Characteristics of B cells and immunoglobulin profile in Takayasu's arteritis

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Abstract

Objective

Disorders of humoral immunity in Takayasu's arteritis (TAK) have not been well explored. This study describes the characteristics of B cells and immunoglobulin (Ig) profile in patients with TAK.

Methods

Peripheral B cell populations assessed using flow cytometry and serum Ig levels assessed using a biochemical analyser in 98 newly diagnosed patients with TAK were analysed and compared with those of 31 patients with systemic lupus erythematosus (SLE) and 60 healthy controls (HCs). CD19+ B cell and IgG infiltration to the aortic tissue was evaluated by immunohistochemical staining.

Results

The proportion of peripheral CD3-CD19+ B cells and levels of serum IgG in TAK were lower than those in SLE, but higher than those in HCs. CD3-CD19+ B cell counts were higher in TAK than in HCs. Serum IgG and IgG1 levels were higher in active TAK than in non-active TAK. In TAK, positive correlations of serum IgG levels with erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, Kerr score, and Indian Takayasu Clinical Activity Score (ITAS2010, ITAS-A) were observed before immunotherapy. After 6 months of immunotherapy, serum Ig levels significantly decreased. Positive correlations between the changes in IgG levels and values of ESR, CRP, Kerr score, and ITAS-A were detected. Immunohistochemical staining confirmed CD19+ B cell and IgG infiltration to the aortic wall in patients with TAK.

Conclusion

Enhanced B cells might contribute to the pathogenesis of TAK, and serum IgG levels could serve as a simple, useful biomarker to assess disease activity and monitor treatment response in TAK.

Key words

B-lymphocyte, disease activity, immunoglobulin, Takayasu's arteritis

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Introduction

Takayasu's arteritis (TAK) is a form of systemic vasculitis with an unclear aetiology, which typically affects the aorta and its primary branches and can cause life- or organ-threatening ischaemic manifestations (1). Although TAK has been reported worldwide, it is a rare disorder and seems to be more prevalent among people of Asian ancestry (2). Previous studies have mainly focused on the role of cellular immunity, while disorders of B cells and humoral immunity in TAK have been ignored (3). Recently, as B cell depletion therapy was reported to be effective in a few patients with active refractory TAK, studies have proposed the impacts of B cell disturbances in the immunopathogenesis of TAK (4, 5). Additionally, the infiltration of $CD20^+$ B cells and $CD79a^+$ B cells has been detected in all three layers of arterial specimens collected from patients with TAK (6). However, studies on B cell-targeted therapy for the management of TAK have been limited (7, 8). The fluctuation of the circulating B cell population and serum immunoglobulin (Ig) profile in patients with TAK has not been well explored. Therefore, in this study, we aimed to describe the characteristics of circulating B cells and serum Ig in patients with TAK before and after immunotherapy.

Materials and methods
Patients

In this retrospective, cohort study, 98 newly diagnosed patients with TAK who were admitted to Capital Medical University affiliated Beijing Anzhen Hospital from January 2015 to December 2021, with lymphocyte subset determination, were consecutively enrolled. The diagnosis of TAK was confirmed by two senior rheumatologists according to the American College of Rheumatology (ACR) criteria (9). Patients with malignant tumours, chronic or ongoing infections, liver or kidney dysfunction, other autoimmune diseases, and pregnancy were excluded. Modified Kerr's criteria combined with the Indian Takayasu Clinical Activity Score (ITAS) were used to evaluate the activity of TAK disease; Kerr score ≥2, ITAS2010 ≥2, or ITAS-A ≥5 indicates active dis-

ease (10, 11). Numano's criteria were used to classify the angiographic findings of TAK (12). For comparison, 31 age- and sex-matched newly diagnosed patients with systemic lupus erythematosus (SLE) who were admitted to Capital Medical University affiliated Beijing Anzhen Hospital from January 2015 to December 2021, with lymphocyte subset determination, were consecutively enrolled, and their data analysed. The diagnosis of SLE fulfilled the 1997 ACR criteria and/or the 2012 Systemic Lupus International Collaborating Clinics criteria (13, 14). SLE Disease Activity Index 2000 (SLEDAI-2K) was used to evaluate SLE disease activity (15). Sixty age- and gender-matched healthy controls (HCs) who underwent medical examinations, including lymphocyte subset determination, were recruited. The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and approved by the Medical Ethics Committee of the Capital Medical University affiliated Beijing Anzhen Hospital (2021173X). Informed consent for participation was provided by all subjects enrolled in this study.

Clinical and laboratory data

Patient demographic data, clinical features, laboratory tests, and immunotherapy strategies at discharge were derived from medical records. Data of HCs were acquired from the medical examination reports. For each participant, a venous blood sample was collected in the morning after fasting for 8–12 h overnight. A second sample was collected from the patients with TAK following immunotherapy for 6 months. The populations of T-, B-, and natural killer (NK)-lymphocytes from peripheral blood were enumerated using the Multitest™ IMK Kit (662965, BD Biosciences, San Jose, CA, USA) with $CD3^+$, $CD3^-CD19^+$, and $CD3^-CD56^+CD16^+$ assays, respectively, and a BD FACSCanto II flow cytometer with BD FACSCanto™ clinical software version 2.1 (BD Biosciences) was used for the analysis. The serum Ig profiles were assessed using an automatic biochemical analyser (AU5400, Beckman Coulter, Pasadena, CA, USA). Subclasses of IgG (IgG1, IgG2, IgG3,

Table I. Clinical and laboratory data of patients with TAK, SLE, and HCs.

Parameters	TAK (n=98)	SLE (n=31)	HCs (n=60)	TAK vs. SLE p-value 1	TAK vs. HCs p-value 2	SLE vs. HCs p-value 3	p-value
Age at baseline [years, median (P25, P75)]	42.00 (29.75, 48.25)	33.00 (26.00, 58.00)	37.50 (31.25, 52.00)	0.941	0.748	0.479	0.864
Male, n (%)	6 (6.1)	1 (3.2)	3 (5.0)	0.868	1.000	1.000	0.801
CD3$^+$ T cells /lymphocytes (%, mean ± SD)	74.19 ± 6.02	67.43 ± 9.77	71.31 ± 5.61	*0.002*	*0.008*	0.136	*<0.001*
CD3$^-$CD19$^+$ B cells /lymphocytes [%, median (P25, P75)]	12.95 (10.18, 16.50)	21.50 (13.90, 29.80)	12.10 (8.80, 14.08)	*<0.001*	*0.013*	*<0.001*	*<0.001*
CD3$^-$CD56$^+$CD16$^+$ NK cells /lymphocytes [%, median (P25, P75)]	10.40 (7.25, 13.63)	5.90 (3.50, 11.60)	15.70 (11.53, 20.10)	*0.001*	*<0.001*	*0.001*	*<0.001*
CD3$^+$ T cells [/µL, median (P25, P75)]	1320.00 (1111.75, 1657.25)	795.00 (440.00, 1185.00)	1286.00 (1056.00, 1446.25)	*<0.001*	0.222	*<0.001*	*<0.001*
CD3$^-$CD19$^+$ B cells [/µL, median (P25, P75)]	232.50 (164.75, 329.50)	247.00 (103.00, 425.00)	201.50 (168.25, 249.00)	0.548	*0.013*	0.592	0.068
CD3$^-$CD56$^+$CD16$^+$ NK cells [/µL, median (P25, P75)]	183.50 (130.00, 252.25)	66.00 (42.00, 129.00)	264.50 (209.25, 369.75)	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Lymphocytes (10^9/L, mean ± SD)	1.94 ± 0.55	1.25 ± 0.75	1.95 ± 0.41	*<0.001*	1.000	*<0.001*	*<0.001*
Immunoglobulin A [g/L, median (P25, P75)]	2.65 (2.01, 3.44)	3.02 (2.19, 4.26)	2.24 (1.75, 2.84)	0.180	0.070	*0.013*	*0.035*
Immunoglobulin G (g/L, mean ± SD)	13.87 ± 4.13	23.23 ± 8.79	12.52 ± 1.82	*<0.001*	*0.023*	*<0.001*	*<0.001*
Immunoglobulin M [g/L, median (P25, P75)]	1.24 (0.93, 1.75)	1.10 (0.81, 2.12)	1.19 (0.88, 1.56)	0.821	0.372	0.709	0.687
ESR [mm/1h, median (P25, P75)]	14.50 (7.00, 36.00)	58.00 (16.00, 75.00)	10.00 (7.00, 12.00)	*<0.001*	*<0.001*	*<0.001*	*<0.001*
CRP [mg/L, median (P25, P75)]	3.17 (0.83, 19.81)	1.60 (0.41, 8.39)	0.71 (0.24, 1.83)	0.193	*<0.001*	*0.014*	*<0.001*

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; HCs: healthy controls; NK cells: natural killer cells; SLE: systemic lupus erythematosus; TAK: Takayasu's arteritis. p-values <0.05 are shown in italics and bold.

IgG4) were measured by nephelometry using an automatic protein analyser (BN II, Siemens, Marburg, Germany). C-reactive protein (CRP) levels were determined using QuikRead CRP test (Orion Diagnostica, Espoo, Finland). Lymphocytes were counted using a Sysmex XE-2100 haematology analyser (Sysmex, Kobe, Japan). The erythrocyte sedimentation rate (ESR) was measured using a Vacuette SRS100/II automatic ESR analyser (Greiner Bio-One, Kremsmunster, Austria). The manufacturer's instructions and standard monitoring approaches were followed in all tests conducted. The missing data of patients with TAK on lymphocyte subsets were 25.5% (12/47) after 6 months of immunotherapy. The missing data of HCs on lymphocyte count and IgA, IgG, IgM, ESR, and CRP levels were 13.3%, 28.3%, 28.3%, 28.3%, 18.3%, and 21.7%, respectively.

Pathology of the aorta samples
Fresh aortic tissues from patients with TAK or atherosclerosis were fixed with 4% paraformaldehyde for more than 24 h, embedded in paraffin, dissected into slices of 4 µm thickness, and dewaxed before staining. For haematoxylin and eosin staining, the sections were stained with haematoxylin (G1005-1, Servicebio, Wuhan, Hubei, China) for 3-5 min and eosin (G1005-2, Servicebio, Wuhan, Hubei, China) for 5 min. For immunohistochemical analysis, the sections were incubated with anti-CD19 primary antibody (EPR5906, ab134114, Abcam, Cambridge, UK) at a dilution rate of 1:250 or working solution of anti-IgG primary antibody (ZA-0448, ZSGB-BIO, Beijing, China) overnight at 4°C. Then, the sections were incubated with a secondary antibody for 50 min at room temperature, followed by incubation with 3,3′-diaminobenzidine.

Each slide was scanned into a whole digital image at ×40 (0.25 µm/pixel) by ScanScope® AT (Aperio Technologies, Inc., San Diego, CA), and the percentage of the immunohistochemical staining positive area in the total area was calculated (area fraction, ×10^{-5}) using NIS-Elements BR software (v. 5.30.02, Nikon, Tokyo, Japan).

Statistical analysis
IBM SPSS Statistics for Windows (version 22.0, IBM Corp., Armonk, NY, USA) was used to perform statistical analysis. Continuous variables are shown as the mean ± standard deviation or median with an interquartile range according to whether the data were normally distributed or not. Categorical variables are described as numbers and proportions. For comparisons among three groups, a one-way analysis of variance with Tamhane's post hoc test

or a Kruskal-Wallis H test was used for quantitative data depending on whether the data were normally distributed. For comparisons between two groups, a Student's paired t-test or group t-test was used for normally distributed quantitative data, and a Wilcoxon test or Mann-Whitney U-test was used for skewed distribution according to whether or not paired samples were included. A likelihood ratio, Fisher's exact test, chi-square test with Yates' continuity correction or Pearson's chi-square test was used to compare qualitative data, where relevant. The relationship of disease activity with B cells and Ig levels was examined using a Spearman correlation coefficient. Statistically significant differences were considered at a two-tailed $p < 0.05$. GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA) was used to create the line art.

Results
Clinical characteristics of the participants
Among the 98 patients with TAK, 92 (93.9%) were women. The median age at baseline was 42.00 (29.75, 48.25) years, onset age was 28.00 (23.00, 41.00) years, and disease duration was 48.00 (12.00, 180.00) months. Seventy-eight (79.6%) patients had active disease. The median disease activity was 2.00 (2.00, 3.00) in Kerr score, 5.00 (1.75, 9.00) in ITAS2010, and 7.00 (2.00, 11.25) in ITAS-A. The Numano classification of type I, type IIa, type IIb, type III, type IV, and type V was 15 (15.3%), 2 (2.0%), 17 (17.3%), 5 (5.1%), 3 (3.1%), and 56 (57.1%), respectively. Of the 31 patients with SLE, the median age was 33.00 (26.00, 58.00) years, 30 (96.8%) were female, and the average score of SLEDAI-2K was 9.90±4.33. Of the 60 HCs enrolled in this study, 37.50 (31.25, 52.00) years was the median age, and 57 (95.0%) were female.

Comparison of peripheral B cells and serum Ig levels among patients with TAK, SLE and HCs
Among the three groups, there was a significant difference in the proportion of peripheral CD3-CD19+ B cells ($p < 0.001$), which was higher in patients with SLE than in patients with

TAK (21.50 (13.90, 29.80)% *vs.* 12.95 (10.18, 16.50)%, $p < 0.001$), and in patients with TAK than in HCs (12.95 (10.18, 16.50)% *vs.* 12.10 (8.80, 14.08)%, $p = 0.013$). The counts of peripheral CD3-CD19+ B cells were also higher in patients with TAK than in HCs (232.50 (164.75, 329.50)/μL *vs.* 201.50 (168.25, 249.00)/μL, $p = 0.013$). Serum IgG levels were significantly different among the three groups ($p < 0.001$); serum IgG levels in patients with TAK were lower than those in patients with SLE (13.87±4.13 g/L *vs.*

23.23±8.79 g/L, $p < 0.001$), but were higher than those in HCs (13.87±4.13 g/L *vs.* 12.52±1.82 g/L, $p = 0.023$). No differences were found in serum IgA or IgM levels between patients with TAK and patients with SLE or HCs ($p > 0.05$; Table I).

Comparison of peripheral B cells and serum Ig levels in patients with active and non-active TAK
Compared to patients with non-active TAK, total serum IgG (14.51±4.08 g/L *vs.* 11.37±3.38 g/L, $p = 0.002$) and IgM

Table II. Clinical and laboratory data of patients with active and non-active TAK.

Parameters	Active TAK (n=78)	Non-active TAK (n=20)	p-value
Age at baseline [years, median (P25, P75)]	41.50 (29.00, 48.25)	42.00 (31.25, 50.50)	0.741
Male, n (%)	3 (3.8)	3 (15.0)	0.182
Onset age of TAK [years, median (P25, P75)]	28.50 (23.50, 40.00)	27.00 (23.00, 41.75)	0.891
Disease duration [months, median (P25, P75)]	42.00 (10.50, 171.00)	78.00 (15.00, 207.00)	0.366
Kerr score [median (P25, P75)]	2.00 (2.00, 3.00)	0.00 (0.00, 1.00)	***<0.001***
ITAS2010 [median (P25, P75)]	6.00 (4.00, 9.00)	0.00 (0.00, 0.75)	***<0.001***
ITAS-A [median (P25, P75)]	8.50 (6.00, 12.00)	0.00 (0.00, 1.00)	***<0.001***
Numano classification, n (%)			
Type I	11 (14.1)	4 (20.0)	0.760
Type IIa	2 (2.6)	0 (0.0)	1.000
Type IIb	15 (19.2)	2 (10.0)	0.521
Type III	4 (5.1)	1 (5.0)	1.000
Type IV	3 (3.8)	0 (0.0)	1.000
Type V	43 (55.1)	13 (65.0)	0.426
CD3+ T cells /lymphocytes (%, mean ± SD)	74.39 ± 6.01	73.40 ± 6.16	0.515
CD3-CD19+ B cells /lymphocytes [%, median (P25, P75)]	13.20 (10.13, 16.85)	11.65 (10.18, 15.08)	0.430
CD3-CD56+CD16+ NK cells /lymphocytes (%, mean ± SD)	10.43 ± 4.58	12.86 ± 5.45	0.078
CD3+ T cells [/μL, median (P25, P75)]	1386.50 (1122.50, 1693.25)	1166.00 (1034.75, 1449.75)	0.086
CD3-CD19+ B cells [/μL, median (P25, P75)]	243.50 (163.00, 353.75)	204.50 (166.50, 276.50)	0.158
CD3-CD56+CD16+ NK cells [/μL, median (P25, P75)]	183.00 (130.75, 240.00)	192.50 (124.75, 283.50)	0.456
Lymphocytes (10⁹/L, mean ± SD)	1.96 ± 0.59	1.84 ± 0.39	0.269
Immunoglobulin A [g/L, median (P25, P75)]	2.66 (2.06, 3.73)	2.46 (1.67, 2.92)	0.191
Immunoglobulin G (g/L, mean ± SD)	14.51 ± 4.08	11.37 ± 3.38	***0.002***
Immunoglobulin M [g/L, median (P25, P75)]	1.39 (0.95, 1.81)	0.99 (0.78, 1.19)	***0.010***
ESR [mm/1h, median (P25, P75)]	22.00 (10.00, 43.00)	6.50 (5.00, 11.75)	***<0.001***
CRP [mg/L, median (P25, P75)]	4.85 (1.49, 22.62)	1.00 (0.24, 2.58)	***<0.001***

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; ITAS: Indian Takayasu Clinical Activity Score; NK cells: natural killer cells; TAK: Takayasu's arteritis.
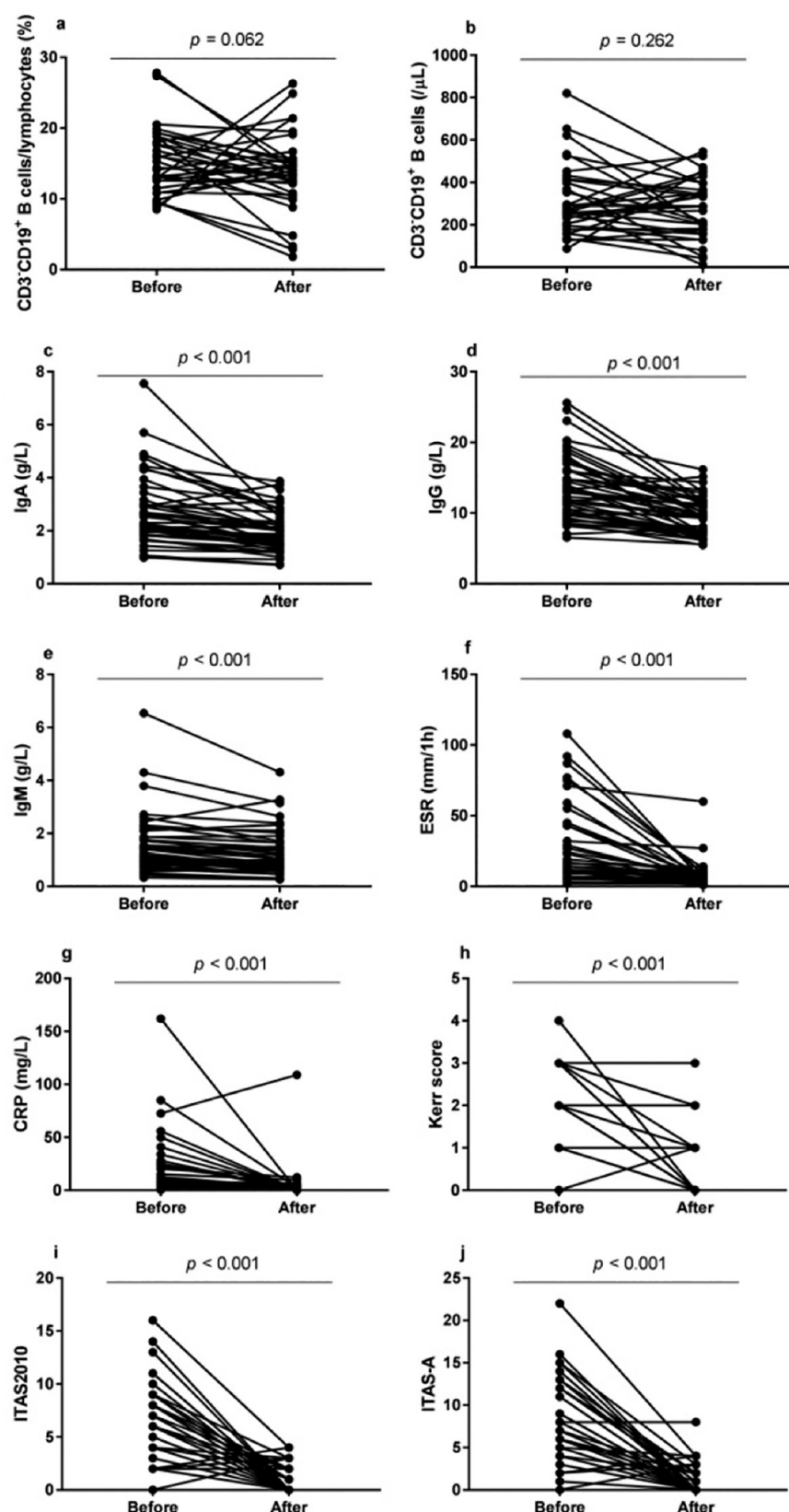p-values <0.05 are shown in italics and bold.

Fig. 1. Peripheral B cells and serum immunoglobulin profile of patients with TAK before and after immunotherapy. Serum IgA, IgG, and IgM levels along with ESR, CRP level, Kerr score, and Indian Takayasu Clinical Activity Score (ITAS2010 and ITAS-A) all decreased after immunotherapy (c-j). No differences were found in the levels of CD3⁻CD19⁺ B cells before and after treatment (a, b). TAK: Takayasu's arteritis; Ig: immunoglobulin; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

levels (1.39 (0.95, 1.81) g/L *vs.* 0.99 (0.78, 1.19) g/L, *p*=0.010) were higher in patients with active TAK. Among the subclasses of serum IgG measured in 22 patients with TAK (17 patients with active TAK and 5 patients with non-active TAK), the concentration of IgG1 (8.44 (6.99, 9.58) g/L) was the highest, and it was higher in patients with active TAK than in those with non-active TAK (8.62 (8.07, 10.37) *vs.* 6.78 (6.01, 7.91), *p*=0.015). No differences were found in the proportion or counts of peripheral CD3⁻CD19⁺ B cells, levels of serum IgA, or levels of IgG2, IgG3, and IgG4 between patients with active and non-active TAK ($p > 0.05$; Table II, Supplementary Table S1).

Comparison of peripheral B cells and serum Ig levels in patients with TAK before and after immunotherapy
Forty-seven patients with TAK were monitored by obtaining a second venous blood sample after receiving immunotherapy for 6.08 (5.10, 6.58) months. The immunotherapy regimen at discharge included glucocorticoids (34, 72.3%), methotrexate (23, 48.9%), cyclophosphamide (11, 23.4%), mycophenolate mofetil (11, 23.4%), azathioprine (3, 6.4%), tocilizumab (22, 46.8%), and adalimumab (1, 2.1%). The average cumulative dosing of glucocorticoids was prednisone 3520.47 ± 1870.82 mg or equivalent. Serum IgA, IgG, and IgM levels significantly decreased after immunotherapy (2.29 (1.95, 3.17) g/L *vs.* 1.83 (1.48, 2.37) g/L, 13.20 (9.86, 17.04) g/L *vs.* 9.53 (7.25, 11.30) g/L, 1.39 (0.90, 1.86) g/L *vs.* 1.01 (0.70, 1.64) g/L, all *p*<0.001). The lymphocytes subsets in 35 of them (74.5%) were enumerated after 6 months of immunotherapy. However, no differences were found in the proportion or counts of peripheral CD3⁻CD19⁺ B cells before and after treatment (*p*>0.05; Fig. 1).

Relationship between peripheral B cells or serum IgG levels and disease activity in patients with TAK
In the 98 patients with TAK, we found no correlation between the proportion or counts of peripheral CD3⁻CD19⁺ B cells and disease activity (ESR, CRP

level, Kerr score, ITAS2010, and ITAS-A) (*p*>0.05). As serum IgG was the only Ig whose levels varied between patients with TAK and HCs, the correlation of IgG levels with disease activity was assessed in this study. Interestingly, positive correlations of serum IgG levels with ESR (*r*=0.476, *p*<0.001), CRP level (*r*=0.364, *p*<0.001), Kerr score (*r*=0.352, *p*<0.001), ITAS2010 (*r*=0.214, *p*=0.034), and ITAS-A (*r*=0.333, *p*=0.001) were detected. In the 47 patients with TAK monitored after 6 months of immunotherapy, changes in IgG levels were positively correlated with changes in ESR (*r*=0.608, *p*<0.001), CRP level (*r*=0.525, *p*<0.001), Kerr score (*r*=0.303, *p*=0.038), and ITAS-A (*r*=0.385, *p*=0.008) values, but we found no correlation with ITAS2010 (*p*>0.05; Fig. 2).

CD19+ B cell and IgG infiltration
to the aortic wall in patients with TAK
Finally, aortic specimens from three patients with TAK and three patients with atherosclerosis (as controls) were collected. H&E staining showed marked thickening of the aortic adventitia and significant lymphocyte infiltration, whereas immunohistochemical staining showed significant CD19+ B cell and IgG infiltration in the aortic wall of patients with TAK (Fig. 3 a). The area fraction of CD19+ B cell (5.00 (3.00, 10.00) ×10^{-5} *vs.* 0.00 (0.00, 0.00), *p*=0.037) and IgG (20.00 (7.00, 20.00) ×10^{-5} *vs.* 0.00 (0.00, 0.01), *p*=0.043) was significantly higher in the aortic wall of patients with TAK compared with that in the aortic wall of HCs (Fig. 3 b).

Discussion
This study describes the characteristics of B cells and Ig profile in patients with TAK. The proportion of peripheral CD3-CD19+ B cells and levels of serum IgG in patients with TAK were lower than those in patients with SLE but higher than those in HCs. Peripheral CD3-CD19+ B cell counts were higher in patients with TAK than in HCs. Serum IgG levels and IgG1 subclass were also higher in active TAK than in non-active TAK. In patients with TAK, positive correlations of serum IgG levels with ESR, CRP level, Kerr score,
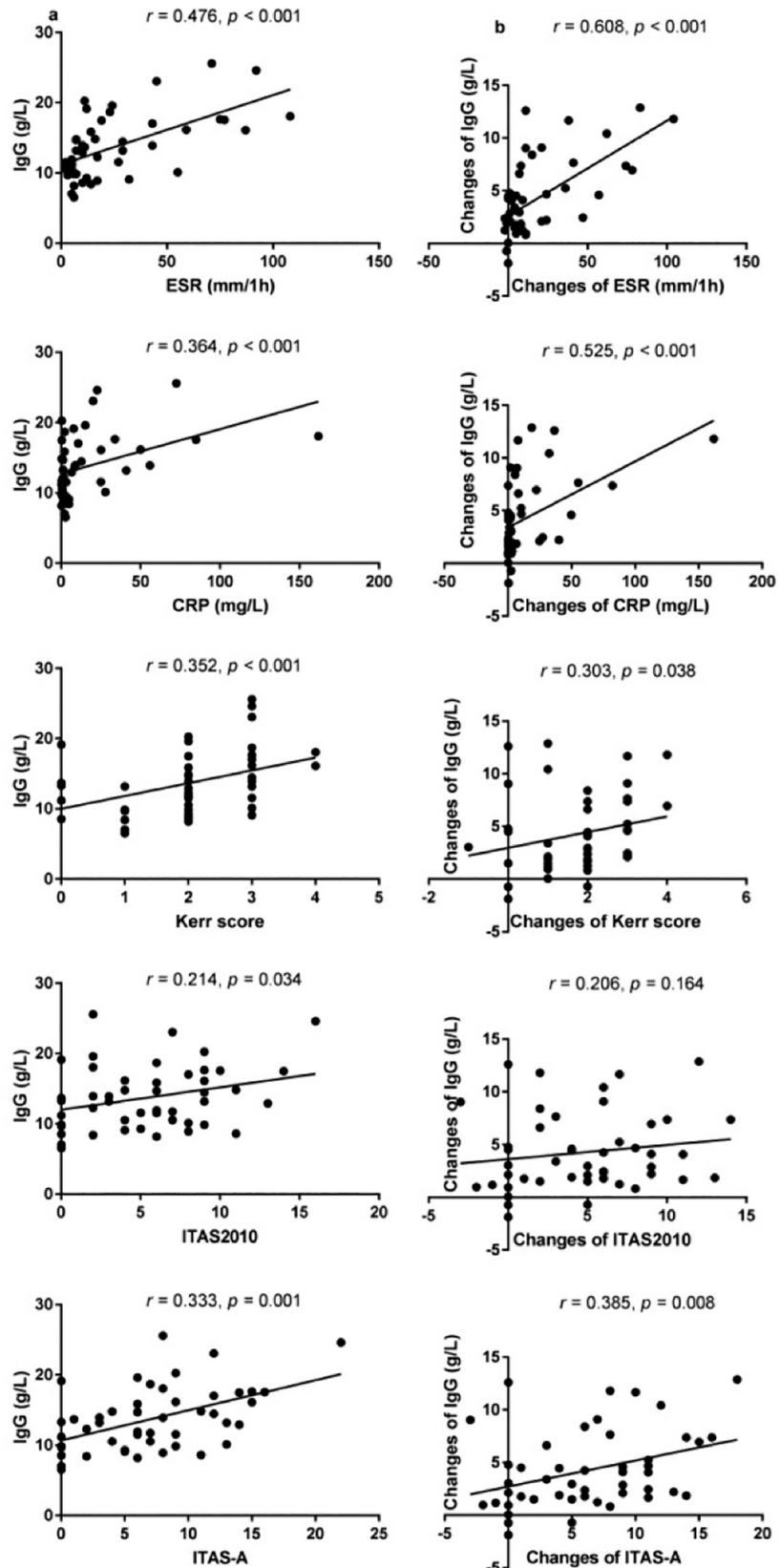

Fig. 2. Relationship between serum immunoglobulin (Ig) G level and disease activity in TAK. Before immunotherapy, positive correlations of serum IgG with ESR, CRP level, Kerr score, and Indian Takayasu Clinical Activity Score (ITAS2010 and ITAS-A) were detected (a). After immunotherapy, changes in IgG levels were positively correlated with changes in ESR, CRP level, Kerr score, and ITAS-A values, but were not correlated with changes in ITAS2010 value (b).
TAK: Takayasu's arteritis; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.
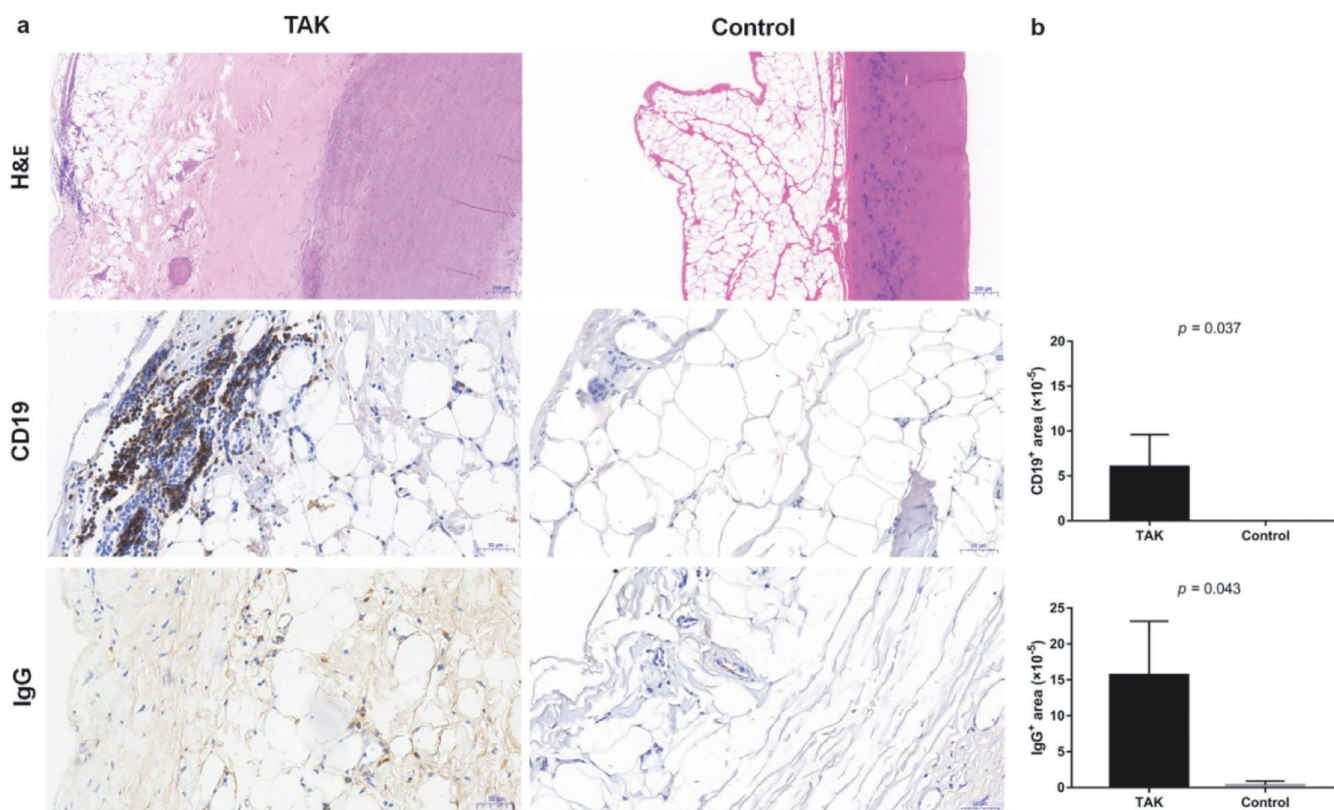
Fig. 3. CD19 and IgG expressions in the aortic specimens of patients with TAK. Marked thickening of the aortic adventitia and significant lymphocyte infiltration (round blue cells, H&E staining, ×40) and CD19+ B cell and IgG infiltration (brown cells, immunohistochemical staining, ×200) in the aortic tissue of a 53-year-old woman with TAK compared with that in a 57-year-old man with atherosclerosis (a). The area fractions of CD19+ B cell and IgG were both higher in patients with TAK (n=3) compared to those in the controls (n=3) (b).
TAK: Takayasu's arteritis.

ITAS2010, and ITAS-A were detected before immunotherapy. After 6 months of immunotherapy, serum IgG levels were significantly decreased. Positive correlations between the changes in IgG levels and values of ESR, CRP, Kerr score, and ITAS-A were detected. Immunohistochemical staining confirmed CD19+ B cell and IgG infiltration to the aortic wall in TAK.

TAK is an autoimmune disease characterised by inflammation within the arterial wall, which can lead to aneurysm, dilation, occlusion, and/or stenosis of the involved large and medium arteries [16]. Activation of T cells, including CD4+ T cells and CD8+ T cells and their subsets, has been shown to play an important role in TAK [3, 17, 18]. Consistently, we found a higher proportion of peripheral CD3+ T cells in patients with TAK compared to that in HCs. Moreover, both the frequency and counts of CD3−CD56+CD16+ NK cells in peripheral blood were lower in patients with TAK than in HCs. Li

et al. [19] suggested that not only the counts of NK cells and their subsets but also their cytotoxicity declined in TAK. Although the immunopathogenesis of TAK has been constantly studied in recent years, it remains to be further elucidated, especially regarding the response of B cells in TAK [20, 21]. SLE is a prototypical autoimmune disease caused by the over-activation and proliferation of B cells and is characterised by the production of numerous autoantibodies and increased serum IgG levels [22]. However, thus far, ideal autoantibodies for identifying TAK have not been identified, and the fluctuation of Ig levels in TAK has been rarely studied [23]. Following stimulation by specific immunogens, B cells can terminally differentiate into plasma cells, which produce Ig or antibodies and participate in humoral immunity [24]. Within the approximately 10 B cell-specific surface molecules that have been identified, CD19 and CD20 have broad expression patterns and a large

overlap between them; CD19 is a glycoprotein of the Ig superfamily, which is expressed by almost all B-lineage cells from the very early stage (pro-B cells) up to essentially all later stages (including mature B cells, most plasmablasts and plasma cells), whereas CD20 is mainly expressed on mature B cells [25]. Therefore, because CD19 not only can help determine B cell proliferation but also may provide useful information on B cell activation, it is preferable when only a limited number of B cell surface markers are available [26]. In our study, although the proportion of peripheral CD3−CD19+ B cells and levels of serum IgG in TAK were lower than those in SLE, they were significantly higher than in HCs. This indicated that B cells underwent proliferation and activation to some extent in TAK. Consistent with this finding, the counts of peripheral CD3−CD19+ B cells were also higher in patients with TAK than in HCs. However, no differences were found in the number of CD3−CD19+ B

cells in peripheral blood between TAK and SLE. We inferred that the high prevalence of lymphopenia in SLE reduced the discrepancy (27). Similarly, a larger extent of CD19$^+$ B cell as well as more marked IgG infiltration to the aortic tissue were detected in patients with TAK compared to the controls, which further indicates that the enhanced B cell populations may contribute to the pathogenesis of TAK. In fact, the results of Desbois *et al.* (5) support our findings at the genetic level; they reported an enrichment in a gene signature in TAK, which is specific for the activation/proliferation of B cells.

Unlike the striking discrepancy in the proportion and/or counts of T, B, and NK cells in patients with TAK compared to HCs, no significant differences were found in them between active and inactive TAK. However, Hoyer *et al.* (4) found that the number of newly generated plasmablasts, CD19$^+$/CD20$^-$/CD27^{high}/HLA-DR$^+$, in the peripheral blood of patients with active TAK was positively correlated with disease activity. We inferred that there might be a positive correlation between serum Ig profile and TAK disease activity. As serum IgG was the only Ig whose levels varied between patients with TAK and HCs, we investigated its association with disease activity. Thus, we found that total serum IgG levels were higher in patients with active TAK than in their non-active counterparts. However, when we further analysed the subclasses of IgG, only the IgG1 levels were higher in active TAK than in non-active TAK. We speculate that this is because the IgG1 subclass accounts for the largest proportion of serum IgG. Additionally, a positive correlation of serum IgG levels with disease activity was detected before immunotherapy in our study. This implies that serum IgG level could serve as a simple and useful biomarker to assess disease activity in patients with TAK.

Thus far, a recommended treatment strategy for TAK involves a combination of glucocorticoids with non-biological immunosuppressive agents (for all cases) or biological agents targeting interleukin-6 or tumour necrosis factor-alpha (for refractory cases) (28, 29). In this study, we monitored peripheral B cell counts, serum Ig levels, and other disease activity parameters after receiving immunotherapy for 6 months. Interestingly, serum IgA, IgG, and IgM levels were all found to be significantly decreased after immunotherapy, but the levels of peripheral CD3$^-$CD19$^+$ B cells were unchanged. This observation suggests that regular long-term immunotherapy could inhibit the activation of plasma cells and ameliorate hyperglobulinemia, while it seemed not to interfere with the proliferation of B cell populations. Furthermore, we found that changes in serum IgG levels were positively correlated with changes in other known parameters of disease activity (*e.g.* ESR, CRP level, Kerr score, and ITAS-A values) after immunotherapy, indicating that serum IgG could also serve as a valuable biomarker to monitor the response of long-term immunotherapy in patients with TAK.

We recognise some limitations in the design of this retrospective observational study. Although patients with TAK received regular immunotherapy, the treatment strategies were not consistent; and as most of the newly diagnosed patients had active disease, the sample size of patients with inactive TAK was relatively small. To address the influence of selection bias, our research questions should be further explored in future prospective, multicentre, and interventional studies.

In conclusion, our results indicated that enhanced B cell populations might contribute to the pathogenesis of TAK, and serum IgG level could serve as a simple, useful biomarker to assess disease activity and monitor treatment response in patients with TAK.

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