Giant Cell Arteritis: Pathogenetic and Epidemiological Aspects

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ABSTRACT
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Giant cell arteritis (GCA) is a chronic inflammatory disorder of medium-size and large arteries which affects people aged 50 years or older with a female preponderance. The two different expressions of GCA are temporal arteritis (TA) and polymyalgia rheumatica (PMR). In recent years, new information about the immunology and morphology of GCA has emerged. However, many details of its pathogenesis remain to be clarified.

The aims of this thesis were a) statistically to investigate a possible connection between GCA and female sex hormone-related factors, b) immunocytologically to assess the expression of interferon (IFN) type 1-associated Myxovirus resistance protein A (MxA protein) in PMR and TA, and c) stereologically to illustrate the neovascularisation in the inflamed arteries.

a) Forty-nine women, aged 50 to 69, with a biopsy-positive diagnosis of GCA replied to a questionnaire regarding estrogen-related factors. As controls served 10,405 age-matched women from the same region, who answered the same questionnaire in connection with mammography screening. Logistic regression analysis, with age as an independent variable, revealed four significant differences. These were further investigated, using multivariate logistic regression analysis. The investigation revealed four independent risk factors for developing GCA: menopause before the age of 43, smoking, low body mass index (BMI) and breast-feeding.

b) The immunocytocchemical expression of the IFN type 1-related MxA protein was mapped in temporal artery biopsies. Non-inflamed biopsies from 11 PMR patients were compared with arteries from 13 patients with other diagnoses. The morphology of arteries from four patients with full-blown temporal arteritis were studied separately. MxA protein expression was significantly more common in vessel walls and vascular dendritic cells in PMR than in controls. The MxA expression in the inflamed arteries was found in smooth muscle cells remote from inflammation. The results indicate that arterial smooth-muscle and dendritic cells from patients with GCA are under influence of IFN type 1.

c) Twenty-one temporal artery biopsies with different inflammatory degrees of GCA were immunocytologically stained for the detection of the endothelium-related CD34 marker. The degree of inflammation was semiquantitatively assessed. A stereological examination of the relation between endothelial surface and tissue volume (surface density) was performed on calibrated photographic enlargements of CD 34-stained arterial cross sections. The degree of vascularisation as well as the degree of inflammation was greatest in the adventitia, smaller in the media, and smallest in the intima. The correlation between the degree of vascularisation (surface density), and the degree of inflammation was assessed in the different layers of the vessel wall. A significant correlation between the two was found in the media and outer and inner halves of the intima. An extended investigation of the intima (n:27) revealed prominent circular microvascular intimal plexa in 16 of the cases. Eight of these lacked connection with microvessels in the media, and another 8 showed only few single connections. There were no connections with the luminal endothelium.

Conclusions. a) Four independent estrogen-related risk factors for developing GCA were detected, namely early menopause, smoking, low BMI, and breast-feeding. The results may indicate a link between estrogen deficiency and GCA. However, the study included relatively young women, aged 50 to 69. To generalise the results, GCA patients of all age groups should be studied. b) The significant expression of the MxA protein in non-inflamed temporal arteries in PMR indicates that they are under the influence of IFN type 1, and that IFN type 1 might play a pathogenetic role in GCA. c) Stereology may be used for the assessment of vascularisation in GCA. The degree of neovascularisation was related to the degree of inflammation. Isolated vascular plexa in the intima may indicate an alternative mechanism of neovascularisation in terms of recruitment of vascular stem/progenitor cells.

Key words: Giant cell arteritis, Temporal arteritis, Polymyalgia Rheumatica, Estrogen, Immunocytocchemistry, MxA protein, IFN type 1, Stereology, Neovascularisation.
LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. Early menopause, low body mass index and smoking are independent risk factors for developing giant cell arteritis. Larsson K, Mélström D, Nordborg C, Odén A, Nordborg E. Ann Rheum Dis 2006 Apr;65(4):529-32


I. ABBREVIATIONS

ACTH= adrenocorticotropic hormone
ACR= American College of Rheumatology
AION= anterior ischemic optic neuropathy
APC= antigen presenting cell
BCP= basic calcium phosphate
BMI= body mass index
CCL= chemokine ligand
CCR= chemokine receptor
CD4+= cluster of differentiation 4 positive
CRP= c-reactive protein
CT= computer tomography
DC= dendritic cell
Ds-RNA= double stranded ribonucleic acid
EPC= endothelial progenitor cell
ER= estrogen receptor
ER-α= estrogen receptor α
ER-β= estrogen receptor β
e-NOS= endothelial nitric oxid synthase
ESR= erytrocyte sedimentation rate
EULAR= the European League against Rheumatism
FGF= fibroblast growth factor
GCA= Giant cell arteritis
GpER= G-protein coupled estrogen receptor
HERS= the Heart and Estrogen/Progestin Replacement Study
HPA= hypothalamic-pituitary-adrenal
HPG= hypothalamic-pituitary-gonadal
HRT= hormone replacement therapy
ICAM= intercellular adhesion molecule
IEM= internal elastica membrane
IFN= interferon
IFN-γ= interferon γ
IgG= immunoglobulin class G
IL= interleukin
IL-1= interleukin 1
IL-2= interleukin 2
IL-6= interleukin 6
IL-8= interleukin 8
LPS= lipopolysackarid
LV-GCA= large vessel giant cell arteritis
MCP-1= monocyte chemoattractant protein 1
MHC= major histocompatibility complex
MMP= metalloproteinase
MRI= magnetic resonance imaging
MxA= Myxovirus resistance protein A
NK-cell= natural killer cell
NO= nitric oxid
PAMP= Pathogen-associated molecular pattern
PCK= Protein kinase C
PDC= plasmacytoid dendritic cell
PDGF= platelet derived growth factor
PET= positron emission tomography
PI3K= phosphatidylinositol 3-OH kinase
PMR= Polymyalgia Rheumatica
PRR= pattern-recognition receptor
RF= reuma factor
SCID= severe combined immunodeficiency
SEM=standard error of the mean
SHBG= sexual hormone binding globuline
TA= Temporalis arteritis
TCR= T-cell receptor
TGF= Tissue growth factor
Th17= T helper cell 17
TLR= toll-like receptor
TNF= tumour necrosis factor
TNF-α= tumour necrosis factor α
VCAM-1= vascular cell-adhesion molecule 1
VEGF= Vascular endothelial growth factor
VLA-4= very late antigen 4
VSMC= Vascular smooth muscle cell
WHI= the Women’s Health Initiative study
II. INTRODUCTION

History and synonyms

In 1890 Sir Jonathan Hutchinson, a British surgeon, published a description of the clinical manifestations and temporal artery involvement in what was probably giant cell arteritis. An 80-year-old man presented with “painful red streaks on his head, which prevented him from wearing his hat”. Hutchinson named the disease “arteritis of the aged” (Hutchinson, 1890). In 1932, Horton was the first to describe its histology and clinical profile, emphasizing the involvement of the temporal arteries, thus contributing to the diagnostic term ‘temporal arteritis’ (TA) (Horton et al. 1932). Gilmour called attention to the histological hallmarks of this disorder, including the presence of multinucleated giant cells and he named the disease giant cell arteritis (GCA). Furthermore, he was the first one to point out that the disease is a generalized vasculitic disorder, which is not limited to the temporal arteries (Gilmour 1941, Östberg 1973). Since then, the names temporal arteritis and giant cell arteritis have been used synonymously. In the 1950s and 60s, muscle pain in the limb girdles which is a cardinal sign in polymyalgia rheumatica (PMR), was recognized as a frequent symptom in biopsy-positive GCA (Hamrin 1964, Paulley 1956, Porsman 1951).

There has been much debate over whether PMR and TA are separate or linked conditions, and whether PMR is a vasculitic syndrome. Recent investigations support the contention that PMR and TA are two different expressions of the same underlying vasculitic disorder. Patients with a diagnosis of PMR without overt vasculitic symptoms may have a positive temporal artery biopsy in up to 40% of the cases (Fauchald et al. 1972, Nordborg et al 1995). The inflammatory lesions of the temporal artery are frequently diminutive in patients with PMR, which may indicate pathogenetic differences between TA and PMR. Weyand et al. (Weyand et al. 1994) demonstrated in situ production of macrophage-specific cytokines (IL-1β, IL-6 and TGF-β1) and T-cell specific interleukin 2 (IL-2) in non-inflamed temporal arteries from patients with pure PMR, indicating vessel wall involvement. However, the cytokine interferon (IFN) γ was detected only in vessels from patients with clinical TA, indicating that IFN γ may be a crucial cytokine for the development of full-blown granulomatous arteritis. PMR might therefore be regarded as a “forme fruste” of GCA. In the present thesis I will use the term “giant cell arteritis” as a common name for temporal arteritis and polymyalgia rheumatica, as is generally the case in Scandinavia.

Clinical features and diagnosis

GCA is a chronic inflammatory disorder of large and medium-sized arteries, which affects people aged 50 years and older with a clear female preponderance. Patients were diagnosed with PMR if they had bilateral pain and stiffness affecting at least two large groups of proximal muscles (i.e. neck-shoulders-upper arms, hips and thighs) with duration of symptoms more than two weeks and with no clinical or laboratory evidence of infection, malignant disease or inflammatory systemic disease. In addition, erythrocyte sedimentation rate (ESR) should be above 40 mm/h and the age of the patient 50 years or more. Furthermore, a prompt and long-lasting relief of symptoms after institution of glucocorticosteroid treatment is required. The European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) are currently jointly responsible for defining new diagnostic criteria for PMR.
Patients were diagnosed as having TA if a temporal artery biopsy showed arteritis, characterised by the histological findings of interruption of the internal elastic membrane with infiltration of mononuclear cells in the arterial wall. Giant cells were often found but their presence was not required for the diagnosis. In patients with negative histological findings, the diagnosis of TA was accepted if they fulfilled the ACR diagnostic criteria (see below). Affection of the cranial arteries may cause headache, scalp tenderness, temporal swelling and tenderness, jaw claudication as well as scalp necrosis. A feared complication is blindness due to anterior ischemic optic neuropathy (AION). Systemic symptoms such as fever, weight loss and malaise are common features and the erythrocyte sedimentation rate is generally high. Frequently, concurrent symptoms of proximal ache and morning stiffness are present. Although the temporal artery and arteries from the aortic arch are held to be the most common locations of this form of vasculitis, arteries throughout the body may be affected (Barack et al. 1999, Nordborg et Nordborg 2004, Salvarani et al. 2008, Weyand et Goronzy 2003b, Östberg 1973). A recent review of GCA emphasizes the frequent involvement of vessels other than the temporal artery, especially the aorta and its primary branches. It is reported that the subset of patients with giant cell arteritis who have aortic aneurysms have a markedly reduced life expectancy. Aortic aneurysms typically occur many years after the onset of the disease and after treatment has been completed (Evans 1995, Nuenninghoff et al. 2003, Schmidt et al. 2008). They are often clinically silent until signs and symptoms of aortic rupture occur. Lately, there has been focus on another less well characterized subgroup of the disease, namely large-vessel GCA (LV-GCA). These patients present with symptoms of large artery involvement (axillary, brachial, femoral, and tibial arteries); contralateral dissimilarities of puls or blood pressure, extremity claudication, transient ischemic attacks, stroke, diplopia, aortic insufficiency murmur are all predictive of LV-GCA (Nuenninghoff et al. 2003). In general, physicians are not familiar with this entity, which may lead to diagnostic delay of this group. Although imaging techniques (duplex sonography, computed tomography, magnetic resonance imaging, positron emission tomography) have improved our ability to detect and study arterial changes in large vessel arteritis there is as yet, no golden standard for the diagnosis of LV-GCA. (Evans et al. 1995, Schmidt et al. 2008, Tatò et al. 2008)

**Diagnostic criteria**

In 1990 the American College of Rheumatology (ACR) defined criteria for the classification of TA:

1. Age at disease onset ≥ 50 yrs
2. Localized new headache
3. Tenderness or diminished pulses of the temporal artery
4. Sedimentation rate ≥ 50 mm/h by the Westergren method
5. Abnormal arterial biopsy: biopsy specimen with artery showing vasculitis characterized by a predominance of mononuclear cell infiltration or granulomatous inflammation, usually with multinucleated giant cells.

The two most widely current used approaches to the nosology of primary vasculitis, The American College of Rheumatology (ACR) (Hunder et al. 1990, Dasgupta et al. 2010) classification system and the Chapel Hill nomenclature system (Jennette et al. 1994) advocate the use of the term ‘giant cell arteritis’ rather than ‘temporal arteritis’ because vasculitides other than giant cell arteritis can cause temporal arteritis and not all patients with giant cell arteritis have temporal involvement.
Epidemiology

The incidence of GCA varies greatly between different geographic areas. The disease is most common among individuals of Northern European descent, in particular those of Nordic heritage, irrespective of their place of residence (Baldursson et al. 1994, Bengtsson et al. 1981, Boesen et al. 1987, Franzen et al. 1992, Nordborg et Bengtsson 1990, Salvarani et al. 1995, Salvarani et al. 2004). An incidence of approximately 20 cases annually per 100,000 persons older than 50 years of age has been reported and the highest figures were documented from Southern Norway (Gran et al. 1997, Haugeberg et al. 2000). Based on the ACR criteria, the annual incidence rates were 32.8/100,000 in individuals older than 50 years, and 29.1/100,000 for biopsy-proven GCA, respectively. The incidence rates are lower in Southern Europe (Gonzalez-Gay et al. 2001, Gonzalez-Gay et al. 2007), scarce in Israel (Fainaru et al. 1979, Friedman et al. 1982) and in black populations (Ballou et al. 1986, Bielory et al. 1989, Liu et al. 2001, Munkacsy et al. 1978) and Arabs (Chaudry et al. 2007, Miller et al. 2007, Shah et al. 2008). It is distinctly infrequent in Asian countries (Hu et al. 2002, Kobayashi 2003, Wang et al. 2009). Recently, a geographical gradient of frequencies in GCA was reported with a statistically verified increase in frequency by latitude north (Lee et al. 2008).

According to some studies, the incidence of GCA has increased in recent years. Three Swedish studies from the periods 1973-1975, 1977-1986, 1976-1995 (Bengtsson et Malmvall 1981, Nordborg et Bengtsson 1990, Petursdottir et al. 1999a) demonstrated a statistically significant increase in incidence of biopsy-positive GCA with time, which is in accordance with a report from the Mayo Clinic (Salvarani et al. 1995). Such observations might reflect genuine changes in morbidity, an increase in the degree of medical attention, or the longer life expectancy of the general population. However, our statistical investigation showed that the increase in GCA incidence was not significantly influenced by the increasing age of the Gothenburg population (Nordborg et al. 2003b). As indicated by the large number of temporal artery biopsies performed, physicians are definitely aware of the disease. On the other hand, taking the data from Östberg (Östberg 1973) into consideration, GCA may be under-diagnosed during life. Investigating transverse sections from the ascending aorta plus the temporal artery in more than 20,000 cases post mortem, she found a prevalence of almost 2% of GCA.

Incidence rates reported for PMR are about two to three times higher than those of biopsy-positive GCA (Doran et al 2002, Schaufelberger et al. 1995). Due to the lack of internationally accepted diagnostic criteria for PMR, the incidence rates are less reliable.

Risk factors

Age

GCA is a disease of elderly people. The likelihood of developing the disorder increases continuously with age. GCA is approximately 20 times more common among people in their ninth decade compared with people aged between 50 and 60 years (Bengtsson et Malmvall 1981). Factors related to the aging of the arterial wall and the immune system may be involved in its pathogenesis (Mohan et al. 2011). A decline in immunocompetence with age has been attributed to deterioration in thymic function, resulting in an increased risk of infections and reduced antitumor immunity. Elderly people also have a higher incidence of
clonal disorders and they more frequently produce autoantibodies (Mohan et al. 2011, Weiskopf et al. 2009). Furthermore, high age is associated with a suppression of the hypothalamo-pituitary-gonadal (HPG) and hypothalamo-pituitary-adrenal (HPA) axes, and a reduction in gonadal hormone biosynthesis and adrenal steroidogenesis resulting in a less efficient anti-inflammatory capability (Cutolo et al. 2000, Straub et Cutolo 2006).

**Gender**

The female predominance of GCA raises the question of whether sex hormones are involved in its pathogenesis. A French epidemiological study claimed that the number of pregnancies is lower among GCA patients than among controls, suggesting that the hyperestrogenic state during pregnancy may protect the arterial wall from future GCA (Duhaut et al. 1999a).

**Genetic factors/ Race**

The genetic background appears to be a predisposing factor in GCA. There is a predominance of HLA-DR4 in patients with both TA and PMR. GCA almost exclusively affects Caucasian populations. The ethnic background seems to be of greater importance than the geography (Bignon et al. 1984, Nordborg and Nordborg 2003a). Familial aggregation has been reported (Bengtsson and Malmvall 1982).

**Environmental factors**

GCA symptoms such as fever, malaise and elevated ESR may mimic an infectious disease. Reported seasonal fluctuations in incidence rates may suggest exogenous etiological factors (Nordborg et al. 2000a, Petursdottir et al. 1999a, Salvarani et al. 1995). Thus, GCA has been suggested to be associated with various infectious agents, but so far there is no proof that GCA is a truly infectious disease (Bacon et al. 1975, Duhaut et al. 1999b, Elling et al. 1980, Elling et al. 1996, Helweg-Larsen 2002, Nordborg et al. 1998, Regan et al. 2002, Staud et al. 1996). Russo and co-workers compared the prevalence of infection in a retrospective review of medical records from 100 patients with biopsy-proven GCA and 100 age- and sex-matched controls which had been subjected to corrective surgery for hip fracture (Russo et al. 1995). The results suggested a correlation between previous infections and the onset of GCA, but no specific agent was pointed out. It may be speculated that various infections may act as triggers by activating the immune system, leading to GCA in genetically susceptible patients with certain predisposing lesions in their arterial walls.

**Hormonal factors**

The female sex steroid estrogen or 17 beta-estradiol (E2) is an important hormone in normal and pathological conditions in human physiology. It regulates a broad array of biological processes throughout the body, including reproduction, cardiovascular function, metabolism, inflammation and neurological function (Gruber et al. 2002). Estrogen displays a wide variety of mechanisms, which could theoretically be related to GCA by affecting the vessel wall or the immune system (Cutolo et Straub 2009, Cutolo et al. 2010, Nordborg et al. 2000a, Straub 2007). The cardiovascular benefits of estrogen have been suggested (Masood et al. 2010). Conversely, the lack of estrogen might, theoretically, contribute to the atrophy and calcification of the arterial wall, which has been considered to be one prerequisite for the disorder.
1. **Genomic effects of estrogen.** Vascular smooth muscle cells (VSMC) have estrogen receptors (ERs) and respond to estrogen stimulation. Estrogen directly inhibits VSMC proliferation—protecting against atherosclerosis. Moreover, it promotes proliferation of endothelial cells and their remodelling, resulting in new vessel formation, neovascularisation/angiogenesis. Estrogen stimulates angiogenic mechanisms in ischemic tissues, and an array of local factors are upregulated by estrogen such as VEGF and its receptors, nitric oxide (NO), adhesion molecules, integrins, and inhibitors of apoptosis. It also augments the telomerase activity of endothelial progenitor cells through activation of phosphatidylinositol 3-OH kinase (PI3K) signalling pathway (Heldring et al. 2007, Masood et al. 2010, Rubanyi et al. 2002, Simoncini 2009). Western blot and molecular genetic investigations revealed no significant aberrations in the ER-α or ER-β genes (Larsson et al. 2006, Petursdottir et al. 1999b, Petursdottir et al. 2001).

2. **Non-genomic effects of estrogen.** The majority of the vascular effects of estrogen are mediated through non-genomic events and membrane receptors. Estrogen plays a significant role in the regulation of the NO synthesis, which results in the induction of rapid vasodilatation through regulation of endothelial NOS (eNOS) enzyme activity. Other important indirect effects of estrogen on the vessel wall include the regulation of the lipoprotein profile, coagulation, fibrinolysis and antioxidan effects. Recent reports suggest that atherosclerotic arteries no longer express ERs. Polymorphisms in genes coding ER and other genes of the coagulation cascade may lead to decrease in cardioprotective effects. Single nucleotide polymorphisms for ER α and ER β are associated with cardiovascular disease (Gungor et al. 2009, Mendelsohn 2009, Meyer et al. 2009, Rauschemberger 2011, Simoncini 2009).

3. **Clinical Implications.** Today, substantial evidence suggests that the vascular functionality of estrogen receptors depends on the length of the period of hypo-estrogenicity and the progression of atherosclerosis. However, it is not known how the cessation of endogenous estrogen production after menopause affects the estrogen receptor signalling in human vascular cells. Such changes were not considered in earlier randomized hormone therapy trials such as the Heart and Estrogen/Progestin Replacement Study (HERS) (Grady et al. 2002, Hulley et al. 1998), and the Women´s Health Initiative study (WHI) (Rossouw et al. 2002) which demonstrated adverse cardiovascular outcome of hormone therapy in late older postmenopausal age. The compounds used in these studies were conjugated equine estrogens derived from horse urine, and synthetic progestins which contain a mixture of at least 10 different estrogens, several androgens, progestins and other substances of unknown vascular and procoagulatory activity. Such substances may result in unpredictable effects on multiple estrogen signalling pathways, especially in the presence of atherosclerosis. Currently, prospective clinical trials are underway to test whether natural estrogen has a therapeutic potential in women with atherosclerosis. Moreover, natural estrogens such as 17β-estradiol in low doses, possibly intermittently administered, will help preserve activating signalling cascades, thus mimicking the normal menstrual cycle. Furthermore, the use of selective estrogen receptor modulators such as raloxifene, which act as G protein-coupled estrogen receptor (gpER)- agonists, might be suitable for the treatment and prevention of atherosclerotic disease (Masood et al. 2010).

**Current pathogenetic aspects**

*Morphological aspects*

The histopathological finding in full-blown GCA is a segmental panarteritis with a mononuclear infiltrate penetrating all layers of the arterial wall (Huston et al. 1978, Lie 1994,
Nordborg et al. 2000b, Parker et al. 1975). A granulomatous macrophage reaction is typical, generally at the border between media and intima. Multinucleated giant cells are seen in approximately 50% of the cases. An intimal hyperplasia leads to advanced luminal stenosis. Although the granulomatous reaction causes extensive damage to the arterial wall, tissue necrosis is not a feature of GCA which is in contrast to other vasculitic conditions such as Wegener’s granulomatosis and Polyarteritis nodosa. Its presence in the arterial tissue should raise doubts about the diagnosis.

According to previous light microscopical, immunocytochemical, electron microscopical and morphometric observations, the inflammatory reaction in GCA appears to start as a foreign-body, giant-cell reaction, directed at small calcifications in the internal elastic membrane (Nordborg et al. 1991, Nordborg et al. 2000b). Such arterial calcifications occur in the general population with an age- and sex distribution, which is the same as the age- and sex distribution of GCA (Nordborg et al. 2001). It may appear that the calcifications are a prerequisite for the development of the disorder. A key question is, why some individuals react against their calcifications, whereas others do not.

The van Kossa staining reveals that the focal calcifications of the internal elastic membrane are not well demarcated, but are surrounded by innumerable smaller granules, measuring approximately 2 µm or less. Their chemical composition remains to be analysed. In vitro studies have demonstrated that basic calcium phosphate (BCP) microcrystals, when internalized into vacuoles of human monocyte-derived macrophages, trigger the secretion of pro-inflammatory cytokines (TNF-α, IL-1, IL-8) and are capable of activating vascular endothelial cells, thus promoting the capturing of flowing cells under shear flow (McCarthy 2009, Morgan et al. 2002). Protein kinase C (PKC)-alpha was identified as the key regulator of BCP-induced TNF alpha release (Nadra et al. 2005). Its activity was inversely related to the size of the crystals. Crystals of 1-2 µm in diameter were the most bioactive. Thus, the response of macrophages to BCP crystals suggests that pathological calcification is not merely a passive consequence of a chronic inflammatory disease but might lead to a pro-inflammatory feed back loop e.g. in GCA (Nadra et al. 2008).

**Immunological key events**

The local inflammatory response in GCA consists of activated CD 68+ macrophages producing pro-inflammatory cytokines (TNF-α, IL-1β, IL-6 and TGF-β), and activated CD4+ T cells that produce IL-2 and IFN-γ, suggesting a Th-1 mediated disease. This assumption is further supported by the granulomatous nature of the disease, suggesting a delayed-type hypersensitivity reaction. The formation of the granulomatous reaction depends strictly on the T-cell production of IFN-γ and is typical for an immune response to indigestible antigens. Deficiency of IFN-γ prevents this reaction.

It is currently believed that the inflammatory process in GCA is the consequence of an antigen-specific immune response, against still unidentified antigens in the arterial wall (Weyand et al. 2004). This contention is supported by the detection of activated dendritic cells, and the oligoclonal expansions of CD4-positive T-cells in arterial lesions. The inflammatory reaction was disrupted in temporal arteries engrafted onto mice with severe combined immunodeficiency (SCID) when exposed to anti-dendritic monoclonal antibodies. Using the same experimental setting, treatment of the SCID mice with antibodies directed at human T-cells caused a reduction in the production of Th1 cytokines in the inflamed temporal arteries (Ma-Krupa et al. 2004, Weyand et al. 1994b). Morphological evidence suggests that
the primary antigen presentation takes place during an initial foreign-body attack on small calcifications in the internal elastic membrane. It is well documented that the foreign body giant cells disintegrate the calcifications (Nordborg et al. 1991 and 2000b). They might thus expose hidden antigens to the immune system.

The CD4+ T-cell population in the inflamed artery is polyclonal and highly heterogeneous and an analysis of Vβ1- Vβ20 elements of the T-cell receptor (TCR) showed that all the Vβ elements were represented (Schaufelberger et al. 1993). Identical TCR clones in different regions in the same inflamed artery and an uneven expression of TCR V genes by T lymphocytes in the inflammatory infiltrates compared to peripheral blood T lymphocytes support the theory that GCA is an antigen-driven disease (Schaufelberger et al. 2008, Weyand et al. 1994b).

1. Cytokines. The study of cytokines in the arterial wall is likely to provide insights into the pathogenetic mechanisms capable of driving the inflammatory response in GCA. Pro-inflammatory cytokines are abundantly produced by activated lymphocytes and macrophages, and are able to maintain and amplify the inflammation in GCA. These cytokines have potent local and systemic effects.

There is a prominent expression of the Th1-derived cytokines IFN-γ and IL-2 in the lesions. IFN-γ is a key cytokine that regulates the differentiation and function of macrophages. It induces the formation of the granulomatous reaction, which is generally found along the media-intima border, often including multinucleated giant cells. The macrophages of the granulomatous infiltrate, in turn, produce growth factors and angiogenic factors which stimulate neovascularisation, and intimal proliferation. IFN-γ also induces the expression of MHC class II molecules and chemokine receptors (Weyand et al. 2004).

In contrast to TA, IFN-γ was absent in non-inflamed vascular tissue from patients with PMR although cytokine mRNAs for TNF-α, IL-1β and IL-6 were detected in the vessel specimens (Weyand et al. 1994a). The profile of T-cell derived cytokines in GCA suggests that IFN-γ is mandatory for the development of a complete vasculitis.

Pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 are abundantly produced by activated lymphocytes and macrophages. They are powerful inducers of acute phase response, which is prominent in GCA, and they have profound effects on inflammatory cells and on vascular wall components (Goronzy and Weyand 2002). TNF-α is able to maintain inflammatory pathways by promoting the expression of other pro-inflammatory cytokines such as IL-1β and IL-6. IL-1 shares multiple pro-inflammatory functions with TNF-α and participates in T cell activation. IL-6 is amply produced in GCA lesions and is characteristically elevated in serum from patients with GCA (Hernández-Rodríguez et al. 2003). IL-6 serum concentration is highest in the patients with a strong systemic inflammatory response which are more resistant to treatment (Hernández-Rodríguez 2004). Moreover, IL-6 is a powerful inducer of systemic inflammatory response which contributes to the anemia of chronic disease by enhancing the hepatic synthesis of hepcidin. Furthermore IL-6 is a multifunctional cytokine with additional immune-modulatory effects which may be relevant in the pathogenesis of chronic inflammatory disease, including B-cell activation and the induction of Th17 functional differentiation which is a relevant pro-inflammatory, autoimmunity-promoting pathway (Deng et al. 2010, Weyand et al. 2011).
2. Chemokines and chemokine receptors. The influx of leukocytes, mainly CD4+ T-lymphocytes and monocytes, in GCA is achieved through the secretion of chemoattractants in vascular lesions. Several chemokines are known to be expressed in the inflammatory infiltrate in GCA. DCs have undergone full maturation, upregulate co-stimulatory molecules (CD80/86), release the chemokines CCL19, CCL21, and express CCR7. Because CCR7 binds CCL19 and CCL21, the dendritic cells are trapped in the vascular infiltrate, initiating an aberrant T-cell response (Krupa WM et al. 2002).

CCL2/MCP-1 is produced by the invading inflammatory cells and by vascular smooth muscle cells. Thus, the infiltrating leukocytes and tissue macrophages amplify the inflammation by additional recruitment of inflammatory cells. It has been shown that CCL-2 (MCP-1) is up-regulated in temporal artery samples from patient with relapsing GCA which may be a marker for persistence of the disease (Cid et al. 2006).

3. Leukocyte/endothelial cell adhesion molecules. Leukocyte integrins are pivotal in mediating firm adhesion to endothelial cells, transmigration and progression through the basement membrane and underlying tissue. VCAM-1 is induced on endothelial cells by pro-inflammatory cytokines. The integrin VLA-4 is strongly expressed by the infiltrating lymphocytes in GCA lesions and co-localizes with VCAM-1 on endothelial cells in vasa vasorum and inflammatory-induced neovessels. The integrin VLA-4 serves as a co-stimulatory molecule by binding to VCAM-1 on antigen-presenting cells (Cid et al 2000, Cox et al. 2010).

4. Innate and adaptive immune response. There are several distinct mechanisms leading to innate DC activation, all of which probably share an evolutionary link to infection. Signals involved in innate dendritic cell activation may be exogenous via the detection of the so-called “pathogen-associated molecular patterns” or PAMPs, or endogenous via infection-induced alterations in self-markers. Either type of signal may be detected by DCs directly or indirectly. The direct pathways involved in the sensing of DCs by infection still remain unclear (Caetano Reis e Sousa, 2004). The indirect pathway involves inflammatory cytokines and other mediators produced by various cell types in response to exogenous or endogenous signals. Microbial components and inflammatory cytokines are known to affect DC phenotype and function.

Cells of the innate immune system, including DCs, possess pattern-recognition receptors (PRRs) that recognise PAMPs from viruses, bacteria, fungi and protozoa. The best studied PAMPs are the Toll-like receptors (TLRs) (Beutler 2009). Engagement of TLRs on DCs leads to an increased expression of MHC-peptide complexes and co-stimulatory molecules, and to the production of immunomodulatory cytokines, all of which have a profound effect on T-cell priming and differentiation. However, for the full DC activation in response to TLRs, a production of IFN-1 is also required. IFN-1 may be produced by virtually any cell type in vivo. It is consequently possible that the activation of DCs is mainly indirect. It has been argued that the expression of alarm signals from virally infected tissues are sufficient to activate DCs. Accordingly, in vitro experiments have shown that keratinocytes which were exposed to a mimic of viral double-stranded RNA (dsRNA) produce cytokines that make DCs competent to prime T helper 1 (Th1) differentiation. Other experiments have shown that DC migration in vivo was attributed to TNF-α and IL-1 rather than to a direct effect of PAMP. Thus, the main function expressed by TLRs may not be to activate the DCs to become immunogenic APCs, but rather to convey information about the nature of the insult, thereby allowing DCs to direct an appropriate class of immune response.
In addition to “alarm” signals and inflammatory cytokines, other alterations of the endogenous milieu may also be sensed by DCs. For example, changes in self-markers can trigger NK-cell activation, which in turn, can promote the activation of DCs and lead to the priming of Th1 responses. These data suggest that the surveillance of infection by innate effector cells can translate into DC activation and the initiation of adaptive immunity.

5. Vascular dendritic cells in giant cell arteritis. The relationship between blood vessels and the immune system is intimate. Immune cells travel with the bloodstream to reach their destination and migrate through the vessel wall to enter the tissue. Even under steady-state conditions, dendritic cells (DCs), monocytes/macrophages, and lymphocytes enter tissues, patrol for damaged cells and structures and respond appropriately. Endothelial cells and resident cells of the blood vessel wall are thus constantly exposed to passing immune cells. The potential for immune recognition events should therefore be particularly high in vascular walls.

In arteries from healthy individuals, dendritic cells reside exclusively in the adventitia. They are immature and inactivated, and have the role of maintaining T-cell unresponsiveness. However, activation of dendritic cells seems to be a prerequisite for subsequent antigen presentation and the triggering of an antigen-specific adaptive immune response.

In GCA, the vascular dendritic cells are activated, mature and antigen-presenting, as exemplified by their expression of CD83 and CD86. According to experimental studies, using human artery-SCID chimeras, the systemic administration of ligands for TLR2 or -4 caused the adventitial DCs to differentiate into chemokine-producing effector cells with high-level expression of both CD83, CD86. They also mediated T-cell regulatory function through the release of interleukin 18, and expressed chemokines and chemokine receptors which enabled their retention in the lesions. Furthermore, they expressed Toll-like receptors (TLRs), particularly TLR2 and TLR4 (Pryschep et al 2008, Weyand et al. 2005).

Angiogenesis in GCA

Neovascularization is a prominent finding in GCA. Inflammation-induced formation of new microvessels express adhesion molecules for leukocytes, providing new sites through which additional inflammatory cells may be recruited into the tissue. Newly formed vessels provide an extensive activated endothelial surface, which may serve as a gateway for cytokines, chemokines and growth factors which amplify and perpetuate the inflammatory process. Several strategies addressed to inhibit angiogenesis have been developed and are currently in clinical trials, mainly in the oncology field. Targeting angiogenesis in GCA requires a better understanding of the functional relevance of the factors known to be expressed in the lesions. Moreover, the vasculitides are unique among inflammatory disorders in that they eventually may lead to vascular stenosis or occlusion; the formation of new microvessels may then compensate for ischemia. GCA patients with ischemic complications have lower tissue expression and circulating levels of IL-6 than patients with no ischemic events (Hernández-Rodriguez 2003). IL-6 has relevant direct effects on vascular wall components that might be protective: IL-6 activates a functional program related to angiogenesis that may compensate for ischemia in patients with GCA.

Vascular endothelial growth factor (VEGF) is an important pro-angiogenic mediator, produced by multinucleated giant cells and CD68-positive macrophages in the inflamed artery
(Kaiser et al. 1999, Rueda et al. 2005). Furthermore, haptoglobin, an acute phase protein, which is increased in patients with GCA has a remarkable angiogenic activity (Cid et al. 1993). Patients with cranial ischemic complications frequently have a less intense acute-phase response than patients without ischemic events (Cid et al. 1998). The reason why a strong inflammatory response is associated with low prevalence of ischemic events is unknown.

**Damage of the normal architecture of the vessel wall**

The inflammatory reaction in GCA remolds the normal architecture of the vessel wall. Enzymes such as metallo-proteinase (MMP) 2, MMP 9, MMP 12 are released and activated by inflammatory cells, leading to the disintegration of vascular smooth-muscle cells and elastic lamellae (Kaiser et al. 1999). In the aorta, this leads to weakening of the media, and to aneurysmal dilatation and/or dissecting bleeding, preferably in thoracic segments. Additionally, in medium size and smaller arterial branches, the hyperplastic thickening of the intima generally dominates over the weakening and dilatation of the media, which, in turn, may lead to severe stenosis and ischaemia. Upon injury, contractile vascular smooth muscle cells acquire a myointimal phenotype which results in proliferation, and migration towards the lumen and production of matrix proteins. Several growth factors, such as PDGF, IL-1β, FGF-2, which stimulate the proliferation of myointimal cells are expressed in temporal arteritis, the most active of which is PDGF (Kaiser et al. 1998, Nordborg et Petursdottir 2000b, Piggott et al. 2009). Although the full-blown inflammation leads to severe stenosis, thrombotic occlusion is rare.
III. AIMS OF THE STUDIES

PAPER I

Giant cell arteritis (GCA) is a vasculitis of large and medium-sized arteries that mainly affects individuals over fifty years of age. The fact that postmenopausal women are affected 2-3 times more often than men raises questions about the connection with sex hormone related factors. In this study we wanted to investigate estrogen-related factors in women diagnosed with GCA during 1991-2000.

PAPER II

GCA appears to have a multi-factorial pathogenesis. Symptoms and epidemiological studies may suggest an association with infectious disease, but no infectious agent has yet been identified. It has been suggested that infections, or other exogenous or endogenous factors, may activate the innate immune system, which in turn may lead to the initiation of an adaptive immune reaction. DNA-containing immune complexes have been reported to act as endogenous inducers of interferon type I (IFN-I) in autoimmune disorders such as systemic lupus erythematosus. Is the interferon type I system activated also in GCA, and if so by an exogenous or endogenous inducer? The aim of this study was to assess the expression of interferon type I-associated MxA protein (myxovirus resistance protein A) in biopsy-negative PMR and in TA.

PAPER III

In the progress of giant cell arteritis, newly formed microvessels are one of the main sites of leukocyte-endothelial cell interaction. Furthermore, it has been shown with semiquantitative methods that an increased amount of new vessels may protect from ischemic complications. The aim of this study was stereologically to map the distribution of microvessels in the temporal arterial wall and to relate it to the degree of inflammation. Stereology is an objective, reproducible and physiologically relevant method not previously applied in GCA.
IV. PATIENTS AND METHODS

PAPER I

Patients

During a ten-year period, 1991-2000, we identified all 320 patients with biopsy-positive GCA from the files of the Department of Pathology, Sahlgrenska University Hospital of Gothenburg. The Department is the only referral centre of the Gothenburg region, with a number of about 433 000 citizens (Petursdottir et al. 1999). We focused on the subpopulation of women aged 50-69 at the time of diagnosis. Apart from a positive temporal artery biopsy, the patients had to fulfill the clinical ACR criteria to be included in the study. For that purpose all clinical records were collected and reviewed. To further explore estrogen-related factors, patients were asked to answer a 10-item questionnaire, sent home by mail. They were also contacted by telephone to avoid misunderstandings.

Sixty-five women were identified during the study period, fulfilling the entry criteria with a positive temporal artery biopsy combined with a clinical diagnosis of GCA (mean age 64.1 years, range 52-69, median 65). A questionnaire was sent to these 65 women in 2003. Forty-nine women replied. Among the drop-outs, five had died and two were demented. Another two did not want to participate. The last seven did not reply despite efforts by telephone and writing. (drop-out mean age 64.8 years, range 51 to 69, median 66).

As controls we included 10,405 women, aged 49-69, who answered the same questionnaire in connection with mammography screening according to the Swedish national programme of breast cancer-prevention).

Questionnaire:

1. Age at menarche?
2. Number of years using contraceptive pills?
3. Number of children born?
4. Total months of breast feeding?
5. Age at menopause?
6. Menopause due to chemotherapy or radiation? (yes/no)
7. Have you been ooforectomized (with or without hysterectomy)? (yes/no) At what age?
8. Use of hormone replacement therapy (HRT) due to menopausal symptoms? (Number of years). Name of pills?
9. Are you a current smoker or have you ever been a smoker for at least 6 months? If so, please answer the following questions:
   a) At what age did you start smoking?
   b) At what age did you stop smoking?
   c) How many cigarettes are you smoking/ did you smoke daily?
   d) Do/did you inhale? (yes/no)
10. Body Mass Index (BMI) [body weight in kg/ (body length in metres)^2] at the time of menopause?
**Statistical Methods**

A difference in age distribution within the 49 to 69 year age range was observed between the patients with giant cell arteritis and the control population. All the analyses were therefore adjusted for age.

As a first step, logistic regression analysis, with age as an independent variable was used to test differences between patients with giant cell arteritis and controls for the set of variables in the questionnaire. Variables showing a significant difference were investigated further, using multivariate logistic regression analysis. In addition to age, the variables included in the latter analyses were smoking, BMI, menopause before the age of 43, and duration of breast-feeding.

The p-values given in table 1 were determined by Fischer’s permutation test, which includes Fischer’s exact test as a special case, and the p-values of table 2 were calculated using the approximate normal distribution of the estimated beta-coefficients.

**PAPER II**

The included temporal artery biopsies, taken 1997-1999, were identified from the files at the Department of Pathology, Sahlgrenska University Hospital of Gothenburg, which is the only referral center for temporal artery biopsies in the Göteborg region. The primary goal was to investigate MxA expression in the early state of the disease. To be included the biopsy specimens had to be of good technical quality. The patients were fulfilling a clinical diagnosis of PMR. The study also included 4 positive temporal arteries from patients with a clinical diagnosis of GCA.

**Patients**

1. Non-inflamed arteries
   Eleven non-inflamed temporal artery biopsies from patients (five women and six men, aged 74±3 years (SEM)) with a clinical diagnosis of PMR were included. The PMR-diagnosis was based on the criteria specified by Bengtsson and Malmvall 1981 (Bengtsson et Malmvall 1981). Ten of eleven PMR patients were on corticosteroid therapy at the time of biopsy. The duration of therapy was 1 day, n=1; 2 days, n=4, and 11 days, n=1. The duration was unknown for the remaining five patients.

2. Inflamed arteries
   Inflamed temporal arteries from four patients (two women and two men) with a clinical diagnosis of TA were collected. Their age was 74.5 ±11.4 years. Three of the four patients were given corticosteroid treatment at the time of biopsy with the duration of 10 days, 1 month and 2 months respectively.

3. Controls
   Thirteen non-inflamed temporal arteries from patients given other diagnosis than PMR or TA (eight women and five men, aged 69±3 years) were controls. The diagnoses were Rheumatoid factor (RF)-negative Rheumatoid Arthritis (RA), n=2; Reuma-factor (RF)-positive RA, n=4; pelvospondylitis, n=1; arthralgia, n=1; gout, n=1; glomerulonephritis, n=1; anaemia and
increased sedimentation rate, n=1; depression, n=1; meningioma, n=1. None of the controls were on corticosteroid treatment at the time of temporal artery biopsy.

Methods

Cross-sections were stained with haematoxylin-eosin and consecutive sections were stained immunocytochemically with a) a monoclonal antibody for the detection of MxA protein (MxA IgG2a, clone M143; 1/100, kindly provided by professor Otto Haller, University of Freiburg, Germany) (Flohr et al. 1999) b) a monoclonal antibody for the detection of activated dendritic cells (Novocastra, Newcastle upon Tyne, UK CD83; clone IH 4b; 1:40), c) a polyclonal antibody for the detection of S100 protein (Dako, Glostrup, Denmark S100; 1:2000), d) with monoclonal antibodies directed at macrophages (CD 68; Clone KP1, 1:1000; Dako, Glostrup, Denmark) and T-cells (CD3; Clone PS1, 1:100; Novocastra, Newcastle upon Tyne, UK). The immunocytochemical sections were counterstained with haematoxylin. Human hepatitis C-infected liver was used as a positive control for MxA and human tonsil was used as a positive control for CD83, S100 protein, CD68 and CD3. Coded sections were screened for the expression of the MxA protein CD83, CD68 and S100 protein in the different layers of the arterial wall.

Sections from the four inflamed temporal artery biopsies from the TA patients were stained with haematoxylin-eosin and stained immunocytochemically for the detection of MxA protein. The sections of inflamed arterial tissue from the four TA-patients were not coded.

Statistical methods

The Chi2-test was used to evaluate the difference in expression of MxA in vascular smooth-muscle cells and MxA and CD83 in adventitial DCs between PMR patients and controls. The Chi2-test was valid according to the minimum expected values.

PAPER III

Patients

Twenty-one positive temporal artery biopsies from the years 1987 to 2002 were collected from the data files at the Department of Pathology, Sahlgrenska University Hospital, the only referral centre for temporal biopsies in Göteborg. The biopsies were chosen with reference to good technical quality and showing varying degree of inflammation. All patients were fulfilling the ACR criteria for GCA (Age 78.8±5.1 years). Concerning corticosteroid treatment, 8 patients were untreated, 12 patients received treatment before biopsy (mean 11 days, range 2-60 days), and for the last patient information was missing. The number of cross-sections per case was 3.8±2.1 SD.

The material was later extended to include positive temporal artery biopsies from 18 women and 9 men, aged 77.7±5.5 SD years which displayed neovascularization of the intima.

Methods

The temporal artery biopsies were fixed in 4 % buffered formaldehyde, cut transversely, dehydrated, and embedded in paraffin. Cross-sections 5 micrometer thick were stained with hematoxylin and eosin and embedded in paraffin.
1. Degree of inflammation
In every cross-section the degree of inflammation was semiquantitatively determined in the adventitia, the media, and the peripheral and luminal halves of the intima. For each layer, two 4-degree scales were used, one to assess the density of the inflammatory infiltrate (0: none, 1: slight; 2: moderate; 3: pronounced) and another to assess the extension of the infiltrate (0: none; 1: <30% of circumference; 2: ≥ 30% and <60% of circumference; 3: ≥ 60% of circumference). The sum of the two scores was calculated for each layer. Mean values for the scores of the adventitia, media, and peripheral and adluminal intima were then calculated for each biopsy.

2. Stereology
After immunohistochemical staining with CD34 antibody, photographs of each cross-section were taken in a Nikon E400 microscope, using a Nikon Coolpix 990 digital camera. Calibrated pictures were magnified and the adventitia, the media, the intima and the half-distance between the outer and inner border of the intima, were delineated on the colour prints. The degree of vascularization was assessed stereologically in each layer. By measurements on the photo prints, a surface density value, i.e. endothelial surface per volume tissue (µm²/µm³) was determined.

A transparent film with a lattice grid with 5x5 mm squares was placed over the calibrated photo print. The surface density was calculated according to the formula:

\[
\frac{\text{Number of intersections between test lines and endothelium} \times 2}{\text{Total length of test lines (in µm)}}
\]

The number of the intersections between test lines and endothelium was multiplied by two and divided by the total length in µm of the grid lines over the investigated area. The latter was achieved by multiplying the number of grid crosses over the investigated area by the double calibrated width in µm of one of the squares of which the lattice grid is composed (Weibel 1979). A surface density value in µm²/µm³ was then achieved.

The mean degree of vascularization was calculated for each layer in each biopsy. The layers were independently investigated for inflammation and vascularization without knowledge of the other measurements of the same vessel.

3. Assessment of the distribution of microvessels in the intima
All the biopsies in the original material did not display intimal vessels. Therefore, additional cases were added. In the extended material of 27 patients paraffin sections were stained immunocytochemically, using the CD34 antibody (clone QBEnd 10; 1:50). The sections were counterstained with hematoxylin. Human tonsil was used as a positive control. In the negative controls, the antibody was replaced by a mouse IgG1 (Dako) of corresponding concentration. Arterial cross-sections were examined for the distribution of microvessels in the two layers of the intima.

For further illustration of the intimal layers, 22 arterial cross-sections were stained with the elastin-van Gieson method. The cross-sections were compared with consecutive sections stained immunocytochemically, using CD34 antibody.
Statistical Methods

The Mann-Whitney rank-sum test was used to investigate differences between the adventitia, media and peripheral and adluminal intima in terms of degree of inflammation and degree of vascularization. The relationship between the degree of inflammation and vascularization in the different wall layers was tested, using ANOVA.

V. RESULTS AND DISCUSSION

PAPER I

Table 1. The variables not contributing significantly to the discrimination between GCA and controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>GCA</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at menarche (years)</td>
<td>13.4</td>
<td>13.7</td>
<td>0.3120</td>
</tr>
<tr>
<td>Number of children born</td>
<td>2.12</td>
<td>2.01</td>
<td>0.5596</td>
</tr>
<tr>
<td>Use of contraceptive pills (%)</td>
<td>32.7</td>
<td>44.9</td>
<td>0.1103</td>
</tr>
<tr>
<td>Oophorectomy (%)</td>
<td>18.4</td>
<td>13.8</td>
<td>0.4602</td>
</tr>
<tr>
<td>HRT (%)</td>
<td>38.8</td>
<td>42.0</td>
<td>0.6570</td>
</tr>
</tbody>
</table>

Five of the nine variables did not differ between the groups (Table 1) whereas four factors differed significantly in GCA patients compared to controls; smoking (current smokers or ex-smokers) was more common, the average BMI was lower, menopause before 43 y was more common, and the average duration of breast feeding was longer. These variables were further analysed, using multivariate logistic regression analysis. Independently of the others, each of them was associated with a significantly increased risk of having GCA. Thus, for women who were smokers or ex-smokers, or who had menopause before 43 years of age, there was an increased risk of having GCA. Likewise, this risk increased 10% when BMI was reduced by 1.0 kg/m² (one unit); one unit corresponds to approximately 3.0 kg of body weight in the present population. Finally, each month of breast feeding implied a 2.9% increased risk of having GCA (Table 2). Depending on the rareness of giant cell arteritis the odds ratios presented in this article almost coincide with hazard ratios.
Table 2. Multivariate analysis and risk factors

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>Standard error</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>1.84437</td>
<td>0.30144</td>
<td>&lt;0.0001</td>
<td>6.324</td>
<td>3.503 – 11.418</td>
</tr>
<tr>
<td></td>
<td>(Never=0) (Now or before=1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.10787</td>
<td>0.03006</td>
<td>0.0003</td>
<td>0.898</td>
<td>0.846 – 0.952</td>
</tr>
<tr>
<td>Menopause before 43 y</td>
<td>1.25875</td>
<td>0.36641</td>
<td>0.0006</td>
<td>3.521</td>
<td>1.717 – 7.220</td>
</tr>
<tr>
<td>(No=0; yes=1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast feeding</td>
<td>0.02827</td>
<td>0.01278</td>
<td>0.0270</td>
<td>1.029</td>
<td>1.003 – 1.055</td>
</tr>
<tr>
<td>(Months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The present study reveals a statistically significant association between biopsy-positive GCA and four factors related to female sex hormones and reproduction, namely early menopause, smoking, low BMI, and breast-feeding.

In the present study, menopause before the age of 43 was associated with an increased risk of having GCA. Likewise, each month of breast-feeding caused a minor increase of this risk. Early menopause and extended breast feeding imply longer periods of reduced influence from female sex hormones (Burger et al. 2002, Mc Neilly 1997, Robertson et al. 2002). The present results may consequently indicate a relationship between low female sex hormone production and the risk of developing GCA.

Shortage of female sex hormones and the suppression of the HPG axis might reduce the responsiveness of the HPA axis in biopsy-positive GCA; whereas high levels of estrogens potentiate the response of the HPA axis, low levels blunt the system (Cutolo et al. 2000, De Leo et al. 1998, Giussani et al. 2000, Straub et al. 2000). The reduced responsiveness of the HPA axis to inflammatory stimuli has been observed in polymyalgia rheumatica (PMR) (Cutolo et al. 2000).

Interleukin 6 (IL-6) and adrenocorticotropic hormone (ACTH) act synergistically to stimulate the direct release of corticosterone from the adrenal gland (Salas et al. 1990). However, despite high serum levels of both IL-6 and ACTH, the serum cortisol level is low in PMR, even before the initiation of corticosteroid therapy, which indicates that in this disorder a reduction of the HPA-axis might augment the inflammatory process (Cutolo et al. 2000). It remains to be shown if this is also the case in biopsy-positive GCA. The relatively high age of the GCA patients might add further to a suppression of the HPA and HPG-axes since the gonadal hormone biosynthesis and the adrenal steroidogenesis decline with age (Cutolo et al 2000, Straub et al. 2000).

In the present study, there was no significant difference between GCA patients and controls, when it came to the number of children born. Duhaut et al. (Duhaut et al 1999a) found fewer
pregnancies among GCA patients than among controls, suggesting that, due to its hyperestrogenic state, pregnancy may protect the arterial wall (Cid et al. 2002b) thereby reducing the risk of GCA. Whereas in the French study, the control group and the GCA patients came from different regions, our patients and controls were from the same geographical area.

Low BMI was associated with an increased risk of having GCA. A low BMI implies a reduction in adipose tissue, which will in turn have impact on estrogen synthesis. Estrone, which is the principal estrogen in post-menopausal women, is formed by the conversion of adrenal androstendione, mainly in adipose tissue (Longcope 1978). Moreover, thinness has been associated with higher levels of SHBG (sexual hormone binding globulin) and lower levels of serum estrone and estradiol than those in heavier women (Hannover Bjarnason et al. 2000). However, in the present study the serum estrogen concentrations were not measured.

Smoking proved to be as strong a risk factor for GCA as it is for pulmonary carcinoma. This is in accordance with two previous studies (Duhaut et al. 1998, Machado et al. 1989). The anti-estrogenic effect of cigarette smoking appears to be related to an increase in the hepatic transformation of estrogens into inactive catechols (Baron et al. 1990). This may, in turn, be related to the induction of estrogen-metabolising cytochrome P450 isoenzymes (Meek et al. 1999). Furthermore, nicotine acts as an aromatase inhibitor (Barbieri et al. 1986). Finally, smoking reduces BMI and the estrone synthesis in adipose tissue; female smokers experience menopause two to three years earlier than non-smokers (Baron 1984, Schmeiser-Rieder et al. 1995). However, according to the multivariate analysis, smoking was a significant risk factor, independent of BMI and age at menopause.

In addition to estrogen-related effects, smoking might directly influence the arterial wall in various ways. Its noxious influence on the endothelium and the increased risk of atherosclerosis are well documented (Blann et al. 1998, Lekakis et al. 1998, Otsuka et al. 2001). However, several arguments speak against an etiological or pathogenetic relationship between atherosclerosis and GCA. Firstly, atherosclerosis is extremely rare in temporal arteries. The two disorders differ in terms of distribution in the arterial tree. Whereas GCA mainly affects the thoracic part of the aorta, atherosclerosis shows a preference for its abdominal segment. Secondly, their histology is different. Atherosclerosis involves intimal lipid accumulation, associated with chronic inflammatory reactions with foam cells, fibrosis and calcification of lipid-rich necrotic atheromatous plaques. The intimal thickening in GCA is strictly associated with, and induced by, the inflammatory reaction (Nordborg et al. 2000b, Kaiser et al. 1998). Lipid accumulation and foam cells are not parts of the process. Finally, its epidemiology is different, especially when it comes to gender distribution (Nordborg et al. 2003a).

The average age at GCA diagnosis is around 70 years in most large series (Nordborg et al. 2003b). In the present study we chose a somewhat younger population of women (median age 65) to match the age of the controls. Therefore, our results may not be applicable to the whole population of GCA patients. Furthermore, a case-control study of this kind may be confounded by selection bias. However, for the investigated period, all 50 to 69-year-old biopsy-positive females in Göteborg were identified, and the median age and age range of the drop-outs were very close to those of the women included.

Recall bias was probably minor in the present study. After answering the questions in writing, each patient was contacted by phone. They did not hesitate regarding the facts asked for. The
relatively young age of the present GCA cohort was probably favourable in terms of recalling
the estrogen-related factors asked for in the questionnaire; the oldest patient was 81 at the
time of the study. One additional explanation may be that the questions concern events which
are central in a woman’s life. The fact that the corticosteroid treatment in GCA leads to
weight gain may explain why the patients were well aware of their body weight.

To conclude, this study shows that smoking, low BMI, early menopause and lactation are
independent risk factors for developing GCA. However, to generalise the results, GCA
patients of all age groups must be studied. Whether these observations may be linked to
estrogen deficiency is an intriguing question which merits further investigations.

PAPER II

No inflammatory reaction was detected in temporal artery biopsies from the PMR patients or
controls, using immunocytochemistry and haematoxylin-eosin staining. A full-blown
panarteritis was seen in all four biopsies from the patients which were given a diagnosis of
temporal arteritis.

The expression of MxA protein in non-inflamed arteries

The MxA protein was focally expressed in the smooth-muscle cytoplasm in the arterial media
and intima in the arteries from PMR patients (Figure 1). Positive staining was found in nine
of the eleven arteries from PMR patients and in four of the thirteen control vessels
(p=0.0124). In all, 74% of the investigated cross-sections expressed MxA in the PMR group,
compared with 30% in the controls.

Figure 1. MxA expression (brown staining) in media smooth-muscle cells in uninflamed temporal artery
tissue from a patient with polymyalgia rheumatica. Note spatially related immunopositivity in endothelium
(arrows) and stippled positivity at the outer margin of the media. A: adventitia. M: media. I: intima.
Arrowheads: internal elastic membrane. (Anti MxA - haematoxylin. Bar: 75 µm)
The expression of S100 protein, CD68, CD83 and MxA protein in dendritic cells in non-inflamed arteries

Dendritic cells, distinctly positive for S100 and CD68, were found in the adventitia in all eleven PMR arteries and in twelve of the thirteen controls. Adventitial DCs with strong MxA expression were found in nine of the PMR arteries and in four of the controls. Their presence was significantly more common in PMR than in the controls (p=0.0124). CD83-expressing, activated DCs were noted in the adventitia in nine of the eleven PMR patients and in nine of the thirteen controls. Two different types of CD83-expressing DC were found, one with a slender cell body, fine processes and a dense elongated nucleus and the other with a larger, round or oval cell body and a round, often eccentric nucleus. The light-microscopic appearance of the latter cell type was reminiscent of a plasma cell, i.e. it was “plasmacytoid”. Such CD83-positive plasmacytoid cells were found in seven of the PMR biopsies and in five of the control arteries.

Endothelial expression of MxA in non-inflamed arteries

MxA expression was seen in the endothelial cells of adventitial microvessels in all cases, except for one control. The expression varied in strength and in the number of vessels included, but there was no obvious difference between PMR and controls. Varying MxA positivity was also seen in the luminal endothelium, apart from one PMR biopsy and two controls.

Figure 2. MxA expression (brown staining) in mononuclear inflammatory cells and giant cells (arrows) in inflamed temporal artery tissue from a patient with temporal arteritis. Note the negative smooth-muscle cells in the inflamed media (M). A: adventitia. I: intima. (Anti MxA - haematoxylin. Bar: 150 µm)
**Inflamed arteries**

MxA positivity was observed in all four biopsies. Focal, restricted MxA expression was noted in smooth-muscle cells, but it did not relate spatially to the inflammation. As a result, in inflamed parts of the vessel wall, the mononuclear inflammatory cells were immunopositive, whereas the smooth-muscle cells were mostly negative (Figure 2).

**Discussion**

The IFN type I-related MxA protein is expressed in arterial smooth-muscle and dendritic cells from patients with PMR, showing that non-inflamed vessel walls in PMR are under the influence of IFN type I. The fact that the MxA expression in the arterial wall was significantly more common in vascular smooth-muscle and dendritic cells in non-inflamed PMR arteries than in controls may indicate that IFN type I plays a pathogenetic role. There is ample evidence to suggest that IFN type I serves as a link between the innate response to infection and the adaptive immune response (Haller et al. 1998, Theofilopoulos et al. 2005, Zhang et al. 2005, Rönnblom et al. 2003, Tough et al. 2004). Further studies are required to elucidate whether IFN type I could play a role in the initiation of PMR/TA, serving as a link between the innate and the adaptive immune responses.

Type I IFNs are expressed in virus-infected cells and they play a major role in their defence against viruses of all kinds (Barchet et al. 2005, Haller et al. 2006, Ito et al. 2005, Liu et al. 2005). The secreted IFNs cause susceptible cells to induce potent antiviral mechanisms such as MxA expression (Haller et al. 2002, Roers et al. 1994). The most potent producer of IFN type I is the plasmacytoid dendritic cell (pDC) which is the only circulating cells to produce IFN type I. They have well-documented migration properties and they produce massive amounts of IFN type I following the detection of the pathogen, mainly viruses, by the Toll-like receptors (TLR)-7 and TLR-9 (Barchet et al. 2005, Haller et al. 2006, Ito et al. 2005, Liu et al. 2005, Theofilopoulos et al. 2005, Zhang et Wang 2005). Their production of IFN type I is more protracted and it is one hundred to one thousand times greater than in other, virus-infected cells (Haller et al. 2006, Liu et al. 2005). It has recently been reported that DNA-containing immune complexes and auto-antibodies also act as endogenous inducers of IFN type I in autoimmune disorders such as systemic lupus erythematosus and Sjögren’s syndrome (Rönnblom et al. 2006). However, this could not be the case in GCA since the inflammation in GCA could be regarded as a delayed hypersensitivity reaction in which immune complexes and auto-antibodies are not involved. The factor that might induce MxA expression in GCA and whether this expression is induced by local triggers in the arterial wall, by circulating IFN type I or through the invasion of the arterial wall by circulating IFN type I-producing cells, remains to be elucidated.

The fact that ten of the eleven PMR patients but none of the controls were being treated with corticosteroids might have influenced the results. Corticosteroid therapy promptly reduces the number of circulating pDCs and the production of IFN type I (Shodell et al. 2003, Boor et al. 2006). Consequently, cortisone therapy might reduce the MxA expression in the vessel wall. The difference in MxA expression might therefore have been even more pronounced if untreated PMR patients had been compared with the untreated controls.

The significant increase in MxA expression in vascular dendritic cells in PMR gives further support to the contention that arteries in GCA are under the influence of IFN type I. However,
further studies are required to elucidate the pathogenetic implications of this finding. Further immunocytochemical examination is needed to determine whether some of the adventitial dendritic cells have a truly plasmacytoid immunophenotype, including the expression of TLR7 and TLR9.

PAPER III

Degree of inflammation

The biopsies revealed a varying degree of mononuclear inflammatory cell infiltration in the vessel wall. Giant cells were found in ten of twenty-one cases. On average, the degree of inflammation was greatest in the adventitia and decreased gradually towards the lumen (Figure 3).

![Figure 3. The inflammation score in the different layers of the arterial wall. (±SEM).](image)

Degree of vascularisation

In ten of forty-four cross-sections, microvessels were only found in the adventitia, while in another five cross-sections they were confined to the adventitia and the media. In the remaining twenty-nine cross-sections, neovascularisation was also seen in the intima. The average degree of vascularisation, expressed as endothelial surface per volume tissue (μm²/μm³), was greatest in the adventitia and decreased gradually towards the lumen of the vessel (Figure 4).

![Figure 4. The degree of vascularisation (μm²/μm³) in the different layers of the arterial wall (±SEM).](image)
Correlation between vascularisation and inflammation

A significant correlation between the degree of inflammation and the degree of vascularisation was observed in the media (p=0.0003), the peripheral half of the intima (p=0.0113) and the adluminal half of the intima (p=0.0099).

There were no significant differences between glucocorticosteroid treated and untreated patients in terms of the degree of inflammation or vascularisation in the temporal artery.

The distribution of microvessels in the intima

In the sections stained with the elastin – van Gieson method, the peripheral part of the intima displayed different degrees of granulomatous inflammation, and the formation of new elastin and collagen. Remnants of the internal elastic membrane (IEM) were found near the inner border of the media smooth muscle. In contrast to the peripheral part, the adluminal part of the intima was looser with sparse collagen and it was free from elastin. The new vessels in the intima were mainly found in the loose adluminal part, and especially at its border towards the denser outer layer (Figure 5). In sixteen of twenty-seven biopsies, prominent circular microvascular plexa were found in this location. Eight of these displayed no apparent connection with microvessels in the adventitia/media. Another eight biopsies showed few, single connections with media microvessels. There was no detectable sprouting from the luminal endothelium to the intimal plexa. Eleven biopsies displayed a more diffuse intimal distribution of microvessels. The mean biopsy length and number of cross sections were somewhat larger in arteries with isolated intimal microvessels than in the other two categories.

Discussion

The present observations support the hypothesis that inflammation is a major inducer of neovascularisation in the arterial wall in GCA. However, no significant correlation between inflammation and vascularity was observed in the adventitia. There may be several reasons for this. Firstly, the adventitia is rich in microvessels, even in uninflamed arteries. Not all the vessels are therefore newly formed, which leads to some overestimation in terms of neovascularisation. Secondly, a proportion of the inflammatory cells which enter the artery via adventitial microvessels migrate from the adventitia into the vessel wall. Their influence on the formation of new microvessels might therefore be greater at their targets in the media and intima than in the adventitia.

The fact that the vascularisation was greatest in the adventitia and decreased gradually towards the lumen indicates that the neovascularisation in GCA proceeds from the adventitia towards the arterial lumen, and that sprouting of adventitial microvessels (angiogenesis) is a major source of new microvessel formation. Accordingly, in fifteen of forty-four vascular cross-sections, microvessels were only found in the adventitia or in the adventitia and media but not in the intima.

The presence of isolated intimal microvascular plexa in almost one third of the biopsies might indicate that microvessels are formed in the intima, later to be connected to a peripheral microvascular plexus by sprouting from one or both plexa. Theoretically, the observations may thus indicate an alternative form of neovascularisation, via the recruitment and migration of stem cells. There is growing evidence that hematopoietic stem cells as well as marrow-
derived and non-marrow-derived mesenchymal stem cells play important roles in vascular biology because of their ability to differentiate into both endothelial and smooth muscle cells (Riha et al. 2005). Endothelial progenitor cells (EPC) may be considered to occupy the middle of the differentiation spectrum between hematopoietic stem cells and endothelial cells. EPCs which are localised in the postnatal bone marrow may be mobilised to induce the formation of microvessels in peripheral tissues. Observations in experimental animals and humans indicate that the recruitment and homing of marrow-derived EPCs is a natural response to tissue ischaemia; EPCs are mobilised following burns and myocardial infarction. Pro-inflammatory cytokines, growth factors such as erythropoietin and vascular endothelial growth factor (VEGF), estrogen and physical activity have been reported to stimulate the recruitment of EPCs (Aicher et al. 2005, Asahara et al. 2005, Hill et al. 2003, Hristov et al. 2003, Masuda et al. 2003).

Cid et al. (Cid et al. 2002, Hernández-Rodríguez et al. 2003) showed that a high serum angiogenic activity and prominent neovascularisation of the temporal arterial wall are associated with a low prevalence of ischaemic complications in GCA. It remains to be shown whether this effect is related to the mobilisation of EPCs. Further efforts should be made to elucidate this question, not only morphologically but also by using other methods; circulating human EPCs may be isolated, quantified and analysed (Masuda et al. 2003, Hristov et al. 2003).
VI. CONCLUSIONS

1. There is a statistically significant association between smoking, low BMI, menopause before 43 years of age, and long periods of breast feeding and the risk of developing GCA in women given the diagnosis before the age of 70.

2. The results may indicate a possible role of estrogen deficiency in the pathogenesis of GCA.

3. The IFN type I-related MxA protein is expressed in non-inflamed arteries from patients with PMR, showing that vessel walls in PMR are under the influence of IFN type I.

4. The fact that the MxA expression in the arterial wall was significantly more common in vascular smooth-muscle and dendritic cells in non-inflamed PMR arteries than in controls may indicate that IFN type I plays a pathogenetic role.

5. Further studies are required to elucidate whether IFN type I could play a role in the initiation of PMR/TA, serving as a link between the innate and the adaptive immune responses.

6. Stereological analysis of the relation between endothelial surface and tissue volume may be used for the determination of neovascularisation in GCA.

7. Inflammation is a major determinant when it comes to neovascularisation in GCA.

8. New microvessels are formed in the inflamed arteries by the budding of adventitial vasa vasorum.

10. The presence of intimal microvascular networks without apparent connection with microvessels in the media might indicate additional influence on the neovascularisation, the nature of which remains to be investigated.
VII. Populärvetenskaplig sammanfattning

Jättecellsarterit: Patogenetiska och Epidemiologiska Aspekter

Karolina Larsson


Syftet med denna avhandling var att a) studera eventuella statistiska samband mellan GCA och östrogen-relaterade faktorer. b) studera förekomst av det interferonrelaterade MxA-proteinet i artärväggen vid PMR och TA. c) kartlägga nybildningen av små blodkärl i den inflammaderade artäväggen.

a) Fyrtionio kvinnliga GCA-patienter mellan 50 och 69 års ålder, vilka hade inflammerade arterbiopsier, svarade på en enkät, gällande östrogenrelaterade faktorer. Som kontroller ingick 10.405 åldersmatchade kvinnor, vilka svarat på samma enkät i samband med mammografi. De två grupperna jämfördes sedan i en statistisk tvästegsanalys. Fyra oberoende faktorer visade sig medföra ökad risk att utveckla GCA, nämligen tidigt upphörd menstruation, rökning, låg BMI samt anning. b) Förekomsten av MxA-protein undersöktes genom mikroskopisk analys av icke-inflammerade arterbiopsier från PMR-patienter, vilka färgats med antikroppar riktade mot proteinet i fråga (immunhistokemi). Man fann härvid att sådant protein förekom signifikant oftare i arter från PMR-patienter än i kontroller, vilket tyder på att PMR-artärerna var påverkade av interferon typ 1. c) Förekomsten av nybildade kapillära blodkärl undersöks i biopsier från inflammerade tinningartärer. Detta skedde genom speciell s.k. stereologisk mätning på tvärsnittade artärer, vilka specialvävdes för påvisande av kapillära blodkärl. Mätningarna gjordes och förarteringar av fotografier som tagits i ljudmikroskop. Genom stereologisk analys fick man ett värde på de kapillära blodkärlens sammanlagda inre yta i relation till vävnadsvolymen (Surface density). Detta värde var relaterat till den inflammatoriska reaktionens styrka i artäväggens skilda lager. I dess innersta lager träffade man även på områden med nätverk av isolerade blodkärl, som inte hade kontakt med blodkärl i andra vägglager.

Slutsatser: a) Fyra oberoende östrogenrelaterade faktorer var kopplade till ökad risk att utveckla GCA, nämligen tidigt klimakterium, rökning, låg BMI samt anning. Resultaten kan tala för en länk mellan östrogenbrist och GCA. Eftersom patienterna i studien var relativt unga (50-69 år), är det dock nödvändigt att utvidga studien till att omfatta alla GCA-drabbade åldersgrupper. b) Förekomsten av MxA-protein i icke-inflammerade temporalarterier vid PMR talar för att dessa står under inflytande av interferon typ 1 och att detta kan ha betydelse för uppkomsten av sjukdomen. c) Stereologi kan användas för att undersöka nybildning av kapillära blodkärl vid GCA. Förekomsten av isolerade kapillära nätverk i kärlväggen vid GCA kan tala för att de nybildade kärlen inte enbart uppstår som förgreningar från omgivande blodkärl, utan möjligen också genom rekrytering av stamceller från benmärgen.
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