Journal of Cardiology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

Profiles of serum cytokine levels in Takayasu arteritis patients: Potential utility as biomarkers for monitoring disease activity

Natsuko Tamura (MD)[a], Yasuhiro Maejima (MD, PhD)[a], Daisuke Tezuka (MD, PhD)[a,b], Chisato Takamura (MD, PhD)[a], Shunji Yoshikawa (MD)[a], Takashi Ashikaga (MD, PhD, FJCC)[a], Kenzo Hirao (MD, PhD)[a], Mitsuaki Isobe (MD, FJCC)[a,*]

[a] Department of Cardiovascular Medicine, Tokyo Medical and Dental University, Tokyo, Japan
[b] Advanced Imaging Center Yaesu Clinic, Department of Cardiovascular Medicine, Tokyo, Japan

ARTICLE INFO

Article history:
Received 17 June 2016
Received in revised form 24 October 2016
Accepted 28 October 2016
Available online xxx

Keywords:
Takayasu arteritis
Cytokine
Biomarker
Autoimmune disease

ABSTRACT

Background: Takayasu arteritis (TA) is an autoimmune arteritis of unknown etiology. Currently, the erythrocyte sedimentation rate (ESR) and serum C-reactive protein (CRP) levels are widely used to monitor disease activity of TA. However, sometimes it is difficult to reflect inflammatory symptoms in either CRP or ESR values, especially in TA patients with immunosuppressive therapies. Therefore, higher-accuracy biomarkers for evaluating disease activity need to be explored.

Methods and results: We examined 21 Japanese patients diagnosed with TA; 17 TA patients were treated with prednisone with or without additional immunosuppressive therapies and the remaining 4 patients were treated with infliximab, a human monoclonal anti-tumor necrosis factor (TNF)-α antibody. In active phase, the serum levels of both TNF-α and interleukin (IL)-6 were significantly higher than in healthy subjects, as is the case with both the levels of CRP and ESR. In contrast, the levels of both IL-12 and IL-23 remained in the normal range. Both TNF-α and IL-6 levels were markedly decreased in response to therapies, on equality with both CRP and ESR levels. Regarding the TA patients treated with infliximab, both CRP and IL-6 levels tended to be decreased after infliximab therapy. Conversely, TNF-α level after infliximab therapy was higher than before therapy.

Conclusion: Both TNF-α and IL-6 levels, but not IL-12 or IL-23 levels, in the serum could be potent biomarkers that can reflect the activity of TA.

Introduction

Takayasu arteritis (TA) is an autoimmune systemic arteritis whose etiology remains unclear. A growing body of evidence suggests that there are particular characteristics of gender, race, and geographic distribution in TA patients. A large majority of TA patients are female and distribution of age of TA onset is from 10 to 40 years [1]. Prevalence of TA is much higher in Asia than in the Western world [2–4]. The annual incidence of TA in Japan is estimated to be around 150 patients per year [5]. The lesions of TA mainly include the aorta and its major arterial branches, including left subclavian artery [6]. As the inflammation of arteries in TA is progressive, all vessels of TA patients would be involved eventually.

Increasing lines of evidence suggest that single nucleotide polymorphisms (SNPs) of some specific human leukocyte antigen (HLA), including HLA-Bw52, HLA-B39, and HLA-B67 are associated with genetic susceptibility to TA [7–9]. Recently, we have demonstrated that SNPs of *IL12B* and *HLA-B* reveal a synergistic role in the susceptibility to TA in Japanese patients [10]. These findings suggest that SNP-mediated alteration of cellular immunity mechanisms would be critical in the prevalence of TA.

Currently, the major way of monitoring TA is based on the evaluation of conventional biomarkers, including serum C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR), and the imaging tests, such as angiography, computed tomography (CT) and magnetic resonance imaging (MRI). However, there are cases that have obvious inflammatory symptoms with no significant increase in either CRP or ESR values, such as relapsed TA patients. We previously reported that there was no significant difference in the values of inflammatory markers, including CRP and ESR,

* Corresponding author at: Department of Cardiovascular Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, 13rd Floor of M&D Tower, Bunkyo-ku, Tokyo 113-8519, Japan. Fax: +81 3 5803 0133.
E-mail address: isobemi.cvm@tmd.ac.jp (M. Isobe).

http://dx.doi.org/10.1016/j.jjcc.2016.10.016
0914-5087/

between the monophasic TA patients and the relapsing-remitting ones [11]. Conventional biomarkers often fail to identify the recurrence of TA activity after intensive drug therapy using steroids and/or other immunosuppressive agents, including methotrexate, azathioprine, anti-tumor necrosis factor (TNF)-α agents (infliximab), and anti-interleukin (IL)-6 receptor (IL-6R) antibody (tocilizumab) [12–17]. Previously, we demonstrated that fluorodeoxyglucose positron emission tomography and computed tomography (FDG-PET/CT), a novel imaging modality, is useful not only for the detection of active inflammatory lesions at diagnosis of TA, but also for the determination of TA recurrence despite receiving immunosuppressive agents [17]. However, it is limited where the FDG-PET/CT examinations can undergo. Thus, more sensitive biomarkers are required to catch the subtle flare up of TA.

We hypothesized that the serum levels of the following cytokines (TNF-α, a target of infliximab; IL-6, a target of tocilizumab; IL-12 and IL-23, the products of *IL12B* gene) could be sensitive biomarkers for the TA patients whose inflammatory status is active even in the absence of an increase in CRP nor ESR.

Methods

Study subjects

Twenty-one Japanese TA patients who visited University Hospital of Tokyo Medical and Dental University between April 2007 and Jan 2014 were investigated. All patients were diagnosed with TA according to the 1990 American College of Rheumatology criteria for classification of TA [1]. Serum levels of CRP, TNF-α, IL-6, IL-12, and IL-23 and/or ESR were evaluated in TA patients in both active and inactive phases. Among 17 TA patients who did not receive infliximab therapy, 12 patients were diagnosed as having new-onset TA, and the remaining 5 patients were diagnosed with flared-up TA. Flared-up TA is defined as follows: The TA patients who (1) showed sustained elevation of CRP concentration which could not be explained by other reasons and/or (2) had clinical signs or symptoms due to arteritis regardless of corticosteroid and/or some immunosuppressive therapies. We defined the "active phase" as the state of disease that are new-onset and flared-up. Concurrently, we defined the "inactive phase" as the state of disease not flared-up. This study was approved by the ethics committee in our university and performed under the informed consent of all patients.

Analyses of blood samples

The measurements of serum CRP level and ESR were conducted at clinical laboratory of University Hospital in Tokyo Medical and Dental University. For the evaluation of serum levels of various cytokines, including TNF-α, IL-6, IL-12, and IL-23, the venous blood was centrifuged at 3000 rpm for 5 min at room temperature. Plasma was stored in microtubes at −20 °C until the assays were performed. The measurements of these cytokines were conducted by SRL (Tokyo, Japan) using enzyme-linked immunosorbent assay kits.

We used the normal levels of typical values in CRP and ESR (CRP is less than 0.3 mg/dl, and ESR is from 2 mm/h to 15 mm/h in women) which are used in our hospital. We used the reference values of TNF-α, IL-6, IL-12, and IL-23 which are provided from SRL laboratory. The normal level of TNF-α (high sensitivity TNF-α) is from 0.6 pg/ml to 2.8 pg/ml, IL-6 is less than 4.0 pg/ml, IL-12 is less than 7.8 pg/ml, and IL-23 is less than 39.0 pg/ml.

Statistical analysis

All results were analyzed by using Wilcoxon signed-rank test. Because all our data were not distributed normally, we analyzed all data in a non-parametric way. The cut-off values were determined for the biomarkers at the points which are the closest to the upper left corner in the ROC curves. All figures were presented as median and interquartile range. The significant difference was set at the 5% level.

Results

Clinical characteristics of TA patients

The clinical characteristics of 21 TA patients are shown in Table 1. Two male and 19 female TA patients were included. The mean age of these patients was 36.6 years in the range of 22–69 years old. All patients received corticosteroid therapy. During active phase, the mean prednisone dosage was 32.1 ± 15.1 mg/day, ranging from 10 to 60 mg/day. During inactive phase, the mean

Table 1
The clinical characteristics of TA patients ($N = 21$).

Pt No.	Age	Sex	Inflammatory bowel disease	Immunosuppressive therapies	Infliximab	Clinical situations
1	24	F	–	–	–	New onset
2	54	F	–	MTX	–	Flare up
3	47	F	–	–	–	New onset
4	53	F	–	–	–	New onset
5	55	F	–	MTX	–	New onset
6	22	F	–	MTX	–	Flare up
7	35	F	–	–	–	New onset
8	40	F	–	–	–	New onset
9	25	F	–	–	–	New onset
10	22	M	+	–	–	Flare up
11	33	F	+	Tacrolimus	–	Flare up
12	43	F	–	–	–	New onset
13	35	F	–	–	–	New onset
14	36	F	–	MTX	–	Flare up
15	44	F	–	–	–	New onset
16	28	M	–	–	–	New onset
17	69	F	–	–	–	New onset
18	24	F	–	MTX	+	Flare up
19	24	F	–	Ciclosporin + MTX	+	Flare up
20	28	F	–	MTX + tacrolimus	+	Flare up
21	28	F	–	MTX + ciclosporin + azathioprine + tacrolimus	+	Flare up

TA, Takayasu arteritis; F, female; M, male; MTX, methotrexate.

Please cite this article in press as: Tamura N, et al. Profiles of serum cytokine levels in Takayasu arteritis patients: Potential utility as biomarkers for monitoring disease activity. J Cardiol (2016), http://dx.doi.org/10.1016/j.jjcc.2016.10.016