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Basic science

Analyses of plasma inflammatory proteins reveal biomarkers predictive of subsequent development of giant cell arteritis: a prospective study

Karin Wadström [1,2,*], Lennart T. H. Jacobsson[1,3], Aladdin J. Mohammad [4,5], Kenneth J. Warrington [6], Eric L. Matteson[6], Magnus E. Jakobsson[7], Carl Turesson [1,4]

[1]Rheumatology, Department of Clinical Sciences, Lund University, Malmö
[2]Center for Rheumatology, Academic Specialist Center, Region Stockholm, Stockholm
[3]Department of Rheumatology and Inflammation Research, The Sahlgrenska Academy, University of Gothenburg, Institute of Medicine, Gothenburg
[4]Department of Rheumatology, Skåne University Hospital, Lund, Sweden
[5]Department of Medicine, University of Cambridge, Cambridge, UK
[6]Division of Rheumatology, Mayo Clinic College of Medicine and Science, Rochester, MN, USA
[7]Department of Immunotechnology, Lund University, Lund, Sweden

*Correspondence to: Karin Wadström, Department of Clinical Sciences, Skåne University Hospital, 205 02 Malmö, Sweden. E-mail: karin.wadstrom@med.lu.se

Abstract

Objective: To investigate the relation between biomarkers of inflammation and subsequent development of GCA.

Method: Participants in the population-based Malmö Diet Cancer Study (MDCS; $N = 30\,447$), established 1991–96, who were subsequently diagnosed with GCA, were identified in a structured process. GCA-free controls, matched for sex, year of birth and year of screening were selected from the study cohort. Baseline plasma samples were analysed using the antibody-based OLINK proteomics inflammation panel (92 inflammatory proteins). Analyses were pre-designated as hypothesis-driven or hypothesis-generating. In the latter, principal component analysis was used to identify groups of proteins that explain the variance in the proteome. Within components selected based on eigenvalues, proteins with a factor loading of >0.50 were investigated.

Results: Ninety-four cases with a confirmed incident diagnosis of GCA (median 11.9 years after inclusion) were identified. Among biomarkers with *a priori* hypotheses, IFN-γ was positively associated with GCA [odds ratio (OR) per SD 1.52; 95% CI 1.00, 2.30]. Eight biomarkers in the hypothesis-generating analyses were significantly associated with development of GCA. Among these, higher levels of IFN-γ (OR 2.37; 95% CI 1.14, 4.92) and monocyte chemotactic protein 3 (MCP3) (OR 4.27; 95% CI 1.26, 14.53) were particularly associated with increased risk of GCA in the subset sampled <8.5 years before diagnosis. Several other proteins known to be important for T cell function were also associated with GCA in these analyses, e.g. CXCL9, IL-2, CD40 and CCL25.

Conclusion: Elevated IFN-γ levels were found years prior to diagnosis of GCA. T cell activation may precede the clinical onset of GCA.

Keywords: GCA, biomarkers, IFN-γ, inflammation, pathogenesis

Rheumatology key messages

- Several potential biomarkers were elevated in cases who later developed GCA compared with controls.
- IFN-γ and MCP3 were significantly elevated in those sampled closest to diagnosis of GCA.
- Our results suggest activation of the adaptive immune system years before GCA onset.

Introduction

GCA is the most common large vessel vasculitis in persons aged >50 years in the western world, affecting medium- to large-sized arteries, with a female predominance [1]. The highest incidence rates have been reported from Scandinavian countries and Minnesota, USA, having incidences of ∼20–30/100 000 among persons aged over 50 years [2, 3].

Factors predicting GCA have been detected years before clinical onset. In a nested case–control study from our group, individuals who subsequently developed GCA had lower blood glucose, cholesterol and triglycerides at baseline, a median of 21 years before disease onset, compared with controls [4]. Earlier studies have found that low body mass index [5–7], current smoking and multiple hormone-related factors were associated with GCA [5].

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The inflammation in the vessel wall has been proposed to be triggered by toll-like receptors (TLRs) on dendritic cells in the adventitia. After activation, two different inflammatory processes, characterized by production of different cytokines, have been identified. The IL-6/IL-17 axis, which drives differentiation of Th17 cells, is important for the acute and early vascular inflammation in GCA. The second process, thought to be important for chronic vasculitis, is the IL-12/IFN-γ axis, which promotes differentiation to Th1 cells producing IFN-γ [8, 9].

Several studies have investigated biomarkers in recent-onset GCA. Levels of IL-6 have been shown to be elevated in newly diagnosed GCA in numerous studies [10–17]. Besides IL-6, several other cytokines have been found to be elevated in active disease [10, 11, 15, 16, 18], including IFN-γ [15, 16].

Vascular smooth muscle cells in the adventitia are activated by IFN-γ, leading to the production of several chemokines, e.g. CCL1, CXCL-9, CXCL-10 and CXCL-11, that affect lymphocyte and monocyte migration. Recruited cells of the monocyte/macrophage lineage that have been activated in the adventitia may fuse into multinucleated giant cells [19]. CXCL9, and other inflammation related proteins, i.e. VCAM, ICAM1, VEGF, MMP1 and MMP9, have been described to be significantly elevated among patients with active GCA [10, 16, 17, 20–23].

In this study, we aimed to investigate inflammation-associated plasma proteins prior to onset of GCA. To our knowledge, this has not been done previously.

Methods

Source population and exposure information

The Malmö Diet Cancer Study (MDCS) is a community-based health survey performed in Malmö in 1991–96, which included 30 447 subjects (12 121 men and 18 326 women). With a total source population of 74 138 persons, this corresponds to a participation rate of 40.8%. Mean age at screening was 58 years in women and 59 years in men. The cohort included residents of Malmö, i.e. all women born 1923–50 and all men born 1923–45. The only exclusion criteria were insufficient Swedish language skills and mental incapacity. Information on lifestyle factors and current health status was collected from all participants using a self-administered questionnaire. Non-fasting blood samples were obtained at the time of inclusion in the health survey in a standardized manner and stored at –80°C. More details on the MDCS are described elsewhere [24].

Cases and controls

The cases were selected on the basis of inclusion in the MDCS before being diagnosed with GCA. Patients were identified based on a registered diagnosis code of GCA in the local outpatient clinic administrative register for Malmö University Hospital or the National Patient Register after inclusion in the MDCS and through 31 December 2011. The medical records of the selected subjects were then reviewed in a structured process, and cases were classified according to the 1990 ACR criteria for GCA [25]. Some cases with typical clinical features were included, based on expert opinion, even if they did not fulfil the classification criteria. In addition, data on visual manifestations, initial dose of glucocorticosteroids, and large vessel involvement and other disease characteristics were collected.

One control for each validated case, matched for sex, year of birth and year of screening, was randomly selected from the MDCS cohort. The controls were alive and free from GCA when the index subject was diagnosed with GCA. Controls were randomly selected among those with preserved blood samples. One control was randomly selected as control for one independent case.

We identified 100 cases with corresponding controls. After excluding those who had no preserved plasma samples, or insufficient sample volume, data were available for 94 cases and 97 controls.

This study was approved by the regional research ethics committee for southern Sweden (registration number 308/2007). When included in the MDCS all participants gave their written informed consent to future use of collected information and samples for research purposes. No additional consent for participating in this study was obtained.

Plasma proteomic biomarkers

A large panel with 92 inflammatory proteins (Olink® Inflammation panel [26]) was used to investigate potential biomarkers of inflammation prior to clinical disease onset in patients developing GCA. All biomarkers are presented in Supplementary Table S1, available at *Rheumatology* online.

Plasma levels of proteins were analysed by the Proximity Extension Assay (PEA) technique using a multiplex reagent kit (O-link Bioscience, Uppsala, Sweden) which is described in detail elsewhere [27]. Briefly, two oligonucleotide-labelled highly specific antibodies bind to each target protein. When both antibodies bind to the same antigen this allows the formation of a unique PCR product sequence that is detected and quantified for every analyte. All data are presented as arbitrary units. As the platform provides relative protein quantification as log2 normalized protein expression (NPX), every unit increase corresponds to a doubling in the relative protein concentration. Patients or public were not involved in study design, recruitment or dissemination of results.

Statistics

Statistical analyses were separated in two categories specified in a study protocol written before obtaining the data. Biomarkers with an *a priori* hypothesis, formulated by the authors, were handled separately from analyses involving all biomarkers. The latter were regarded as hypothesis-generating analyses.

Variables that were not normally distributed were log-transformed using the natural logarithm. Variables were considered skewed in cases with Shapiro–Wilk statistics of <0.85. Those with values of 0.85–0.90 were graphically assessed. To allow for logarithmic computation without censoring individuals with negative NPX values, the smallest possible constant was added to the arbitrary values.

Biomarkers with a priori hypothesis

Eight biomarkers were selected for evaluation of *a priori* hypothesis biomarkers (Supplementary Table S2, available at *Rheumatology* online). Six of them were assumed to be elevated: IFN-γ, IL-6, CXCL-10, CXCL-11, CASPASE 8 and LIF; and two of them were predicted to be reduced: FGF-21 and PD-L1. These assumptions were based previous studies on GCA pathogenesis [16, 28, 29].

Conditional logistic regression was used to examine potential biomarker predictors, with case status as outcome. Each case and its corresponding control were given a group number. This number was entered in the logistic regression models as a categorical variable. Odds ratios (ORs) were calculated per S.D. to enable comparisons of effect sizes.

All analyses were stratified by time from screening to GCA diagnosis (by quartiles) in years. Trends of associations across quartiles were assessed by examining the interactions between quartile of time to diagnosis and biomarker levels in separate logistic regression models. Furthermore, we stratified the analyses by visual symptoms, temporal artery biopsy (TAB) results, age at screening and age at diagnosis.

Multiple hypothesis testing was handled using the Holm correction approach [30], and both corrected and original *P*-values are presented.

All biomarkers, hypothesis-generating analysis

To enable comparability, z-scores were computed for all biomarker values and included in the principal component analysis (PCA). PCA was used to identify groups of proteins that explain variance in the proteome. Assumptions were fulfilled for Kaiser–Meyer–Olkin (KMO) test for sampling adequacy and Bartlett's test for sphericity, respectively. To reduce the number of components, the preliminary cut-off for the eigenvalue was set to >2.0, yielding nine components. PCA components 7–9 were considered to add a minor contribution, based on the scree plot (Supplementary Fig. S1, available at *Rheumatology* online) and limited numbers of variables with high factor loading (Supplementary Table S3, available at *Rheumatology* online). Due to the design of the PCA, individuals with missing data for one or more variable were automatically excluded from the analyses. Twelve individuals had incomplete data for IL-24 alone; therefore, IL-24 was dropped from the analysis. This reduced the number of individuals excluded due to missing values from 20 to 8.

Differences between cases and controls in these components, and in the biomarkers with a loading of >0.5 within in each component (1–6), were investigated using the same statistical protocol as for the *a priori* analyses. Correction for multiple testing was not performed for these analyses, due to the hypothesis-generating approach.

All statistical analyses were performed using the Statistical Package for Social Sciences (version 24.0; IBM, Armonk, NY, USA).

Results

Incident cases of GCA

A total of 94 cases were identified with a confirmed incident diagnosis of GCA and available results from the plasma proteome analyses. Of these, 77 (82%) of cases were female, 64% were biopsy positive and 90% fulfilled the ACR classification criteria for GCA. The mean age at diagnosis was 73.6 years (range 56.9–85.8). The median time from screening to diagnosis was 11.9 years (range 0.32–19.11) (Table 1). Only one case was screened <1 year before diagnosis.

Proportions with a self-reported history of comorbidities (malignancy, diabetes, myocardial infarction, stroke and recent respiratory infection) were similar in cases and controls (Supplementary Table S4, available at *Rheumatology* online).

Testing of a priori hypotheses

IFN-γ was higher in cases compared with controls [mean (NPX) 7.27 *vs* 7.07] (Table 2) [OR (per S.D.) 1.52; 95% CI 1.00, 2.30, $P = 0.048$)]. In analysis stratified by time from screening to diagnosis (quartiles), the highest OR was found in the subset sampled closer to GCA diagnosis (0.32–8.48 years) (OR 2.37; 95% CI 1.14, 4.92) with a decreasing trend by quartile (Table 3). Similarly, CXCL-10 levels were higher in cases compared with controls in the quartile closest to diagnosis (OR 3.34; 95% CI 1.03, 10.89, $P = 0.045$). Holm's corrected *P*-values were all >0.05. CXCL-11 had significantly higher levels in quartile 2 (8.49–11.92 years prior to diagnosis) (OR 2.63; 95% CI 1.04, 6.64, p 0.045) (Table 3). In the fourth quartile (15.5–19.1 years before diagnosis), there were negative associations for IL-6 and Caspase-8 with GCA (Table 3). Additional stratification revealed significant associations with GCA with visual symptoms and TAB negative GCA for IFN-γ, CXCL-10, CXCL-11 and FGF-21 (Supplementary Table S5, available at *Rheumatology* online).

Hypothesis-generating results

In the PCA, we identified six components with eigenvalues above 2.5. Factor loadings for every protein for each of these components are shown in Supplementary Table S3, available at *Rheumatology* online. The component that explained the greatest proportion of the total variation of protein concentrations was largely driven by markers of vascular endothelial activation. The two other components with eigenvalues >3.5

Table 1. Characteristics of patients with GCA at diagnosis

N	94
Female sex [*n* (%)]	77 (82)
Age at GCA diagnosis (years) (mean)	73.6 (S.D. 6.0; range 56.9–85.8)
Time from screening to GCA diagnosis (years) (median)	11.9 (range 0.32–19.11)
Biopsy positive [*n* (%)]	60 (64)
Fulfilled ACR classification criteria for GCA [*n* (%)]	85 (90)
Visual impairment at diagnosis [*n* (%)]	39 (42)
Large vessel involvement [*n* (%)]	13 (14)
ESR at diagnosis (mm/h) (mean)[a]	80 (S.D. 31)
CRP at diagnosis (median)[a]	88 (IQR 50–142)
Initial glucocorticoid dose (mg prednisolone) (median)[b]	40 (IQR 40–60)

[a] Missing data: ESR $n = 25$, CRP $n = 8$.
[b] Seven patients initially treated with i.v. glucocorticoids. IQR: interquartile range.

Table 2. Baseline values for biomarkers with *a priori* hypothesis, in subsequent GCA cases compared with controls

	Cases ($n = 94$)	Controls ($n = 97$)
IFN-γ	7.27 (0.90)	7.07 (0.79)
IL-6	2.12 (0.82)	2.14 (0.70)
CXCL10	9.47 (0.90)	9.33 (0.83)
CXCL11	10.51 (0.62)	10.42 (0.51)
Caspase 8	2.58 (0.70)	2.58 (0.81)
FGF-21	4.80 (1.12)	4.81 (1.16)
PD-L1	5.30 (0.47)	5.24 (0.40)
LIF	–0.31 (0.25)	–0.33 (0.34)

All values are in arbitrary units, NPX. Means (S.D.). NPX: normalized protein expression.

Table 3. Potential biomarkers of subsequent GCA, overall and stratified for time from screening to diagnosis

	All			Quartile 1 (0.3–8.5 years) (N^a =47)			Quartile 2 (8.5–11.9 years) (N^a =48)			Quartile 3 (11.9–15.5 years) (N^a =47)			Quartile 4 (15.5–19.1 years) (N^a =49)		
	OR (95% CI)	*P* value	*P* value (corr)	OR (95% CI)	*P*-value	*P*-value (corr)	OR (95% CI)	*P*-value	*P*-value (corr)	OR (95% CI)	*P*-value	*P*-value (corr)	OR (95% CI)	*P*-value	*P*-value (corr)
IFN-γ	1.52 (1.00, 2.30)	0.048	0.38	2.37 (1.14, 4.92)	0.021	0.17	1.72 (0.78, 3.77)	0.18	1.00	1.09 (0.39, 3.06)	0.87	1.00	0.60 (0.21, 1.75)	0.35	1.00
IL-6	0.91 (0.60, 1.38)	0.67	1.00	1.37 (0.65, 2.88)	0.41	1.00	1.15 (0.32, 4.20)	0.83	1.00	1.45 (0.57, 3.64)	0.44	1.00	0.37 (0.15, 0.95)	0.038	0.27
CXCL10	1.52 (0.92, 2.50)	0.10	0.71	3.34 (1.03, 10.89)	0.045	0.27	2.11 (0.83, 5.40)	0.12	0.82	0.82 (0.32, 2.14)	0.69	1.00	0.89 (0.29, 2.78)	0.85	1.00
CXCL11	1.39 (0.90, 2.16)	0.14	0.82	2.17 (0.83, 5.63)	0.95	0.95	2.63 (1.04, 6.64)	0.041	0.43	0.58 (0.25, 1.34)	0.20	1.00	1.36 (0.51, 3.60)	0.54	1.00
Caspase 8	0.90 (0.54, 1.50)	0.68	1.00	1.37 (0.47, 4.00)	0.57	0.57	1.38 (0.63, 3.05)	0.42	1.00	0.99 (0.26, 3.85)	0.99	1.00	0.11 (0.02, 0.66)	0.016	0.13
FGF-21	1.04 (0.71, 1.51)	0.85	1.00	1.60 (0.68, 3.77)	0.29	0.29	0.86 (0.39, 1.88)	0.70	1.00	0.68 (0.35, 1.31)	0.25	1.00	1.71 (0.73, 4.03)	0.22	1.00
PD-L1	1.30 (0.86, 1.98)	0.22	1.00	3.92 (1.09, 14.11)	0.037	0.26	0.74 (0.37, 1.48)	0.40	1.00	0.90 (0.26, 3.10)	0.86	1.00	2.61 (0.58, 11.80)	0.21	1.00
LIF	1.17 (0.79, 1.75)	0.44	1.00	1.16 (0.62, 2.17)	0.64	0.64	0.63 (0.24, 1.64)	0.35	1.00	1.56 (0.67, 3.65)	0.30	1.00	1.64 (0.59, 4.59)	0.35	1.00

[a] Total of cases and controls. Conditional logistic regression analysis of biomarkers with *a priori* hypothesis. Odds ratios (OR) per S.D. with 95% CI. *P*-values with and without Holm correction for multiple testing.

were characterized by markers of T cell and macrophage activation and cell migration, and by apoptosis, respectively.

The six components included 38 variables with a factor loading of >0.50, which were further investigated as potential predictors of GCA in logistic regression models.

Descriptive data and conditional logistic regression models using these six components with the greatest explanatory power from the PCA analysis as independent variables are shown in Table 4. None of these tests demonstrated a significant difference between cases and controls for the component variables. There was trend towards an association with component number 6 (Table 4), which was mainly driven by IL-2 (Supplementary Table S3, available at *Rheumatology* online).

Analysis of biomarkers with a loading >0.5 in the components listed above revealed significantly higher concentrations per S.D. of SCF, IL-10RB, CXCL9, MCP3, CD40, CCL25 and IL-2, as well as IFN-γ as in the *a priori* analyses (Table 5).

Stratification of the significant findings from this analysis by time from screening to diagnosis (quartiles) in years, similar to IFN-γ, MCP3 had significantly higher values among cases *vs* controls in the quartile closest to diagnosis (OR 3.74; 95% CI 1.26, 11.07) with a decreasing trend with longer time to GCA onset. CXCL9 and SCF had the highest ORs in quartile 2 (Table 6). Further stratification revealed associations for all these biomarkers with GCA with visual symptoms except IL-2 and SCF (which had a borderline association) (Supplementary Table S6, available at *Rheumatology* online).

In a *post hoc* sensitivity analysis of IFN-γ and MCP3 excluding cases and controls with self-reported recent respiratory infection or unknown recent infection status, MCP3 but not IFN-γ remained significantly associated with subsequent development of GCA (Supplementary Table S7, available at *Rheumatology* online).

Discussion

Results of the current study indicate that some of the processes in the pathogenesis of GCA may begin years before clinical onset. We found that circulating levels of IFN-γ were significantly elevated years before diagnosis. The highest levels were found in the quartile closest to diagnosis with a decreasing trend further from diagnosis. These results did not reach significance with Holm correction for multiple testing, although IFN-γ was also identified as a predictor in the hypothesis-generating analysis, underlining its potential importance as a very early biomarker of GCA. IFN-γ, which is mainly produced by T cells and NK cells, was originally called macrophage-activating factor and is still widely recognized as one of the most important factors for macrophages activation.

Table 4. PCA component characteristics, and relation to risk of GCA (conditional logistic regression)

PCA			Logistic regression	
	Eigenvalue	% of variance	OR (95% CI)	*P*-value
Component 1	21.8	23.9	1.06 (0.70, 1.62)	0.77
Component 2	4.7	5.2	1.18 (0.70, 1.98)	0.53
Component 3	3.7	4.0	1.27 (0.76, 2.13)	0.37
Component 4	3.3	3.7	1.45 (0.92, 2.31)	0.11
Component 5	2.8	3.1	1.08 (0.70, 1.66)	0.72
Component 6	2.6	2.9	1.54 (0.98, 2.41)	0.06

PCA: principal component analysis; OR: odds ratio.

Table 5. Biomarkers with a loading >0.5 in PCA, baseline values, and relation to GCA risk

	Baseline values (NPX) [mean (S.D.)]		Conditional logistic regression	
	Cases ($n = 94$)	Controls ($n = 97$)	OR (95% CI)	*P*-value
PCA 1				
VEGFA	10.31 (0.35)	10.27 (0.31)	1.29 (0.84, 1.98)	0.25
SCF	9.24 (0.36)	9.14 (0.37)	1.84 (1.20, 2.82)	0.005
IL-6	2.12 (0.82)	2.14 (0.70)	0.91 (0.60, 1.38)	0.67
IL-10RB	6.07 (0.34)	6.00 (0.36)	1.63 (1.01, 2.61)	0.045
HGF	7.49 (0.40)	7.44 (0.33)	1.37 (0.88, 2.12)	0.16
IL-18R1	8.05 (0.42)	8.05 (0.43)	1.09 (0.71, 1.68)	0.71
FGF-23	1.67 (0.71)	1.56 (0.43)	1.41 (0.92, 2.15)	0.11
PCA 2				
CXCL11	10.51 (0.62)	10.42 (0.51)	1.39 (0.90, 2.16)	0.14
CXCL10	9.47 (0.90)	9.33 (0.83)	1.52 (0.92, 2.50)	0.10
CXCL9	7.23 (0.80)	7.02 (0.66)	2.17 (1.31, 3.59)	0.003
IFN-γ	7.27 (0.90)	7.07 (0.79)	1.52 (1.00, 2.30)	0.048
MCP3	3.34 (0.69)	3.21 (0.40)	2.01 (1.24, 3.25)	0.004
MCP1	11.56 (0.37)	11.59 (0.32)	0.79 (0.49, 1.28)	0.34
MCP2	8.92 (0.54)	8.91 (0.45)	1.12 (0.72, 1.75)	0.61
PCA 3				
AXIN1	2.79 (0.58)	2.73 (0.67)	1.16 (0.75, 1.80)	0.50
SIRT2	2.71 (0.78)	2.64 (0.95)	1.11 (0.68, 1.82)	0.67
ST1A1	2.55 (1.01)	2.51 (1.17)	1.03 (0.61, 1.71)	0.92
STAMBP	3.85 (0.61)	3.75 (0.65)	1.34 (0.84, 2.13)	0.23
CXCL5	10.10 (0.86)	10.04 (1.17)	1.17 (0.75, 1.83)	0.49
TNFSF14	3.75 (0.51)	3.69 (0.44)	1.25 (0.83, 1.89)	0.29
Caspase 8	2.58 (0.70)	2.58 (0.81)	0.90 (0.54, 1.50)	0.68
CD40	11.24 (0.43)	11.14 (0.49)	1.66 (1.02, 2.70)	0.043
PCA 4				
DNER	8.57 (0.43)	8.53 (0.25)	1.34 (0.85, 2.11)	0.21
TWEAK	8.42 (0.32)	8.36 (0.28)	1.50 (0.93, 2.43)	0.096
uPA	9.76 (0.31)	9.76 (0.31)	1.05 (0.67, 1.62)	0.84
LIFR	3.87 (0.27)	3.84 (0.27)	1.44 (0.91, 2.30)	0.12
FGF-5	1.04 (0.29)	1.00 (0.22)	1.46 (0.91, 2.35)	0.11
CCL11	8.87 (0.29)	8.89 (0.22)	0.94 (0.60, 1.47)	0.78
FLT3L	9.23 (0.42)	9.26 (0.44)	0.91 (0.59, 1.41)	0.66
CCL25	6.11 (0.60)	6.02 (0.52)	1.67 (1.04, 2.67)	0.034
PCA 5				
CD5	5.42 (0.38)	5.40 (0.43)	1.10 (0.72, 1.69)	0.65
CD6	6.07 (0.53)	6.03 (0.52)	1.23 (0.79, 1.91)	0.36
TNF-β	4.87 (0.43)	4.86 (0.37)	1.02 (0.68, 1.53)	0.91
IL-12B	5.93 (0.67)	5.96 (0.65)	0.91 (0.61, 1.36)	0.65
CSF1	10.02 (0.29)	9.97 (0.22)	1.29 (0.89, 2.18)	0.15
PCA 6				
IL-2	0.37 (0.26)	0.31 (0.26)	1.52 (1.02, 2.27)	0.040
IL-13	0.45 (1.24)	0.40 (1.07)	1.19 (0.78, 1.81)	0.43
ARTN	0.62 (0.49)	0.52 (0.37)	1.41 (0.97, 2.16)	0.072

Baseline values are in arbitrary units, NPX. NPX: normalized protein expression; OR: odds ratio.

Table 6. Conditional logistic regression for hypothesis-generating biomarkers, stratified for time from screening to diagnosis (years)

	Quartile 1 (0.3–8.5) (N^a=47)		Quartile 2 (8.5–11.9) (N^a=48)		Quartile 3 (11.9–15.5) (N^a =47)		Quartile 4 (15.5–19.1) (N^a=49)		
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	*P* for trend[b]
IFN-γ	2.37 (1.14, 4.92)	0.021	1.72 (0.78, 3.77)	0.18	1.09 (0.39, 3.06)	0.87	0.60 (0.21, 1.75)	0.35	0.03
MCP3	3.74 (1.26, 11.07)	0.017	2.31 (0.94, 5.64)	0.067	1.15 (0.41, 3.20)	0.79	1.44 (0.51, 4.02)	0.49	0.13
CXCL9	2.22 (0.82, 5.98)	0.12	5.67 (1.83, 17.56)	0.003	1.00 (0.42, 2.39)	0.99	1.92 (0.48, 7.77)	0.36	0.27
IL-2	1.65 (0.83, 3.28)	0.15	1.23 (0.49, 3.10)	0.66	1.09 (0.37, 3.21)	0.88	1.82 (0.88, 3.80)	0.11	0.88
SCF	1.93 (0.81, 4.59)	0.14	3.43 (1.35, 8.76)	0.010	0.52 (0.19, 1.44)	0.21	2.36 (0.99, 5.61)	0.052	0.63
IL-10RB	2.40 (0.92, 6.24)	0.072	1.94 (0.74, 5.09)	0.18	1.21 (0.49, 3.02)	0.68	1.18 (0.43, 3.24)	0.75	0.24
CD40	4.27 (1.26, 14.53)	0.020	0.78 (0.34, 1.80	0.56	1.10 (0.49, 2.48)	0.83	8.17 (1.74, 38.25)	0.008	0.75
CCL25	1.35 (0.03, 73.52)	0.88	2.52 (0.90, 7.04)	0.078	0.91 (0.34, 2.44)	0.85	1.46 (0.52, 4.14)	0.47	0.35

[a] Total of cases and controls.
[b] Based on interaction between quartile and biomarkers level; logistic regression models. Odds ratios (OR) per S.D. with 95% CI.

However, it has many other known functions, e.g. attraction of leukocytes, differentiation of many cell types, enhancement of NK cell activity and regulation of B cell functions [31].

Our results indicate that the IL-12/IFN-γ axis, which promotes differentiation to Th1 cells and upregulation of macrophage activity [28, 32], and is thought to be responsible for the chronic aspect of GCA, might have low-level activity years before clinical presentation. In contrast to the elevated levels of IFN-γ, levels of IL-6 were not found to be elevated prior to GCA diagnosis in this study. This suggests that activation of the IL-12/IFN-γ axis, but not of the IL-6/IL-17 axis, precedes clinical disease onset.

Another cytokine, IL-2, which is also produced by Th1 cells within the same axis as IFN-γ, was found to be elevated among subsequent cases compared with controls. This cytokine explained the greatest part of variation within component 6 in the PCA analysis, which nearly reached significance as a predictor of GCA in logistic regression. In analysis stratified by time to diagnosis, associations with IL-2 did not reach significance in any of the subsets. This might reflect loss of power due to the limited number of cases in the stratified analysis.

MCP3 was significantly elevated in the quartile closest to diagnosis in our analysis. MCP3 has been shown to be involved in T cell migration and to induce monocyte/macrophage migration [33]. It partly overlaps with the functions of MCP1 in activating mononuclear phagocytes, T cells, NK cells and basophiles. In addition, MCP3 also activated eosinophils and dendritic cells [34]. IFN-γ is known to induce MCP3 expression in human mononuclear cells as well as in monocytes [34].

Other early biomarkers of GCA, identified through the PCA analysis, were CD40, a receptor expressed on monocytes/macrophages whose interaction with activated T cells results in pro-inflammatory cytokine production [35], and CXCL-9, which stimulates immune cells through Th1 polarization and activation [36].

The elevated cytokines found prior to disease onset in our study might reflect that either Th1 and/or macrophage activation precede the clinical features. These are mainly cytokines/biomarkers linked to the chronic axis of the pathogenesis. It has been shown that Th1 cells may persist during treatment with glucocorticoids [28]. There is a great variety of potential future target therapies currently in clinical trials; the demonstration of such a Th1 cell signal may have implications for the use of targeted therapy [37].

Although stratification by GCA subphenotype should be interpreted with caution due to limited power, the associations between several biomarkers and GCA with visual symptoms are of particular interest and potential clinical relevance.

Different microbes have been proposed as triggers of GCA. Possibly, an activation of the immune system leading to IFN-γ production as a response to these microbes could play a part in the activation of this axis. Indeed, it has been shown that infections are more common overall in patients subsequently diagnosed with GCA compared with controls. One study from the same region as the current study showed such an association for upper respiratory tract infections, influenza and pneumonia with biopsy-proven GCA [38], while no such associations for skin infections or gastrointestinal infections were evident [38]. In *post hoc* analyses in the present study, excluding individuals with recent respiratory infections, there was no association between IFN-γ and subsequent GCA, further underlining the potential role of such events in this context. A number of microbes have been investigated as potential triggers of GCA, e.g. mycoplasma pneumoniae, chlamydia pneumoniae, parvovirus B19 and varicella zoster virus (VZV) [39, 40]. VZV antigens have been detected in positive TAB specimens from patients with GCA [41], and IFN-γ has been shown to be important in the short- and long-term immune response to VZV [42], mycoplasma [43], *Chlamydia pneumoniae* [44] and parvovirus B19 [45].

Although findings implicating specific microbes as a trigger have been difficult to reproduce, the shared triggering of IFN-γ production by microorganisms might contribute to activation of the the IL-12/IFN-γ axis in subsequent development of GCA.

Our results suggest that activation of some inflammatory pathways important in GCA might start years before clinical disease onset. Both in our *a priori* analysis as well as in the hypothesis-generating analysis, many of the biomarkers that are thought to reflect the chronic pathway of vasculitis were elevated. Even though the results did not reach significance when adjusted for multiple testing, the fact that several biomarkers are involved in the same inflammatory pathways increase the likelihood that they reflect important biologic mechanisms. Also, the inflammatory biomarkers included in the OLINK panel have been selected based on their potential role in different inflammatory and autoimmune diseases.

A limitation of our study is the relatively small sample size. As samples were only obtained at a single time point, the changes in biomarkers over time could not be assessed. The results cannot be generalized beyond the investigated age groups of mainly Scandinavian ethnicity.

The antibody-based OLINK proteomics assay reports protein abundances in arbitrary units (NPX), which are not readily convertible to absolute concentrations. Thus, the obtained results are difficult to compare with previous studies on biomarkers in active disease. Moreover, the reported NPX values can only be used to compare the relative abundance of each biomarker across the sample cohort, and not for ranking the relative abundance of biomarkers within samples.

Aspects of pre-analytic handling and analytic procedures can always be a source of bias.

Pre-analytical procedures of blood sample collection and storage, and storage time in the freezer may all be sources of bias. The risk of such bias was minimized by matching cases and controls at the time point for inclusion.

In this study, we have analysed plasma levels of biomarkers, which do not necessarily reflect levels in relevant tissues, i.e. lymphoid organs or arteries [46]. Furthermore, the candidate biomarkers were selected based on knowledge on established GCA as there are no previous studies on prediagnostic samples, and the OLINK platform only picks up the proteins to which the antibodies are directed. Lastly, a validation cohort was not accessible in this study set up and further studies on this matter on other cohorts are needed to show generalizability.

A strength of this study as a well-defined cohort is the case validation through a structured review process. Blood samples were obtained in a standardized manner and stored as appropriate for later analysis.

For plasma protein biomarker discovery, the main alternative to our utilized antibody-based OLINK approach is mass spectrometry–based proteomics [47]. The two approaches are largely complementary. Mass spectrometry–based methods

are biased to quantify the most abundant plasma proteins, but on the other hand give direct evidence for proteins through protein-specific, or peptide-specific, mass spectra [48]. In contrast, antibody-based platforms, such as the OLINK inflammation panel this study extends on, enable targeted analysis of defined protein groups that include those with low concentrations [49].

The matching of controls on age and sex is a strength in comparing levels of biomarkers, as concentrations of various biomarkers change during a lifetime, and there are known differences in biomarkers between sexes [50].

In conclusion, in this nested case–control study, elevated IFN-γ levels were found years prior to diagnosis in individuals subsequently diagnosed with GCA. It cannot be excluded that this finding is due to multiple testing. PCA revealed that T cell related proteins that explain a major part of the variance in the proteome were associated with subsequent GCA, suggesting that activation of the adaptive immune system may precede the clinical onset.

Supplementary data

Supplementary data are available at *Rheumatology* online.

Data availability statement

The key data underlying this article will be shared on reasonable request to the corresponding author.

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