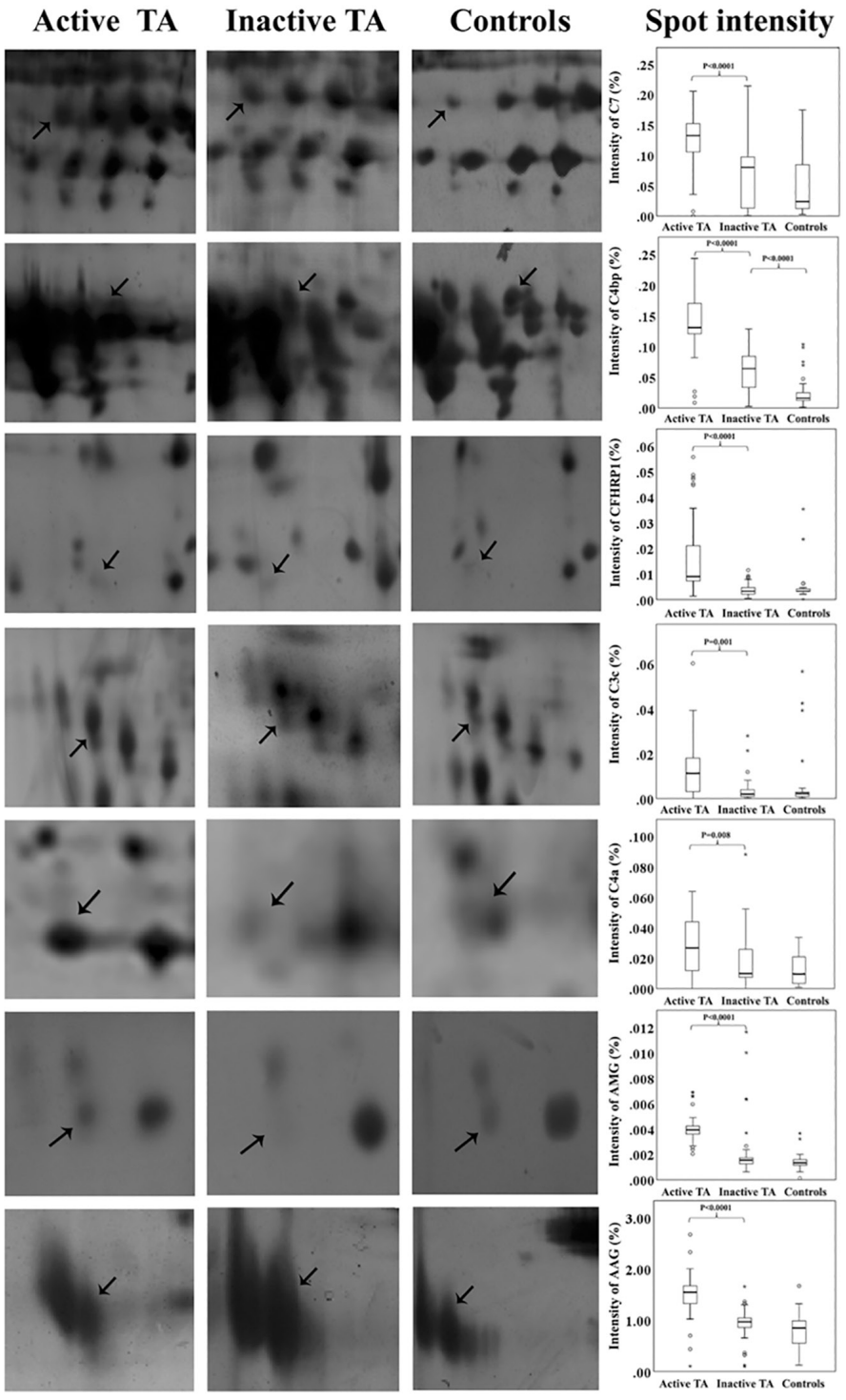

Fig. 3. The spot intensity of complement C7 (C7), complement C4a (C4a), alpha-1-microglobulin (AMG), alpha-1-acid glycoprotein 2 (AGG), complement factor H-related protein 1 (CFHRP1), complement C3c (C3c).

The immunologic mechanism of TAK is not clear. Some evidences show T cell immunity may participate in TAK (26, 27). In this study, differentially expression of RAG protein, C3c, C4a, C7, C4bp, CFHRP-1 indicated that antibody production or lymphatic cell receptors, complement activating, com-

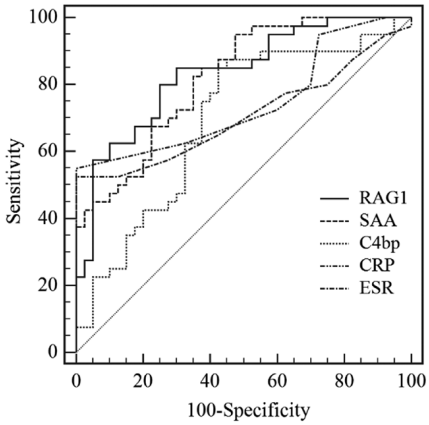

Fig. 4. Receiver operating characteristic curves of serum amyloid A(SAA), recombination activating gene-1 protein (RAG1), C4b-binding protein(C4bp), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR).

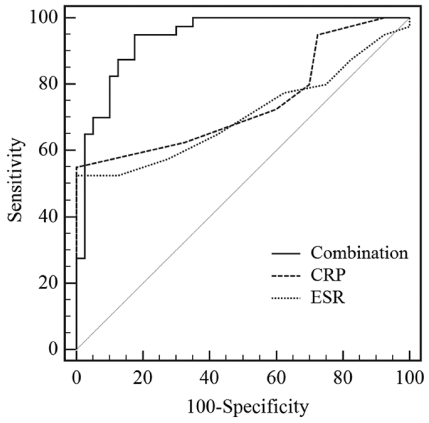

Fig. 5. Receiver operating characteristic curve of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and composite curve (Combination) of serum amyloid A(SAA), recombination activating gene-1 protein (RAG1) and C4b-binding protein(C4bp).

plement modulation may be involved in immune mechanism of TAK.

Up to now, there have been no reports about the relation between RAG proteins and Takayasu arteritis. Proteins RAG1 and RAG2 form the RAG complex and are encoded by recombination activating genes (RAGs) (28). RAGs are usually expressed in the nucleus of B and T cells and play an essential role in the production of antigen receptor diversity (29). Circulating B-lymphocytes producing autoantibodies to endothelial cells played an essential role in the pathogenesis of TAK (30). Other studies observed a higher frequency of RAG positive peripheral B cells in systemic lupus erythematosus and the synovial tissue of rheumatoid arthritis patients (31, 32). The study of the T-cell receptor repertoire of circulating T-cells in TAK showed that active TAK had a higher number of gamma delta T-cells and alpha beta T-cells compared to inactive TAK (33). These studies suggest that RAG might be involved in cellular or humoral immunity in TAK. The role of RAG in auto-antibody or change of B cell receptor or T cell receptor in TAK deserves further study. RAG protein might be a promising biomarker of the immunity reaction in TAK.

C4bp is a soluble complement regulator inhibiting classical and lectin pathways of the complement system, which could be detected in tissues of human atherosclerotic coronary lesions (34, 35). Martin *et al*. reported that C4bp plasma levels increased with the degree of tissue necrosis and severity of critical limb ischaemia (36). C4bp might be a useful biomarker of tissue injury in TAK. However, little is known about the role of C4BP in TAK.

In this study, inactive patients and healthy controls had similar levels of ESR and CRP, but the circulation levels of SAA, RAG1, and C4bp were significantly higher in inactive patients compared to healthy controls. Thus, they might be more sensitive biomarkers than ESR and CRP. These proteins might be promising biomarkers of different stages of Takayasu arteritis such as immunity or systemic inflammation, tissue damage, tissue repair or remodelling. Furthermore, a combination of SAA, RAG1, and C4bp levels might be a more reliable tool for assessing disease activity.

A limitation of this study is that it is cross-sectional and the results need the validation of a longitudinal study. Also, this is observatory clinical data, and it would be useful to examine these proteins in an experimental animal model.

In conclusion, the comparison of plasma proteomic profiling showed that active Takayasu arteritis differentially expressed some acute-phase proteins and immunity-related proteins. Differential proteins such as SAA, C4bp, and RAG1 may serve as new potential biomarkers of disease activity. Their roles in the pathology of this disease and potential treatment deserve further investigation.

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