Original article

Markers of angiogenesis and macrophage products for predicting disease course and monitoring vascular inflammation in giant cell arteritis

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Abstract

Objective. GCA, a systemic vasculitis, is characterized by an IL-6-dependent acute-phase response. This response is typically suppressed by treatment rendering CRP/ESR unreliable for monitoring vascular inflammation. Also, there are no accurate biomarkers predicting a non-favourable disease course. Here we investigated macrophage products and markers of angiogenesis as biomarkers for prognosis and monitoring of vascular inflammation.

Methods. Forty-one newly diagnosed, glucocorticoid-naive GCA patients were prospectively followed for relapses and glucocorticoid requirement for a median of 30 months (range 0–71). Serum markers at baseline and during follow-up were compared with 33 age-matched healthy controls and 13 infection controls. Concentrations of IL-6, serum amyloid A, soluble CD163, calprotectin, YKL-40, VEGF, angiopoietin-1 and -2 and sTie2 were determined by ELISA/Luminex assay.

Results. Serum concentrations of all markers, but not angiopoietin-1, were elevated in GCA patients at baseline when compared with healthy controls. High VEGF ($P = 0.0025$) and angiopoietin-1 ($P = 0.0174$) and low YKL-40 ($P = 0.0369$) levels at baseline were predictive of a short time to glucocorticoid-free remission. Elevated angiopoietin-2 levels were associated with an imminent relapse during treatment ($P < 0.05$). IL-6 correlated strongly with acute-phase markers and soluble CD163 but not with markers of angiogenesis, YKL-40 or calprotectin. Glucocorticoid treatment down-modulated all markers except for calprotectin and YKL-40. Tissue expression of markers in temporal arteries was confirmed.

Conclusion. Markers of angiogenesis at baseline and during treatment predict GCA disease course, suggesting utility in patient stratification for glucocorticoid-sparing therapy. Calprotectin and YKL-40 are candidate markers for monitoring vessel wall inflammation.

Key words: angiogenesis, angiopoietin-2, biomarkers, calprotectin, giant cell arteritis, glucocorticoids, IL-6, macrophages, VEGF, YKL-40

CLINICAL SCIENCE

Rheumatology key messages

- A serum profile of angiogenesis predicts disease course in giant cell arteritis patients.
- CRP and ESR do not predict disease course and lose value during glucocorticoid treatment.
- Calprotectin and YKL-40 qualify as candidate markers for monitoring vascular inflammation.

Introduction

GCA is the most common inflammatory disease of medium and large arteries [1]. Involvement of cranial arteries in GCA (C-GCA) can lead to symptoms like headache, jaw claudication and vision loss [2]. Signs and symptoms of inflammation of the aorta and its branches [large vessel GCA (LV-GCA)] are less specific and include weight loss and low-grade fever. Ultimately, LV-GCA can lead to the formation of aneurysms and aortic dissection [3, 4].

The most common treatment for GCA remains high-dose and long-term glucocorticoid (GC) monotherapy. However, many patients relapse, and the burden of GC treatment adds to that of the disease itself, with a great impact on patients' quality of life [5–7]. Still, a subset of patients experience a more favourable, non-relapsing

disease course requiring short-term GC treatment. To prevent GC toxicity and the risk of relapse, there is an urgent need for biomarkers that, either at baseline or during treatment, can predict disease course in GCA. Recently IL-6 receptor (IL-6R) blocking therapy (tocilizumab) has become available as GC-sparing treatment [8].

Classically, IL-6-dependent acute-phase markers CRP and ESR are used in the diagnosis and monitoring of GCA [6, 7]. However, in 5–16% of newly diagnosed GCA patients, CRP and ESR levels are within the normal range [9, 10]. In addition, although both GC and tocilizumab treatment strongly suppress the synthesis of these markers [7, 8], disease activity may persist [7, 11, 12]. In line with this notion, recent studies have shown ongoing vessel inflammation, despite normalization of CRP and ESR, under GC treatment [13] as well as under tocilizumab treatment [11, 12]. Recent meta-analyses on serum markers in GCA concluded that there are no reliable serological markers for monitoring or prognosis [14, 15]. Thus there is an unmet need for IL-6-independent biomarkers that accurately reflect disease activity and vessel wall inflammation during treatment with GCs or tocilizumab.

In an effort to identify prognostic biomarkers and biomarkers for monitoring disease activity, we took clues from GCA characteristic pathogenic processes at the tissue level; these include vessel wall granulomatous infiltrates and neoangiogenesis [16–18]. Consequently, we hypothesized a role for macrophage products and markers of angiogenesis as novel candidate biomarkers. Monocytes and macrophages are capable producers of IL-6 [19], a cytokine known to stimulate hepatocytes to produce acute-phase response markers, including serum amyloid A (SAA) [20, 21]. During inflammation, monocytes/macrophages also release calprotectin, sCD163 and YKL-40 [22–24]. Inflamed GCA vessels are characterized by new vessel formation involving VEGF, angiopoietin-1 and -2 and sTie2 as key regulators in this process [25–27].

We thus compared the performance of these nine soluble markers at baseline with those of CRP and ESR using serum samples prospectively collected over 7 years in our GCA cohort. We established their association with the IL-6-driven acute-phase response. Next we investigated these markers for prediction of disease course and analysed the effects of GC treatment on these markers to identify candidates for monitoring of ongoing vascular inflammation.

Methods

Baseline

Forty-one newly diagnosed, treatment-naive GCA patients participated in the study (Table 1). Patients were diagnosed based on clinical signs and symptoms in combination with either a positive temporal artery biopsy (TAB) and/or a positive ^{18}F-fluorodeoxyglucose PET-CT scan. In this study, 27 of 41 GCA patients fulfilled the 1990 ACR criteria. The ACR criteria are useful in the diagnosis of C-GCA rather than LV-GCA. Blood samples were obtained before noon and all donors were non-fasted. Thirty-three age- and sex-matched healthy controls (HCs) and 13 age-matched infection controls (INFs) were included as well. HCs were screened for past and present morbidities. Hospitalized INFs were included only if diagnosed with pneumonia or a urinary tract infection. They were excluded in case of comorbid diseases such as cancer or diabetes and/or treatment with immunosuppressive drugs. Written informed consent was obtained from all study participants. All procedures were in compliance with the Declaration of Helsinki. The study was approved by the institutional review board of the University Medical Center Groningen (METc2010/222 for GCA and INFs and METc2012/375 for HCs).

Follow-up

GCA patients were prospectively followed, during which time they visited the outpatient clinic according to a fixed study protocol. In case of reappearance of clinical signs and symptoms, a relapse visit was planned. Remission or relapse was defined based on clinical signs and symptoms of GCA. CRP or ESR levels were not taken into

TABLE 1 Baseline characteristics of GCA patients and controls

Characteristics	HCs	GCA	INFs
n	33	41	13
Age, years, median (range)	67 (50–83)	71 (52–89)	74 (47–97)
Female, *n* (%)	22 (67)	28 (68)	4 (31)[a]
GCA diagnosis (TAB/PET-CT/both), *n*	NA	13/19/9	NA
GCA symptoms (cranial/systemic/combined), *n*	NA	11/8/22	NA
Fulfilled ACR criteria, *n* (%)	NA	27 (66)	NA
PMR clinic, *n* (%)	NA	10 (24)	NA
Ischaemic ocular involvement, *n* (%)	NA	11 (27)	NA
Claudication, *n* (%)	NA	22 (54)	NA
Follow-up, months, median (range)	NA	30 (0–71)	NA

[a]The three groups did not significantly differ in age, but significantly fewer IFNs were female compared with the other groups (χ^2 test, $P < 0.05$). NA: not applicable.

account, in line with the analysis of the GiACTA trial [8]. At 3 months (range 4 weeks; $n = 30$), 6 or 9 months (S.D. 10 weeks; $n = 5$) and 12 months (S.D. 10 weeks; $n = 29$), follow-up samples were collected as per protocol (Supplementary Fig. S1 and Table S1, available at *Rheumatology* online).

To investigate differences in biomarker levels in remission patients who would or would not relapse within a time frame of 4 months, samples were identified, grouped and compared (Supplementary Fig. S1 and Table S1, available at *Rheumatology* online).

Treatment

All patients were treated with GCs, which were tapered in agreement with the British Society for Rheumatology guidelines [28]; in short, a starting dose of 40–60 mg/day and tapering by 10 mg every 2–3 weeks to 20 mg/day, followed by more gradual tapering. Tapering was done when clinical signs and symptoms of disease activity were absent, preferably with normalization of CRP and ESR. In case of a relapse, the GC dose was increased and/or a conventional synthetic DMARD was added (MTX or LEF). GC-free remission was defined as an absence of signs and symptoms, no GC use and no return of active disease within at least 6 months of follow-up. Treatment-free remission was defined as no signs and symptoms, no GCs or other DMARDs and no return of active disease for a period of at least 6 months of follow-up. Serum marker levels were assessed in samples of eight patients having achieved treatment-free remission.

Laboratory measurements

Serum marker levels were determined by ELISA or Luminex. Immunohistochemistry was performed on five GCA-positive TABs. A more detailed description of the methods can be found in the supplementary material, section Supplementary Methods, available at *Rheumatology* online.

Statistical analysis

Non-parametric tests (two-tailed) were used to analyse the data (differences between groups). Comparisons between baseline patients and control groups were done by Kruskal–Wallis and Mann–Whitney U tests. Also, the Mann–Whitney U test was used for comparison of follow-up samples with HCs, comparison of samples from active patients and patients in remission during treatment and comparison of remission patients who would or would not relapse within 4 months. Paired testing was performed to compare follow-up samples and baseline samples using the Wilcoxon signed rank test. Correlations between biomarkers were assessed using Spearman's rank correlation coefficient. To compare the time to GC-free remission of patients with high levels of serum markers at baseline to patients with low levels, the log rank test was used. The log rank test was used as well to calculate hazard ratios for long-term GC requirement. Analyses were performed with SPSS 23 (IBM, Armonk, NY, USA) and GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA) software.

Results

Follow-up patient characteristics

The median follow-up duration of GCA patients was 30 months (range 0–71). Of 41 patients in the cohort, 15 reached GC-free remission in a median of 21 months (range 8–47) (Supplementary Table S1, available at *Rheumatology* online).

Elevated levels of inflammatory and angiogenesis serum markers in newly diagnosed GCA patients

Macrophage products (calprotectin, YKL-40, sCD163) and markers of angiogenesis (VEGF, angiopoietin-2, sTie2) were significantly higher in newly diagnosed, GC treatment-naive GCA patients ($n = 41$) compared with age- and sex-matched HCs ($n = 33$) (Fig. 1, Supplementary Fig. S2 and Table S2, available at *Rheumatology* online). In contrast, angiopoietin-1 levels were not elevated. As expected, ESR and acute-phase markers (CRP, IL-6 and SAA) were also elevated. Most markers were also found to be elevated in INFs ($n = 13$), indicating that these markers are not disease specific. Interestingly, ESR and angiopoietin-2 levels were clearly elevated in four of five patients with low CRP levels, suggesting that these markers could add to diagnosis (Supplementary Table S3, available at *Rheumatology* online).

Acute-phase response and GCA clinic

Patients with combined cranial and systemic symptoms had a significantly higher acute-phase response compared with patients with isolated cranial or systemic symptoms (Table 1, supplementary material, section Supplementary Methods, available at *Rheumatology* online). CRP and ESR were significantly higher in the combined group ($n = 22$) when compared with the isolated cranial group ($n = 11$, $P < 0.05$) or the isolated systemic group ($n = 8$, $P < 0.05$). IL-6, SAA and sCD163 were also significantly higher in the combined group compared with the isolated cranial group ($P < 0.05$). No differences were found for the other macrophage and angiogenesis markers.

Levels of acute-phase markers were lower in patients with ischaemic ocular involvement ($n = 11$, $P < 0.05$ for IL-6 and SAA). Interestingly, this was not typical for patients with other ischaemic symptoms in both C- and LV-GCA (e.g. jaw claudication and limb claudication; $n = 22$). No differences were found for the other markers.

Baseline inflammatory and angiogenesis serum marker correlations

Next we investigated serum marker correlations. As expected, levels of CRP, ESR and SAA were strongly correlated with IL-6 in newly diagnosed GCA patients ($n = 41$; Fig. 2A). Also, we found a strong correlation of sCD163 with IL-6 and the acute-phase markers. In contrast, YKL-40 correlated only weakly with IL-6 and SAA. No correlations were found for IL-6 and macrophage marker

Fig. 1 Serum marker levels in newly diagnosed GCA patients compared with INFs and HCs
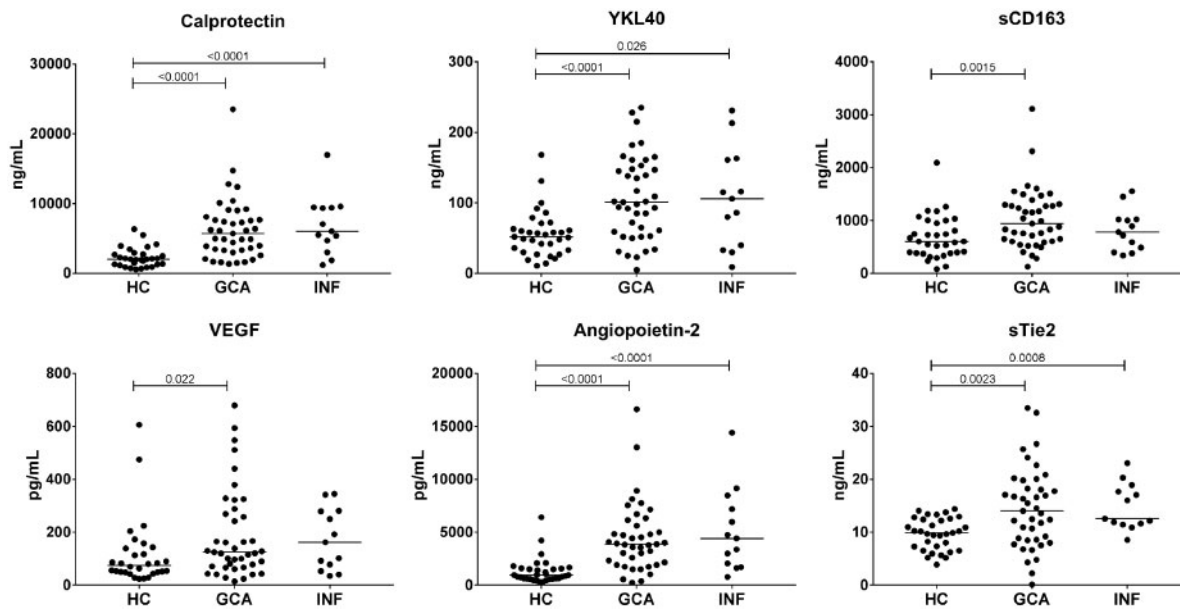
Serum levels of calprotectin, YKL-40, sCD163, VEGF, angiopoietin-2 and sTie2 in newly diagnosed GCA patients compared with IFNs and HCs. All markers were significantly higher in newly diagnosed, treatment-naive GCA ($n = 41$) and in INFs ($n = 13$) as compared with age-matched HCs ($n = 33$). Since the Kruskal–Wallis test showed a significant difference between groups ($P < 0.05$), differences between individual groups were tested with the Mann–Whitney U test. The horizontal line represents the median. Statistical significance is indicated by P-values in the graphs.

calprotectin. No or weak correlations were seen for markers of angiogenesis with IL-6 or the acute-phase response.

Interestingly, in INFs, IL-6 correlated strongly with calprotectin, YKL-40 and angiopoietin-2, whereas correlations of IL-6 with SAA and sCD163 were absent ($n = 13$; Fig. 2B).

Expression of macrophage and angiogenesis markers in TABs at diagnosis

To confirm that markers of macrophages are expressed at the site of GCA pathology, consecutive TAB sections ($n = 5$) were stained for calprotectin and YKL-40 by immunohistochemistry (IHC; Fig. 3). To identify macrophage-rich areas, sections were stained with CD68 and CD163. In addition, we stained for VEGF and angiopoietin-2 as markers of angiogenesis. Newly formed vessels were identified by staining of $CD34^+$ endothelial cells. Expression of IL-6 and SAA was assessed as markers of the acute-phase response. We did not investigate the expression of angiopoietin-1, as serum levels were not modulated in GCA, nor sTie-2, as there are no IHC reagents available.

All markers were found to be expressed in the tissue. Massive staining was observed for YKL-40 and angiopoietin-2. As expected, expression of all markers was found mostly in macrophage-rich areas, but endothelial cells also appeared to express IL-6, VEGF and angiopoietin-2. As calprotectin may also be expressed by neutrophils,

we checked their presence by CD15 staining. Few $CD15^+$ cells were found (data not shown).
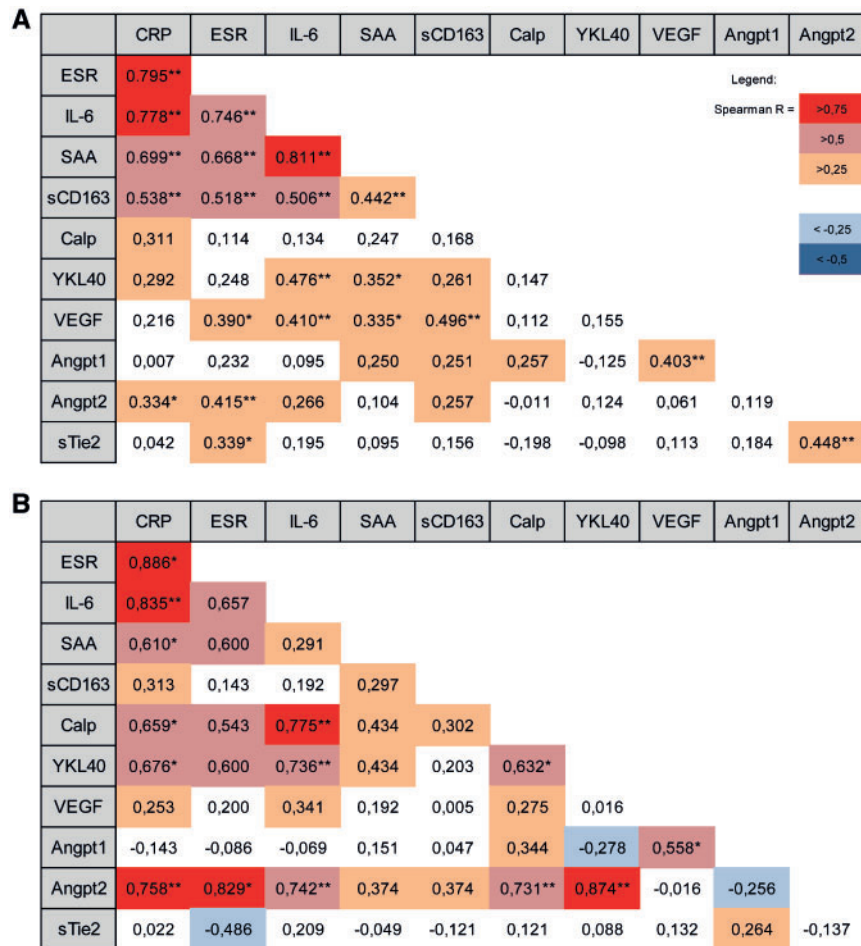
Angiogenic markers at baseline predict time to GC-free remission

Next we determined if baseline serum marker levels could predict disease outcome. To that end we compared the time to GC-free remission in patients with serum levels below the median (low) and above the median (high). High relative levels of VEGF and angiopoietin-1 and low relative levels of YKL-40 at baseline were found to predict a short time to GC-free remission (Fig. 4). In addition, a strong trend was seen for low levels of angiopoietin-2, predicting a short time to GC-free remission. The hazard ratio for long-term GC requirement per biomarker was calculated: 5.5 for lower than median VEGF (95% CI 2.0, 15.3), 3.5 for lower angiopoietin-1 (95% CI 1.3, 9.8), 2.8 for higher YKL-40 (95% CI 1.0, 8.2) and 2.9 for higher angiopoietin-2 (95% CI 1.1, 8.0).

Calprotectin and YKL-40 remain elevated during GC treatment

To identify markers associated with ongoing vascular inflammation in spite of GC treatment, we investigated the effects of GC treatment on all candidate markers. After 3 and 12 months ($n = 30$ and $n = 29$, respectively; Fig. 5A, B) of GC treatment, levels of most markers were found to be decreased compared with baseline, even though many remained significantly elevated compared with HCs.

Fɪɢ. 2 Baseline inflammatory and angiogenesis serum marker correlations

A

	CRP	ESR	IL-6	SAA	sCD163	Calp	YKL40	VEGF	Angpt1	Angpt2
ESR	0.795**									
IL-6	0.778**	0.746**								
SAA	0.699**	0.668**	0.811**							
sCD163	0.538**	0.518**	0.506**	0.442**						
Calp	0,311	0,114	0,134	0,247	0,168					
YKL40	0,292	0,248	0.476**	0.352*	0,261	0,147				
VEGF	0,216	0.390*	0.410**	0.335*	0.496**	0,112	0,155			
Angpt1	0,007	0,232	0,095	0,250	0,251	0,257	-0,125	0.403**		
Angpt2	0.334*	0.415**	0,266	0,104	0,257	-0,011	0,124	0,061	0,119	
sTie2	0,042	0.339*	0,195	0,095	0,156	-0,198	-0,098	0,113	0,184	0.448**

Legend:
Spearman R =
>0,75
>0,5
>0,25
<-0,25
<-0,5

B

	CRP	ESR	IL-6	SAA	sCD163	Calp	YKL40	VEGF	Angpt1	Angpt2
ESR	0,886*									
IL-6	0,835**	0,657								
SAA	0,610*	0,600	0,291							
sCD163	0,313	0,143	0,192	0,297						
Calp	0,659*	0,543	0,775**	0,434	0,302					
YKL40	0,676*	0,600	0,736**	0,434	0,203	0,632*				
VEGF	0,253	0,200	0,341	0,192	0,005	0,275	0,016			
Angpt1	-0,143	-0,086	-0,069	0,151	0,047	0,344	-0,278	0,558*		
Angpt2	0,758**	0,829*	0,742**	0,374	0,374	0,731**	0,874**	-0,016	-0,256	
sTie2	0,022	-0,486	0,209	-0,049	-0,121	0,121	0,088	0,132	0,264	-0,137

Depicted are Spearman's correlation coefficients for all markers in (**A**) newly diagnosed, treatment-naive GCA patients and (**B**) INFs. The strength of correlation is indicated by cell colours. Statistical significance is *$P < 0.05$ or **$P < 0.01$.

Importantly, calprotectin and YKL-40 levels remained mostly unaffected by GCs and could thus reflect asymptomatic smouldering vessel wall inflammation. Angiopoietin-1 levels were significantly higher in active patients compared with patients in remission at 12 months ($P < 0.05$; Fig. 5B). YKL-40 correlated with the treatment-reduced ESR, CRP and IL-6, suggesting that YKL-40 may identify ongoing subclinical inflammation in spite of treatment (Supplementary Fig. S3, available at *Rheumatology* online).

Angiopoetin-2 elevated in remission patients with an imminent relapse

Next we assessed marker levels associated with future relapses in remission patients (all within 12 months of GC treatment; see Supplementary Fig. S1 and Table S1, available at *Rheumatology* online). To this end we compared remission patients that would relapse within a period of 4 months ($n = 14$, future relapse) with those that would not relapse in 4 months ($n = 35$). Angiopoietin-2 levels were significantly higher in the future-relapsing group (Fig. 5C). The data are in line with increased hazard ratio's for long-term GC requirement associated with high angiopoietin-2 levels (see above, Fig. 4).

### Extended elevation of markers in treatment-free remission

There is a paucity of data on serum markers in treatment-free remission, as these samples are rarely available. To answer the question of whether treatment leads to normalization of serum markers in treatment-free remission, we assessed serum marker levels in a small group of patients ($n = 8$; Supplementary Fig. S1, available at *Rheumatology* online). Levels of IL-6, ESR, sCD163, angiopoietin-1 and -2 and calprotectin remained significantly elevated compared with HC levels (Fig. 5D). Calprotectin levels were persistently high throughout the whole disease course, while angiopoietin-1 levels

FIG. 3 Representative IHC stainings of consecutive sections in a positive TAB of a treatment-naive GCA patient
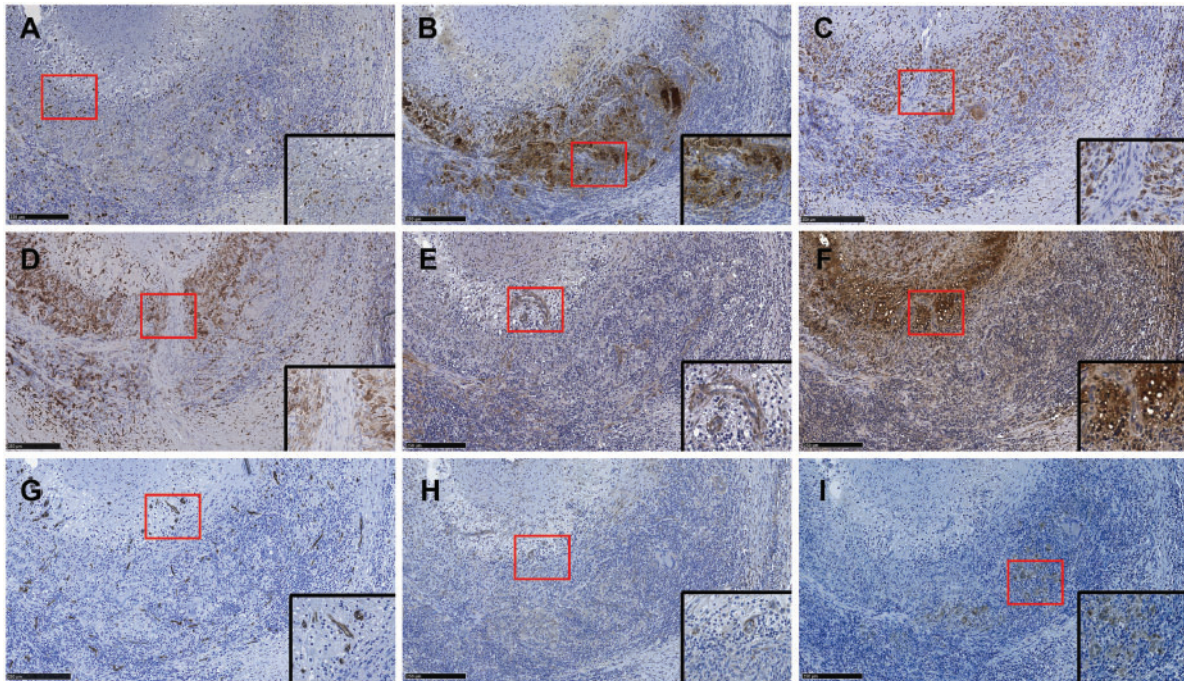

Paraffin-embedded tissues were stained with antibodies against (**A**) calprotectin, (**B**) YKL-40, (**C**) CD68, (**D**) CD163, (**E**) VEGF, (**F**) angiopoietin-2, (**G**) CD34, (**H**) IL-6 and (**I**) SAA. Regions of interest (red) are magnified and are shown in the lower right corner.

FIG. 4 Angiogenesis markers and YKL-40 at baseline predicted short-term GC treatment in GCA
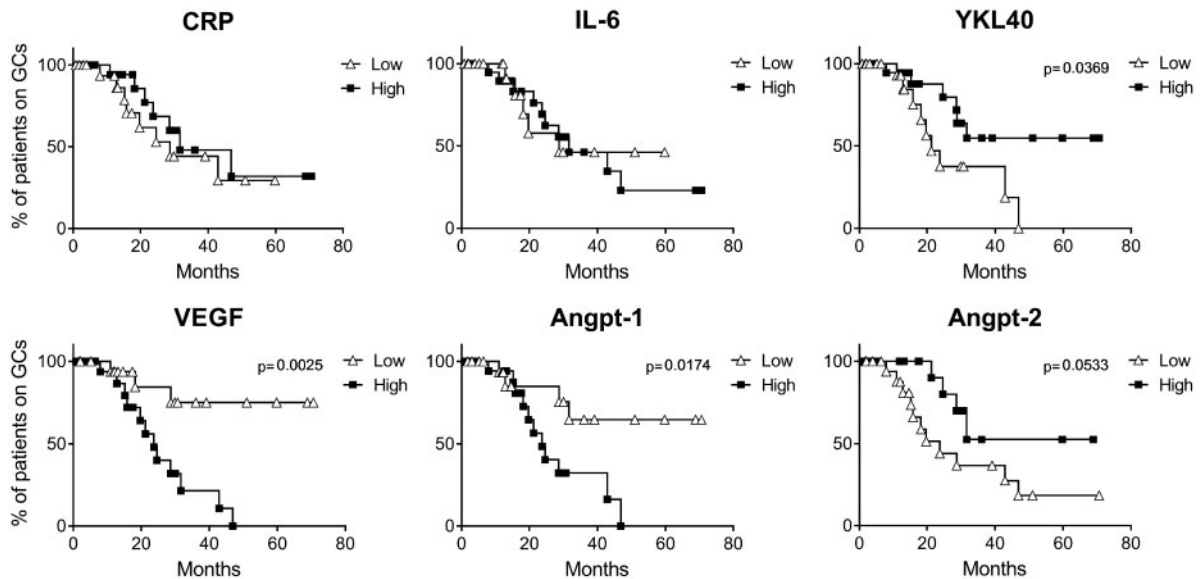
Baseline serum marker levels were split in GCA patients by low and high levels (based on the median) and were plotted in a Kaplan–Meier curve against time to GC-free remission. Strong trends and significant differences of the log rank test are indicated as *P*-values in the graphs. Like CRP and IL-6, baseline levels of ESR, SAA, sCD163, calprotectin and sTie2 were not predictive of time to GC-free remission (data not shown).

Fig. 5 Changes in serum biomarker concentrations during and after treatment
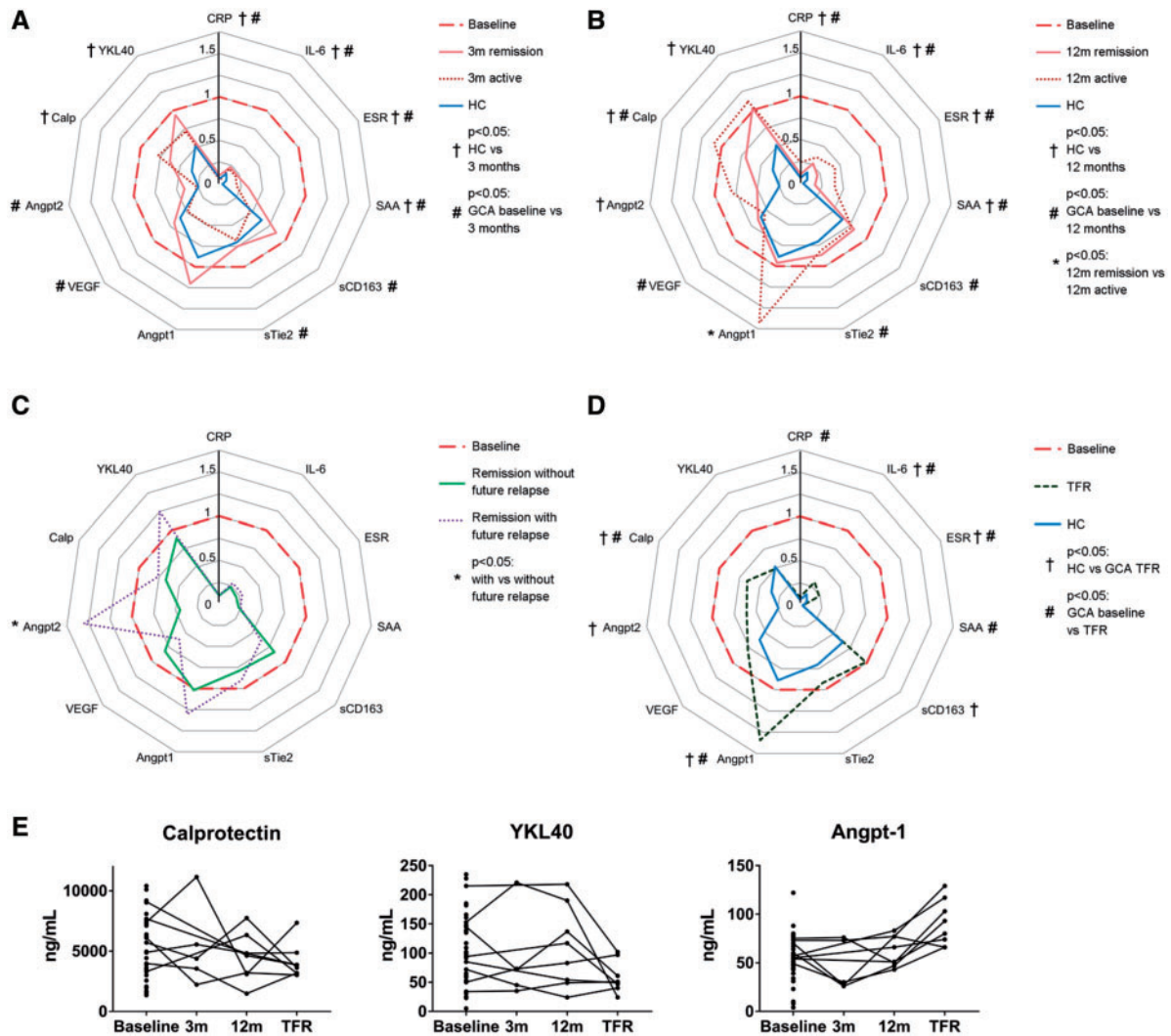
Radar plots showing biomarker levels expressed as fold changes compared with GCA baseline values. (**A**) Patients in remission (*n* = 24) or active disease (*n* = 6) at 3 months after the start of treatment. (**B**) Patients in remission (*n* = 20) or active disease (*n* = 9) at 12 months. (**C**) Samples from remission patients who would (*n* = 14) or would not relapse (*n* = 35) within 4 months. (**D**) Patients in treatment-free remission (TFR; *n* = 8). (**E**) Serum markers over time in GCA patients in treatment-free remission (*n* = 8). HCs, *n* = 33. Statistical significance by Mann–Whitney *U* test.

increased only after at least 12 months of treatment (Fig. 5E). Interestingly, YKL-40 levels remained elevated during 12 months of treatment but eventually normalized in treatment-free remission. Fluctuations of the other markers over time are shown in Supplementary Fig. S4, available at *Rheumatology* online.

Discussion

GCA remains difficult to treat, as the current treatment strategy with GCs is a trade-off between their variable efficacy and their side effects. The goal of treatment in GCA is to reach stable GC-free remission as quickly as possible. Currently only scarce evidence suggests that serum markers may predict disease course in GCA [14]. In this study,

we found that serum markers of angiogenesis at baseline predicted not only time to GC-free remission (VEGF, angiopoietin-1 and YKL-40), but were also associated with an imminent relapse while on treatment (angiopoietin-2). Thus these markers may aid the stratification of patients eligible for a quick or slow GC tapering scheme. In addition, we identified macrophage products as markers of vessel wall inflammation that may be used for monitoring vascular disease during and after treatment.

We found several markers of angiogenesis (VEGF, angiopoietin-2 and sTie2) to be upregulated in GCA. Neoangiogenesis is instigated by disruption of homeostatic angiopoietin-1–Tie2 signalling by angiopoietin-2 (competing for binding the Tie-2 receptor) and sTie2 (as decoy receptor) in the presence of VEGF [26, 27]. In GCA

TABs, we indeed found VEGF and angiopoietin-2 expressed in neoangiogenic areas, likely triggered by hypoxia [29]. Our findings highlight the importance of new vessel formation at the site of inflammation in GCA to fuel the ongoing inflammatory process and are in line with previous studies reporting on elevated levels of VEGF in GCA. However, these studies did not investigate this marker in longitudinal follow-up studies [25, 30, 31].

We found that high levels of serum VEGF and angiopoietin-1 at baseline were predictive of a short time to GC-free remission. In contrast, high levels of angiopoietin-2, tended to be predictive of a non-favourable disease course. Moreover, elevation of angiopoietin-2 preceded relapses during treatment. Thus markers of angiogenesis impact the disease course in GCA. The protective effect of VEGF may be explained by its potential to repress CD4$^+$ T cell proliferation and activation. CD4$^+$ T cells, key players in GCA pathogenesis, express VEGFR2, but not the angiopoietin receptor Tie2 [32]. However, the notion of protective features of VEGF was not substantiated in another study in which VEGF was reported to amplify T cell pathogenic effector functions in GCA [33].

Worldwide, CRP is increasingly being used for GCA diagnosis instead of ESR, however, diagnosis is typically difficult in patients with low CRP [9, 10]. We propose that elevated angiopoietin-2 may have utility in diagnosis of a small subset of GCA patients with low CRP. Angiopoietin-1 levels were not altered at baseline and during treatment. In treatment-free remission patients, however, increased levels of angiopoietin-1 were found, which may suggest a role in microvessel stabilization. So far, serum levels of angiopoietins and their decoy receptor sTie2 [27] have not been documented in GCA. Clearly, more fundamental studies are needed to elucidate the role of angiopoietins in GCA.

In this study we provide evidence for the notion of IL-6-independent biomarkers of vessel inflammation at baseline and during treatment. The monocyte/macrophage products calprotectin and, to a lesser extent, YKL-40 are IL-6-independent. Moreover, both markers remain elevated in spite of treatment and thus qualify as candidate biomarkers of smouldering vessel inflammation. Our findings are in line with the notion that GCs do not sufficiently suppress vascular inflammation [13]. Calprotectin (MRP8/14 or S100A8/9) is a calcium binding protein that acts as a damage-associated molecular pattern signal on the TLR4 and RAGE receptors [34]. It is released by monocytes and neutrophils after interaction with endothelial cells during migration [35]. Importantly, calprotectin levels did not correlate with IL-6 and the acute-phase response. Calprotectin levels remained high in treated patients, suggesting ongoing monocyte/neutrophil tissue migration and innate immune activation. Surprisingly, calprotectin levels remained elevated in treatment-free remission. YKL-40 is a marker expressed by mature macrophages, thought to be involved in tissue remodelling and angiogenesis [36, 37]. It is expressed by non-classical monocytes in the blood and by macrophages and giant cells in GCA TABs [24, 38]. *In vitro*, YKL-40 production by macrophages is sensitive to GCs [39]. In our study, however, long-term high-dose GC treatment did not lead to a direct decrease in serum YKL-40 levels, suggesting that YKL-40-producing cells are GC resistant. High YKL-40 levels at baseline predicted a long time to GC-free remission. Our observation of strong YKL-40 expression in TABs in the intima-media border region suggests that this protein is mainly released in fully developed GCA with transmural inflammation, which may be more GC resistant. Interestingly, YKL-40 levels were clearly decreased in treatment-free remission, which may point towards resolution of inflammation.

We found a strong correlation between IL-6, CRP/ESR and SAA at baseline in GCA patients. This was not the case in infection controls, where levels of SAA were not correlated with IL-6, implying that other cytokines stimulate hepatocytes to produce SAA, such as IL-1β or TNFα, cytokines that are reportedly not increased in GCA [20, 40]. We found SAA also expressed at the tissue level. SAA may amplify the local inflammatory response, as O'Neill *et al.* [21] showed that stimulation with SAA induced the production of IL-6, VEGF and angiopoietin-2 in TAB explants.

We observed a stronger acute-phase response in patients with overlapping C-GCA and LV-GCA compared with patients with C-GCA or LV-GCA alone. Recent reviews addressed the similarities and differences between C-GCA and LV-GCA patients [2, 3, 14]. It is currently still debated which patient group expresses the strongest acute-phase response: C-GCA, LV-GCA or patients with overlapping symptoms [3, 41]. High levels of acute-phase proteins in patients with overlapping symptoms may be due to a higher inflammatory load (more inflamed vessels) and consequently greater net IL-6 production and ensuing acute-phase response.

Patients with ischaemic ocular involvement presented with a weaker acute-phase response, in line with previous reports [42–44]. In contrast, we did not observe a weak acute-phase response in patients presenting with other ischaemic symptoms, such as claudication. It has been suggested that high levels of IL-6 are protective against ischaemic events by promoting neoangiogenesis [44]. Thus it could be expected that IL-6, via a similar mechanism, is protective against claudication as well, which was not the case in our cohort. Therefore it is more likely that patients with visual symptoms present earlier in the disease course not yet having developed more extensive vessel wall inflammation.

This study has several strengths. The selection of biomarkers was based on a strong rationale—their potential involvement in GCA immunopathogenesis. Also, we included newly diagnosed GCA patients before the start of GC treatment. This is an important strength, as we observed a strong effect of GCs on most serum markers. Furthermore, newly diagnosed, treatment-naive patients were prospectively followed for up to 7 years and samples were taken at fixed time points. Patients were intensively monitored by frequent follow-up visits according to protocol and extra visits in between in case of suspicion of a relapse. This allowed us to calculate the exact time to

GC-free remission. Due to the already long-standing follow-up, we were also able to include treatment-free remission samples. This revealed that many serum markers are still elevated for an extended period of time. Another advantage of our study design is the inclusion of two control populations: age- and sex-matched HCs and age-matched INFs, allowing us to discriminate between disease-specific and non-specific events.

Our study has the following limitations: low numbers of patients with active disease during treatment and low numbers of patients in treatment-free remission (both $n < 10$). The latter limitation is obviously due to the length of the disease course. This implies that the data in active disease and in treatment-free remission should be taken with caution. Data from this study cannot yet be extended to GCA patients treated with tocilizumab.

The serum markers in this study may aid in designing personalized medicine for easy- (short-term GC requiring) and difficult-to-treat (long-term GC requiring) GCA patients. Patients at baseline may be stratified based on VEGF, angiopoietins and possibly YKL-40 levels for a quick or a slow GC tapering scheme. It is yet unclear whether these markers have a similar predictive value in patients on IL-6R blockade treatment. The predictive values of angiogenesis-related serum markers require further confirmation. Future studies on tissue inflammation markers may focus on calprotectin or YKL-40, especially to prevent aneurysms and aortic dissection. PET-CT or follow-up biopsies would allow us to determine whether these markers correlate with silently ongoing tissue inflammation. If calprotectin and/or YKL-40 are confirmed as markers of tissue inflammation, monitoring their levels would be implied to prevent recurrence of disease in GCA patients in remission.

To conclude, this prospective study identified a profile of angiogenic and macrophage serum markers that predict disease course in GCA. This profile outperformed the classic GCA biomarkers CRP and ESR. In addition, calprotectin and/or YKL-40 may prove useful as IL-6-independent biomarkers monitoring vessel inflammation during treatment.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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