Concise report

Serum markers associated with disease activity in giant cell arteritis and polymyalgia rheumatica

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Abstract

Objective. To compare multiple serum markers for their ability to detect active disease in patients with GCA and in those with PMR.

Methods. Twenty-six markers related to immune cells that may be involved in GCA and PMR were determined by ELISA and multiplex assay in the serum of 24 newly diagnosed, untreated GCA/PMR patients, 14 corticosteroid (CS)-treated GCA/PMR patients in remission and 13 healthy controls. Receiver operating characteristic analysis with area under the curve and Spearman’s correlation coefficients were performed.

Results. Serum B-cell activating factor (BAFF), CXCL9 and IL-6 were increased in newly diagnosed GCA and PMR patients. Serum CCL2, CCL11, IL-10 and sIL-2R were modulated in GCA patients only and CXCL10 in PMR patients only. BAFF, CXCL9 and IL-6 accurately distinguished newly diagnosed GCA and PMR patients from healthy controls, as shown by area under the curve > 0.80. Upon CS-induced remission, serum BAFF and IL-6 decreased significantly in both GCA and PMR patients, whereas CXCL9 remained high. Serum BAFF and IL-6 correlated strongly with ESR and CRP in GCA and PMR patients.

Conclusion. Among the serum markers tested, BAFF and IL-6 showed the strongest association with disease activity in both GCA and PMR patients. The diagnostic value of these markers should be evaluated in larger, longitudinal studies with GCA and PMR patients, and in patients with infections or other inflammatory conditions.

Key words: giant cell arteritis, polymyalgia rheumatica, BAFF, biological markers, IL-6, chemokines, cytokines.

Rheumatology key messages

- Serum BAFF and IL-6 modulate closely with disease activity in GCA and PMR.
- GCA and PMR may be characterized by distinct changes in serum markers.

Introduction

GCA and PMR are closely related inflammatory diseases that frequently co-occur [1]. GCA involves inflammation of large- and medium-sized arteries and gives rise to classic symptoms such as headache, blindness and stroke. PMR is a rheumatic disease characterized by pain and stiffness of shoulders and hips.

Besides careful examination of clinical signs and symptoms, the assessment of GCA and PMR patients typically relies on measurement of the ESR and serum levels of CRP. The ESR has been considered the gold standard inflammation marker in GCA and PMR for many years, and is also part of the classification criteria for GCA and PMR [1]. Although the ESR and CRP are increased in the majority of GCA and PMR patients with active disease [2, 3], these inflammatory markers remain normal in a
subpopulation of patients [2, 3]. As objective measures for disease activity are important not only for daily clinical practice, but also for evaluating treatment outcomes in clinical trials, novel markers for active GCA and PMR are needed [4, 5].

As a first step towards identifying alternative markers for active GCA and PMR, we performed a comprehensive analysis of 26 serum markers in well-defined cohorts of GCA and PMR patients. These markers, which were related to immune cells that may be involved in the immunopathology of GCA and PMR [6, 7], were studied both before and after initiation of CS treatment. In essence, we searched for markers that not only discriminated newly diagnosed GCA and PMR from healthy controls, but also modulated with disease activity upon CS-induced remission.

Methods
Study population
In a prospective study [7], 24 patients with newly diagnosed GCA (n = 12) or PMR (n = 12) were enrolled (supplementary Table S1, available at Rheumatology Online). None of these patients received CSs or DMARDs at the time of blood withdrawal. GCA patients fulfilled the ACR criteria and PMR patients the Chuang/Hunder criteria [8, 9]. An 18F-fluorodeoxyglucose-PET/CT scan with similar or enhanced glucose uptake by large vessels in comparison with the liver was considered equal as a diagnostic criterion to a positive temporal artery biopsy. Follow-up samples were obtained from 14 patients with GCA (n = 7) or PMR (n = 7) who were in remission after 3 months of CS treatment. Remission was defined as absence of symptoms and a normal ESR (<30 mm/h). Control samples were collected from 13 age-matched healthy individuals. All procedures were in compliance with the Declaration of Helsinki. Written informed consent was obtained from all study participants, and the study was approved by the medical ethics committee of the University Medical Center Groningen (UMCG).

Measurement of serum markers
Serum samples were stored at −40°C and used for analysis immediately after thawing. Serum B-cell activating factor (BAFF) was measured with an ELISA kit from R&D systems. The other 25 serum markers were measured with the 25-plex Human Cytokine 25-plex (Life Technologies). The limits of detection of all measurements are provided in supplementary Table S2, available at Rheumatology Online. All procedures were performed according to the manufacturer’s instructions.

Statistical analysis
The Mann–Whitney U-test was used to compare serum markers between groups. The Wilcoxon Signed Rank test was used to compare serum markers within groups over time. Receiver operating characteristic (ROC) analysis with area under the curve (AUC) was performed for non-paired samples only, i.e. patients vs healthy controls. Optimal cut-off points were identified by assessing the maximum of the sum of sensitivity and specificity, according to the Youden Index. Correlations were determined with Spearman’s rho correlation coefficient. Two-tailed P-values < 0.05 were considered statistically significant. Statistics were performed in IBM SPSS Statistics 20 and Graphpad Prism 5.0.

Results
Serum markers discriminating newly diagnosed GCA and PMR patients from healthy controls
Eight out of 26 serum markers were significantly modulated in newly diagnosed GCA and/or PMR patients vs healthy controls (Table 1). Serum BAFF, CXCL9 and IL-6 were increased in both newly diagnosed GCA and newly diagnosed PMR patients. Serum CCL2 and CCL11 were decreased in GCA patients only, whereas serum IL-10 and sIL-2R were increased. In contrast, serum CXCL10 was increased in PMR patients only. Thus, both overlapping and specific changes in serum markers were observed in GCA and PMR patients (supplementary Fig. S1, available at Rheumatology Online).

In a ROC analysis, we next determined the diagnostic accuracy of individual markers in distinguishing newly diagnosed patients from healthy controls. Serum CXCL9 and IL-6 provided excellent discrimination of newly diagnosed GCA and PMR patients from healthy controls, as indicated by AUCs > 0.90 (Table 1 and supplementary Table S3, available at Rheumatology Online). Serum BAFF also accurately distinguished newly diagnosed GCA and PMR patients from healthy controls, with AUCs > 0.80. Serum CXCL10, which was not modulated in GCA, could discriminate between PMR patients and healthy controls, as shown by an AUC > 0.80. None of the other markers provided an AUC > 0.80. Taken together, CXCL9, IL-6 and BAFF were the most accurate markers for discriminating newly diagnosed GCA and PMR patients from healthy controls. Of interest, these three serum markers were increased in most GCA and PMR patients in whom ESR levels did not fulfill the classification criteria for GCA or PMR [1], respectively (supplementary Table S4, available at Rheumatology Online).

Serum markers modulating upon CS-induced remission in GCA and PMR
Next, we evaluated the eight serum markers that were significantly modulated in patients at baseline, for changes upon remission after 3 months of CS treatment. Serum levels of BAFF and IL-6 were significantly decreased in GCA and PMR patients in remission, whereas CCL11 was increased in both patient groups (Fig. 1A). In addition, serum CCL2 was increased in GCA patients in remission, but not in PMR patients. No significant modulation of CXCL9, CXCL10, IL-10 or sIL-2R was observed upon CS-induced remission. Among the serum markers that were not modulated at baseline, only a slight increase in CCL4 was observed in GCA patients in remission (data not shown).
TABLE 1 Serum marker levels in newly diagnosed GCA and PMR patients

<table>
<thead>
<tr>
<th>Marker</th>
<th>Serum marker levels, pg/ml</th>
<th>Area under the curve</th>
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<tbody>
<tr>
<td></td>
<td>HC</td>
<td>New GCA</td>
</tr>
<tr>
<td>BAFF</td>
<td>1013 (765-3794)</td>
<td>1321 (1019-1578)**</td>
</tr>
<tr>
<td>CCL2</td>
<td>612 (445-924)</td>
<td>461 (295-960)*</td>
</tr>
<tr>
<td>CCL3</td>
<td>74 (61-85)</td>
<td>74 (60-135)</td>
</tr>
<tr>
<td>CCL4</td>
<td>77 (48-149)</td>
<td>79 (29-191)</td>
</tr>
<tr>
<td>CCL5</td>
<td>6.6 (4.9-6.6)</td>
<td>6.6 (2.6-6.6)*</td>
</tr>
<tr>
<td>CCL11</td>
<td>164 (88-414)</td>
<td>118 (49-463)*</td>
</tr>
<tr>
<td>CXCL9</td>
<td>35 (18-51)</td>
<td>88 (21-704)**</td>
</tr>
<tr>
<td>CXCL10</td>
<td>65 (38-99)</td>
<td>68 (48-114)</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>11 (7-29)</td>
<td>11 (5-2457)</td>
</tr>
<tr>
<td>IFN-α</td>
<td>39 (35-51)</td>
<td>39 (35-51)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>131 (87-153)</td>
<td>142 (114-170)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>5.2 (0.1-56.6)</td>
<td>2.0 (0.1-84.2)</td>
</tr>
<tr>
<td>IL-2</td>
<td>5 (1.3-11.4)</td>
<td>3.5 (2.0-51.2)</td>
</tr>
<tr>
<td>IL-4</td>
<td>183 (142-236)</td>
<td>178 (146-346)</td>
</tr>
<tr>
<td>IL-5</td>
<td>7.1 (5.3-10.9)</td>
<td>7.1 (5.3-9.0)</td>
</tr>
<tr>
<td>IL-6</td>
<td>2 (1-6)</td>
<td>15 (4-494)***</td>
</tr>
<tr>
<td>IL-7</td>
<td>13 (1-48)</td>
<td>13 (1-257)</td>
</tr>
<tr>
<td>IL-8</td>
<td>20 (8-239)</td>
<td>19 (0-41)</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.7 (0.4-1.7)</td>
<td>2.1 (0.7-29.0)*</td>
</tr>
<tr>
<td>IL-12</td>
<td>796 (574-964)</td>
<td>804 (634-1094)</td>
</tr>
<tr>
<td>IL-13</td>
<td>4.2 (0.0-11.0)</td>
<td>7.7 (4.2-11.0)</td>
</tr>
<tr>
<td>IL-15</td>
<td>22 (17-34)</td>
<td>23 (15-31)</td>
</tr>
<tr>
<td>IL-17</td>
<td>65 (61-76)</td>
<td>72 (61 80)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>10 (7-12)</td>
<td>11 (8-12)</td>
</tr>
<tr>
<td>sIL-1Ra</td>
<td>163 (125-305)</td>
<td>182 (107-1140)</td>
</tr>
<tr>
<td>sIL-2R</td>
<td>773 (638-980)</td>
<td>911 (722-1085)*</td>
</tr>
</tbody>
</table>

Serum levels of 26 markers were measured in 12 newly diagnosed GCA patients, 12 newly diagnosed PMR patients and 13 age-matched healthy controls. Values are presented as median (range). Statistical significance of patients vs healthy controls is indicated as *P < 0.05, **P < 0.01, ***P < 0.001. *ng/ml.

Serum markers correlating with the ESR and CRP in GCA and PMR

Finally, we studied the serum markers that were initially modulated in newly diagnosed GCA and PMR patients for correlations with the ESR and CRP. Serum BAFF provided correlation coefficients of >0.75 with the ESR and CRP in both GCA and PMR patients (Fig. 1B and Supplementary Fig. S2, available at Rheumatology Online). Serum IL-6 correlated strongly with the ESR and CRP in PMR patients and moderately in GCA patients, as indicated by correlation coefficients of >0.75 and >0.50, respectively. Serum CCL2 and CCL11 showed a moderate to strong inverse correlation with the ESR and CRP in GCA patients, but not in PMR patients. Serum CXCL9 correlated moderately with the ESR and CRP in PMR patients only. Serum CXCL10, IL-10 and sIL-2R, which were modulated at baseline, did not correlate with ESR and CRP in patients with GCA or PMR. Taken together, serum BAFF and IL-6 showed stronger correlations with ESR and CRP in GCA and PMR patients than any other serum marker studied.

Discussion

This is the first study to compare serum levels of 26 markers in GCA and PMR patients with those in healthy controls. Three serum markers (i.e. BAFF, CXCL9 and IL-6) were increased in both newly diagnosed GCA and newly diagnosed PMR patients. Moreover, these markers discriminated well between patients and healthy controls. Serum BAFF and IL-6, but not CXCL9, were attenuated upon CS-induced remission and showed the strongest association with disease activity in both GCA and PMR patients.

Objective markers that reflect active inflammation in GCA and PMR patients are important not only for daily clinical practice, but also for therapeutic trials with these patients [4, 5]. The ESR and CRP, which are the most commonly used markers in the diagnosis of active GCA and PMR [1], remain normal in a subgroup of GCA and PMR patients with active disease [2, 3]. Consequently, active disease may go undetected in these patients and disease-related complications can arise [10]. Among the serum markers tested here, serum BAFF and L-6 showed the strongest association with
Fig. 1 Correlation of serum markers with disease activity in GCA and PMR

(A) Serum levels of the eight markers that were initially modulated in newly diagnosed GCA and PMR patients (nGCA and nPMR) are shown in combination with the serum levels of the same patients in remission (rGCA and rPMR) after 3 months of corticosteroid treatment. Statistical significance is indicated as *P < 0.05. (B) Correlation coefficients between these eight serum markers and the ESR and CRP. Cell colours indicate the strength of the correlations. Statistical significance is indicated as *P < 0.05 and **P < 0.01.
disease activity in GCA and PMR patients. Markers that are modulated in both diseases are attractive, because GCA and PMR frequently co-occur [1]. In addition, other serum markers were modulated either in newly diagnosed GCA patients (i.e. CCL2, CCL11, IL-10 and sIL-2R) or in newly diagnosed PMR patients (i.e. CXCL10). Although none of these markers showed diagnostic potential comparable to that of BAFF and IL-6, the differential modulation of these serum proteins would suggest that distinct pathological processes may be involved in GCA and PMR.

As in other inflammatory conditions [11], serum IL-6 is increased in active GCA and PMR [12, 13]. IL-6 has been linked to the enhanced Th17 response in GCA and PMR and is locally expressed in inflamed arteries of GCA patients [6]. It has been proposed that serum IL-6 may be a better marker for disease activity in GCA and PMR than the ESR and CRP [1, 13]. We here confirm that serum IL-6 is increased in newly diagnosed GCA and PMR patients and decreases upon remission. Serum IL-6 accurately discriminated GCA and PMR patients from healthy controls and correlated strongly with the ESR and CRP in both patient groups. Thus, our findings confirm that IL-6 may be a valuable marker for diagnosing active GCA and PMR [1, 13].

Serum BAFF also distinguished newly diagnosed GCA and PMR patients from healthy controls and correlated strongly with disease activity in both diseases. BAFF is an important regulator of B cell responses and has been linked to the development of many autoimmune diseases [14]. Serum BAFF is constitutively produced by stromal cells, but this cytokine can also be produced by monocytes upon exposure to pro-inflammatory cytokines such as IFN-γ [14]. BAFF is therefore regulated in a different way from IL-6 and CRP. It remains to be elucidated to what extent BAFF is expressed in inflamed arteries of GCA patients. In addition, serum BAFF levels are influenced by the presence of circulating B cells, which are decreased during active GCA and PMR [7]. Although elevated serum levels of BAFF have also been observed in patients with infectious diseases or cancer [14], our findings indicate that serum BAFF is a promising marker for active GCA and PMR.

To the best of our knowledge, we are the first to show that serum CXCL9 is increased in GCA and PMR, and may provide excellent discrimination between newly diagnosed patients and healthy controls. CXCL9 is involved in the migration of Th1 cells, which are abundant in inflamed arteries of GCA patients [15–17]. Interestingly, we observed that serum levels of CXCL9 remained relatively high during remission. CXCL10, a Th1 cell chemo-attractant that was increased in PMR patients only, also remained high during remission. Although were are not aware of prior studies on local expression of CXCL9 and CXCL10 in inflamed tissues of GCA and PMR patients, the persistently high serum levels of these Th1 cell chemokines during CS-induced remission seem in accordance with the prior observation that Th1 responses are resistant to CS-mediated suppression [16].

We here observed decreased serum levels of CCL2 and CCL11 in newly diagnosed GCA patients. Although we are not aware of prior studies on CCL11 in GCA, Cid et al. [18] have also found decreased serum levels of CCL2 in GCA patients. In contrast, Ellingsen et al. [19] reported higher serum levels of CCL2 in GCA and PMR patients when compared with healthy controls. However, the healthy controls in the latter study were markedly younger than the patients, and CCL2 serum levels are known to increase with age [20]. Interestingly, both Cid et al. [18] and Ellingsen et al. [19] observed substantial expression of CCL2 in temporal arteries of GCA patients. In our study, both CCL2 and CCL11 were markedly increased in serum of GCA patients that were in CS-induced remission. Consequently, CCL2 and CCL11 showed a moderate to strong association with disease activity in GCA. CCL2 and CCL11 share the chemokine receptor CCR2, which is highly expressed by activated monocytes. To what extent the numbers of activated monocytes are altered in the circulation of GCA and PMR remains unknown.

A limitation of our study is the relatively small number of patients that were included. As a result, subtle changes in serum markers may have gone unnoticed. However, biomarkers should clearly modulate with disease activity in order to be useful in daily clinical practice. We here identified markers that would fit this profile. In addition, CS treatment may have directly affected serum markers of patients in remission. It would therefore be interesting to study serum markers in relapsing and non-relapsing patients using similar doses of CS during follow-up.

In conclusion, our study shows that serum levels of BAFF and IL-6 are strongly associated with disease activity in GCA and PMR patients. The diagnostic value of BAFF and IL-6, both separately and in combination, should be further evaluated in larger cohorts of GCA and PMR patients, as well as in patients with infections or other inflammatory conditions.

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Supplementary data

Supplementary data are available at Rheumatology Online.
References