
Serum cytokine profiles in Takayasu's arteritis: search for biomarkers

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ABSTRACT

Objective. Assessment of disease activity is one of the major difficulties in patients with Takayasu arteritis (TA) during follow-up. To date, no biomarker is universally accepted to be a surrogate for active disease in TA. In this study, we aimed to investigate levels of various pro- and anti-inflammatory molecules including serum granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-6, IL-8, IL-10, IL-18 and IL-23 in patients with TA.

Methods. The study included 51 patients (age: 40.6±12.2 years, F/M: 45/6) with TA and 42 age- and sex-matched healthy controls (age: 38.1±7.4 years, F/M: 38/4). All patients fulfilled the criteria of the American College of Rheumatology (ACR). TA patients were evaluated by physician's global assessment (PGA; active/inactive) and ITAS2010 (Indian Takayasu Arteritis Clinical Activity Score) in terms of clinical activity in baseline and follow-up visits. Commercial enzyme linked immuno-sorbent assay (ELISA) kits were used for measurements of serum cytokine levels.

Results. At baseline, 21 (41.2%) patients were active according to PGA and 8 (15.7%) according to ITAS2010. Serum IL-6, IL-8 and IL-18 levels were significantly higher in patients with TA, whereas GM-CSF, IL-10, IL-23 levels were similar to healthy controls. IL-8 significantly decreased in the follow-up, associated with a decrease of clinical activity, whereas IL-23 level significantly increased. When assessed by ITAS2010 active patients had significantly higher IL-18 levels.

Conclusion. We found significantly increased IL-6, IL-8 and IL-18 levels in patients with TA compared to healthy controls. Only IL-18 level was significantly higher in active patients assessed by ITAS2010. IL-18 was also the only cytokine in our study that correlated with CRP. These findings suggest

that cytokines associated with neutrophilic, pro-inflammatory responses such as IL-6, IL-8 and IL-18 can be potential biomarkers for the assessment of disease activity in TA and warrant further studies in larger series.

Introduction

Takayasu's arteritis (TA) is a rare, chronic, large-vessel vasculitis that predominantly affects aorta, its major branches and the pulmonary arteries (1). The etiology of the disease is still unknown, but infectious agents (2) and genetic factors are implicated (3, 4). Histological findings showing inflammatory cell infiltrations and necrosis of the arterial vascular wall strongly suggest that cell-mediated immunity plays an important role in the pathogenesis of TA (5). Vascular inflammation of TA possibly originate in the vasa vasorum, followed by infiltration of inflammatory cells with the production of inflammatory and Th1-type cytokines, such as tumour necrosis factor (TNF)- α , interferon (IFN)- γ , interleukin (IL)-12 and IL-18 leading to the formation of granulomas (6). Th17 cells induced by IL-6 and IL-23 in the microenvironment, possibly also contribute to the vascular lesions through the recruitment of infiltrating neutrophils. IL-12-IFN- γ -associated Th1 and IL-6-IL-17 and IL-23-dependent Th17 pathways are more clearly defined in giant-cell arteritis (GCA), a similar large-vessel vasculitis (7).

Currently, clinical activations of TA are monitored by acute-phase reactants ESR and CRP. However, various studies demonstrated the limitations of this approach, especially during the indolent course of TA and a significant subset of flares show no acute-phase response (8). In a search to find better biomarkers for disease assessment, in various previous studies, serum concentrations of IL-6, Regulated on ac-

Competing interests: none declared.

tivation, normal T cell expressed and secreted (RANTES) (9), IL-8 (10), IL-12 (11) and IL-18 (12) were studied and some appear to correlate with disease activity. In this study, we aimed to replicate some of these results and investigated serum levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, IL-8, IL-10, IL-18 and IL-23 and their correlations with disease activity in patients with TA.

Methods

The study included 51 patients (age: 40.6±12.2 years, F/M: 45/6) with TA and 42 age- and sex-matched healthy controls (age: 38.1±7.4 years, F/M: 39/3) and was performed between January 2012 to August 2013. All patients with TA fulfilled the criteria of American College of Rheumatology (ACR) (13). TA patients were evaluated by physician's global assessment (PGA: active/inactive) and ITAS2010 (Indian Takayasu Arteritis Clinical Activity Score) (14) in terms of clinical activity at baseline and 4–6 months of follow-up.

Venous blood samples were obtained from patients and controls at baseline and at the follow-up visit. Serum specimens were separated in an hour and stored at -80°C until assayed. Commercial enzyme linked immunosorbent assay (ELISA) kits were used for the measurement of serum GM-CSF, IL-6, IL-8, IL-10 (Peprotech, UK), IL-18 (MBL, Japan) and IL-23 (eBioscience, Austria) levels. ESR (modified Westergren method) and C-reactive protein (CRP) levels were measured at the same time point as the collection of samples for the measurement of cytokine levels.

The study was performed according to the Declaration of Helsinki and all subjects gave informed consent before participation. Statistical data were performed with Statistical Package for the Social Sciences 16.0 (SPSS, Chicago, IL, USA). Results were expressed as means and standard deviations or as median (minimum-maximum) according to the distribution of data. Mann-Whitney U-test, independent-samples *t*-test, Wilcoxon test and χ^2 test were used for comparisons of data. Spear-

Table I. Baseline ESR, CRP and cytokine levels in patients with Takayasu's arteritis and healthy controls.

	Takayasu's Arteritis (n=51)	Healthy controls (n=42)	<i>p</i> -value
ESR (mm/hour)	25.6 ± 17		
CRP (mg/l)	9.5 (0.4-90)		
GM-CSF (pg/ml)	7.9 ± 27 (0-181)	4.7 ± 16.8 (0-96)	0.088
IL-6 (pg/ml)	194.7 ± 485 (0-2555)	64.3 ± 156.8 (0-748)	0.036
IL-8 (pg/ml)	49.4 ± 189 (0-1349)	8.4 ± 23.8 (0-97)	<0.001
IL-10 (pg/ml)	57.5 ± 243 (0-1735)	137.8 ± 432.5 (0-2451)	0.144
IL-18 (ng/ml)	535.1 ± 252 (109-1301)	268.8 ± 216.2	<0.001
IL-23 (pg/ml)	20.2 ± 45 (0-215)	63.9 ± 195.2 (0-1254)	0.136

Table II. Comparison of ESR, CRP and cytokine levels between active and inactive patients with Takayasu's arteritis (assessed with PGA).

	Active TA patients	Inactive TAK patients	<i>p</i> -value
ESR (mm/hour)	42.4 ± 14.9	15.7 ± 9.8	<0.001
CRP (mg/l)	16.4 ± 20.1 (1.1-90)	4.2 ± 3 (0.1-14.7)	<0.001
GM-CSF (pg/ml)	4.2 ± 10.6 (0-44)	16.9 ± 89.6 (0-728)	0.352
IL-6 (pg/ml)	200.8 ± 436 (0-2327)	154.1 ± 554 (0-3714)	0.059
IL-8 (pg/ml)	21.6 ± 29.2 (0-104)	40.9 ± 170 (0-1349)	0.009
IL-10 (pg/ml)	26.8 ± 46 (0-214)	44.5 ± 210 (0-1735)	0.032
IL-18 (ng/ml)	502.3 ± 420	463.3 ± 394	0.149
IL-23 (pg/ml)	18.3 ± 45 (0-219)	45.5 ± 63 (0-227)	0.023

man correlation test was used to analyse correlations.

Results

According to the angiographic classification, 26.9% (n=25) of the study group had type I, 21.5% (n=20) had type V, 1.1% (n=1) had type IV and 5.4% (n=5) had type IIb disease (15). Mean disease duration of the patients were 4.5±4 years. Forty-four patients (86.3%) were on oral methylprednisolone therapy. As additional immunosuppression, 28 (54.9%) patients were on azathioprine, 16 (31.4%) were on methotrexate, 5 (9.8%) were on leflunomide and 1 (2%) was on infliximab. At baseline, 21 (41.2%) patients were active according to PGA and 8 (15.7%) patients were active according to ITAS2010. Baseline ESR, CRP and cytokine levels were given in Table I. Serum IL-6, IL-8 and IL-18 levels were significantly higher in patients with TA ($p=0.036$ for IL-6, $p<0.001$ for IL-8 and IL-18), whereas serum GM-CSF, IL-10, IL-23 levels were similar in patients compared to healthy controls (Table I). IL-18 level correlated significantly with CRP ($r=0.521$, $p<0.001$). There were no correlations between

age, disease duration, treatments (including corticosteroids) and serum cytokine levels.

In follow-up visits (4-6 months after baseline), clinical activity decreased from 41.2% (n=21) to 19.6% (n=10) according to PGA, and from 15.7% (n=8) to 5.9% (n=3) according to ITAS2010. In serial assessment, IL-8 level significantly decreased (basal vs. follow-up: 49.4±189.5 (0-1349) vs. 20.5±67.8 (0-454), $p=0.017$), whereas IL-23 level significantly increased in the follow-up visit compared to the baseline (20.1±45.2 (0-215) vs. 51.8±66.5 (0-227), $p=0.004$).

For the assessment of serum cytokine levels with clinical activity (according to PGA), we combined and pooled baseline and follow-up visits and re-analysed. However, although acute-phase response fell significantly supporting our clinical observations, no single cytokine measurement seemed useful to predict "remission". Only IL-6 levels were increased, not reaching to statistical significance, in active patients ($p=0.059$). When we compared activity according to ITAS2010 assessment, only IL-18 level was significantly higher in active patients (397.4±320 vs.

253±83, $p=0.031$). CRP significantly correlated also only with IL-18 levels ($p<0.001$, $r=0.422$). Interestingly, in serial assessments, IL-8, IL-10 and IL-23 levels were observed to be significantly higher in inactive patients.

Discussion

Assessment of disease activity is one of the major difficulties in clinical follow-up of Takayasu's arteritis. To date, no biomarker showing a reliable correlation with clinical activity have been identified in TA. Although, acute phase response (APR) is frequently advocated for disease assessment, it was shown to be neither sensitive nor specific enough to monitor disease activity (8). Serum biomarkers such as IL-6, IL-8, IL-12 and IL-18 (8, 9, 10, 11) matrix metalloproteinase-9 and recently pentraxin3 (16) have been suggested to be related to active disease in TA, however, these data require confirmatory studies.

IL-18, a member of the IL-1 family, is known to play an important role in Th1 polarisation. Levels of IL-18 is reportedly elevated in serum and synovial fluids of patients with rheumatoid arthritis, adult-onset Still's disease and psoriatic arthritis (17, 18). Similar to our results, Park *et al.* observed high serum IL-18 levels in patients with TA, associated also with active disease (12). Although we could not find difference between active and inactive patients according to PGA, IL-18 levels were higher in the subset of active patients assessed by ITAS2010. This new assessment tool developed by Misra *et al.*, only includes physical signs and symptoms of active disease with elevated APR. It has a lower sensitivity compared to PGA, but possibly have a higher specificity for active disease. So, IL-18 can be a specific biomarker for activity in TA and warrant further studies. Correlation of CRP only with IL-18 also supports this observation. Studies of IL-18 in GCA is limited, but an IL-18 gene polymorphism is suggested to be associated with GCA (19). IL-8, also known as CXCL8, is a chemokine functioning to recruit neutrophils and T lymphocytes to inflammatory tissues (20). In a recent study, increased IL-8 levels were observed in GCA. Nadkarni *et al.* also reported

potential involvement of neutrophils in GCA pathogenesis and relapse, suggesting that disease process is incompletely controlled by CS therapy as CS tapering lead to the loss of a neutrophil-suppressor T-cell subset (21). In our study, serum IL-8 was significantly elevated in TA compared to controls, however was not associated with disease activity. In contrast to our result, Tripathy *et al.* previously observed higher plasma IL-8 levels associated with clinical activity in TA (10).

IL-6 is a pro-inflammatory cytokine mainly synthesised by activated monocytes, macrophages and T cells and have an important role in Th17 pathway (22). Serum IL-6 levels have previously been shown to be raised in active GCA and TA patients, correlating with disease activity (23). IL-6 receptor blockage seem also the most promising new treatment option for large-vessel vasculitis (24), (25). IL-6 receptor blocker, tocilizumab, was also found effective in patients with inflammatory aortitis refractory to CSs or to other biologic immunosuppressive drugs (26). In our study, IL-6 levels were also significantly higher in TA compared to controls. IL-6 level was also higher in active patients, but it did not reach to statistical significance. Our results should be confirmed in a larger cohort.

GM-CSF was originally defined as a haemopoietic growth factor that promotes myeloid cell development, maturation and dendritic cell differentiation (27). However, it can act also on mature myeloid cells and accepted to be a pro-inflammatory cytokine (28). In GCA, GM-CSF was shown to be associated with disease activity (29), however it has not been studied in TA previously. In our study, serum GM-CSF levels were similar in patients with TA compared to healthy controls and showed no consistent association with activity.

IL-10 is an important immunoregulatory cytokine with anti-inflammatory properties. IL-10 is known to suppress the release and function of a number of proinflammatory cytokines, including IL-1 β , TNF- α and IL-6 (30). Samson M *et al.* reported increased serum IL-10 levels in GCA, which were also significantly lower at the time of relapses (31).

However, we could not demonstrate a role of IL-10 in our patient group.

IL-23 is a member of the IL-12 family which is important for the generation and maintenance of Th17 cells. IL-23 has been associated with the generation of a particularly proinflammatory subset of Th17 that expresses both IL-17 and IFN- γ (32). Espígol-Frigolé *et al.* observed that IL-17A expression was higher in temporal artery biopsy specimens of GCA patients and was a predictor of response to CS therapy (33). In our study, serum IL-23 levels was similar to healthy controls in patients with TA. However, IL-23 levels stayed high in inactive disease, suggesting that it might be a factor for disease relapses, similar to the observations in GCA.

The main limitation of our study is its cross-sectional nature and a high use of immunosuppressive therapies in our patient group. Some of our results such as IL-6 levels also seem to be affected by a Type II statistical error with low number of patients in our study group. However, we still think our results are valuable and show the limitations of current biomarker evaluation of cytokine measurements in TA. As ESR and CRP highly correlated with disease activity, we think, none of the cytokines in our study seems to surpass APRs for the assessment of TA.

In conclusion, we found significantly increased IL-6, IL-8 and IL-18 levels in patients with TA compared to healthy controls. IL-18 levels were significantly higher in active patients assessed by ITAS2010. IL-18 was also the only cytokine in our study, correlating with CRP. These findings suggest that cytokines associated with neutrophilic, pro-inflammatory responses such as IL-6, IL-8 and IL-18 can be potential biomarkers for the assessment of disease activity in TA and warrant further studies in larger series.

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