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ORIGINAL RESEARCH

Biomarker Changes and Molecular Signatures Associated with Takayasu Arteritis Following Treatment with Glucocorticoids and Tofacitinib

Xiaojuan Dai[1], Jinghua Wang[1], Xiao Zhang[1], Li Wang[1], Sifan Wu[1], Huiyong Chen[1], Ying Sun[1], Lili Ma[1], Lingying Ma[1], Xiufang Kong[1], Lindi Jiang[1,2]

[1]Department of Rheumatology, Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China; [2]Center of Clinical Epidemiology and Evidence-Based Medicine, Fudan University, Shanghai, People's Republic of China

Correspondence: Lindi Jiang; Xiufang Kong, Email zsh-rheum@hotmail.com; kongxiufang2007@163.com

Objective: This study aimed to analyze biomarker changes in patients with TAK following treatment with glucocorticoids (GCs) and tofacitinib (TOF).

Methods: Seventeen patients from a prospective TAK cohort treated with GCs and TOF and 12 healthy individuals were recruited. TAK associated cytokines, chemokines, growth factors, and MMPs were analyzed in these patients before and after GCs and TOF treatment, and healthy controls. Molecular signatures associated with clinical features were evaluated.

Results: Patients' cytokines (PTX3, IL-6, IFN-γ), chemokines (IL-16, CCL22, CCL2), growth factors (VEGF), and MMP9 levels were significantly higher at baseline (all $p < 0.05$), while patients' FGF-2 levels were significantly lower ($p = 0.02$). After treatment, IL-10 was significantly increased at 6 months ($p=0.007$), and inflammatory cytokines such as PTX3, IL-6 demonstrated a downward trend. Patients without vascular occlusion had higher baseline CCL22 levels than patients with it ($p = 0.05$), which remained persistently higher after treatment. Radar plot analysis demonstrated that PTX3 was closely correlated with disease activity. In addition, patients without imaging improvement had relatively higher baseline levels of CCL22, FGF-2, and PDGF-AB ($p = 0.056$, $p = 0.06$ and $p = 0.08$ respectively) and lower baseline levels of TNFα, ESR, and CRP ($p=0.04$, $p=0.056$, $p=0.07$, respectively) compared with patients without it.

Conclusion: GCs and TOF are effective in decreasing inflammatory molecules but have limited efficacy in regulating multiple other markers involved in TAK. PTX3 is a prominent marker for disease activity, and CCL22 may have a predictive value for vascular progression.

Keywords: Takayasu arteritis, tofacitinib, molecular signature, biomarker changes

Introduction

Takayasu's arteritis (TAK) is a chronic, non-specific, granulomatous macrovasculitis that mainly affects the aorta and its branches.[1,2] Although the pathogenesis of TAK remains unclear, it is established that multiple pathological processes are involved in the development of TAK.

There are many biomarkers representative of different pathological processes in TAK. Most of the identified biomarkers are inflammatory cytokines, such as pentraxin 3 (PTX3), interleukin 6 (IL-6), interferon-γ (IFN-γ), IL-8, IL-17, and YKL-40.[3–6] Elevation of matrix metalloproteinases (MMP), including MMP2, 3, and 9, have also been reported in active TAK,[7] which is thought to indicate an inflammatory response. Since MMPs are also involved in tissue fibrosis, elevated levels of MMPS in TAK may also contribute to vascular fibrosis. In addition, chemokines such as RANTES and CCL2 have been reported to associate with TAK disease activity.[8,9] Recently, we investigated chemokine profiles in TAK,[10] and we identified a potential role for IL-16 and CCL22 in the pathogenesis of TAK. Moreover, angiogenic factors such as vascular endothelial growth factor (VEGF) may be involved in vascular endothelial damage

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and neovascularization in TAK.[11] However, it is unknown which specific biomarkers that closely reflect disease activity or vascular changes in TAK and whether these abnormal processes can be prevented by current therapies.

According to guidelines set by the American College of Rheumatology (ACR) in 2021, glucocorticoids (GCs) and immunosuppressants (IS), such as methotrexate (MTX), are recommended as initial treatment regimens in TAK. However, only about 58% patients achieve clinical remission on these treatments, and relapses are common under current practices of GCs tapering.[12] Recently, studies of janus kinase (JAK inhibitors) especially JAK1/3 inhibitor, have shown their treatment effects in multiple autoimmune diseases or inflammatory diseases.[13] In our previous research, we found that JAK1 signaling participated in vascular fibrosis mediated by IL-6 in TAK.[14] Tofacitinib (TOF) is a JAK inhibitor preferentially acting on JAK1 or JAK3, which has shown a promising treatment efficacy and favorable safety profile in TAK.[15] Our former research also reported that over 85% patients could achieve clinical remission after 12 months of TOF treatment, and experienced a low relapse rate in TAK.[16] Moreover, animal models of TAK also revealed that TOF could suppress microvascular angiogenesis.[17] However, changes in levels of serum cytokines, chemokines, and growth factors in TAK patients after TOF treatment remain unknown. But changes in levels of serum cytokines, chemokines, and growth factors in TAK patients after TOF treatment remain unknown.

Thus, the purpose of this study was to analyze changes in serum biomarkers including inflammatory cytokines, MMPs, chemokines, and growth factors in patients after TOF treatment and to discover potential molecular signatures correlated with various disease characteristics of TAK.

Materials and Methods

Patients

This study was performed on patients recruited to a prospective TAK cohort, named East China Takayasu Arteritis (ECTA). This cohort was established beginning in 2009 by the Department of Rheumatology, Zhongshan Hospital, Fudan University. All patients in this cohort met the 1990 ACR classification criteria.[18] Patients were followed up and assessed according to a predesigned protocol.[16] All the clinical data were uniformly recorded in a database. The establishment of this cohort was approved by the Ethics Committee of Zhongshan Hospital Affiliated to Fudan University ((B-2016-168 (2) R). Recruitment and study of this cohort was conducted in accordance with the Declaration of Helsinki 1964 and its later amendments. All patients provided written informed consent.

In this cohort, a subgroup of patients, who were in active disease phase defined by National Institutes of Health (NIH) criteria ≥2 points[19] and had no concurrent tumors, infections or other autoimmune disease, was prospectively enrolled and treated with GCs and TOF (5 mg b.i.d.) since May 1st, 2019. Magnetic Resonance Angiography (MRA) was performed at baseline and then every 6 months during the follow-up. The clinical assessment of these patients following treatment with GCs and TOF has been reported recently.[16] To evaluate molecular changes in these patients, 17 patients with serum samples at 0, 6, and 12 months of treatment were included. The other 10 patients were excluded due to incomplete samples, since they received followed-up care at local centers during the pandemic period of COVID-19. All the 17 patients enrolled in this study have completed 12 months' clinical and MRA assessment. Among the 17 patients, six patients were treatment-native, while 11 patients were treatment-refractory (defined as a failure to respond to ≥2 conventional immunosuppressive agents in their previous treatment).

In addition, 12 subjects with matched gender and age were also included for serum examination in the current research. These healthy controls were recruited from subjects for health checkup in our center during the same study period and no disease records such as autoimmune disease, malignant tumors were found among them.

Detection of Serum Molecular Biomarkers

In this study, potential molecular biomarkers of TAK were evaluated in healthy controls and in TAK patients at baseline or after TOF treatment (6 and 12 months), including cytokines (PTX3, IL-6, IFN-γ, IL-17, TNF-α, YKL40, IL-10), chemokines (IL-16, CCL22, CCL2, CCL5), growth factors (VEGF, FGF-2, PDGF-AB), and MMPs (MMP1, MMP2, MMP3, MMP9). Among these, 12 molecules including TNF-α, IFN-γ, IL-6, IL-8, IL10, MMP1, MMP2, MMP9, VEGF, PDGF-AB, CCL5, and FGF-2 were evaluated by a customized magnetic Luminex assay (R&D Systems, Inc.,

Minneapolis, MN, USA) according to the manufacturer's instructions. The other molecules, including IL-17, PTX3, YKL40, MMP3 and CCL2, CCL22, and IL-16 were detected using ELISA (enzyme-linked immunosorbent assay)) kits (R&D Systems, Minneapolis, MN, USA).

Clinical Assessment

To evaluate potential relationships between serum biomarkers and clinical characteristics of TAK, the clinical data of these 17 TAK patients at baseline and after TOF treatment were retrieved from the database. Clinical data retrieved included symptoms, signs, lab results, and imaging data. In addition, the disease condition of patients after treatment was assessed according to the following characteristics: (1) disease activity (NIH score ≥2);[19] (2) complete remission (CR) and partial remission (PR); (3) imaging changes: imaging progression was defined as occurrence of new lesions/vascular stenosis or ≥20% progression of wall thickening of the original lesions confirmed by MRA. Imaging improvement was defined as a ≥20% increase of the lumen of the original lesion confirmed by MRA. The definitions of CR, PR are the same used in our previous cohort study.[16]

Statistical Analyses

Categorical variables were described as frequency and percentage. Continuous variables with a normal distribution were expressed as mean ± standard deviation (SD), while continuous variables with skewed distribution were represented as median and interquartile range (IQR). The Student's *t*-test or Mann Whitney's test was used to compare baseline molecular levels of TAK patients with those of healthy controls, as appropriate. The paired *t*-test or Wilcoxon rank test was applied to compare the molecular levels of patients at baseline with levels at 6 or 12 months after treatment, as appropriate. In addition, Spearman correlation analysis was used to assess the correlation between potential factors and ESR or CRP. Finally, radar chart analysis was used to evaluate molecular signatures related to clinical characteristics of TAK, such as disease activity and treatment. SPSS 23.0 (IBM, Armonk, NY, USA) and Prism 9.0.0 (GraphPad, La Jolla, CA, USA) were employed for statistical analyses. A p-value of $p < 0.05$ was considered to indicate a statistically significant difference.

Results

Patients' Characteristics

The demographic and clinical characteristics of the patients with TAK are shown in Table 1. The average age of the patients was 28.12 ± 8.99 years; there were 14 female and 3 male patients. Median disease duration among these patients was 15.00 (IQR: 12.00, 35.5) months. All patients were in the active stage of the disease; six (35.29%) patients took GCs and TOF as their initial treatment. In addition, seven (41.18%) patients had vascular occlusion on culprit arteries. There were no significant differences between TAK patients included in this study and TAK patients excluded from this study due to incomplete samples regarding treatment with GCs and TOF in this cohort (Supplementary Table S1). The gender ratio of healthy controls was 9:3 (female: male), and their average age was 28.25 ± 1.76 years.

Baseline Levels of Cytokines, Chemokines, Growth Factors, and MMPs

The levels of serum cytokines, chemokines, growth factors, and MMPs in TAK patients and healthy controls were evaluated, and differences between them were determined (Figure 1). Levels of cytokines (PTX3, IL-6, IFN-γ) (Figure 1A), chemokines (CCL22, IL-16, CCL2) (Figure 1B), growth factor (VEGF) (Figure 1C VEGF), and MMP9 (Figure 1D) were significantly higher in TAK patients at baseline than in healthy controls (all $p < 0.05$). Levels of FGF-2 were significantly lower in patients with TAK than in healthy controls ($p = 0.02$, Figure 1C FGF-2). No differences were observed regarding other factors (Figure 1).

Disease Evaluation After Treatment with GCs and TOF

Clinical characteristics of TAK patients after treatment are reported in Table 2. Patients' ESR and CRP levels were significantly reduced at 6 months ($p = 0.02$, $p = 0.047$ respectively, Table 2). After 6 and 12 months of treatment, 1 (5.88%) and 1 (5.88%) patient were still in active disease respectively. Meanwhile, 16 (94.12%) and 16 (94.12%)

Table 1 Patient Clinical Characteristic

Parameters	Results
General information	
Females: males (n)	14:3
Age (mean ± SD, years)	28.12±8.99
Disease duration (median, IQR, months)	15.00 (12.00, 35.50)
Disease activity, n (%)	17 (100)
Naive patients: refractory patients	6: 11
Imaging type, n (%)	
I	3 (17.65)
II	3 (17.65)
III	3 (17.65)
IV	1 (5.88)
V	7 (41.18)
Symptoms and signs, n (%)	
Systemic symptoms	13 (76.47)
Fever	3 (17.65)
Weakness	11 (64.71)
Weight loss	1 (5.88)
Ischemia symptoms or signs	15 (88.24)
Headache/dizziness	9 (52.94)
Chest pain/distress	4 (23.53)
Vascular murmur	9 (52.94)
Neck pain	6 (35.29)
Laboratory results (median, IQR)	
ESR (mm/H)	21.00 (2.50, 53.50)
CRP (mg/dl)	6.30 (0.30, 38.80)
Previous treatment, n (%)	11 (64.71)
GCs alone	3 (17.65)
GCs + MTX	1 (5.88)
GCs + LEF	4 (23.53)
GCs + MMF	2 (11.76)
GCs + TCZ	1 (5.88)

Abbreviations: SD, standard deviation; IQR, interquartile range; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; MTX, methotrexate; GCs, glucocorticoids; LEF, leflunomide; MMF, mycophenolate mofetil; TCZ, tocilizumab.

patients achieved CR at 6 and 12 months, respectively. After 12 months' treatment, 6 (35.29%) of patients exhibited improvement in vascular lesions, while 1 (5.88%) patient presented vascular imaging progression (Table 2). No patients experienced side effects such as gastrointestinal discomfort, liver and kidney function injury or other adverse reactions.

Changes of Cytokines, Chemokines, Growth Factors, and MMPs After Treatment

Molecular changes in TAK patients were analyzed over the 12 months of treatment with GCs and TOF (Figure 2). As a whole, PTX3, IL-6, YKL-40 and VEGF showed a downward trend, while IL-8, CCL22, FGF and MMP2 presented an upward trend over the course of treatment. In detail, IFN-γ, TNF-α, IL-10, PDGF-AB and MMP9 demonstrated an increase trend in the first 6 months and then a decrease during 6 to 12 months, while IL-17 and MMP3 presented a decrease trend in the first 6 months and then an increase trend during 6 to 12 months.

The specific data were shown in Supplementary Figure S1. Compared with baseline, IL-10 was significantly increased at 6 months ($p < 0.01$, Supplementary Figure S1A IL-10). No statistical differences were observed in other factors at 6 or 12 months in contrast with their baseline levels. In comparison with 6 months, TNF-α was significantly reduced (p =

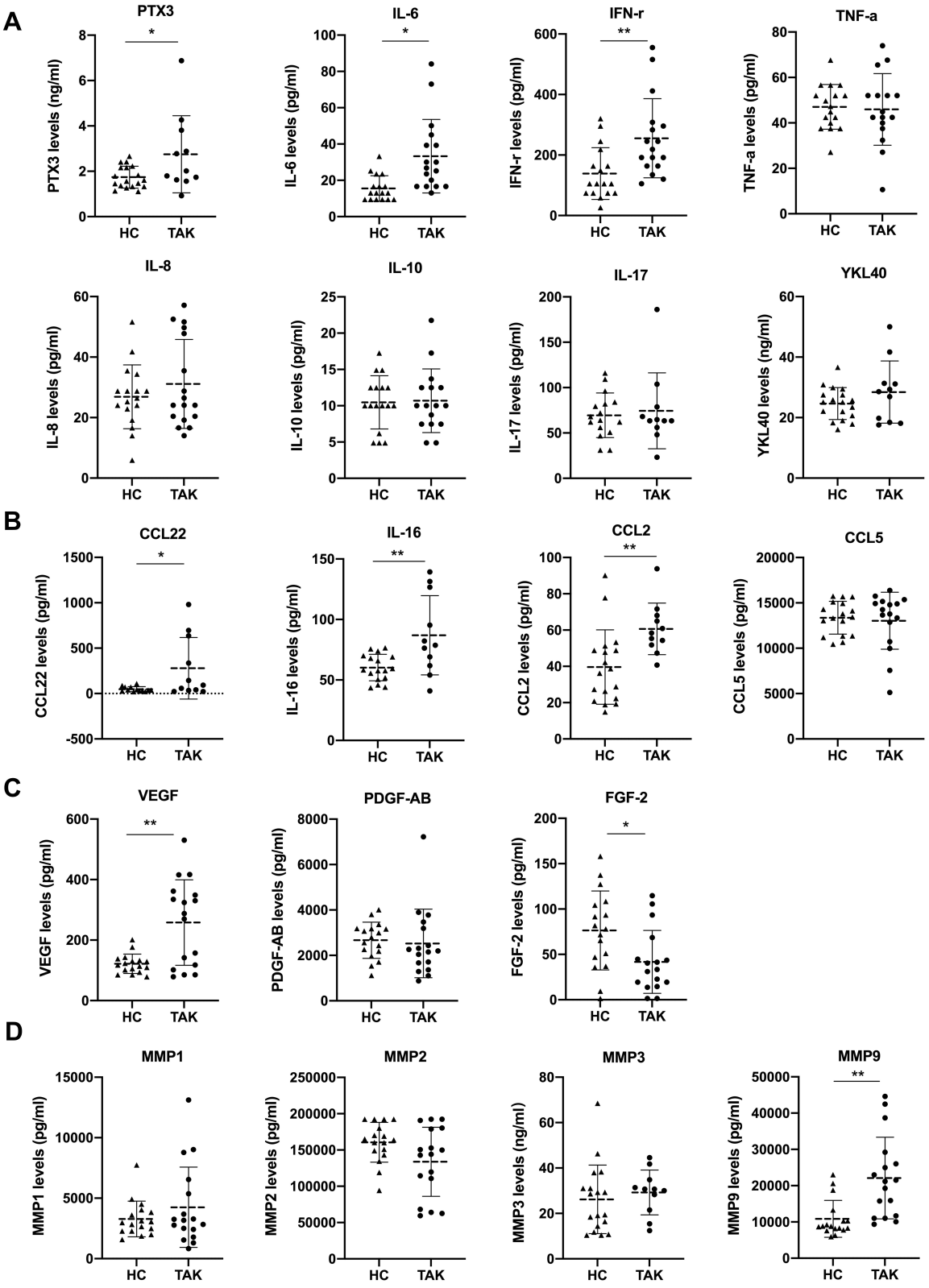

Figure 1 Baseline levels of cytokines, chemokines, growth factors Baseline levels of plasma cytokines (**A**), chemokines (**B**), growth factors (**C**), and MMPs (**D**) in healthy controls and patients with TAK. *P < 0.05, **P < 0.01.

Abbreviations: HC, healthy control; TAK, Takayasu arteritis.

Table 2 Disease Evaluation After Treatment with GCs and TOF

	6 Months	12 Months
Disease activity, n (%)		
NIH ≥2	1 (5.88)	1 (5.88)
NIH<2	16 (94.12)	16 (94.12)
Laboratory results (median, IQR)		
ESR (mm/H)	8.00 (4.00, 17.00)*	11.00 (2.50, 21.00)
CRP (mg/dl)	3.30 (0.55, 7.15)*	5.59 (0.35, 8.95)
Imaging evaluation after treatment, n (%)		
Stable lesions	13 (76.47)	14 (82.35)
Imaging improvement	3 (17.65)	3 (17.65)
Imaging progression	1 (5.88)	0
Evaluation of treatment efficacy, n (%)		
Complete remission	16 (94.12)	16 (94.12)
Partial remission	1 (5.88)	1 (5.88)

Note: *P<0.05, baseline vs 6 months.
Abbreviations: GCs, glucocorticoids; TOF, tofacitinib.

0.03, Supplementary Figure S1A TNF-α), and IFN-γ levels also showed a decreasing tendency at 12 months (p = 0.08, Supplementary Figure S1A IFN-γ). No differences were found among other factors between 6 and 12 months.

Potential Biomarkers Related with TAK Disease Activity

To identify biomarkers that might be related to TAK disease activity, different panels of molecules were evaluated in the TAK patients with NIH scores at baseline using radar plot. Patterns of changes in each molecular panel from baseline to 6 or 12 months of treatment were also analyzed (Figure 3). The radar plot indicates that the levels of PTX3 presented an increasing trend as the increase in NIH scores at baseline (Figure 3A). Similarly, levels of PTX3 were reduced at 6 and 12 months, when patients' NIH scores were also decreased (Figure 3B). However, the other cytokines were not closely related to changes in NIH score. In addition, CCL22 demonstrated persistent high levels despite changes in NIH score or treatment (Figure 3C and D). VEGF (Figure 3E and F) and MMP9 (Figure 3G and H) were higher when patients' NIH scores were 3 or 4, but did not decrease significantly after treatment.

To further clarify the relationship between PTX3 and TAK disease activity, the correlation between PTX3 levels and ESR, CRP, or IL-6 levels were analyzed. PTX3 was moderately correlated with IL-6 levels (p = 0.04, rho = 0.50; Supplementary Figure S2A) and CRP (p = 0.08, rho = 0.62; Supplementary Figure S2B), but not with ESR (Supplementary Figure S2C).

Molecular Signature Associated with Vascular Lesions and Imaging Changes

To evaluate potential biomarkers that may relate to vascular lesions, baseline biomarker levels were compared between patients with and without vascular occlusion (Table 3). Patients without vascular occlusion had significantly higher CCL22 (p = 0.05). To explore potential markers that can predict vascular imaging changes after treatment, baseline molecular levels were compared between patients with and without imaging improvement (Table 3). Patients with imaging improvement had relatively higher baseline inflammatory markers, including TNFα, ESR and CRP (p = 0.04, p = 0.056, and p = 0.07, respectively), while patients without imaging improvement had relatively higher baseline profibrotic factors, including CCL22, FGF-2 and PDGF-AB (p = 0.056, p = 0.06, and p = 0.08, respectively). Besides, we also compared the molecular characteristics of vascular lesions or imaging changes in native patients and refractory patients separately (Supplementary Tables S2 and S3). Among refractory patients, relatively higher baseline CCL22 and PDGF-AB levels were also observed in patients without vascular improvement in contrast to patients with vascular improvement (p = 0.11, p = 0.12, respectively). It is worth noting one patient with persistent CCL22 levels under normal median value demonstrated improvement in vascular imaging after treatment (Figure 4A), while in two other patients

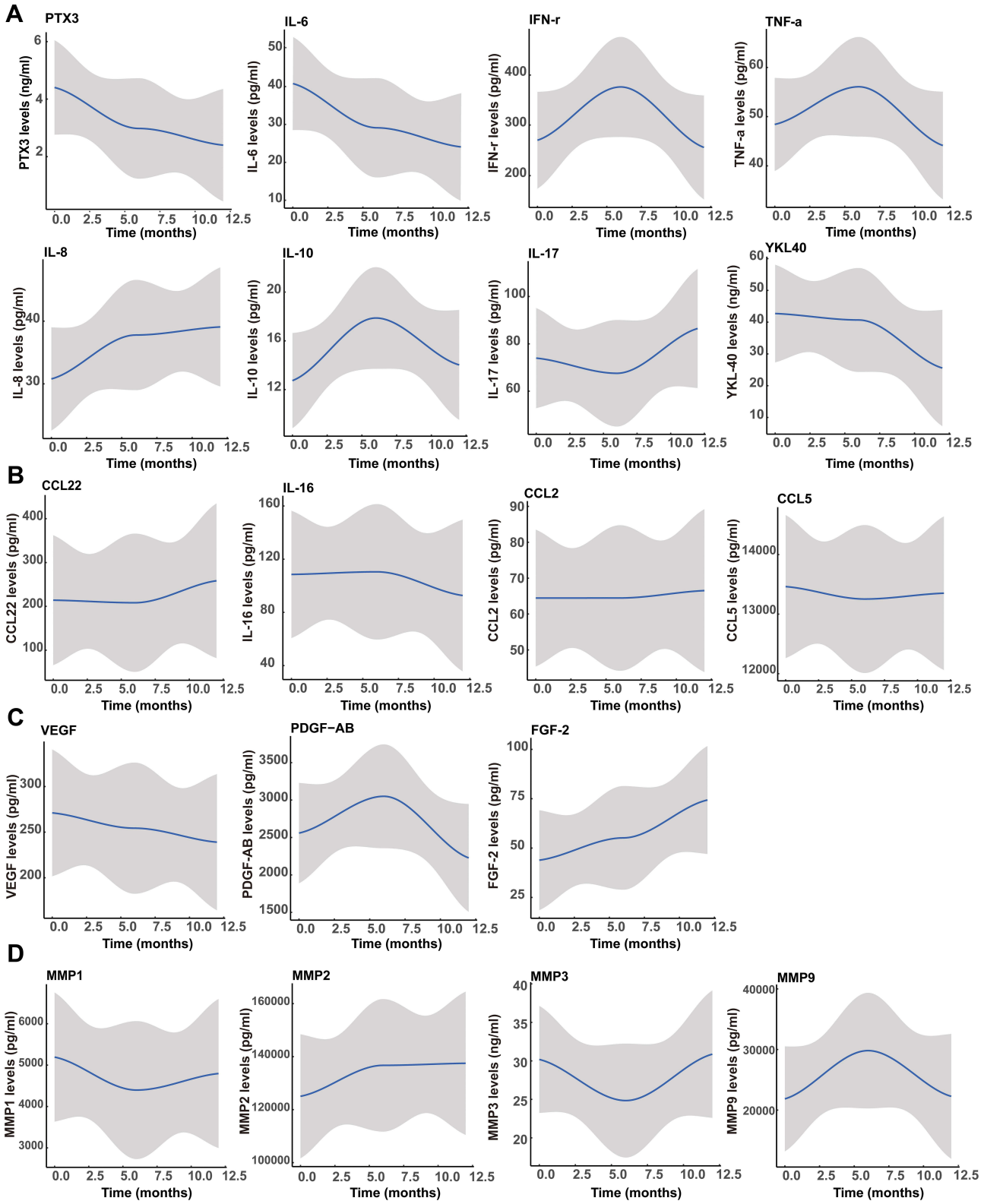

Figure 2 Changes in cytokines, chemokines, growth factors, and MMPs after treatment with GCs and TOF in TAK patients. Changes in the levels of cytokines (**A**), chemokines (**B**), growth factors (**C**), and MMPs (**D**) at 6 and 12 months after treatment with GCs and TOF.

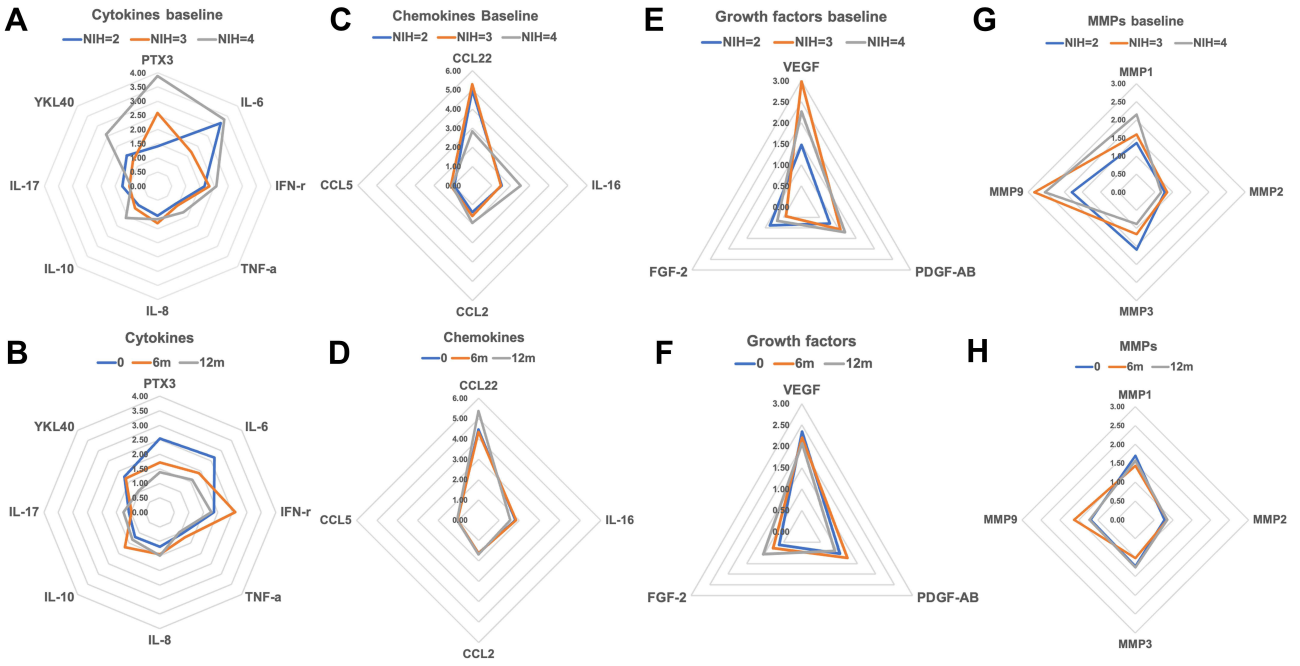

Figure 3 Potential biomarkers related with disease activity in TAK. **(A, C, E**, and **G)**: Evaluation of different groups of factors that may be related to NIH score at baseline by radar plot. (**B, D, F**, and **H)**: Changes in each group of factors at baseline, 6 months, and 12 months after treatment visualized by radar plot.

with continuously higher levels of CCL22 or FGF-2 demonstrated deteriorated vascular lesions after treatment, presenting as a thicker vascular wall or more stenotic vascular lumen (Figure 4B and C).

Discussion

The present study comprehensively evaluated the changes of peripheral cytokines, chemokines, growth factors, and MMPs in TAK patients after GCs and TOF treatment, and explored the relationships between different molecular profiles with TAK disease activity or vascular imaging characteristics. The results indicated that GCs and TOF treatment were effective in suppressing the expression of certain inflammatory parameters, such as PTX3 and IL-6, but was incapable of inhibiting many other factors that are related to chemotactic or fibrotic processes, such as CCL22 or FGF-2. Moreover, PTX3 was also identified as a prominent marker for TAK disease activity, and CCL22 might have a predictive value for vascular imaging progression following treatment with GCs or TOF.

Table 3 Molecular Signatures Associated with Vascular Lesions or Imaging Changes in TAK

Baseline Biomarkers	Baseline Vascular Occlusion			Post-Treatment Vascular Imaging Improvement		
	No (n=10)	Yes (n=7)	P value	No (n=11)	Yes (n=6)	P value
CCL22 (pg/mL)	315.80±341.90	69.00±68.17	0.05	289.14±323.19	69.91±87.66	0.056
IL-17 (pg/mL)	79.74±41.18	65.74±24.25	0.44	75.84±20.35	57.64±22.20	0.11
TNF-a (pg/mL)	49.31±18.92	46.93±12.87	0.79	42.11±15.95	59.29±12.27	0.04
MMP9 (pg/mL)	21647.50±13107.26	22150.71±9880.51	0.93	18419.8±9977.65	28226.83±12309.10	0.10
FGF-2 (pg/mL)	51.85±41.17	23.92±17.58	0.10	52.60±40.32	22.85±15.63	0.06
PDGF-AB (pg/mL)	2763.02±1893.23	2510.40±291.77	0.49	3202.01±1778.96	2046.66±457.61	0.08
ESR (mm/H)	38.20±41.55	29.57±33.76	0.66	21.09±33.75	58±35.28	0.056
CRP (mg/dL)	39.80±67.89	21.84±27.19	0.52	15.03±30.31	64.25±75.69	0.07
PTX3 (ng/mL)	4.26±4.97	4.70±4.62	0.86	3.75±3.77	6.34±5.94	0.28

Abbreviation: TAK, Takayasu arteritis.

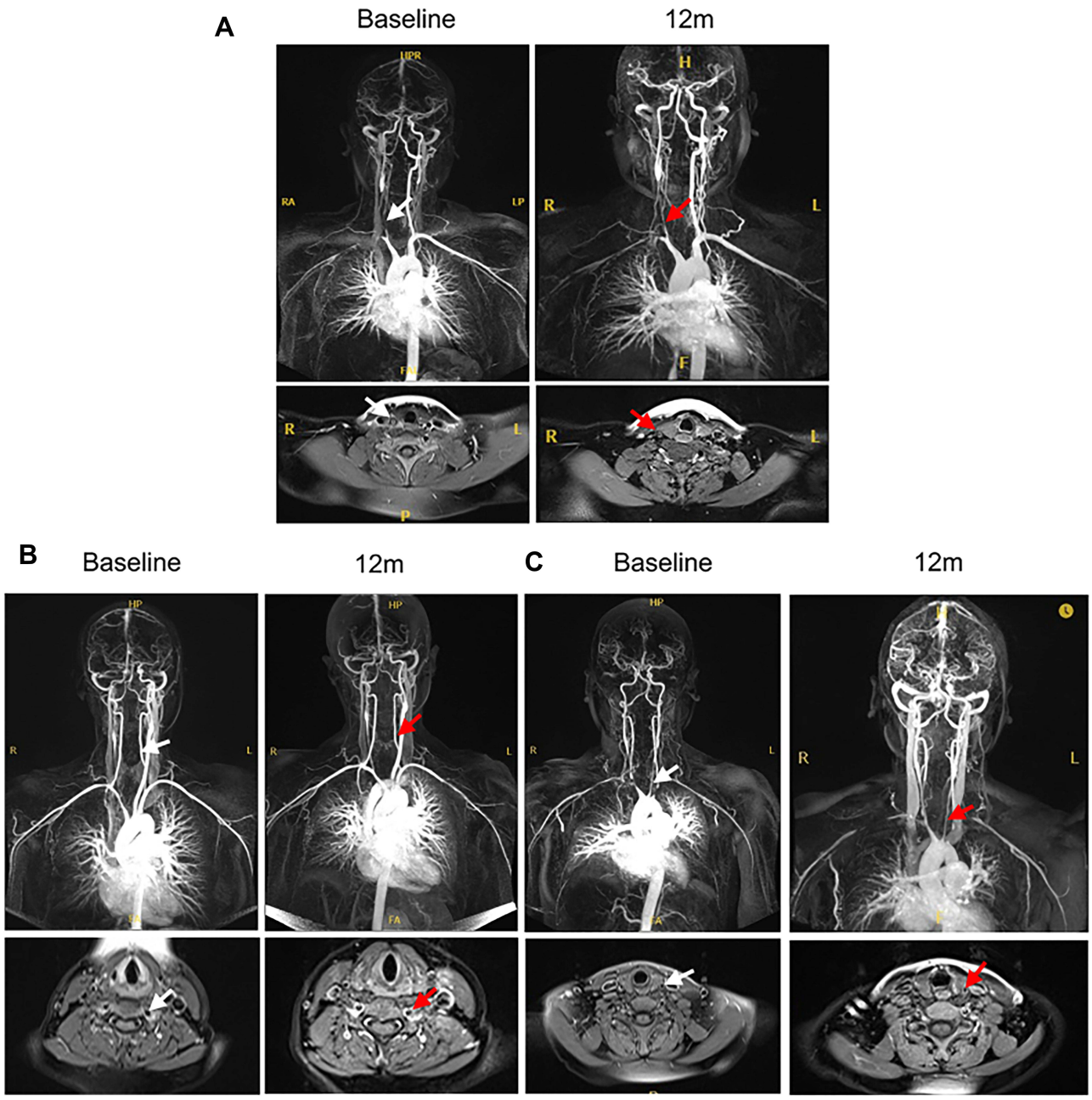

Figure 4 Imaging changes after 12 months of treatment. (**A**). A 28-year-old female patient with a disease course of 29 months. Before GCs and TOF treatment, the CCL22 levels were 40.83 pg/mL. MRA indicated that the involved vessels exhibited stenosis and occlusion of the right common carotid artery. After 12 months of treatment, the CCL22 levels were 29.22 pg/mL, and MRA suggested that the wall of the right common carotid artery was improved. White arrow: right common carotid artery before treatment, red arrow: right common carotid artery after GCs and TOF treatment. (**B**). A 31-year-old woman with a disease course of 24 months. The CCL22 levels before and after treatment were 981.05 pg/mL and 1085.58 pg/mL, respectively, and MRA showed thickening of the left vertebral artery was worsened after treatment. White arrow: left vertebral artery before treatment, red arrow: left vertebral artery after GCs and TOF treatment. (**C**). A 32-year-old woman with a disease course of 30 months. The levels of FGF-2 before and after treatment were 19.41 pg/mL and 133.86 pg/mL, respectively. MRA showed thickening of left common carotid artery wall after treatment. White arrow: left common carotid artery before treatment, red arrow: left common carotid artery after GCs and TOF treatment.
Abbreviations: HPR, head posterior right; RA, right anterior; LP, left posterior; FA, foot anterior; R, right; L, left; F, foot; P, posterior; H, head; HP, head posterior; 12m, 12 months; MRA, Magnetic Resonance Angiography.

TAK is a disorder with a complicated pathogenesis. In this study, by comparing the levels of various molecules between patients with TAK and healthy subjects, we found that multiple factors, including cytokines (PTX3, IL-6, IFN-γ), chemokines (CCL22, IL-16), growth factors (VEGF), and MMPs (MMP9) were elevated in patients with TAK, suggesting that a variety of pathophysiological processes are involved in the development of TAK. TOF is an effective

agent in TAK treatment, but little attention has been paid to how these specific biomarkers might change after TOF treatment.

Among the cytokines, PTX3 is a soluble pattern recognition receptor. It is mainly induced under immune-mediated inflammatory conditions. It has reported that PTX3 levels were increased among patients with TAK and closely related with vascular inflammation.[20,21] Higher levels of PTX3 may increase the risk of vascular involvement in TAK and GCA.[20] Moreover, PTX3 has been reported to be superior to CRP as a disease activity biomarker in TAK.[21] In the present study, PTX3 was also significantly higher in TAK patients, which was consistent with previous reports.[3] In addition, the close relationship between PTX3 and NIH score also demonstrated it as a biomarker for TAK disease activity. After 12 months of treatment, PTX3 levels showed an overall downward trend, indicating that GCs and TOF treatment was helpful in suppressing PTX3.

IL-6 has both pro-inflammatory and pro-fibrotic effects, and is involved in the pathogenesis of multiple rheumatic diseases.[22] It has also been reported to be a biomarker reflecting disease activity in TAK.[23,24] Our previous research identified an important role for IL-6 in vascular inflammation and fibrosis associated with TAK.[14,25] In the current study, higher baseline levels and a decreasing trend post treatment of IL-6 were seen in TAK patients, which was similar to PTX3. Since both PTX3 and IL-6 are induced in the acute phase of inflammation, the decrease of these two biomarkers indicated the anti-inflammatory effects of GCs and TOF treatment.

IFN-γ is mainly secreted by Th1 cells and has a variety of physiological functions in the immune activation. Saadoun et al found that stimulation of healthy human $CD4^+$ T cells with TAK patient serum in vitro led to a significant increase in IFN-γ levels, and treatment with GCs inhibited this process.[6] In line with previous reports, IFN-γ was also increased in patients with TAK in this study. However, it did not respond well to GCs and TOF during the first 6 months of treatment, and even exhibited an increasing trend. After 6 months, however, IFN-γ levels began to decrease. This phenomenon indicated that there might be other factors indirectly stimulating the production of IFN-γ, which lead to a delayed inhibitory effect of GCs and TOF.

IL-10 is a well-known anti-inflammatory cytokine. It was significantly reduced in active patients compared with stable patients, and higher IL-10 levels was correlated with a long-term remission of TAK.[26,27] In the present study, there was no significant difference in serum IL-10 levels between TAK patients at baseline and healthy controls. However, after 6 months of GCs and TOF treatment, IL-10 was significantly increased in TAK patients, indicating that GCs and TOF could enhance the anti-inflammatory responses. Although a downward trend in IL-10 levels was observed after 6 months, it was still higher at 12 months than its baseline level. This decrease of IL-10 may indicate a feedback to the suppression of systematic inflammation, as represented by the decrease of IL-6 and PTX3 levels.

Chemokines are signaling molecules for immune cells migration and trafficking. Higher levels of proinflammatory chemokines, such as CCL2 and CCL5, have been reported in patients with active TAK.[28] In this study, we also found higher levels of CCL2 in patients, but no difference was observed in CCL5 levels. In addition, CCL22 is a chemokine mainly produced by M2 macrophages,[29] which can promote the migration and growth of fibroblasts.[30] Our previous research found that leflunomide inhibits the secretion of CCL22 by M2 macrophages in vitro.[31] In the present study, a significantly higher CCL22 levels at baseline and an increasing trend of it after treatment were observed in patients with TAK. Importantly, it was significantly higher in patients with vascular occlusion than in patients without it, and patients with higher baseline CCL22 levels tended to have more imaging progression after follow-up. These data suggested that increased CCL22 levels were likely related with vascular progression. Since CCL22 is also a pro-fibrotic factor that has been implicated in other fibrotic diseases, such as pulmonary fibrosis,[32] it was assumed that CCL22 may play a role in vascular fibrosis of TAK.

VEGF is a growth factor predominantly involved in angiogenesis. In TAK, VEGF is closely related to an increase of neovascularization and inflammation in the vascular wall.[33] VEGF levels are elevated in patients with TAK,[11] which is consistent with the findings found in this study. After TOF treatment, VEGF demonstrated a decreasing trend. Since inflammatory cytokines, such as IL-6 and TNF-α, are potent inducers of VEGF expression,[34] remission of inflammation after treatment with GCs and TOF might largely contribute to the inhibition of VEGF. On the other hand, the suppression of VEGF could further dampen vascular inflammation by decreasing the infiltration of immune cells through microvessels.

FGF-2 is another growth factor involved in multiple processes, such as angiogenesis and wound repair. Fukui et al found that FGF-2 levels are significantly higher in TAK patients than patients with GCA.[35] However, in the present study, FGF-2 levels were lower in TAK patients at baseline, and appeared to continuously increase after treatment. This inconsistency might be due to different disease status and factors that affect FGF-2 expression in different patients. Since FGF-2 is also an important protein in tissue repair, the increase of FGF-2 after treatment may benefit to vascular healing after inflammatory injury. Although patients without imaging improvement had relatively higher baseline FGF-2 levels, and one patient with increased FGF-2 levels presented vascular progression, whether FGF-2 participates in vascular fibrosis in TAK needs further research.

MMPs are enzymes capable of degrading extracellular matrix. They play important roles in the development of TAK.[36,37] In previous reports, MMP2, MMP3, and MMP9 were elevated in patients with TAK, and MMP3 and MMP9 were associated TAK disease activity.[7] In this study, only MMP9 was significantly elevated in TAK patients. It is mainly derived from activated macrophages and T lymphocytes, and its increase indicates severe inflammation and the destruction of vascular structures. After treatment with GCs and TOF, MMP9 showed a trend of increasing first and then decreasing; interestingly, the trend of MMP3 levels was the opposite. MMP-3 belongs to stromelysin and can degrade a variety of collagen types, including type II, III, etc.[38] MMP9 is a gelatinase, and can regulate extracellular matrix (ECM) remodeling.[39] It has been reported that MMP3 can also activate MMP-9.[38] Thus, the opposite change trend of MMP3 may be the indirect feedback in response to MMP9 changes. However, how the expression of MMP3 and MMP9 are regulated after GCs and TOF treatment remains to be further clarified.

Among these factors, we found slight increases of IFN-γ, TNF-α, PDGF-AB at the first six months followed by decrease after six months. They are all direct or indirect activators of JAK signaling pathway.[40–42] When their signaling pathway were inhibited by TOF, IFN-γ, TNF-α and PDGF-AB might be increased in a reflective way to antagonize the inhibitory effects. This probably explained the slight increase of them at first 6 months. After 6 months, when patients' systematic inflammation was suppressed, the factors that promoted IFN-γ, TNF-α, PDGF-AB expression might be controlled. This may lead to a new decrease of these factors. However, since the regulation of these molecules in the human body was complicated and resulted from combined effects of promoting and inhibitory factors, the underlying rational for their changes needs further investigation.

This study identified that treatment with GCs and TOF differentially impacted the levels of different biomarkers associated with TAK. They were more effective in down-regulating inflammatory cytokines, such as PTX3, IL-6, and other inflammation related factors such as VEGF, but were not as effective in inhibiting chemokines or pro-fibrotic factors, such as CCL22 and FGF-2. This phenomenon suggests that combined therapies with different mechanisms are required in future treatment.

However, this study had some limitations, such as small sample number and short follow-up time. In addition, molecular changes in patients may be delayed after treatment due to complicated regulatory mechanisms, and longer follow-up periods may be required to clarify the effectiveness of TOF on changes in biomarker levels. Furthermore, the human body is in a state of dynamic equilibrium, and the levels of these molecules could be in a state of fluctuation. Therefore, it would be better to evaluate these molecules in an integral condition in future studies. Finally, it was hard to clarify the regulatory effects of a specific medication on these factors since patients received GCs as well as TOF in this study.

Conclusion

Multiple factors, representative of different pathological process including inflammation, cell recruitment, growth, and extracellular remodeling, participate in the pathogenesis of TAK. GCs and TOF are effective in decreasing inflammatory molecules but have limited efficacy in regulating multiple other markers involved in TAK. PTX3 is a prominent marker for TAK disease activity, and CCL22 may have predictive value for vascular progression.

Ethics Approval and Consent to Participate

The study protocol was approved by the Ethics Committees of Zhongshan Hospital (B-2016-168 (2) R). Recruitment and study of this cohort was conducted in accordance with the Declaration of Helsinki 1964 and its later amendments. All patients provided written informed consent.

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Disclosure

The authors report no conflicts of interest in this work.

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