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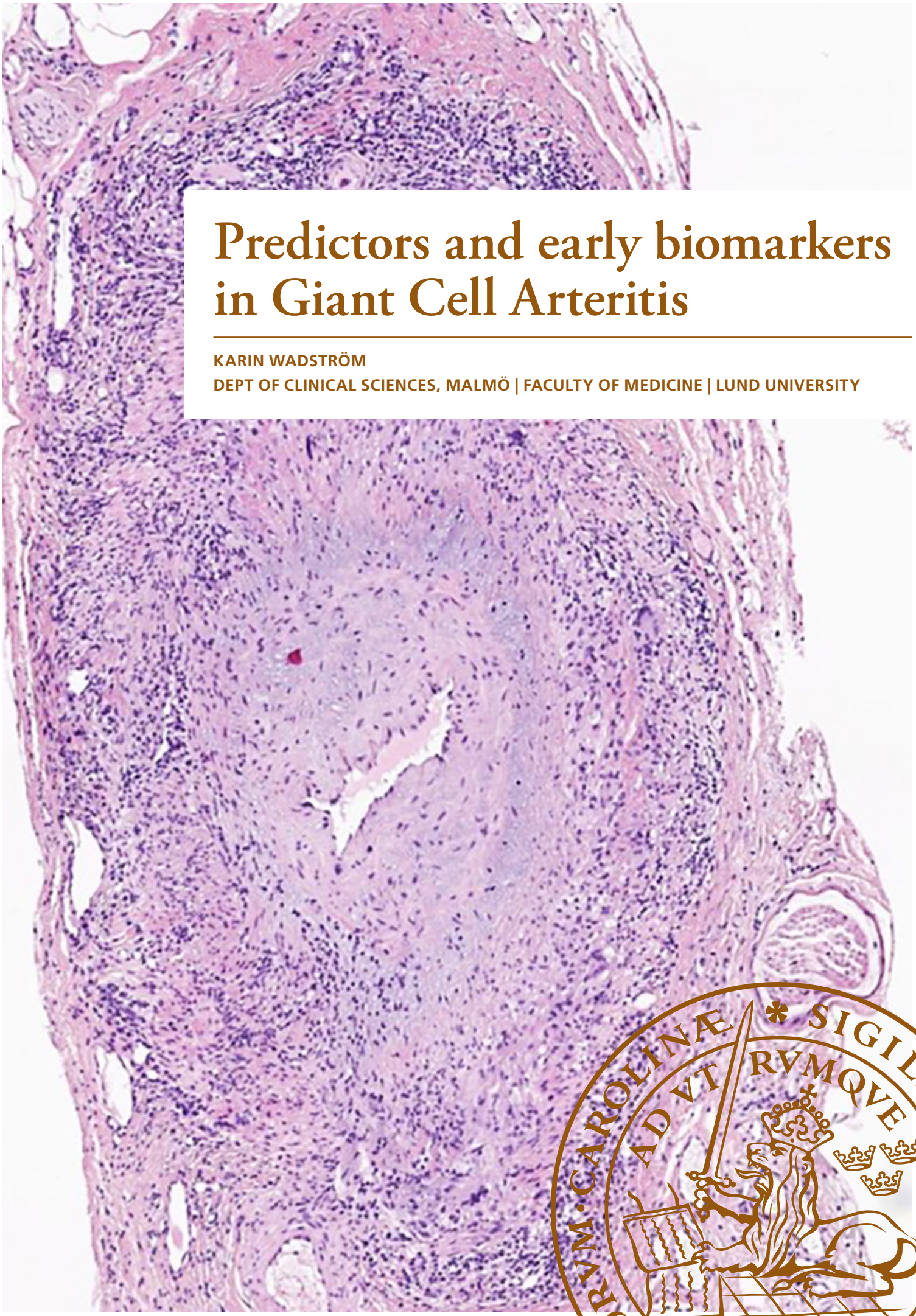

Predictors and early biomarkers in Giant Cell Arteritis

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Title and subtitle
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Abstract
Giant Cell Arteritis (GCA) is the most common vasculitis in adults among people in the western world. The pathogenesis and etiology are not fully understood.

In Study I, we investigated potential predictors for developing GCA, i.e. Body Mass Index (BMI), smoking and hormone-related factors, in a nested case control study. We identified cases and matched controls (4/case) who previously had participated in two health surveys performed in Malmö, Sweden, between 1974 and 1996, the Malmö Preventive Medicine Program (MPMP) and the Malmö Diet and Cancer Study (MDCS). We found a significant association between a higher BMI and reduced risk of subsequent development of GCA (Odds ratio 0.91 per kg/m2; 95 % confidence interval 0.84-0.98).

In Study II the objective was to investigate how duration of glucocorticosteroid treatment affects TAB findings. Previous studies have shown conflicting results regarding the effect of treatment prior to biopsy. We found that biopsies taken 1-4 weeks after initiating of treatment still yield clinically useful information for the diagnosis of GCA.

Study III aimed to investigate the role of metabolic factors as predictors of GCA. Validated cases from the MPMP cohort were compared with matched controls. We found that cases had significantly lower fasting blood glucose, total cholesterol levels and triglyceride levels a median of 20.7 years before diagnosis of GCA compared to controls.

In Study IV we investigated the relation between inflammatory biomarkers and subsequent development of GCA. With cases and controls from the MDCS cohort and a median time from screening to diagnosis at 11.9 years. Our results showed that IFN-γ levels were elevated years before onset of GCA, in particular among those sampled closer to diagnosis. In addition, several other T-cell related proteins were also elevated prior to diagnosis, suggesting that activation of the adaptive immune system may precede the clinical onset of GCA.

In conclusion a lower BMI and better metabolic control were identified as predictors of GCA. Our results suggest that features of cellular inflammation may be detected years before disease onset. TABs seem to yield useful information when taken 1-4 weeks after treatment initiation with glucocorticoids.

Key words Giant Cell Arteritis, Epidemiology, BMI, Biomarkers

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Cover photo: Light microscopy photograph of an artery from a GCA patients with typical histopathological findings. Photo by Nazanin Naderi.

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Table of Contents

Abstract

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In **Study IV** we investigated the relation between potential biomarkers associated with inflammation and subsequent development of GCA. With cases and controls from the MDCS cohort and a median time from screening to diagnosis at 11.9 years. Our results showed that IFN-γ levels were elevated years before onset of GCA, in particular among those sampled closer to diagnosis. In addition, several other T-cell related proteins were also elevated prior to diagnosis, suggesting that activation of the adaptive immune system may precede the clinical onset of GCA.

In **conclusion** a lower BMI and better metabolic control were identified as predictors of GCA. Our results suggest that features of cellular inflammation may be detected years before disease onset. TABs seem to yield useful information when taken 1-4 weeks after treatment initiation with glucocorticoids.

List of abbreviations

ACR	American College of Rheumatology
AION	Anterior Ischemic Optic Neuropathy
BAFF	B-cell activating factor
BMI	Body Mass Index
BSR	British Society for Rheumatology
CAD	Coronary artery disease
CCL	C-C motif chemokine ligand
CDS	Color duplex sonography
CDU	Color doppler ultrasound
C-GCA	Cranial Giant Cell Arteritis
CI	Confidence interval
CRP	C-reactive protein
CT	Computed tomography
CTLA-4lg	Cytotoxic T-lymphocyte associated protein 4 immunoglobulin
CXCL	C-X-C motif chemokine ligand
DNA	Deoxyribonucleic acid
DC	Dendritic Cell
DCVAS	The diagnostic and classification criteria in vasculitis
DM	Diabetes mellitus
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
fB-glucose	Fasting blood glucose
FDG-PET	Fluorodeoxyglucose-positron emission tomography
FOU	Fever of unknown origin
GCA	Giant Cell Arteritis
GiACTA	Giant cell arteritis Actemra trial
GM-CSF	Granulocyte macrophage colony stimulating factor
HLA	Human leukocyte antigen
HSV	Herpes simplex virus
ICAM-1	Intracellular adhesion molecule 1
IEL	Internal elastic lamina
IFN-γ	Interferon gamma
IL	Interleukin
IL12RB2	Interleukin 12 receptor subunit beta 2
IRR	Incidence Rate Ratio
JAK	Janus kinase
LV-GCA	Large-vessel Giant Cell Arteritis
LVI	Large vessel involvement
MCP-1	Monocyte chemoattractant protein 1
MDCS	Malmö Diet and Cancer Study
MHC	Major histocompatibility complex

MMP 9	Matrix metallopeptidase 9
MPMP	Malmö Preventive Medicine Program
MPO	Myeloperoxidase
MTX	Methotrexate
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
NOS	Nitric oxide synthase
OR	Odds ratio
PCR	Polymerase chain reaction
PD1	Program death receptor 1
PD-L1	Program death-ligand 1
PDGF	Platelet derived growth factor
PMR	Polymyalgia Rheumatica
RA	Rheumatoid Arthritis
RCT	Randomized controlled trial
RP1	Retinitis pigmentosa 1
SCID	Severe combined immunodeficiency
TA	Temporal arteritis
TAB	Temporal artery biopsy
TABUL	Temporal artery biopsy vs ultrasound
TC	Total cholesterol
TG	Triglyceride
TLR 4	Toll-like receptor 4
TNF	Tumour necrosis factor
Th1	T helper cell 1
Th17	T helper cell 17
TLR	Toll-like receptor
US	Ultrasound
VEGF	Vascular endothelial growth factor
VSMC	Vascular smooth muscle cell
VZV	Varicella zoster virus

List of scientific papers

I. **Jakobsson K**, Jacobsson L, Warrington K, Matteson E, Liang K, Melander O, Turesson C. Body mass index and the risk of giant cell arteritis – results from a prospective study. Rheumatology (Oxford). 2015 Mar 54(3):433-440

II. **Jakobsson K,** Jacobsson L, Mohammad AJ, Nilsson J-Å, Warrington K, Matteson E, Turesson C. The effect of clinical features and glucocorticoids on biopsy findings in giant cell arteritis. BMC Musculoskeletal disord. 2016 Aug 24;17(1):363

III. **Wadström K,** Jacobsson L, Mohammad AJ, Warrington K, Matteson E, Turesson C. Negative associations for fasting blood glucose, cholesterol and triglyceride levels with the development of giant cell arteritis. Rheumatology (Oxford). 2020 Nov 1;59(11):3229-3236

IV. **Wadström K,** Jacobsson L, Mohammad AJ, Warrington K, Matteson E, Jakobsson M, Turesson C. Analyses of Plasma Inflammatory Proteins Reveal Biomarkers Predictive of Subsequent Development of Giant Cell Arteritis; a Nested Case-Control Study (manuscript)

Preface

When I first got in contact with Carl Turesson who would become my main supervisor. I was about to start my medical studies in Odense, Denmark. I needed a job over the summer, and I got the opportunity to review medical records for a project that was intended for a Ph.D. student coming from North America. This student had a change of plans and decided not to come. I spent several summers reviewing medical records and reading up on GCA, and eventually, I was asked if I wanted to continue as a Ph.D. student.

At this point I was in the middle of my medical studies, and I had reflected on different clinical pathways. I knew for me that personal contact with patients in combination with an investigative specialty were my top priorities. Therefore, I knew Rheumatology was a possible path, and as I also enjoyed the work as a research assistant and enjoyed working with Carl the answer was simple. Yes!

The journey began.

Thesis at a glance

Study I

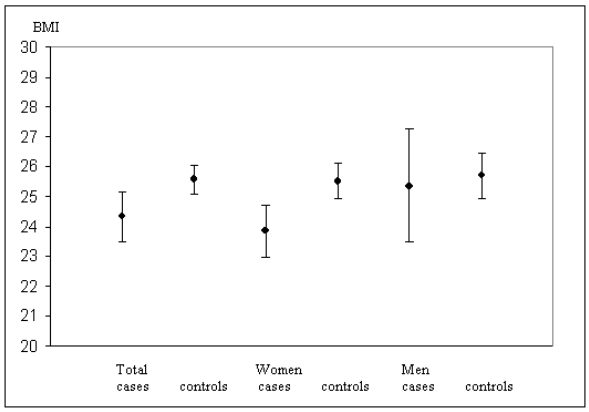

Figure 1.
Higher BMI was associatied with decreased risk for subsequent GCA (OR 0.91/kg/m^2, 95 % CI 0.84-0.98). Stratified by sex the association remained significant in women (OR 0.89/kg/m^2, 95 % CI 0.81-0.97), but not in men (OR 0.97/kg/m^2, 95 % CI 0.85-1.11).

Study II

Table 1.
Features recorded in pathology reports, stratified by time from initiation of glucocorticoid treatment to biopsy

Time from treatment start to biopsy	≤ 0 days (n=44)	1-3 days (n=74)	4-6 days (n=43)	7-28 days (n=20)	p	p for trend *
Inflammatory infiltrates	89 %	70 %	81 %	75 %	0.12	0.95
Biopsy positive	86 %	69 %	79 %	80 %	0.17	0.64
Fragmented internal elastic lamina	45 %	45 %	49 %	50 %	0.86	0.65
Giant cells	48 %	31 %	53 %	40 %	0.10	0.73
Granuloma	20 %	8 %	9 %	15 %	0.19	0.28
Fibrosis	9 %	20 %	19 %	20 %	0.47	0.24

*The Mann-Whitney U test was used to assess the trend of differences in the distribution
There were no major differences in the proportions with various pathology features between the groups with different time on glucocorticoids.

Study III

Table 2.
Potential predictors of giant GCA in bivariate analyses using conditional logistic regression models, stratified by sex.

	All		Women		Men	
	OR	95 % CI	OR	95 % CI	OR	95 % CI
BMI (per kg/m^2)	0.90	0.82-0.98	0.86	0.76-0.98	0.95	0.82-1.10
fB-glucose (per SD)	0.35	0.17-0.71	0.67	0.35-1.25	0.07	0.01-0.30
Cholesterol (per SD)	0.58	0.42-0.81	0.55	0.35-0.85	0.63	0.38-1.03
Triglycerides (per SD)	0.45	0.27-0.74	0.34	0.15-0.75	0.55	0.30-1.01

Standard deviation: fB-glucose 1.5 mmol/l, cholesterol 1.0 mmol/l, triglycerides 0.8 mmol/l,
There were negative associations for BMI, fB-glucose and lipid levels with subsequent GCA.

Study IV

Table 3.
Proteins of inflammation significantly associated with subsequent GCA, overall and stratified by time (years) from screening to diagnosis with GCA. Conditional logistic regression.

	All		Quartile 1 (0.3-8.5) (N=47)		Quartile 2 (8.5-11.9) (N=48)		Quartile 3 (11.9-15.5) (N=47)		Quartile 4 (15.5-19.1) (N=49)	
	OR (CI)	**P**	**OR (CI)**	**P**	**OR (CI)**	**P**	**OR (CI)**	**P**	**OR (CI)**	**P**
IFN-γ	1.52 (1.00-2.30)	**0.048**	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
MCP3	2.01 (1.24-3.25)	**0.004**	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
CXCL9	2.17 (1.31-3.59)	**0.003**	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
IL2	1.52 (1.02-2.27)	**0.040**	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
SCF	1.84 (1.20-2.82)	**0.005**	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
IL10RB	1.63 (1.01-2.61)	**0.045**	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
CD40	1.66 (1.02-2.70)	**0.043**	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**
CCL25	1.67 (1.04-2.67)	**0.034**	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

Proteins from the Olink® Inflammation panel, selected based on principal component analysis and identified to be significantly associated with subsequent GCA. IFN-γ and MCP3 showed signifiantly higher values among cases in the quertile closest to diagnosis compared to controls with a decreasing trend in those samples taken with longer time to diagnosis.

Background

Brief History

Cases with probable Giant Cell Arteritis (GCA) were first published in the late 19th century, when Jonathan Hutchinson reported a case of an older man with inflamed temporal arteries (1) but it was not until Horton described it in the 1930s and other authors followed in the 1940s that GCA started to be recognized as a specific disease. Horton obtained the first temporal artery biopsy (TAB) and described the association between clinical findings such as jaw claudication and histopathology of GCA (2). The association with Polymyalgia Rheumatica (PMR), a closely related disease, was first described in the 1960s (3).

The nomenclature for GCA has varied over time, as described in a historical review by Gene Hunder (3): from thrombotic arteritis of the aged by Hutchinson when first described in 1889, to temporal arteritis by Horton in 1932, to cranial arteritis, Horton's disease, senile arteritis, granulomatous arteritis, and finally "giant cell arteritis" by Gilmour in the 1950s (4). Today the terms giant cell arteritis and temporal arteritis are used to an equal extent in the literature. Giant cells are not mandatory in the pathology and are only present in approximately half of the temporal artery biopsies taken (5, 6).

Before glucocorticoid usage there were limited treatment options for GCA. Different approaches were used; sectioning of the involved temporal artery seem to relieve the headache but did no good for the other manifestations (7). In 1950, glucocorticoids were reported to relieve both local and systemic symptoms (8) as well as to reduce the risk of visual loss (9). This was just after glucocorticoids had been found to be effective in rheumatoid arthritis (RA)(10).

By the 1970s most investigators in the medical field had been convinced that GCA and PMR were somehow related to each other (3) and current literature describes an overlap between GCA and PMR. Approximately 15 % of PMR patients are found to have a positive TAB (11) and about 40-60 % of patients with GCA may have signs or symptoms of PMR at diagnosis (12-16). However, it is still unclear how the two conditions are linked and why some, but not all, patients present with both features of PMR and GCA.

Large vessel involvement (LVI) might have been described for the first time in 1937 by Sproul and Hawthorne. Post mortem findings in two cases without clinical

vasculitis during their lifetimes included chronic diffuse inflammation with giant cells in the aorta and the iliac arteries in both and in the carotid arteries in one (17).

A quarter century later, in 1963, the Swedish rheumatologist Bengt Hamrin published observations connecting PMR with large vessel involvement (18).

In his historical review (3), Hunder examined reports regarding GCA and PMR until the 1970s and concluded that it is not easy to provide a clear answer whether these are diseases of modern times or if they existed before they were first described. Some early-culture findings imply they did exist even in early historic times. For example, a carving from the Egyptian tomb of Pa-Aton-Em-Heb dating back to 1350 BC show a blind harpist with swollen eyelids and prominent temporal arteries (19). But if they did exist, it would be surprising that other cases were not recorded and described earlier, considering the conspicuous presentation, including prominent, tender temporal arteries, severe headache, and visual disturbances. On the other hand, factors such as prolonged life expectancy would likely have made the diseases more common in modern times.

Epidemiology

Incidence

The highest reported incidence of GCA has been found in the Scandinavian countries and in Minnesota, USA (20). These populations have incidence rates of 19.1/100 000 aged over 50 in Minnesota (21), 14-33/100 000 in Sweden (22-24) and 7.5/100 000 in Finland (25). Iceland, has the highest incidence reported, with 43.6/100 000 (26), followed by Norway with 32.8/100 000 (27).

In other European regions, the incidence has been reported to be approximately 10/100 000 in northwestern Spain (28), 6.9/100 000 in Italy and 11.2/100 000 in the United Kingdom (29). There have only been a few such studies from other regions of the world: for example, different studies in Jerusalem (Israel) reported roughly 10/100 000 over time (30, 31) and a report of 3.2/100 000 came from south Australia (32). A small case series from Saudi Arabia indicated a low incidence of GCA in the Arab population (33).

To some extent the differences in incidence can be explained by the fact that the studies use different methods to identify cases. Whereas some studies only include TAB-verified cases, others include cases based on classification criteria after medical records review, diagnostic codes and/or hospital records.

In a historical review over the epidemiological progress in PMR, Rooney et al. (34) discuss an interesting view of the geographic distribution of PMR. The highest incidence rates have been reported in the Scandinavian countries, but the other areas with high incidence rates correspond quite well with the Viking invasions that

occurred around the end of the first millennium AD. This might be a coincidence, or it might give us a clue on the etiology of PMR and also that of GCA.

Minnesota, which along with the Scandinavian countries reports one of the top incidences of GCA worldwide, has a community of primarily Scandinavian descent. Possible explanations for the common patterns of GCA epidemiology would be genetic factors, climate, other similar exposure factors between the regions, or chance.

Prevalence

Few studies have been published on the prevalence of GCA. These show similar trends as for the incidence, with higher prevalence rates found in northern Europe and Minnesota compared to southern Europe (35-39). The highest reported prevalence in persons aged over 50 was found in the UK (250/100 000 (35)) and the lowest in Japan (1.47/100 000 (40)). In a study on TAB-confirmed cases from southern Sweden the point-prevalence was 76/100 000 in persons aged over 50 (41).

The Swedish study above highlights some of the problems with prevalence estimates in GCA. The reported prevalence of 76/100 000 only includes patients receiving glucocorticoids or other immunosuppressive therapy at the time. Estimate rates irrespective of current treatment in the same cohort was 127/100 000 in people over 50 years of age.

Incidence trends over time

Some studies have reported on changes in incidence over time. In 2004, Salvarani et al. (21) published incidence rates between 1950 and 2000 in Olmsted County, Minnesota. There was a significant progressive increase in incidence rates observed from 1950 to 1979. The incidence rates peaked in the beginning of the 1980s and then remained stable over the following three decades, at 25-30/100 000 in people aged ≥50.

In western Norway, an increased incidence rate between 1972-1992 was observed, after which incidence levelled out, with a peak incidence of 32.8, in 2007 (42). More recently, a review that included two Swedish cohorts with TAB-confirmed GCA cases looked at incidence trends over a 40-year period, between 1973 and 2019 were estimated. A peak incidence of 22.2 per 100 000 inhabitants aged ≥50 was seen during 1976-1995, followed by a decrease to on average 13.3 per 100 000 inhabitants aged ≥50 between 1997 and 2019 (43).

Seasonal variation

Studies have shown conflicting results regarding the seasonality of GCA onset. In Sweden two studies have indicated seasonality: a Gothenburg study showed significantly higher incidence in March, September and October (44) and a study

on a southern Sweden-based cohort showed higher incidence rates in spring and summer compared to winter (41). Both studies included biopsy proven GCA. Several studies have seen a higher incidence in the summer months, in Jerusalem (30, 31), the UK (45), and Denmark (46). In Australia, a peak was seen in December/January (47). Some European studies have seen a peak in the winter season e.g., Scotland (48). On the other hand, a number of studies did not confirm any seasonal variation (28, 49-53).

In a systematic review and meta-analysis by Hysa et al. published in 2020 (54), the pooled analysis did not confirm a significant seasonal variation for the onset of GCA. The incidence rate ratio (IRR) estimate for the cold period versus the warm period showed a non-significant trend towards a higher frequency of disease onset in the warm season (1.13; 95 % confidence interval 0.89,1.36).

In conclusion, the highest incidence rates have been reported in Caucasian populations. This might be due to genetics, or to similarities in environmental factors. Incidences seem to have increased over time in several populations in the second half of the 20th century. Several factors might contribute to this pattern, such as increased awareness among clinicians, difference in biopsy referral, and difference in biopsy assessment as well as improved imaging. Regarding prevalence, the fact that GCA often is a self-limiting disorder makes this estimate less topical. Studies of seasonal variation show highly discordant results between various cohorts.

Clinical features

Vasculitis indicates an inflammation of blood vessel walls, and GCA typically affects medium to large vessel. It usually affects the aorta and/or its branches, typically branches of the carotid and vertebral arteries. The temporal artery is often but not always involved (55).

The disease rarely occurs before the age of 50. Incidences increase with age to reach a peak in the age group 71-80 years (21, 22, 37). GCA affects women more commonly than men with a ratio of 3:1 (22, 37, 47, 56).

Headache

A new-onset headache or a change in headache pattern in a patient aged 50 years or older should always lead to suspicion of cranial GCA.

Headache is present in >60 % of patients. Typically, but not always, it is a new-onset headache located in the temporal regions but may also be frontal, occipital, or generalized headache. It might be persistent or intermittent (57). Cranial GCA can mimic other primary headache disorders such as migraine, cluster headache or

stabbing headache (57-59). While headache is the most common symptom of GCA (47, 60), jaw claudication is more specific and predictive for a positive TAB (61, 62).

Visual symptoms

One of the most serious complications in GCA is visual loss. It has been reported to occur in up to 10-30 % of cases (47, 63-65). Visual manifestations might be transient visual loss (amaurosis fugax); diplopia; permanent visual loss, most often due to anterior ischemic optic neuropathy (AION); or partial visual field deficit (64, 66). Amaurosis fugax is thought to be more associated with development of other ischemic visual complications than diplopia (64). Permanent visual loss is rarely reversible, even when treated with high doses of glucocorticoids (67).

In a study of 161 patients by Gonzalez-Gay et al., patients with permanent visual loss were identified to have significantly lower frequency of constitutional symptoms, less fever and higher mean hemoglobin value. Also, they had no significant difference in classical features of GCA such as headache, jaw claudication, abnormal temporal arteries on physical examination, or symptoms of PMR. Genetically they were significantly more likely to be HLA-DRB1*04 positive (64).

Visual manifestations have also been associated with higher age (64, 66), lower CRP (65) and concomitant treatment with β-adrenergic inhibitors (65). Moreover, a study from Australia showed that males were significantly more likely than females to have symptoms of visual disturbance (47).

In a cross-sectional analysis of patients with prevalent GCA, when asked about visual symptoms (response rate about 60 %), 28 % reported current visual symptoms. Significant differences between the cases reporting visual symptoms and cases not reporting visual symptoms included pre-GCA diplopia, temporary or permanent visual symptoms, and hoarseness, all of which were more common among the cases who later reported visual symptoms after treatment initiation (68).

One of health professionals' major concerns are that delayed treatment for GCA patients will lead to permanent visual damage. However, a delay of diagnosis has not been associated with visual symptoms at diagnosis (64), nor at a later stage of the disease (68). In 1997 Font et al. described that prior to vision loss due to GCA, patients had PMR in 34 % of cases for approximately 10 months and 65 % of the patients had visual symptoms for 8.5 days before diagnosis and treatment (63).

Systemic features

Systemic features of GCA include low-grade fever, fatigue, anorexia, and weight loss (56).

In a study by Calamia et al. 15 out of 100 patients with GCA had "fever of unknown origin" (FUO) as the initial manifestation. In 4 out of these 15 patients, TAB were obtained despite lack of symptoms of cranial arteritis (69).

Out of patients with long-term FOU overall, approximately 30 % are eventually diagnosed with rheumatic disease. In one Western population, the most frequent diagnoses among such patients are adult Still's disease (5 %) and large-vessel vasculitis (5 %), followed by SLE and sarcoidosis (70).

PMR and GCA

PMR is characterized by pain and morning stiffness, with bilateral involvement of the neck and pelvic girdle. Similar to GCA, PMR predominantly affects people older than 50, with an incidence peaking in individuals aged 70 years or older. PMR can occur independently or in association with GCA and may be diagnosed prior to, at the same time as, or after GCA diagnosis.

A study in Olmsted County, Minnesota including 245 incident cases of PMR reported that 13-17 % (varying over 21 years) of the patients had biopsy-proven GCA (11). Among patients with primarily GCA, approximately 40-50 % had symptoms of PMR (12-15).

A German study that sought to determine the incidence of GCA in patients with PMR included 127 patients, 102 with no clinical features of GCA (pure PMR). Out of these patients with pure PMR, 8 % had ultrasonography findings suspicious for GCA, whereas 7 % had specific halo sign and/or positive TAB (71). In a smaller German study of 22 patients with clinically pure PMR, the incidence of GCA was higher, 21 % according to TAB and 32 % according to ultrasonography findings of halo-sign (72).

In patients with a clinical diagnosis of PMR, GCA should be ruled out if the patient has constitutional symptoms, elevated acute phase reactants or doesn't respond as expected to glucocorticoid treatment, even in the absence of typical cranial symptoms. Imaging of the aorta and its major branches should be considered in such cases to catch any form of large vessel involvement.

Different clinical phenotypes of GCA

GCA is a heterogeneous disease with different phenotypes, associated with different clinical manifestations. The two major phenotypes are cranial GCA (C-GCA) and large vessel GCA (LV-GCA). A retrospective cohort study that included 116 patients, with the aim to compare diagnostic criteria, showed that 76.7 % of the patients had C-GCA; 3.4 %, LV-GCA: and 18.9 % were classified as constitutional GCA. These results indicate that C-GCA is the dominating phenotype (73), confirming previous studies on this topic (74).

Whereas signs and symptoms of PMR seem to occur in similar frequency in these two different phenotypes (75), some other features are more common in one or the other.

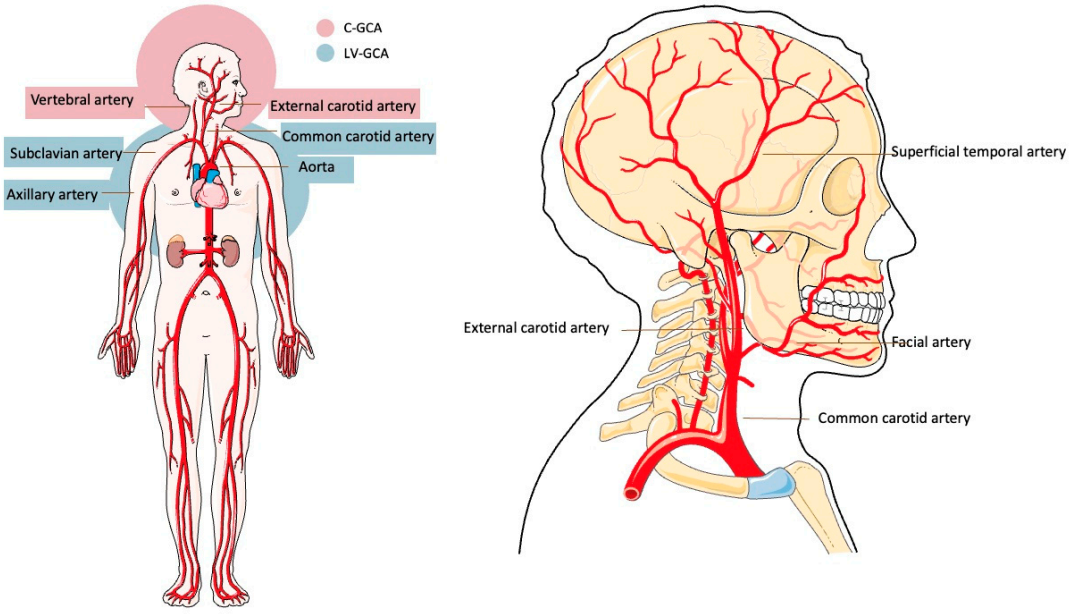

Figure 2.
Illustration of affected arteries in different phenotypes of GCA (to the left) and illustration of external carotid artery with its branches (to the right). Picture source: smart.servier.com.

Cranial GCA (C-GCA)

Patients with cranial GCA more commonly have the typical clinical manifestations of GCA. For example, onset of a new type of headache, temporal artery swelling and tenderness, jaw claudication and visual disturbance are symptoms characterizing this group. These symptoms are caused by inflammation in cranial arteries leading to local ischemia.

In a 2008 study, Schmidt et al. found that C-GCA patients compared to those with predominantly LVI were more commonly men, older at diagnosis, and significantly more likely to have symptoms like headaches, jaw claudication, and AION. In addition, they had a significantly shorter time from symptom onset to diagnosis (76) and studies have shown that patients with C-GCA are more likely to have a positive TAB (75, 76).

Large vessel GCA (LV-GCA)

Large vessel giant cell arteritis (LV-GCA) is an extracranial arteritis. Vessels involved might include the aorta and its major branches such as subclavian, vertebral, carotid, axillary, iliac and femoral arteries. Among these, the proximal branches of the aorta are more commonly involved than the branches of the abdominal aorta and arteries of the lower extremities (75, 77, 78).

In a study comparing 120 patients with LV-GCA with 212 C-GCA patients, the LV-GCA patients were younger and had a longer duration of symptoms at the time of diagnosis. They had a history of PMR to a greater extent but had less commonly cranial features of GCA, such as headaches, jaw claudication or scalp tenderness. They were less likely to have a positive TAB and had a lower risk of visual loss.

Moreover, they also tended to relapse more frequently and needed a longer duration of therapy than cranial GCA patients (79). Female sex, younger age and arm claudication were positively associated with LV-GCA, and there was a negative association with cranial symptoms, in a study by Brack et al. (75). A smaller study (n=62) did not see a difference in age at diagnosis between the different phenotypes of GCA, but it confirmed the positive association for LV-GCA with female sex and the negative association with cranial features (80).

Table 4. Selected studies on LVI estimates in GCA
Studies presenting estimates of large vessel involvement (LVI) using different methods for LVI identification.

Author	n of patients	GCA diagnosis	Method for LVI identification	Estimated proportion of LVI	Type of LVI
Ostberg 1973 (81)	13	Medical history and/or autopsy	Post-mortem autopsy	92 %	Aortic inflammation
Blockmans et al. 2006 (78)	35	TAB- positive (94 %) or PMR + typical GCA symptoms	18F-FDG PET at diagnosis	83%	Aorta main upper and lower branches
Prieto-Gonzales et al. 2012 (82)	40	TAB-positive	CT Angiography at diagnosis	67.5 %	Aorta and main branches
Naderi et al. 2017 (83)	164	TAB-positive	Medical records – long-term follow-up	15 %	Aorta and main branches

The studies with the highest proportion of LVI include one post-mortem study from 1973 of only 13 patients (81) and a second study using 18-flurodeoxyglucose (18F-FDG) positron emission tomography (PET) at diagnosis. However, this latter study used liberal criteria and any degree of FDG uptake was considered positive (78).

Regarding aortic involvement in the studies above, 15 % of the cases had a thoracic aorta dilatation at time of diagnosis in the study by Prieto-Gonzalez et al. (82). In the study by Naderi et al., aortic involvement was identified in 10 % of patients. Symptoms of PMR were more common among those with LVI and the presence of giant cells in TAB was less common (83). In a subanalysis of a cohort of LV-GCA patients, Muratore et al. focused on those with subclavian artery vasculitis and found thoracic aorta involvement in 66 patients (56 %), including aneurysm in 14 patients (12 %) (79).

Relapse

The European League Against Rheumatism (EULAR) recommendations for management of large vessel vasculitis divide relapses into major or minor relapses. A major relapse is defined as the recurrence of active disease with either clinical features of ischemia or evidence of active aortic inflammation. Minor relapse is defined as recurrence of active disease without fulfilling the criteria above for major relapse (84). The majority of relapses have been reported to occur during

glucocorticoid treatment, and normal erythrocyte sedimentation rate (ESR) and normal C-reactive protein (CRP) do not exclude a relapse (85).

PMR is the most common symptom of GCA relapse (46-51 %) (85-87), followed by cranial symptoms (31-41.9 %) (86, 87), such as headache (85). Ischemic manifestations such as visual disturbance, and claudication in the jaw or limbs have been described in 29 % of relapses (85). Systemic manifestations have been found to be present in 18 % of relapses (86).

Relapses are common in GCA, with risk varying in different studies depending on duration of follow-up time (85-88). For example, one showed a 23 % risk during a mean follow-up of 21.4 months (85) and another 64 % during on average 7.8 years (86). This indicates that many patients have late-occuring relapses.

Relapses occur more commonly in patients with LV-GCA than C-GCA (79). A recent retrospective study confirmed an association between LV-GCA and the risk of relapse (89). Other findings at diagnosis that have been associated with later relapse are fever (87), established hypertension or diabetes mellitus at baseline, and female sex (88).

According to a recently published meta-analysis of 34 studies, the overall pooled prevalence of relapse was 47.2 % (95 % CI 10.0, 54.3). A significant difference was seen depending on tapering study protocol in risk of relapse: those who had a target GC tapering within 12 months had higher relapse rates compared to those with target tapering >12 months. However, no associations with initial dose, age at diagnosis or sex were seen (90).

Biomarkers associated with relapse

To some extent, biomarkers at diagnosis have been investigated to see if they might be able to predict risk of relapse. Some of the published results in this area are discussed below.

Visvanathan et al. found statistically elevated baseline serum concentrations of ESR, CRP, ICAM-1 and PDGF in patients relapsing versus remitting (91). Baseline levels of osteopontin have been found to be significantly higher in relapsing patients compared to non-relapsing patients (92). In addition, in 2019, Van Sleen et al. published a study reporting that investigation of biomarkers at baseline and during follow-up revealed that angiopoietin-2 was predictive for imminent relapse (93).

Diagnosis of GCA

GCA can either begin abruptly or gradually, over weeks to a few months, before becoming clinically recognizable. Due to risk for severe complications, i.e., loss of vision, in untreated patients, it is important to diagnose GCA early.

As there are no diagnostic criteria for GCA, the diagnosis is usually made by collecting information on clinical manifestations and measuring laboratory

parameters of systemic inflammation (ESR and CRP), and then confirmed by typical histopathologic findings on TAB or by imaging (i.e., ultrasound of temporal arteries demonstrating halo sign indicating vasculitis, or other typical imaging modalities). TAB is still considered the gold standard for the diagnosis of GCA.

In a recent review and meta-analysis by Van der Geest et al. symptoms like scalp tenderness, weight loss, vision loss, headache and fevers were all associated with GCA but not as specific as limb claudication, jaw claudication, temporal artery (TA) abnormalities (TA thickening, loss of TA pulse, temporal tenderness), AION and previous diagnosis of PMR. The later ones should upgrade the suspicion of GCA in combination with laboratory features (see below) (94).

Laboratory tests

In contrast with many other rheumatic diseases, no useful serology test for GCA has been identified. GCA is typically associated with an elevated ESR, CRP, platelets, immunoglobulins, and alkaline phosphatase as well as with normocytic anemia (95). However, ESR and CRP can be normal in GCA cases. In a study by Kermani et al. 7 out of 177 patients (4.0 %) had normal ESR and CRP at time of diagnosis even in absence of glucocorticoid treatment. The study concluded a sensitivity for elevated CRP and ESR of 86.9 % and 84.1 % respectively for a positive TAB. CRP was a more sensitive marker than ESR for positive TAB (96).

At time of a clinical relapse the number of cases with normal ESR and CRP has been reported to be 21 %, with the majority of relapsing patients currently on glucocorticoids (85). The study was conducted before treatment with IL-6 was approved for treatment of GCA.

In the recently published meta-analysis by Van der Geest et al., mentioned above, pooled analysis showed that an elevated ESR of >60 mm/h gives increased likelihood for GCA, as did an elevated platelet count of > $400x10^9$/l (94).

Temporal artery biopsy (TAB)

TAB has been the gold standard for GCA diagnosis for decades. However, patients with GCA can have negative TAB results. It is known that the inflammatory changes in GCA are usually segmental along the vessels leading to skip lesions. This may be one of many causes resulting in a negative TAB in a patient with GCA.

Several studies have looked at the sensitivity of TAB with various results, A positive TAB has been found in 39-85 % of GCA cases overall and in less than 60 % of cases with LV-GCA (75, 97-100). However, the proportion of TAB-positive GCA cases is sensitive to case selection.

Three important factors that may affect the outcome are timing of biopsy, the location of the biopsy and the length of the artery specimen.

Timing

Several studies have investigated how treatment with glucocorticoids might affect biopsy outcome. An early study from the 1980s showed a rapidly decreasing trend in positivity of biopsies after initiation of treatment. Of a total of 132 patients, 82% had a positive TAB of those biopsied before treatment start, if biopsy was taken within a week of starting treatment, 60 % were positive, and with >1week of treatment only 10 % had positive TAB (101).

Achkar et al. published a paper in 1994 reporting that TAB may show arteritis even after more than 14 days of treatment (102). In 2007, Narváez et al., concluded from their study performed on 78 GCA patients (73 TAB positive) that TAB information was valuable even after 4 weeks of high-dose treatment with glucocorticoids. The number of positive TAB were 78 % when tested after <14 days of treatment, 65 % at 15-28 days, and 40 % after >28 days (103).

Naraváez et al. also concluded that in patients with a prior history of PMR, who had been on long-term treatment with low doses of glucocorticoids, a TAB might be informative. Of 8 cases, seven had a positive biopsy after a median treatment duration of 180 days (103).

In one study, 40 patients were randomized to have a second TAB after having a positive TAB at time for diagnoses. The second biopsy was taken 3, 6, 9, or 12 months after diagnosis; the results revealed signs of vasculitis in 7/10 (70 %) patients with the second biopsy taken after 3 months, 9/12 (75 %) at 6 months, and 4/9 (45 %) at both 9 and 12 months. This indicates that even though clinical manifestations are suppressed, vascular changes might persist for a longer time (104).

Font et al. investigated which histopathological features were reliable in patients after treatment initiation. They concluded that changes in the histopathologic findings were detected at the end of the first week of treatment, but the findings were not difficult to recognize within 2-3 months after treatment initiation. In treated patients they found that the most reliable features were a mix of lymphocytes with mononuclear cells and epithelioid histiocytes located in the outer muscle layer and the adventitia followed by fragmentation of the elastic lamina. In addition, the multinucleated giant cells were less common after treatment (105).

Location

One debated question is if the TAB should be taken unilaterally or bilaterally.

Studies indicate that the yield may increase by 5-10 % with bilateral biopsy (106). In one study of 186 patients having bilateral TAB, 6 of these cases had unilateral arteritis. Five of these 6 patients eventually got a GCA diagnosis and represented 11 % of the total number of GCA patients who were diagnosed through biopsy (showing active or healed GCA) (106). Another study included 250 patients, of which 24 % had subsequently confirmed diagnosis of GCA. All underwent bilateral biopsies. Eleven cases showed unilateral arteritis, representing 18 % of the total biopsy-positive group. The rate of discordant biopsy in this study was 4.4 % (107).

In a study by Germano et al., 112 patients with suspected GCA were randomized to undergo standard TAB or color duplex sonography (CDS)-guided TAB. The results concluded that CDS-guided TAB did not improve the sensitivity of TAB for diagnosing GCA (108).

The recommendation from the American College of Rheumatology (ACR) is unilateral over bilateral TAB. Exceptions are if the symptoms are not clearly localized to one temporal artery or if a unilateral biopsy could not confirm diagnosis even though the clinical suspicion was high (109).

Length

The appropriate length of the biopsy has been debated. Both the ACR recommendation and the British Society for Rheumatology (BSR) guidelines recommend a longer temporal artery biopsy (>1 cm) even though the evidence for this is not high-quality (109, 110). However, they do not specify if the recommended length is before or after fixation. A study investigating length of TABs before excision, directly after excision, and after fixation with formalin, showed a significant contraction directly after excision by 12 % (range 0-30 %) but no further shrinkage after fixation with formalin (111).

The recommendations above are based on experience indicating that the added morbidity from a more extensive biopsy is low, and a longer specimen is thought to increase the diagnostic yield, given the background known about skipped lesions of inflammation (109). An often-cited rule has been that a TAB should not be less than 2 cm, and it had been argued that an adequate biopsy should be at least 2-3 cm of length (5).

Studies that have published data on the length of biopsy affecting the biopsy outcome did not see any significant difference (98, 112) However, it is hard to draw a conclusion regarding which lengths of the specimen matter. These studies indicate however that > 1cm it is not likely to make a significant difference.

TAB - summary

In conclusion, current findings are conflicting on the optimal timing, location and length of TABs, and more data are needed. It is most likely better to perform the biopsy as soon as possible, even though up to several weeks of treatment might not affect the usefulness of the TAB. The histopathological features may differ between treated and not treated patients. Biopsy logistics should, however, not delay treatment in a case with high suspicion for GCA. Regarding the unilateral versus bilateral biopsy, the increase in diagnostic yield is a fact but it is modest. It is reasonable to follow the pragmatic guidelines from the ACR and go for bilateral biopsy only when needed. The fact that the biopsy can still yield information regarding arteritis, possibly after several weeks, strengthens this line of reasoning.

Histopathological features

GCA is often a granulomatous vasculitis. However, this does not necessarily mean that giant cells nor granulomas must be present. Whereas granulomas are defined as nodular formation of epithelioid cells, and sometimes contain giant cells, granulomatous inflammation is indicated by cellular infiltrates with epithelial cells in any configuration (113). In the ACR 1990 classification criteria the specification is that the histopathologic feature should show vasculitis described as a predominance of mononuclear cells or granulomatous inflammation, usually also containing multinucleated giant cells (16)

The name giant cell arteritis is somewhat misleading, as giant cells are in fact only seen in approximately 50 % of biopsies (5).

The inflammatory lesions in GCA might be focal and concentrated to one or two layers of the arterial wall or such lesions might be a panarteritis (transmural arteritis) involving all three artery wall layers. However, if inflammatory infiltrates are localized, they are typically found in the media near the internal elastic lamina (IEL) (5, 113).

Additionally, a circumferential band of fibrinoid necrosis might be present in a positive biopsy (5). Regarding the fragmentation of the IEL state, it has been suggested that even partial damage might indicate GCA (114). It is a common feature in GCA patients, and if found, it persists long after the active phase of the disease (5). However, fractionation of the IEL has also been identified as a progressive process with increasing age (115).

In conclusion, histological features that might be seen in active GCA and to some extent in healed arteritis are the following: giant cells, inflammatory cells such as epithelioid cells, lymphocytes, plasma cells, eosinophils and fibroblasts. Other features include either transmural or focal inflammation, an occlusion of lumen (due to intima hyperplasia) and possible necrosis. Small vessel inflammation might be present and the IEL fragmented, reduplicated or even absent in parts (113).

An additional factor that might influence biopsy outcome is the pathology assessment. In the TABUL study, Luqmani et al. asked 14 different pathologists to examine and assess 30 TABs. In only 11/30 cases did all 14 pathologists agree on the outcome. This highlights the subjectivity of interpretation of TAB specimens (116).

Why do pathologists disagree upon diagnosis? The interpretation contains some uncertainties regarding the degree of inflammation, the importance of a fragmented IEL, and what role the involvement of small vessels might play. Added to this the biopsy can also be assessed as “healed arteritis” which makes it even more complex. In addition, artery wall changes due to age or other diagnoses might make the interpretation more difficult. Some non-GCA diseases that are likely to affect the temporal artery and could mimic GCA are ANCA-associated vasculitides, polyarteritis nodosa and mixed cryoglobulinemia (117).

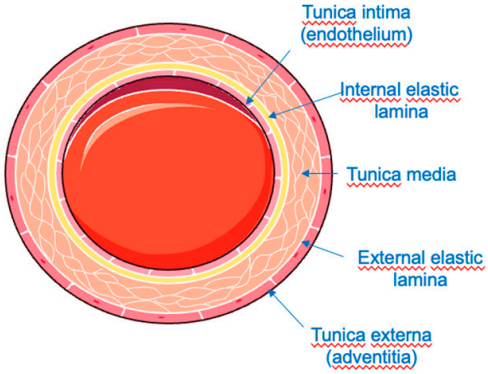

Figure 3a

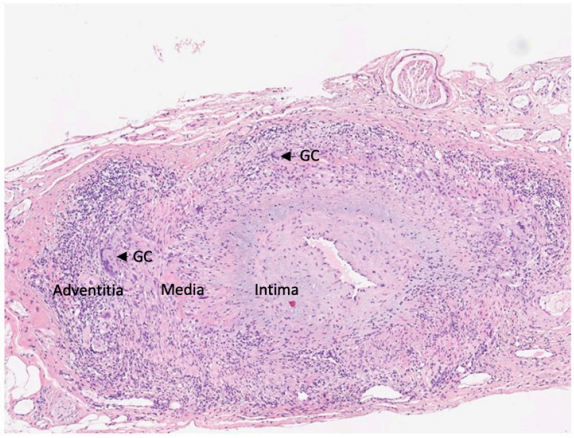

Figure 3b

Figure 3a.
Anatomic picture of the artery wall anatomy. Picture source: smart.sevier.com.

Figure 3b
Light microscopy photograph of an artery from a GCA patients with typical histopathological findings. Present are: giant cells (GC), extensive inflammatory infiltrates, constriction of lumen (intima hyperplasia) and fragmentation of the internal elastic lamina. Photo by Nazanin Naderi.

Imaging in GCA

The quality of imaging techniques and equipment has evolved rapidly in recent years. Combined with greater availability, this has made imaging a cornerstone in the diagnosis of GCA, for both C-GCA and LV-GCA.

Ultrasound

As a non-invasive imaging method with broad availability in the everyday practice is the Color Doppler Ultrasound (CDU), which is widely used to support the diagnosis of GCA. Relevant ultrasound findings include a non-compressible hypoechoic ‘halo sign’, as well as occlusion or stenosis of the temporal artery. The typical halo sign surrounding a perfused lumen was first described by Dr Schmidt in Germany in the 1990s (118). Furthermore, examination of the axillary and common carotid arteries may also reveal typical GCA findings, although the increment in sensitivity from adding such investigation appears to be limited (97).

Thereafter, a debate began over whether CDU could eventually replace the TAB as the gold standard for diagnosing GCA (119, 120).

In the latest recommendations on imaging in this context, published in 2018, the EULAR recommended temporal artery ultrasound as first-line imaging for patients with suspected GCA, providing that it is available and can be performed with high quality (121). In contrast, the ACR recommendations are that TAB is recommended over ultrasound for GCA diagnosis with the motivation that US rheumatologists and radiologists are less experienced in using ultrasound compared to their counterparts in Europe (109).

In a study by Schmidt et al. in 1997, the halo sign disappeared a mean of 16 days after treatment initiation with glucocorticoids (119). However, a more recent study indicated that the sensitivity of ultrasound might decrease from 92 % when scanned the first day of glucocorticoid treatment to 80 % when treated for 2-4 days, and to 50 % when scanned >4 days after treatment initiation (122).

Earlier meta-analyses of ultrasound for the diagnosis of GCA by Arida et al. and Ball et al. concluded specificity at 91 % and 83 % respectively, and sensitivity at 68 % and 75 % respectively (123, 124). The first meta-analysis used the ACR criteria as reference and the second TAB as reference.

A more recent meta-analysis found a pooled specificity of 96 % and sensitivity of 77 % for ultrasound compared to clinical diagnosis of GCA (125). Bilateral halo sign has been reported to reach a specificity of 100 % (126).

In conclusion, ultrasound has many advantages. It is widely available in many clinical units, can be used as a part of the clinical exam, does not need referral, and is non-invasive, fast, inexpensive and repeatable. However, it is operator-dependent and therefore needs an experienced user.

CT Angiography

Prieto-Gonzalez and colleagues investigated 40 newly diagnosed patients with biopsy-proven GCA, all of whom were imaged within 3 days of treatment initiation with glucocorticoids. Within the group that had not yet started treatment, 79 % had large vessel involvement, 15 % of all had aortic dilatation and in the whole cohort 67.5 % were diagnosed with large vessel involvement (82).

In a CT angiography, vessel dilatation and stenotic lesions can be evaluated. However, this modality cannot assess active inflammation in the vessel wall and therefore is not diagnostic, but it is valuable in addressing GCA complications. Pros are that it is fast, inexpensive and readily available in many centers.

High resolution MRI/ MRI angiography

High-resolution MRI of cranial arteries is recommended by EULAR as an alternative to ultrasound for GCA diagnosis (121).

One study containing 28 patients with either GCA or PMR used magnetic resonance angiography (MRA) as the imaging method, where masked readers detected extracranial vasculitis in 67% of patients and zero controls (127). In a prospective study including 64 patients, MRI with contrast on superficial cranial arteries has showed a sensitivity of 80.6 % and a specificity of 97.0 %, comparing with rheumatologists` diagnoses of GCA (128).

More recently pooled analysis of MRI showed a sensitivity of about 73 % and a specificity of 88 % when used to evaluate cranial arteries compared to clinical diagnosis of GCA. When comparing MRI with TAB sensitivity increased to 93 % and specificity decreased to 81 % (125).

In addition to vascular dilatation and stenosis also seen in CT angiography, MRI/MRA gives the opportunity to measure mural thickening and enhancement which makes it more useful in making diagnosis.

It has been proposed that an MRI should be taken within 5 days of treatment initiation (129).

Disadvantages for MRI/MRA include limited availability; they are time consuming and have possible adverse effects from contrast reagents. However, there are continuous technical advances, and the availability has improved in many centers. Advantages are that the test is non-invasive, repeatable, entails no radiation exposure can be used for diagnosis.

PET-CT

Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET) detects inflammation such as vasculitis by showing increased glucose uptake reflecting metabolic activity. It is combined with computer tomography in order to demonstrate the location of uptake (PET-CT).

One advantage with the modality is the possibility to assess aortitis. Until recently PET-CT was not able to evaluate cranial arteries (130) and was used exclusively for assessing large vessel involvement or to exclude differential diagnoses, i.e., infections and malignancies. However, a recently developed method has now improved the ability to evaluate temporal arteries. In a study of 64 patients in which PET-CT was compared to TAB, the sensitivity was 92 % and specificity 85 % for GCA diagnosis. PET-CT also had a negative predictive value of 98 %, indicating that the modality worked well for excluding GCA in low-risk patients (131).

A study by Lehmann et al. followed 17 patients with clinical GCA fulfilling the ACR criteria and who had a positive PET scan at diagnosis, as well as 3 patients with Takayasu arteritis. The researchers calculated a 65 % sensitivity and 80 % specificity for vasculitis when comparing with age and gender-matched controls (132).

PET-CT is recommended to be performed within 72 hours of glucocorticoid treatment, as ^{18}F-FDG uptake decreases after glucocorticoid exposure. Diagnosis of LV-GCA was shown to be accurate in 100 % of patients after 3 days of treatment, but only in 5 of 14 patients after 10 days of treatment (133).

A difficulty with PET-CT is that there is no consensus regarding scoring of the increased ^{18}F-FDG uptake. Vascular aging, atheroma plaques and other inflammatory processes may be hard to distinguish from arteritis in some cases. Other disadvantages of this modality include high cost, limited availability of new generation imaging, and uncertainties of using it in follow-up or in cases of clinical relapse because of the decreased ^{18}F-FDG uptake while on glucocorticoids.

Imaging - summary

Regarding imaging in GCA, a lot of different modalities are available each with their pros and cons. Firsthand non-invasive modalities are recommended. However, the choice must be costume-made for each patient taking different features into

account, such as clinical manifestation, age, initiation of treatment, suspicion of large vessel involvement, and finally local expertise with different modalities.

Classification criteria

The 1990 ACR classification criteria

In 1990 the American College of Rheumatology published classification criteria for GCA (16). The criteria, also summarized in Table 5 are: 1. Age of onset ≥50 years. 2. A new headache including a new onset or new type of localisation. 3. Temporal artery abnormality as in tenderness to palpation or decreased pulsation unrelated to arteriosclerosis. 4. ESR ≥50 mm/h. 5. Abnormal TAB showing vasculitis. A patient with vasculitis is classified to have GCA if at least three of the five criteria are present, this has been associated with a sensitivity of 93.5 % and a specificity of 91.2 %. The intention was that the criteria should discriminate between GCA patients and those with other systemic vasculitis. They were not designed to distinguish from other common diseases. Therefor the sensitivity and specificity of these criteria can only be extrapolated to the group they are intended for, and they need a high pre-test probability. The ACR classification criteria focus on cranial symptoms and therefore they do not recognize large vessel involvement which is a weakness. In addition, they do not incorporate modern imaging techniques such as PET-CT and ultrasound which are now widely used in clinical management.

Table 5.
The 1990 ACR classification criteria

Criteria	Definition
Age at onset ≥50 years	Development of symptoms or findings beginning at age 50 or older
New headache	New onset of or new type of localized pain in the head
Temporal artery abnormality	Temporal artery tenderness to palpation or decreased pulsation, unrelated to arteriosclerosis of cervical arteries
Elevated erythrocyte sedimentation rate	Erythrocyte sedimentation rate ≥50 mm/h by the Westergren method
Abnormal artery biopsy	Biopsy specimen with artery showing vasculitis characterized by a predominance of mononuclear cell infiltration or granulomatous inflammation, usually with multinucleated giant cells

Adapted from reference number 16

Suggested expansion of ACR 1990 classification criteria – 2016

In 2016 Dejaco et al. (134) suggested an expansion of the existing ACR 1990 criteria (Table 6). Suggested expansion (marked in bold in the table below) included visual manifestations, PMR, constitutional symptoms, and other ischemic manifestations such as jaw and/or tongue claudication which are less common but more specific

for GCA compared to headache. Also, extra-cranial artery abnormalities, CRP levels and other imaging modalities such as US, MRI, and FDG PET were suggested to be incorporated. To fulfill the criteria, it was suggested that three out of five criteria should be present if one of the present criteria were either TAB and/or imaging compatible with diagnosis of GCA. By making criteria nr 5 obligate the diagnosis of vasculitis is objectively verified. This reflects the inclusion criteria in randomized controlled trials (RCTs), i.e. The Giant Cell Arteritis Actemra (GiACTA) Study including only patients with either a positive TAB or evidence of large vessel vasculitis on imaging (135). These suggestions are based on the concept that the original classification criteria focus on cranial manifestations and therefor are not good at capturing patients with large vessel phenotypes. Highly sensitive imaging techniques has been developed since 1990 and are now widely available in clinical practice which makes it easier to diagnose large vessel involvement.

Table 6.
The 1990 ACR classification criteria and suggested expansion (marked in bold)

Original criteria	Suggested expansion (In bold)
Age at onset ≥50 years	Age at onset ≥50 years
New onset of or new type of localized pain in the head	New onset of or new type of localized pain in the head, **Visual symptoms, sight loss, PMR, Constitutional symptoms, Jaw and/or tongue claudication**
Temporal artery abnormality (tenderness to palpation or decrease pulsation unrelated to arteriosclerosis)	Abnomrality of emporal artery **and/or extra-cranial arteries** (tenderness to palpation or decrease pulsation, **bruits of exra-cranial arteries** unrelated to arteriosclerosis)
Erythrocyte sedimentation rate ≥50 mm/h	Erythrocyte sedimentation rate ≥50 mm/h **and/or CRP levels ≥10 mg/l**
Abnormal artery biopsy	Abnormal artery biopsy **and/or abnormal imaging results (US, MRI and/or ^{18}F-FDG PET)**

Adapted from reference number 134

DCVAS - upcoming classification criteria

The DCVAS (Diagnostic and Classification Criteria in Vasculitis) network is founded by the American College of Rheumatology, the European League Against Rheumatism and the Vasculitis Foundation. It is sponsored by the University of Oxford and supported in the UK by the NIHR Clinical Research Network.

An attempt to improve classification criteria for six different forms of vasculitis, including GCA, subjects have been enrolled between 2010 to 2017. The initial draft of new classification criteria for GCA were presented in the ACR conference in Chicago, USA in 2018.

The rationale for development of new improved criteria was partly a reduced sensitivity of the ACR 1990 criteria in modern cohorts. The sensitivity was found to be higher (93.5% vs 81.1 %) when investigated in the DCVAS cohort compared to the original ACR 1990 cohort. In addition, a lot has happened in the field of imaging in the past 20 years. For example, the availability of MRI scanners has

grown, new imaging modalities such as FDG PET CT are used, and ultrasound has become part of the daily routine in many rheumatology units.

All the above in combination with the fact that the ACR criteria focused on the phenotype of cranial GCA and to lesser extent captured those patients with large vessel involvement makes improved classification criteria needed.

The DCVAS proposal has been incorporated in a EULAR process for new criteria for large vessel vasculitis and a draft is currently under review for official EULAR approval.

Pathogenesis

GCA is a large vessel vasculitis, and it affects medium-size and large arteries. Usually, the third to fifth branches of the aorta are affected, although the aorta and its primary branches may also be affected.

Whereas some other forms of vasculitis are induced by circulating antibodies, i.e. ANCA-associated vasculitis, where antibodies directed against proteinase-3 or myeloperoxidase activate neutrophile granulocytes that initiate vessel inflammation, the inflammation in GCA starts in the adventitia around the vasa vasorum.

Previously, researchers have proposed that GCA is triggered by toll-like receptor (TLR) activation of dendritic cells (DCs) in the adventitia. The TLRs might be triggered by factors such as pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) released from damaged tissue (136). Once activated the DCs release chemokines CCL19 and CCL21, and attract, retain, and activate T-cells (137). The produced chemokines recruit CD4+ T-cells which infiltrate the adventitia, via vasa vasorum (138). The CD4+ T-cells then get activated by DCs, through production of cytokines (IL-12, IL-18, IL-23, IL-6, and IL-1β) and a differentiation toward Th1 and Th17 cell phenotypes takes place (139). In Figure 4, two major inflammatory processes in GCA are illustrated. They are thought to play different roles in the inflammatory process (140). The differentiation towards Th17 T-cells is a result of the IL-6/Th17 axis, which is known to be activated in the acute phase and early pathogenesis of GCA. This leads to systemic features such as fever and weight loss, and an inflammatory response with elevated ESR and CRP, anemia and thrombocytosis. The second process involves the IL-12-IFN-γ axis, and results in IL-12 promoting differentiation to Th1 cells which produce IFN-γ, leading to upregulation of macrophage activation. This axis is thought to be responsible for Th1-driven chronic aspects of vasculitis (138, 141).

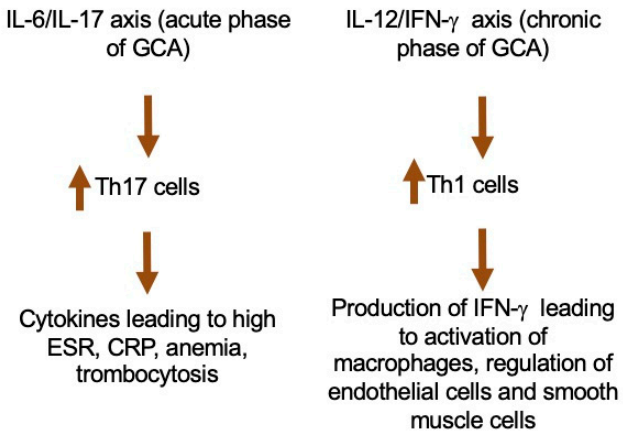

Figure 4.
Illustration of two major inflammatory processes in GCA

The multinucleated giant cells results from fusion of recruited monocytes (142). These monocytes are recruited by chemokines (CCL2, CXCL9, CXCL10, CXCL11) produced by vascular smooth muscle cells (VSMC) when activated by IFN-γ (143). Elevated GM-CSF has been found in active GCA compared to patients in remission (144). In combination with IFN-γ, GM-CSF has been shown to significantly increase macrophage fusion into giant cells (145) and is likely to play a crucial role in the differentiation of macrophage subgroups and the distribution of macrophage phenotypes in GCA (146). Recent observations showing that GM-CSF expression decreases rapidly upon glucocorticoid treatment suggest it to be a possible novel therapeutic target in GCA (147).

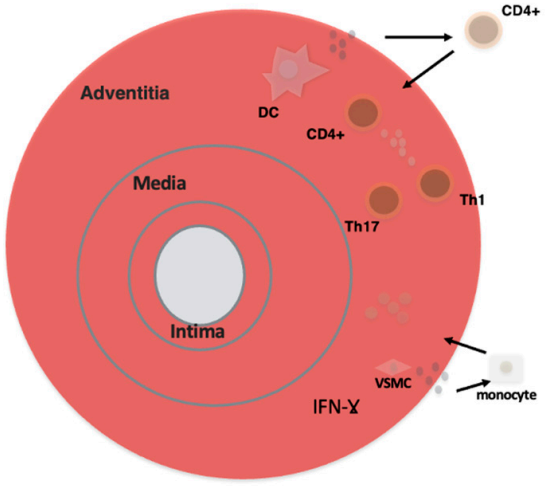

Figure 5. Overview of important steps in the pathogenesis of GCA in the arterial wall.
Dendritic cells (DCs) get activated by some exogen or endogen trigger. Activation leading to production of CCL19 and CCL21, these chemokines attract and retain T-cells. CD4+ T-cells migrate to the adventitia where they are activated by dendritic cells through actions of IL-12, IL-18, IL-23, IL-6, and IL-1β. A differentiation towards Th1 and Th17 phenotypes take place. In parallel, IFN-γ stimulates vascular smooth muscle cells (VSMC) in the adventitia which upon activation produce chemokines (i.e. CCL, CXCL9, CXCL10, CXCL11) recruiting monocytes to the adventitia, where they mature into macrophages and fuse into giant cells.

As one of the main pro-inflammatory cytokines, IL-6 correlates well with disease activity (148-151) and activates hepatocytes, leading to production of acute phase proteins. IL-6 is also known to promote the differentiation towards Th17 cells and has been implicated in regulating induction of T regulatory (Treg) cells. The Treg cells are anti-inflammatory and have been reported to be downregulated in GCA patients (140).

Inhibition of IL-6 by tocilizumab treatment seems to correct the TH17/Treg imbalance. The treatment decreases the amount of Th17 T-cells and increases T reg cells, as shown in rheumatoid arthritis (RA) and later also in GCA (152, 153).

Additionally, T-cell checkpoint dysregulation may play a part in the pathogenesis of GCA (Figure 6). A higher expression of programmed cell death receptor-1 (PD-1) on T-cells and lower expression of programmed death ligand-1 (PD-L1) on DCs have been reported in TABs from patients with GCA when compared with non-inflamed arteries (154). Normally the binding between PD-1 on activated B and T-cells and PD-L1 on DC leads to immunosuppression by suppression of T-cell activity, apoptosis and promotion of regulatory T-cells (154). In addition, studies have shown that in vivo blocking PD-1 in human-artery severe combined immunodeficiency (SCID) mice chimaeras, reconstituted with peripheral blood mononuclear cells from GCA patients resulted in exacerbated vascular inflammation and amplified tissue production of cytokines including IFN-γ, IL-17 and IL-21 (154).

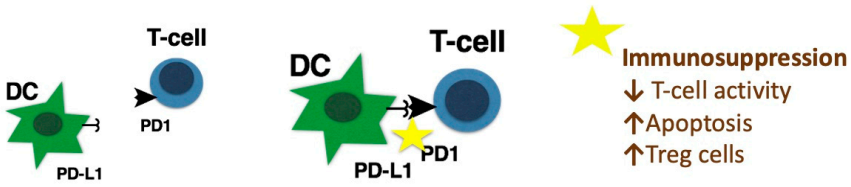

Figure 6.
Illustration of PD1 receptors on T-cells binding to PD-L1 on Dendritic cells (DCs), resulting in immunosuppression.

Infections/antigens

The inflammatory process in GCA is likely to be antigen-driven (155). Specific antigens have not yet been identified, and they might be exogenous and/or endogenous.

Infection as a trigger for GCA development has been discussed. Although there are conflicting results the fact that some epidemiological studies, as described above, have shown a seasonal variation of disease incidence could strengthen this hypothesis (11, 32), Furthermore, the role of TLR discussed under pathogenesis is also compatible with this concept.

Several infectious pathogens have been discussed as potential triggers, some discussed below:

For *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and parvovirus B19, an increased risk of GCA incidence closely associated with infection outbreaks in various regions in Denmark have been observed (156).

In addition, *C. pneumoniae* has been identified more commonly in TAB specimens from GCA patients compared to controls using immunohistochemistry and PCR methods (157). Parvovirus B19 DNA has been reported more commonly represented in GCA TABs than in non-GCA TABs in studies using PCR (158, 159). Powers et al. found an association between *Herpes simplex virus* (HSV) in TAB specimens in GCA cases compared to controls (160). However, another study could not confirm previous findings of either parvovirus B19, *C. Pneumoniae*, or HSV in TAB specimens from GCA patients (161). In some (162) but not all (159) studies *Varicella zoster virus* (VZV) antigens in TAB specimens have been found more frequently both in TAB-positive and TAB-negative compared to controls. A more recent study including 41 TAB and 47 specimens from ascending aorta found no evidence of VZV with immunohistochemistry analysis in any of the specimens (163).

Two studies have taken a more epidemiologic approach, investigating the relationship between prior infections and subsequent GCA; both demonstrated a significant association between prior infections and subsequent GCA (164, 165). The first study, conducted by Rhee et al., was a nested case-control study design using a validated algorithm identifying 4559 GCA patients in the UK, compared to 22 795 matched controls. The results showed significant associations with prior infections overall, with a higher number of infections associated with a greater risk. In subgroup analyses of different infection types, respiratory tract, urinary tract, gastrointestinal tract, conjunctiva and skin and soft tissue infections all remained significantly associated with subsequent GCA independently (164). A later study from southern Sweden, included 1005 TAB positive GCA patients, which were compared to 10 050 matched controls. This second study found significant associations for upper respiratory tract infections, influenza and pneumonia, but not for skin and gastrointestinal tract infections, with later GCA diagnosis (165).

In conclusion, there seems to be an association between previous infections and subsequent GCA diagnosis in studies with an epidemiological approach. However, in studies that investigated TAB specimen aiming to detect antigens there are conflicting results. This might reflect coincidental findings, it might be due to several possible antigens, or there might be methodological problems with these studies. For example, it has been described that a false-positive VZV antigen detection can be the result of antibodies cross-reacting with monocytes and/or calcified tissue (166, 167).

Biomarkers

Several studies regarding biomarkers and active GCA have been published. In the table below some of these, based on analyses in plasma or serum samples, are summarized.

Table 7.
Circulating biomarkers investigated in GCA patients with active disease

	GCA patients/controls	Detection method	Significantly increased analytes	Significantly decreased analytes	Determined not significant
Burja 2019 (168)	97 GCA / 46 healthy blood donors (HBD)	Multiplex protein analysis (serum)	SAA, IL-23, CHI3L1, IFN-γ, IL-1 beta, IL-8, IL-10, IL-18, IL-23, IL-27, IL-31, M-CSF, MMP-1, MMP-9, protein C, resistin, tenascin C (TNC), TNF R1, VCAM-1, VEGF, IL-6, BAFF (50/8), MARCO (75/8)	Alpha-fetoprotein,IL-13, MMP-2	IL-2, IL-9, IL-17A, TNF-alfa
	(GCA/HBD) BAFF (50/8) MARCO (75/8)	ELISA (serum)			
	AGP (81/47) Hemopexin (81/47)	Immunonephelometry (serum)	SAA, AGP *(81/47), hemopexin (81/47)*		
Van der Geest 2015 (149)	12 GCA/13 HC	ELISA (serum) Multiplex protein analysis (serum)	BAFF IL-6, CXCL9, IL-10, sIL-2R	CCL11, CCL2	CCL3, CCL4, CCL5, CXCL-10, GM-CSF, IFN-α, IFN-γ, IL-1beta, IL-2, IL-4, IL-5, IL-7, IL-8, IL-12, IL-13, IL-15, IL-15, TNF-α, sIL-1Ra
O´Neill 2015 (169)	16 GCA/5HC	ELISA (serum)	SAA		
Zerbini 2018 (170)	12 GCA/12 HC	ELISA (plasma)	IL-22		
Prieto-González 2017 (92)	76 GCA/25 HC	Immunoassay	Osteopontin (sOPN)		
Samson 2016 (171)	24 GCA/ 21 HC	Multiplex protein analysis (serum)	CXCL 9,-10, -11		
Van der Geest 2014 (172)	14 GCA/ 16 HC	ELISA	BAFF		
Baldini 2012 (173)	75 GCA/35 HBD 75 GCA/24 HBD	ELISA (plasma) ELISA (serum)	PTX3 VEGF		
Deng 2010 (139)	6 GCA/ 6 HC	ELISArray (plasma)	TNF-γ, IL-17, IL-6, IL-1β, IL-12		

	GCA patients/controls	Detection method	Significantly increased analytes	Significantly decreased analytes	Determined not significant
Remahl 2007 (174)	35 GCA/ 40 HC	ELISA (serum)	sICAM-1, sVCAM-1		sE-selectin
Hernández-Rodríguez 2002 (150)	62 GCA/ 16 HC	ELISA (serum)	IL-6, TNF-α		IL-1beta
Coll-Vinent 1999 (175)	64 GCA/ 35 HBD	ELISA (serum + plasma)	sICAM-1		sICAM-3, sVCAM-1, sE-selectin, sL-selectin
Roche 1993 (151)	19 GCA/ 20 HBD	ELISA (plasma)	IL-6		TNF-α
Nordborg 1991 (176)	63 GCA/ 201 controls from gen-pop health-survey	ELISA (plasma)	vWF:Ag		Plasminogen activator inhibitor activity
Blain 2002 (177)	7 GCA/15 non-GCA (nor vasculitis but other diseases)	ELISA (plasma)		TNF-α,IL-6 soluble receptor	IL-2, IL-6, IFN-γ, RIL-2
Garcia-Unzueta 2006 (178)	3 GCA/ 14 HC	Radioimmunassay (plasma)	adrenomedullin		
Van Sleen 2019 (93)	41 GCA/33 HC	ELISA/Multiplex, protein analysis (serum)	Calprotectin, YKL-40, sCD 163, VEGF, angiopoietin-2, sTie2, CRP, IL-6, SAA		Angiopoietin-1
Sorbi 1996 (179)	12 GCA/ 12 HC	ELISA (serum)	MMP-9		
Springer 2018 (180)	106 GCA (inactive+ active)/ 35 HC	ELISA (serum)	S100A8/S100A9, S100A12		
	50 active GCA compared to 56 inactive	ELISA (serum)	S100A8/S100A9, S100A12		
Foell 2004 (181)	42 GCA/ 25 HC	ELISA (serum)	S100A8/S100A9, S100A12		
Ellingsen 2000 (182)	33 GCA/ 12 HC	ELISA (plasma)	MCP-1		

Several studies have demonstrated higher levels of IL-6 in active GCA, (139, 144, 149-151, 168, 183). Decreasing levels with glucocorticoid treatment have been demonstrated (139, 149, 172).

In addition, other biomarkers identified to decrease after initiation of glucocorticoid treatment are: BAFF (149), osteopontin (92), soluble Th17 (171), ICAM-1 (175).

Findings reported by Deng et al. indicated that glucocorticoid treatment seems to suppress the Th17 axis of inflammation (measures by IL-1β and IL-6 levels) but not the Th1 axis (measures by IL-12) (139). It has also been concluded, that elevated levels of CXCL9 seem to be maintained after treatment with glucocorticoids (149).

Van der Sleen et al. published in 2017 study showing that the number of circulating monocytes were higher in newly diagnosed GCA as well as PMR patients compared to healthy controls. After three months of treatment the monocyte counts were normalized in PMR patients but not in GCA patients (184).

Co-morbidities prior to diagnosis

Diabetes Mellitus (DM)

In a meta-analysis published in 2015 by Ungpresart et al. reviewed five studies, including a total of 903 patients. Pooled analysis gave a significantly lower prevalence of DM at diagnosis among patients with GCA (OR 0.74, 95 % CI 0.57-0.97) (185). However, a year later, a study published by Abel et al. reported opposite results. The study design included identifying patients with DM (type 2) diagnosis in Medicare, a national health insurance program in the United States. Controls without DM were selected using propensity score matching. In the statistical analysis an increased risk for developing GCA was found in the DM group (186).

Malignancies

A problem with studying the relation between malignant disease and GCA is that the timing of tumorigenesis is unclear. Results are conflicting in studies investigating the risk of malignancies in GCA patients. Even though several of studies concluded no increased risk (187-191), some have reported the opposite (192, 193).

One meta-analysis at first indicated a significant but marginally increased risk of cancer in GCA and PMR patients. However, after sensitivity analysis, a study with potential selection bias was excluded and the risk was no longer significant (194).

A population-based case-control study investigating malignancies preceding GCA indicated that GCA patients had significantly fewer malignancies prior to index date compared with controls (195). Another population-based cohort study, which included 830 patients with biopsy-proven GCA concluded that the overall risk for

cancer was not increased in GCA patients after diagnosis compared to the general population. However, a subanalysis showed an increased risk for leukemia and a decreased risk for breast cancer and upper gastrointestinal tract carcinomas (196).

Other co-morbidities prior to disease onset

Dunstan et al. listed co-morbidities at diagnosis in their GCA cohort as follows: hypertension (66.9 %), ischemic heart disease (35.8 %), cancer (34.9 %), chronic lung disease (26.5 %), asthma (17.9 %) and diabetes (17.3 %) (47).

A nested case-control study (4559 cases and 22795 controls) comparing GCA patients to controls revealed that prior to index date GCA patients had significant increased likelihood to have cerebrovascular disease (9 % vs 7 %), chronic pulmonary disease (22 % vs 15 %), mild liver disease (0.6 % vs 0.4 %), and peptic ulcer disease (6 % vs 4 %), as well as peripheral vascular disease (5 % vs 3 %), renal disease (13 % vs 11 %). However, they were less likely to have pre-existing rheumatic disease (3 % vs 5%) and dementia (1 % vs 2 %) (164).

Predictors

Genetic

Familial aggregation has been seen in GCA, indicating a genetic predisposition (197). Several studies have found association between polymorphisms in the human leukocyte antigen (HLA) region, i.e., HLA-DRB1*04, more specifically DRB1*0401, DRB1*0404 or DRB1*0408, and GCA (198). The corresponding gene products are known to play a crucial role in the selection of antigens presented to CD4+ T-cells (138).

These results, indicate that the MHC class II was the genomic region with strongest association to GCA, and have later been confirmed by a genome-wide association study (GWAS) (199). In addition, a study by Mackie et al. that include meta-analysis and geo-epidemiological data confirmed the association between GCA susceptibility and HLA-DRB1*04, and also indicated possible protective effects of HLA-DRB*01 and HLA-DRB*15. And an investigation with geo-epidemiological data found GCA to be independently associated with both population frequency of HLA-DRB1*04 and with latitude itself (200), suggesting that geography may play a role beyond that of population genetics.

Moreover, several studies have investigated gene polymorphism located outside of the HLA and susceptibility for GCA, as summarized in a review by Carmona et al. (201). In summary, gene variants found to be associated with susceptibility to GCA include polymorphisms affecting the following proteins: TNF, IL-10, IL-4,

IL-18, MCP-1, endothelial nitric oxide synthases 2 and 3 (NOSs), MMP 9, TLR 4, Fc-γ, myeloperoxidase (MPO), NLR family pyrin domain containing 1 (NLRP1), IL-12 receptor beta 2 (IL12RB2) and VEGF for GCA overall. On the other hand, some genetic variants, those affecting IFN-γ and IL-2/IL-21, were only associated with ischemic manifestations, i.e., visual symptoms, jaw claudication, etc. Conflicting results gave been published regarding associations with genetic variations for the IL-6 and ICAM-1 regions (201).

However, most of the studies mentioned above were performed in Spanish and Italian populations. Replication in other cohorts is needed. Many of the studies lack sufficient power due to small sample size, and more higher-powered studies are necessary for confirming existing associations as well as identifying new genetic variations that might contribute to the risk of GCA.

Smoking

Evidence is conflicting on the role of smoking in GCA. However, a meta-analysis published in 2018, including eight prospective and five retrospective case-control studies concluded that GCA was associated with a smoking history (OR 1.19, 95 % CI 1.01-1.39), as well as current smoking at diagnosis (OR1.18, 95 % CI 1.01-1.38) (202). A later study by Tomasson et al. found a possible protective association for smoking in men but not in women (26).

BMI and hormone-related factors

Larsson et al. were the first to report a significant association between lower BMI and risk for developing GCA. In their retrospective case-control study 49 women answered a questionnaire on hormonal and reproductive factors; the authors found an independent significant association between GCA and early menopaus, longer duration of breast feeding and smoking (203).

Findings regarding BMI (three studies, including one from the present thesis) were included in a pooled analysis by Ungprasert et al., demonstrating a significant inverse relationship between BMI and subsequent GCA (204). In addition, others have confirmed such an association more recently, in a study from Iceland published in 2019 (26).

Co-morbidities after GCA onset and mortality

A population-based identified co-morbidities diagnosed after GCA onset, for 768 biopsy-proven GCA patients compared to 3066 matched controls. A significant elevated risk was found for several co-morbidities, e.g. osteoporosis, venous

thromboembolic diseases, severe infections, thyroid disease, cerebrovascular accidents, and DM (205). Moreover, meta-analyses have found an increased risk for stroke (206) but not for CAD (207).

Many of the observed co-morbidities in newly diagnosed patients might be due to glucocorticoid treatment. In a Danish study that used health care registries to identify 1682 GCA patients, with a median follow-up of 6.5 years, saw a significantly increased risk of new-onset DM within the first year of treatment. However, they observed no increased risk during later follow-up (208).

Several published studies did not find an increased risk of mortality in GCA patients compared to controls (21, 37, 209). In a population-based study in southern Sweden, comparing 840 biopsy-proven cases with the general population, observed that mortality rates were significantly increased the first two years after GCA diagnosis, in particular in patients <70 years of age at diagnosis. However, the mortality was not increased when studying a longer follow-up (22).

Treatment

Glucocorticoids

Glucocorticoids have been the main treatment for GCA since first found effective in the 1950s. Until recently, other treatment options have been limited.

Glucocorticoid treatment usually gives a dramatic relief of symptoms clinically and since treatment became standard in clinical practice the risk of permanent visual loss has decreased dramatically (210). A multicenter retrospective study demonstrated that if glucocorticoids are initiated within 24 h of onset of visual symptoms, the symptoms might partially improve (210). The optimal dose has not been investigated, though one study with a retrospective design including 286 TAB-positive patients, demonstrated that higher initial prednisolone dose (>40 mg/day) enabled faster tapering (88). High doses of glucocorticoids, i.e., 40-60 mg of oral prednisolone or equivalent per day are recommended for initial treatment (84, 109, 110, 211, 212). If there were visual symptoms at diagnosis it is usually recommended to initially start with pulse therapy with methylprednisolone intravenously for three days (212).

Once symptoms are under control, current practice is to taper the glucocorticoids slowly, with the target to taper down to 15-20 mg/day within 2 to 3 months. Patients usually need treatment for a minimum of 2 years.

In recent years, there have been advances in the understanding of how glucocorticoids affect the disease processes in GCA.

A study published last year investigated the glucocorticoid treatment effects on granulomatous infiltrates and peripheral DCs in GCA patients (147). The researchers concluded that a rapid time-dependent reduction of DCs was observed

in temporal arteries. In addition, the expression of granulocyte-macrophage-colony-stimulating factor (GM-CSF) was also significantly decreased after therapy initiation. (147).

As described under the section of biomarkers, Deng et al. presented results indicating that glucocorticoids might have an effect on downregulating the Th17 axis of inflammation but not the Th1 axis (139).

Glucocorticoid toxicities

Glucocorticoids adverse events appear to be dependent on dose and duration of treatment. Possible adverse events of glucocorticoid treatment are numerous; only several are discussed below. Underlying conditions such as DM, hypertension, peptic ulcer disease and psychosis might be exacerbated by treatment. Furthermore, additional side effects that might be seen include cushingoid appearance, adrenal insufficiency and osteonecrosis. Increased susceptibility to infections can be a major problem in elderly patients treated for GCA with long-term high doses of glucocorticoids.

Some side-effects can appear after some delay or due to high cumulative dose, including cataracts, fatty liver, osteoporosis, skin atrophy, glaucoma, pancreatitis.

Some early adverse events such as emotional lability, enhanced appetite leading to weight gain, or insomnia, might have less impact in health care use, but have a huge effect on the patients` quality of life (213, 214).

In a study, of GCA patients by Dunstan et al., information on side effects was available for 122 patients. Approximately 11 % of patients reported no side effects, while 34 % reported five side effects or more (47).

Table 8.
Side-effects reported in the study by Dunstan et al. (47), ranked according to frequency

Bruising	45 %
Cataracts	41 %
Proximal weakness	34 %
Sleep disturbance	28 %
Oedema	27 %
Depression	26 %
Moon facies	26 %
Weight gain	25 %
Gastrointestinal disturbance	25 %
Fragility fracture	22 %
High blood pressure	20 %
Increased appetite	19 %
Mood disturbance	18 %
Osteoporosis	10 %
High blood sugar	8 %
Oral thrush	8 %
New onset diabetes	7 %
Vaginal thrush	5 %

Because of the side effects of glucocorticoid treatment and high frequency of relapse when tapering, there has been extensive search for other therapies. Major advances have been made in the last few years, discussed below.

IL-6 inhibitors

The utility of IL-6 inhibitors as a glucocorticoid sparing agent in GCA has been widely recognized and use of Tocilizumab is now part of routine management guidelines. In Swedish guidelines Tocilizumab is recommended as additional therapy to glucocorticoids in patients who are at high risk for side-effects to glucocorticoid treatment, who have pronounced clinical or laboratory signs or to treatment-resistant patients (212); this is in line with the EULAR recommendations from 2018 (84). However, the ACR recommendations from 2021 recommend the use of combined therapy with IL-6 inhibition to all patients over glucocorticoids alone (109). However, according to the ACR recommendations, the choice should be based on clinicians' expertise as well as the patients' clinical condition.

The IL-6 inhibitors Tocilizumab for GCA has been investigated in a phase 2 study (215) and in a large phase 3 study (GiACTA). The GiACTA study included 251 patients with GCA who were randomized into four different arms with a ratio of 2:1:1:1: either Tocilizumab weekly or every other week (both combined with prednisolone for 26 weeks) or prednisolone alone for 26 or 52 weeks respectively. Glucocorticoid tapering was conducted to schedule. The rate of sustained remission without glucocorticoids was measured at week 52. For the adjuvant Tocilizumab groups the rates were 56 % and 53 % respectively, compared to the prednisolone alone-treated groups, which had remission rates of 14 % and 18 %, respectively. In addition, the cumulative prednisolone doses were significantly lower with adjuvant tocilizumab; serious adverse events were more common in the prednisolone-alone groups compared to the groups with adjuvant Tocilizumab (135). In the three-year follow-up, the cumulative doses of glucocorticoids were significantly lower among those treated with Tocilizumab, with the lowest cumulative dose in those treated with Tocilizumab weekly (216).

Methotrexate (MTX)

Three placebo-controlled RCTs have been carried out investigating MTX as glucocorticoid sparing treatment, with conflicting results (217-219). A meta-analysis of these three trials, including a total of 161 patients, demonstrated a moderate glucocorticoid sparing effect as well as reduced risk of first and second relapse (220). In these studies, a maximum dose of MTX of 15 mg per week was used. Higher doses have not been investigated.

Recent guidelines suggest that MTX may be used as an alternative to IL-6 inhibitors as glucocorticoid sparing agent in GCA. However, it is not the first choice (84, 109).

Other biologic agents

Based on what is currently known about the pathogenesis of GCA several biologic agents have been proposed as possible treatment alternatives.

A placebo-controlled RCT investigated intravenous **Abatacept (CTLA-4Ig)** vs placebo in 49 patients. The results showed some reduction in relapse rate in the abatacept-treated patients, as well as a longer median duration of remission before relapse (221).

Two open-label, single-armed studies, investigated the use of **Ustekinumab** (**monoclonal antibody to IL12/23 p40**) as treatment in GCA, included 25 and 13 patients respectively. The first study suggested a glucocorticoid sparing effect in patients who had failed to taper glucocorticoids despite use of another immunosuppressive agent (222), whereas the second one did not confirm these findings, as it was stopped prematurely due to high frequency of relapse among the treated patients (223).

RCTs on **TNF-α inhibitors** and GCA have not demonstrated any benefits of treatment with either **Infliximab** (224) or **Adalimumab** (225). An RCT on **Etanercept** with a limited sample size (cases=8, controls=9) showed significantly lower cumulative dose of accumulated prednisolone during first year of treatment but no significant difference in disease control after 12 months (226).

Considering the **IL-17A inhibitor Secukinumab** (SEC), a phase 2 placebo-controlled RCT is ongoing (NCT 03765788). Preliminary results of this study were presented at the ACR congress in 2021, showing higher sustained remission rate (59.3 % vs 8.0 % until week 52) and longer duration until first flare in those receiving SEC versus placebo. The presented results included 52 patients, with 27 receiving SEC and 25 receiving placebo (227).

A phase 3 double-blind placebo-controlled study on SEC for GCA is registered and enrolling patients, with a planned completion date in March 2022 (NCT 02902731).

Possible treatment advances at a glance

CSF-2 (GM-CSF)

As both Th1 and Th17 cells play a role in the pathogenesis of GCA it has been proposed that Mavrilimumab could be a drug of interest. It is an IgG4 humanized monoclonal antibody neutralizing the CSF-2 receptor alpha chain. The expression of CSF-2, its receptor and associated biomarkers have been identified in GCA TAB

specimens. Furthermore, in an ex-vivo study on temporal arteries from patients with GCA treatment with Mavrilimumab resulted in significantly decreased levels of biomarkers associated with active disease (228).

Preliminary results of a phase 2 RCT study (NCT 03827018) were presented at the EULAR congress in 2021, showing promising results. The included 70 patients were randomized to Mavrilimumab (N=42) or placebo (N=28). Sustained remission was achieved at week 26 of the study in 83 % of patients receiving Mavrilimumab and 50 % of patients receiving placebo (p=0.0038) (229).

JAK inhibitors

Several JAK inhibitors on the market are approved to treat various rheumatic and hematologic disorders.

In one study, mice engrafted with human inflamed arteries were treated with tofacitinib. Results showed immunosuppression partly by efficiently reduction of the proliferation of T-cells as well as a decrease in effector molecules such as IFN-γ, IL-17 and IL-21 (230).

Results from Baricitinib treatment are from an open-label, pilot study including 15 patients who had relapsed during treatment (no control group). Only 1/14 patients relapsed during the study period (52 weeks) and the results indicate that Baricitinib appears to be both safe and effective in patients with relapsing GCA (231).

An ongoing clinical trial (NCT03725202) with planned completion in 2024, comparing Upadacitinib with placebo as adjuvant therapy to glucocorticoids in GCA.

Many studies will present more data soon and several of the agents discussed above have shown promising results in preliminary data sets.

Overall Aim

To contribute to increase knowledge on predictors of GCA, possibly adding insights on the pathogenesis with relevance to clinical management

Specific aims of the studies

- To examine potential risk factors for GCA such as BMI, hormone-related factors (early menopause and total duration of breastfeeding) and level of education
- To investigate the effect of baseline clinical characteristics and glucocorticoid treatment on temporal artery biopsy (TAB) findings in patients with GCA
- To investigate metabolic features that may predipose to GCA such as fB-glucose (FBG), triglycerides (TG) and total cholesterol (TC)
- To investigate the relation between proteins associated with inflammation and subsequent development of GCA

Setting

Study area and population

Malmö is the third largest city in Sweden with a population of 347 949 as of December 2020. Three out of the four papers in this thesis used information from two health surveys in Malmö performed between 1974 and 1996. The population during this screening period was between 229 000 and 247 000.

The Swedish personal identification number

Everyone born or registered in Sweden receives a personal identity number (PIN) and is registered by this number in the Swedish national population register. The number is given by the Swedish Tax Agency and once obtained, individuals usually keep the same number for the rest of their lives. The first part s date of birth, and the second part contains four unique numbers resulting in a PIN for every individual. It can therefore be used in research for identifying people and for linkage between different registers.

Cohorts

In study I, II and IV, cases were identified based on previous participation in two population-based health surveys performed in Malmö between 1974 and 1996.

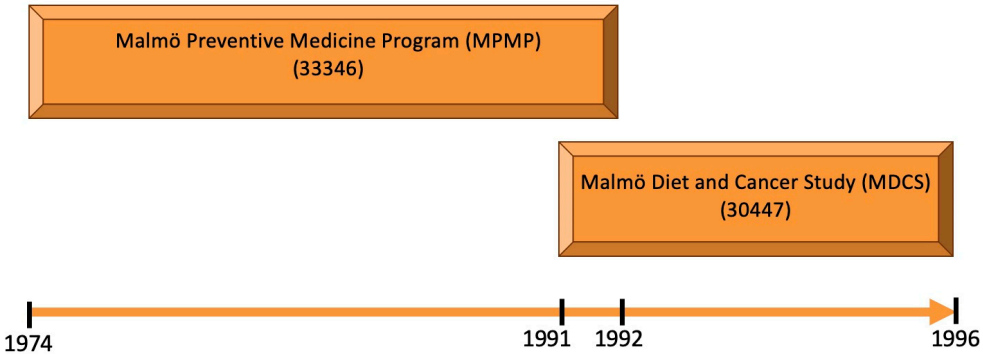

Figure 7.
Study cohorts from populations-based surveys

Malmö Preventive Medicine Program (MPMP)

The Malmö Preventive Medicine Program (MPMP) was started by the Department of Preventive Medicine in Malmö in 1974 with the main aim of studying cardiovascular risk factors, alcohol consumption, glucose intolerance, and breast cancer. The goal was to find out if and how screening and preventive interventions in high-risk individuals might affect mortality. Different selected age-groups were invited, and city residents who were not invited to participate were used as a control group.

Malmö Diet and Cancer Study (MDCS)

The Malmö Diet and Cancer Study (MDCS) is a community-based health survey performed in Malmö between 1991 and 96. The total source population of 74 138 people corresponds to a participation rate of 40.8%. The main goal was to study the impact of diet on cancer risk and mortality. Details of the study are described elsewhere (232).

Identification of patients:

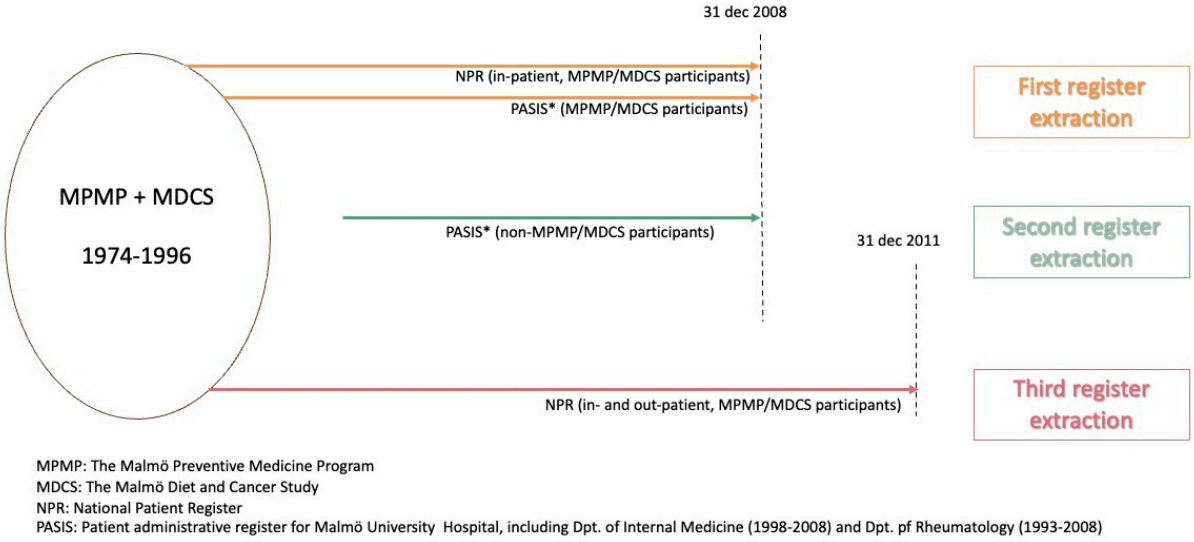

Figure 8.
Overview of register extractions

First register extraction *(cases study I)*

People who had previously participated in MPMP or MDCS and later been diagnosed with GCA in in-patient care were identified from the National Patient Register (NPR) (233). Other previous participants in the MPMP or MDCS were diagnosed in the local outpatient clinic administrative register for Malmö University Hospital (PASIS). The cutoff date for both groups to be included was 31 December 2008.

Second register extraction *(additional cases study II)*

We identified people diagnosed with GCA in the local patient administrative register for Malmö University Hospital (PASIS), including all patients at the Department of Rheumatology, between 1993 and 2008, and at the Department of Internal Medicine, between 1998 and 2008. Patients added after this register extraction had not previously participated in MPMP or MDCS.

Third register extraction *(additional cases study III, IV)*

In the last linkage to registers, new cases were added by identifying people with GCA diagnosis codes through 31 December 2011 in the NPR, now including both in-patient and out-patient diagnoses.

The National Patient Register (NPR)

The National Board of Health and Welfare started collecting information regarding in-patient cases at public hospitals in the 1960s; this became the foundation of the National Patient Register (NPR). In 1984, participation became mandatory for all county councils, and since 1987, the NPR includes all in-patient care in Sweden. In addition, since 2001, the register also covers out-patient visits. However, primary care is not included in the NPR.

In this thesis, the first linkage using the NPR included only information on in-patient care. The third linkage (the second using NPR) out-patient information was added.

The local patient administrative register (PASIS)

The local patient clinic administrative register for Malmö University Hospital (PASIS) included information on both outpatient and inpatient care at the hospital. The present study used this register between 1993 and 2008 on visits and admissions at the Department of Rheumatology, Malmö, and between 1998 and 2008 at the Department of Internal Medicine, Malmö.

Table 9.
Overview of included patients in the different papers

Source	MPMP/MDCS	Paper I	Paper II	Paper III	Paper IV
NPR (inpatient) **PASIS,** Malmö	MPMP – diagnosis until 31 Dec 2008	X	X	X	
	MDCS – diagnosis until 31 Dec 2008	X	X		X
NPR (in- and outpatient)	MPMP – diagnosis until 31 Dec 2011			X	
	MDCS until 31 Dec 2011				X
PASIS, Malmö	Non . MPMP/MDCS		X		
	Non - MPMP/MDCS		X		

NPR: National Patient Register
PASIS: Patient administrative register for Malmö University Hospital, including Dpt. of Internal Medicine (1998-2008) and Dpt. pf Rheumatology (1993-2008)
MPMP: The Malmö Preventive Medicine Program
MDCS: The Malmö Diet and Cancer Study

Study design

Nested case-control study design

A nested case-control study implies that cases of a certain disease in a defined cohort are identified. It is alternatively called the case-control in a cohort design. When cases have been identified a certain number of matched controls is selected from the same cohort. These controls should not have the disease at the time when their corresponding case gets the diagnosis. The nested case-control study design can be either retrospective or prospective.

An advantage in the nested case-control study design compared with a traditional case-control study design is that data have been obtained prior to disease onset. This usually improves accuracy and diminishes recall bias problems. The controls are selected from the same defined cohort as the cases which limits the problem with control selection bias. A nested case-control has some of the advantages of a cohort design, such as collecting exposure data before disease onset.

In addition, a cohort study also provides you the opportunity to calculate incidence rates, which facilitates external comparisons. Negative aspects of strict cohort designs are that they are costly, and time-consuming. From a resource perspective, it may be inefficient to obtain exposure information, blood samples, etc., for so many people who do not develop the disease of interest.

Study population and methods

Study I, III, IV

Study I, III and IV all aimed to investigate predictors of developing GCA with different aspects. Study I aimed to find out if previous results regarding associations between BMI, smoking and hormone-related factors as predictors for GCA could be reproduced in another cohort and with prospectively collected data. Study nr III focused on investigating if metabolic features, i.e. f-blood glucose, cholesterol, or triglycerides, could predict GCA independently of BMI years before disease onset. In addition, in study IV we analyzed frozen plasma samples taken from patients many years before disease onset to investigate if biomarkers of inflammation might reveal specific patterns in those with subsequent GCA compared to controls.

All the studies mentioned above used a retrospective nested case-control study design. The cohorts from which cases have been identified are described below. As described, in a nested case-control study, cases are identified in a defined cohort. In this thesis two cohorts from the same catchment area have been used, the MPMP (1974-92) and the MDCS (1991-1996).

We identified subsequent cases of GCA by linking register ICD-codes (ICD-9 446F and ICD-10 M31.5/M31.6) with the health surveys. We performed a structured review for all medical records after identification through register linkage. Cases were confirmed and classified according to the 1990 ACR criteria (16). The ACR criteria were not mandatory for inclusion. Some cases were included based on expert opinion due to typical clinical features. These cases might have had limited data on some parameters, which resulted in not fulfilling the ACR criteria.

Matching of controls

For every confirmed case of GCA, in study I and III, four controls were randomly selected from the corresponding health survey population. In study IV one corresponding control for every confirmed case was selected. The controls were matched for sex, year of birth and year of screening and they were alive and free of GCA when the index person was diagnosed. Any GCA subjects remained in the pool of possible controls until the time they themselves became cases and were randomly selected, using a specially designed software. If cases were included in first the MPMP and later the MDCS before GCA diagnosis, information and controls from the MDCS was used.

The cohort

The Malmö Preventive Medicine Program - MPMP

Between 1974 and 1992 a total of 22 444 men and 10 902 women entered the screening program. There was an attendance rate of 71.2 % (range 64-78 %) among the invited. During the first period, 1974-1982 mostly men were screened and during the second half 1981-1992, more women. This resulted in a difference in follow-up time between the sexes. Non-participants had a higher total and cause-specific mortality, they had lower socioeconomic status and the attendance rate was lower towards the end of the screening period when the invited participants were younger (234).

Exposure information

Physical examination, included height without shoes, weight with indoor clothing and blood pressure measurement (performed twice). For the present study, hypertension was classified as high blood-pressure or recorded medication for high blood pressure at health survey inclusion.

Fasting blood samples were taken and analyzed for serum cholesterol, triglycerides and blood glucose. All women above 45 years of age were offered screening for breast cancer by mammography.

In addition, all participants filled out a self-administered questionnaire using a computer. The questionnaire contained 260 questions regarding family health history, smoking habits, alcohol consumption, physical activity, dietary habits, present symptoms or signs of cardiovascular disease, diabetes and history of malignancies, among other questions.

The Malmö Diet and Cancer Study - MDCS

A total of 30 447 subjects (12 121 men and 18 326 women) were recruited between 1991 and 1996 (28 098 had complete data). Residents of Malmö - all women born between 1923 and 50 and all men born between 1923 and 45 were invited. The only exclusion criteria were inadequate Swedish language skills and mental incapacity. The attendance rate was 40.8 % and mean age at baseline 58.1 (SD 7.6). Non-participants had higher mortality during recruitment and follow-up. Socio-demographic structures, prevalence of smoking and obesity where similar in participant compared to non-participants (232, 235, 236).

Exposure information

All participants provided information on lifestyle factors and current health status using a self-administered questionnaire. The questionnaire was given at the first visit and looked for missing values at the second visit, usually two weeks later.

Among questions asked were if the participant was born in Sweden, highest level of education achieved, type of education, marital status, and questions regarding smoking, alcohol, previously weight, diet and medical conditions, as well as global health condition, among others.

In addition, weight and height were measured and blood samples were obtained at the time of inclusion in a standardized, non-fasting manner, and stored in -80°C.

OLINK

Using the panels of protein profiling available from O-link, a large number of proteins can be detected using a small blood sample. We decided to focus on the Inflammation panel, containing 92 biomarkers, in this study. Further information regarding the specific proteins analysed are presented in supplementary material in study IV.

Plasma levels of proteins were analysed by the Proximity Extension Assay (PEA) technique using a multiplex reagent kit (O-link Bioscience, Uppsala, Sweden). Two oligonucleotide-labelled highly specific antibodies for each target protein were used. This allows the formation of a polymerase chain reaction (PCR) sequence that can be detected and quantified. All data are presented as arbitrary units. Validation data and more technical information about the assays are available on the O-link homepage (http://www.olink.com).

Study II

The second study has an observational study design. We investigated the impact of glucocorticoid treatment in TAB findings and features in GCA validated cases.

Additional study population

In addition to cases previously participating in MPMP and/or MDCS identified through register linkage, we also identified individuals who were diagnosed with GCA at the department of rheumatology in Malmö 1993-2008 and the department of internal medicine in Malmö 1998-2008. Validation through medical records were performed.

These cases were added to the participants in MPMP and MDCS diagnosed after screening through 31 dec 2011 to make up the cohort of study II.

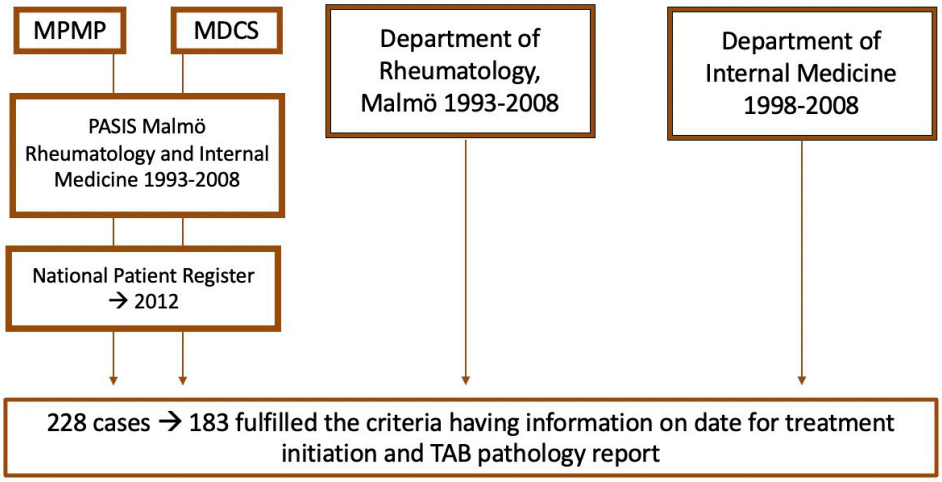

Figure 9.
Included cases in study II

Statistical analysis

Study I and III

In the first and third study, we used conditional logistic regression to examine potential predictors for GCA. Each identified case was matched to four controls from the corresponding health survey, described above. Each group including every validated case and its corresponding controls was given a group number. This number was entered into the logistic regression model as a categorical variable.
Analysis was initially done bivariately and in a second stage with multivariate adjustment for the other potential risk factors. BMI was analyzed per unit and, in study III continuous variables for metabolic factors were transformed into z-scores to enable comparisons of effect sizes. Analyses were stratified by time from screening to GCA diagnosis (divided by median) and by BMI category (WHO definition).

In study III, interactions between BMI category and each of the variables FBG, TG and TC was tested. Correlations were tested using Spearman's rank test and Pearson's test, as appropriate. In cases with $r>0.3$, the covariate with the weaker association with GCA was excluded from the multivariate analysis. The multivariate analysis contained all variables with a significant association in the univariate analysis.

Study II

Study II aimed to investigate the relationship between glucocorticoid treatment and biopsy findings. All included cases had information regarding date for treatment initiation and date for TAB. Information from pathology reports.

Baseline parameters in TAB positive vs. TAB negative cases were compared using chi-square test, Student's T test and the Mann-Whitney U test as appropriate. Relationships between overall positive biopsy and different reported histopathology features in those biopsied before treatment start or on the same day, after 1-3 days, after 4-6 days or after 7-28 gays after glucocorticoids treatment was analysed using chi-square test. To asses differences in distribution of categories of time on treatment we used the Mann-Whitney U test. Furthermore, we used logistic regression models to analyze reported histopathological features as predictors for a positive vs negative TAB.

Study IV

In study IV frozen plasma samples were analyzed for 97 cases and 94 controls using panels of protein profiling available from O-link (described above). Analyses were separated into two categories, a priori analysis including 6 biomarkers with a pre specified hypothesis of being either increased or decreased in subsequent GCA cases compared to controls and hypothesis generating analysis. All protein values were z-transformed before analyses, to enable comparisons of effect sizes.

Proteins with a priori hypothesis were examined as predictors in conditional logistic regressions, with case status as outcome. All analyses were stratified by time from screening to GCA diagnosis (by quartiles) using Holm correction to account for multiple testing (237).

In the hypothesis generating part of the analyses, 91 proteins (IL-24 excluded due to high number of missing values) were included in principal component analysis (PCA). Proteins were selected for further analysis based on their contribution to the overall variation in protein concentrations. Selected based on Eigenvalues >2.0 yielded 9 components. Based on the scree plot, components 7-9 were considered to add minor contribution. Component 1-6 as well as all biomarkers with a factor loading of >0.5 within these components were analyzed as potential predictors using conditional logistic regression. Study protocol for study IV is attached as appendix I.

Ethical considerations

The research ethics committee for southern Sweden has approved all included studies in this thesis (registration numbers 308/2007 and 2010/517). When included in the MPMP and the MDCS all participants gave their informed consent to future use of collected information and samples for research purposes. No additional consent for participating in the studies included in this thesis was obtained. However, before study initiation we advertised in local newspapers, with a notice informing that study subjects could contact the investigators if they didn't want to participate or if they had questions on the study.

Results

Incident cases (all studies)

Table 10.
Characteristics of incident cases of GCA in the separate studies

	Study I	Study II	Study III	Study IV
Cases, n	83	183	76	94
Female sex, n	58 (70 %)	134 (73 %)	46 (61 %)	77 (82 %)
Age at GCA diagnosis, years, mean (S.D.; range)	71.0 (6.6; 56-83)	74.3 (8.97; 49-95)	70 (6.4; 56-83)	73.6 (6.0; 57-86)
Time from screening to diagnosis	10.6 (0.3-28.2)	NA	20.7 (3.0-32.1)	11.9 (0.32-19.11)
Positive biopsy, n	53 (64 %)	141 (77 %)	49 (65 %)	60 (64 %)
Fulfilled 1990 ACR criteria, n (%)	79 (95 %)	175 (96 %)	72 (95 %)	85 (90 %)

In Table 10 the characteristic of cases in study I-IV are presented. The difference in sex distribution reflects the health survey populations from which cases have been identified in Study I, III and IV. The proportions fulfilling the 1990 ACR criteria are similar. A higher rate of biopsy positivity is seen in paper II, reflecting that only patients with information on pathology reports were included.

Estimated Incidence (Study I)

In the first study estimated incidence rates per year were calculated to 15/100 000 in people >50 years of age (18/100 000 for women and 10/100 000 for men) in the MDCS and corresponding estimate was 7/100 000 (12/100 000 for women and 5/100 000 for men) for the MPMP. Incidence rates were calculated by dividing all new confirmed GCA cases with the total follow-up time of people included in the corresponding health survey through 31 December 2008.

Predictors (Study I + III)

As presented in Table 10, study I included a total of 83 validated cases and the median duration between screening and diagnosis was 10.6 years (range 0.3-28.2). In study III, 76 verified cases were included, and the median follow-up time was 20.7 years (range 3.0-32.1)

Table 11.
Potential predictors of GCA in bivariate analyses using conditional logistic regression, for all and stratified by sex

	All		Women		Men	
	OR	95 % CI	OR	95 % CI	OR	95 % CI
Study I						
BMI per kg/m^2	0.91	0.84-0.98	0.89	0.81-0.97	0.97	0.85-1.11
Study III						
BMI per kg/m^2	0.90	0.82-0.98	0.86	0.76-0.98	0.95	0.82-1.10
fB-glucose per SD	0.35	0.17-0.71	0.67	0.35-1.25	0.07	0.01-0.30
Total cholesterol per SD	0.58	0.42-0.81	0.55	0.35-0.85	0.63	0.38-1.03
Triglycerides per SD	0.45	0.27-0.74	0.34	0.15-0.75	0.55	0.30-1.01

BMI

In study I, higher BMI was associated with a reduced risk of subsequent GCA OR 0.91/kg/m^2 (95 % CI 0.84-0.98). These results remained significant in analysis stratified for those with a median time for screening to diagnosis above median (10.6 years) OR 0.30 (95 % CI 0.12-0.77) and in women, but not in men (Table 11). The association between higher BMI and a reduced risk for GCA remained significant in multivariate analysis adjusting for smoking and level of education OR 0.91 (95 % CI 0.85-0.99).

Study III confirmed that higher BMI was associated with a reduced risk for subsequent GCA (OR 0.90/kg/m^2; 95 % CI 0.82-0.98). In analysis stratified by sex there was a significant negative association in women, but not in men (Table 11). In analysis stratified by median time from screening to diagnosis (20.7 years) the results remained significant in those screened <20.7 years prior to GCA diagnosis (OR 0.81; 95 % CI 0.70-0.95) but not in those screened >20.7 years prior to diagnosis (OR 0.96; 95 % CI 0.86-1.08).

Smoking

In study I, we did not find a significant association between current smoking and subsequent GCA (OR 1.36, 95 % CI 0.77-2.57). Stratified by sex there seemed to be a tendency towards an increased risk for women current smoking at screening, although this did not reach significance (OR 2.14, 95 % CI 0.97-4.68) Ever smoking was not associated with GCA in either sex.

In study III, current smokers had a reduced risk of GCA (OR 0.35, 95 % CI 0.18-0.70). This association reached significance in men but not in women in stratified analyses (OR 0.24; 95 % CI 0.09-0.80 and OR 0.48; 95 % CI 0.19-1.19).

Hormone-related factors and level of education

We did not find any significant association between early menopause (before age 46) and subsequent GCA (OR 1.76, 95 % CI 0.71-4.39). Neither total amount of breastfeeding (OR 1.00, 95 % CI 0.95-1.06) nor low level of formal education (OR 0.72, 95 % CI 0.39-1.33) predicted GCA.

Metabolic factors

Cases had significantly lower fB-glucose, total cholesterol and triglycerides compared to controls (Table 11), all associations remained significant when adjusted for current smoking at screening. The association for fB-glucose and subsequent GCA was stronger in men compared to women (Table 11).

Patterns where similar stratifying for time from screening to diagnosis (above vs below median of 20.7 years) (see Paper III).

In study III, there was no association between hypertension (yes vs no) OR 1.15; 95 % CI 0.64-2.06) or Triceps skinfold index (per SD) OR 1.04; 95 % CI 0.72-1.51 and subsequent GCA.

Rates of self-reported co-morbidities (76 cases and 304 controls, median time 20.7 years before diagnosis) were low overall. However, proportions with self-reported cancer (0 % cases, 2 % controls), diabetes (0 % cases, 2.3 % controls) and cardiovascular disease (cases 2.7 %, controls 4.7 %) were slightly lower among cases.

Proteins related to inflammation preceding clinical onset of GCA (Study IV)

Investigations of potential biomarkers were handled as a priori hypothesis or hypothesis generating analysis, as previously described and described in detail in the study protocol of study IV (appendix I). The eight proteins handled as a priori hypothesis proteins are tabulated in Table 12 with information on whether increased or decreased levels were expected. In Table 13 proteins identified through PCA are presented.

A priori analyses

Table 12.
Relation between plasma biomarkers with a priori hypothesis, with information regarding a priori hypothesis. Conditional logistic regression.

	OR (CI)	P value	P value (corr)	A priori hypothesis
IFN-γ	1.52 (1.00-2.30)	0.048	0.38	↑
IL-6	0.91 (0.60-1.38)	0.67	1.00	↑
CXCL-10	1.52 (0.92-2.50)	0.10	0.71	↑
CXCL-11	1.39 (0.90-2.16)	0.14	0.82	↑
Caspase-8	0.90 (0.54-1.50)	0.68	1.00	↑
FGF-21	1.04 (0.71-1.51)	0.85	1.00	↓
PD-L1	1.30 (0.86-1.98)	0.22	1.00	↓
LIF	1.17 (0.79-1.75)	0.44	1.00	↑

In our a priori hypothesis analyses, IFN-γ was significantly elevated in cases compared to controls. Similarly, CXCL-10 and CXCL-11 were also higher in subsequent GCA cases although not reaching significance overall, CXCL-10 was found to be significantly increased in the quartile closest to diagnose (0-8.5 years) (OR 3.34; 95 % CI 1.03-10.89, p=0.045), and CXCL-11 in quartile 2 (8.5-11.9 years) (OR 2.63; 95 % CI 1.04-6.64, p = 0.045), in analysis stratified by time from screening to diagnosis. Holm's corrected values were all >0.05.

Hypothesis generating analyses

Table 13.
Proteins of inflammation significantly associated with subsequent GCA, overalll and stratified by time (years) from screening to diagnosis with GCA. Conditional logistic regression.

	All		Quartile 1 (0.3-8.5) (N=47)		Quartile 2 (8.5-11.9) (N=48)		Quartile 3 (11.9-15.5) (N=47)		Quartile 4 (15.5-19.1) (N=49)	
	OR (CI)	**P**	**OR (CI)**	**P**	**OR (CI)**	**P**	**OR (CI)**	**P**	**OR (CI)**	**P**
IFN-γ	1.52 (1.00-2.30)	**0.048**	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
MCP3	2.01 (1.24-3.25)	**0.004**	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
CXCL9	2.17 (1.31-3.59)	**0.003**	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
IL2	1.52 (1.02-2.27)	**0.040**	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
SCF	1.84 (1.20-2.82)	**0.005**	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
IL10RB	1.63 (1.01-2.61)	**0.045**	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
CD40	1.66 (1.02-2.70)	**0.043**	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**
CCL25	1.67 (1.04-2.67)	**0.034**	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

Through PCA analysis of 91 proteins, 6 components were identified with Eigenvalues above 2.5. All proteins with a factor loading of >0.5 were further analysed in logistic regression models (n=38). Among these 91 proteins, eight were found to be significantly associated with subsequent GCA (Table 13).

Significantly higher concentrations per SD were found for IFN-γ, MCP-1, CXCL9, IL2, SCF, IL10RB, CD40 and CCL25. They were further analyzed and stratified by time from screening to clinical diagnosis, Table 13. IFN-γ was found to be associated with subsequent GCA in both the a priori analysis and the hypothesis generating analysis.

IFN-γ and MCP-1 were significantly elevated in those sampled closest to clinical diagnose with a decreasing trend if sampled with a longer duration to diagnosis.

Effect of glucocorticoids on biopsy findings (Study II)

In a total of 183 cases with available information on date of treatment initiation and TAB were analyzed to investigate if glucocorticoid treatment affects the biopsy outcome and/or specific biopsy features (Table 14).

Table 14.
Features recorded in pathology reports, stratified by time from initiation of glucocorticoid treatment to biopsy

Time from treatment start to biopsy	≤ 0 days (n=44)	1-3 days (n=74)	4-6 days (n=43)	7-28 days (n=20)	P	P for trend *
Inflammatory infiltrates	39 (89 %)	52 (70 %)	35 (81 %)	15 (75 %)	0.12	0.95
Biopsy positive	38 (86 %)	51 (69 %)	34 (79 %)	16 (80 %)	0.17	0.64
Fragmented internal elastic lamina	20 (45 %)	33 (45 %)	21 (49 %)	10 (50 %)	0.86	0.65
Giant cells	21 (48 %)	23 (31 %)	23 (53 %)	8 (40 %)	0.10	0.73
Granuloma	9 (20 %)	6 (8 %)	4 (9 %)	3 (15 %)	0.19	0.28
Fibrosis	4 (9 %)	15 (20 %)	8 (19 %)	4 (20 %)	0.47	0.24
Minor inflammatory infiltrates [a]	13 (30 %)	27 (36 %)	14 (33 %)	6 (30 %)	0.87	0.94
Major inflammatory infiltrates [b]	23 (52 %)	23 (31 %)	21 (49 %)	9 (45 %)	0.09	0.90

a Lesions described as “limited infiltrates”, “minor inflammation” etc. were classified as minor inflammatory infiltrates.
b Lesions described as “massive inflammation”, “typical GCA” or using similar wording were classified as major inflammatory infiltrates.
* The Mann-Whitney U test was used to assess differences in the distribution of category of time on treatment with glucocorticoids among those with vs. without specific histopathology features.

The median time from start to glucocorticoid treatment to TAB was 3 days (IQR 2-5). Two cases with long duration between time from initiation of treatment to TAB, 35 and 253 days, were excluded from the analysis (i.e. not shown in Table 14). Both these patients had a positive TAB and fulfilled the 1990 ACR classification criteria.

When comparing pathology report findings depending on time from treatment initiation to TAB, and subgrouping cases into patients starting treatment with glucocorticoids after TAB or on the same day, patients treated with GC for 1-3 days, 4-6 days and 7-28 days (Table 14). There was no significant difference in distribution of proportions of positive TAB results between the four groups. Moreover, no significant difference in number of TABs reported inflammatory infiltrates, fragmented elastic lamina, giant cells, granuloma was found between these groups. Neither was there and significant trend for histopathology findings per quartile with longer time on GC treatment (Table 14).

We found that female sex (OR 2.01; 95 % CI 0.97-4.20) tended to be predictive for a positive TAB outcome. There was a progressively increased chance of positive TAB with higher quartiles of ESR (p for trend: 0.01) and CRP (p for trend: 0.07).

Conclusions

Our results have added insights into the pathogenesis of GCA indirectly through identifying various potential predictors of GCA, including differences in the proteome years before clinical onset. More specifically, this thesis has found that:

- A lower BMI is an independent risk factor for developing GCA.
- There was no significant association between a history of early menopause nor duration of breastfeeding and subsequent GCA.
- A low level of formal education did not predict GCA.
- Subsequent GCA was associated with lower FBG, TC and TG levels at a median of 20.7 years before diagnosis, all adjusted for current smoking habits.
- IFN-γ and MCP-1 were elevated in plasma samples taken before clinical onset (median time 11 years). Results were significant in those sampled closest to diagnosis with a decreasing trend if sampled with a longer duration to diagnosis.
- Several potential biomarkers (IFN-γ, MCP-1, CXCL9, IL2, SCF, IL10RB, CD40 and CCL25) were significantly elevated in cases compared to controls in samples taken at a median of 11 years prior to clinical diagnosis. This suggests activation of the adaptive immune system years before clinical onset.
- TABs still yield clinically useful information when taken up to four weeks after initiation of glucocorticoids.

Discussion

The diagnosis

In this thesis, GCA has been investigated as a single disease, although it is not a single entity and includes a broad spectrum of symptoms that can be categorized into different phenotypes, as described in the background. These phenotypes have different characteristics; for example, LV-GCA affects younger people more. Variation in management may influence the difference in phenotypes by different choices in imaging modality, TAB performance, etc. Also, it is important to keep in mind that inclusion criteria in different studies might affect the generalizability of study results.

Phenotypes might have slightly different pathogenesis; therefore, it is not farfetched that they also have differences in predictors and triggers which need further investigation.

The incidence of GCA varies greatly in published studies. Possible explanations might be differences in study design such as selection of cases (TAB positive cases, diagnoses code linkage, medical record review, etc.). Incidence over time might be affected by clinical awareness, biopsy routines (i.e., accessibility and routines for referral), imaging access and advances in imaging techniques over time.

With the highest incidence rates identified in Caucasian populations, our estimated incidence rates in paper I are in line with what would be expected in this study's particular set-up. The cohort investigated is overall younger in age than the general population aged over 50, with an even a lower age among those participating in MPMP compared to MDCS. When looking at the incidence rates among individuals ages 60-69, these were approximately 30 % lower than what has been reported from a population-based study in Minnesota (21). Additional factors that may have affected the incidence in our study are that; we did not capture a) patients exclusively treated in primary care b) subjects who had moved away from Malmö and developed GCA without hospitalization.

Predisposition to GCA

Genetically, HLA-DRB1*04 regions have been associated in several studies with the susceptibility for GCA (198, 200), and familial aggregation has been observed (197). However, one study presenting geo-epidemiological data suggested that GCA was independently associated with both population frequency of HLA-DRB1*04 and with latitude itself (200). This indicates that the high incidence rates in northern

Europe are not exclusively explained by genetic factors. Possible additional explanations for the geographic differences besides genetics are a geographic variance in metabolic factors such as fB-glucose, cholesterol, and triglycerides. Also, geographic differences in BMI might affect incidence rates. Other potential explanations are geographic differences in other expositions, e.g., infections.

Aging

Aging affects both the innate and adaptive immune systems leading to several manifestations including a decreased ability to fight infections, increased cancer incidence, higher prevalence of autoimmunity, and a low grade of persistent systemic inflammation. As for T-cells, a contracted repertoire with age may be associated with expansion of clones with higher affinity for self-antigens, leading to autoreactivity in older individuals. Latent virus infections, accumulated over time, might cause expansion of certain clones of memory T-cells. Also, intrinsic defects such as changes in cell surface glycosylation, defects in cytoskeletal signaling, inability to adjust for threshold of response to type 1 IFN receptor signaling and increase/decrease in expression of certain proteins, among others might contribute to impairment of normal immune responses and promotion of autoimmunity (238).

Furthermore, epigenetic changes that come with age and anatomical changes in the vessel walls, i.e., the lamina elastica (internal and external) dividing the different layers in the temporal arteries becoming more permeable with age (5), may contribute to increased susceptibility to GCA in older individuals.

BMI and GCA

The rationale behind the first study in this thesis was a previous retrospective case-control study from Gothenburg which indicated that lower BMI, smoking and several hormone-related factors were associated with biopsy-proven GCA (203). Using a nested case-control design we investigated these factors in a larger prospective cohort including patients with a GCA diagnosis, verified through review of medical records. Our findings confirmed an association between lower BMI and a significantly increased risk for subsequent GCA.

BMI was associated with GCA in both study I (median time from screening to diagnosis 10.6 years), and in study III (median time from screening to diagnosis, 20.7 years, with a partly overlapping cohort. In addition, the group with the longer time span from screening to diagnosis gave significant and similar results when stratifying by median time from screening to diagnosis in study I. This indicates that these observations are not due to inflammation-driven weight loss close to diagnosis.

The association between BMI and GCA has later been confirmed by a meta-analysis (including a total of three studies: study I in this thesis, the previously mentioned study by Larsson et al. from Gothenburg and a third study based on register linkage to the Danish National Birth Cohort (239)). Also, this association has more recently been confirmed in two additional, independent cohorts (26, 240).

Together these results strengthens the hypothesis that BMI might, directly or indirectly, contribute to the risk of subsequently developing GCA.

An unknown confounder that is associated with both the risk of GCA and having a lower BMI cannot be excluded. This confounder could be either genetic or environmental. However, the observed association between BMI and the risk for GCA might be explained by a protective effect of adipose tissue, possibly through effects on estrogen synthesis and related anti-inflammatory pathways (241-244). It might also be due to production of adiponectin and other inflammation-related proteins (cytokines, chemokines etc). Smaller adipocytes produce more adiponectin than hypertrophic ones (245). A negative association between BMI and serum levels of adiponectin has been demonstrated (246). Adiponectin is known to have many anti-inflammatory properties but also pro-inflammatory properties, such as upregulation of NF_KB (247). One study showed that adiponectin can activate DCs leading to a Th1 and Th17 response (248)

Adipose tissue might also be associated with a hyperactivity of the hypothalamic-pituitary-adrenal axis (249). In theory an increased release of endogenous corticosteroids due to such activation might have an anti-inflammatory effect.

Hormone-related factors and level of education

In this thesis we did not find any association between neither history of early menopause nor total duration of breastfeeding and subsequent GCA. Our findings are in contrast with Larsson et al. (203) who found that early menopause, and longer duration of breastfeeding were associated with increased risk for GCA. The conflicting results could be explained by differences in study design, our study having a prospective cohort study design and the Gothenburg study a retrospective design. There is also a difference in sample size (83 vs 49 cases) which gives our study greater power. Other potential explanations for the discrepancy could be differences in geography or case selection (biopsy positive vs all cases verified by medical records review). Hormone-related factors have been associated with other autoimmune diseases e.g., RA (250, 251). Further studies on this topic are needed. The fact that 2/3 of the affected patients are women indicate that sex somehow matters, whether this is linked to hormones, genetics or other factors we are still to figure out.

We also concluded in the first study that a low level of formal education did not predict GCA. Such an association has been found in e.g. RA (252, 253). One possible explanation for the lack of association in GCA might be that education early in life has less impact on diseases with a clinical onset late in life compared to diseases which are clinically diagnosed at an earlier age.

Metabolic predictors and potential underlying mechanisms

Study III presented significant association between lower levels of fB-glucose, TG and TC and increased risk for subsequent GCA. These results are in line with results showing a lower prevalence of diabetes in GCA cases at time of diagnosis compared

to controls. Lower levels of fB-glucose and cholesterol 5 years prior to disease onset have been confirmed in another cohort recently (240). One study showing discrepant findings with an increased risk of DM associated with GCA was based on exclusively Claims data with no validation of diagnosis (186). Strengths of our study design include the prospective design, with blood samples taken with a median of 20.7 years prior to clinical onset, as well as the structured review of cases for validation.

A dysregulation of the PD1/PD-L1 checkpoint as part of the GCA pathogenesis has been identified by Weyand et al. The function of PD1/PD-L1 interaction is to regulate T-cell activation. A higher expression of PD-1 on T-cells and a decreased level of PD-L1 on DCs have been identified in TABs from patients with GCA compared to non-inflamed human arteries (154). The PD1/PD-L1 checkpoint leads to a T-cell receptor activating cascade resulting in immunosuppression (254). A suggested connection between the PD1/PD-L1 checkpoint and glucose metabolites has been proposed. One study showed a positive association between mitochondrial pyruvate and the expression of PD-L1 on macrophages (255) This is a potential pathway in which fB-glucose levels might influence the GCA pathogenesis.

The possible dysregulation of the PD1/PD-L1 checkpoint in GCA patients might also be reflected by studies indicating that GCA patients have fewer malignancies than controls prior to diagnosis (195).

Another possible explanation for the correlation between fB-glucose levels preceding GCA are that IFN-γ/LPS-induced M1 macrophages are known to increase glucose consumption and lactate release (256). The correlation might therefore reflect a very early activation of M1 macrophages in the pre-clinical phase of GCA.

Again, it cannot be excluded that these findings are caused by a confounder associated both with these metabolic factors and with GCA independently. It is also possible that glucose metabolites or lipids have other effects on the immune system that are not yet known.

Biomarker patterns prior to disease onset

As summarized in the background biomarkers in active GCA have been extensively investigated. However, to our knowledge no one has previously investigated proteins of inflammation (potential biomarkers) prior to disease onset. These results add possible new insights in the pathogenesis including possible early disease mechanisms of GCA, starting years before clinical disease onset.

Analysis of frozen plasma samples obtained a median of 11.9 years before clinical diagnosis revealed that several T-cell related proteins were elevated in cases with subsequent GCA compared to controls. Among these proteins found to be significantly associated with subsequent GCA in logistic regression analysis were IFN-γ, MCP-1, CXCL9, IL2, SCF, IL10RB, CD40 and CCL25. In analysis stratified for time from screening to clinical diagnosis IFN-γ and MCP-1 had the strongest associations in the quartile closest to diagnosis with and decreasing trend for those

sampled with a longer duration from screening to diagnosis. All predictive proteins, except IFN-γ, were identified through hypothesis generating analysis.

Such biomarker patterns, and related pathomechanisms, may be regulated by the metabolic factors discussed above, or by genetics. They may also be influenced by other exposures, such as infections.

Several of the inflammation-related proteins we found to be elevated prior to clinical onset of GCA have gene polymorphisms that have been associated with GCA. A dinucleotide (CA) repeat within the beginning of the IFN-γ gene has been associated with visual manifestations of GCA, but not with GCA overall (257).

Two independent studies in different cohorts indicated that variations in the IL10 promotor region might be involved in the genetic susceptibility to GCA (258, 259). Differences in SNP variants in the MCP-1 gene have been associated with GCA (260). Although IL-2/IL-21 have not been statistically associated with GCA overall, but a SNP located in the region for these genes was more common in individuals with GCA and ischemic complications, reaching significance in patients with jaw claudication (261). As discussed in the background, many of these studies lack sufficient power. However, our observations of differences for some of these proteins years before clinical onset might partly be due to variations in the genetics encoding these proteins.

Infections have been discussed as a possible trigger of GCA although no consistent evidence of any particular microorganism has been presented. Epidemiological studies indicate an overall increased incidence of infections prior to GCA onset (164, 165), which supports a role of infections as a possible trigger for GCA. In addition, this concept is also supported by the role of TLRs in the pathogenesis and by studies indicating seasonal variation. Our results in study IV indicate a T-cell activation and with a pattern that includes increased levels of IFN-gamma prior to clinical onset, an increased possibly triggered by infections in predisposed individuals. Some of the microorganisms that have been suggested as triggers for GCA are also associated with a high IFN-γ response i.e., VZV (262), *Mycoplasma* (263), *C. pneumoniae* (264) and parvovirus B19 (265).

Yet again, it is possible that there are confounding factors explaining the association between infections and subsequent GCA. One possible confounder could be a common dysfunction in the immune system leading to an increased risk of or more fulminant infections and independently an increased risk of GCA.

In addition to previously discussed genetic predictors, a polymorphism within the TLR4 gene has been significantly associated with GCA (266). A meta-analysis has concluded that this variant may be a risk factor for vasculitis in general but with a higher specificity for GCA (267). TLR4 can induce signal transduction when activated by Gram negative bacteria (through LPS), fungal, mycobacterial pathogens or endogenous ligands (268), possibly triggering vascular inflammation in predisposed individuals.

The impact of glucocorticoids on TAB outcome

As TAB is still the gold standard for GCA diagnosis, it continuous to be a matter of debate as to which extent the biopsy yields clinical information once the patient is on glucocorticoid treatment. Previous studies have shown somewhat conflicting results regarding glucocorticoids effect on TAB findings (101-105). The updated ACR guidelines from 2021 (109) recommend that a biopsy should be taken within 2 weeks of treatment initiation which is compatible with the results of this thesis. In our study, there was no significant difference in overall biopsy positivity nor specific histopathological findings between patients biopsied prior to or on the same, patients treated for 1-3, 4-6 and 7-28 days in relation to initiation of treatment with glucocorticoids. We assessed biopsy information from pathology reports which might have resulted in missing information in some cases. However, the fact that two patients, treated for 35 and 253 days respectively still had TAB findings suggestive for GCA strengthens the hypothesis that biopsy findings can persist for a long time.

Discrepancy of previous results regarding GCs effect on TAB findings might partly be due to relatively short biopsies (mean 0.6-0.8 cm) in some studies (101). Serial sectioning of longer biopsies might have yielded evidence of residual inflammatory lesions in some of the patients biopsied after >1 week of GC treatment.

Less impact of GCs on TAB findings have been seen in other studies where TABs were on average longer (mean 1.6 cm) (103). Although the lack of data on TAB length in our study is a limitation, clinical practice in our centre during the study period was to obtain TABs with length of >1 cm.

As ultrasound is becoming more available and clinicians' experience and habit increase the use of this tool, ultrasound and TAB are equally recommended for diagnosing GCA in the updated EULAR guidelines from 2018 (121). However, there are still major regional differences in expertise and some studies indicate that typical findings from ultrasound might disappear within days (122). Taken together even if ultrasound, with its many advantages become more commonly used in the diagnostic workup, TAB may still be the first choice for diagnosing patients who have already initiated treatment as our results indicate that TAB still yield clinical useful information when taken up to four weeks (possibly even longer) after initiation of glucocorticoids.

As it is expected that we during the coming decades we will have a longer duration of life, the total number of GCA cases is expected to increase. In addition, advances in imaging techniques may contribute to detection and diagnosis of more cases.

On the other hand, a higher BMI, and metabolic factors such those investigated in study III, is associated with a decreased risk for GCA. With increasing BMI in many populations around the world (269), these effects might counteract those of increasing age on the incidence rates the upcoming decades.

Strengths

We have used community-based cohorts with a good coverage rate from the same well-defined catchment area. This limits the possibility of selection bias. The external validity is strengthened by our incidence estimates in study I, which are as would be expected in our population. Other strengths are the use of the Swedish identification number system in combination with registers with diagnosis codes, i.e. the National Patient Register (NPR) and the Patient Administrative Register (PASIS) in Malmö which enables identification of cases by linking our health surveys to these registers.

All patients who received a GCA diagnosis code were validated through a structured review of medical records, which is not always done in register based studies.

In addition, due to our nested case-control study design approach we limit the risk of recall bias, as information on exposures was collected before disease onset in a standardized manner.

Limitations

Limitations include the relatively small number of cases which limits the power of the studies. In study I and III, the lower participation rate in the MDCS compared to the MPMP may affect the ability to generalize these results.

Our data on exposures are from a single time-point. Some of the exposures measured, such as lifestyle factors that smoking, as well as concentrations of biomarkers in blood samples, might change over time. This should be taken into account when analyzing and interpreting the results.

In study II we collected information from the pathology reports which in some cases gave missing information depending on the level of detail and the clarity of the reports.

Future research (bullet points)

Local research community

- Further studies should investigate proteins associated with metabolism as potential biomarkers predicting GCA (project initiated)
- Further investigation of infections as possible triggers for GCA, with particular focus on certain group of infections with similarities in immune response (IFN-γ response) are of particular relevance. This may involve both epidemiologic and experimental studies.
- Studies of genetics associated with plasma levels of potential biomarkers may reveal important patterns.

Global research community

- Larger studies with greater power regarding biomarkers (in particular those associated with inflammation or metabolism) prior to disease onset are required.
- Investigation of biomarkers as potential predictors for clinical outcome (with subgrouping of phenotypes) and treatment response, may contribute to the emergence of precision medicine for GCA. They may also be a potential for preventive medicine?
- It would be of interest with more studies on phenotype subgroups regarding differences in proteome expression and pathogenesis.

Populärvetenskaplig sammanfattning

Giant cell arteritis (jättecellsarterit), eller temporalisarterit som är den vanligaste svenska benämningen på sjukdomen är en relativt sett vanlig kärlinflammatorisk sjukdom på våra breddgrader. Det finns geografiska skillnaderna i insjuknande och möjliga förklaringar till dessa geografiska skillnader är genetisk variation och geografiska skillnader vad gäller potentiella riskfaktorer. Omgivningsfaktorer såsom miljö och infektioner kan också tänkas spela en roll. Personer under 50 års ålder drabbas ytterst sällan och högst andel insjunkande ser man kring 70-80 års ålder, vilket indikerar att åldrande av vårt immunförsvar kan spela en viktig roll.

Våra studier syftar till att öka kunskapen om vem som senare i livet kan komma att insjukna. Detta har vi gjort genom att jämföra personer som deltagit i hälsoundersökningar i Malmö (1974–1996). Genom att identifiera vilka som flera år senare fått diagnosen har vi kunnat gå tillbaka och jämföra personer som insjuknat med personer som inte insjuknat. På det sättet har vi kunnat se vad som kännetecknar de som senare i livet blir sjuka många år innan diagnos.

Sammanfattningsvis så talar våra resultat (studie I och III) för att personer som får temporalisarterit senare i livet flera år tidigare i lägre utsträckning är överviktiga eller obesa (dvs har lägre body mass index, BMI) och mer gynnsamma metabola värden avseende blodsocker och blodfetter jämfört med en kontrollgrupp.

I det andra arbetet tittade vi på hur behandlingsstart av kortison påverkar vad man kan se i ett vävnadsprov (biopsi) från temporal artären. En sådan biopsi är ofta grundläggande för att ställa en definitiv diagnos och därför viktig i det kliniska arbetet. Ofta har man sagt att en biopsi måste tas inom två veckor efter behandlingsstart, I vår studie kunde vi se att en biopsi tagen upp till 4 veckor efter behandlingsstart inte visade signifikant lägre sannolikhet för att ge ett positivt resultat.

I det fjärde och sista arbetet tittade vi på olika proteinmarkörer i blodet från frysta prover och jämförde dessa markörer hos våra patienter och friska kontrollpersoner. Vi kunde då identifiera flera markörer som visade sig vara förhöjda hos våra fall jämfört med kontroller. Dessa proteiner är kopplade till inflammation generellt och specifikt till aktiv temporalisarterit. Detta talar för att det finns en aktivering av immunförsvaret flera år innan sjukdomsdebut.

Våra resultat överensstämmer med andra forskare som visat att dessa patienter tycks ha ett lägre BMI innan sjukdomsdebut och lägre sannolikhet att ha diabetes vid tidpunkten för diagnos. Man har sett att ”kontrollstationer” för T-celler, de immunceller som driver sjukdomen i hög grad, möjligen kan regleras av

blodsockernivåer. Detta kan vara en bidragande förklaring till hur de skillnader i blodsockernivåer vi fann långt innan sjukdomsdebut kan spela en roll i sjukdomsuppkomsten senare i livet. Förhoppningen är att våra studier tillsammans med andra som bidrar till ökad kunskap om sjukdomsmekanismerna för temporalisarterit i förlängningen kan leda till bättre handläggning och behandling.

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Appendix I
- Study protocol (study IV)

Biomarkers as predictors for development of GCA

Investigators

Karin Wadström, MD, PhD student - will we responsible for the main parts if the study including study design, interpretation of results, writing the initial manuscript draft.
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Lennart Jacobsson, MD, PhD, professor – will participate in study design, interpretation of results and drafting the manuscript.

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Aladdin Mohammed, MD, PhD, associate professor. Co-supervisor for KW. Will participate in study design, interpretation of results, drafting the manuscript.
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Eric Matteson, MD, MPH, professor. Will participate in study design, interpretation of results, drafting the manuscript.
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Kenneth Warrington, MD, professor. Will participate in study design, interpretation of results, drafting the manuscript.
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Jan-Åke Nilsson, statistician. Will participate in study design, statistical analysis and interpretation of results.
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Magnus Jakobsson, PhD, associate senior lecturer - Expert on proteomics. Will participate in study design, interpretation of results and will also contribute with input on limitations and strengths of the laboratory methods used in this study.
Affiliations: Lund University, Department of Immuntechnology

Carl Turesson, MD, PhD, professor. Main supervisor for KW. Will be responsible for the main parts if the study including study design, interpretation of results, writing the initial manuscript draft.
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Background

Giant cell arteritis (GCA) is the most common large vessel vasculitis among people in the western world. The highest incidence rates have been reported from Scandinavian countries and Minnesota, USA. In these areas, the incidence is approximately 20-30/100 000 among people aged over 50. The aetiology and pathogenesis is not fully understood even though some advances have been made the last couple of years. Genetic factors i.e. HLADRB1 alleles has been associated with the disease in some, but not all populations.

There is little known about the triggers and predictors of developing GCA. A case-control study from Gothenburg, which included women with biopsy-proven GCA and matched controls, showed that smoking, low BMI and multiple hormone related factors were associated with GCA [1]. In a nested case-control study, our group demonstrated that a lower BMI predicted disease development later in life. although we did not find any association regarding hormone related factors [2]. In a later study, we found that individuals who subsequently developed GCA had lower blood glucose, cholesterol and triglycerides at baseline, with a median of 21 years before disease onset, compared to controls [3].

Biomarkers in GCA

Regarding biomarkers, several studies have been done on recent-onset GCA, both treatment-naïve and glucocorticosteroid treated patients, compared to controls. Most studies have investigated circulating protein markers.

In summary, several studies have shown elevated levels of IL-6 in newly diagnosed GCA [4-11] and one study saw a decrease of the soluble IL-6 receptor (sIL-6R) [12]. Besides IL-6, other interleukins, i.e. IL-8, IL-10, IL-12, IL-17, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, have been found to be elevated in active disease [4, 5, 9, 10, 13].

Other cytokines such as CXCL9, and other proteins such as VCAM, ICAM1, VGEF, as well as matrix metalloproteinase 1 and 9 has been described to be significantly elevated among patients with GCA in an active stage [4, 10, 11, 14-17].

Some studies have found increased levels of TNF-alpha [6, 9], although there are conflicting results [12]

Acute phase protein acute serum amyloid A (A-SAA) seems to be elevated in active disease along with other acute phase proteins such as ESR and CRP [10, 11, 18]. However, A-SAA has been shown to be more sensitive than CRP in determining disease activity and more specific than ESR in determining inactive disease [19].

Circulating B cells seems to be decreased in active disease and the B-cell stimulating factor BAFF has shown to be increased in the same populations [20], as

well as in other populations [4, 10]. Significantly decreased levels of cytokines CCL1, CCL2 have been identified [4].

Monocyte counts have been shown to be increased in both newly diagnosed GCA and PMR patients. In remission, monocyte levels decreased in PMR patients but not in GCA patients [21].

In addition, endothelial cell activating proteins such as S100A8/S100A9 and S100A12 have been elevated in active GCA compared to healthy controls [22, 23] as well as active GCA compared to inactive GCA [22].

Some other biomarkers that have been found increased in active GCA are vWF:Ag [24], serum osteopontin (sOPN) [25], adrenomedullin [26], calprotectin, YKL40, sCD163, angiopoietin-2, sTie2 [11], ferritin peptide autoantibodies [27], CHI3L1, M-CSF, protein C, resistin, tenascin C, TNF R1, AGF, hemopexin, MARCO [10], IFN-gamma, IL-1beta [9, 10], GM-CSF [9] and sIL-2R [4].

To our knowledge no one has investigated biomarkers prior to onset of GCA.

O-link and laboratory methods

Using the panels of protein profiling available from O-link, a large number of proteins can be detected using a small blood sample. We have decided to focus on two panels, the Inflammation panel and the Metabolism panel, containing 92 biomarkers each, in this study. Further information regarding the specific proteins analysed are well described on the O-link homepage (http://www.olink.com).

Plasma levels of proteins will be analysed by the Proximity Extension Assay (PEA) technique using a multiplex reagent kit (O-link Bioscience, Uppsala, Sweden) which uses two oligonucleotide-labelled highly specific antibodies to bind to each target protein. This allows the formation of a polymerase chain reaction sequence that will be detected and quantified. All data are presented as arbitrary units. Validation data and more technical information about the assays are available on the O-link homepage (http://www.olink.com).

This method is patented by O-link and has been used in similar types of research, leading to several recent publications in well-renowned journals [28-33].

Summary of possible outcomes of the panels that will be analysed

As GCA in active stage is an inflammatory disease shown by both the clinical presentations of the disease as well as elevated acute phase reactants such as CRP and ESR routinely used in the clinic. Studies of the pathogenesis and comparing inflammatory biomarkers in active GCA patients compared to healthy controls confirm this.

The full pathogenesis is not well understood even though advances are made. Therefore, investigating early inflammatory biomarkers prior to disease onset is both unique and highly interesting. Maybe some inflammatory processes begin long

before clinical onset? Several of the inflammatory biomarkers in the o-link inflammation panel has been described to be involved in other inflammation-related diseases such as RA and several of them has been described to be elevated in active GCA but no one has to our knowledge looked at biomarkers prior to disease onset. This may result in further knowledge of disease activation and pathogenesis.

O-link Inflammation panel

This panel includes a large number of inflammation related proteins some more known than others. The ones that we think might of interest in the following:

Chemokines, analysed i.e. CCL3, 4, 19, 20, 23 and 25, have been described to be involved in several inflammation-related diseases such as rheumatoid arthritis systemic lupus erythematosus, asthma, ulcerative colitis among others. This is also the case for CXCL-family which has been described to be involved in partly the same inflammation-related diseases. Specifically, CXCL10 and CXCL 11 have been found to be elevated in active GCA, and are induced by elevated levels of IFN-gamma. IFN-gamma itself plays a crucial part of the innate and adaptive immunity and is known to be produced by Th1-cells in active GCA. Deng et al showed that IL-17, but not IFN-gamma, decreased with glucocorticoid treatment [5]. Circulating IFN-γ may thus reflect mechanisms central to the chronic disease process. It is unknown whether elevation of circulating IFN-γ may predate clinical disease onset.

Caspase 8, a cysteine protease in apoptosis involved in macrophages differentiation and the activation of T, B and NK cells. Both T-cells and macrophages play a crucial part in GCA pathogenesis and the amount of circulating B cells has been reported to be decreased in active disease. Fibroblast growth factor 21, stimulates glucose uptake in differentiated adipocytes via induction of glucose transporter expression. Might be of interested regarding previous findings that lower fasting blood glucose and lower BMI has been shown to predict later development om GCA [3].

Fms-related tyrosine kinase 3 ligand, has been shown to play a role in dendritic cell development and has been described to be involved in other inflammation-related diseases.

A variety of Interleukins and subunits will be measured. Many of them play a part in the active disease and early differences between cases and controls could be a sign of an early inflammatory process. IL-6 is known to have a crucial role as it has been found elevated in many studies and is since a couple of years a target for therapy.

The Matrix metalloproteinases, especially number 2 and 9, have been found to be important in active stages of GCA. Firstly, these two are not measured in the o-link panel and secondly they play a part in the destruction of the arterial walls

in active disease which make them less likely to be elevated so many years prior to disease onset.

Other biomarkers that are known to be involved in other inflammatory diseases i.e. RA and plays important role in the immune system are Macrophage colony-stimulating factor 1 (CSF-1), Oncostatin-M (OSM), Signaling lymphocytic activation molecule (SLAMF1), T-cell surface glycoprotein CD6 isoform (CD6), TNF-beta (TNFB), TNF-related activation-induced cytokine (TRANCE), TNF-related apoptosis-inducing ligand (TRAIL), Tumor necrosis factor (TNF) among others.

Programmed cell death ligand 1 PD-L1 has been described to be involved in various inflammation-related diseases. In active GCA it has been shown to be downregulated on dendritic cells when the PD1 receptor on the T cells were up-regulated [34] which makes it an interesting marker to investigate prior to disease onset.

Aims

The aim of this study is to investigate early biomarkers that may predispose GCA years before diagnosis, focusing on metabolic and inflammatory biomarkers and comparing individuals who later develop disease with non-GCA controls. Furthermore, relations between early biomarker patterns and phenotypes among those diagnosed with GCA will be investigated.

Analyses will be performed:

- Using GCA as the main endpoint, investigating differences in biomarkers prior to diagnosis overall, comparing cases and non-GCA controls and stratified by time from screening to diagnosis (main analysis)
- Using clinical features as endpoints, investigating if biomarkers can predict different features in GCA prior to diagnosis i.e. visual loss, signs of PMR and extra-cranial large vessel involvement (exploratory analysis)
- Using histopathology features as endpoint, investigating if early biomarker patterns are different for individuals who will develop biopsy positive vs biopsy negative GCA, or for those with vs. without giant cells in the biopsy (exploratory analysis)

Participating sites

List the sites and departments that will be involved

- Lund University, Rheumatology, Department of Clinical Sciences, Malmö
- Skåne University Hospital, Department of Rheumatology
- Mayo Clinic, Rochester, Minnesota
- Lund University, Department of Immunotechnology

Study design

Study design

In this nested case-control study individuals were identified who developed GCA after inclusion in a population-based health survey, the Malmö Diet Cancer Study (MDCS), performed in the city of Malmö.

Cases and controls

MDCS is a community-based health survey performed in Malmö between 1991-96. The study included 20447 subjects (12 121 men and 18326 women). The total source population of 74 138 persons corresponds to a participation rate of 40.8%. Mean age at screening was 58 years in women and 59 years in men. Details of the study are described elsewhere [35]. The cases were selected on the basis that they had been included in either the MDCS before being diagnosed with GCA. Patients were identified using a registered diagnosis code of GCA in the local outpatient clinic administrative register for Malmö University Hospital and the National Hospital Discharge Register (National patient register) after inclusion in the MDCS and through December 31, 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 [36].

One control for every validated case, matched for sex, year of birth and year of screening, was 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 blood sample preserved.

Blood samples were obtained at the time of inclusion in the health survey in a standardized, non-fasting manner, and stored in -80°C.

We have identified 100 cases with corresponding controls, 98 cases had preserved blood samples that can be analyzed in the present study, all of them with corresponding controls. In a total 195 blood samples, will be analyzed. One control was randomly selected as control for two independent cases.

Laboratory testing

For this study, analyses of the blood samples have been performed using O-link, method described above.

Statistical analysis

The statistical analysis will be performed by KW mainly, with supervision by CT and in close dialogue with Jan-Åke Nilsson. Assistance from others in the project group when necessary.

All data are presented as arbitrary units from O-link. All non-normally distributed variables will be ln-transformed prior to analysis. All arbitrary units will be transformed into z-values to facilitate comparison between them.

The inflammatory and metabolism panels will be analyzed separately. For the main analysis, markers with an a priori hypothesis will be analyzed separately. The analyses that involve all biomarkers, including those without a priori hypothesis latter will be regarded as hypothesis generating analyses, and so will the exploratory analyses.

Descriptive statistics for all proteins, by case/control status, and by exploratory outcomes, will be tabulated.

Biomarkers with a priori hypothesis (Table 2, Table 3) - analysis

1. Non-normally distributed variables will be ln-transformed
2. Transformation to z-scores
3. Cox regression, using GCA case status and time to GCA or censoring, if assumption for proportional hazards are fulfilled – taking into account the matched design of the study.
4. Conditional logistic regression using case status as outcome
5. T-test for comparing means between cases and controls
6. Multiple testing will be handled using the Holm correction for all p-values. Both corrected, original p-values and confidence intervals will be presented in tables

All biomarkers, hypothesis generating analysis

1. Non-normally distributed variables will be ln-transformed
2. Transformation to z-scores
3. Principal component analysis will be used to identify groups of proteins that explain the variance in the proteome. Proteins will be selected for further statistical analyses based on factor loading.
4. Selected proteins in step 3 will be further analyzed with the same statistical methods as above. Cox regression, using GCA case status and time to GCA or censoring, if assumption for proportional hazards are fulfilled. Conditional

logistic regression using case status as outcome. T-test for comparing means between cases and controls.

5. Factors from step 3 will be used for analyses of the types described in step 4.

In Cox regression analyses, Shoenfeld residuals will be used to test proportional hazard assumptions.

Relevant assumptions for logistic regression models will be tested, including linearity for continuous variables to the logit of the dependent variable.

Table 1. Biomarkers analyzed using the Inflammation panel, with a priori hypotheses

Biomarker	Hypothesis
IFN-γ	Elevated in pre-GCA cases
IL-6	Elevated in pre-GCA cases
CXCL-10	Elevated in pre-GCA cases
CXCL-11	Elevated in pre-GCA cases
Caspace-8	Elevated in pre-GCA cases
FGF-21	Reduced in pre-GCA cases
PD-L1	Reduced in pre-GCA cases
LIF	Elevated in pre-GCA cases
S100A12	Elevated in pre-GCA cases

Ethical considerations

This study has been approved by the regional ethic committee in southern Sweden (registration number 38/2007).

All cases have been given a study number and cannot be identified in the file used for statistical analysis. All data, including those obtained from medical records are managed according to standard procedures and stored on a password protected server.

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