Persistence of inflammatory activity in giant cell arteritis: Role of the angiotensin II system

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PERSISTENCE OF INFLAMMATORY ACTIVITY IN GIANT CELL ARTERITIS: ROLE OF THE ANGIOTENSIN II SYSTEM

Tesi presentada per

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CERTIFICA:

Que la present tesi doctoral ha estat realitzada per **Marco Antonio Alba Garibay** sota la seva direcció en el Departament de Medicina de la Facultat de Medicina de la Universitat de Barcelona. El treball reuneix les condicions necessàries per aspirar al grau de Doctor en Medicina en el marc del programa de doctorat "*Agresió biològica i Mecanismes de Resposta*" de la Facultat de Medicina de la Universitat de Barcelona i està en condicions de ser defensada davant del tribunal corresponent.

Barcelona, Setembre de 2014.

Dra. Maria Cinta Cid Xutglà

A la atenció de la comissió de Doctorat del Departament de Medicina de la Universitat de Barcelona.

La doctora **Maria Cinta Cid Xutglà**, professora associada del Departament de Medicina de la Universitat de Barcelona

CERTIFICA:

Que la present tesi doctoral ha estat realitzada per **Marco Antonio Alba Garibay** sota la seva direcció en el Departament de Medicina de la Facultat de Medicina de la Universitat de Barcelona. La presentació de la Tesi s' ha realitzat de d'acord amb la normativa vigent de la Universitat en la última actualització, seguint el model de presentació com compendi d'articles científics.

D'acord amb la normativa, a continuació es detallen les característiques dels articles científics que conformen la tesi, incloent el factor d'impacte, així como la informació requerida sobre la participació del doctorand en els articles amb autoria compartida.

Article 1. *Giant cell arteritis in Mexican patients*. **Alba MA**, Mena-Madrazo JA, Reyes E, Flores-Suárez LF. J Clin Rheumatol. 2012 Jan;18(1):1-7. IF (JCR 2014): 1.245.

En a quest treball realitzat a Mèxic el doctorand troba una prevalença molt baixa d'arteritis de cèl·lules gegants (ACG) i es pregunta si a banda de factors ètnics hi ha una manca de detecció per desconeixement de la malaltia.

Article 2. *Relapses in Patients With Giant Cell Arteritis: Prevalence, Characteristics, and Associated Clinical Findings in a Longitudinally Followed Cohort of 106 Patients.* **Alba MA**, García-Martínez A, Prieto-González S, Tavera-Bahillo I, Corbera-Bellalta M, Planas-Rigol E, Espígol-Frigolé G, Butjosa M, Hernández-Rodríguez J, Cid MC. Medicine (Baltimore) 2014 Jul; 93 (5): 194–201. IF (JCR 2014): 4.867.

El doctorand investiga el seguiment dels pacients en el nostre mitjà i analitza la prevalença i característiques de les recurrències que seran utilitzades en els treballs següents com a mesura d'eficàcia.

Article 3. *Treatment with angiotensin II receptor blockers is associated with prolonged relapse-free survival, lower relapse rate, and corticosteroid-sparing effect in patients with giant cell arteritis.* **Alba MA**, García-Martínez A, Prieto-González S, Espígol-Frigolé G, Butjosa M, Tavera-Bahillo I, Rodríguez-Pintó I, Hernández-Rodríguez J, Cid MC. Semin Arthritis Rheum. 2014 Jun;43(6):772-7. IF (JCR 2014): 3.62

El doctorand investiga, en la cohort de l'estudi anterior, l'influencia potencial del tractament amb bloquejants del receptor de l'angiotensina II (ARB) sobre el curs evolutiu dels pacients i els resultats demostren associació entre l'ús de ARB i menor incidència de recurrències i menor dosi acumulada de corticoides, suggerint participació del sistema angiotensina en la persistència de la malaltia.

Article 4. *Expression of the Angiotensin II system in temporal artery inflammatory lesions from patients with giant cell arteritis.* **Alba MA**, Planas-Rigol E, Corbera-Bellalta M, Terrades N, García-Martínez A, Prieto-González S, Lozano E, Hernández-Rodríguez J, Grau JM, Cid MC. (Submitted)

El doctorand investiga l'expressió d'angiotensinogen, enzims convertidors i receptors d'angiotensina en les lesions vasculars dels pacients amb ACG. Al mateix temps investiga quines cèl·lules produeixen cada un dels components del sistema mitjançant microscòpia confocal i sistema de co-cultiu de cèl·lules mononuclears de sang perifèrica i cèl·lules musculars llises obtingudes de la paret arterial de les artèries dels pacients. En el sistema de co-cultiu el bloqueig del receptor d'angiotensina disminueix la producció de citocines pro-inflamatòries. L'article està enviat a Ann Rheum Dis. Es tracta d'una revista molt competitiva i no tenim garanties d'acceptació.

Informació sobre la co-autoria

En l'article numero 3, ""Treatment with angiotensin II receptor blockers is associated with prolonged relapse-free survival, lower relapse rate, and corticosteroid-sparing effect in patients with giant cell arteritis", el doctorand te autoria compartida amb la Dra. Ana García Martínez. El doctorand ha realitzat al major part de tasques relacionades amb l'article, inclosa la redacció del mateix, si be la participació de la Dra. Ana García Martínez va ser també crucial en la caracterització de la cohort d'estudi. La Dra. Ana García Martínez és ja doctora i aquest article no ha format part de la seva tesi doctoral.

Barcelona, setembre de 2014

Dra. Maria Cinta Cid Xutglà

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- Dra Cid (*Mariona*), esta tesis está dedicada a usted. Gracias por ser para mi no sólo una gran mentora, sino también una amiga, una confidente y muchas veces una madre. Trabajar con usted es poder ver un día tras otro lo que una mente genial puede hacer.

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ABBREVIATIONS

ACE-Angiotensin-converting enzyme

ACR-American College of Rheumatology

AGTR-Angiotensin II receptor

ANCA-Anti-neutrophil cytoplasmic antibody

ATII-Angiotensin II

CD-Cluster designation

CRP-C-reactive protein

CTA-Computed tomography angiography

DCVAS-Diagnosis and classification of vasculitis study

DNA- Deoxyribonucleic acid

EGF-Epidermal growth factor

ESR-Erythrocyte sedimentation rate

EULAR-European League Against Rheumatism

FGF-Fibroblast growth factor

GC-Glucocorticoids

GCA-Giant cell arteritis

IL-Interleukin

IFN-γ-Interferon-*gamma*

ICAM-Intercellular adhesion molecule

MCP-Monocyte chemotactic protein

MMPs-Metalloproteinases

MRI- Magnetic resonance imaging

MS-Multiple sclerosis

NF-κB-Nuclear factor *kappa* B

PBMC-Peripheral blood mononuclear cells

PCR- Polymerase chain reaction

PET-Positron emission tomography

PDGF-Platelet-derived growth factor

PDN-Prednisone

PMN-Polymorphonuclear leukocytes

PMR- Polymyalgia rheumatica

RAS-Renin-angiotensin system

RNA-Ribonucleic acid

SIR-Systemic inflammatory response

SSc-Systemic sclerosis

TAB-Temporal artery biopsy

TGF-β-Transforming growth factor-*beta*

Th-T-helper

TLR-Toll like receptor

TNF-α-Tumor necrosis factor-*alpha*

VCAM-Vascular cell adhesion protein

VEGF-Vascular endothelial growth factor

vDC-Vascular dendritric cells

VSMC-Vascular smooth muscle cells

ABSTRACT

Giant cell arteritis (GCA) is a systemic vasculitis of unknown aetiology that affects medium-sized and large arteries. GCA may deeply impair the patient's quality of life as a result of systemic smouldering activity, disease progression or adverse effects related to therapy.

The present thesis is a compendium of four articles assessing clinical and immunopathological characteristics associated with persistence of chronic inflammation in GCA patients. In the first publication, we described the clinical course of a small series of GCA patients derived from Latin America, a geographic area where data regarding this disease were virtually absent. We found that major features observed in this population were similar to those reported in series of Caucasian origin.

The second article described the prevalence, predictors, and main features of relapses, one of the many faces of the persistent inflammatory activity that characterizes GCA. This study, performed in a large cohort of patients uniformly treated and followed, demonstrated that disease flares are frequent and, importantly, that a relapsing course was associated with higher and prolonged GC requirements and increased morbidity.

The third publication was oriented to the need of identifying adjuvant therapies for GCA and deals with the potential benefit of adding angiotensin II receptor blockers (ARB) to standard prednisone treatment. The study rationale was based on the pleiotropic anti-inflammatory effects recently described for this drug class. Our results suggest that ATII receptor blockade may help to maintain remission, reduce the relapse rate, and spare glucocorticoids in patients with GCA.

Following this clinical observation, we further investigated a potential contribution of the angiotensin II (ATII) system in the maintenance of inflammatory activity in GCA. In the fourth paper we showed that ATII system is overexpressed in GCA inflammatory lesions. In addition, we confirmed that ATII exhibited pro-inflammatory properties in co-cultures of lymphocytes, monocytes and temporal artery-derived vascular smooth muscle cells which were reversed by the angiotensin II receptor blocker losartan.

RESUMEN

La arteritis de células gigantes (ACG) es una vasculitis sistémica idiopática que afecta principalmente arterias de mediano y gran calibre. La calidad de vida de estos pacientes puede verse seriamente afectada debido a múltiples complicaciones inmediatas y tardías.

Esta tesis es un compendio de cuatro artículos que evalúan características clínicas e inmunopatológicas de la persistencia de la inflamación crónica en la ACG. En la primera publicación, se describen las características y la evolución de la enfermedad en una pequeña serie de pacientes latinoamericanos, concluyéndose que las manifestaciones y el comportamiento de esta vasculitis es similar a la reportada en otras cohortes, principalmente de origen caucásico.

En el segundo artículo se describe la prevalencia, características y factores asociados con las recaídas o rebrotes, una manifestación de la constante actividad inflamatoria de la ACG. Nuestro estudio demostró que los pacientes con esta vasculitis sufren recaídas de manera frecuente, lo que se asoció a largo plazo con mayor requerimiento de esteroides sistémicos y mayor prevalencia de efectos adversos.

La tercera publicación tuvo como punto de partida la necesidad de identificar tratamientos alternativos para lograr un mejor control de la ACG. El punto de partida de este estudio fueron los efectos anti-inflamatorios vinculados a los antagonistas de los receptores de angiotensina (ARA). Los resultados mostraron que la adición de ARA al tratamiento habitual de la ACG estaba asociado con un menor número de recaídas y un efecto ahorrador de corticoesteroides (CE).

A partir de esta observación clínica, investigamos si la angiotensina II (ATII) podría desempeñar un papel en el mantenimiento de la actividad inflamatoria crónica de la ACG. En el cuarto manuscrito mostramos que el sistema de la ATII esta sobre-expresado en las arterias temporales de pacientes con esta vasculitis. Además, corroboramos que la ATII es capaz de inducir citoquinas pro-inflamatorias en un sistema de co-cultivo de linfocitos, monocitos y células musculares lisas vasculares derivadas de arteria temporal, efecto inhibido mediante el bloqueante del receptor de angiotensina II, losartán.

I. INTRODUCTION

1. Giant cell arteritis

1.1. Definition.

Giant cell arteritis (GCA) is a large and medium size vessel vasculitis, often granulomatous in nature, that predominantly affects the aorta and/or its major branches, with a predilection for the branches of the carotid and vertebral arteries (1).

1.2. Epidemiology.

GCA affects individuals aged >50 years, mostly woman (female to male ratio 2-3:1) (2). Worldwide, GCA is considered the most frequent primary systemic vasculitis in western countries in people older to 50 years old (2, 3). The mean age at diagnosis is 73 years (4). The highest incidence rates are found in northern Europe (5-7), in Scandinavian populations (17 cases per 100,000 individuals aged more than fifty years) and in North American inhabitants of similar ancestry (8). In Northwestern Spain, an annual incidence rate of 10.13 per 100,000 inhabitants older than 50 years has been reported; this increases to 23.16 in the 70-79 year age-group (9). Remarkably, in the past two decades an increased incidence of GCA has been observed, possibly reflecting higher physician awareness or improvement in accessibility to diagnostic methods, such as imaging techniques (2).

Giant cell arteritis is infrequent in Asia (10, 11) and Arabic countries (12) and seems to be far less common in Latin-America (13-15). Data of this condition in so-called Hispanic populations is derived from small case series where histological confirmation was not always obtained (13-15). Geographic variation of GCA has been attributed to several factors, including genetic predisposition. In this sense, several genetic variants have been proposed to influence disease susceptibility, i.e., HLA-DRB1*04 or PTPN22 alleles (16, 17) and tumor necrosis factor (TNF-α) microsatellite polymorphisms (18, 19). In addition, a very recent study that included a large number of biopsy-proven GCA patients (n=1266) demonstrated an interesting association between polymorphisms at the IL-17A gene and increased risk for developing GCA (20).

On the other hand, environmental factors have also been investigated as potential determinants of the particular geoepidemiology observed in GCA. Based on cyclic fluctuations in incidence, with peaks every 8-10 years and clear seasonal distribution, infectious agents have been hypothesized as potential triggers (21, 22). Previous studies investigating microbial infections as possible causal agents for GCA have reported the presence of *Chlamydia* species, *herpes virus 6* and *7, varicella zoster virus, Epstein Barr virus, human parainfluenzae* and *parvovirus B19 virus* in temporal arteries of affected patients (23-26). However, involvement of these microorganisms in early phases of vascular inflammation has not been confirmed (23-26). For instance, DNA sequencing performed in 17 temporal artery biopsy specimens did not show significant differences between the microbiomes of GCA (n=12) and control (n=5) patients (27).

1.3. Pathogenesis.

Vascular inflammation is the pathological substrate of GCA. Inflammatory cells infiltrate the arterial wall and cause structural damage that eventually leads to the vascular complications. The current hypothesized model about the generation of inflammatory lesions in GCA is based on low levels of experimental evidence. In fact, most information has been obtained from comparison between inflamed and normal arteries and extrapolation of known biologic activities of certain cytokines, proteases, or growth factors differentially expressed in GCA temporal biopsies versus control arteries.

It has been proposed that inflammation in GCA temporal arteries initiates with the activation of vascular dendritric cells (vDC) located at the adventitial layer through toll-like receptors (TLR) 2, 4 and 5 (28). The stimuli that activate vDC are currently unknown, although a number of pathogens have been proposed (24-26). Different vessel segments harbour distinct TLR that might explain the tropism of GCA for selected arteries (29).

Fully activated dendritic cells release chemokines CCL-18, CCL-19, CCL-20 and CCL-21, which probably contribute to the recruitment or trapping of other vDC in the arterial wall (30).

Next, vDC may act as antigen-presenting cells, activating and recruiting CD4+ lymphocytes into the vessel wall. It has been proposed that the initial immunologic injury occurs in the adventitia, with T lymphocytes probably getting into the artery through the *vasa vasorum* and not from the main lumen. Possible explanations for this phenomenon includes the lack of vascularization of the media and intima layers in addition to the high velocity of blood flow observed in the lumen, thus preventing cell adhesion (31).

In the adventitial layer, T cells are exposed to stimulatory signals, expand clonally, and release several pro-inflammatory cytokines (32). Recent evidence has demonstrated that two separate lineages of CD4+ T cells are involved in GCA pathogenesis, Th1 and Th17 (20). Th1-mediated response, characterized by the production of IL-2 and interferon-gamma (IFN-γ), seems to participate in granuloma formation and chronic persistence of GCA. Th17 cells are characterized by the production of IL-17A, which, in contrast to IFN-γ is rapidly downregulated by high-dose glucocorticoid (GC) therapy (32). Of note, expression of IL-17A in GCA arteries inversely correlated with relapses and GC requirements according to a recent study (32).

The major target cells for IFN-γ are macrophages, which are recruited into the vessel wall to form the prominent granulomatous reaction. Macrophages promote inflammatory cascades through the production of IL-1β, IL-6 and TNF-α and also participate in the process of vascular injury and remodeling through the release of cytokines, growth factors, reactive oxygen species and matrix metalloproteinases (33, 34). The release of inflammatory mediators by macrophages probably elicits the prominent systemic inflammatory response characteristic of GCA (35).

In addition to the production of pro-inflammatory cytokines, macrophages secrete metalloproteinases (MMP-2 and MMP-9) or platelet-derived growth factors (PDGFs), likely involved, respectively, in the destruction of elastic membranes and proliferation and migration of vascular smooth muscle cells (VSMC) (36, 37) towards the intima, and leading to intimal hyperplasia, vascular stenosis and occlusion.

In addition to PDGFs, intimal hyperplasia is also promoted by other mesenchymal growth factors expressed in temporal arteries, such as transforming growth factor-beta (TGF-β), fibroblast growth factor (FGF-2), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) (33, 38, 39). The combination of reactive oxygen species, reactive nitrogen intermediates and MMPs contributes to the induction of VSMC apoptosis and destruction of the internal elastic lamina.

In addition, alterations in peripheral blood B lymphocyte distribution have been recently described in active GCA and polymyalgia rheumatica (PMR) patients (40). These patients exhibited reduced number of circulating B-lymphocytes (by redistribution or marginalization) and interestingly, their $B_{effector}$ cells showed enhanced production of IL-6 (40).

In a related matter, some authors have described the presence of certain auto-antibodies in patients with GCA, i.e anti-cardiolipin antibodies (41, 42), anti-endothelial cell antibodies (43), anti-smooth muscle cells (44) and most recently, anti-human ferritin-heavy chain antibodies (45). Currently, the diagnostic usefulness of these antibodies as well as their contribution to the pathogenesis of GCA remain unclear and further investigation is needed.

1.4. Histopathology.

Typical findings observed in temporal arteries from GCA patients include dense transmural inflammatory infiltrate of the vessel wall associated with marked disruption of the internal elastic lamina, intimal hyperplasia and multinucleated giant cells (found near to bordeline intima-media territory) (46, 47). Along the biopsed arteries, focal and segmental inflammation is usually observed.

The inflammatory infiltrate is mainly constituted by macrophages and CD4+ lymphocytes (47). Scattered CD8+ lymphocytes, B cells and eosinophils can be also observed (47). Giant cells are detected in only half of the analyzed specimens (47).

In a small proportion of patients, temporal artery biopsy discloses a small-vessel vasculitis affecting the *vasa vasorum* or small vessels surrounding a normal temporal artery as the only abnormal finding (48). In these cases, the existence of other systemic vasculitis primarily involving small vessels needs to be excluded.

1.5. Clinical manifestations.

GCA presentation includes a broad spectrum of clinical abnormalities that are secondary to tissue ischemia and/or local or systemic inflammation. Headache is the main symptom in almost all series, presenting in approximately 86% of patients (49). This is sudden in onset, severe and usually located in the temporal territory, although other localizations are not so uncommon (occipital, frontal, parietal). Headache is usually resistant to standard analgesia. Other classic features include scalp tenderness, recorded in 50% of individuals and jaw claudication (30-50%) (49). Jaw claudication has been reported as the most specific symptom of GCA (50). In patients with GCA, physical examination of temporal area could reveal tender, thickened, nodular or erythematous arteries. Also, pulses may be decreased or absent.

Importantly, severe ischemic complications are currently present in 20% to 30% of patients. Ischemic complications include permanent visual loss (15-21%) (51, 52), *amaurosis fugax* (10%) or diplopia in addition to stroke (3-6 %), particularly involving the vertebro-basilar territory (53). Visual manifestations are secondary to ischemia of the optic nerve due to vasculitis of the ciliary or ophthalmic arteries. Less frequently, retinal artery occlusion is the main cause (51). In previous series, ocular ischemic lesions consisted in anterior ischemic optic neuropathy in 94.4% of patients (with the characteristic chalky white optic disk edema observed in 69%) and central retinal artery occlusion or cilioretinal artery occlusion in 10% (51, 52). Unfortunately, severe ischemic complications are a major source of chronic disability with small chances of improvement after they occur (54).

It is estimated that 10% to 15% of patients with visual symptoms experience progression of visual loss within the first 2 weeks after the initiation of therapy (51, 52). Risk factors for the development of severe ischemic complications include previously transient ischemic episodes (54, 55), classic risk factors favoring atherosclerosis (56), low inflammatory systemic response (54, 57), scalp necrosis (58), jaw claudication (59) and thrombocytosis (60). Anecdotic reports of other major ischemic manifestations include tongue, lip or scalp necrosis (61).

Systemic manifestations are present in 50% of patients and include fever, fatigue, weight loss and/or chronic anemia. Fever is usually low grade but in 15% of patients, temperature could exceed 38^{o}C, leading to misdiagnoses of infection (62). In addition, in approximately 10% of cases, constitutional symptoms or fever of unknown origin are the only clues to the presence of GCA.

Inflammatory involvement of the extracranial arteries, particularly the aorta and its main tributaries seems to be common in GCA patients (63, 64). In a previous prospective study of patients with recent-onset GCA, large vessel arteritis was detected by CT-angiography in almost 70% (63). Although aortic inflammation is usually asymptomatic, it may lead to delayed structural damage (65). In fact, GCA related large vessel complications of GCA are reported in 20% to 25% of patients, presenting as thoracic aortic aneurysms (22% after a median follow of 5.4 years), abdominal aneurysm (6.5%) or vessel stenosis manifested clinically as limb claudication (13.5%) (65-67).

In addition to these main clinical features, PMR can be present in 40% to 60% of patients with GCA (68). Also, 15% to 20% of patients with isolated PMR develop GCA (68). PMR is a synovitis of the proximal joints and periarticular structures (bursitis, tenosynovitis), clinically characterized by aching and morning stiffness in the shoulders, hip girdle, and/or neck (68). Pain is characteristically prolonged and bilateral, with morning stiffness usually lasting more than 30 minutes (68).

Constitutional symptoms are observed in 40% of patients. Peripheral arthritis, particularly affecting the knees and wrist, ocurr in a small fraction of cases. The arthritis is asymmetric and nonerosive.

1.6. Diagnosis.

Giant cell arteritis is suspected on the basis of clinical evaluation in combination with laboratory and imaging tests, and it is confirmed by histologic examination (68). Laboratory results usually disclose elevated acute phase reactants (ESR, C-reactive protein-CRP-, haptoglobin) and normochromic anemia related with chronic disease (69, 70). Thrombocytosis, elevated serum levels of hepatic enzymes and low serum albumin concentration occur in 25% to 35% of patients (69, 70). CRP is probably more sensitive than ESR in assessing disease activity in both, GCA and PMR (71).

Temporal artery biopsy (1.5-2 cm length) is the gold standard to establish the diagnosis of GCA (72). Due to the patchy distribution of inflammatory lesions, temporal artery biopsy can be negative or non-conclusive in 10% to 20% of patients with characteristic clinical manifestations (73). Complications of temporal biopsy are extremely uncommon.

In recent years, several imaging modalities have been investigated for clinical use in GCA, i.e. doppler ultrasound, CTA, magnetic resonance imaging/angiography (MRI), and PET (74). Until now, these techniques have not supplanted TAB as the critical test in the evaluation of a patient with possible GCA.

Colour Doppler ultrasonography may be helpful in diagnosing GCA (75). The halo sign (hypoechoic rim surrounding the arterial lumen) reflecting artery wall edema has a sensitivity of 69% and a specificity of 82% for the diagnosis of biopsy-proven GCA (75). The specificity is close to 100% when the halo sign is bilateral (75). CTA and MRI can also be used to diagnose large-vessel involvement in GCA. Both may disclose findings consistent with aortitis, i.e. aortic wall thickening, wall oedema or increased mural contrast enhancement (76).

^{18}F-PET has been demonstrated to be useful in the diagnosis of early large vessel involvement in GCA by showing increased uptake by metabolically active cells infiltrating the arterial wall (77). Recently, it has been suggested that PET can also be used for GCA diagnosis (78) and disease activity follow-up (74).

In addition to their role in diagnosis, late complications such as vascular stenosis or dilations can be also recognized with imaging techniques (66). As described in recent years, development of aortic aneurysms is a late and potentially serious complication of GCA (65, 66). It should be stressed that imaging findings are not always associated with disease activity or development of new lesions and therefore, they are not reliable as the sole factor to guide treatment decisions.

1.7. Classification criteria.

The American College of Rheumatology (ACR) criteria developed in 1990 are used for classification purposes, i.e. for inclusion of GCA patients in clinical or translational research studies (79). At least three of the following five must be fulfilled: 1) Age at disease onset >50 years, 2) New onset headache, 3) Abnormal temporal artery on physical examination, 4) ESR >50 mm/hr by Westergren method and 5) Biopsy specimen of temporal artery disclosing vasculitis with mononuclear cell infiltration with multinucleated giant cells (observed frequently but not always). When used to discriminate between different types of vasculitis, these criteria have a sensitivity of 93.5% and a specificity of 91.2%.

As ACR criteria were designed and are useful for standardizing cases for clinical investigation only, a major international effort is now under way to develop diagnostic criteria for temporal arteritis and other systemic vasculitis (DCVAS, diagnosis and classification of vasculitis) (80).

Regarding PMR, provisional classification criteria have been recently published thanks to the coordinated efforts of the European League Against Rheumatism (EULAR) and the ACR (81).

According to these criteria, patients >50 years, with new bilateral shoulder pain and elevated ESR can be classified as having PMR if they score ≥4 or ≥5 points if ultrasound findings are included.

Scoring algorithm includes 1) Morning stiffness lasting >45 minutes (2 points), 2) Hip pain or restricted range of motion (1 point), 3) Absence of rheumatoid factor and antibodies to citrullinated peptides (2 points), 4) Absence of other joint involvement (1 point). Complementary ultrasound findings (1 point each): 1) At least one shoulder with subdeltoid bursitis, biceps tenosynovitis or glenohumeral synovitis and at least one hip with synovitis or throchanteric bursitis or 2) Both shoulders with subdeltoid bursitis, biceps tenosynovitis or glenohumeral synovitis.

1.8. Treatment.

High dose glucocorticoids with initial doses of 1 mg/kg/d up to 60 mg/d of prednisone or its equivalent are considered the cornerstone of treatment to induce remission (67, 72). Steroids cause a prompt resolution of symptoms and, in patients with partial or transient visual impairment, prevent further visual loss in 85% to 90% of cases, although they usually do not reverse established damage (82). Intravenous pulses of 1 gr methylprednisolone have been recommended to treat impending visual loss (72), although no randomized controlled trial have demonstrated superiority of pulse vs oral GC for preventing ischemic complications (83, 84). Tapering schedules are heterogeneous but aimed to achieve 10 to 15 mg of prednisone (PDN) per day at 3 months and 5 to 7.5 mg at 8 to 12 months (85). The British Society of Rheumatology recommended maintain high-dose GC for 3 to 4 weeks, then reduced by 10 mg every 2 weeks to 20 mg, then by 2.5 mg every 2 to 4 weeks to 10 mg and then 1 mg every 1 to 2 months if no flare occurs (86).

Although the initial response to treatment is rapid and satisfactory, GCA may deeply impair the patient's quality of life as a result of systemic smouldering activity, disease complications or adverse effects related to therapy.

It has been estimated that 10%-15% of patients cannot reduce prednisone below 10 to 15 mg/d without experiencing disease flares or low-grade persistent activity (87). In addition, relapses are observed in 40% to 80% of patients, particularly during the first 1.5 years after diagnosis (88). Consequently, approximately 50% of the patients cannot tolerate complete PDN withdrawal after 2 to 3 years of treatment (87, 89).

As a result of long term GC treatment, adverse effects occur in nearly 86% of patients during long term follow-up (90). Importantly, a fraction of patients may develop major steroid related complications such serious infections or fractures (91).

Immunosuppressive agents have been used in an effort to reduce the cumulated GC exposure and to treat cases not adequately controlled by GC alone. Unfortunately, only methotrexate (10-15 mg/w) has demonstrated modest efficacy in reducing relapse rate and lowering cumulative prednisone dose (92-95). However, addition of methotrexate did not decrease GC-related side effects. In spite of these limited benefits, methotrexate is the only immunosuppressant recommended by the EULAR as adjunctive therapy for GCA (72).

Azathioprine (2 mg/kg/d) treatment resulted in a significant but small reduction in median prednisone dose at 1 year in a small trial of mixed patients with GCA and PMR (96). Infliximab has been reported to be useful in case reports and small series of patients with GC resistant GCA or marked glucocorticoid-related toxicity. However, a randomized clinical trial with infliximab in newly diagnosed patients yielded disappointing results (97). Etanercept, another TNF-inhibitor, demonstrated significant but slight reduction in cumulated prednisone dose in relapsing patients or patients with GC-related side effects but the number of patients included precluded strong conclusions (98). More recently, retrospective case series have suggested efficacy for cyclophosphamide in patients with GC-dependent GCA at the expenses of infrequent but serious side effects (99).

In addition, tocilizumab, an IL-6 receptor blocking humanized monoclonal antibody, has been shown to elicit remission in GC-dependent GCA patients in several case reports and small case series and, a large multi-center, international randomized, double-blind controlled trial with this drug is currently underway (100).

Other adjuvant measures recommended in GCA patients include anti-platelet therapy in patients with severe ischemic complications, as the addition of low-dose aspirin may decrease the development of new ischemic complications once high-dose GC treatment has been started (101) and, the use of calcium, vitamin D and bisphosphonates for the prevention of glucocorticoid-induced osteoporosis (85).

2. The systemic inflammatory response in giant cell arteritis.

2.1. *Intensity of systemic inflammatory response.*

The intensity of the initial systemic inflammatory reaction (SIR) is highly variable among patients (102). In fact, the intensity of SIR has been demonstrated to be an important prognostic factor in GCA (54, 103, 104). In previous studies, the presence of a strong initial acute-phase response, defined as having both fever and weight loss in addition to ESR >85 mm/h and a hemoglobin level <11.0 gm/dl, was associated with lower risk of developing cranial ischemic complications (54) but also with a relapsing course with prolonged duration of GC therapy (103).

In this sense, it has been hypothesized that inflammation-induced angiogenesis may have an initial protective role by reducing the prevalence of ischemic complications (105). However, in the long-term, the same phenomenon could have a role in disease perpetuation as newly formed vessels could be the main site at which adhesion molecules for leukocytes are expressed and the main sites through which leukocytes invade the vessel wall (103, 106).

2.2. Involved molecules.

The intense systemic inflammatory response that characterizes GCA is driven by both tissue and circulating pro-inflammatory cytokines acting in target tissues, such as the liver, hypothalamus and bone marrow (103). The main sources of these cytokines are probably the infiltrating macrophages located in inflammatory foci of affected vessels although activated bloodstream monocytes may also contribute to their production.

Studies performed on TAB obtained from GCA patients have demonstrated that local production of several pro-inflammatory molecules correlate with the initial clinical manifestations and major disease outcomes (102, 103, 107-110). In this sense, lower tissue mRNA expression of IL-6 has been associated with the development of disease-related ischemic events (107). In addition, it has been reported that expression of IL-1b, TNF-α, IL-6 and IL-33 correlates with the intensity of the initial SIR (103, 111).

Regarding to disease course, patients with an elevated expression of IL-6, TNFα and, IL-1β not only exhibit an intense SIR but also develop refractory, long-lasting disease that requires prolonged GC treatment (103, 108). In the same line, up-regulation of CCL2 (MCP-1), an important chemokine involved in monocyte recruitment, has been demonstrated in temporal artery samples from relapsing individuals (109). By contrast, increased IL-17A expression has been reported to be a predictor of sustained response to GC treatment (110).

On the other hand, results of previous studies that investigated the relevance of circulating cytokines have demonstrated that patients with strong SIR who have elevated serum levels of TNF-α and IL-6 have higher and more prolonged GC requirements, and experienced more disease flares during follow-up (103, 106). Notably, elevated IL-6 serum concentrations have been reported in 92% of active untreated patients and in 89% of disease flares (112). In addition, as demonstrated for tissue expression of IL-6 in TAB, reduced circulating levels of this cytokine were identified in patients with ischemic complications (107).

Of note, although IL-1β mRNA can be detected in temporal artery lesions from patients with GCA, serum levels usually remain below the detection threshold (103).

2.3. Persistence of chronic inflammation.

Persistence of chronic inflammation is an important unsolved issue in GCA. Short GC courses and low maintenance doses did not eradicate essential inflammatory pathways, allowing subsequent reactivation of GCA (67). Supporting this concept, increased circulating cytokine (TNF-α, IL-6) or von Willebrand's factor concentrations have been observed in a substantial proportion of patients with apparent clinical remission (108, 112-114). Similarly, almost one third of PMR patients remained with elevated serum levels of IL-6 during the first year of treatment, despite clinical remission (115). Of interest, in patients with subclinical inflammatory activity, ESR values may remain within the normal range despite persistent elevation of other inflammatory markers (112).

Although the source of elevated cytokines in patients with smouldering activity has not been clarified, intriguing reports show persistent inflammatory infiltrates in some aortic specimens obtained from necropsies or from patients with aneurysms that have undergone surgery years after GCA diagnosis (116). Interestingly, specimens from long-term treated patients showed extensive vascular remodelling in addition to small foci of inflammatory cells (116). Inflammatory cells may not be the only source of cytokines since VSMC are also able to produce substantial amounts of cytokines when exposed to an inflammatory microenvironment (38, 117).

In addition to clinical evidence, experimental data also supports the ineffectiveness of standard GCA therapy to completely abrogate inflammatory infiltrates found in GCA lesions (118). Analysis of temporal artery biopsies engrafted onto SCID mice reveals down-regulation of IL-2, IL-6 and IL-1β mRNAs after 3 weeks of treatment with high-dose dexamethasone. In contrast, synthesis of IFN-γ was only slightly decreased, and expression of TGF-β1 remained unaffected (118). These results suggest that relevant inflammatory pathways are only partially affected by GC and their persistent activity results in the chronicity observed in a substantial proportion of patients (118).

2.4. Clinical consequences of persistent vascular inflammation.

Which are the clinical consequences of persistent vascular inflammation in GCA? Relapses or flares are the most evident clinical repercussion of the chronic inflammatory activity observed in this systemic vasculitis. Clinically evident relapses occurred in 40% to 80% of GCA treated patients (88, 90, 95, 97). Former studies have reported that patients with recurrent GCA have higher overall concentrations of inflammatory cytokines and adhesion molecules, such as TNF-α, IL-6, and soluble ICAM-1 (108, 119, 120). In addition, elevated IL-12p40 and IFN-γ mRNA concentrations have been observed after 1 year of treatment in second temporal artery biopsies of relapsing patients (119).

In line with results observed in GCA, persistently elevated levels of CRP and IL-6 were related to higher risk of flares in PMR patients (115).

On the other hand, chronic large-vessel inflammation may lead to the development of aneurysms, dissections or stenosis. About 10% to 22% of GCA patients develop delayed vascular complications in the form of aortic aneurysms or dissections, and large-artery stenosis (65, 121, 122). The incidence of aortic structural damage is maximal within the first 5 years after diagnosis but continues developing over time, affecting approximately to 30% of patients after long term follow-up (66). It should be stressed that the association of delayed GCA-related complications with persistence of chronic inflammation remain speculative, as no definitive relationship has been demonstrated. Other factors such as the aggressiveness of the initial inflammatory injury, hemodynamic factors and the intensity of remodelling responses in target tissues may have an important influence (108).

At last, accelerated atherosclerosis could be a complication of long lasting inflammation, as described in various forms of vasculitis (123). In this regard, results of a recent study confirmed that GCA is associated with an increased risk of atherosclerotic disease, in the form of myocardial infarction, peripheral vascular disease and stroke (124).

3. The angiotensin II system

3.1. Definition and general characteristics.

Angiotensin II (ATII) is the most important effector peptide of the renin-angiotensin system (RAS), the hormonal axis that regulates arterial blood pressure and salt balance.

The classic RAS has been configured as a multiple-step cascade initiated by secretion of renin, the rate-limiting enzyme of the system, by the granular cells of the renal juxtaglomerular apparatus in response to glomerular hypoperfusion (125, 126). Renin is primary formed as preprorenin, cleaved in the endoplasmic reticulum to from preprorenin, which is packaged in the Golgi apparatus, where it is further processed to active renin (127).

Renin mediates the cleavage of angiotensinogen (AGT, primarily formed and secreted by hepatic cells) into angiotensin I (125, 126). AGT can also be synthetized in other tissues such as the heart, kidneys and adipose tissue (127). The level of renin activity determines the rate of angiotensin I formation in plasma (126). In addition, angiotensin I can also be synthesized from AGT by non-renin pathways involving tonin A, cathepsin D, and tissue plasminogen activator (128).

Angiotensin I (1–10) is a decapeptide with not known bioactive functions that undergo cleavage into smaller fragments by angiotensin-converting enzyme (ACE). ACE is a zinc dipeptidyl carboxypeptidase, that is largely responsible for the conversion of angiotensin I into angiotensin II (ATII) (1-8), the final effector molecule of the RAS (125). This enzyme is most abundant in the lungs, intestine, kidneys, brain, aorta, and adrenal medulla (129). ACE is attached to the plasma membrane where it acts as an ectoenzyme that hydrolyses circulating peptides as angiotensin I and bradykinin (129). The enzyme may also be present in soluble form, although its physiological relevance is not well understood (127). Of relevance, alternative enzymatic pathways not involving ACE can contribute to ATII production (i.e., chymase, kallikrein, cathepsin G, chymostatin-sensitive ATII generating enzyme and elastase-2) (130).

Once produced, ATII binds to its specific receptors and participates in the regulation of arteriolar vasoconstriction and sodium reabsorption in the renal tubules (125). Unlike renin and AGT, which have long plasma half-lives, ATII is degraded within seconds by angiotensinases, acting at different amino acid sites (127, 131).

Two major categories of ATII receptors have been identified, type 1 (AGTR1) and type 2 (AGTR2) (132). AGTR1, the main receptor for angiotensin II, is a seven-transmembrane G-protein–coupled receptor widely expressed in cardiovascular and renal tissues (126, 132). In contrast, AGTR2 is highly expressed during fetal life but decreases dramatically after birth (126). It has been reported that AGTR2 protein is detectable in the adult human diseased heart (133, 134). However, as AGTR2 mRNA has been difficult to demonstrate, receptor expression is poorly characterized (127). In this line, the precise function of AGTR2 is controversial, although it has been suggested that it counteracts AGTR1 effects. For example, in blood vessels, AGTR2 actions may include vasodilatory properties and antiproliferative and apoptotic effects in VSMC counteracting the neointimal formation induced by ATII activation of AGTR1 (127, 135).

In contrast to the traditional concept of a unique and widespread RAS, a paradigm shift has occurred in recent years as recent evidence suggests the presence of local RAS systems in several organs of human body, including the heart, adipose tissue, kidneys, brain, adrenal glands, and pancreas (136-138). These local systems would function in an autocrine or paracrine fashion and would be compartmentalized and regulated independently from the plasma circulation (136, 137). The heart is an interesting example of these novel findings with complete RAS components being present although with heterogeneous distribution (127). AGT is primarily distributed in atrial muscle and the conduction system (139). In contrast, the conduction system contains little ACE, but the enzyme is highly expressed by coronary endothelial cells and cardiac fibroblasts (140). Interestingly, overactivation of local ATII system has been considered of relevance in the pathogenesis of chronic kidney damage secondary to hypertension and diabetes mellitus (141, 142).

In addition to the identification of local production of angiotensin II, another major advance in the current understanding of the RAS has been the discovery of additional components of the RAS, including ATIII (AT 2-8), ATIV (AT 3-8), AT (1-7), ACE2, (pro) renin receptors, pro-angiotensin-12 and angiotensin (1-7)-specific G protein-coupled Mas receptor (143-145).

Of relevance, ACE2 (a mono carboxypeptidase related to ACE, predominantly expressed in the heart, intestine, lungs, intra-renal vessels and placenta) generates the bioactive AT (1-7) by cleaving C-terminal phenylalanine from ATII (129). This enzyme also catalyzes to a lesser extent the conversion of angiotensin I to inactive AT (1-9), which can be further hydrolyzed to AT (1-7) by ACE (127). Of special interest is the fact that although similar to ACE, ACE2 is not inhibited by commonly used ACE inhibitors. It is clear that further investigation is required to fully elucidate this complex system.

3.2. The role of angiotensin II in inflammation.

Over the past years ATII has emerged as a powerful pro-inflammatory peptide (125). Investigation exploring atherosclerosis pathogenesis, one of the most prevalent chronic inflammatory diseases, have allowed us to know that ATII is able to stimulate the secretion of several pro-inflammatory cytokines and chemokines that may regulate critical steps of leukocyte recruitment into the vessel wall (146, 147). Of note, many of the ATII induced molecules are overexpressed in GCA involved arteries or increased in patient's sera and are thought to participate in pro-inflammatory amplification cascades (105, 108, 119, 120, 148).

Vascular smooth muscle cells. In human VSMC extracted from saphenous veins, ATII induced concentration and time-dependent increments of IL-6 both at mRNA and protein level (149). Similarly, the addition of ATII to cultured VSMC increased the production of pro-inflammatory molecules IL-1, IL-8, IL-18 and osteopontin (149-151). In addition, ATII downregulates peroxisome proliferator-activated receptors in mice vascular wall and these receptors have anti-inflammatory effects (152).

Of relevance, pharmacological blockade of AGTR1 or ACE with losartan or captopril/ramipralat respectively, resulted in reduced levels of these cytokines (149). ATII also enhances the expression of MCP-1 (monocyte chemotactic protein 1) (150, 153, 154). MCP-1 is one of the major chemokines involved in macrophage infiltration of the vessel wall. This molecule has important role in the pathogenesis of atherosclerosis and probably of GCA (109, 155).

The dynamic of AGTR1 expression in VSMC has been investigated in previous studies, although with inconclusive results (156-158). In cultured VSMC obtained from rats, angiotensin II induced a strong reduction of AGTR1 gene expression levels (156). This negative feedback has also been reported in human platelets, cells functionally similar to VSMC, where elevated concentrations of ATII downregulate AGTR1 mRNA concentration (158). In contrast, an earlier study found a reciprocal relationship between plasma ATII concentration and the number of AGTR1 in VSMC preparations (157). Similar results have been published in human mononuclear leukocytes, where expression of AGTR1 transcripts increases when exposed to high ATII concentration (158).

Monocytes/macrophages. Pro-inflammatory activity is enhanced by ATII in peripheral blood mononuclear cells. In this sense, human cultured peripheral blood monocytes stimulated by angiotensin II increment gene expression and protein release of TNF-α (159), MCP-1/MCP-2 (160) and IL-12(161). As in the case of VSMC, increased production of these molecules is dose-dependent, can be saturated and is sensitive to inhibition by AGTR1 blockers (159). Of interest, not only AGTR1 is present in monocytes (160); AGTR2 gene expression has also been identified during differentiation of monocytes into macrophages (162).

Lymphocytes. Evidence of the effects of ATII in lymphocytes is scarce. It has been reported that, *in vitro*, ATII stimulates the proliferation of CD3+ T cells and natural killer lymphocytes (163). Yet, angiotensin II can directly induce IFN-γ and TNF-α production by peripheral human T lymphocytes via AGTR1 (164, 165).

In this regard, AGTR1 has been demonstrated in the surface of human T cells (CD3+), B cells (CD19+), monocytes (CD14+) and polymorphonuclear leukocytes (PMN, CD16+) (158, 166, 167). In these leukocyte subsets, the receptor 1 of ATII was demonstrated in 87% of PMN, 71% of monocytes and 68% of B-lymphocytes but only in 3.5% of T-cells (167). Particularly for the minor subset of T-lymphocytes that express AGTR1, a specific functional role, probably with T*reg* properties has been hypothesized (165). Until now, the physiologic relevance of AGTR1 in immune cells remains poorly understood.

Endothelial cells. VCAM-1 gene and protein expression are upregulated by ATII in rat cultured aortic endothelial cells (168). The same results have been reported with other adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and E-selectin (169, 170).

In addition to its described pro-inflammatory functions, ATII is also involved in vascular remodeling. In mouse models, chronic infusion of ATII promotes aortic wall inflammation (171), and thoracic aneurysms or dissections (172). The effects observed in vascular tissue are mediated by up-regulation of VEGF, EGF receptor, PDGF receptor and insulin-like growth factor receptor (171, 173-176).

Angiotensin II-induced inflammatory pathways. Nuclear factor *kappa* B (NF-κB) activation has been repeatedly demonstrated as a critical element through which angiotensin II exerts its inflammatory effects (154, 169, 177-180). This transcription factor is of particular importance in vascular disease, as it controls the expression of a variety of cytokines (IL-1β, IL6, TNF-α, and IL-8), chemokines (MCP1, CCL5), and adhesion molecules (VCAM-1, ICAM-1) that promote mononuclear infiltration and inflammation (149, 154, 177, 181-183). Interestingly, NF-kB also stimulates ATII and angiotensinogen gene expression, thereby amplifying the angiotensin II-mediated inflammatory cascade (169).

3.3. Angiotensin II system and autoinmune disorders.

Alterations in the ATII system have been described in inflammatory diseases affecting the brain and vasculature. Yet, certain genetic polymorphisms in the RAS system have been associated with increased susceptibility to developing autoimmune diseases (184-187). Of particular importance is the ACE gene, as plasma and tissue concentrations of ACE, and therefore levels of angiotensin II, are determined in part by insertion (I) or deletion (D) polymorphisms of this gene (188).

Multiple Sclerosis. Increased evidence relates multiple sclerosis (MS), the most common autoimmune inflammatory demyelinating disease of the central nervous system, with abnormalities in RAS. It has been suggested that ACE may play a role in the pathogenesis and progression of multiple sclerosis, as increased activity of this enzyme has been identified in blood and cerebrospinal fluid of affected patients (189). According to this observation, ACE DD gene polymorphisms has been associated with an increased risk of developing MS in men from Eastern European origin (184). In addition, in experimental animal models of MS, inhibition of ATII actions by lisinopril or losartan reversed the clinical manifestations of the disorder (190, 191).

Systemic Sclerosis (SSc). Serum level of ATII is elevated in SSc patients and this peptide probably plays a role in the characteristic vascular injury and tissue fibrosis observed in affected individuals (192). In addition, agonistic auto-antibodies against AGTR1 have been identified in the sera of SSc patients (193). These antibodies are able to induce inflammatory reactions as demonstrated in a very recently publication (194). In this study, performed on 18 patients with the diffuse form of systemic sclerosis, SSc-IgG antibodies directed against AGTR1 induced *in vitro* T-cell migration as well as IL-8 and CCL18 secretion from peripheral blood mononucleated cells. These effects were significantly reverted by AGTR1 pharmacological antagonism (194).

Of note, AGTR1 density was significantly decreased on CD3+ T-cells and CD14+ monocytes of affected patients, which was probably related to chronic activation induced by both elevated ATII levels and agonistic antibodies.

Systemic Vasculitis. ACE I/D polymorphisms have been associated with increased susceptibility to systemic vasculitis (185), in particular IgA vasculitis (186) and Behçet's disease (187). In addition, as reported in SSc (194), reduced AGTR1 expression has been reported in VSMC of arteries from nasal biopsies of patients with granulomatosis with polyangitis (GPA, Wegener´s), an ANCA-associated systemic vasculitis (195). Based on these data, some authors have suggested that RAS may play a role in the pathogenesis of vascular inflammation in certain vasculitides (185).

3.4. Angiotensin II system and Giant cell arteritis (GCA).

Three previous publications have partially explored some aspects of the ATII system in patients with GCA (196-198). In an earlier study performed in the 80s decade, serum ACE activity was found to be significantly lower in GCA than in control patients, although it increased during GC treatment (196).

More recently, the expression of AGTR1 and AGTR2 was investigated by immunohistochemistry in temporal arteries of 10 GCA patients and 10 controls (198). Results of this study showed a mild increase of AGTR1 in VSMC of GCA patients. In addition, AGTR1 immunostaining was observed in lymphocytes, histiocytes and multinucleated giant cells of inflammed lesions. Only faint immunostaining was seen for AGTR2, primarily in the endothelial cell layer (198).

3.5 The ACE2/AT (1-7)/Mas receptor pathway.

In addition to the ACE/ATII/AGTR1 pro-inflammatory axis described in the previous paragraphs, a major counter-regulatory pathway with anti-inflammatory properties (i.e., ACE2/AT (1-7)/Mas receptor) has gained attention in recent years (129, 145).

Evidence now suggests that a balance between the activation of both divergent systems plays an important role in the function of several organs and that an imbalance in these opposing pathways towards the ACE/ATII/AGT1R axis may predispose to cardiovascular and renal disorders (199). In fact, most of the information about the ACE2 alternative RAS pathway derive from experimental and clinical studies investigating renal diseases which include diabetic and hypertensive nephropathy (200). In the next lines we summarize some of the available data regarding the role of the ACE2/AT (1-7)/Mas receptor axis in inflammation.

AT (1-7) may be formed from the hydrolysis of angiotensin I by several tissue endo-peptidases (i.e., prolyl-endopeptidase, neutral endopeptidase, and oligopeptidase) and most importantly, by the cleavage of ATII by ACE2 (200, 201). The Mas receptor, a G-protein coupled receptor highly expressed in testis, hypothalamus, and amygdala, has been recently identified as the protein transducing the actions of AT (1-7)(202, 203). The ACE pathway appears to be an important mode of inactivation of circulating AT (1–7) and, on the other hand, ACE 2 is an inhibitor of the formation of ATII by stimulating alternative pathways for angiotensin I degradation (127, 129). Based on this dynamic interaction, the ratio of ACE/ACE2 seems to be of relevance.

Former studies suggested that AT (1-7) pathway expression and activity is reduced in some inflammatory conditions (200). In this sense, experimental evidence using genetic deletion of ACE2 in ApoE gene knockout mice demonstrated an increase in vascular inflammation by enhancing the expression of IL-1β, IL-6, MCP-1 (CCL-2), vascular cell adhesion molecule 1, and matrix metalloproteinases in excised aortic tissue (204, 205). Furthermore, ACE2 deficiency led to greater increases in ATII-mediated NADPH oxidase activity and superoxide/peroxynitrite production in the aortic tissue of this animal model (205).

The signaling mechanisms by which AT (1-7) exerts their pleiotropic anti-inflammatory actions remain under investigation (200).

One possible mechanism is through the ERK1 and NF-κB signalling pathways, as activation of the Mas receptor by AT (1-7) reduces the phosphorylation of c-Src, and IκBα, an inhibitor of NF-κB (129, 161, 206, 207). In addition, in VSMC, AT (1-7) inhibited ATII stimulation of mitogen-activated protein kinase activities (ERK1/2) (208). Also, in apoE knockout mice, AT (1–7) diminished NADPH oxidase vessel expression, which is involved in the production of reactive oxygen species, and reduced the expression of co-stimulatory molecules on antigen-presenting cells (209, 210). In addition, AT (1-7) seems to enhance the vasodilator and metabolic actions of bradykinin (211). Moreover, *in vitro* treatment of mouse peritoneal macrophages with AT (1-7) induced a reduction in the release of pro-inflammatory cytokines IL-6 and TNF-α after lipopolysaccharide stimulation (212). Reduction of pro-inflammatory molecules has also been observed in a mouse experimental model of inflammatory arthritis (213). In this model, treatment with AT (1-7) ameliorates synovial tissue expression of IL-1β and CXCL1 (213). Other anti-inflammatory actions of AT (1-7) include its direct antagonism on AGTR1, as demonstrated in rat renal cortex (214).

Remarkably, some of the anti-inflammatory effects observed with ACEI or ARB could be related to alterations in the balance of ACE/ACE2. In this regard, ACE inhibitors increase plasma concentration of AT (1–7), due in part to the increase in angiotensin I and in part to reduced AT (1–7) degradation by ACE (215). More importantly, pharmacological blockade of AGTR1 increases the expression and activity of ACE2, and, consequently, circulating levels of AT (1-7) (205). This effect may be due to prolyl-endopeptidase-mediated AT (1–7) formation from ATII (216). In contrast to the anti-inflammatory properties described in the cardiovascular system, the role ACE2/Ang-(1-7)/Mas receptor in other tissues such as kidneys is controversial. Recent data in cultured renal cells demonstrated that ACE2 activity may exhibit pro-inflammatory effects, as the stimulation of ACE2 axis in these cells activated NF-κB (217). More information is needed to elucidate the functional role of the ACE2/Ang-(1-7)/Mas receptor pathway in other organs of human body.

In summary, the RAS components not only undergo a profound alteration in inflammation but also have a functional role in this scenario (129). As a consequence, there is a potential for the ATII system to offer molecular targets for therapeutic intervention in inflammatory diseases.

II. HYPOTHESIS

In giant cell arteritis, many important questions still await satisfactory answers. Which factor triggers this disease? What are the essential pathways involved in maintaining inflammatory activity? Which ones are efficiently suppressed by glucocorticoids? Does apparent clinical remission always imply disease suppression? It is conceivable that different patterns of inflammation are associated with particular disease presentations and outcomes?

Currently, no therapeutic intervention has been found to achieve the clinical benefit elicited by glucocorticoids in GCA, although this remains unsatisfactory. Therefore, a better understanding of the molecular mechanisms involved in the pathogenesis of GCA should provide new targets for therapy.

Based on this scenario, our initial working hypothesis was:

1. Clinical, laboratory and outcome of GCA is similar in different ethnic populations and is characterized by an intense inflammatory activity and relapsing course.
2. Persistence of disease activity is associated with increased morbidity.
3. Concomitant administration of commonly used drugs with anti-inflammatory pleiotropic effects might facilitate sustained clinical remission in GCA patients.
4. Angiotensin II system is involved in pro-inflammatory pathways relevant to persistence of disease activity in GCA and, therefore, blockade of angiotensin II effects might be potentially useful as adjunctive therapy.

III. OBJECTIVES

General objective.

To investigate the main clinical and biochemical characteristics derived from persistent chronic inflammation in GCA and to identify essential pathways involved in maintaining disease activity, which may lead to the develop of new therapeutic approaches.

Specific objectives.

- First article. To describe the clinical presentation, laboratory, and follow-up findings of a Mexican Mestizo population with GCA.

- Second article. To investigate the prevalence, timing, predictors and characteristics of relapses in a longitudinally followed cohort of patients with GCA with long-term follow-up. In addition, we analyzed whether a relapsing course was associated with disease-related complications, increased glucocorticoid doses, and treatment-related adverse effects.

- Third article. To determine whether concomitant treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers is associated with changes in the outcome of patients with giant cell arteritis (GCA).

- Fourth article. To investigate the expression and functional activity of the angiotensin II system in temporal artery lesions from GCA patients, and its relationship with disease outcome.

IV. RESULTS

The present doctoral thesis is a compendium of the following articles:

I. **Giant cell arteritis in Mexican patients**. J Clin Rheumatol. 2012 Jan;18(1):1-7. (IF: 1.245)

II. **Relapses in Patients With Giant Cell Arteritis: Prevalence, Characteristics, and Associated Clinical Findings in a Longitudinally Followed Cohort of 106 Patients.** Medicine (Baltimore). 2014 Jul; 93(5):194–201. (IF 4.23)

III. **Treatment with angiotensin II receptor blockers is associated with prolonged relapse-free survival, lower relapse rate, and corticosteroid-sparing effect in patients with giant cell arteritis.** Semin Arthritis Rheum. 2014 Jun;43(6):772-7. (IF: 3.62)

IV. Expression of the angiotensin II system in temporal artery lesions from patients with giant cell arteritis. (Submitted)

Giant Cell Arteritis in Mexican Patients

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Background: Giant cell arteritis (GCA) is the most common primary systemic vasculitis worldwide, although it seems to be very rare in some areas, such as Latin America.

Objectives: The objective of the study was to describe the clinical, laboratory, and treatment features in a Mexican Mestizo population with GCA.

Methods: Retrospective data chart review (1989–2010).

Results: Twenty-two patients with GCA were identified, 18 women and 4 men. Mean age was 73 (SD, 7.9) years. Diagnosis was made at a mean of 67 (SD, 83.6) days from symptom onset. Most frequent presenting symptoms included headache (90%), constitutional symptoms (86%), and polymyalgia rheumatica (59%). Severe cranial ischemic complications were present in 32%. Amaurosis fugax and blindness were present in 36% and 27%, respectively. High erythrocyte sedimentation rate was present in 89% of patients. Rapid response to prednisone treatment was seen, but in 10 patients, relapse occurred, possibly related to fast tapering. Additional treatment was methotrexate (n = 8), azathioprine (n = 5), and cyclophosphamide (n = 3). Median follow-up was 242 (SD, 214) weeks.

Conclusions: Giant cell arteritis is rarely recognized in Latin America. We report on characteristics of GCA in a population of Mexican Mestizos, as ours is the largest series to be reported from Latin America so far. When compared with other series, age at onset is similar, females are more affected, and although a good response to corticosteroid treatment was seen, a higher frequency of amaurosis fugax and blindness was observed, accounting for an unfavorable functional outcome in 6 (27%) of 22 patients.

Key Words: giant cell arteritis, cranial ischemia, amaurosis fugax, Mexico, Mestizos

(*J Clin Rheumatol* 2012;18: 1–7)

Giant cell arteritis (GCA), a vasculitis that involves large and medium arteries, especially the extracranial branches of the external carotid artery, is considered worldwide to be the most common primary systemic vasculitis. It has the highest incidence in northern Europe,[1] as compared with the South,[2] with the lowest rates found in Asian and Arabic countries.[3,4] Data of this condition in a so-called Hispanic population are scarce. In a series of 121 patients with GCA suspicion in California, no case was observed in 40 "Hispanics."[5] A recent study in Florida[6] found 65 "Hispanics" of 257 (25%) GCA patients within a span of 7 consecutive years. However, only 13 (20%) had biopsy-proven GCA, the majority being Cuban-American. Therefore, data on this ethnic group are based on figures from the United States. There is only 1 study about GCA in Latin America, which reported on 11 patients from Puerto Rico.[7] As a result, data on the disease including its clinical behavior are virtually absent from that geographic area. This is complicated by the fact that symptoms of the disease and laboratory findings are nonspecific. For this reason, the purpose of the present report was to describe the clinical presentation, laboratory, and follow-up findings in a Mexican population attending a nationwide referral institution that mainly serves patients from the central and southern regions of the country.

PATIENTS AND METHODS

A retrospective review of medical records from patients with GCA seen from 1989 to 2010 at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán in Mexico City was done. Retrieved data included sex, age at diagnosis, cardiovascular risk factors (diabetes mellitus, arterial hypertension, dyslipidemia, chronic renal failure, smoking, and cardiac arrythmias), initial manifestations (systemic symptoms, polymyalgia rheumatica [PMR], headache, cranial ischemic events, abnormal temporal arteries on physical examination), and the disease symptoms throughout (recurrences, aneurysm development, or late ischemic complications). Treatment, response to it, and therapy-related complications were also assessed. Laboratory information recorded was erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), hemoglobin (Hb) value, white blood cells (WBCs), and platelet counts. Definitions of the 1990 American College of Rheumatology Criteria (ACR) for the classification of GCA were used, and diagnosis was made if patients fulfilled at least 3 of these criteria, coupled with exclusion of other causes explaining symptoms.[8] All diagnoses were made by rheumatologists. Systemic symptoms included fever, anorexia, and weight loss of at least 10% the total body weight within 6 months. Polymyalgia rheumatica was also sought and recorded.

Data on the following cranial ischemic manifestations were recorded: scalp tenderness, jaw claudication, amaurosis fugax, permanent visual loss, diplopia, stroke, and transient ischemic attacks. Patients were considered to have severe permanent cranial complications (SCICs) if they developed permanent visual loss, diplopia, or stroke. The occurrence of other type of ischemic complications (i.e., limb claudication, intestinal ischemia) was also retrieved. Based on previous studies,[9–11] neuroophthalmologic manifestations were considered GCA related if they occurred between disease onset and 2 weeks after the diagnosis. Later cranial ischemic complications, as during tapering or discontinuation of prednisone, were considered GCA associated only when concurrent with clear-cut disease activity plus rise of ESR or CRP. Time and cumulative dose of prednisone received until the patients reached a maintenance dose of less than 10 mg/d not followed by a relapse during at least 3 months were calculated. Anemia was defined by Hb levels of less than 12 g/dL, leukocytosis as WBCs of greater than 11,000/μL, and thrombocytosis as platelets of greater than 450,000/μL, and the initial ESR was considered elevated if 50 mm/hr or higher, as

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TABLE 1. Individual Data of 22 Mexican Mestizo GCA Patients

	Sex	Age, y	No. Fulfilled ACR Criteria	Symptoms Duration, d	Systemic Symptoms	Presence of New-Onset Headache	Initial Symptoms	Severe Ischemic Complications	Abnormal TA on Physical Examination[a]	ESR, mm/hr	Temporal Biopsy
1	F	70	3	60	Fever, anorexia weight loss	Y	Jaw claudication, odynophagia, blurred vision	None	N	NR	Positive
2	M	84	3	28	N	N	Blurred vision	Amaurosis fugax, blindness	Y	9	Positive
3	F	62	4	30	Fever, weight loss	Y	Persistent cough	None	N	146	Positive
4	F	78	3	60	Fever, weight loss	N	PMR, jaw claudication, ear pain	None	N	105	Positive
5	F	77	3	ND	N	Y	PMR	N	N	NR	Positive
6	M	82	5	150	Weight loss	Y	Diplopia	Amaurosis fugax, diplopía, transitory ischemic attack	Y	55	Positive
7	F	77	4	45	Fever, anorexia	Y	PMR, jaw claudication, scalp tenderness, ocular pain, blurred vision	N	Y	40	Positive
8	M	66	4	8	Anorexia, weight loss	Y	New-onset headache	Amaurosis fugax, blindness	Y	23	Positive
9	F	84	4	ND	Anorexia, weight loss	Y	PMR, jaw claudication, scalp tenderness, ocular pain, teeth pain, blurred vision	N	Y	46	Positive
10	F	77	3	ND	N	Y	PMR, jaw claudication, blurred vision	N	N	NR	Positive
11	F	74	4	4	Fever	Y	Blurred vision	N	Y	34	Positive
12	M	75	3	12	Fever	Y	PMR	N	N	106	Negative
13	F	66	3	365	Weight loss	Y	PMR	N	N	50	Negative
14	F	60	3	60	Fever, weight loss	Y	PMR, jaw claudication, ear pain	N	N	75	Negative
15	F	73	3	60	Weight loss	Y	PMR, jaw claudication, scalp tenderness	Amaurosis fugax, blindness	N	116	Negative
16	F	77	3	7	N	Y	New-onset headache	N	N	50	Negative
17	F	78	3	18	N	Y	Scalp tenderness, blurred vision	Amaurosis fugax, blindness	Y	2	Negative
18	F	74	4	45	Weight loss	Y	PMR	None	Y	104	Not performed
19	F	72	3	150	N	Y	PMR, scalp tenderness, ocular, ear and teeth pain, hearing loss	Amaurosis fugax, blindness	Y	34	Not performed
20	F	57	3	21	Fever, weight loss	Y	Ear pain, odynophagia, cough	Amaurosis fugax, blindness	Y	36	Not performed
21	F	61	4	90	N	Y	PMR, scalp tenderness, ocular pain	N	Y	50	Not performed
22	F	83	4	60	Fever	Y	PMR, jaw claudication, ear pain, hearing loss	Amaurosis fugax	Y	108	Not performed

Y, Yes; N, no; ND, not detailed; NR, not recorded.

[a]Tenderness or decreased pulse of the temporal artery on physical examination.

per the ACR criteria.[8] Clinical and laboratory characteristics of patients with and without severe permanent cranial ischemic complications were compared. The study was conducted in accordance with the 1964 Declaration of Helsinki standards.

Statistical Analysis

Continuous variables are presented as mean (SD) and categorical data as percentages. Association between ischemic events and selected covariates was analyzed using Student t test for quantitative variables and χ^2 test for categorical data. Statistical significance was defined as $P < 0.05$. Calculations were performed with the statistical package PAWS statistics version 18 (SPSS, Inc., 2009, Chicago, IL).

RESULTS

Clinical and Laboratory Characteristics at Diagnosis

Between January 1989 and June 2010, 22 patients with GCA were identified. Ten were born in Mexico City, and 2 in Tabasco, a state located in Southeast Mexico (related patients, mother, and daughter), whereas the rest were from other states through Mexico (one each). All, except one (whose father was Italian) had Mexican parents and grandparents and were therefore Mexican Mestizos, showing black lank hair, colored skin, high cheekbones, and shovel incisive teeth. Mean age at diagnosis was 73 (SD, 7.9) years with a male-female ratio of 1:4.5 (our country's life expectancy for males is 73 years, and for females, 78 years). Mean time between the first symptom and diagnosis was 67 (SD, 83.6) days. The most common symptom at presentation was headache (91%). Constitutional symptoms and PMR were present in 86% and 59% of patients, respectively. Physical examination of temporal arteries was abnormal in 12 patients (54.5%). Seventeen patients had unilateral temporal artery biopsy, with 11 presenting typical findings of GCA with giant cells, granulomas, elastic laminae fragmentation, myointimal hyperplasia, and/or mononuclear cell infiltration.

The initial levels of Hb, ESR, and platelet counts were available in 19 patients, and the WBC count in 18 patients. Anemia, thrombocytosis, and leukocytosis were detected in 32%, 37%, and 55% of patients, respectively. Erythrocyte sedimentation rate of greater than 50 mm/hr was found in 58%. C-reactive protein was done in only 7 cases, being high in 4. Table 1 shows the relevant data of each patient, whereas Table 2 compares the

TABLE 2. Baseline Characteristics and Clinical Presentation of GCA Patients According to Biopsy Status

	Positive Biopsy Findings	Negative Biopsy Findings	Biopsy not Performed	Whole Group
	n = 11	n = 6	n = 5	n = 22
General characteristics				
Age, mean (SD) (range), y	75 (7) (62–84)	71 (7) (60–78)	69 (10) (57–83)	73 (7.9) (57–84)
Sex, no. male/female (%)	3/8 (27/73)	1/5 (17/83)	0/5 (0/100)	4/18 (18/82)
Symptoms duration, mean (SD), d	48 (46)	87 (138)	73 (49)	67 (83.6)
Cardiovascular risk factors, n (%)	10 (53)	4 (21)	5 (26)	19 (86)
No. fulfilled GCA ACR criteria, n (%)				
3	5 (45.5)	6 (100)	2 (40)	13 (59%)
4	5 (45.5)	0 (0)	3 (60)	8 (36%)
5	1 (9)	0 (0)	0 (0)	1 (4.5%)
Clinical symptoms at diagnosis, n (%)				
Headache	9 (82)	6 (100)	5 (100)	20 (91)
Jaw claudication	5 (45.5)	2 (33)	1 (20)	8 (36)
Scalp tenderness	2 (18)	2 (33)	2 (40)	6 (27)
Amaurosis fugax	3 (27)	2 (33)	3 (60)	8 (36)
Facial pain[a]	5 (45.5)	1 (17)	4 (80)	10 (45.5)
PMR	5 (45.5)	4 (67)	4 (80)	13 (59)
Systemic manifestations, n (%)				
Fever	5 (45.5)	2 (33)	2 (40)	9 (41)
Anorexia	4 (36)	0	0	4 (18)
Weight loss	6 (54.5)	3 (50)	2 (40)	11 (50)
Severe permanent cranial ischemic complications, n (%)	3 (27)	2 (33)	2 (40)	7 (32)
Abnormal temporal artery on examination,[b,c] n (%)	6 (54.5)	1 (17)	5 (100)	12 (54.5)
Baseline abnormal laboratory parameters, n (%)				
Elevated ESR (n = 19)	7 (87.5)	5 (83)	5 (100)	17 (89)
Elevated CRP (n = 7)	3 (100)	0 (0)	2 (67)	5 (71)
Anemia (n = 19)	3 (37.5)	2 (33)	1 (20)	6 (32)
Thrombocytosis (n = 19)	5 (62.5)	2 (33)	0 (0)	7 (37)
Leukocytosis (n = 18)	5 (62.5)	2 (40)	3 (60)	10 (55)

[a]Includes lingual, ocular, ear and tooth pain, odynophagia, carotidynia, and persistent cough.

[b]Includes artery tenderness or hardness, weak or absent pulse, or other inflammatory signs.

[c]No differences were found between the groups except for abnormal temporal artery on physical examination ($P = 0.02$).

TABLE 3. Percentage Comparison of Clinical Manifestations and Laboratory Parameters in Our Series and Others (Round Numbers)

Clinical/ Laboratory Data	Present Series n = 22	González-Gay et al[12,13] n = 240	Cid et al[14] n = 200	Liozon et al[15] n = 175	Berger et al[9] n = 85	Huston et al[16] n = 42
Headache	91	84.5	76	77	87	90
Jaw claudication	36	41	43	39	53	67
Scalp tenderness	27	34	40	51	32	69
PMR	59	40	49	26	62	48
Fever	41	9.5	50	54	21	21
Weight loss	50	NR	55	51	34	55
Amaurosis fugax	36	12	10	19	11	12
Blindness	27	13	14	12	32	10
ESR >20 mm/hr	89	100	NR	93	96	NR
Anemia	32	55	NR	64	NR	NR
Thrombocytosis	37	49	NR	84	53	NR
Leukocytosis	55	28	NR	NR	NR	NR

NR, Not reported.

baseline characteristics of patients according to temporal artery biopsy status. In Table 3, a comparison of the clinical and laboratory findings with other series[9,12–16] is shown (in all tables, percentages have been rounded off).

Severe Ischemic Complications

Seven patients (32%) developed SCICs, 6 with permanent blindness, 5 of them unilateral, and one, who initially had monocular blindness, went on to bilateral visual loss despite glucocorticoid (GC) treatment. The other patient had permanent diplopia. Although 1 patient had a transitory ischemic attack, he recovered completely.

When comparing patients with and without SCICs, we found no differences in age, cardiovascular risk factors, presence of constitutional symptoms, or time between beginning of symptoms and diagnosis. The most important difference between both groups was the presence of amaurosis fugax, which developed in all patients with SCICs versus only one of patients without these complications ($P < 0.0001$). The mean value of ESR was also different between both groups, 39.2 (SD, 38.1) mm/hr in patients with ischemia compared with 76.1 (SD, 36.2) mm/hr in nonischemic patients ($P = 0.05$). Patients with SCICs received a mean dose of 58.5 (SD, 3.7) mg of prednisone for initial treatment compared with 45.7 (SD, 10.9) mg of patients without severe ischemia ($P = 0.008$). Trends to differences in presence of PMR and abnormal temporal arteries on examination were also seen between groups but were not statistically significant ($P = 0.07$). Table 4 shows the characteristics of patients presenting with and without severe cranial ischemic events. We also found that patients with jaw claudication had thrombocytosis (83% vs. 15%, $P = 0.01$) and leukocytosis (100% vs. 33%, $P = 0.01$) more frequently than those without it.

Follow-Up

Total mean follow-up was 242 (SD, 214) weeks. Ten patients had a relapse (45.5%). Interestingly, all patients in whom no biopsies were done had a relapse (100% vs. 27% of those with positive biopsy vs. 33% of patients with negative biopsy, $P = 0.02$). Two patients died, none due to GCA-related complications. The whole group received prednisone with a mean initial dose of 50.2 (SD, 10.9) mg/d. Intravenous methylprednisolone was prescribed in 3 patients because of anterior ischemic optic neuropathy (confirmed by ophthalmologists). Of 22 patients, 10 achieved sustained remission without the need of GC or immunosuppressive therapy at a mean of 94.8 (SD, 65.7) weeks. Other treatments given are shown in Table 5. Adverse

TABLE 4. Clinical Characteristics of Patients Presenting With Severe Permanent Cranial Ischemic Events (Group 1) and Patients Without Ischemic Complications (Group 2)

Clinical Characteristics	Group 1 n = 7	Group 2 n = 15	*P*
General characteristics			
Age, mean (SD) (range), y	73 (9)	73 (7)	NS
Sex, no. male/female	3/4	1/14	0.07
Cardiovascular risk factors, n (%)	7 (100)	12 (80)	NS
Clinical symptoms at diagnosis, n (%)			
Headache	6 (86)	14 (93)	NS
Jaw claudication	1 (14)	7 (47)	NS
Scalp tenderness	3 (43)	3 (20)	NS
Amaurosis fugax	7 (100)	1 (7)	<0.0001
PMR	2 (29)	11 (73)	0.07
Abnormal temporal artery, n (%)	6 (86)	6 (40)	0.07
Baseline laboratory parameters, mean (SD)			
ESR, mm/hr	39.2 (38.1)	76.1 (36.2)	0.05
Corticosteroid requirements, n (%)			
Initial prednisone dose, mean (SD), mg	58.5 (3.7)	45.7 (10.9)	0.008

There were no significant differences between both groups in duration of symptoms, percentage of patients with diabetes mellitus, hypertension, chronic renal failure, hypercholesterolemia, smoking, facial pain, blurred vision, fever, anorexia, weight loss, time of follow-up, number of relapses, late ischemic cranial or vascular complications, use of additional immunosuppressive therapy, treatment-related adverse effects, or initial levels of CRP, Hb, platelets, and white cells.

NS, Not significant.

TABLE 5. Clinical Characteristics During Follow-Up

General characteristics	
Follow-up time, mean (SD), wk	242 (214)
Relapses, n (%)	10 (45.5)
No. relapses, mean (SD) (range)	1.8 (1.13) (1–4)
Deaths, n (%)	2 (9)
Vascular complications	
Aneurysm development[a]	0
Limb claudication (arterial stenosis), n (%)	2 (9)
Corticosteroid requirements	
Initial prednisone dose, mean (SD), mg/d	50.2 (10.9)
Time to reach a maintenance prednisone dose <10 mg/d, mean (SD), wk	41.6 (18.4)
Cumulative dose of prednisone at time of reaching <10 mg/d, mean (SD), mg	6464 (3954)
Cumulative dose of prednisone at 1 y of follow-up, mean (SD), mg	7560 (3582)
Time to stop prednisone, mean (SD), wk	94.8 (65.7)
Cumulative dose of prednisone at time 0, mean (SD), mg	7661 (2793)
Immunosuppressive therapy,[b] n (%)	13 (59)
Methotrexate, n (%)	8 (36)
Azathioprine, n (%)	5 (23)
Cyclophosphamide, n (%)	3 (14)
Treatment-related adverse effects, n (%)	11 (50)
Cushing syndrome	7 (32)
Osteoporosis	2 (9)
Diabetes mellitus	2 (9)

[a]Not intentionally sought; no clinical or paraclinical evidence of aneurysms from available data.

[b]Some patients were prescribed more than 1 immunosuppressive drug.

effects were reported in 50% of patients. Table 5 displays follow-up characteristics.

DISCUSSION

Giant cell arteritis is a complex disease, in which not only genetic traits but also environmental factors may explain the differences observed concerning geoepidemiology of the disease. It does seem very rare in Latin America, a region where most of the European heritage is of Spanish origin, but in contrast to Spain, GCA is uncommon. The influence of the native genetics in tempering the development of GCA is to be considered, as the interplay between Amerindian and white genes seems to influence the susceptibility to certain autoimmune diseases.[17] In an attempt to know the characteristics of GCA in our country, we retrospectively reviewed the information of all cases attending our referral center, which constitutes the largest series in Latin Americans outside the United States, with all except one, being Mexican Mestizos.

When compared with other series,[9,12–16] we want to highlight the following differences: a greater proportion of the disease was found in women, and scalp tenderness was less reported by our patients (27%) as compared with 33% to 69% observed by others.[15,16] These slight differences are similar to those from a comparative study between regions in Northwestern Spain and Northern Italy in which few clinical differences were found despite dissimilar geographic and genetic factors.[18] As for laboratory parameters, increased ESR levels were slightly less frequent, compared with numbers up to 100% of patients having high ESR. It is worth mentioning that normal levels have also been reported.[19] We shall mention that before ESR measurements, 3 patients were prescribed GC. This was due to amaurosis fugax and a high suspicion of GCA by the referring physicians. Of the remaining 16 patients, all but 5 had initial ESR of greater than 50 mm/hr.

The most common and important vascular complications in GCA are cranial ischemic events.[14] In fact, one of the treatment aims is to prevent further ischemia and long-term vascular complications.[20] The reported frequencies of GCA ischemic complications lay between 16% and 73%.[9,14,21–24] Our findings are in agreement with this, and 32% of our patients developed a cranial ischemic event. Most relevant, we observed amaurosis fugax and permanent visual loss more frequently and as part of the initial manifestations. This does not seem to be related to delayed treatment initiation because, although GCA is rare in our population, the interval between the initial symptoms, diagnosis, and institution of GC therapy was similar to that reported in countries where the disease is more frequent.[12–14] Because SCICs are a major source of chronic disability[14] with small chances of improvement after they occur, there have been attempts to identify their risk factors in GCA. They include previously transient ischemic episodes,[14,25] atherosclerosis risk factors,[26] low inflammatory response,[14,27] scalp necrosis,[28] jaw claudication,[10] presence of giant cells in temporal artery biopsy,[29] trombocytosis,[30] and ESR between 70 and 100 mm/hr.[23] In the present series, amaurosis fugax was reported in all individuals with SCICs except one. This suggests that some patients may be at particular risk for developing ischemic complications, and in our population, this needs to be identified.

In general, despite the initial good response to GCs, chronic complications are common in GCA, and increasing frequency of vascular complications such as aortic aneurysms and symptomatic stenosis of large vessels develops in approximately 11% to 22% and 5% to 15% of patients, respectively.[31–34] We did not find aortic aneurysms, although they were not intentionally sought, whereas 2 patients developed limb claudication related to arterial stenosis. Very importantly, clinical flares are seen in 50% to 90% of patients, occurring mostly in the first year after treatment start.[35–38] We found that 45.5% of patients had at least 1 relapse when prednisone was being tapered. A recent study identified that the median dose of prednisone at time of first relapse was 5 mg every day, and at time of diagnosis, the presence of anemia conferred relapse risk.[39]

We found a difference in the initial prednisone dose used to treat patients with and without SCICs, probably related to a more aggressive treatment in the hope to prevent further ischemic events. Of interest, although GCs are the mainstay of treatment, approximately 86% of patients experience at least 1 adverse effect over a median follow-up period of 10 years.[37] In our series, 50% of patients developed Cushing syndrome, osteoporosis, or diabetes mellitus.

Concerning the treatment with other immunosuppressive agents (Table 5), we need to recall the retrospective nature of the data, and thereof, from the chart reviews, the prescription's decisions seem to have been to taper corticosteroids faster or as adjuvant therapy in cases where disease was severe. Their contribution to the disease course is impossible to determine.

We are much aware of the limitations of our report: its retrospective nature and, because of that, the lack of standardized data collection and the fact that different physicians were responsible for the care of the patients, with no uniform diagnostic, treatment, and follow-up data retrieval set. As examples, not all patients had baseline ESR or CRP, nor did we have complete information of all the variables sought. Five patients had no

temporal artery biopsy done, but from those in whom it was performed, half had positive temporal artery biopsy, a percentage higher than the one reported from the Florida study[6]; despite this, all patients fulfilled at least 3 (or more) of the 1990 ACR classification criteria for GCA, all responded well to corticosteroid treatment, and the follow-up did not reveal alternative diagnosis. Finally, its small number, which precludes more firm comparisons with populations in which prevalence and incidence of GCA are known, suggests some differences that may be important for physicians in our region.

In conclusion, this is the largest series known from Latin America, suggesting the rarity of GCA in more austral countries, with a female predominance but similar age at onset as in the rest of the world. The appearance of the disease was marked by amaurosis fugax and permanent visual loss from the beginning, whereas the remaining course was similar to other reports. Response to prednisone was also comparable, with rapid improvement. Although we acknowledge our results and comparisons with other populations should be cautiously interpreted, the main aim was to describe the characteristics of an extremely rare, although possibly underrecognized, disease in our area, especially by physicians not familiar with its clinical spectrum. We hope this will foster further interest to GCA and its related condition, PMR, in our region, as there are no epidemiologic data on them.

KEY POINTS

(1) Giant cell arteritis is infrequent in Latin America, specifically in Mexican Mestizos, but it does occur as shown in this series, the largest so far from the region.
(2) It may have different clinical characteristics, some of them severe even at onset (amaurosis fugax and blindness), but if these are readily treated, they can respond favorably to corticosteroids, as in larger series.
(3) We hope that more frequent and earlier recognition of this disease in our countries, otherwise the most frequent vasculitis worldwide, stimulates research in our area, especially to identify different patterns of the disease. This may have local implications for diagnosis, treatment, and outcome.

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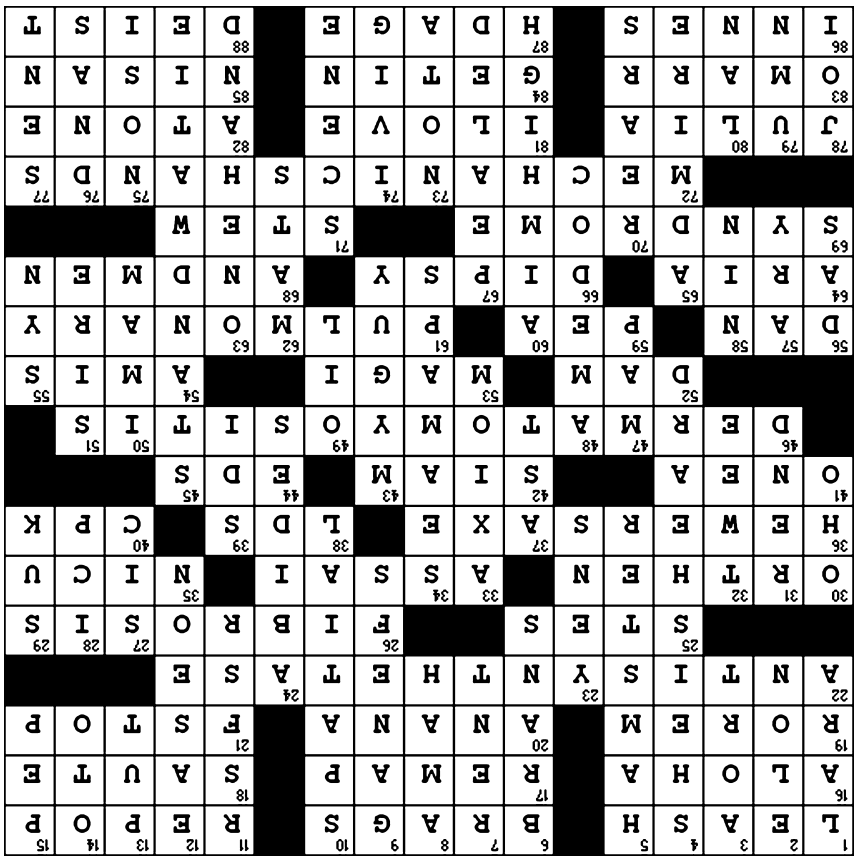

Relapses in Patients With Giant Cell Arteritis

Prevalence, Characteristics, and Associated Clinical Findings in a Longitudinally Followed Cohort of 106 Patients

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Abstract: Giant cell arteritis (GCA) is a relapsing disease. However, the nature, chronology, therapeutic impact, and clinical consequences of relapses have been scarcely addressed. We conducted the present study to investigate the prevalence, timing, and characteristics of relapses in patients with GCA and to analyze whether a relapsing course is associated with disease-related complications, increased glucocorticoid (GC) doses, and GC-related adverse effects. The study cohort included 106 patients, longitudinally followed by the authors for 7.8 ± 3.3 years. Relapses were defined as reappearance of disease-related symptoms requiring treatment adjustment. Relapses were classified into 4 categories: polymyalgia rheumatica (PMR), cranial symptoms (including ischemic complications), systemic disease, or symptomatic large vessel involvement. Cumulated GC dose during the first year of treatment, time required to achieve a maintenance prednisone dose <10 mg/d (T10), <5 mg/d (T5), or complete prednisone discontinuation (T0), and GC-related side effects were recorded. Sixty-eight patients (64%) experienced at least 1 relapse, and 38 (36%) experienced 2 or more. First relapse consisted of PMR in 51%, cranial symptoms in 31%, and systemic complaints in 18%. Relapses appeared predominantly, but not exclusively, within the first 2 years of treatment, and only 1 patient developed visual loss. T10, T5, and T0 were significantly longer in patients with relapses than in patients without relapse (median, 40 vs 27 wk, $p < 0.0001$; 163 vs 89.5 wk, $p = 0.004$; and 340 vs 190 wk, $p = 0.001$, respectively). Cumulated prednisone dose during the first year was significantly higher in relapsing patients (6.2 ± 1.7 g vs 5.4 ± 0.78 g, $p = 0.015$). Osteoporosis was more common in patients with relapses compared to those without (65% vs 32%, $p = 0.001$). In conclusion, the results of the present study provide evidence that a relapsing course is associated with higher and prolonged GC requirements and a higher frequency of osteoporosis in GCA.

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Abbreviations: CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, GC = glucocorticoids, GCA = giant cell arteritis, Hb = hemoglobin, IQR = interquartile range, PDN = prednisone, PMR = polymyalgia rheumatica, SD = standard deviation, SIR = systemic inflammatory response, TNF = tumor necrosis factor.

INTRODUCTION

Giant cell arteritis (GCA) is a granulomatous arteritis predominantly affecting large and medium-sized vessels.[18,27] Treatment with high-dose glucocorticoids (GC) results in prompt and remarkable improvement of symptoms and reduces the risk of ischemic complications.[2] However, reduced GC doses do not completely abolish essential pathways involved in disease persistence, and consequently, the course of GCA may be troubled by relapses.[5,8,27] Recrudescence of GCA activity is common, occurring in at least 43% of patients in population-based studies[3,26] and up to 80% in clinical trials with adjuvant therapies.[15,16,19,20,22] The remarkable variability in the reported prevalence of relapses may be related to heterogeneity in the definition of relapses and to variability in the GC-tapering schedules. Definition of relapse, flare, or recurrence considerably varies across different studies.[16,21–23,26] While in some publications definition of relapse has been based on clinical grounds,[15,16,19] in others, isolated increases in acute-phase reactants have been considered disease flares.[24]

In addition, although this has not been formally evaluated, initial doses and tapering schedules seem to influence relapse rate in GCA.[17,20] In this regard, it is noteworthy that the higher relapse rates have been observed in the context of clinical trials with adjuvant therapies where GC tapering is more aggressive than in standard of care settings, and when alternate-day GC tapering is applied.[15,16,19,20,22] Consistently, a detailed review of treatments received by patients with isolated polymyalgia rheumatica (PMR) suggests that starting with lower GC doses is associated with higher relapse rates.[13]

Relapse rate is a commonly used primary endpoint in clinical trials with patients with GCA. However, although frequency of relapses has been reported in various studies,[15,16,19,20,22,26] limited information exists regarding the

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clinical characteristics and predictors of relapses, and accompanying blood test abnormalities, which have been only specifically addressed in a previous study.[23] Moreover, it has not been clearly demonstrated whether a relapsing course results in increased disease or treatment-related morbidity in these patients. Therefore, we conducted the present study to investigate the prevalence, timing, predictors, and main features of relapses in a longitudinally followed cohort of patients with GCA with long-term follow-up. In addition, we analyzed whether a relapsing course was associated with disease-related ischemic complications, higher cumulated GC doses, more prolonged treatment periods, and/or higher frequency of GC-related adverse effects.

PATIENTS AND METHODS

Between 1995 and 2007, 187 individuals were diagnosed with biopsy-proven GCA at our institution (Hospital Clínic, Barcelona, Spain). Among them, patients treated by the authors who underwent a regular follow-up for at least 4 years were selected. From the initial 187 patients diagnosed, 81 were excluded for the following reasons: 31 were subsequently treated at other departments or institutions, 19 died early during follow-up, 14 were transferred to nursing homes for advanced dementia, and 17 moved to other regions or had deficient compliance with the scheduled follow-up visits.

The remaining 106 patients were uniformly evaluated, treated, and longitudinally followed by the authors for an average of 7.8 ± 3.3 years (range, 4–15 yr). Clinical and laboratory findings at disease diagnosis were recorded. A combination of clinical and blood test abnormalities was used to evaluate the intensity of the systemic inflammatory response (SIR) as previously reported.[6,7,14] These included fever $>38\,^{\circ}C$, weight loss ≥ 4 kg, hemoglobin (Hb) <11 g/L, and erythrocyte sedimentation rate (ESR) ≥ 85 mm/h. Patients with 3 or 4 of these items were considered to have a strong SIR, whereas patients with ≤ 2 were considered to have a weak SIR. Patients underwent clinical assessments in our outpatient facility every 3 months for the first 2 years after diagnosis and approximately every 4–6 months thereafter. ESR, C-reactive protein (CRP), blood cell counts, and Hb concentration were determined at each visit. The treatment protocol consisted of an initial prednisone (PDN) dose of 1 mg/kg per day (up to 60 mg/d) for 1 month. Intravenous methylprednisolone pulse therapy (1 g daily for 3 d) was initially administered to patients with recent (<48 h) visual loss. PDN was subsequently tapered at 10 mg/wk. When reaching 20 mg/d, this dose was maintained for 1–2 weeks and then reduced to 15 mg/d, which was maintained for 1 month. A further reduction to a maintenance dose of 10 mg/d was attempted. If tolerated, a reduction to 7.5 mg/d was tried after 3–6 months. Subsequent tapering was more variable. In general, a reduction to 5 mg/d was attempted approximately 3–6 months later and maintained for 1 year, after which a reduction of 1.25 mg/d was attempted every 6 months. Methotrexate at 15 mg/wk was added when patients experienced ≥ 2 relapses or had developed GC side effects. Reduction in PDN dose was performed 1 month before the scheduled follow-up visit to evaluate tolerance to the adjustment and to avoid severe relapses. If disease-related symptoms (cranial manifestations or PMR), fever, weight loss, or anemia not attributable to other reasons after the necessary work-up occurred, PDN dose was increased by 10–15 mg/d above the previous effective dose. If asymptomatic increases in acute-phase reactants were detected, PDN dose was held until the next visit. When a relapse could be defined, patients were managed as discussed above. If not, a reduction was attempted regardless of the ESR or CRP levels.

We used a consensus definition of relapse established in the context of international multicenter clinical trials.[15,16,19] Relapse or recurrence were indistinctly defined as reappearance of disease-related symptoms, usually accompanied by elevation of acute-phase reactants that required treatment adjustment. Relapses were categorized according to the clinical manifestation into 4 categories: 1) PMR, 2) cranial symptoms (headache, scalp tenderness, jaw claudication, cranial ischemic complications), 3) systemic disease (anemia, fever, and/or weight loss), or 4) symptomatic large vessel involvement (extremity claudication). Cranial ischemic manifestations included stroke, transient ischemic attacks, amaurosis fugax, GCA-related visual loss, or diplopia. Number of relapses, time (in weeks) from the initiation of treatment to first relapse, time required to reach a PDN maintenance dose <10 mg/d (T10), <5 mg/d (T5), and time required to complete GC withdrawal (T0), not followed by a relapse for at least 3 months, were recorded. Cumulated PDN doses received after the first year of treatment were calculated. For each episode of relapsing activity, the ESR, serum CRP, and Hb concentrations were determined, as well as the PDN dose received at that time. In addition, GC-related adverse effects including new or worsening hypertension, diabetes mellitus, hypercholesterolemia, osteoporosis, cataracts, and Cushing appearance were recorded. Measurement of bone mineral density with dual energy X-ray absorptiometry was performed at disease diagnosis and thereafter approximately every 2 years. Osteoporosis was diagnosed using the World Health Organization criteria–that is, bone mineral density T-score of 2.5 standard deviations (SDs) or more below the young adult mean.[4] For screening of diabetes and hyperlipidemia, patients had blood tests prior to each visit, and blood pressure was periodically assessed both at their scheduled visits and by their primary care physicians. We recorded events as adverse events when they appeared or worsened after GC treatment and required new treatment or intensification of previous therapy. The study was approved by the Ethics Committee of Hospital Clínic (Barcelona, Spain).

Statistical Analysis

Continuous variables are presented as mean±SD and/or median and interquartile range (IQR) and categorical data as percentages. Association between relapses and selected covariates was analyzed using the T-test (paired and unpaired) for quantitative variables and the chi-square test for categorical data. Time required to achieve maintenance PDN dose <10 mg/d, <5 mg/d, and time to treatment discontinuation were compared between patients with and without relapses by the Kaplan-Meier survival analysis method. Statistical significance was defined as $p < 0.05$. Calculations were performed with the statistical package PAWS statistics v 18 (SPSS Inc, Chicago, IL) and GraphPad Prism v 5.04 for Windows (GraphPad Software, La Jolla, CA).

RESULTS

We analyzed 106 patients. Mean age at diagnosis was 75 ± 7 years (range, 58–89 yr) with a male to female ratio of 1:2.6. Demographic data and main clinical features at disease onset are depicted in Table 1.

TABLE 1. Baseline Characteristics at Diagnosis (*n=106*)

General Characteristics:	
Age, mean ± SD (range), years	75 ± 7 (58–89)
Sex, No. Male/female (%)	29/77 (27/73)
Duration of Symptoms, Mean ± SD, Weeks	16 ± 21
Cranial Symptoms at Diagnosis, N (%)	
Headache	83 (78)
Jaw Claudication	47 (44)
Scalp Tenderness	49 (46)
Facial Pain[+]	50 (47)
Cranial Ischemic Complications[†]	26 (24.5)
Polymyalgia Rheumatica	54 (51)
Systemic Manifestations, N (%)	
Fever	40 (38)
Anorexia	12 (11)
Weight Loss	54 (51)
Laboratory Parameters	
ESR, mm/hour	90 ± 30.2
CRP, mg/dL	11.2 ± 17.6
Haptoglobin, g/L	3.6 ± 1.5
Hemoglobin, g/dL	11.3 ± 1.4

[+]Includes ocular pain, tongue pain, toothache, earache, odynophagia and carotidynia.

† Includes stroke, transitory ischemic attacks, amaurosis fugax, blindness and diplopia.

Chronology and Characteristics of Relapses

Sixty-eight patients (64%) relapsed during follow-up (mean, 7.8 ± 3.3 yr; range, 4–15 yr) (Figure 1). Mean time to first relapse was 79 ± 75 weeks (range, 11–339 wk) with a median of 51 (IQR, 89) weeks. Thirty-four of the 68 patients (50%) relapsed during the first year after diagnosis (Figure 2A).

PMR was the most frequent clinical manifestation observed during the first flare (Figure 2B). Of note, severe ischemic complications were not developed by any patient as part of the first relapsing episode. Most patients relapsed with the same features originally present at GCA diagnosis (n = 52, 78%). For those who developed a different clinical manifestation, PMR was the most frequent new feature (n = 9, 75%). No patients in this series relapsed with symptomatic large vessel involvement.

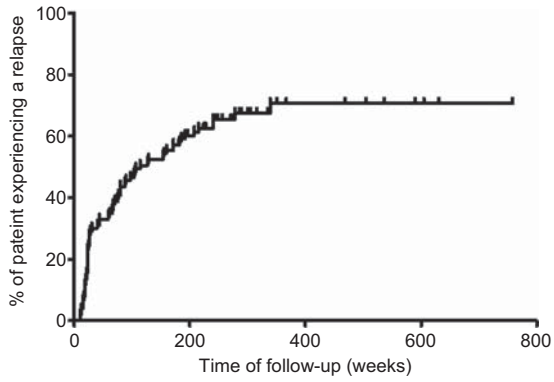

FIGURE 1. Kaplan-Meier plot of the entire series showing the probability of relapse over time.

Figure 2A shows the mean±SD and median (IQR) of PDN used by patients at the time of relapse during the first 5 years of follow-up. Mean PDN dose received by the 68 patients at the first relapse was of 5.3 ± 6.5 mg/d with a median of 2.5 (IQR, 7.5) mg/d. Fifty-two percent were receiving doses ≤2.5 mg/d (Figure 2C). PDN doses at the time of relapse tended to decrease over time (Figure 2A). Patients who relapsed during the first year received 8.3 ± 8.2 mg/d, median 7.5 mg (IQR, 15), whereas patients who relapsed during the second year were receiving 3.7 ± 2.3 mg, median 5 mg (IQR, 2.5).

Mean ESR, CRP, and Hb levels at the time of the first relapse were 61 ± 29 mm/h, 4.0 ± 3.8 mg/dL, and 12 ± 1.4 g/L, respectively. The inflammatory response at that time was comparatively lower than that observed at GCA onset (ESR 88 ± 33 vs 61 ± 29 mm/h, $p < 0.0001$; CRP 11 ± 19 vs 4.0 ± 3.8 mg/dL, $p = 0.001$; and Hb 11.3 ± 1.6 vs 12 ± 1.4 g/L, $p < 0.0001$). We observed an increase of 33 ± 14 mm/hr in ESR level, an increase of 2.9 ± 2.2 mg/dL in CRP concentration, and a decrease of 0.7 ± 0.1 g/L for Hb values between the previous laboratory tests while in remission and the ones performed at disease relapse.

There were significant differences in the PDN doses used to treat relapses according to the type of recurrence (Table 2). The lowest doses were used to treat PMR symptoms (14.5 ± 6.8 mg/d) whereas higher doses were employed to treat cranial manifestations (23.7 ± 12.9 mg/d). ESR levels and Hb concentrations were significantly more deviated from normal values in patients who relapsed with systemic manifestations (see Table 2).

Thirty-eight patients (36%) had 2 or more relapses. Distribution of relapse types was similar to that observed at the first episode (Figure 2D). One patient developed a severe ischemic complication (anterior ischemic optic neuritis) as part of her second recurrence. This patient was treated for 3.5 years and regularly followed for 5 years. She subsequently abandoned regular visits and presented with a relapse including malaise, ischemic optic neuritis, and elevation of ESR, 4 years later. The mean PDN dose at the second flare was 4.3 ± 2.7 mg/d with a median of 5 (IQR 2.5) mg. ESR (mm/h), CRP (mg/dL), and Hb (g/L) levels were 55 ± 30, 3.9 ± 6.1, and 12.1 ± 1.4 respectively.

Predictors of Relapse

To search for predictors of relapses, we compared initial clinical and laboratory findings between patients with or without recurrent disease. At disease onset, PMR and scalp tenderness were more frequently observed in relapsing patients (Table 3). Acute-phase reactants tended to be higher in patients with recurrences, but only haptoglobin reached statistical significance (3.8 ± 1.6 g/L vs 3.0 ± 1.3 g/L, $p = 0.042$). When the intensity of the SIR was evaluated combining clinical and laboratory findings, patients with multiple relapses were significantly more frequent among those with a strong SIR: 23 of 27 (85%) patients with a strong SIR relapsed 2 or more times compared with 15 of 41 (37%) of those with a weak SIR. No other predictors of recurrences could be identified.

Glucocorticoid Requirements and Side Effects According to Disease Recurrences

Treatment requirements were different in both groups of patients (Table 4). Patients with relapses required

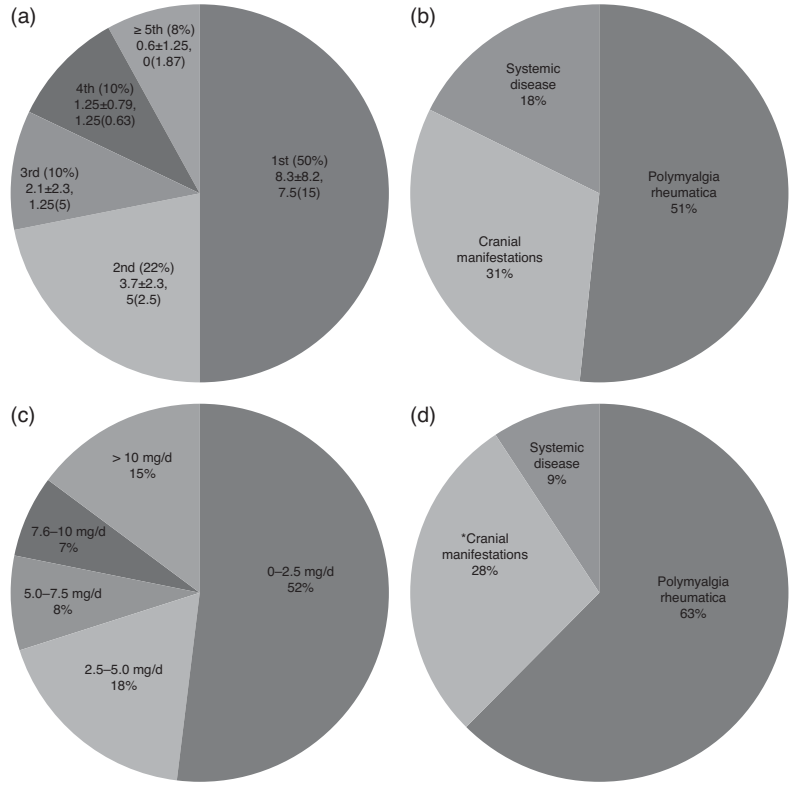

FIGURE 2. Characteristics and timing of flares in relapsing patients (n = 68). A) Percentage of patients relapsing per year of follow-up and mean±SD and median (IQR) prednisone dose (mg/d) received at the time of relapse. B) Percentage of relapsing patients with a given clinical type of relapse. C) Percentage of patients receiving the indicated dose of prednisone (mg/d) at the time of the first

significantly longer periods of time to reach a maintenance dose of PDN <10 mg/d, <5 mg/d, and to completely discontinue GC therapy (Figure 3). Cumulated PDN dose during the first year was significantly higher in relapsing patients (6.2 ± 1.7 g vs 5.4 ± 0.78 g, $p = 0.015$). Relapsing patients had an increased prevalence of osteoporosis (65% for relapsing patients vs 32% for nonrelapsing, $p = 0.001$). Other adverse effects also tended to be more frequent in patients with relapses, but differences did not reach statistical significance. As expected, methotrexate was administered more frequently in patients with relapses than in patients in sustained remission (22% vs 3%, $p = 0.009$).

DISCUSSION

Limited information about the characteristics of recurrences occurring in patients with GCA is available.[23] Here, we present detailed data about clinical and laboratory characteristics of relapses from a cohort of uniformly treated patients with GCA with long-term follow-up. The definition of relapse used in the present study was similar to that used in randomized, controlled clinical trials evaluating adjunctive therapies for GCA.[15,16,19]

In spite of the satisfactory initial response to GC treatment, 64% of patients relapsed in the present series. This percentage is somewhat higher than that reported in population-based studies,[3,26] possibly due to the more extended follow-up of our patient cohort, but some selection bias cannot be excluded. Although most relapses occurred within the first 2 years of treatment, recurrences also developed subsequently. PMR was the most frequent symptom (51%) at the time of relapse, followed by cranial manifestations (31%). In previous studies[16,22,23] headache was the leading feature (44%-60%), followed by PMR (19%-30%),[16,23] and constitutional syndrome (28%).[23] Therefore, the distribution found in the current cohort is close to that found in other studies. It is noteworthy that disease-related

TABLE 2. Laboratory Characteristics and PDN Dose at Each Relapse Type

	Polymyalgia Rheumatica	Cranial Manifestations	Systemic Disease	*p*
ESR (mm/h)	55 ± 28	59 ± 28	80 ± 26	0.038
CRP (mg/L)	3.9 ± 3.5	4.5 ± 4.2	3.6 ± 4.1	ns
Hemoglobin (g/L)	12.2 ± 1.3	12.3 ± 1.4	11.7 ± 1.2	0.017
Dose of prednisone (mg/d) used to treat relapse	14.5 ± 6.8	23.7 ± 12.9	17.9 ± 5.6	0.002
Increment in prednisone dose (mg/d)	10 ± 6.5	17 ± 12	14 ± 11	0.009

TABLE 3. Clinical Manifestations at Diagnosis in Patients With and without Relapses

Clinical characteristics	*Relapse (n=68)*	*No relapse (n=38)*	*p*
General characteristics:			
Sex, no. female/male	52/16 (76.5/23.5)	25/13 (66/34)	ns
Age, mean, yr	74 ± 6.5	76 ± 8	ns
Cranial symptoms, n (%)			
Headache	56 (82)	27 (71)	ns
Jaw claudication	34 (50)	13 (34)	ns
Scalp tenderness	40 (59)	9 (24)	0.001
Ischemic complications	17 (25)	9 (24)	ns
Stroke	2 (3)	0 (0)	ns
Transitory ischemic attack	4 (6)	0 (0)	ns
Amaurosis fugax	9 (13)	6 (16)	ns
Diplopia	9 (13)	2 (5)	ns
Permanent visual loss	6 (9)	6 (16)	ns
Polymyalgia rheumatica	40 (59)	14 (37)	0.042
Systemic manifestations, n (%)			
Fever	24 (35)	16 (42)	ns
Anorexia	7 (10)	5 (13)	ns
Weight loss	36 (53)	18 (47)	ns
Baseline laboratory parameters, mean ± SD			
ESR, mm/hour	88 ± 33	92 ± 24	ns
CRP, mg/dL	11.4 ± 18.6	10.7 ± 15.9	ns
Haptoglobin, g/L	3.8 ± 1.6	3.0 ± 1.3	0.049
Hemoglobin, g/dL	11.3 ± 1.5	11.2 ± 1.2	ns

ischemic complications seem to be extremely infrequent in the context of controlled relapses. In previous studies the occurrence of ischemic manifestations has been also found to be infrequent during follow-up (0%-6%).[1,16,23] Only 1 patient in the current series suffered anterior ischemic optic neuropathy in the context of a delayed relapse, but this patient had interrupted regular control visits at the time of disease recurrence. No patient in our series relapsed with symptomatic involvement of large vessels.

Relapses were usually accompanied by elevated levels of ESR and CRP that were, nevertheless, lower than those observed at disease onset. In accordance, PDN doses much lower than the starting doses were usually effective for treating controlled relapses. However, it must be stressed that the reported features were obtained from patients who were closely followed with reassessments performed approximately every 3 months during the first 2 years after diagnosis. We cannot exclude that severe relapses requiring higher GC doses may occur in patients controlled less tightly. These findings indicate that patients with GCA need to be indefinitely observed even after successful GC discontinuation.

As for the time at greatest risk for relapse, in 50% of patients who relapsed, recurrences occurred during the first year. Mean time to first relapse was 19.7 ± 18.7 months, similar to what has been reported by others.[21,23,26] However delayed relapses also occurred.

As shown in Figure 2, PDN dose received at the time when relapses occurred decreased over time, suggesting that

TABLE 4. Treatment Requirements and Side Effects During Follow-up

Clinical characteristic	*Relapse (n=68)*	*No relapse (n=38)*	*p*
Treatment requirements			
*Intravenous methylprednisolone pulse**	3 (4)	4 (10.5)	ns
Cumulated dose first year, mean ± SD (g)	6.2 ± 1.7	5.4 ± 0.78	0.015
Methotrexate (15 mg/week)	15 (22)	1 (3)	0.009
Glucocorticoid related adverse effects n, (%)†			
Diabetes mellitus	7 (10)	2 (5)	ns
Systemic hypertension	37 (54)	17 (45)	ns
Hypercholesterolemia	42 (62)	26 (69)	ns
Osteoporosis	44 (65)	12 (32)	0.001
Cushing appearance	8 (12)	1 (3)	ns
Cataracts	16 (23.5)	3 (8)	ns

* At disease onset.
† 100% of patients presented at least 1 side effect.

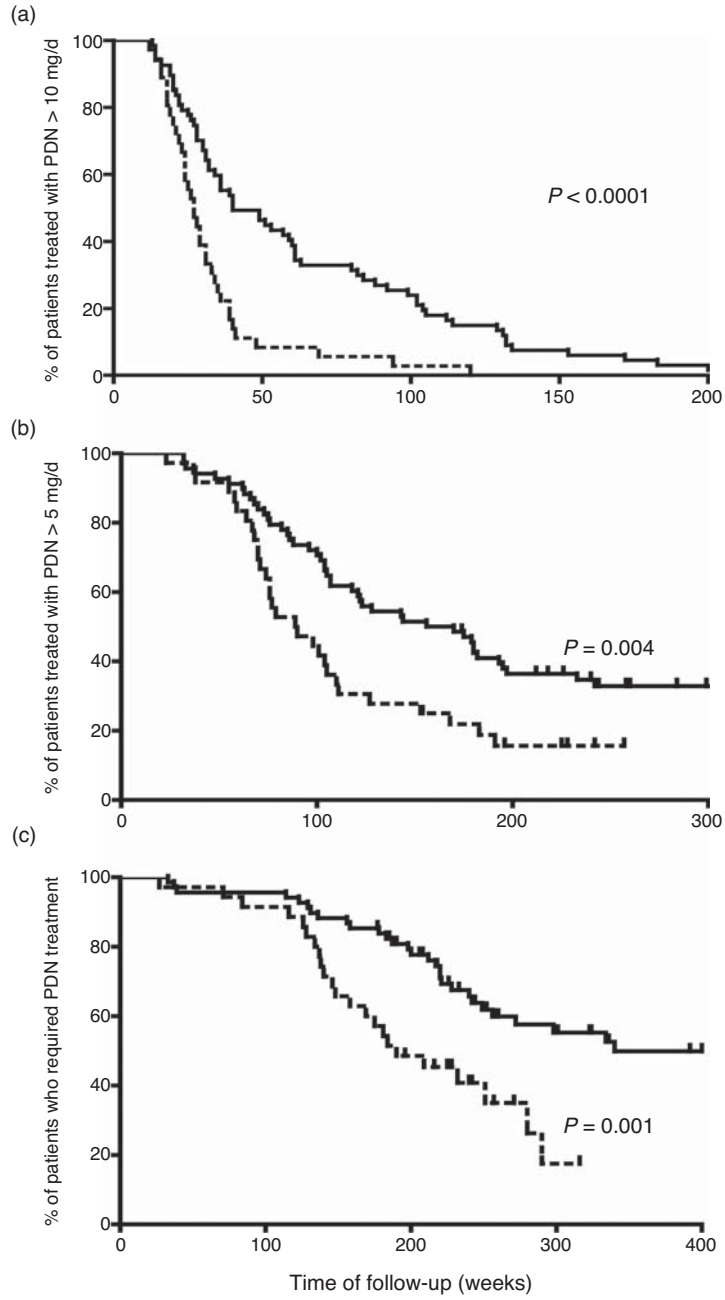

FIGURE 3. Survival curves showing the time required to reach a stable dose of prednisone <10 mg/d (A), <5 mg/d (B), and 0 mg/d (C) in patients with relapses (solid line) and with sustained remission (broken line). [Note the scale for time of follow-up is different among the 3 figure parts].

disease activity progressively decreases and, over the years, lower PDN doses are required to maintain remission. Overall, relapses occurred when patients were receiving a mean PDN dose of 5.3 ± 6.5 mg/d with median 2.5 mg/d (IQR, 7.5). This dose is lower than that reported in other series. This may be due to variability in the rate of initial PDN tapering across different studies or to other reasons. No patient in our cohort relapsed with PDN higher than 25 mg/d.

Patients with relapses required longer periods of treatment and were exposed to higher cumulated PDN doses, similar to what was found in a previous study.[23] All patients in our cohort experienced at least 1 GC-related side effect. Other studies have reported GC adverse effects in 90%-95% of GCA patients within the first 3 years of therapy.[24,26] These include new or worsening hypertension (22%-84%),[19,24,26] infections (22%-56%),[19,22,24,26] osteoporosis and bone fractures (8%-38%),[19,22–24,26] new or worsening diabetes mellitus (7%-37%),[19,22–24,26] and cataracts (4%-41%).[19,24,26] The higher frequency of side effects in our patient cohort may be related to the longer follow-up period. We observed that patients with

recurrences presented more GC-related toxicity, in particular osteoporosis despite the administration of calcium supplements, vitamin D, and bisphosphonates. These data highlight the need for more efficient and safer therapies.

In the current cohort, relapses could not be attributed to insufficient treatment because relapsing patients received more GC and for more extended periods of time. This observation indicates that some patients have a more resistant disease. From the clinical standpoint, an intense acute-phase response was associated with higher risk of relapse. Other investigators have also observed that abnormalities related to the acute-phase response are predictors of relapse.[6,9,12,14,23,25,28] Among other findings, only scalp tenderness and PMR were slightly more frequent in relapsing patients. Although this association may be spurious, a similar trend has been observed in other studies.[23] Previous studies have investigated tissue and serum biomarkers associated with persistent disease activity and relapsing course. Elevated serum concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-6, and soluble intercellular adhesion molecule (ICAM)-1 are associated with relapsing disease.[10,12,28] Increased expression of TNF-α or chemokine (C-C motif) ligand (CCL)-2 mRNA in involved arteries is associated with recurrent disease and higher GC requirements. However, TNF-α blockade was not sufficient to reduce relapses and spare corticosteroids,[16] indicating that association does not imply causality and suggesting that TNF-α effects may be compensated by other cytokines and that upstream mediators may be more relevant to perpetuate disease activity. Elevated mRNA concentrations of Th1 cytokines IL-12/23p40 and interferon (IFN)-γ have been observed after 1 year of treatment in second temporal artery biopsies of relapsing patients, suggesting reactivation of initial events able to drive subsequent inflammatory cascades.[28] In contrast, increased IL-17 expression in GCA lesions is a predictor of sustained response to GC.[11] Further research is needed to elucidate the mechanisms involved in disease persistence, to enable the design of more specific and efficient targeted therapies.

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Treatment with angiotensin II receptor blockers is associated with prolonged relapse-free survival, lower relapse rate, and corticosteroid-sparing effect in patients with giant cell arteritis

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ABSTRACT

Objective: To determine whether concomitant treatment with angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) is associated with changes in the outcome of patients with giant cell arteritis (GCA).

Methods: A study cohort of 106 patients with biopsy-proven GCA was longitudinally followed up for 7.8 ± 3.3 years. Patients were stratified according to their treatment with ACEI, ARB, or no ACEI/ARB. Time to first relapse, number of flares, time to achieve a stable prednisone dose < 10 mg/day and < 5 mg/day with no relapses, time required to completely discontinue prednisone, cumulative dose of prednisone received during the first year, and concentrations of acute-phase reactants at pre-defined time points (baseline, 6, 12, 18, and 24 months) were compared among the 3 groups. Cox proportional hazards models were performed to adjust for potential confounders.

Results: Patients receiving ARB presented a significantly longer relapse-free survival than patients treated with ACEI or patients not receiving ACEI/ARB ($p = 0.02$). The adjusted hazard ratio for relapses in patients treated with ARB was 0.32 (95% CI: 0.12–0.81, $p = 0.017$). In addition, patients who received ARB achieved a prednisone maintenance dose < 10 mg/day faster than all other patients ($p = 0.0002$). No significant differences were observed among groups in acute-phase reactant levels during follow-up. However, patients not receiving ACEI/ARB had significantly higher C-reactive protein and haptoglobin concentrations than those receiving ACEI or ARB at various time points.

Conclusions: Addition of ARB to glucocorticoids is associated with lower relapse rate and more prolonged disease-free survival in patients with GCA.

Introduction

Giant cell arteritis (GCA) is a granulomatous arteritis predominantly affecting large- and medium-size vessels [1,2]. About 15–20% of patients experience partial or complete visual loss, 3–6% experience stroke, and 5–15% experience symptomatic stenosis of large vessels as a consequence of vascular occlusion derived from inflammation-induced vascular remodeling [2–4]. Glucocorticoid (GC) treatment induces a dramatic symptomatic improvement and reduces the risk of ischemic complications; but the clinical course of GCA may be troubled by disease- and treatment-related complications that may severely impair patients' quality of life [2–4]. Nearly 10–15% of patients cannot reduce prednisone below 10–15 mg/day without experiencing disease flares or low-grade persistent activity [5,6]. Relapses are frequent, occurring in up to 60% of patients, particularly during the first 1.5 years after the initiation of therapy [5,7]. Consequently, half of the patients cannot tolerate complete withdrawal after 2–3 years of treatment [6,8]. As a result of long-term GC treatment, adverse effects occur in nearly 90% of patients during long-term follow-up [9], with infections and fractures occurring in more than 50% [10]. Moreover, GCs are not able to prevent the development of significant aortic structural

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damage, resulting in aneurysm or dilatation in approximately 22% of cases during the first 5 years of follow-up [11].

In this context, several randomized controlled trials with adjuvant therapies have been conducted in an effort to reduce exposure to GC and its associated side effects. Unfortunately, only methotrexate has demonstrated a modest efficacy in reducing relapse rate and lowering cumulative prednisone dose [12–15]. In a small trial of mixed patients with GCA and isolated polymyalgia rheumatica (PMR), azathioprine treatment resulted in a significant but small reduction in median prednisone dose at 1 year [16]. A trial with infliximab in newly diagnosed patients yielded disappointing results [17], and a small trial of etanercept in relapsing patients or in patients with GC-related side effects resulted in a significant but slight reduction in cumulated prednisone dose [18]. Recently, retrospective case series have suggested efficacy for cyclophosphamide in patients with GC-dependent GCA at the expense of infrequent but serious side effects [19]. Based on several case reports and small case series where tocilizumab, an IL-6 receptor blocking humanized monoclonal antibody, elicited remission in GC-dependent GCA patients, a large multicenter, international, randomized, double-blind controlled trial has been designed [20].

Over the past years, angiotensin II (ATII) has emerged as a powerful pro-inflammatory peptide and anti-inflammatory properties have been demonstrated for angiotensin-converting enzyme inhibitors (ACEI) and ATII receptor blockers (ARB) [21]. In this regard, several studies have addressed the potential benefits of blocking the renin–angiotensin–aldosterone system (RAAS) in autoimmune conditions. An open study performed with 15 rheumatoid arthritis (RA) patients with active disease showed that 48 weeks of treatment with captopril decreased articular pain (measured by Ritchie articular index) and plasma concentrations of C-reactive protein (CRP) in two-thirds of the subjects [22]. Also, in a small, randomized, double-blind study of 11 patients, addition of ramipril to concomitant RA therapy significantly reduced plasma concentrations of pro-inflammatory cytokines TNF-α and soluble CD40, although other inflammation parameters like CRP, IL-1, IL-6, myeloperoxidase, and erythrocyte sedimentation rate (ESR) were not influenced [23]. A retrospective analysis of a large cohort of patients with systemic lupus erythematosus (SLE) ($n = 378$) demonstrated that the use of ACEI was associated with a decreased probability of renal involvement and decreased risk of disease activity measured by the SLAM-R score [24]. Furthermore, monotherapy with losartan significantly ameliorated the clinical course and reduced activity markers in animal models of autoimmune nephritis [25] and multiple sclerosis [26].

Based on the chronic inflammatory nature of GCA, we hypothesized that concomitant use of ACEI or ARB might facilitate sustained remission. Therefore, we conducted the present study in order to evaluate whether GCA patients who received ACEI or ARB in addition to GC presented reduced frequency of relapses, corticosteroid requirements, or changes in biomarkers of disease activity.

Patients and methods

The study cohort was selected among the 187 individuals diagnosed with biopsy-proven GCA at our institution (Hospital Clínic, Barcelona, Spain) between 1995 and 2007. Inclusion criteria consisted of treatment by the authors according to a standardized protocol and regular follow-up for at least 4 years. From the initial 187 patients, 31 were excluded because they were treated and followed up at other departments or institutions, 19 because of early death before completing follow-up, 14 because of transfer to nursing homes for advanced dementia or dependency, and 17 because of incomplete follow-up due to a variety of reasons, including moving to other regions. The study cohort consisted of the remaining 106 patients who fulfilled the inclusion criteria.

All patients included were prospectively evaluated at baseline and treated by the authors according to a uniform protocol consisting of an initial prednisone dose of 1 mg/kg/day (up to 60 mg/day) for 1 month with subsequent tapering of prednisone dose (10 mg/week). Intravenous methylprednisolone pulse therapy (1 g daily for 3 days) was initially administered to patients with recent (< 48 h) visual loss. When reaching 20 mg/day, this dose was maintained for 1–2 weeks and subsequently reduced to 15 mg/day, which was maintained for 1 month. A further reduction to a maintenance dose of 10 mg/day was attempted. If tolerated, a reduction to 7.5 mg/day was tried after 3–6 months. Subsequent tapering and discontinuation was more variable and left to the judgment of treating physicians. Roughly, a reduction to 5 mg/day was attempted approximately 3–6 months later and maintained for 1 year, after which a reduction of 1.25 mg/day was attempted every 6 months. Methotrexate at 15 mg/week was added when patients had experienced ≥ 2 relapses or had experienced GC side effects. GCA relapse was defined as the reappearance of disease-related symptoms including cranial manifestations, PMR, fever, or anemia not attributable to other reasons, which resolved by increasing the prednisone dose 10–15 mg/day above the previous effective dose. Relapses were usually accompanied by a rebound in erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) of variable intensity, but this was not a mandatory requirement. If asymptomatic increases in acute-phase reactants occurred, the prednisone dose was held until the next visit. If a relapse could be defined, patients were managed as discussed above. If not, a reduction was attempted regardless of the ESR or CRP levels. A patient suffering from 3 or more recurrences was considered as a frequent relapser.

Patients were divided in 3 groups. Group 1 included those who had received an ACEI, group 2 those who had received ARB, and group 3 patients who had received neither ACEI nor ARB. We included in group 1 patients treated with an ACEI for at least 1 year before GCA diagnosis or those who started treatment with ACEI during the first year after diagnosis and continued its regular use for at least 12 more months. The same criteria were applied to patients receiving ARB (group 2).

End points compared between the 3 groups were (1) time (in weeks) to first relapse and number of relapses, (2) time (in weeks) required to reach a prednisone maintenance dose < 10 mg/day, < 5 mg/day, and completely discontinue treatment not followed by a relapse for at least 3 months, and cumulative dose of prednisone received until that moment and during the first year, and (3) inflammatory biomarkers including erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP) and haptoglobin, as well as hemoglobin and alkaline phosphatase concentrations, and white blood cell and platelet counts determined at predefined time points (baseline, 6 months, 12 months, 18 months, and 24 months).

Statistical analysis

Continuous variables are presented as mean ± SD and categorical data as percentages. Association between ACEI or ARB therapy and selected covariates was analyzed using ANOVA test for quantitative variables and Pearson's chi square test for categorical data. Bonferroni correction was used to adjust for multiple comparisons. Time required to achieve maintenance prednisone dosage < 10 mg/day and time to first relapse were compared between the 3 groups by the Kaplan–Meier survival analysis method. In order to determine the risk of relapses as a function of treatment with ACEI, ARBs, or no ACEI/ARBs as co-variables, Cox

regression models were performed to adjust for potential confounders (sex, age at diagnosis, co-therapy with aspirin, methotrexate, statins, and methylprednisolone pulses). One-time inclusion of these variables, forward selection, and backward elimination were done. Results are shown as hazard ratio (HR) with 95% confidence intervals (CI). Statistical significance was defined as $p < 0.05$. Calculations were performed with the statistical package PASW statistics version 18 (SPSS, Inc. 2009, Chicago, IL) and GraphPad Prism version 5.04 for Windows (GraphPad Software, La Jolla, CA).

Results

Patient characteristics

The 106 patients included (mean age = 75 ± 7 years, 73% women) were longitudinally followed up for 7.8 ± 3.3 years. At diagnosis, 17 patients received an ACEI and 10 were treated with ARB. After the first year of follow-up, these numbers increased to 36 and 14, respectively. Indication for therapy was systemic hypertension in all cases with enalapril (10–20 mg/day) being the most frequently prescribed drug. Table 1 shows the type and doses of ACEI and ARB drugs used at disease onset and during the first year after diagnosis.

Table 2 compares clinical manifestations and laboratory results at diagnosis between the patients under therapy with ACEI, ARB, or no ACEI/ARB. Disease presentation was not different between the 3 groups with respect to age, sex, and presence of systemic manifestations or PMR. As expected, cardiovascular risk factors were present in all patients receiving ACEI and ARB and in 71% of patients not receiving these drugs ($p = 0.02$). Interestingly, permanent visual loss tended to be more frequent in patients treated with ARB before the diagnosis of GCA (40%) than in those receiving ACEI (12%) and those receiving none of these treatments (8%) ($p = 0.05$).

Relapses

Relapse-free survival was significantly longer in patients treated with ARB compared with the other 2 groups (Fig. 1). After adjusting for covariates that were potential confounders (sex, age at diagnosis, treatment with aspirin, methotrexate, statins, or methylprednisolone pulses), the HR for relapses developing in patients who received ARB relative to that in patients who were not receiving ARB was 0.32 (95% CI: 0.12–0.81, $p = 0.017$). Moreover, there were no frequent relapsers (patients who relapsed 3 times or more) among patients receiving ARB compared with 6% among patients treated with ACEI and 21% among those with no ARB/ACEI ($p = 0.02$).

Table 1
Type and dosage of ACEI/ARB

	At diagnosis	First year[a]	Dosage (mg/day)
ATII enzyme-converting inhibitors	$n = 17$	$n = 36$	
Enalapril	12	30	10–20
Captopril	2	3	25–50
Perindopril	2	2	4–8
Fosinopril	1	1	20–40
ATII receptor blockers	$n = 10$	$n = 14$	
Irbesartan	4	4	150–300
Losartan	4	7	50–100
Candesartan	1	1	16–32
Eprosartan	1	1	600–800
Valsartan	0	1	160

[a] Patients who used ACEI/ARB at diagnosis and those who initiated these drugs during the first year after GCA diagnosis.

Glucocorticoid requirements

Time required to reach a maintenance prednisone dose < 10 mg/day, not followed by a relapse during at least 3 months ($T < 10$), was significantly shorter for patients receiving ARB than for patients receiving ACEI or not receiving ACEI/ARB ($p = 0.0002$) (Fig. 2). There were no differences in time (weeks) required to achieve a prednisone dose < 5 mg/day (median survival 123 weeks for ACEI vs 102 weeks for ARB vs 104 weeks for no ACEI/ARB, $p =$ NS) or time required to complete prednisone withdrawal (166 weeks for ACEI vs 131 weeks for ARB vs 203 weeks for no ACEI/ARB, $p =$ NS). Cumulated prednisone dose measured until $T < 10$ tended to be different among groups (2.4 ± 2.2 g for patients receiving ACEI, 1.0 ± 0.9 g for patients treated with ARB, and 1.6 ± 1.8 g for patients with no ACEI/ARB, $p = 0.05$), as was the cumulated prednisone dose during the first year (6.1 ± 1.5 g for ACEI group, 5.1 ± 0.65 g for ARB, and 6.0 ± 1.5 g for no ACEI/ARB, $p = 0.08$). There were no differences in adjuvant therapies received among groups, including intravenous methylprednisolone pulses (3 g) in patients with recent visual loss at diagnosis or addition of methotrexate during follow-up (Table 2).

Inflammatory biomarkers

Levels of ESR, CPR, haptoglobin, hemoglobin, alkaline phosphatase, and leukocyte and platelet counts recorded every 6 months for the first 2 years of follow-up did not differ among patients with regular use of ACEI and ARB or those not receiving these treatments (data not shown). However, concentrations of CRP and haptoglobin were significantly higher in patients not receiving ACEI/ARB than in those receiving either ACEI or ARB at several time points (Fig. 3).

Discussion

In this observational study, we show that patients with GCA receiving treatment with angiotensin II receptor blockers exhibit significantly longer disease-free survival, significantly reduced relapse rate, significant reduction in the time receiving more than 10 mg of prednisone/day, and a trend to decreased cumulated GC doses during the first year. Unfortunately, GC side effects were not systematically recorded in this study.

Comparison of GC treatment duration and doses among published series is difficult due to the heterogeneity of treatment schedules and the lack of a uniform definition regarding when to measure cumulative doses of prednisone or its equivalent [7–9,13]. However, when comparing our data with a similarly treated cohort of GCA patients in northwestern Spain [7], the reported mean time to first relapse (71 weeks) was remarkably similar to that observed in patients treated with ACEI or not treated with ACEI/ARB in our cohort (60 and 68 weeks, respectively) but clearly shorter than that observed in patients receiving ARB (153 weeks). In this sense, the observation that ARB treatment is associated with more sustained remission may be relevant.

The potential adjuvant effect of ARB was more evident during the first year of treatment. This may be due to the fact that while the initial tapering protocol was uniform, tapering beyond 7.5 mg/day or the time of complete GC discontinuation was far less uniform and left to the discretion of treating physicians. Alternatively, ARB might boost the effect of GC at certain doses or disease stages.

During the past decade, several studies have shown that commonly used drugs such as statins [27], pentoxifylline [28], antimalarials [29], and ACEI or ARB [22–24,30,31] elicit anti-inflammatory pleiotropic effects. In particular, blockade of angiotensin II (ATII) exhibits immune-modulatory properties and may improve clinical manifestations and inflammatory biomarkers in RA and SLE [22–24].

Table 2
Clinical and biochemical findings at diagnosis in patients under therapy with ACEI, ARB, or no ACEI/ARB

Clinical characteristics	ACEI (n = 17)	ARB (n = 10)	No ACEI/ARB (n = 79)	p
General characteristics				
Age, mean ± SD, years	77 ± 7	79 ± 6	74 ± 7	ns
Sex, no. of male/female	3/14	1/9	25/54	ns
Duration of symptoms, mean, weeks	18 ± 27	22 ± 28	15 ± 19	ns
Cardiovascular risk factors[a], n (%)	17 (100)	10 (100)	56 (71)	0.02
Clinical symptoms at diagnosis, n (%)				
Headache	12 (71)	6 (60)	65 (82)	ns
Jaw claudication	9 (53)	5 (50)	33 (42)	ns
Scalp tenderness	3 (18)	3 (30)	43 (54)	0.012[b]
Polymyalgia rheumatica	10 (59)	7 (70)	37 (47)	ns
Systemic manifestations, n (%)				
Fever	7 (41)	4 (40)	29 (37)	ns
Anorexia	2 (12)	1 (10)	9 (11)	ns
Weight loss	8 (47)	4 (40)	42 (53)	ns
Ischemic complications, n (%)				
Stroke	0 (0)	0 (0)	2 (2.5)	ns
Transitory ischemic attack	1 (6)	0 (0)	3 (4)	ns
Amaurosis fugax	4 (23.5)	3 (30)	8 (10)	ns
Diplopia	3 (18)	0 (0)	8 (10)	ns
Permanent visual loss	2 (12)	4 (40)	6 (8)	0.01[c]
Laboratory parameter (mean ± SD)				
ESR, mm/h	92.3 ± 32.3	106.4 ± 16.2	87.4 ± 30.6	ns
CRP, mg/dl	13.6 ± 17.4	10.1 ± 4.2	10.8 ± 18.9	ns
Haptoglobin, g/l	3.7 ± 1.7	4.6 ± 0.96	3.3 ± 1.5	ns
Adjuvant treatments (disease onset)				
Intravenous methylprednisolone pulse	1 (6)	2 (20)	4 (5)	ns
Adjuvant treatments (follow-up)				
Methotrexate	8 (22)	1 (7)	6 (11)	ns
Aspirin	20 (56)	9 (64)	24 (43)	ns
Statins	13 (36)	4 (29)	23 (41)	ns

ESR = erythrocyte sedimentation rate; CRP = C-reactive protein.
[a] Type 2 diabetes mellitus, arterial hypertension, and smoking.
[b] p = 0.048 after Bonferroni's correction.
[c] p = 0.05 after Bonferroni's correction.

The mechanisms through which ATII blockade may ameliorate inflammatory diseases have been explored in several studies but are not completely understood. ATII stimulates the production of pro-inflammatory molecules IL-1, IL-6, IL-8, and osteopontin by human vascular smooth muscle cells (VSMC) [32–34] and up-regulates the expression of adhesion molecules VCAM-I, ICAM-1, and E-selectin by cultured endothelial cells [35,36]. Many of these molecules are expressed in GCA-involved arteries or increased in patient's sera and are thought to participate in pro-inflammatory amplification cascades [37–41]. In addition, ATII promotes the generation of reactive oxygen species in cultured VSMC and in aortic adventitial fibroblasts [42]. Moreover, in animal models [43] ATII promotes abnormal vascular remodeling by inducing the expression and synthesis of extracellular matrix proteins such as fibronectin, laminin, and collagens. In addition, ATII induces hypertrophy and hyperplasia of cultured VSMC [44,45].

Interestingly, recent contributions demonstrate that certain genetic polymorphisms in the RAAS system are associated with increased susceptibility to developing several autoimmune diseases including multiple sclerosis [46] and, importantly, systemic vasculitis [47], specifically IgA vasculitis [48] and Behçet's disease [49].

In GCA patients, expression of angiotensin type 1 receptor has been demonstrated by immunohistochemistry in vascular smooth muscle cells of temporal arteries and infiltrating leukocytes [50]. Therefore, increased activity of the angiotensin system may play a role in the persistence of chronic inflammation in GCA. In addition

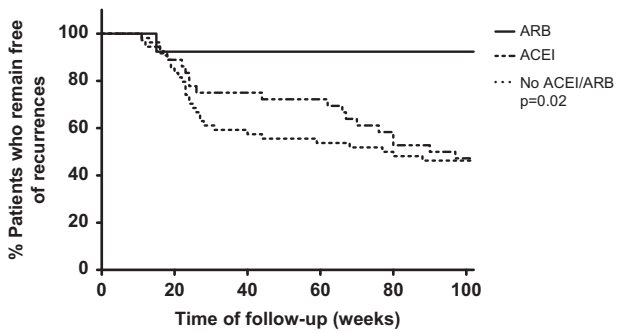

Fig. 1. Kaplan–Meier estimate of the probability of relapse depending on treatment with ACEI, ARB, or no ACEI/ARB.

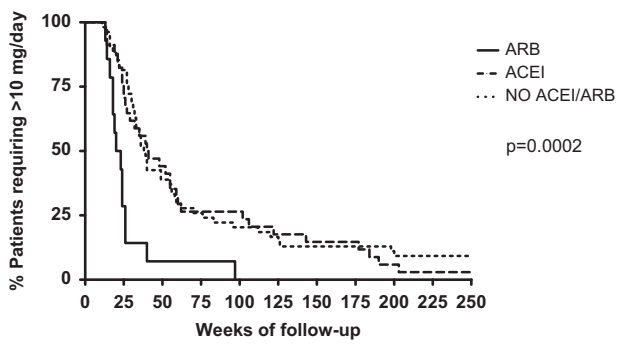

Fig. 2. Percentage of patients requiring a prednisone dose > 10 mg/day over time.

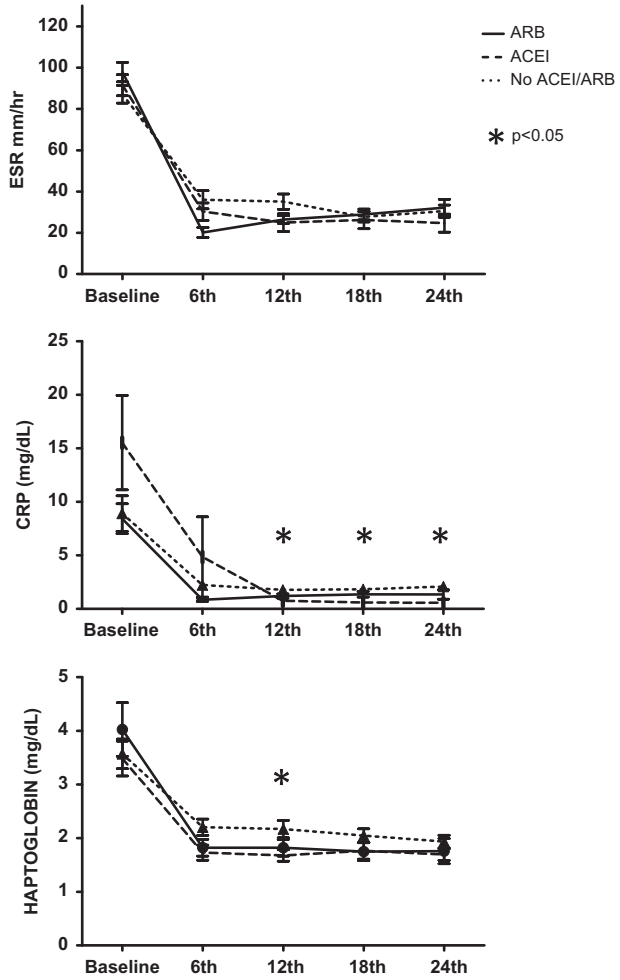

Fig. 3. Serial measurement of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, and haptoglobin level during follow-up according to treatment with ARB or ACEI. * $p < 0.05$ comparing patients receiving either ARB or ACEI versus those not receiving ARB/ACEI.

to the above-mentioned pro-inflammatory effects of ATII, an additional intriguing effect of this peptide has been recently described. In animal models, ATII stimulates the production of IFN-γ and TNFα by peripheral T cells [51] and is capable of stimulating a Th1 response in ApoE-deficient mice [52]. More importantly, blocking the actions of ATII with ACEI or ARB suppresses the production of auto-reactive Th1 and Th17 cells and promotes the expression of antigen-specific T*reg* cells in an experimental animal model of multiple sclerosis [53]. Since GCA is characterized by a Th1/Th17 response [54,55], this particular effect might be relevant to patients with GCA.

Interestingly, patients receiving ARB before being diagnosed with GCA tended to have higher frequency of visual loss when developing GCA. In some studies [56], GCA-related ischemic complications are more frequent among patients with traditional cardiovascular risk factors. ARB were prescribed to our patients because of arterial hypertension and, consequently, ARB-receiving patients had increased cardiovascular risk. However, this association was not observed in patients in whom hypertension was treated with ACEI or other drugs. If confirmed in larger series, this finding may indicate that, in the context of active inflammation, ARB may influence inflammation-induced vascular remodeling and facilitate vascular occlusion. At present, the strength of this association is too weak to draw definitive conclusions.

It was surprising that ARB but not ACEI treatment was associated with reduced relapse rate. One possible explanation for this finding could be that in blood vessels (as it happens in the kidney and heart) production of ATII from angiotensinogen can be catalyzed by enzymes other than ACE (i.e., chymase) and therefore, the effects of angiotensin II produced by this alternative way can be inhibited by ARB but not completely by ACE inhibitors [57].

Limitations of the present study include the relatively low number of patients treated with ARB and the retrospective design, precluding homogeneity in type, dose, and duration of ACEI/ARB treatment. However, our data was obtained from a longitudinally followed up and uniformly treated cohort where concomitant medications were systematically recorded. Our findings suggest that ATII receptor blockade may help to maintain remission, reduce the relapse rate, and spare glucocorticoids in patients with GCA. However, the observational design of our study prevents any conclusion beyond association and this interesting possibility needs to be tested in randomized controlled trials.

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Expression and functional activity of the angiotensin II system in temporal artery lesions from patients with giant cell arteritis

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Short title: *The angiotensin II system in GCA*

ABSTRACT

Background. A previous observational study suggests that treatment with angiotensin II blockers (ARB) is associated with lower relapse rate and glucocorticoid-sparing effect in giant-cell arteritis (GCA).

Objective. To investigate the expression and function of the angiotensin II system in temporal artery (TA) biopsies from GCA patients.

Methods. 40 patients and 18 controls were included. Angiotensinogen (AGT), angiotensin II receptor 1 (AGTR1) and angiotensin converting enzyme (ACE) mRNA expression was investigated by quantitative real-time PCR in TA biopsies. Localization of the components of the ATII system was assessed by confocal microscopy. The effects of ATII or AGTR1 blockade on pro-inflammatory cytokine production were assessed in co-culture systems of peripheral blood mononuclear cells (PBMC) and TA-derived vascular smooth muscle cells (VSMC).

Results. Local production of the major components of the ATII system was demonstrated in GCA and control arteries. ACE mRNA was increased in TA lesions from GCA patients whereas ATII and ATG was decreased, probably indicating VSMC loss. While VSMC were the main cells expressing ATII and AGTR1, ACE was predominantly expressed by inflammatory cells with scattered T lymphocytes and macrophages expressing the AGTR1. ATII induced expression of pro-inflammatory molecules IL-1β, IL-6, TNF-α, IFNg and CCL-2 in PBMC and IL-1β, IL-6, TNF-α, and CCL-2 in VSMC co-cultured with PBMC and this increase was abrogated by the AGTR1 blocker losartan.

Conclusion. These data suggest that the angiotensin II system may play a role in sustaining inflammatory pathways in GCA and may explain the apparent clinical benefit of adjuvant treatment with ARB.

Key words: angiotensin II, giant cell arteritis, inflammation, cytokines

INTRODUCTION

Giant cell arteritis (GCA) is a large and medium size -vessel vasculitis, often granulomatous, that predominantly affects the aorta and its major branches [1]. Worldwide, GCA is considered the most frequent primary systemic vasculitis in people >50 years old [2].

The quality of life of patients with GCA may be deeply impaired by acute and chronic complications of the disease or its treatment [3]. At diagnosis, partial or complete visual loss is exhibited by 15-20% of patients whereas stroke is observed in 3-6% [4-6]. During follow up, the course of GCA may be troubled by relapses, occurring in 40-60% of patients [7, 8] and large vessel structural damage in the form of aortic aneurysms (18-33%) [9, 10] or stenoses of the aortic primary or secondary branches (5-15%) [11]. In addition, chronic administration of systemic glucocorticoids (GC) is required in a significant proportion of cases [12, 13]. Approximately 10-15% of patients cannot reduce prednisone (PDN) dose below 10-15 mg/d without experiencing flares or smoldering activity [12] and 40-50% of those who can achieve a tapering of PDN to physiologic levels cannot tolerate complete withdrawal after 2-3 years of therapy [12, 13]. More importantly, long-term therapy with GC is associated with adverse effects in 86-95% of patients [14, 15].

These data highlight that, although treatment with high-dose GC results in prompt symptomatic improvement and decreases the risk of ischemic complications [16], lower maintenance PDN doses do not completely abrogate essential pathways involved in disease persistence [11]. In this sense, the search for complementary therapies for GCA has been unsuccessful [17, 18]. In fact, only methotrexate has demonstrated a modest efficacy in reducing cumulative GC dose and relapse rate in affected patients and it's the only adjuvant agent recommended by European League Against Rheumatism (EULAR) [19-23]. Clearly, new therapeutic alternatives are needed for these patients.

The renin-angiotensin system (RAS) has been understood as an endocrine axis that regulates blood pressure and electrolyte balance [24]. The classic RAS has been configured as a multiple-step cascade initiated by secretion of renin, the rate-limiting enzyme of the system, by the granular cells of the renal juxtaglomerular apparatus in response to glomerular hypoperfusion. Renin mediates the cleavage of angiotensinogen (AGT), primarily synthesized

by hepatocytes, into angiotensin I which undergo cleavage into smaller fragments, including angiotensin II (ATII), by angiotensin-converting enzyme (ACE) [24]. In addition to the classical systemic understanding of the RAS, recent evidence indicates the presence of local angiotensin systems in several organs [25-27]. These local systems would function in an autocrine/ paracrine manner and its regulation would be independent of the systemic RAS. In recent years, ATII, the major effector peptide of the RAS, has emerged as a powerful pro-inflammatory peptide and pharmacological blockade of angiotensin-converting enzyme (ACE) or ATII receptor 1 (AGTR1) has been found to result in anti-inflammatory effects. [28].

Previously, we have reported that the addition of ATII receptor blockers (ARB) to standard prednisone therapy was associated with longer relapse-free survival, reduced relapse rate and glucocorticoid-sparing effect in patients with biopsy-proven GCA [29]. Noteworthy, a previous study performed in inflamed GCA temporal arteries showed an increased AGTR1 immunostaining in vascular smooth muscle cells (VSMC) and infiltrating leukocytes [30].

Based on these results, we hypothesized that GCA lesions may contain a local and functional angiotensin system. In the present study we investigated whether the angiotensin system (AGT, ATII, ACE, AGTR1 and AGTR2) is expressed in inflammatory lesions of GCA. In addition we performed functional studies in order to assess potential pro-inflammatory functions of ATII and their reversal by AGTR1 blockers in a co-culture model mimicking vascular inflammation.

PATIENTS AND METHODS

Patients. The study group consisted of 40 patients, aged 77 years (range 60–89). These patients were selected among patients diagnosed with biopsy-proven GCA between 1995 and 2007 [29], on the basis of availability of remaining frozen tissue to perform molecular pathology studies. General characteristics of these patients are similar to previously published cohorts [15, 18-20] indicating an unbiased selection (*see online supplementary table S1 for general characteristics*). We included as controls 18 uninvolved temporal artery biopsies obtained from patients in whom GCA was initially suspected but not confirmed, being subsequently diagnosed with other conditions. The ultimate diagnoses in these patients were isolated polymyalgia rheumatica (PMR, n=3), non-vasculitic anterior ischaemic neuropathy (n=6), migraine or other headache syndrome (n=4) and pluripathologic chronic conditions with constitutional symptoms (n=5). Except for the patients with isolated PMR, who received low-dose prednisone, none of the controls received long-term GC treatment. The study was approved by the local Ethics Committee of Hospital Clínic of Barcelona.

Isolation and culture of vascular smooth muscle cells from human temporal arteries. Description of the procedure has been previously reported [31]. Briefly, serial, 1 mm thick sections from temporal artery fragments, obtained for diagnostic purposes, were placed onto reconstituted basement membrane *Matrigel* (*Collaborative Biomedical Products, Bedford, Massachusetts, USA*) and cultured in Dulbecco modified Eagle medium (DMEM)/10% foetal bovine serum (FBS, *Invitrogen, Carlsbad, CA, USA*). Vascular smooth muscle cells (VSMC) began to sprout from the artery ring after 5-7 days and became confluent after 3 weeks, these VSMC were released with trypsin/EDTA (*Invitrogen*), transferred to uncoated flasks, and split 1:2 upon confluence. Phenotypic homogeneity was confirmed by a-smooth muscle actin expression by flow cytometry [32].

Co-culture of VSMC and peripheral blood monocyte cells (PBMCs). In an attempt to mimic some of the characteristics found in pathological lesions of GCA, VSMC were co-cultured with T-lymphocytes and monocytes, the major components of vessel wall infiltrates observed in GCA biopsies [33]. VSMC (3 x 10^5, passage 5-7) and PBMCs (1 x 10^6) were exposed to angiotensin II (1000 nM, *Abcam, Cambridge, UK*) or AGTR1 blocker losartan (100 μM, *Abcam*)

for 24 or 48 hours. These doses were selected on the basis of former literature review, and after exploration of 3 logarithmic doses (data not shown) [34, 35].

Isolation of mononuclear cells from human peripheral blood (PBMC) was done by density gradient centrifugation *Ficoll-Paque*™ technique. Experiments were performed with whole PBMCs or isolated CD4+ T-cells or CD14+ monocytes. Isolation of subpopulations was performed by magnetic separation technology with anti-CD4+/CD14+ coated Dynabeads™ (*Invitrogen*). In co-culture experiments, the term *attatched* is used to designate leukocytes that remained adhered to VSMC after incubation. These cells were separated from VSMC by a gentle non-enzymatic cell EDTA dissociation with Versene™ (*Life Technologies, Grand Island, NY, USA*). Lymphocytes or monocytes that remained in the co-culture supernatant were designated as *non-attached.*

Ex-vivo temporal artery culture. As previously described [36], freshly removed temporal artery sections from patients with histologically confirmed GCA were placed in RPMI 1640 medium (*Lonza, Verviers, Belgium*) supplemented with 10% FBS, 2 mM L-glutamine (*Invitrogen*), amphotericin B at 2.5 µg/ml (*Invitrogen*) and gentamycine (*Braun, Germany*) at 200 µg/ml. Twenty-four-well tissue culture plates were coated with a 25 µl *Matrigel* drop per well, which was allowed to solidify at 37°C for 30 min. One temporal artery section per well was dipped in the *Matrigel* coating and covered with 1 ml medium.

In this tri-dimentional matrix culture model, pharmacological intervention was performed to compare the expression of AGT, ACE and AGTR1 between GCA artery sections cultured with medium alone and cultured in the presence of dexamethasone (*Sigma, Ayrshire, UK*) at 0.5µg/ml. Each condition was tested in 3–4 replicate wells. Sections were incubated al 37°C in 5% CO2 for 5 days and were subsequently lysed in TRIzol reagent (*Invitrogen*). Replicates of biopsy sections were pooled.

Cytokine mRNA measurement by real-time quantitative PCR. Three to four temporal artery sections or 3 x 10^5 cultured vascular smooth muscle cells (VSMC) or 1 x 10^6 peripheral blood mononuclear cells (PBMC) were homogenised in 1 ml TRIzol reagent. RNA extraction was performed according to the chloroform-isopropanol precipitation method. Total RNA

(1μg) was reverse transcribed to cDNA using Archive kit (*Applied Biosystems, Life Technologies, Carlsbad, CA, USA*) in a final volume of 100 μl, employing random hexamer priming.

cDNAs of the angiotensin system components AGT, ACE, AGTR1, AGTR2 and pro-inflammatory cytokines IL-1β, IL-6, IL-6 receptor (IL-6R), IL-17, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and chemokine ligand 2/monocyte chemoattractant protein (CCL-2/MCP-1) were measured by quantitative real-time PCR using specific predeveloped *Taqman* probes from Applied Biosystems (*Taqman Gene Expression Assays, see online supplementary methods*). Human liver tissue was used as positive control for AGT determination.

The reaction was monitored by measuring the fluorescence signal after each cycle with ABI Prism 7900 Sequence Detection System (*Applied Biosystems*) and results were analysed with the Sequence Detection Software V.2.3 (*Applied Biosystems*). All samples were normalised to the expression of the endogenous control GUSb and values were expressed as *relative units*. Comparative Ct method was used to assess the relative gene expression. Detailed methodology has been described in previous studies [37, 38].

Confocal microscopy. Qualitative assessment of the distribution of the ATII-system elements at the cellular level was performed in inflammatory lesions of one GCA temporal artery and one control artery. Sections were prepared as previously described [39]. Briefly, temporal artery biopsy fragments were fixed in 4% paraformaldehyde with increasing concentrations of sucrose, frozen with OCT and stored at −80°C. Cryostat 10 μm sections were fixed with 4% paraformaldehyde, permeabilised with Triton 0.1% and immunostained with the following primary antibodies: mouse monoclonal anti-alpha smooth muscle actin (α-SMA, *Abcam*), goat polyclonal anti-ATII (*Santa Cruz biotechnology, Santa Cruz, CA, USA*), goat anti-ACE (*Santa Cruz biotechnology*), rabbit polyclonal anti-AGTR1 (*Abcam*), mouse anti-CD3+ (clone PS1, *Novocastra, Leica Microsystems, Newcastle, UK*) and mouse anti-CD68+ (clone KP1, *Dako, Glostrup, Denmark*). Secondary antibodies consisted of Alexa Fluor 488 donkey anti-rabbit IgG, Alexa Fluor 555 donkey anti-goat IgG and Alexa Fluor 647 donkey anti-Mouse IgG (*Life Technologies*) at 1: 300 dilution. Negative control was performed using nuclear

staininig and secondary antibodies only. Nuclei were stained with *Hoechst dye* (*Molecular Probes, Life Technologies Ltd, Paisley, UK*) at 1:1000. Slides were mounted in *Mowiol 4-88 Reagent* (*Merck4Biosciences, Nottingham, UK*) and examined using a laser scanning confocal *Leica TCS SP5* microscope (*Leica Microsystems, Heildelberg, Germany*). Images were processed with *Leica application suite X* software.

Western blot analysis. Assessment of AGTR1 protein expression by Western blot was performed as described [40]. Briefly, cell lysates were obtained in modified radioimmunoprecipitation assay (RIPA) buffer and supplemented with protease inhibitors (*Complete, Boehringer Mannheim, Mannheim, Germany*). Twenty micrograms of protein per lane were subjected to SDS-PAGE and transferred onto nitrocellulose membranes (*Invitrogen, Carlsbad, California, USA*). Blocked membranes were incubated overnight at 4°C with polyclonal rabbit antihuman AGTR1 (*Abcam*). Immunodetection was performed with horseradish peroxidase conjugated goat anti-rabbit antibody (*Cell Signaling Technolgy, Danvers, MA, USA*) at 1:2000 dilution.

Statistical analysis. Continuous variables are presented as mean ± SEM or median (interquartile range, IQR) and categorical data as percentages. Mann–Whitney test Wilcoxon matched-pairs signed rank test and Spearman's rho correlation coefficient were used for statistical analysis. Calculations and graphics were performed with the statistical package IBM SPSS Statistics for Mac OS (*Version 20.0. Armonk, NY: IBM Corp*) and GraphPad Prism version 6.0 for Mac OS (*GraphPad Software, La Jolla California USA*).

RESULTS

Expression of the ATII system in GCA and control temporal arteries. The main components of the ATII system were constitutively expressed in both normal and GCA–involved temporal arteries at the mRNA level. However, there were remarkable differences. As shown in figure 1, ACE transcripts (median [IQR]) were significantly more abundant in GCA samples than in normal specimens (46.5 [IQR 40.0] vs 23.3 [IQR 23.9] *relative units*; p=0.003). In contrast, expression of AGT and AGTR1 was lower in inflammatory lesions of GCA than in control arteries (10.2 [IQR 20.9] vs 27 [IQR 28.6], p=0.041 and 0.35 [IQR 1.0] vs 2 [IQR 2.7], p=0.017, respectively). Of note, we observed that expression of AGT and AGTR1 significantly correlated to each other (r=0.547, p=0.001). Remarkably, AGTR2 mRNA was not detected in any of the evaluated temporal artery biopsies.

AGT, ACE and AGTR1 are differently expressed in constituent cells of inflamed lesions of GCA. We next assessed how ATII-system key elements were distributed in cellular populations considered of relevance to GCA pathogenesis. For this purpose, the expression of AGT, ACE, AGTR1 and AGTR2 was assessed in isolated and co-cultured VSMC, CD4+ T-cells and CD14+ monocytes.

AGT and AGTR1 gene expression was observed in isolated VSMC but not in isolated CD4+ T-cells or CD14+ monocytes (figure 2). In contrast, ACE transcripts were identified in all the studied cell populations, being comparative higher in macrophages (figure 2). In VSMC, co-culture (24 hr) with CD4+ T-cells or CD14+ monocytes enhanced the expression of AGT and ACE (figure 2). In the case of CD4+ T-cells and CD14+ monocytes, 24 hr co-culture with VSMC up-regulated gene expression of AGT and ACE, and AGT and AGTR1, respectively (figure 2). The same trend was observed during extended incubation (48 hr and 72 hr, data not shown). Again, gene expression of AGTR2 was not observed.

ATII, ACE and AGTR1 expression is qualitatively higher in inflamed lesions of GCA. To gain a better understanding of the expression of the ATII system key elements at the protein level, temporal artery sections obtained from one control patient and one untreated case were analysed by immunofluorescence and confocal microscopy. We found that ATII and AGTR1 were present in both, GCA and control arteries, although intensity of immunostaining was

clearly higher in GCA sections. In contrast, ACE stained in GCA inflammatory lesions only (figure 3).

Figures 4 and 5 show the distribution of ATII, ACE and AGTR1 in a GCA-involved temporal artery. Antibodies against α-SMA, CD3+ and CD68+ were used for identification of VSMC, T-cells and macrophages, respectively.

In VSMC we observed an intense immunostaining for ATII and AGTR1 but not for ACE (figure 4). This result confirmed the mRNA findings, where VSMC were the main source of AGT and AGTR1. In contrast, ACE protein was not observed in VSMC, which contrast to that found at the mRNA level.

Regarding infiltrating leukocytes and in agreement with the mRNA results, ACE expression was present in the surface of a fraction of CD3+ and CD68+ cells (figure 5). Of note, AGTR1 was clearly identified in some T-cells and macrophages. Although no AGTR1 mRNA expression was observed in isolated T-cells or monocytes, it was slightly induced when co-cultured with VSMC cells (figure 5). In order to confirm these results, the presence of AGTR1 in isolated VSMC, PBMC, CD4+ T-cells and CD14+ monocytes was assessed by Western-blot. As depicted in figure 5, AGTR1 was strongly expressed by VSMC and slightly expressed by PBMC, CD4+ T-cells and monocytes.

Interestingly, a fraction of CD3+ and CD68+ cells stained for ATII, probably due to the presence of ACE in their surface. In addition, intense expression of the AGTR1 was observed in endothelial cells of the inflamed artery (figure 3).

ATII stimulates the release of pro-inflammatory cytokines in VSMC and PBMC. We next tested the effect of ATII and AGTR1 blockade in VSMC extracted from temporal arteries, in PBMC, and in co-culture systems. For this purpose VSMCs and PBMC were incubated with ATII (1000 nM) or specific AGTR1 antagonist losartan (100 μM). After 24 or 48 hrs, mRNA expression of pro-inflammatory cytokines IL-1β, IL-6, IL-6R, IL-17, TNF-α, IFN-γ and CCL-2 (MCP-1) was determined in both isolated and co-cultured cells. These molecules were selected according to their previously demonstrated expression in GCA and their association with clinically relevant outcomes [39, 41-46].

We observed that ATII did not modify the expression of the selected molecules in isolated VSMC (figure 6 and *online supplementary figure S1*). In contrast, ATII strongly induced the expression of IL-1β, IL-6, CCL2 (MCP-1) and TNF-α in VSMC co-cultured with PBMC (figure 6). As expected, losartan produced the opposite effect with reduction of the ATII up-regulated molecules (figure 6). Interestingly, losartan also reduced baseline expression of IL-1β and IL-6 by VSMC. Basal expression of IL-6R, IL-17 and IFN-γ was minimal or absent in isolated and co-cultured VSMC and was not modified in presence of ATII or losartan (data not shown).

With respect to PBMC, ATII induced an important increase in mRNA level of IL-1β, IL-6, TNF-α, IFN-γ and CCL2 (MCP-1) in isolated cells. In addition, gene expression of IL-1β, IL-6, and CCL2 (MCP-1) was up-regulated in co-cultured PBMC with VSMC (figure 6). Again, losartan minimized the basal expression of these cytokines (figure 6). Expression of IL-6R in isolated and co-cultured PBMC was not modified after ATII or losartan incubation (data not shown). IL-17 was not expressed by PBMC in this system.

Similar results were obtained after extended stimulation for 48 hrs in both VSMC and PBMC (data not shown).

Influence of dexamethasone on the expression of the ATII system in ex-vivo cultured GCA temporal arteries. We next assessed whether ATII system was influenced by the administration of GC. For this purpose, temporal artery sections obtained from 7 GCA patients were cultured on *Matrigel* for 5 days in the presence of dexamethasone (0.5 μg/ml). As depicted in figure 7, we found that dexamethasone significantly down-regulated mRNA concentration (median [IQR]) of ACE, from 52.3 (IQR 71) to 31.75 (IQR 32.4, p=0.032) *relative units* and increased AGRTR1 expression, from 0.261 (IQR 0.794) to 0.868 (IQR 1.69, p=0.031). Although AGT transcripts increased in presence of GC, the difference was not significant (figure 7).

DISCUSSION

The present study demonstrates that a local ATII system is present in GCA inflammatory lesions. The functional pro-inflammatory relevance of this system is demonstrated in a co-culture model mimicking the vascular inflammatory infiltrates in GCA. Moreover, in this model, the ARB losartan is able to down-regulate the expression of baseline and ATII-induced cytokines, which, according to previous studies, are known to be expressed in GCA lesions and correlate with clinically relevant outcomes.

The expression of the ATII system appears to require multi-cellular cooperation in GCA since VSMC mainly expressed AGTR1 and AGT whereas infiltrating leukocytes contributed ACE. Generation of active ATII from constitutive AGT might then require ACE expression by infiltrating leucocytes. Both cell types may have the capacity to respond to ATII: VSMC strongly expressed ATGR1 but lower AGTR1 expression could also be observed in infiltrating leucocytes. These results are in line with those reported in a previous study, where, by using classical immunohistochemistry, AGTR1 immunostaining was observed in VSMC, infiltrating leucocytes and multinucleated giant cells in GCA arteries [30].

As mentioned, over the past years, local ATII systems have been identified in several organs, such as the heart, adipose tissue, brain, or kidneys [24-27]. Although the physiological relevance of these autonomous systems is not completely understood, overactivation with augmented local production of ATII has been described as a pathogenic mechanism in animal models of chronic renal failure associated to hypertension and diabetes mellitus [47, 48]. Altogether, these findings suggest participation of the ATII system in vascular inflammatory lesions of GCA.

ATII is currently considered an important pro-inflammatory peptide [24]. Of relevance, our results demonstrated that *in vitro* ATII is able to enhance the production of several pro-inflammatory molecules overexpressed in GCA involved arteries or increased in patient's sera [39, 41-46]. Former data in human VSMC from other origins (i.e. saphenous vein) showed that ATII up-regulates the expression of IL-1β, IL-6, IL-8, CCL2 (MCP-1) and, osteopontin [34, 35, 49, 50].

The same effect has been reported for TNF-α, CCL2 (MCP-1) and IL-8 in blood monocytes [51, 52]. Furthermore, ATII up-regulates the expression of VCAM-I, ICAM-1 and E-selectin by cultured endothelial cells [53, 54].

Interestingly, in our model, ATII enhanced the release of pro-inflammatory cytokines in VSMC obtained form temporal arteries only when co-cultured with PBMC. This finding suggests that, in GCA, infiltrating leukocytes may exert phenotypic modulations that switch VSMC into pro-inflammatory cells, able to secrete and react to other inflammatory molecules, such as ATII [55]. Although our results do not definitely demonstrate a direct participation of the ATII system in the generation of vascular inflammation in GCA, these findings suggest that ACE/ATII/AGTR1 axis regulates interplays between inflammatory cells and vascular wall components.

The exposed experimental evidence is consistent with a previous clinical observation, suggesting that ARB may have relevant anti-inflammatory effects in GCA patients [29]. In an observational study performed on a longitudinally followed cohort of biopsy-proven GCA patients, addition of ARB to standard prednisone therapy was associated with glucocorticoid-sparing effect in addition to lower relapse rate [29]. In our experimental functional conditions, AGTR1 blockade was accompanied by reductions in mRNA concentration of IL-1β, IL-6, IFN-γ and CCL-2 (MCP-1) in VSMC and PBMC. In the same way, previous studies performed on rat and human cultured VSMC showed that losartan resulted in significant reduction of IL-6 and CCL-2 (MCP-1) gene expression [34, 35]. Interestingly, in our system, losartan had some effect in down-regulating expression of IFN-γ, a crucial cytokine in Th1 differentiation which has been considered to have a seminal role in GCA chronicity since its expression is less dramatically reduced by GC treatment than the expression of other relevant cytokines, including IL-1b, IL-6 or IL-17 [36, 44, 56].

An interesting observation was the fact that, in cultured GCA-involved temporal arteries, dexamethasone down-regulated the expression of ACE but has the opposite effect on AGTR1. These findings indicate that the modulation of the ATII system by GC is partial and suggests that blockade of AGTR1 with ARB may contribute additional effects to GC

treatment. Of note, ARB anti-inflammatory effects may derive not only from the blockade of ATII functions, but also as a consequence of its ability to promote increased expression of the ACE2/angiotensin (1-7)/Mas receptor axis [57]. In experimental conditions, activation of this pathway has been associated with anti-inflammatory, anti-proliferative, anti-fibrotic, and anti-thrombotic effects [58-61]. Whether this axis is expressed in GCA remains to be investigated.

In summary, our findings suggest that angiotensin II system may play a role in the persistence of chronic inflammation in GCA. Interference with this system may, then, be a potential therapeutic option for patients with GCA. However the observational nature of our results does not allow drawing strong mechanistic conclusions about the specific role of the ATII system in sustaining vascular inflammation in GCA and further investigation is needed. The potential efficacy of ARB, low cost commonly used drugs with a security profile widely known, would make them attractive as an adjuvant treatment for patients with GCA.

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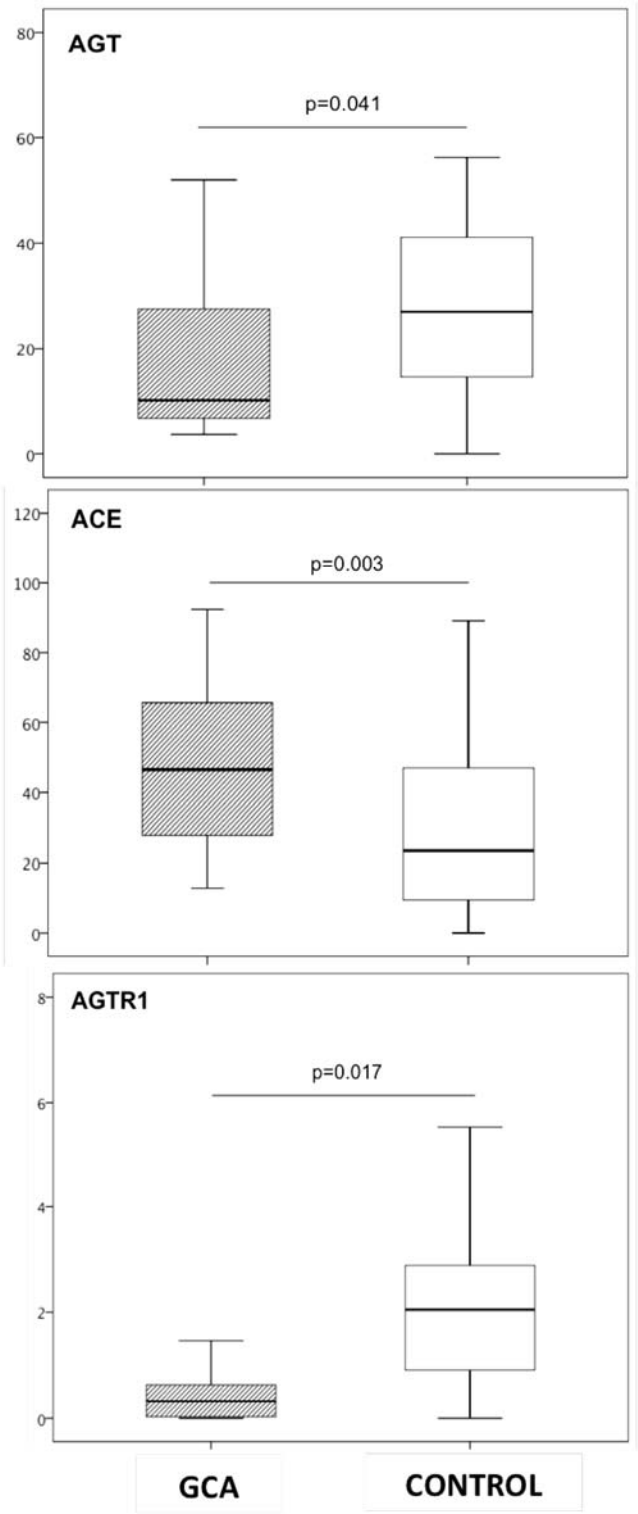


Figure 1. Comparison of mRNA concentration (*relative units*) of angiotensinogen (AGT), angiotensin II-converting enzyme (ACE) and angiotensin II receptor 1 (AGTR1) in temporal artery sections from giant cell arteritis (dashed bars) and control patients (white bars). Figure shows the results of 38 GCA vs 17 control arteries in the case of AGT and AGTR1 and, 40 vs 18 in the case of ACE. [Note the scale is different among the 3 figure parts].

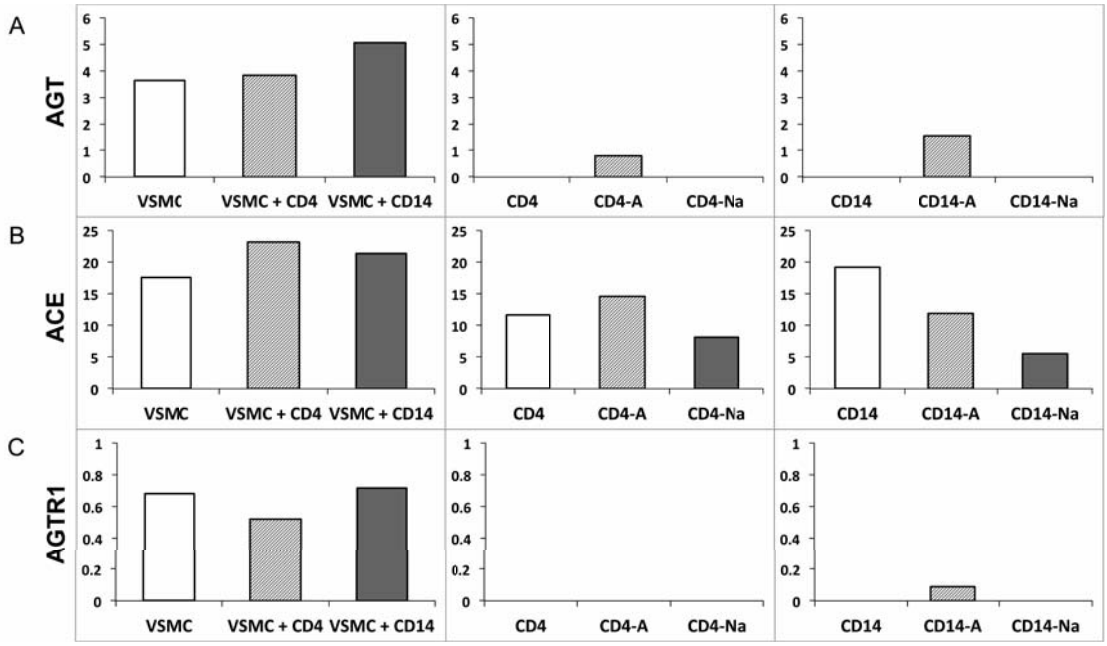


Figure 2. Differential expression of (A) angiotensinogen (AGT), (B) angiotensin-converting enzyme (ACE) and (C) angiotensin II receptor 1 (AGTR1) mRNA (*relative units*) by isolated smooth muscle cells (VSMC), CD4+ T-lymphocytes and CD14+ monocytes. Expression in co-cultured cells (24 h) is depicted as VSMC + CD4 or VSMC + CD14. Co-cultured CD4+ or CD14+ that remained adhered to VSMC are labeled as CD4-A and CD14-A (*attached*), respectively. NA (*non-attached*) refers to CD4+ T-cells and monocytes remaining in the supernatant. The experiment was reproduced two times with similar results and a representative experiment is displayed.

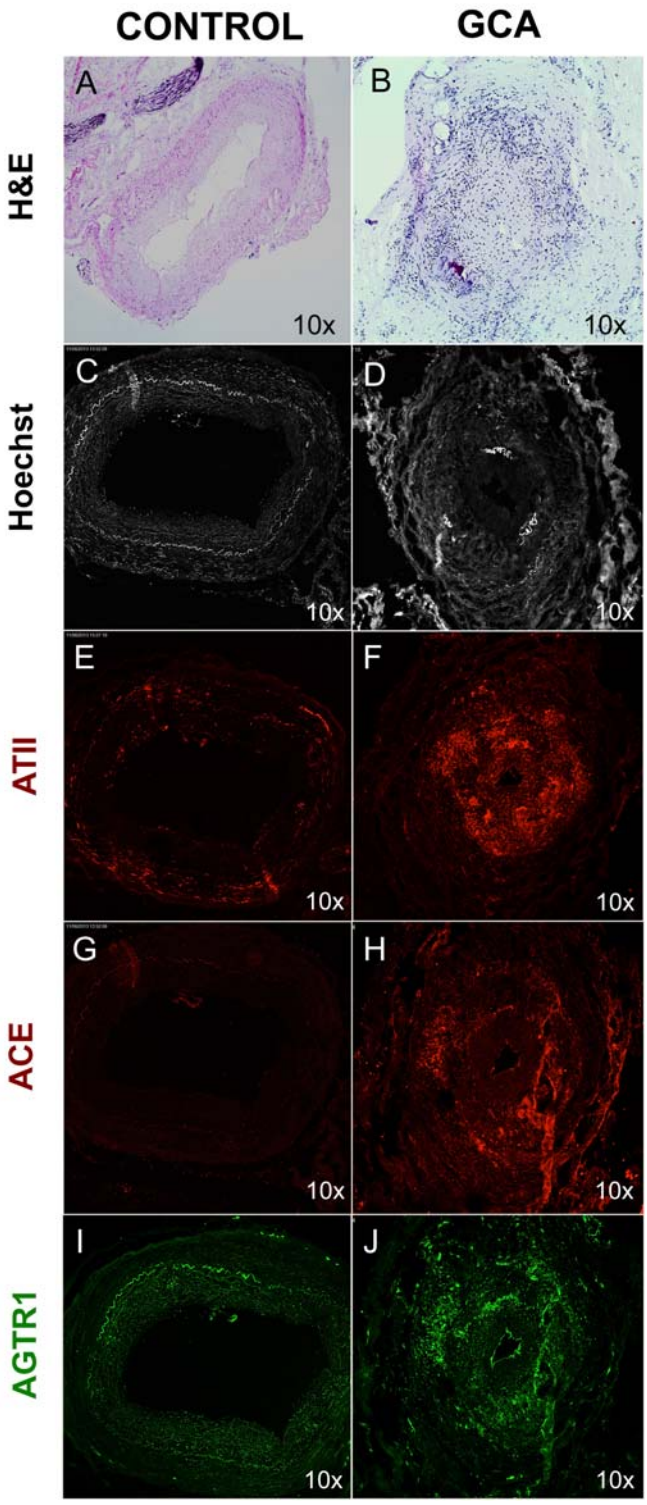


Figure 3. Expression of the main components of the ATII system in temporal artery sections from a control (*left column*) and an active GCA (*right column*) patient. (A and B) Hematoxylin and eosin (H&E)-stained sections. (C and D) Nuclear staining (Hoechst). (E and F) Angiotensin II expression (red colour). (G and H) Angiotensin II converting enzyme expression (red colour). (I and J) Angiotensin II receptor 1 expression (green colour). Intense signal expression of ATII system was observed in GCA sections.

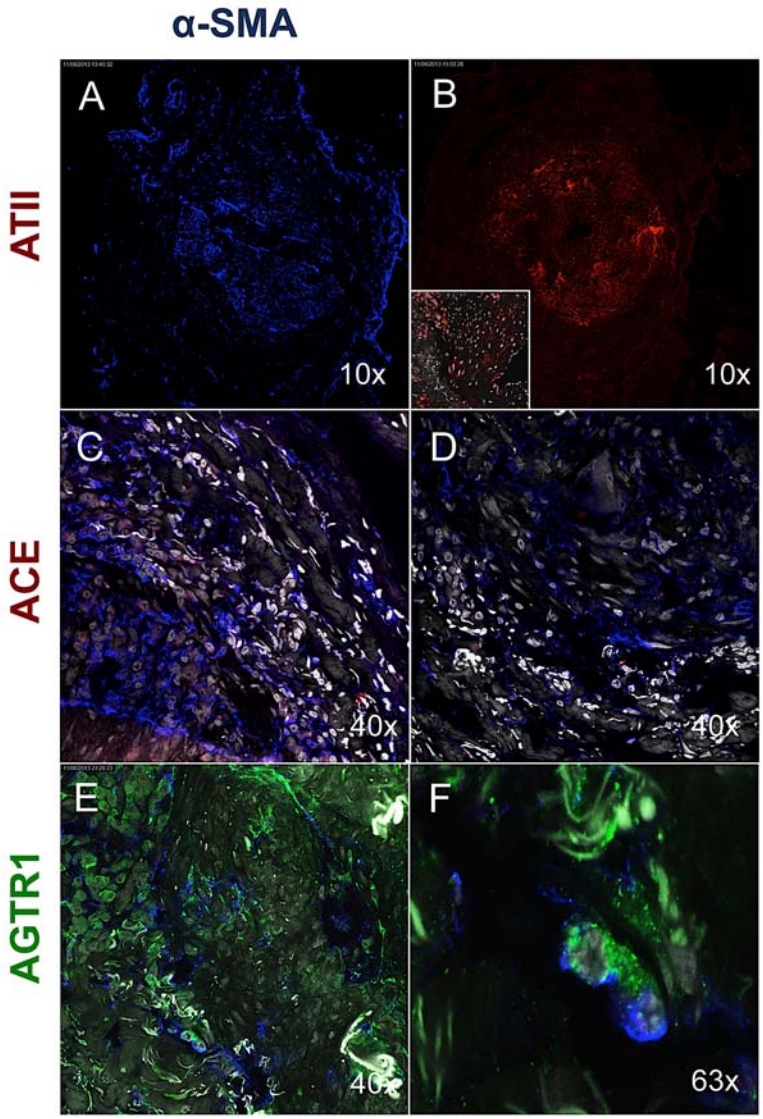


Figure 4. Expression of ATII system in vascular smooth muscle cells (VSMC) in an inflamed GCA temporal artery. Staining for alpha-smooth muscle actin (α-SMA, blue colour) was used to identify VSMC. (A-B) Angiotensin II (ATII) expression (red colour). An intense ATII signal co-localized with VSMC . (C-D) ACE (red colour) expression. Note the absence of ACE co-location with α-SMA in the intimal-medial layers. (E-F) AGTR1 (green colour) expression. Magnified medial layer cells intensively expressing both α-SMA and AGTR1 (green over blue).

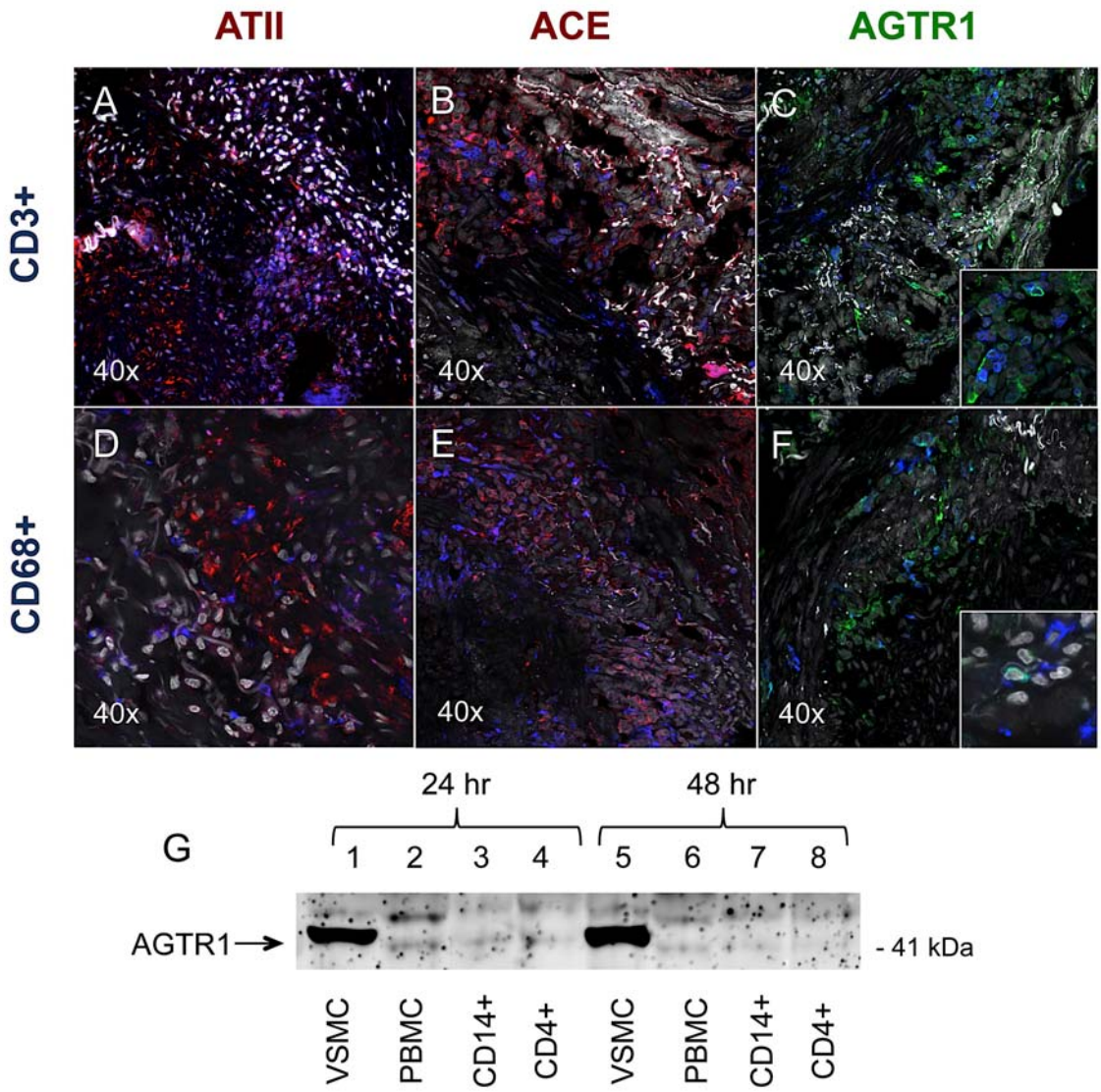


Figure 5. Expression of the ATII system in T-lymphocytes and macrophages in an inflamed GCA temporal artery. Immunostaining for CD3+ and CD68+ (blue colour) were used to identify T-cells and macrophages, respectively. (A) ATII expression (red colour) co-localized with a fraction of CD3+ positive cells in the adventitial layer. (B) ACE expression (red colour) observed in a group of T-lymphocytes. (C) AGTR1 expression (green colour) in clear co-location with CD3+. Inset shows a closer examination of CD3+ cells with AGTR1 signal. (D) Clear ATII expression (red colour) in a fraction of CD68+ macrophages. (E) ACE (red colour) expression co-localizing with CD68+ cells in the adventitial layer. (F) AGTR1 (green colour) observed over macrophages. Magnified cells intensively expressing both AGTR1 and CD68+ (green over blue) are shown in the inset image. (G) Detection of AGTR1 in isolated VSMC, PBMC, CD4+ and CD14+ subpopulations by western blot.

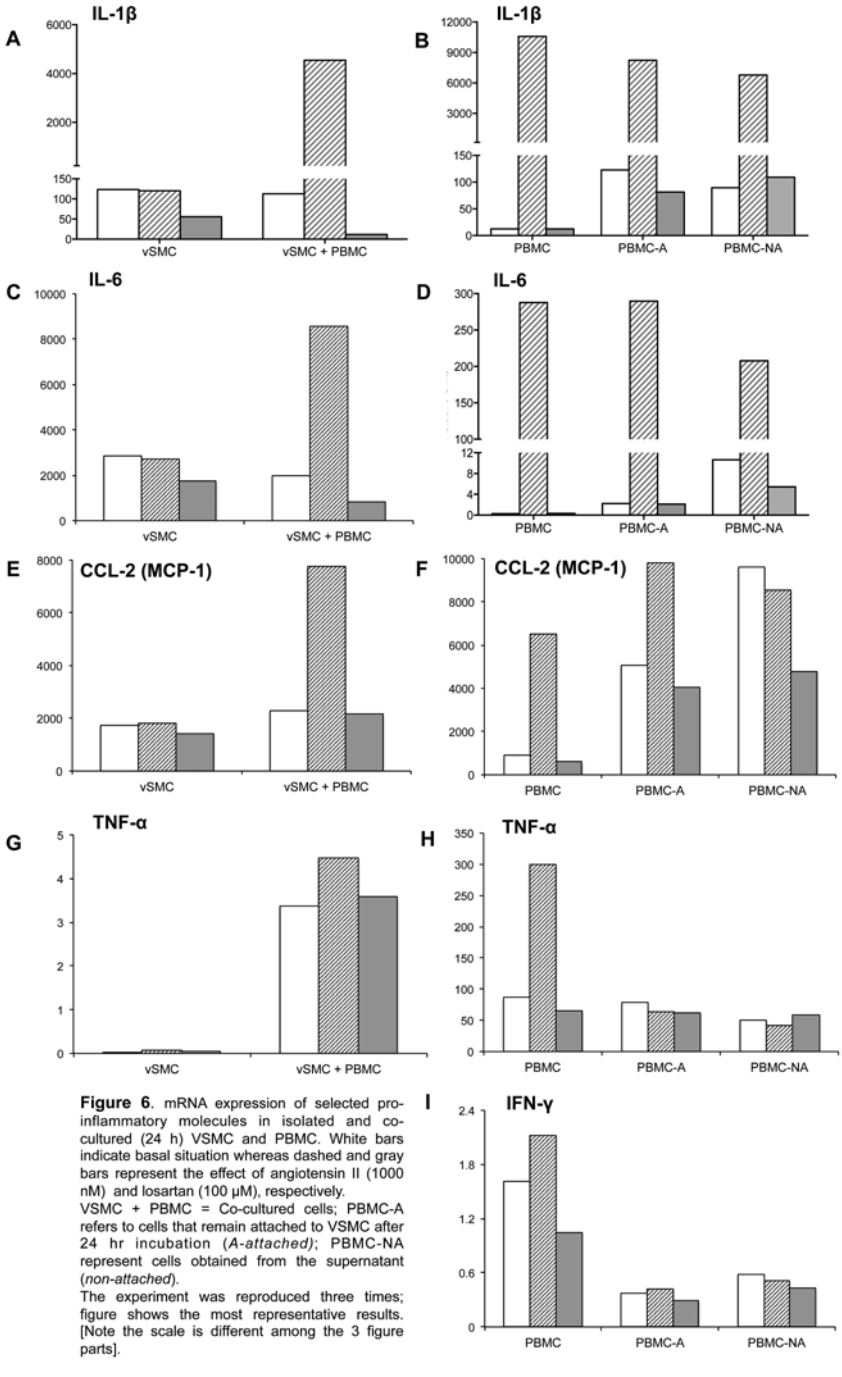


Figure 6. mRNA expression of selected pro-inflammatory molecules in isolated and co-cultured (24 h) VSMC and PBMC. White bars indicate basal situation whereas dashed and gray bars represent the effect of angiotensin II (1000 nM) and losartan (100 μM), respectively.
VSMC + PBMC = Co-cultured cells; PBMC-A refers to cells that remain attached to VSMC after 24 hr incubation (*A-attached)*; PBMC-NA represent cells obtained from the supernatant (*non-attached*).
The experiment was reproduced three times; figure shows the most representative results. [Note the scale is different among the 3 figure parts].

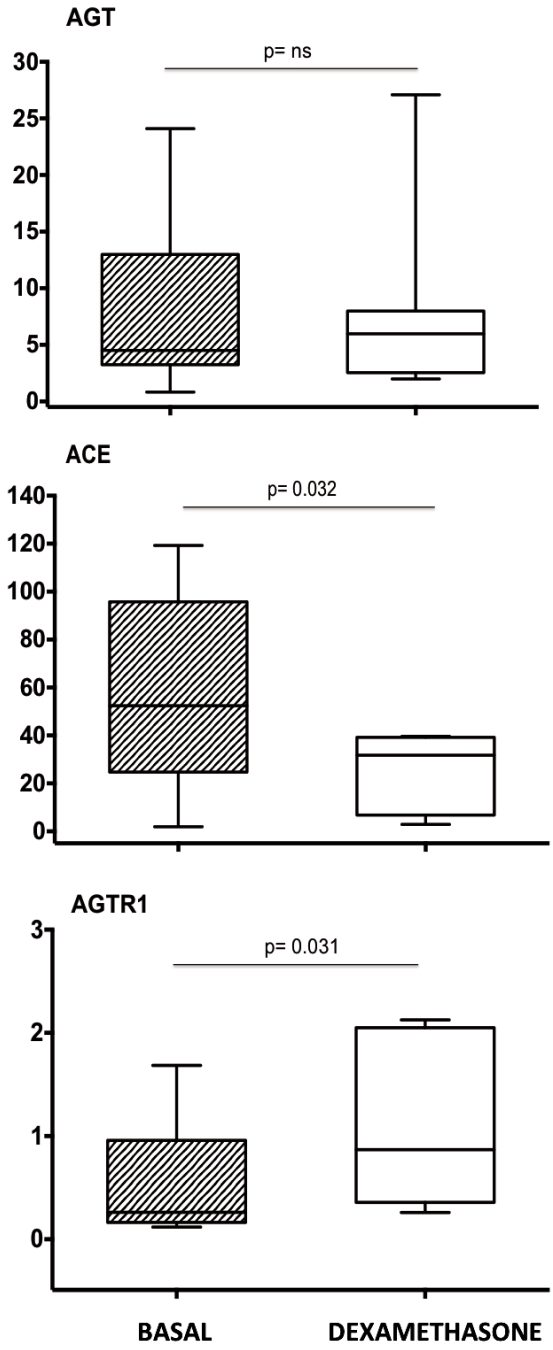


Figure 7. Changes in mRNA concentration (*relative units*) of angiotensinogen (AGT), angiotensin II-converting enzyme (ACE) and angiotensin II receptor 1 (AGTR1) induced by dexamethasone treatment (0.5 μg/ml). Comparison was performed in 7 cultured temporal arteries from patients with active GCA. [Note the scale is different among the 3 figure parts].

Supplementary methods.

1) *For description of cohort characteristics see table S1.*

2) Probes used for real-time PCR experiments.

ACE: Hs00174179_m1
AGT: Hs00174854_m1
AGTR1: Hs00258938_m1
AGTR2: Hs00169126_m1
CCL2 (MCP-1): Hs00234140_m1
GUSb: Hs99999908_m1
IFNγ: Hs00174143_m1
IL-1β: Hs01555413_m1
IL-6: Hs00985639_m1
IL-6R: Hs01075667_m1
IL-17A: Hs00174383_m1
TNFα: Hs00174128_m1

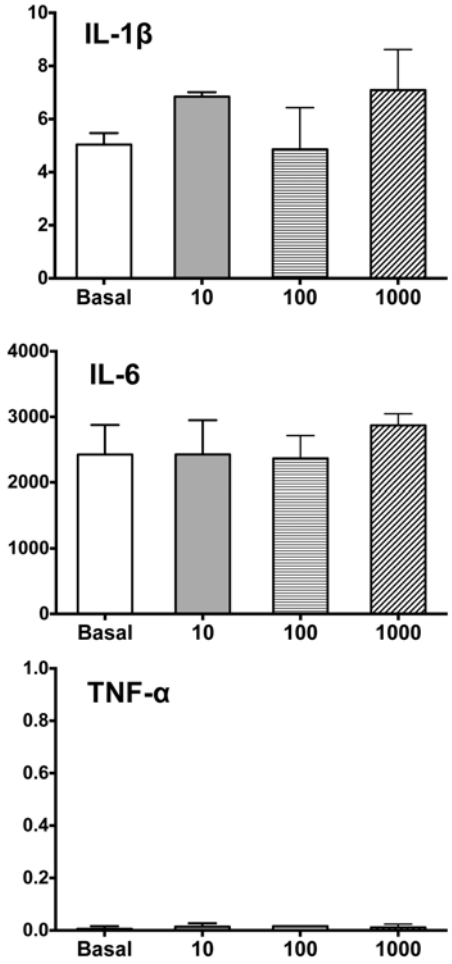
3) Prednisone administration

In 12 of the 40 GCA patients, the temporal artery was removed after the administration of 1-2 doses of prednisone (1 mg/Kg/day up to 60 mg/day). High dose glucocorticoids did not influenced mRNA concentration of AGT, ACE, or AGTR1.

SUPPLEMENTARY TABLE 1. Clinical features of the study cohort (n=40)

Characteristic	*n (%)*
Sex, no. female/male	28/12 (70/30)
Cranial symptoms, n (%)	
Headache	31 (77.5)
Jaw claudication	21 (52.5)
Scalp tenderness	21 (52.5)
Systemic manifestations, n (%)	
Fever	8 (20)
Anorexia	4 (10)
Weight loss	22 (55)
Ischemic complications, n (%)	
Amaurosis fugax	5 (12.5)
Permanent visual loss	6 (15)
Diplopia	5 (12.5)
Stroke	0 (0)
Polymyalgia rheumatica, n (%)	17 (42.5)
LABORATORY PARAMETERS, MEAN ± SD	
ESR, mm/hour	86 ± 28
CRP, mg/dl	10.1 ± 13.7
Haptoglobin, mg/dl	3.8 ± 1.6
Haemoglobin, gm/l	11.4 ± 1.5

CRP= C-reactive protein; ESR= erythrocyte sedimentation rate



Supplemental figure S1. Vascular smooth muscle cell gene expression (mean ± SEM, *relative units*) of selected pro-inflammatory cytokines after stimulation (6 hr) with three different ATII concentrations (nM). [Note the scale is different among the 3 figure parts]. The experiment was reproduced two times with similar results and a representative experiment is displayed.

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Practical Reports on Rheumatic & Musculoskeletal Diseases

Giant Cell Arteritis in Mexican Patients

Marco A. Alba, MD, MSc, Jorge A. Mena-Madrazo, MD,† Edgardo Reyes, MD,‡ and Luis Felipe Flores-Suárez, MD, PhD**

First Article: main results.

1. Only 22 patients with GCA were identified in a span of 21 years, underlining the rarity of the disease in Mexican population.

2. Clinical manifestations were similar to those reported in large series, with headache being the most frequent presenting symptom.

3. Severe cranial ischemic complications were identified in 32% of cases, mostly in the form of unilateral blindness.

4. Most patients developed an intense systemic inflammatory response with elevated serum concentration of C-reactive protein (CRP) and high erythrocyte sedimentation rate (ESR).

5. Although prompt initial response to high prednisone treatment was observed, relapses were recorded in 45.5% of patients.

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Relapses in Patients With Giant Cell Arteritis

Prevalence, Characteristics, and Associated Clinical Findings in a Longitudinally Followed Cohort of 106 Patients

Marco A. Alba, MD, Ana García-Martínez, MD, Sergio Prieto-González, MD, Itziar Tavera-Bahillo, MD, Marc Corbera-Bellalta, PhD, Ester Planas-Rigol, PhD, Georgina Espígol-Frigolé, MD, Montserrat Butjosa, MD, José Hernández-Rodríguez, MD, and Maria C. Cid, MD

Second article: main results.

1. Sixty-four percent of patients experienced at least 1 relapse and thirty-six percent 2 or more. Mean time to first relapse was 79 ± 75 weeks (range, 11 to 339 weeks). Mean prednisone dose at relapse was of 5.3 ± 6.5 mg/d with a median of 2.5 (IQR 7.5).

2. Polymyalgia rheumatica was the most frequent manifestation during relapses, while severe ischemic complications were observed in one patient only.

3. Relapses were accompanied by elevated levels of ESR (mean 61 ± 29 mm/h) and CRP (4.0 ± 3.8 mg/dL) that, nevertheless, were lower than those observed at disease onset.

4. Patients with multiple relapses were significantly more frequent among those with a strong systemic inflammatory response (SIR): 85% of patients with intense SIR relapsed 2 or more times compared with 37% of those with a weak response.

5. Patients with relapses required longer periods of glucocorticoid treatment, were exposed to higher cumulated prednisone doses and experienced more adverse effects.

SEMINARS IN ARTHRITIS & RHEUMATISM

Treatment with angiotensin II receptor blockers is associated with prolonged relapse-free survival, lower relapse rate, and corticosteroid-sparing effect in patients with giant cell arteritis

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Third article: main results.

1. Patients receiving angiotensin receptor blockers (ARB) presented a significantly longer relapse-free survival than patients treated with angiotensin converting enzyme inhibitors (ACEI) or patients not receiving ACEI/ARB (p=0.02).

2. The hazard ratio for relapses developing in patients who received ARB relative to that in patients who were not receiving ARB was 0.32 (IC95% 0.12-0.81, p=0.017).

3. Time required to reach a stable maintenance prednisone dose <10 mg/day (T<10) was significantly shorter for patients receiving ARB than for patients receiving ACEI or not receiving ACEI/ARB (p=0.0002).
4. Cumulated prednisone dose measured until T<10 tended to be different among groups (p=0.05), as was the cumulated prednisone dose during the first year.

5. Concentration of CRP was significantly higher in patients not receiving ACEI/ARB than in those receiving either ACEI or ARB (12, 18 and 24 months). This is in contrast to other inflammatory markers, such as ESR or hemoglobin, which levels were similar between the three groups during the follow-up.

Expression and functional activity of the angiotensin II system in temporal artery lesions from patients with giant cell arteritis

Marco A Alba*[1], MD, Ester Planas-Rigol*[1], PhD, Marc Corbera-Bellalta[1], PhD, Nekane Terrades[1], PhD, Ana García-Martínez[1], MD, Sergio Prieto-González[1], MD, Ester Lozano[1], PhD, José Hernández-Rodríguez[1], MD, Josep M. Grau[2], MD, and Maria C Cid[1], MD.

Fourth article: main results.

1. Gene expression of angiotensin converting enzyme (ACE) was higher in GCA temporal arteries than in normal specimens (median 46.5 [IQR 40.0] vs 23.3 [IQR 23.9] *relative units*; p=0.003). In contrast, mRNA levels of angiotensinogen (AGT) and angiotensin II receptor 1 (AGTR1) were significantly reduced in GCA (median 10.2 [IQR 20.9] vs 27 [IQR 28.6], p=0.041 and 0.35 [IQR 1.0] vs 2 [IQR 2.7], p=0.017, respectively).

2. Qualitative assessment of ATII-system by confocal microscopy revealed a strong expression of angiotensin II (ATII), ACE and AGTR1 protein in inflamed lesions of GCA.

3. In vascular smooth muscle cells (VSMC) co-cultured with peripheral blood mononuclear cells, ATII enhanced the production of IL-6, IL-6R, IL-1β and CCL2. Similarly, mRNA levels of IL-6, IL-1β, IFN-γ and CCL2 were increased by ATII in cultured peripheral blood mononuclear cells (PBMC).

4. Losartan reduced the production of pro-inflammatory molecules in co-cultured VSMC and PBMC.

5. In cultured temporal arteries, dexamethasone significantly downregulates the mRNA concentration of ACE, from a median of 52.3 (IQR 71) to 31.75 (IQR 32.4, p=0.032) *relative units* and increased AGTR1 expression, from a median of 0.261 (IQR 0.794) to 0.868 (IQR 1.69, p=0.031).

V. DISCUSSION

The present research line focused on the persistence of inflammatory activity in patients with giant cell arteritis. As part of this thesis project we characterized some of the major clinical manifestations and blood test abnormalities associated with persistent chronic inflammation. In addition, we investigated potential mechanisms participating in GCA inflammatory activity, which may lead to identification of potential new immunopathogenic pathways involved in disease persistence.

GCA is the most frequent systemic vasculitis in western countries (2, 3). However, it seems to be infrequent in geographic areas such Asia (10, 11) or Latin America (13-15). In the latter, ANCA-associated vasculitis are far more common (218, 219). To gain insight on this rare and possibly under-recognized disease in Hispanic patients, we retrospectively analyzed the characteristics of 22 Mexican patients, diagnosed over 21 years.

We found that, despite genetic and environmental differences, age at diagnosis, clinical presentation and disease course were similar to those reported in large cohorts of Caucasian patients (49, 54, 62, 70, 220, 221). As reported in all series, headache was the most frequent initial symptom, recorded in 91% of patients. In addition, PMR was observed in 59%.

Interestingly, most patients exhibited an intense systemic inflammatory response (SIR) clinically manifested as fever, anorexia or weight loss (86%). This was accompanied by laboratory abnormalities typically reported in active patients, i.e., elevated levels of ESR and CRP (recorded in 89% and 71% of patients, respectively), anemia and thrombocytosis. In this regard, the intensity of SIR has demonstrated to be a major prognostic factor in GCA (54, 103, 104). Previous studies have demonstrated that a strong initial SIR is associated with lower risk of ischemic complications (54) but with a more relapsing course and prolonged glucocorticoid (GC) requirements (54, 103).

Severe cranial ischemic complications (SIC), the most threatening manifestation of GCA, ocurred in 7/22 cases (32%). This prevalence was slightly higher to that found in European countries but similar to that reported in Brazilian patients (15).

Although GCA is rare in Latin-American countries, it seems that delayed treatment initiation was not the main causal factor of this finding, as the interval between the initial symptoms, diagnosis, and institution of GC therapy was similar to that reported in countries where the disease is more frequent. Probably, traditional risk factors of atherosclerosis, highly prevalent in Mexican population (222), play a role in the development of ischemic complications in some of the affected patients (56).

Of relevance, patients with ischemic complications displayed significant lower ESR levels than their non-ischemic counterpart (39.2 ± 38.1 vs 76.1 ± 36.2 mm/h, p=0.05). The relation of a weak initial acute-phase response and the development of GCA-related ischemic complications has been reported by our group (54, 106) and confirmed by others (57). In line with this, when SIR was categorized (strong vs weak) as in previous studies (54, 106, 111), we observed that seven of the eight ischemic patients (87.5%) exhibited a poor systemic inflammatory reaction.

As previously reported (88), relapses were frequent in this series, presented in 45.5% of patients. This percentage is similar to that reported by others (88, 90, 95, 97). Although these patients were not uniformly treated, episodes of disease recrudescence (range 1-4) occurred when prednisone was being tapered, confirming that high dose GC only transiently down-regulate proinflammatory activity.

In spite of its clear limitations, i.e., not biopsy-proven diagnosis in all cases, non-homogeneous treatment protocol and laboratory data not available for the entire series, we observed in this study of Latin American patients that manifestations related to intense initial and persistent inflammatory response were similar to those found in other regions around the world.

As a next step, we decided to characterize one of the most important consequences of persistent chronic inflammation in GCA: flare episodes.

This was considered of relevance because limited information existed regarding clinical characteristics, associated long-term morbidity or predisposing factors (88). Of relevance, our group addressed in previous studies the incidence and prognosis of GCA-associated delayed large vessel complications, another possible consequence associated with persistent activity and maladaptative remodelling (65, 66).

The second study included in the present thesis was performed in a cohort of 106 biopsy-proven GCA patients that were uniformly evaluated, treated and followed for an average of 7.8 ± 3.3 years (range 4-15). In this large cohort we found that 64% of the patients (n=68) relapsed at least once, usually in the first 1.5 years of follow up. Most patients relapsed with the same clinical features presented at diagnosis and importantly, only one patient developed an episode of severe cranial ischemia. Similarly, former studies documented flares in approximately 40% of patients in population-based series (89, 90) and in up to 85% in clinical trials with adjuvant therapies where glucocorticoids are quickly tapered (92, 93, 95, 97, 223). In these studies, relapses occurred commonly in the first 12 to 16 months after the onset of treatment. These data indicate that a large proportion of individuals suffering from GCA are predisposed to develop a sustained, self-perpetuating disease.

Relapses were accompanied by elevated levels of ESR and CPR; these inflammatory markers varied with particular clinical manifestations. Lower ESR and CRP concentrations were observed in patients with PMR and cranial manifestations whereas an intense acute phase reaction characterized cases with more systemic involvement. Of note, the inflammatory reaction of flares was comparatively lower than that observed at GCA onset. This was an interesting finding, which suggested that inflammatory activity progressively decreases but not completely abrogates in treated patients.

In this sense, as reported elsewhere (88-90, 224) and observed in the present study, PDN doses below 7.5 mg were usually not able to maintain sustained remission.

Previous clinical and experimental evidence have demonstrated that low GC doses did not completely abrogate all the inflammatory pathways involved in GCA (67). For instance, plasma IL-6 and acute phase reactants have been found to remain slightly or moderately elevated for several months after the beginning of corticosteroid treatment, even in patients with apparent clinical remission (108, 112-115, 225, 226). In addition, experimental data obtained from GCA temporal arteries engrafted into SCID mice demonstrated that after 1 week of dexamethasone treatment, tissue concentrations of IL-2, IL-1β, and IL-6 mRNA were reduced, which suggest a partial suppression of T cell and macrophage function. In contrast, synthesis of IFN-γ was only slightly decreased, and expression of TGF-β1 remains essentially unaltered even after 3 weeks of GC administration (118).

We observed that, as a consequence of the chronic nature of their illness, relapsing patients were dependent on long-term administration of GC and were exposed to higher cumulated PDN doses. In contrast to previous studies (88) that were unable to show that a relapsing course resulted in increased disease or treatment-related morbidity, we found that these individuals developed more GC-related toxicity, in particular osteoporosis.

Finally, we tried to identify some clinical or laboratory clues that could predict the development of relapses. In this regard, an intense acute-phase response was associated with higher risk of relapse. Consistent with this finding, other investigators have reported that strong acute phase response was associated with recalcitrant GCA (88, 106, 108, 109, 111, 119, 227). In this regard, elevated serum concentrations of TNF-α, IL-6 and soluble ICAM-1 have been associated with relapsing disease (108, 119, 120). In addition, studies performed in temporal arteries of relapsing patients have disclosed an intense initial mRNA expression of TNF-α and CCL-2 and of IL-12/23p40 and IFN-γ in second biopsies after 1 year of treatment (103, 109, 119). By contrast, increase IL-17 expression in GCA lesions is a predictor of sustained response to GC (228).

In summary, we found that although flares are frequent in GCA, they often respond to slight increases in the dose of GC. More importantly, ischemic complications are an extremely uncommon manifestation of controlled relapses. In addition, an intense inflammatory response may predict the development of recurrences and, as expected, a relapsing course was associated with prolonged GC requirements and higher prevalence of adverse effects. Taken together these results indicate that patients with GCA need to be indefinitely observed even after achieving stable minimal PDN doses.

As previously shown, in spite of the initial satisfactory response to high dose GC experienced by the majority of patients, remission maintenance is more problematic and long-term response to treatment is quite heterogeneous (85). Unresolved challenges that GCA poses to affected individuals and their physicians are diverse: some patients are mainly afflicted by excessive vascular destruction leading to aortic dilatation or aneurysm (22% after a median follow of 5.4 years and 33% after 10.3 years) (65, 229), other patients suffer from the consequences of vascular occlusion (5-15%) and in others, iatrogenic complications arise (230). Approximately 10% to 15% of patients cannot reduce corticosteroids below 10 mg/day without experiencing disease flares or smouldering activity (87, 88). And, about 40% to 60% of those who are able to taper corticosteroids to physiologic or near to physiologic levels cannot tolerate complete withdrawal after 2 to 3 years (87, 89). In addition, treatment related adverse events cumulate over the years and ~90% of patients experience at least one side effect over a median follow-up period of 10 years (90).

As it can be expected, morbidity resulting from these complications may deeply impair patient's quality of life (231). In addition, although some studies indicated that GCA does not reduce the life spans of affected individuals (232, 233), others have shown that GCA patients are more likely to die within the first years after diagnosis than age- and gender-matched controls (234, 235).

Not surprisingly, numerous efforts have been made to identify adjuvant treatments to reduce cumulative glucocorticoid exposure and more efficient therapies for patients with resistant disease (67, 236). During the past two decades, this subject has been addressed in retrospective case series, open-label trials and clinical-trials, although with disappointing results.

Methotrexate has demonstrated a modest efficacy in reducing relapse rate and lowering cumulative prednisone dose (92-95) and, is the only immunosuppressive drug recommended as adjuvant to GC in the treatment of large vessel vasculitis (72). In a small trial of mixed patients with GCA and PMR, azathioprine treatment resulted in a significant but small reduction in median prednisone dose at 1 year (96). A trial with infliximab in newly diagnosed patients yielded frustrating results (97) and a small trial of etanercept in relapsing patients or patients with GC-related side effects resulted in a significant but slight reduction in cumulated prednisone dose (98). Recently, retrospective case series have suggested efficacy for cyclophosphamide in patients with GC-dependent GCA at the expenses of infrequent but serious side effects (99). However, a substantial proportion of patients included in this study were not biopsy-proven. In addition, therapy targeted to disrupt IL-6 function is currently being evaluated in a large multicenter, randomized, placebo-controlled trial (100). Background for this study includes several case reports and small case series where tocilizumab, an IL-6 receptor blocking humanized monoclonal antibody, elicited remission in GC-dependent GCA patients (237). In summary, there is a clear unmet need for more efficient and safer therapies for the management of GCA.

During the past decade several studies have shown that commonly used drugs such as statins (238), pentoxifylline (239), antimalarials (240) and angiotensin converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ACEI) (241-245) elicit anti-inflammatory pleiotropic effects. Most of the beneficial effects of these compounds were observed in patients with atherosclerosis, the most common vascular chronic inflammatory disease.

Atherosclerosis and GCA may share some immune-inflammatory pathways; therefore, some of these drugs have been evaluated as potential adjunctive therapies in the management of patients with GCA. However, retrospective series did not found any significant benefit derived from the use of statins (246-248) or hydroxychloroquine (249, 250) on disease outcome.

In the search for new therapeutic options, we investigated whether the addition of ACEI or ARB to standard prednisone therapy had any significant impact in the outcome of patients with GCA in terms of frequency of relapses, corticosteroid requirements, and disease activity markers.

Again, data was obtained from our longitudinally followed and uniformly-treated cohort of 106 biopsy-proven GCA patients. As described in detail in the *methods* section of the third publication included in this thesis, patients were categorized in three groups: those treated with ACEI or ARB were included in group 1 (n=36) and group 2 (n=14), respectively; patients who had received neither ACEI nor ARB constituted group 3 (n=56). Pre-established end point variables reflecting chronic inflammatory GCA activity were compared between the three groups. In this observational study, ARB treatment was associated with reduction in two relevant primary outcome measures in large vessel vasculitis (251): relapses and glucocorticoid requirements. We found that patients receiving treatment with ARB in combination with standard GC therapy exhibited significantly reduced relapse rate (hazard ratio 0.32, IC95% 0.12-0.81, p=0.017) and longer disease free survival. In addition, mean time (in weeks) to first relapse was significantly longer (153 weeks for patients receiving ARB vs 60 weeks for those treated with ACEI vs 68 weeks for patients treated only with GC, p=0.012). In addition to the differences observed among our study groups, when we compared our data with that published in a similarly treated-cohort of Spanish patients (88), we observed that the reported mean time to first relapse (71 weeks) was remarkably similar to that recorded in patients from group 1 and 3 but, clearly shorter than that observed in patients receiving ARB (group 2).

Regarding GC requirements, therapy with ARB was associated with significant reduction in the time receiving more than 10 mg of prednisone/day (T<10, median survival 21 weeks for ARB vs 39 for ACEI vs 34 for no ACEI/ARB, p=0.0002) and its accompanying cumulated dose (1.0 ± 0.9 gm vs 2.4 ± 2.2 gm vs 1.6 ± 1.8 gm, p=0.05). Cumulated prednisone dose during the first year in ARB treated patients was 5.1 ± 0.65 gm, far lower than doses reported in another population-based study (mean 7.4 ± 4.3 gm) (88) and close to that published in clinical-trials, ranging from 3.7 to 5.2 gm (92, 95).

Unfortunately, as GC side effects were not systematically recorded in our study, we were unable to analyze whether reduced prednisone exposure was accompanied also by a decrease in incidence of GC toxicity.

Previous studies have reported the potential benefits of blocking the RAS in autoimmune conditions. An open study performed in 15 patients suffering from rheumatoid arthritis (RA) with active disease showed that 48 weeks of treatment with captopril decreased articular pain and plasma concentrations of CRP in ~60% of the subjects (244). Also, in a small randomized, double blind study of 11 patients, addition of ramipril to concomitant RA therapy significantly reduced plasma concentrations of pro-inflammatory cytokines TNF-α and soluble CD40 although other inflammatory markers such as CRP, IL-1β, IL-6, myeloperoxidase, and ESR were not influenced (245). At last, a retrospective analysis of a large cohort of patients with systemic lupus erythematosus (SLE) (n=378) demonstrated that the use of ACEI was associated with a decreased probability of renal involvement and decreased risk of disease activity measured by SLAM-R score (243). In contrast to some of these studies, we did not find any beneficial effect with ACEI administration. We hypothesize that in blood vessels (as it happens in the kidney and heart) production of ATII from angiotensinogen can be catalyzed by enzymes other than ACE (i.e., chymase, kallikrein, cathepsin G, and elastase-2) and therefore, the effects of angiotensin II produced by this alternative way could be inhibited by ARB but not completely by ACE inhibitors (130).

Limitations of the discussed study included the relatively low number of patients treated with ARB and the retrospective design, precluding homogeneity in type, dose and duration of ACEI/ARB treatment. In addition, the observational design of our study prevents any conclusion beyond association. In spite of these limitations, our findings suggested that ATII receptor blockade may help to maintain remission, reduce the relapse rate and spare glucocorticoids in patients with GCA.

Based on this clinical observation, we next explored whether the angiotensin II system was expressed in GCA lesions and whether angiotensin II or ARB influenced the expression of inflammatory biomarkers in functional models. The study population included 40 GCA patients derived from the 106 patient-cohort previously described. These patients were selected on the basis of availability of sufficient remaining frozen temporal artery biopsies to perform molecular pathology studies. Also, we included as controls 18 temporal artery biopsies from patients diagnosed with conditions other than GCA.

In the first phase of this translational research, we found that the main elements of angiotensin II (ATII) system, i.e., angiotensinogen (ATG), angiotensin II converting enzyme (ACE) and ATII receptor 1 (AGTR1) were expressed in both GCA inflammatory lesions and control arteries. Analysis of gene expression revealed that ACE expression was significantly more abundant in GCA samples than in normal specimens. In contrast, expression of AGT and AGTR1 was lower in pathological arteries. AGTR2 mRNA was not detected in any of the evaluated specimens. Decreased AGT and AGTR1 mRNA expression in GCA lesions was an unexpected finding. However, constitutive mRNA expression by normal arteries with no translation into protein and decreased mRNA in inflamed arteries with increased protein expression by inflammatory cells has been observed with other molecules relevant to both inflammation and vascular remodeling (252). We hypothesize that loss of VSMC in inflamed arteries accounts for the observed decrease in constitutively expressed mRNA.

To gain a better understanding of these differences, we next determined the expression of the same molecules in the major cellular fractions found in GCA inflammatory lesions: vascular smooth muscle cells, T-lymphocytes and macrophages. Our results showed that isolated VSMC expressed AGT, AGTR1 and ACE mRNAs. In contrast, isolated T lymphocytes and monocytes expressed only ACE but not AGT or AGTR1. However, when T lymphocytes or monocytes were co-cultured with VSMC, expression of AGTR1 was mildly expressed, particularly in monocytes.

These data suggested that infiltrating leukocytes were mainly responsible for the higher ACE expression observed in GCA arteries. On the other hand, destruction or dysfunction of VSMC, the main cellular component of the artery wall, might explain, as mentioned, reduced AGT/AGTR1 levels found in inflamed GCA samples. In this sense, loss of VSMC may be mediated by cytotoxicity, or oxidative damage (253-255).

We further investigated these molecules, at the protein level, through confocal microscopy. Images revealed that angiotensin II and AGTR1 immunofluorescence staining was clearly higher in GCA inflamed lesions compared to control samples. Of relevance, ACE was only demonstrated in pathological biopsy specimens. These results show that in comparison to controls, angiotensin II, AGTR1 and ACE proteins are overexpressed in GCA. Consistent with these findings, a previous study showed increased immunostaining for AGTR1 in VSMC, histiocytes and multinucleated giant cells in temporal artery lesions form patients with GCA (198). As mentioned, although AGTR1 gene expression was not detected in T-cells and monocytes in basal conditions it was slightly upregulated in co-culture systems with VSMC. Moreover, confocal microscopy and Western-blot demonstrated some protein expression by inflammatory cells. Previous studies have reported that the expression pattern of AGTR1 is widely different across leukocyte subsets being higher in polymorphonuclear and B-cells than in monocytes or T-lymphocytes (167).

Interestingly, in the later study, mRNA expression of AGTR1 in T-cells was almost null. In addition, receptor protein expression assessed by flow cytometry was detected in a very limited percentage of T-cells (3.5% of studied cells) (167). Therefore, it is probable that only a small fraction of temporal artery infiltrating leukocytes express AGTR1.

We next investigated the effect of GC treatment in ATII system and found that in *ex vivo* cultured temporal arteries, dexamethasone downregulated the expression of ACE, molecule mainly found in the inflammatory infiltrates. In contrast, steroid treatment has the opposite effect in constitutively expressed AGTR1. These findings indicate a partial modulation of the ATII system by GC and, consequently, blockade of AGTR1 with ARB might provide additional effects to GC treatment.

Based on these translational data and our previous clinical results it was attractive to hypothesise that the ATII system might be involved in maintaining disease activity in GCA patients. The rationale behind this idea comes from several studies that demonstrated that ATII exhibits pro-inflammatory effects (125).

To test this hypothesis, we performed *in vitro* functional studies with cultured peripheral blood mononuclear cells and VSMC obtained from temporal arteries. We observed that ATII is able to enhance the expression of pro-inflammatory molecules IL-6, IL-1β, TNF-α and CCL2 (MCP-1) in VSMC and IL-6, IL-1β, IFN-γ and CCL2 in PBMC. Remarkably, ATII enhanced the expression of the studied pro-inflammatory molecules in VSMC co-cultured with leukocytes, but not in isolated VSMC. This finding suggests that infiltrating leukocytes may induce a pro-inflammatory phenotype in VSMC, rending them able to produce pro-inflammatory cytokines and to respond to other inflammatory molecules, such as ATII (256). In fact, there is some evidence that, in GCA, VSMC are not only a target of the inflammatory process, but are also active players in its generation and maintenance, through the production of pro-inflammatory cytokines and metalloproteinases (37, 257, 258).

In accordance with this findings, previous studies have shown that ATII induces expression of IL-1β, IL-6, IL-8, CCL2 (MCP-1) and, osteopontin (149-151, 153) by human VSMC. The same effect has been reported for TNF-α, CCL2 and IL-8 in blood monocytes (159, 259). Of relevance, overexpression of these pro-inflammatory cytokines/chemokines in GCA involved arteries or in patient's sera has been previously associated with disease persistence (105, 108, 119, 120, 148). The source of elevated cytokines in patients with recalcitrant GCA is unknown. Surgical or necropsy specimens from long-term treated patients with GCA have disclosed extensive vascular remodelling with persistent, small foci of inflammatory cells (116, 260). However, inflammatory cells may not be the only source of cytokines as VSMC are also able to produce substantial amounts of inflammatory cytokines, as we and others have demonstrated (38, 117).

Next, we confirmed that treatment of cultured VSMC and PBMC with AGTR1 blocker losartan down-regulated all aforementioned cytokines. Former experimental studies have demonstrated that ARB may reduce the expression of IL-6 and MCP-1 in rat and human cultured VSMC (149, 150). In addition to the direct effects of blockade of AGTR1, ARB may also increase the expression and activity of ACE2, and in consequence the circulating levels of AT (1-7) (205). This last angiotensin peptide has been recently described as potent vasodilator, with anti-inflammatory, anti-proliferative, anti-fibrotic, and anti-thrombotic effects (129, 161, 200, 207).

Previous experimental studies have reported that activation of the Mas receptor by AT (1-7) reduced the production of reactive oxygen species and the expression of co-stimulatory molecules on antigen-presenting cells (209, 210). In addition, AT (1-7) decreased the release of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in an animal model of inflammatory arthritis (213).

In addition, ARB are able to suppress the production of auto-reactive Th1 and Th17 cells and promote the expression of antigen specific T*reg* cells, as demonstrated in an experimental animal model of multiple sclerosis (190). All these data support a potential benefit of blocking the ATII system in chronic inflammatory disorders such as GCA.

In the last part of this study, we investigated the relationship between ATII system expression in temporal arteries and disease outcome (data not showed in the final version of article 4). For this purpose, the same end-points measured in our clinical study with ARB were analyzed: relapses, GC requirements and level of selected inflammatory markers (ESR, CRP, haptoglobin).

The most relevant finding was that patients with elevated expression of both AGT and ACE required longer GC treatment. Specifically, patients with AGT and ACE mRNA above the median value had a longer delay to reach a stable maintenance daily prednisone dosage <10 mg/d. In addition, initial concentration of AGT and AGTR1 correlated significantly with cumulative PDN dose during follow-up. In addition, we found that 70% (n=14/20) of patients with ACE mRNA level below the median (46.5 *relative units*) were able to completely discontinue prednisone during long-term follow up in contrast to only 25% (n=5/20) of those with higher ACE levels (p=0.01). Remarkably, no differences in ACE, AGT or AGTR1 transcripts level was found in patients with relapses and those with sustained remission or those with a strong versus weak systemic inflammatory response. Also, no correlation was found with ATII system molecules and serum acute reactant proteins ESR, CRP and haptoglobin or haemoglobin.

We are aware that some of the associations detected between clinical end-points and the ATII system could be spurious, given the multiple comparisons that were performed. In addition, the high level of constitutive expression of some of these molecules could impact the identification of subtle but relevant differences.

Moreover the quantitative mRNA of a given mediator may not reflect the resulting biological activity and this may be particularly relevant in this complex system with such a remarkable post-trascriptional and post-translational modulation leading to the generation of multiple active peptides with sometimes opposite effects. Therefore, the relation of ATII system with diverse clinical outcomes in GCA patients deserves further investigation.

In summary, our findings suggest that angiotensin II system may play a role in the persistence of chronic inflammation in GCA. Interference with this system may, then, be a therapeutic option for patients with GCA. Given the predominantly observational nature of our results, which prevents strong mechanistic conclusions, this interesting hypothesis needs to be studied in more detail in the future.

VI. CONCLUDING REMARKS AND RESEARCH AGENDA

Giant cell arteritis is a chronic inflammatory disease with a broad spectrum of clinical manifestations and complications, as well as therapeutic challenges. Relevant issues in GCA management include the inability of GC to completely reverse and prevent disease-derived damage and the failure to go beyond symptomatic relief and efficiently abrogate disease activity, as well as the elevated frequency of GC-related side effects. Therefore, a better understanding of the pathogenic mechanisms involved in GCA is of paramount importance to design better therapeutic approaches.

Research into the development of new therapeutic approaches for this disease should be actively pursued by targeting key steps in the pathogenesis or maintenance of inflammatory cascades. Interfering with early events leading to vessel wall inflammation, avoiding vascular destruction or limiting vascular occlusion, have the potential to be a major achievement in the treatment of GCA.

The validity of the proposed therapeutic targets can only be confirmed with properly designed and conducted clinical trials, underlining the need of co-ordinated international collaboration.

In the study of ATII system, future prospects include investigation on specific cellular signaling pathways activated by ATII or suppressed by ARB; potential impact in long-term consequences of large-vessel involvement by exploring its role in vascular remodeling; and assessment of its relationship with other relevant inflammatory/remodeling systems, such as interferons or endothelin. In addition, we will try to explore the ACE2/AT (1-7)/Mas receptor axis and their anti-inflammatory properties. At last, search for specific auto-antibodies directed against AGTR1, recently described in vasculopathy associated with systemic sclerosis, could be of interest (194).

VII. REFERENCES

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VIII. ADDENDUM. RELATED PUBLICATIONS

Treatment of Large Vessel Vasculitis

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Abstract: Giant-cell arteritis (GCA) and Takayasu's arteritis (TAK) are chronic and relapsing inflammatory diseases involving large and medium sized arteries. While symptoms derived from large-vessel involvement characterize TAK clinical presentation, cranial symptoms and complications usually dominate the clinical picture of GCA. However, delayed consequences of large-artery involvement are being increasingly recognized. Glucocorticoids are the mainstay of treatment for both conditions but flares and relapses are common when glucocorticoids are tapered or discontinued and adverse effects are frequent. Methotrexate has shown modest efficacy as glucocorticoid-sparing agent in GCA whereas infliximab did not demonstrate benefit in a randomized clinical trial. Adjuvant treatment for TAK is only supported by open-label trials and observational studies and small series, and methotrexate is the most widely used immunosuppressive agent. Infliximab shows promise for refractory/ relapsing TAK as supported by an open label study with long-term follow up. Abatacept is currently being tested for both diseases in a randomized controlled trial. Other investigational agents in the horizon such as rituximab and tocilizumab have been anecdotally used but their efficacy needs to be confirmed. Revascularization procedures, mainly angioplasty, play a crucial role in the management of patients with TAK.

Keywords: Giant-cell arteritis, Takayasu's disease, treatment.

Primary large vessel vasculitides are granulomatous disorders and include two closely related but distinct entities: giant-cell arteritis (GCA) and Takayasu arteritis (TAK) [1, 2]. These conditions have overlapping anatomical, histopathological, and clinical features but also have substantial differences, particularly in epidemiology [3, 4] and disease outcome [1, 2]. GCA occurs predominantly in Caucasians >50 years of age. TAK preferentially, but not exclusively occurs in young women and is more frequent among individuals of Asian or Hispanic ethnicity. TAK targets the aorta and/or its primary and secondary branches. The spectrum of vascular involvement in GCA ranges from the aorta to small arteries (small vessels surrounding the temporal artery and small arteries supplying the optic nerve and retina) [5, 6] (Fig. **1**). Large-vessel involvement in GCA appears to be common but it is frequently subclinical at disease presentation although delayed complications (aneurysm or stenosis) may occur (Fig. **2**). Patients usually present with a combination of cranial, polymyalgic and/or systemic symptoms [1]. In contrast, characteristic features of TAK are usually related to symptomatic involvement of the aorta and its primary or secondary branches (Figs. **3**, **4**) [2, 7].

Both conditions were considered self-limiting in the pre-steroid era albeit with the potential for major discomfort and severe vascular complications [2, 8]. The introduction of glucocorticoid therapy in the 1950's provided substantial relief in affected patients and decreased the development of vascular complications, particularly in GCA. The initial response to treatment is rapid and perceived by patients and physicians to be satisfactory. However, substantial difficulties arise during follow-up and most patients have a relapsing course [2, 7, 9]. There are many unsolved issues in the treatment of large-vessel vasculitis, especially in TAK but also in GCA. Through substantial efforts, advances in the pharmacotherapy of these diseases have occurred and will be the main subject of this review.

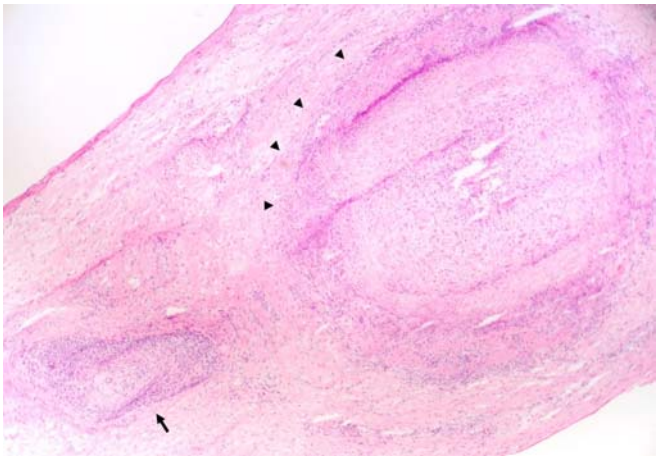

Fig. (1). Inflammatory involvement of a small cranial artery (arrow) in the vicinity of an inflamed temporal artery (arrowheads). Haematoxylin-eosin staining.

GLUCOCORTICOID THERAPY

Giant Cell Arteritis

Glucocorticoid treatment is invariably followed by a remarkable improvement of symptoms. It is effective in reducing the risk of visual loss, the most frequent ischaemic complication of GCA. The use of glucocorticoid therapy has

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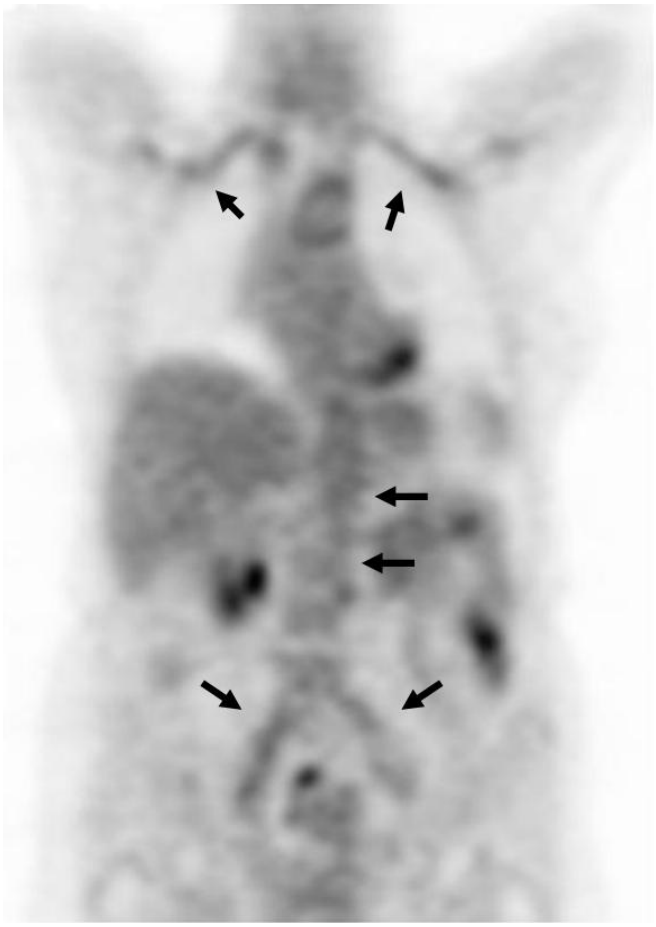

Fig. (2). Positron emission tomography disclosing ^{18}FDG uptake in the aorta, subclavian, and iliac arteries (arrows) of a patient with newly diagnosed giant-cell arteritis.

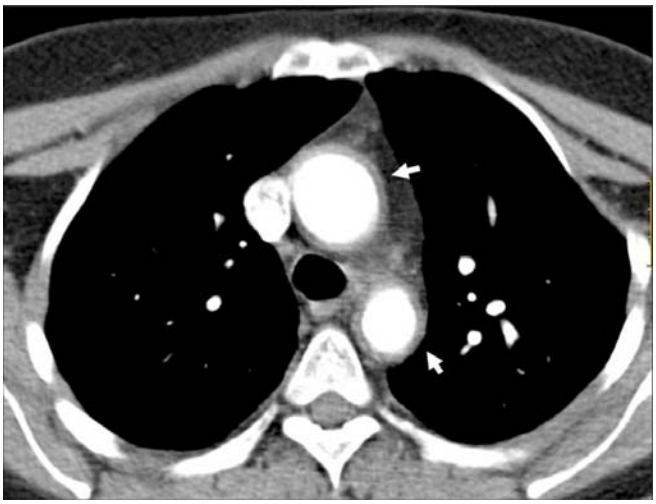

Fig. (3). Computed tomography of the thoracic aorta disclosing aortic wall thickening in the ascending and descending aorta (arrows) in a patient with Takayasu's arteritis.

decreased the incidence of visual impairment from 60% [10] to about 1% at 5 years in treated patients [11]. In patients with partial or transient visual impairment, glucocorticoids prevent further visual loss in 85-90% of patients [12-14]. However, when visual loss has occurred, objective recovery of visual acuity or visual field defects occur in only 4% of patients [13, 15]. Subjective improvement may be experienced by higher proportion of patients due to a learned ability to more efficiently use the remaining vision. Due to the potential of glucocorticoid therapy to limit additional ischemic lesions and the small but real likelihood of significant improvement, immediate treatment of patients with visual symptoms and suspected GCA is essential. Chances of recovery may be higher when treatment is started within the first 24-48 hours after the occurrence of the event [16].

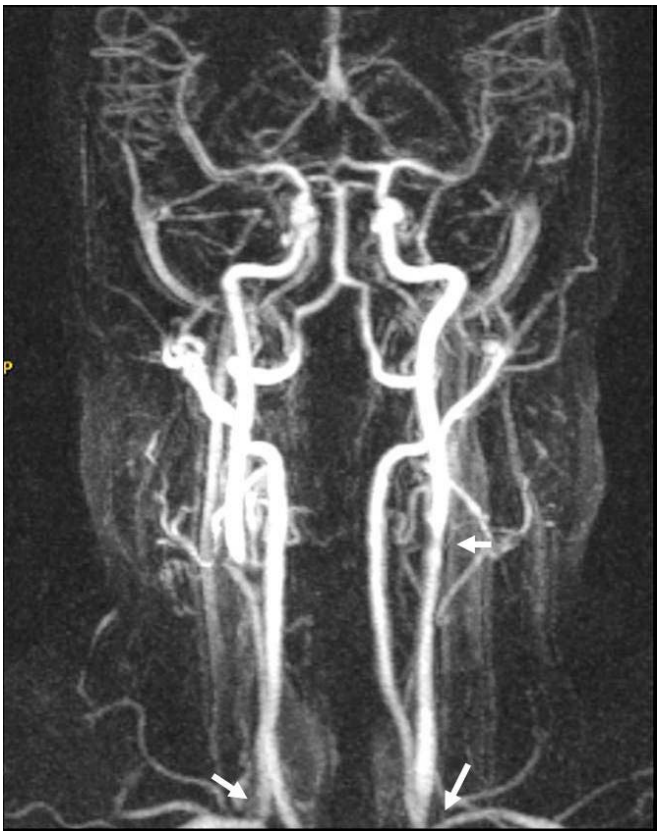

Fig. (4). Multiple stenoses in the carotid and vertebral branches (arrows) in a patient with Takayasu's arteritis disclosed by magnetic resonance angiography.

The optimal initial glucocorticoid dose has not been established but commonly ranges from 40 to 60 mg oral prednisone or equivalent per day [1]. Some investigators have used doses between 30-40 mg/day [17-19] whereas others, particularly in Ophthalmology departments, have used doses of 80 mg/day, in patients with visual symptoms [12, 15]. A recent guideline endorsed by the European League Against Rheumatism (EULAR) recommends an initial dose of prednisone of 1 mg/kg/day (maximum 60 mg/day), maintained for 1 month and tapered gradually [20]. Alternate day reduction of glucocorticoid may increase the probability of relapse [21-23] and perhaps disease-related visual impairment during treatment. Tapering schedules used are heterogeneous but aim to achieve a dose of 10-15 mg/day at month 3 and a maintenance dose of 5 to 7.5 mg of prednisone per day at 8 to 12 months [9, 20, 24]. Although this protocol is generally accepted and induce remission in the majority of cases, the risk and benefits of a given dose

must be weighed carefully for each patient during the entire follow-up.

For patients presenting with visual symptoms intravenous methylprednisolone (1000 mg/day for 3 days) followed by standard initial doses can be considered [20]. The rationale for this tactic relies on the ability of high-dose glucocorticoid to elicit rapid non-genomic effects by interfering with multiple signalling pathways [25]. However, there is no proof that this approach is clinically more effective than the standard treatment. About 10-15% of patients with visual symptoms experience progression of visual loss within the first 1-2 weeks after the initiation of glucocorticoid treatment [11, 14, 15]. There is no proven effective solution to prevent disease progression in this sub-group of cases.

Takayasu Arteritis

Glucocorticoid is the mainstay of the initial treatment of TAK. The initial dose ranges from 0.5 to 1 mg/Kg/day of oral prednisone or equivalent [26-32]. The EULAR guidelines recommend an initial dose of 1 mg/Kg/day (max 60 mg/day) maintained for 1 month and gradually tapered [20]. With this treatment, 93% of patients achieve disease remission. However, remission is sustained in only 20% of patients [7, 26]. More than 70% of patients need adjuvant therapy and long-term low-dose glucocorticoid is required by most patients [7, 32].

LIMITING GLUCOCORTICOID EXPOSURE

Long term glucocorticoid therapy carries the risk of toxic side effects. In an effort to reduce the cumulative glucocorticoid exposure and to treat cases with disease which was not adequately controlled by glucocorticoid therapy, there have been several open studies and clinical trials. We will discuss the evidence for the different modalities.

Immunosuppressive Agents

Giant Cell Arteritis

In a meta-analysis [33] of three randomized, double blind, placebo-controlled trials [23, 34, 35], methotrexate (10-15 mg/week) demonstrated a modest effect in reducing relapse rate and lowering cumulative glucocorticoid dose but not glucocorticoid-related side effects. The addition of methotrexate was estimated to reduce prednisone exposure by 800 mg per patient. Azathioprine (100-200 mg/day) was tested in a randomized, double-blind, placebo-controlled clinical trial in 31 patients with glucocorticoid-related complications [36]. This study included a mixed population of patients with GCA and polymyalgia rheumatica, making disease specific conclusions difficult to draw. Patients in the placebo arm were receiving a mean of 4.2 mg prednisolone per day at week 52, compared to patients in the azathioprine arm who were receiving 1.9 mg/day. The clinical significance of the modest prednisolone-sparing effect demonstrated in this trial is questionable and the potential role of azathioprine requires more robust confirmation. The use of other immunosuppressive or immunomodulatory agents including cyclosporine A, cyclophosphamide, hydroxychloroquine or dapsone has been anecdotal and information is too limited to draw any conclusion about their risk/benefit for GCA patients [37-42].

Takayasu Arteritis

Several immunosuppressive agents have been tried in patients with TAK but there are no randomized clinical trials performed with TAK patients. Recommendations are based on retrospective studies, small open-label studies or expert opinion. Cyclophosphamide was the first adjuvant therapy given to patients with TAK based on its benefit in necrotizing systemic vasculitis [43]. Due to the severe side effects of long-term use of cyclophosphamide and the fact that TAK is not acutely life-threatening in most instances but a chronic and relapsing disease with the potential of requiring long-term therapy, cyclophosphamide is not generally recommended and should only be considered in selected severe situations or patients refractory to other therapies [2]. Methotrexate and azathioprine have better risk/benefit profiles. Addition of MTX starting at 15 mg/week and escalating up to 25 mg/week when necessary resulted in remission in 81% of 18 patients with persistent/refractory disease and remission was sustained in 50% of patients during a mean follow-up of 18 months [44]. Azathioprine at 2 mg/Kg/day was tested in an open-label study including 15 patients with newly diagnosed TAK. Systemic symptoms and acute phase reactants improved in all patients. Although lost pulses or differences in blood pressure were not modified, no new lesions assessed by aortography developed during a follow-up of 12 months [45]. The use of mycophenolate mofetil (MMF) has been reported in small case series [46], a retrospective study on 21 patients [47] and in a small open-label study including 10 patients [48]. In about 50% of the patients treated, MMF was the first immunosuppressive drug used as glucocorticoid-sparing agent. The remaining patients had active disease in spite of receiving other drugs including methotrexate and azathioprine. Except for one patient in each study who could not tolerate MMF, all patients experienced improvement in disease activity scores, in systemic symptoms when present and in inflammatory markers [46, 48]. In a recent review of 75 patients treated at the Cleveland Clinic, 20% received glucocorticoid alone, 43% received methotrexate, 7% azathioprine, 7% mycophenolate, 13% cyclophosphamide, and 37% anti-TNF agents [7]. The addition of an immunosuppressive agent did not fully prevent subsequent flares, still occurring in 63% of patients in this series, particularly when prednisone was reduced around 10 mg/day [7]. The use of other medications (i.e. leflunomide) has been anecdotal [49]. It is not uncommon that patients are switched from one drug to another due to side effects or partial efficacy.

Biologic Therapies

Giant Cell Arteritis

Infliximab had been reported to be useful in case reports and small series of patients with glucocorticoid resistant GCA or marked glucocorticoid-related toxicity [50, 51]. However, in an international, multicenter, randomized, double-blind, placebo-controlled trial [52], 44 newly

diagnosed patients in glucocorticoid induced remission were randomized to infliximab (5mg/kg) or placebo. Infliximab was well tolerated, but did not prevent disease relapse. Its use cannot be routinely recommended in patients with GCA. TNF α blockade with etanercept has also been subjected to a randomized, double blind-placebo-controlled trial in patients with GCA with glucocorticoid-related side effects. Unfortunately only 17 patients were recruited and only 6 completed the trial. Although 50% of patients in the etanercept arm and 22.2% in the placebo group were able to discontinue prednisone at 12 months, this difference was not significant. Patients receiving etanercept cumulated a significantly lower prednisone dose. Unfortunately, the number of patients included was too small to draw conclusions [53]. Blocking TNF α with the fully human monoclonal antibody adalimumab, supported so far by a single case report [54], is currently being tested as a glucocorticoid-sparing intervention in a double blind placebo-controlled randomized clinical trial (www.clinicaltrials.gov).

It has been stated that B lymphocytes do not play a role in the pathogenesis of GCA [55]. However, 2 cases responding to B-cell depletion with the chimeric monoclonal antibody rituximab have been published [56]. In these patients the indication for anti-CD20 therapy was inadequate control of disease activity with glucocorticoid (with concurrent neutropenia in one of them). Although these results are intriguing and may be worth to be further investigated, the existing evidence is too weak to support efficacy of B-cell depletion in GCA.

Interfering with early events in antigen presentation and T cell activation might abrogate vascular inflammatory lesions in GCA. Targeting activated dendritic cells with a monoclonal antibody against CD86 has been shown to reduce T-cell infiltrates in temporal arteries from patients with GCA engrafted into NOD-SCID mice, although the number of samples tested was too small to draw definite conclusions [57]. Interfering with T cell activation is currently feasible with abatacept, a fusion protein consisting of an immunoglobulin Fc fragment coupled to the extracellular domain of CTLA. Abatacept efficiently blocks interactions between CD80/86 molecules on antigen-presenting cells and co-stimulatory CD28 molecule on the T cell membrane. It has demonstrated efficacy in several chronic inflammatory diseases and is presently approved for the treatment of rheumatoid arthritis resistant to TNF blockade [58]. Abatacept is currently being tested in a clinical trial for patients with giant cell arteritis and Takayasu disease (www.clinicaltrials.gov).

The spectrum of targeted therapies for chronic inflammatory diseases is progressively increasing. The paucity of functional models in GCA implies that future testing of additional targeted therapies will be essentially driven by results obtained in more prevalent inflammatory diseases. Immunopathologic studies have shown expression of several cytokines for which blocking interventions are available or in development but their specific role in maintaining inflammation in GCA is mostly extrapolated from their known biologic functions. Potential targets considered for GCA patients include IFN γ, IL12/IL23, IL-17, IL-6 [59-62]. The conceptual rationale for targeting some of these molecules in GCA has been recently reviewed [59, 63]. Anti-IL6 receptor blockade with tocilizumab has been reported to be useful in a case report and a short series [64, 65].

Takayasu Arteritis

Based on the granulomatous nature of TAK and the expression of TNF α demonstrated in lesions, TNF α blockade has been attempted in patients with TAK [66]. TNF α blockade with etanercept or infliximab, seems to be useful for patients with TAK in whom other immunosuppressive agents have failed to elicit sustained remission [66, 67]. In a small open-label series of 15 patients refractory to immunosuppressive agents and requiring a median dose of 20 mg prednisone/day achieved remission with more than 50% reduction in the glucocorticoid dose in 4 patients and complete glucocorticoid withdrawal in the remaining [66]. In 10 out of these 15 patients remission was sustained for a median of 1-3.3 years. In a retrospective study of 25 patients refractory to other therapies, blocking TNF α for a median of 28 months resulted in remission and complete glucocorticoid withdrawal in 60% and glucocorticoid tapering below 10 mg/day in an additional 28% [65]. In 50% of the 18 patients who were simultaneously receiving immunosuppressive agents, these could be discontinued [67].

Anti-IL-6 receptor tocilizumab has been tried in a few patients and remarkable improvement in clinical and laboratory findings was observed [65, 68]. Abatacept is being tested in a randomized clinical trial in patients with large-vessel vasculitis including TAK and GCA. When completed, this will be the first clinical trial ever performed in TAK.

OTHER PHARMACOLOGICAL OPTIONS

Statins in Giant Cell Arteritis

Based on the anti-inflammatory effects of statins, their potential benefit in maintaining glucocorticoid-induced remission has been evaluated in 2 retrospective analyses [69, 70]. In both studies the statin doses prescribed were within the low-moderate range. No effect was observed on relapse rate, duration of glucocorticoid treatment, or cumulative glucocorticoid dose. No differences in the levels of acute phase reactants over time were evidenced between patients according to statin use. Whether statins at higher doses with a more aggressive glucocorticoid tapering schedule would allow an earlier reduction or discontinuation of glucocorticoid is not known. It has been suggested that statin treatment may reduce the incidence and the growth rate of abdominal aortic aneurysms, which would be interesting in the context of large-vessel vasculitis, but this issue remains controversial [71-73].

Aspirin in Giant Cell Arteritis

GCA-related stroke occurs in about 4-6% of patients [5, 74, 75]. Not exceptionally, cerebrovascular accidents appear after the initiation of glucocorticoid treatment (Fig. **5**). Autopsy studies in patients dying from GCA-related stroke have shown thrombotic occlusion with distal embolization as a precipitating event, supporting the adjuvant use of anti-

platelet therapy [5, 76, 77]. Based on 2 retrospective studies showing that patients treated with anti-platelet therapy (primarily aspirin) for other conditions had a lower frequency of ischemic events at the time of developing GCA, the use of low-dose aspirin has been recommended to prevent further ischemic events [20, 78, 79]. It is important to remark that these studies do not demonstrate that aspirin adds an additional effect to glucocorticoid therapy in preventing ischemic complications. Moreover, subsequent retrospective studies have not been able to support a protective effect of previous anti-platelet therapy [80-82]. However, GCA-related ischemic complications may be more frequent among patients with traditional cardiovascular risk factors which are relatively frequent among the population targeted by GCA [83]. For these reasons recommending aspirin seems reasonable although the risk/benefit must be weighted in individual patients.

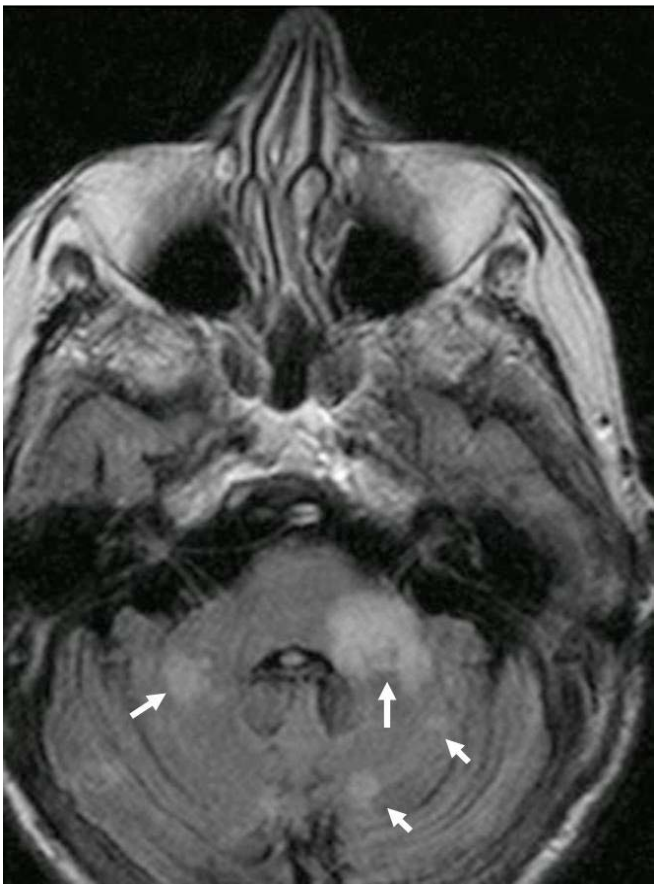

Fig. (5). Magnetic resonance imaging disclosing multiple infarcts in the cerebellum and brainstem (arrows) of a patient with giant-cell arteritis.

REVASCULARISATION

Fixed vascular stenosis may not respond to medical treatment (Fig. **6**) and revascularisation is required in up to 70% of patients with TAK, but it also has a role in a minority of patients with GCA. In the presence of irreversible arterial stenosis causing significant ischemic symptoms, arterial bypass surgery and percutaneous transluminal angioplasty have been tried. Most of the revascularization experience is in TAK. Angioplasty, alone or with stenting, has an initial success of 85-100% of cases [7, 26-29, 32, 84]. Within 2 years, the rate of re-stenosis is about 16-20% [32]. With extended follow-up, re-stenosis may occur in 78% of initially successful procedures [7, 26, 32, 84]. Arterial bypass grafting achieves successful revascularization in the majority of patients with a reported rate of restenosis or occlusion of approximately 20% to 30% during variable follow-up periods (months to years). It is, therefore, the most successful treatment modality [7, 26-29, 32, 43-46, 48, 49, 66-68, 85]. Complex vascular reconstructions (i.e. complete aortic arch replacement) may be need by patients with multiple stenoses [86]. It is recommended that elective procedures are performed during periods of inactive disease [7, 26, 87, 88].

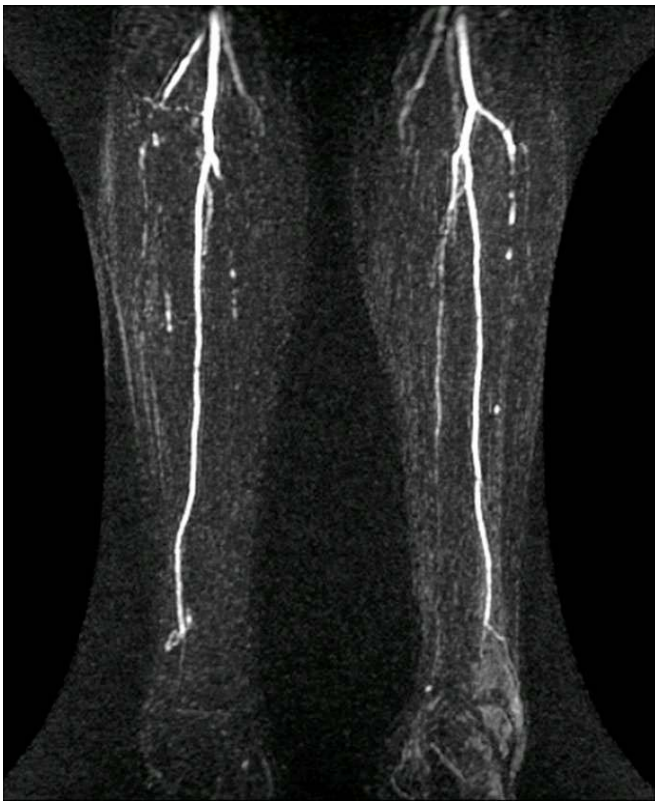

Fig. (6). CT angiogram disclosing infrapopliteal artery stenoses in a patient with giant-cell arteritis and no additional vascular risk factors who developed intermittent leg claudication at the time of giant-cell arteritis diagnosis.

FUTURE AVENUES FOR RESEARCH

Small artery vasospasm may contribute to optic nerve ischemia in GCA as suggested by the typical amaurosis fugax that often precedes permanent visual loss [1, 72]. Increased expression of the potent vasoconstrictive agent endothelin has been demonstrated in GCA lesions and it is not rapidly downregulated by glucocorticoids [89]. Elevated plasma concentrations of endothelin have been found in GCA patients with visual complications [89]. Endothelin receptor antagonists may potentially help patients who continue deteriorating vision in spite of glucocorticoid treatment, but this remains hypothetical.

Recently, aortic involvement in GCA has received a great deal of attention due to the severity of its potential

complications (i.e. aneurysm, dissection or aortic valve insufficiency) [73, 90-93] (Fig. **7**). Glucocorticoid treatment is not able to prevent significant aortic dilatation in about 22.2% of patients after a median follow-up of 5.4 years according to a prospective screening [73]. It is not clear at present whether these complications, result from vessel wall destruction by persisting subclinical inflammation, are due to defective vascular remodelling after the initial inflammatory injury, or to a combination of factors. Whether aortic dilatation can be prevented by new therapeutic options is unknown but new treatments entering clinical trials should be also evaluated for their ability to prevent late aortic complications as a relevant outcome.

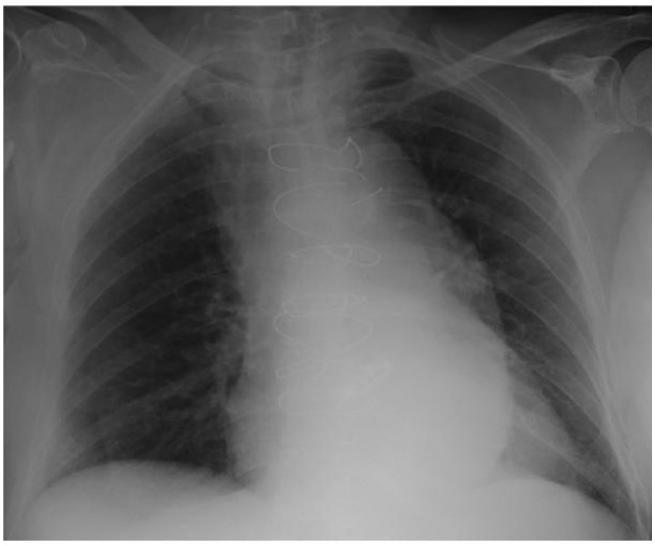

Fig. (7). Aortic aneurysm apparent in a simple chest X-ray in a patient with giant-cell arteritis.

Vascular stenoses occur in large vessel vasculitis as a consequence of hyperplasia of the intimal layer [94]. Intimal hyperplasia results from myointimal cell proliferation, migration of cells towards the lumen and excessive matrix production [94]. Among several growth factors known to be produced in GCA, platelet-derived growth factor (PDGF) is the most potent inducer of myointimal cell proliferation and migration in vessels targeted by GCA [95]. Imatinib mesylate, a potent inhibitor of the tyrosine kinase activity of the PDGF receptor, has been shown to inhibit myointimal cell outgrowth from explanted temporal arteries. Whether Imatinib mesylate would be able to prevent or reverse vascular stenosis in large-vessel vasculitis is hypothetical but underlines that targeting the vaso-occlusive component independently or in addition to the inflammatory component may be feasible. This is an interesting concept that should be pursuit, not only for GCA patients but, more importantly, for patients with TAK.

CONCLUDING REMARKS

The current treatment of large-vessel vasculitis is far from being optimal. Glucocorticoids induce dramatic symptomatic relief but do not completely suppress disease activity in most cases. Glucocorticoid related adverse effects are an important cause of morbidity. The addition of other immunosuppressive agents adds beneficial effects but relapses and smouldering activity still occur. The current realistic therapeutic goal for patients with large-vessel vasculitis is disease control rather than disease cure, especially for patients with TAK. There is hope that with better knowledge of the key pathogenic elements triggering or perpetuating the inflammatory response, new therapeutic tools able to improve the outcome of patients with large-vessel vasculitis will be identified.

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EXTENDED REPORT

Changes in biomarkers after therapeutic intervention in temporal arteries cultured in Matrigel: a new model for preclinical studies in giant-cell arteritis

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ABSTRACT

Background Search for therapeutic targets in giant-cell arteritis (GCA) is hampered by the scarcity of functional systems. We developed a new model consisting of temporal artery culture in tri-dimensional matrix and assessed changes in biomarkers induced by glucocorticoid treatment.

Methods Temporal artery sections from 28 patients with GCA and 22 controls were cultured in Matrigel for 5 days in the presence or the absence of dexamethasone. Tissue mRNA concentrations of pro-inflammatory mediators and vascular remodelling molecules was assessed by real-time RT-PCR. Soluble molecules were measured in the supernatant fluid by immunoassay.

Results Histopathological features were exquisitely preserved in cultured arteries. mRNA concentrations of pro-inflammatory cytokines (particularly IL-1β and IFNγ), chemokines (CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES) and MMP-9 as well as IL-1β and MMP-9 protein concentrations in the supernatants were significantly higher in cultured arteries from patients compared with control arteries. The culture system itself upregulated expression of cytokines and vascular remodelling factors in control arteries. This minimised differences between patients and controls but underlines the relevance of changes observed. Dexamethasone downregulated pro-inflammatory mediator (IL-1β, IL-6, TNFα, IFNγ, MMP-9, TIMP-1, CCL3 and CXCL8) mRNAs but did not modify expression of vascular remodelling factors (platelet derived growth factor, MMP-2 and collagens I and III).

Conclusions Differences in gene expression in temporal arteries from patients and controls are preserved during temporal artery culture in tri-dimensional matrix. Changes in biomarkers elicited by glucocorticoid treatment satisfactorily parallel results obtained in vivo. This may be a suitable model to explore pathogenetic pathways and to perform preclinical studies with new therapeutic agents.

INTRODUCTION

Giant-cell arteritis (GCA) is a granulomatous arteritis of the elderly, targeting the aorta and its branches with a striking tropism for the cranial arteries.[1] Although most patients with GCA experience a remarkable relief with high-dose glucocorticoids (GC), treatment has proven to be unsatisfactory. GC fail to prevent further sight deterioration in 10%–17% of patients presenting with visual impairment and are unable to avoid large vessel damage leading to aortic dilatation in about 22.5% of patients.[2 3] Moreover, more than 50% of patients relapse when GC are tapered[4 5] and GC-related adverse events occur in a more than 80% of patients with GCA.[6] There is an unmet need for more effective and specific therapies.

Search for therapeutic innovation in GCA is difficult due to the limited understanding of pathogenesis and the scarcity of functional models where the impact of therapeutic interventions can be assessed. The pathogenesis model of GCA is based on the identification of particular cell types (CD4T lymphocytes, macrophages, dendritic cells, endothelial cells),[7 8] cell activation and differentiation markers,[7–9] and inflammatory mediators in lesions.[9–13] The interpretation of immunopathology findings is often extrapolated from basic immunology principles, and the role of infiltrating cells and their products is assumed from their known biological activities and association with particular phenotypes,[10 11 13] histopathological changes or outcomes.[12–14] Proof of concept is weak for the majority of grounds on which the current pathogenetic model is sustained.

The frustrating experience with anti-tumour necrosis factor (TNF) therapy in GCA underlines the crucial need for functional systems. TNFα was considered a potential therapeutic target based on its strong upregulation in lesions[13] and correlation of tissue and serum TNFα levels with GC requirements and relapsing course.[13 15] In spite of these observations and in spite of the therapeutic efficacy of TNF blockers in other granulomatous diseases, neutralising TNFα with infliximab did not seem sufficient to abrogate inflammatory activity in GCA.[5] Blocking IL-6 receptor is currently being considered as a therapeutic option.[16] This and other interventions could benefit from preclinical functional testing.

A functional model was created by Brack *et al*[17] subcutaneously engrafting fragments of human temporal arteries into severe combined immunodeficiency (SCID) mice. This pioneer model has been useful to detect changes in cytokine expression in temporal artery tissue after pharmacological

treatment of engrafted mice[18 19] or after selective depletion of specific cell types with antibodies injected to animals,[8 17] providing proof of concept of some of the basic pathogenic principles. However, this model is complex, expensive and not widely available. Moreover, monitoring of successful engraftment is difficult and the accessibility of therapeutic agents administered to the mice cannot be controlled. Due to its complexity, the majority of published experiments have been performed with only 1–3 temporal arteries split into several mice.[8 18 19]

The Engelbreth-Holm-Swarm sarcoma-derived tri-dimensional matrix, Matrigel,[20] provides anchorage and survival signals for vascular smooth muscle cells (VSMC).[21] Based on these findings, we developed a new model to assess changes in lesions after therapeutic intervention, consisting of culture of temporal artery sections embedded in Matrigel. We found that cultured arteries remained viable for at least 2 weeks with exquisitely preserved morphology. Moreover, this system was sensitive enough to demonstrate clear differences in cytokine expression between normal and inflamed arteries as well as changes induced by therapeutic intervention.

METHODS

Patients

Temporal artery biopsies were performed to 50 consecutive patients with suspected GCA for diagnostic purposes. A 5–15 mm segment was saved for this study and the remaining fragment was processed for histopathological diagnosis. The study was approved by the Ethics Committee of the Hospital Clínic of Barcelona and patients signed informed consent.

A total of 28 biopsies disclosed histopathological features of GCA and 22 revealed no inflammatory infiltrates. Patients with a negative temporal artery biopsy were eventually diagnosed with other conditions (see online supplementary methods).

Temporal artery culture

Temporal artery fragments were placed in RPMI 1640 medium (Lonza; Verviers, Belgium) supplemented with 10% foetal bovine serum (Invitrogen, Carlsbad, California, USA), 2 mM L-glutamine (Invitrogen), amphotericin B at 2.5 µg/ml (Invitrogen) and gentamycine (Braun, Germany) at 200 µg/ml. An average of 10.79±2.91 (mean±SEM) 0.8–1 mm sections per specimen were cut in a tissue culture hood. Matrigel (Collaborative Biomedical Products, Bedford, Massachusetts, USA) was allowed to thaw on ice and 24-well tissue culture plates were coated with a 25 µl Matrigel drop per well, which was allowed to solidify at 37°C for 30 min. One temporal artery section per well was dipped in the Matrigel coating and covered with 1 ml medium. Dexamethasone (Sigma, Ayrshire, UK) at 0.5 µg/ml was added to selected wells. Each condition was tested in 3–4 replicate wells. Sections were incubated al 37°C in 5% CO_2 for 5 days. Replicates of supernatant fluids and biopsies were respectively pooled. Biopsies were frozen in TRIzol reagent (Invitrogen) and stored at −80°C. Random specimens were cultured for 2 weeks in order to assess morphology preservation after extended culture periods and fixed in 10% formalin for H&E staining and histopathological examination.

Immunostaining

Cultured temporal artery sections were de-paraffinised, washed in phosphate-buffered saline (PBS) and endogenous peroxidase was blocked with H_2O_2. Slides were incubated with mouse antihuman CD3 (clone PS1, Leica Microsystems, Wetzlar, Germany, at 1:60 dilution) or undiluted mouse antihuman CD68 (clone KP1 from Dako, Glostrup, Denmark, ready to use). Optimal dilutions were tested on human tonsils (positive control). Isotype-matched mouse immunoglobulins served as negative controls. Immunodetection was performed with a HRP-labelled polymer conjugated to a secondary antibody (EnVision, Dako) using 3-3′-diaminobenzidine as a chromogen.

Cytokine mRNA measurement by real-time quantitative RT-PCR

Three to four temporal artery sections per condition were homogenised in TRIzol reagent. RNA extraction was performed according to the chloroform-isopropanol precipitation method. Total RNA (1 µg) was reverse transcribed to cDNA using Archive kit (Applied Biosystems, Life Technologies, Carlsbad, California, USA) in a final volume of 100 µl, employing random hexamer priming. Samples were stored at −80°C until use.

Gene expression of pro-inflammatory cytokines (IL-1 β, IL-6, TNFα, interferon (IFN)γ), chemokines (chemokine ligand (CCL)2/monocyte chemoattractant protein (MCP)-1, CCL3/MIP-1α, CCL4/MIP1β, CCL5/regulated upon activation normal T cell expressed and secreted (RANTES) and CXCL8/IL-8), metalloproteases (matrix metalloproteinases (MMP)-2, MMP-9) and their inhibitors (tissue inhibitor of metalloproteinases (TIMP)-1 and TIMP-2), growth factors (platelet derived growth factor (PDGF) A and B) and vascular matrix components (collagen I, collagen III) was assessed using specific predeveloped Taqman probes from Applied Biosystems (Taqman Gene Expression Assays; see online supplementary methods). Fluorescence was detected with ABI PRISM 7900 Sequence Detection system and results were analysed with the Sequence Detection Software V.2.3 (Applied Biosystems). Comparative Ct method was used to assess the relative gene expression. All samples were normalised to the expression of the endogenous control GUSb and values were expressed as relative units.

Detection of inflammatory mediators in the supernatant fluid by immunoassay

Pro-inflammatory cytokines (IL-6, TNFα, IL-1β, IFNγ), chemokines (CCL2/MCP-1 and CCL3/MIP-1α), metalloproteases (MMP2 and MMP-9) and growth factors (PDGF AB) were detected by enzyme-linked immunoassay (Quantikine ELISA kits from R&D Systems, Minneapolis, Minnesota, USA) in the culture supernatants form all patient and control arteries.

CCL4/MIP-1β and CXCL8/IL-8 were assessed by the Multiplex Luminex system (Life Technologies, Paisley, UK) in the supernatant fluid from 10 patients and six controls.

Statistical analysis

Mann–Whitney test was applied for statistical analysis.

RESULTS

Viability of the system and preserved morphology

Arterial sections were daily monitored under an inverted microscope. In GCA arteries white blood cells were visible in the periphery of the artery and remained bright and viable as assessed by Trypan blue exclusion throughout the duration of the experiment (figure 1A). After 1 week, VSMC began to spread and extend towards the matrix, further supporting the viability of this system (figure 1B).

As shown in figure 1C and 1D histopathological examination of cultured GCA arteries disclosed that morphological details including distinct arterial layers, inflammatory infiltrates, internal elastic lamina fragments and giant-cells were perfectly preserved. Over time, the intensity of inflammatory infiltrates decreased in cultured arteries, as described in arteries engrafted into SCID mice.[17] As shown in figure 1E–H, inflammatory

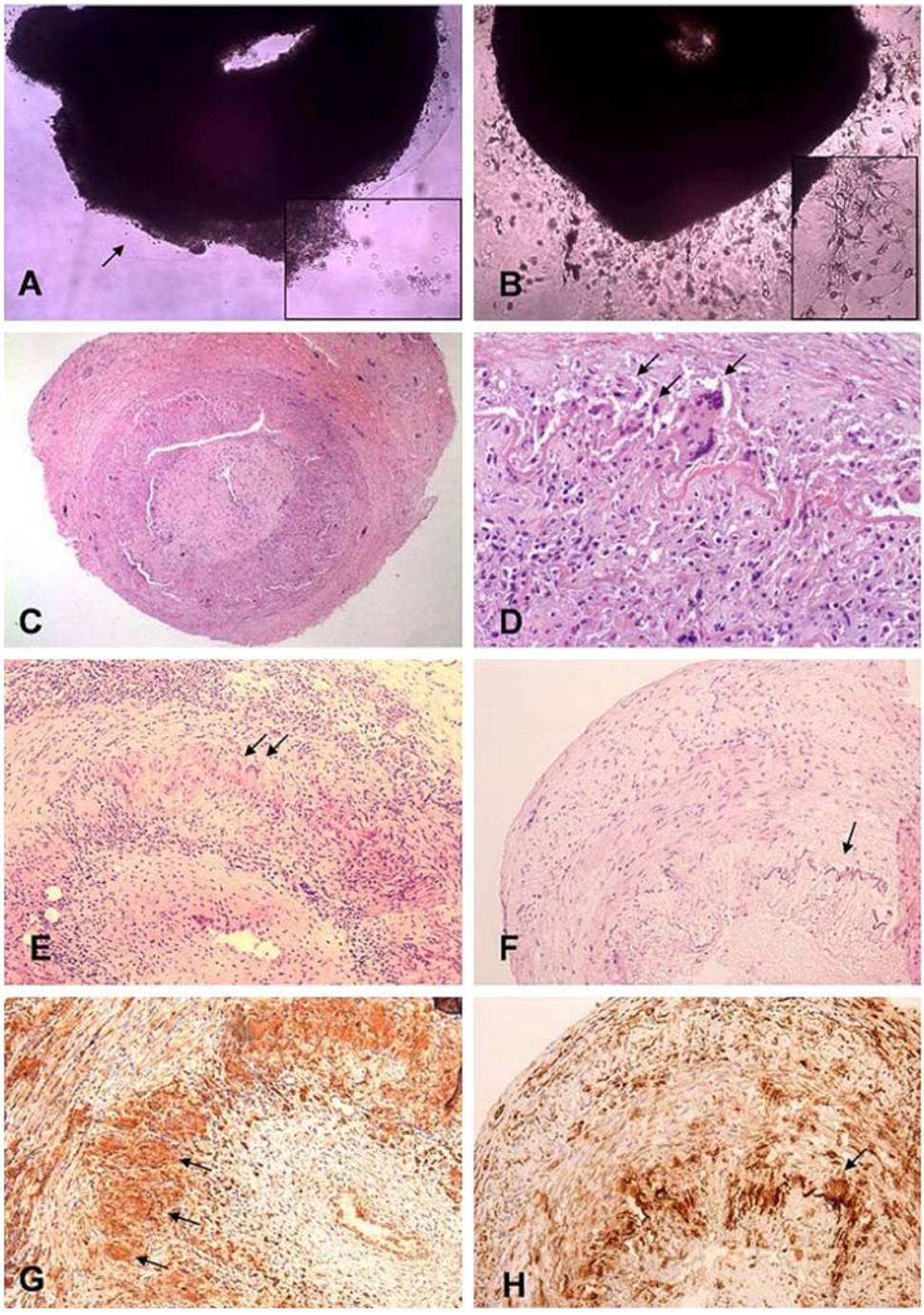

Figure 1 Histopathological findings in temporal artery sections from patients with giant-cell arteritis (GCA) cultured in Matrigel. (A) Temporal artery section from a patient with GCA cultured for 24 h and observed under an inverted, phase-contrast microscope. The arrow shows bright leukocytes cumulating in the periphery of the artery (inset shows a closer view). (B) Temporal artery section from a patient with GCA after 7-day culture. Vascular smooth muscle cells (VSMC) sprout from the artery and leukocytes migrate outwards (inset shows a closer view). Identity of these cells as VSMC has been previously demonstrated.[21] (C) H&E staining of a temporal artery section cultured for 2 weeks showing exquisite preservation of morphology. (D) Closer view of another temporal artery section cultured for 2 weeks displaying giant cells (arrows) along fragments of the internal elastic lamina. (E) H&E staining of a section of a freshly removed artery. Arrows show giant-cells. (F) Serial section of the artery disclosed in E cultured for 2 weeks showing a reduction in inflammatory infiltrates. The arrow indicates typical internal elastic lamina fragments. (G) Macrophages and numerous giant-cells (arrows) identified by anti-CD68 immunostaining in a freshly removed artery. (H) Anti-CD68 immunostaining of a serial section cultured for 2 weeks. Giant-cells (arrow) are dramatically reduced.

infiltrates, including giant-cells, decreased after 2-week culture. Examination of the cultured arteries under an inverted microscope disclosed that, over time, some inflammatory cells migrated along the outgrowing VSMC (figure 1B).

Differences in expression and release of relevant molecules between cultured GCA and control arteries

To assess the model reliability we investigated expression of pro-inflammatory cytokines, chemokines, metalloproteinases and

growth factors largely known to be expressed in GCA lesions and thought to be relevant to pathogenesis. We also explored some additional chemokines, such as CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES and CXCL8/IL-8, not previously investigated in GCA.

After a 5-day incubation period, remarkable differences in the spontaneous expression and release of various relevant factors were detected between GCA and control arteries, underlining the accurate sensitivity of the system to distinguish between non-inflamed and inflamed arteries (table 1). Differences in gene expression were particularly significant for IL-1β, IFNγ, chemokines CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES, and MMP-9. Less marked or no differences were observed for other factors known to be upregulated in GCA lesions including IL-6, TNFα and CCL2. Intense expression by cultured control arteries probably minimised differences.

Of interest, MMP-9, mainly produced by inflammatory cells, was overexpressed in patient versus control specimens whereas MMP-2, constitutively expressed by VSMC, was similar between patients and controls, paralleling again what has been observed in uncultured temporal artery biopsies.[22] As previously observed in freshly removed arteries, TIMP-1 and TIMP-2 mRNAs were decreased in inflamed arteries, leading to increased proteolytic balance.[22] Vascular remodelling factors PDGFs, CCL2, MMP-2 and collagens were strongly expressed in cultured arteries with no relevant differences between patients and controls.

Variations in the secretion of various markers were observed. TNFα and particularly IL-6 were remarkably released in the supernatant fluid (table 1). However, IFNγ and IL-1β, markedly expressed at the mRNA level, were secreted in small amounts. This parallels what happens in vivo where circulating TNFα and IL-6 are increased in sera of patients whereas IL-1β and IFNγ are not easily secreted and remain around the detection threshold in human serum. Therefore, this system allows evaluation of cytokine expression and investigation of cytokine secretion.

Similarly, while there were significant differences in chemokines CCL3/MIP1-α, CCL4/MIP-1β and CCL5/RANTES between patients and controls at the mRNA level, differences in released chemokines were less apparent.

Effect of the culture system on gene expression in cultured arteries

Since control arteries notably expressed various mediators we next investigated whether the culture system itself influenced gene expression. Frozen tissue from the original artery was available for six of the GCA patients and five controls and the expression of selected markers was compared between sections of the same specimen before and after 5-day culture in Matrigel. With the exception of IFNγ, the culture system upregulated expression of pro-inflammatory cytokines, chemokines CCL2 and CXCL8, and MMP-9 in both patients and controls. PDGFs and collagen III were markedly increased in control arteries whereas IFNγ and collagens decreased in GCA specimens (figure 2). In general, the culture system minimised differences between patients and controls.

Effect of dexamethasone on inflammatory infiltrates and on the expression and release of inflammatory and vascular remodelling markers

To assess whether this ex vivo system allowed accurate detection of changes induced by pharmacological intervention, we compared expression and release of inflammatory markers between artery sections cultured with medium alone and sections from 10 patients cultured in the presence of dexamethasone. A marked decrease in cytokine production was observed (figure 3 and table 2). Chemokines were downregulated at the mRNA level but changes in chemokine release were, again, less apparent (table 2). Vascular remodelling factors such as CCL2, MMP-2 and PDGF as well as collagens I and III were not downregulated by dexamethasone.

Dexamethasone treatment for 2 weeks induced a decrease in macrophage infiltration as assessed by CD68 mRNA expression and immunohistochemistry (figure 4). No effect on T cells was observed during the same treatment period.

Table 1 Differences in biomarker mRNA expression (*relative units*) and protein secretion (*pg/ml*) between cultured temporal artery sections from GCA patients and controls

	mRNA concentration (relative units)			Protein concentration (pg/ml)		
	GCA biopsies	Control biopsies	p Value	GCA biopsies	Control biopsies	p Value
IL-1β	35.91±8.80	14.22±2.86	**0.047**	5.61±1.33	−0.85±0.58	**0.000**
IL-6	448.54±86.88	380.04±68.37	0.543	25583±9404	7805.4±2685	0.076
TNFα	4.70±2.26	6.69±1.24	0.420	21.25±5.09	8.42±2.59	0.053
IFNγ	0.805±0.257	0.012±0.011	**0.010**	7.75±2.41	7.75±2.41	0.764
CCL-2/MCP-1	648.72±155.21	729.5±201.42	0.758	11268±1903	5850.91±4316.1	0.227
CXCL-8/IL-8	2287.9±619.9	4346.5±1092.4	0.095	81403±25050	34778.2±18253.9	0.157
CCL-3/MIP-1α	86.31±16.9	20.16±.5.10	**0.002**	25.617±2.503	26.62±4.25	0.834
CCL-4/MIP-1β	28.21±6.13	5.36±1.13	**0.003**	12.61±3.1	6.15±1.3	0.087
CCL-5/RANTES	139.83±37.34	16.42±5.58	**0.007**	21.43±5.19	17.33±6.54	0.633
MMP-2	2097.4±276.9	3450.9±1143.4	0.297	39125±11144	15250±8280	0.192
MMP-9	1283.85±408.2	304.21±90.70	**0.039**	48913±10740	7825±3512.4	**0.006**
TIMP-1	11813±3550	15126.8±5893.7	0.613	Not done	Not done	–
TIMP-2	586.68±87.77	2798.1±1135.2	0.074	Not done	Not done	–
COL I	1545.6±284.61	1065.34±196.7	0.175	Not done	Not done	–
COL III	3674.4±637.07	3979.2±991.5	0.789	Not done	Not done	–
PDGF A	71.14±24.75	163.55±40.13	0.056	Not done	Not done	–
PDGF B	40.78±7.78	43.807±7.992	0.806	Not done	Not done	–
PDGF AB	Not applicable	Not applicable	–	23.375±3.245	41.50±15.34	0.325

Values in bold are statistically significant (p<0.05). mRNA expression was detected in the entire cohort of 28 GCA patients and 22 controls. CCL3/MIP-1α, CCL4/MIP-1β and CXCL8/IL-8 protein concentrations were detected by Luminex in 10 patients and six controls. The remaining proteins were detected by ELISA in the entire cohort.
COL, Collagen; GCA, giant-cell arteritis.

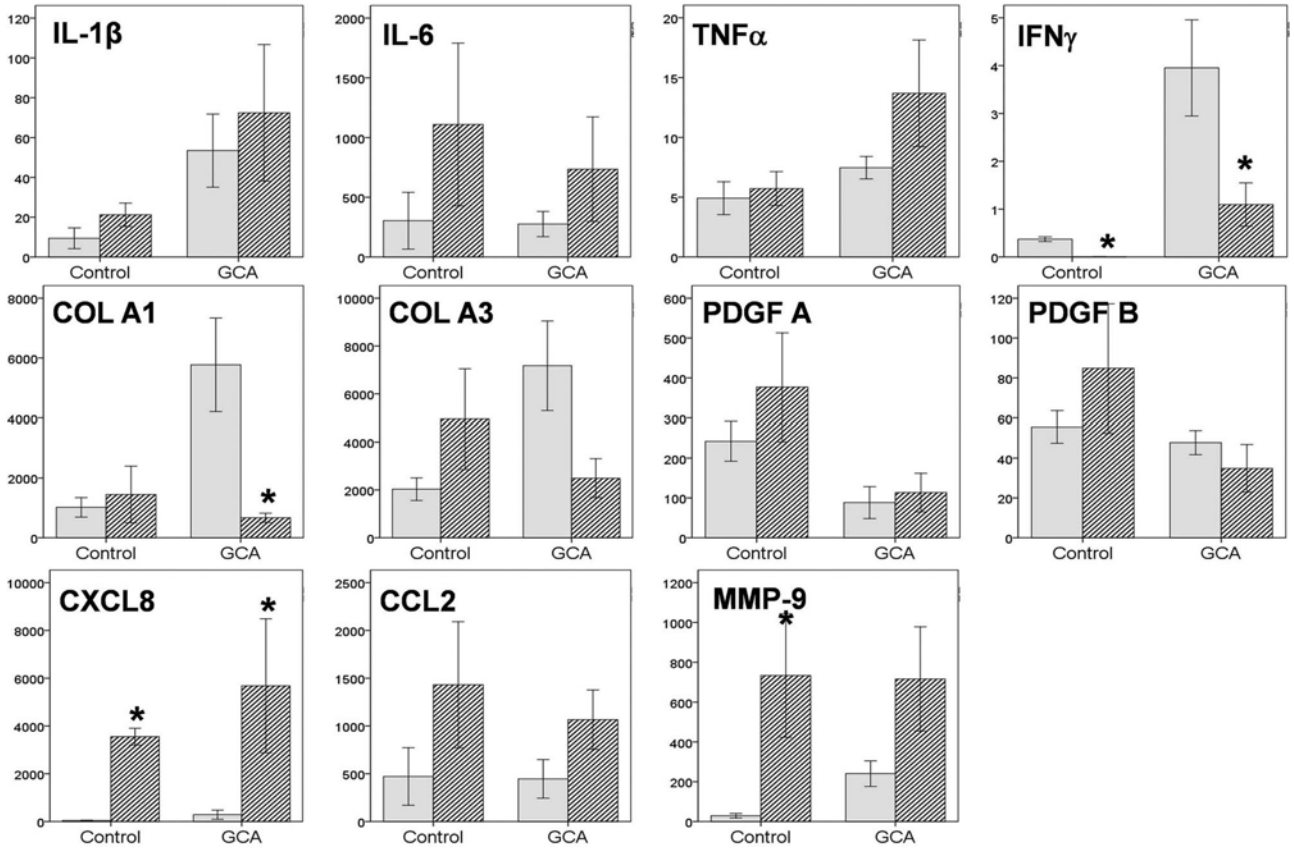

Figure 2 Effects of the culture system on biomarker expression. mRNA concentration (*relative units*) of pro-inflammatory cytokines, chemokines, vascular remodelling factors and matrix proteins in freshly removed (white bars) versus cultured, untreated, serial temporal artery sections (dashed bars) from six giant-cell arteritis patients and five controls (mean±SEM). *p<0.05 comparing fresh versus cultured arteries. Statistics are only indicative given the low number of samples studied.

DISCUSSION

Functional models are essential to explore pathogenic pathways and to test therapeutic intervention in diseases. We developed a new model of temporal artery culture in tri-dimensional matrix to perform functional studies in GCA. Short-term explant culture of involved tissue has been previously used in other conditions such as rheumatoid arthritis and has provided useful insights into involved immunopathogenic pathways.[23] A previous attempt of culturing temporal artery explants was tried by Blain *et al*.[24] However, without the use of a supporting matrix, the specimen remained viable for a short period of time. Specimens were cultured for 20 h only and the release of mediators in the supernatant fluid had to be induced with lypopolysaccharide which is an important exogenous manipulation.

The main innovation of our culture system is the embedding of the specimen in Matrigel which supports viability with active production of inflammatory mediators and their spontaneous release into the culture medium. In addition to provide an anchorage system for the wounded VSMC medial layer of the excised sections, Matrigel provides survival and proliferation signals for VSMC[21] which, in turn, may promote survival of infiltrating lymphocytes and macrophages. In this model, morphology was excellently preserved within 2-week culture.

There was a remarkable variability in the spontaneous production of inflammatory mediators, reflecting the notable differences in the density of inflammatory infiltrates and individual variation in cytokine production existing among patients with GCA. This observation underlines the need of a suitable model where testing specimens from multiple donors is feasible.

Spontaneous expression of IL-1β, IFNγ, MMP-9 and chemokines CCL3, CCL4 and CCL5 was significantly higher in explants from patients compared with controls and closely paralleled what has been described in immunopathology studies of freshly removed GCA arteries.

GC substantially reduced the production of pro-inflammatory cytokines IL-1β, IL-6 and TNFα both at the mRNA and protein level and also IFNγ mRNA. Expression of chemokines was also markedly decreased. These changes were similar to what has been observed in cross-sectional comparisons in biomarker expression between biopsies obtained from untreated patients and biopsies from patients who have already received GC,[7 22] in sequential biopsies obtained in four patients before and after 1 year of GC treatment,[14] or results obtained in temporal artery biopsies engrafted in the SCID mice.[18] GC treatment induced also a decrease in macrophage infiltration, whereas virtually no effect was observed on T cells, suggesting that T cell infiltration may be more resistant to GC therapy.

An interesting contribution of this study is that the expression of vascular remodelling factors such as CCL2/MCP-1, MMP-2, PDGFs and collagen I and III is not influenced by GC. A previous study comparing sequential biopsies obtained in four patients before and after 1 year of GC treatment showed, indeed, that vascular remodelling factors increased after long-term GC treatment.[14] This may explain why some patients

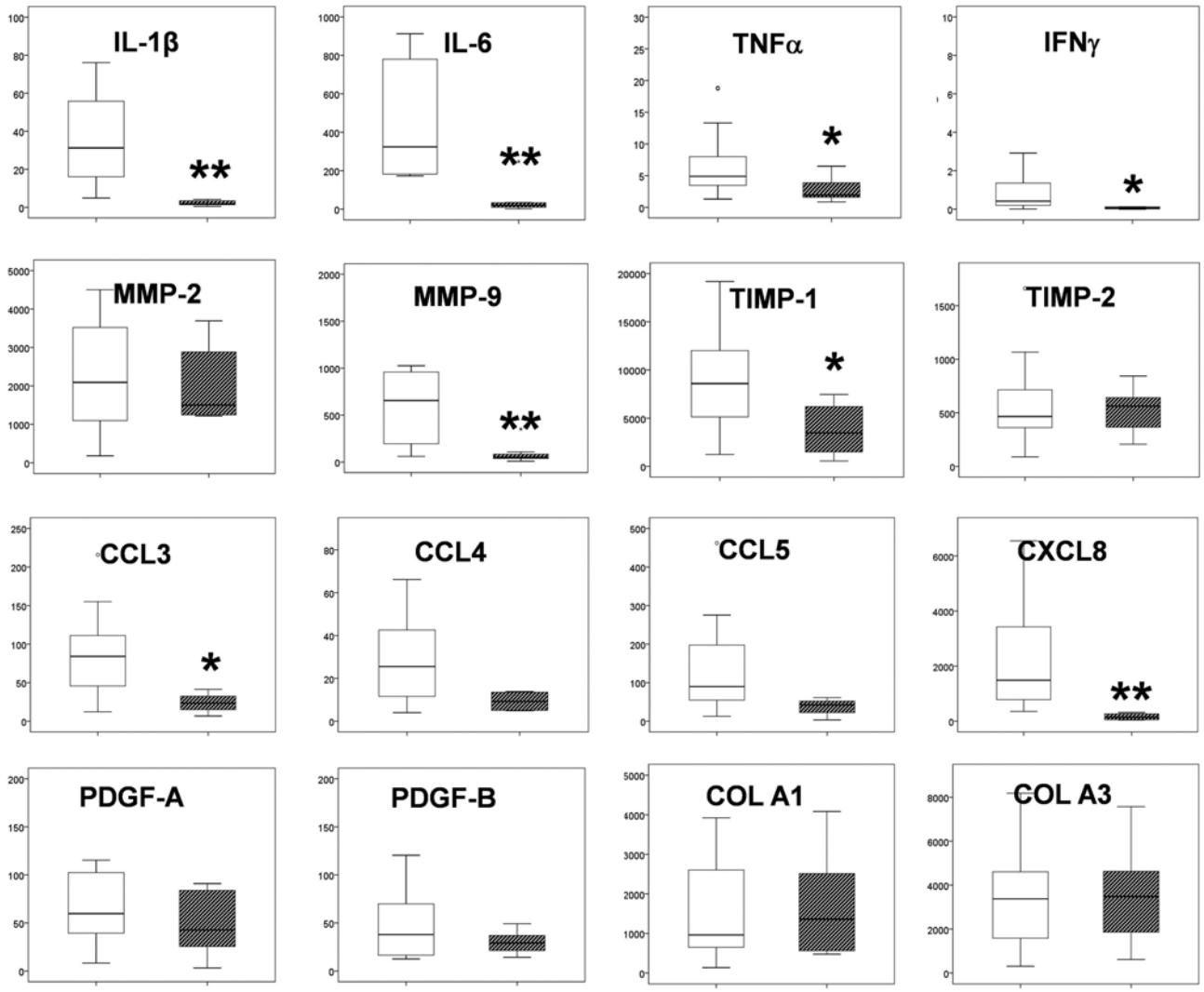

Figure 3 Changes in biomarker mRNAs induced by dexamethasone treatment. Comparison in mRNA concentration of selected biomarkers between untreated temporal artery sections from the giant-cell arteritis cohort (white box) and temporal artery sections from 10 of the patients subjected to dexamethasone at 0.5 mg/ml (grey box). *p<0.05; **p<0.005.

Table 2 Changes in biomarker protein concentration (*pg/ml*) in the supernatant fluid from untreated cultured GCA temporal artery sections and cultured GCA sections exposed to dexamethasone

	Protein concentration (pg/ml)		
	Untreated artery sections (mean ±SEM)	Dexamethasone-treated artery sections (mean±SEM)	p Value
IL-1β	6.06±1.32	2.41±0.76	**0.017**
IL-6	31059.8±10600.6	4796.5±1968.4	**0.020**
TNFα	27.043±6.398	2.22±2.104	**0.041**
IFNγ	4.901±1.8	4.792±1.66	0.961
CCL-2/ MCP-1	11759±2679.5	5130.2±598.83	0.921
CCL3/ MIP-1α	24.11±9.05	19.41±11.38	0.399
CXCL8/ IL-8	132670±41358.1	2465.5±631.76	0.056
MMP-2	39.125±11.14	27.5±7.79	0.204
MMP-9	48.91±10.74	8.64±1.89	**0.003**
PDGF AB	23.38±3.25	20±2.83	0.394

GCA, giant-cell arteritis.

continue to develop vascular occlusive events in spite of GC treatment.[2]

A limitation of this model is that Matrigel itself, by promoting survival and proliferation of smooth muscle cells, may directly influence the expression or detection of some products introducing a bias in the results. The culture system downregulated IFNγ and collagen I expression in GCA arteries and, conversely, upregulated the expression IL-6, CCL2/MCP-1, MMP-9, CXCL8/IL-8, PDGFs and collagen III in control arteries. These molecules may be part of vascular remodelling/repair programme stimulated by surgical injury and facilitated by attachment to the matrix. These observations indicate that some differences in gene expression observed between patients and controls are minimised by the culture system but, at the same time, enhance the significance of the differences observed. Furthermore, this finding underlines the need of investigating how the culture system influences the expression of any factor to be tested in this model.

Another limitation is that detection of some mediators such as chemokines in the culture medium may not accurately reflect their actual production. Chemokines act in an autocrine/paracrine manner and interact with matrix proteins to create a local

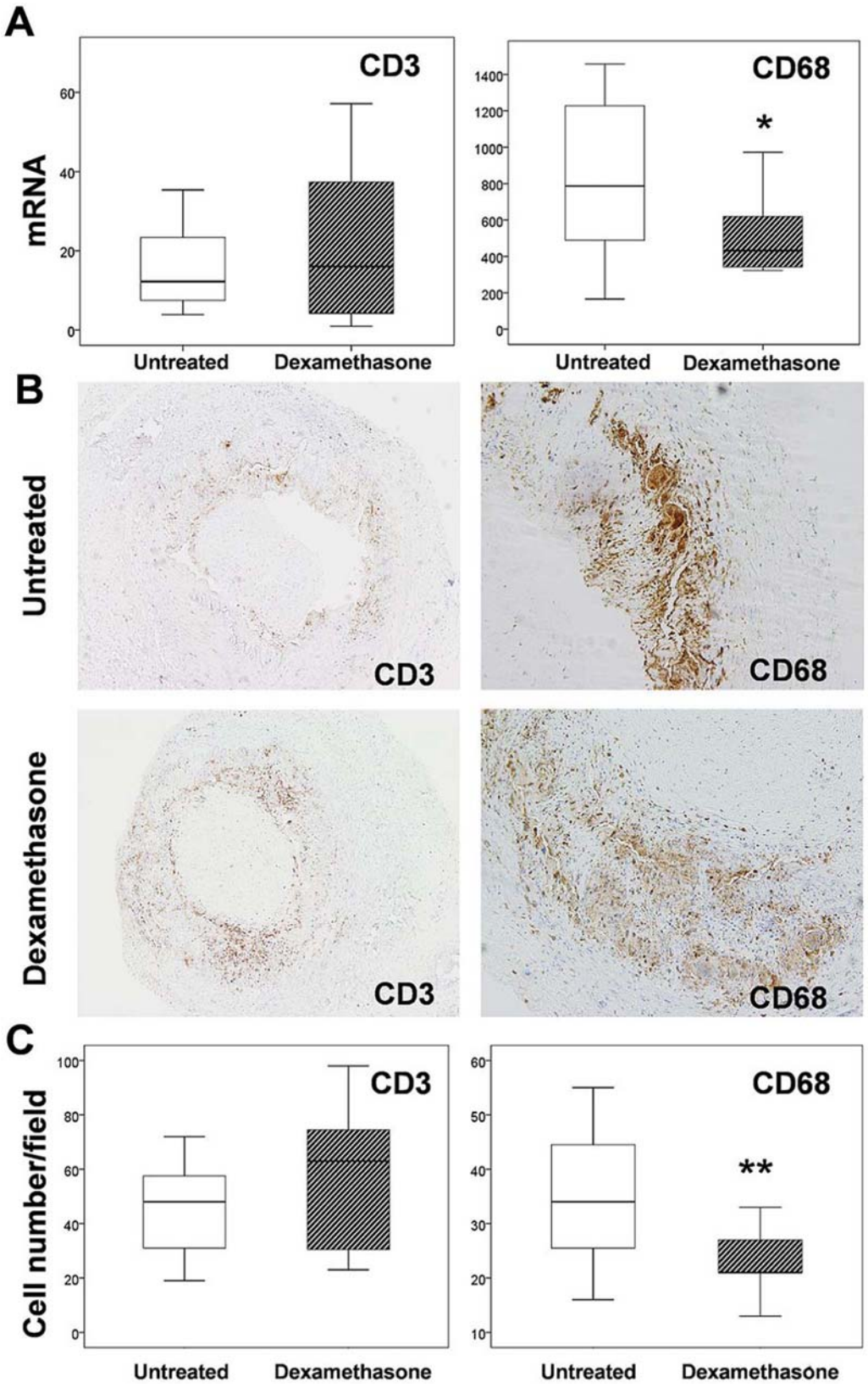

Figure 4 Effects of dexamethasone treatment on the density of infiltrating T lymphocytes and macrophages. (A) Differences in mRNA concentration of CD3 (T lymphocyte marker) and CD68 (macrophage marker) between 28 untreated giant-cell arteritis (GCA) temporal artery sections and 10 GCA sections exposed to 0.5 μg/ml dexamethasone. *p=0.059. (B) Changes in infiltrating T lymphocytes (identified by anti-CD3 immunostaining) and macrophages (identified by anti-CD68 immunostaining) upon dexamethasone treatment. (C) CD3 or CD68 cell number per field in three paired arteries cultured with or without dexamethasone. **p=0.004.

gradient. Therefore, chemokines may be retained in the artery and surrounding proteoglycan-rich matrix, according to their physiological function.[25] Dissociation between tissue and serum concentrations of relevant chemokines has been observed in several chronic inflammatory conditions.[12]

Our model overcomes some of the limitations of the temporal artery engraftment into the SCID mice. It allows daily monitoring of viability, it ensures direct accessibility of the molecules tested, it allows serial detection of proteins secreted into the culture medium and morphology is better preserved. Since retrieval of the cultured specimens is direct and simple, very thin sections can be used, allowing the assessment of replicates to assure consistency, and the testing of various conditions per specimen. This is very important given the remarkable variability in the intensity of inflammatory infiltrates and cytokine production among patients. In addition, it is cheap, easy, spares animals and does not require special equipment besides tissue culture facilities. In fact, since the initial communication of preliminary results,[26 27] this model is being used by other investigators.[28] It shares with the SCID mice model the limitation that only changes in biomarkers can be assessed and true, clinically relevant, disease outcomes cannot be investigated.

In summary, we developed an artery explant culture system based on the unique properties of Matrigel in creating a tridimensional matrix support and promoting VSMC survival. This method is sensitive enough to detect changes after intervention and may be useful to explore pathogenic pathways and to assess the impact of new therapeutic agents.

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Contributors MCC and MUR designed the study. J-MD contributed important input to its design. MC-B, AG-M, EL, EP-R and PR-L performed the experimental work. GE-F, SP-G, MAA, JH-R and MB contributed to clinical selection and contributed to the experimental work, PLF supervised the immunopathology studies. All authors evaluated and criticised the data and J-MD provided important contributions to their interpretation. MC-B and MCC wrote the manuscript. All authors read, made improvements and approved the final version.

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EXTENDED REPORT

Prospective long term follow-up of a cohort of patients with giant cell arteritis screened for aortic structural damage (aneurysm or dilatation)

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Handling editor Tore K Kvien

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The results were presented in part at the 72nd ACR Annual Scientific Meeting (San Francisco, 2008), the 14th International Vasculitis and ANCA Workshop (Lund, 2009) and the Annual European Congress of Rheumatology (Berlin, 2012).

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ABSTRACT

Background Aortic structural damage (ASD) may complicate the course of patients with giant cell arteritis (GCA). However the frequency and outcome of ASD has not been assessed in long term prospective studies.

Methods In a previous screening of 54 biopsy proven GCA patients, significant ASD was detected in 12 (22.2%) after a median follow-up of 5.4 years. These patients were periodically evaluated (every 4 years) over a median of 10.3 years (range 4–16.6 years) in order to investigate the development of new ASD and the outcome of previously detected abnormalities.

Results 18 of the 54 patients abandoned the study due to death or other reasons. The remaining 36 patients were subjected to a second screening and 14 to a third screening. 12 (33.3%) of the 36 patients re-screened and 16 (29.6%) of the initial cohort developed ASD, all but one in the thoracic aorta. Aortic diameters at the ascending and descending aorta significantly increased over time. One patient (1.9% of the initial cohort) died from aortic dissection. Surgery was advised in eight (50%) patients with ASD but could only be performed in three patients (37.7%). The development of ASD was not associated with persistence of detectable disease activity.

Conclusions The incidence of ASD is maximal within the first 5 years after diagnosis but continues developing over time, affecting up to 33.3% of individuals after long term follow-up. Once ASD occurs, dilatation increases over time, underlining the need for periodic evaluation. Surgical repair is feasible in about one-third of candidates.

INTRODUCTION

Giant cell arteritis (GCA) is a large vessel vasculitis in the elderly, typically targeting the cranial arteries.[1 2] Aortic involvement by GCA has been sporadically reported in small necropsy studies or surgical series for decades[3–5] but the prevalence of aortic inflammation in GCA has remained undefined until recently when the development of imaging techniques has allowed detection of aortitis at early disease stages, before the development of aortic complications requiring surgery or death.[6–8] Using positron emission tomography or CT angiography, up to 45–65% of GCA patients can be identified exhibiting radiological signs of aortic inflammation at the time of diagnosis.[6–8] Aortitis is usually asymptomatic but may lead to subsequent aortic structural damage (ASD) with the potential for catastrophic events, such as aneurysm rupture or dissection.[9–12]

In a retrospective, population based study, Evans *et al*[9] found that GCA patients were 17.3 times more likely to develop thoracic aortic aneurysms and 2.4 times more likely to develop abdominal aortic aneurysms than individuals of similar age and gender from the general population. Subsequent studies have estimated that 9.5–18% of GCA patients develop aortic aneurysm or dissection.[10 11] However, the retrospective nature of these studies where patients were not routinely imaged might have underestimated the prevalence of aortic complications. In 54 GCA patients systematically subjected to a defined screening protocol, significant ASD was detected in 12 (22.5%) after a median follow-up of 5.4 years (range 4–10.5 years).[12]

The development of significant ASD is challenging due to the life threatening nature of its complications[13] and the difficulties in performing aortic reconstructive surgery in aged individuals. The natural history of aortic dilatation in GCA is not well known and has not been prospectively investigated. According to retrospective studies, although aortic dissection may occur early in the course of the disease, aortic dilatation appears to be more frequently a delayed complication.[9–12] The frequency of aortic related life threatening complications is not known, and therefore, whether or not aortic dilatation conveys an increased mortality in aged patients with a naturally limited lifespan has not been clearly defined. Moreover, feasibility and outcome of reconstructive surgery in these patients has not been evaluated. Answers to these questions are seminal to establish recommendations about systematic population screening.[14]

The aim of this study was to perform a prospective longitudinal evaluation of a patient cohort previously screened for ASD in order to assess the development of new ASD and the outcome of previously detected abnormalities over an extended follow-up period.

PATIENTS AND METHODS

Study population and screening protocol

The study group comprised 54 patients (14 men and 40 women) with biopsy proven GCA systematically screened for ASD, 5.4 years (range 4.0–

10.5 years) after diagnosis. The results of the first screening have been published previously.[12] After this initial assessment, patients were subjected to a longitudinal, prospective follow-up and the same screening protocol was performed approximately every 4 years. If relevant ASD was detected in the initial screening, additional imaging was performed when judged appropriate by the consultant cardiovascular surgeon. All patients were treated and followed by the authors according to uniform criteria.[12] The screening protocol consisted of a medical evaluation and routine blood tests, including acute phase reactants. Serum concentrations of proinflammatory cytokines (interleukin 6 and tumour necrosis factor α) were measured by immunoassay according to the manufacturer's instructions (R&D Systems, Minneapolis, USA). A chest x-ray was performed in all patients and compared with that obtained at the time of diagnosis. When aortic dilatation or changes with respect to previous radiographs were suspected, even minimal or questionable, a contrast enhanced spiral chest CT scan was performed and aortic diameters were measured at different segments. The abdominal aorta was evaluated by ultrasonography (US). CT and ultrasonography results were compared with those obtained at the initial screening. As previously stated, significant ASD was defined as focal dilatation (saccular or fusiform aneurysm) or, in the case of diffuse dilatation, when the aortic diameter exceeded 4 cm at the ascending aorta or reached at least 4 cm in the aortic arch/descending aorta or 3 cm at the abdominal aorta.[8 15] Clinical data recorded included number of relapses, time to first relapse, time required to achieve a maintenance prednisone dose <10 mg/day and time to complete prednisone withdrawal. Relapses were defined as recurrence of cranial, polymyalgic or systemic symptoms, including anaemia not attributable to other causes, which completely resolved by increasing prednisone 10 mg above the previously effective dose. Relapses were usually accompanied by mild to marked increases in acute phase reactants. However, isolated variations in erythrocyte sedimentation rate or C reactive protein were not considered relapses unless the above mentioned disease features appeared. Number and cause of deaths and survival time from GCA diagnosis were also recorded.

Statistical analysis

Comparisons between groups were performed using a t test for continuous variables and Fisher's exact test for categorical variables. Time to first relapse, time required to achieving a maintenance prednisone dose <10 mg/day, time to complete prednisone withdrawal and time to death were analysed by Kaplan–Meier survival analysis and compared using the log rank test.

RESULTS

Development of aortic dilatation over time

The initial cohort of 54 patients was prospectively followed for a median of 10.3 years (range 4–16.6 years), until death, dropout, the third screening or March 2013. During the study period and before the second screening, 18 of the 54 patients died or abandoned the study for major logistical difficulties in continuing visits at a referral centre (figure 1). A second screening was completed in the remaining 36 patients (10 men and 26 women; 66.6% of the initial cohort) after a median follow-up of 8.7 years (range 6.9–13.6 years). Seven of these patients dropped out before the third screening (figure 1) which, to date, has been completed in 14 patients (median follow-up 12.8 years, range 10.3–16 years). The remaining 15 patients

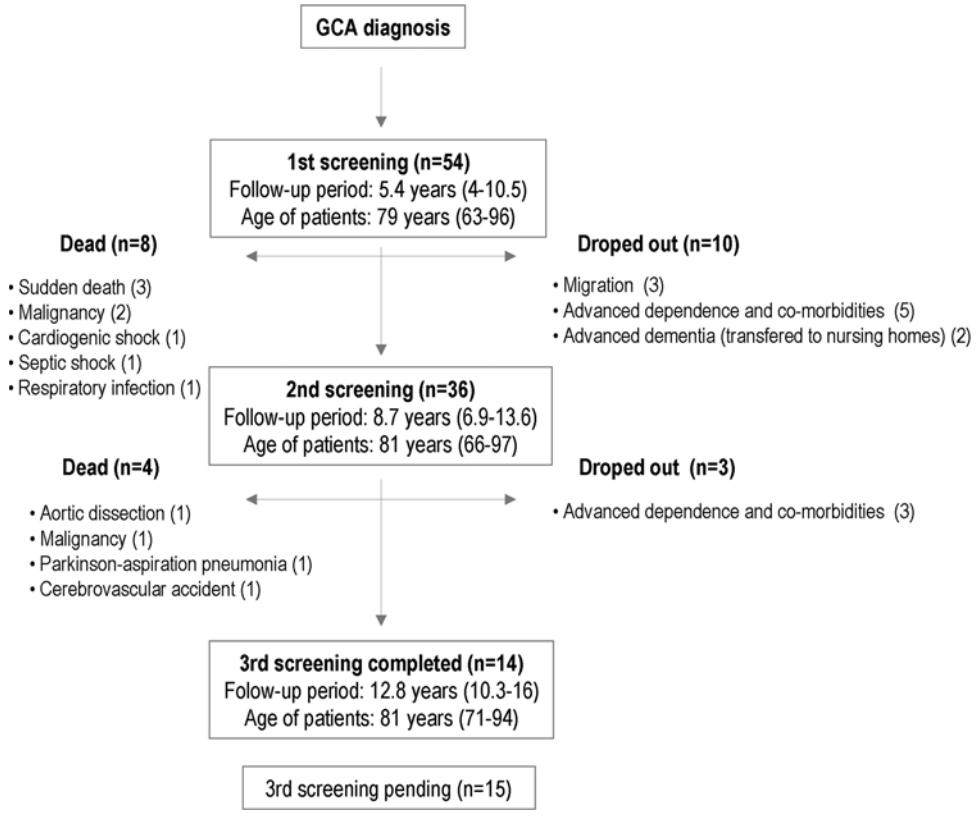

Figure 1 Flowchart of the outcome of the study cohort. A remarkable drop out occurred during the study period, due to advanced age of the study population. GCA, giant cell arteritis.

will be eventually re-screened within the next 2 years if they continue their scheduled appointments.

During the overall study duration, 12 (33.3%) of the 36 patients who completed the second or third screenings and 16 (29.6%) of the 54 patients encompassing the initial cohort, developed ASD. In 12 patients, ASD was detected at the first screening, in three at the second assessment and in one at the third evaluation. Figure 2A shows the percentage of patients who developed ASD over time.

Table 1 summarises the characteristics and outcome of ASD in these patients. In 15 of the 16 patients with ASD, dilatation was found in the thoracic aorta, and only one patient had a small fusiform aneurysm located at the abdominal aorta that remained stable over time. This aneurysm was detected at the first screening. It is unclear whether this aneurysm was related to GCA or to atherosclerotic disease. No abdominal aneurysms were detected in additional patients during follow-up.

Outcome of aortic dilatation over time

According to the screening protocol designed for potential general use, assessment of the thoracic aorta was initially based on a chest x-ray. However, 44 (81.5%) of the 54 patients underwent a CT scan at some point during follow-up because of a slight, moderate or high suspicion of aortic dilatation, allowing accurate measurements of the aortic diameters. As shown in table 1, aortic diameters increased over time in patients with ASD. Table 2 shows aortic diameters at various segments of the thoracic aorta in 17 patients who underwent a CT scan in both the first and second screenings. A significant increase in the diameters of the ascending and descending aorta was observed. This was at the expenses of patients with ASD in the first CT scan, indicating progressive dilatation over time of the damaged aortic segments.

Aortic reconstructive surgery

At the end of the follow-up period, eight (50%) of the 16 discovered dilatations were candidates for elective surgery, according to general consensus guidelines for surgical repair of aneurysmal disease.[16] However, aortic reconstructive surgery was performed in only three patients (37.5% of candidates for surgery, 18.8% of those with dilatation and 5.5% of the entire patient cohort). In the remaining five, surgery was not advised because of advanced age and comorbidities (three patients) or patients themselves declined intervention after being informed of the potential risks (two patients). One of the three patients who underwent surgical repair of an ascending aortic aneurysm exhibited diffuse dilatation of the aortic segments distal to the aortic prosthesis at the second screening. Another patient suddenly died 6 months after surgery.

Patient mortality according to ASD

Forty-one of the 54 patients were followed until the end of the study or death, and 13 abandoned the study (figure 1). During the study period, at least 12 patients (22.2% of the initial cohort) died.

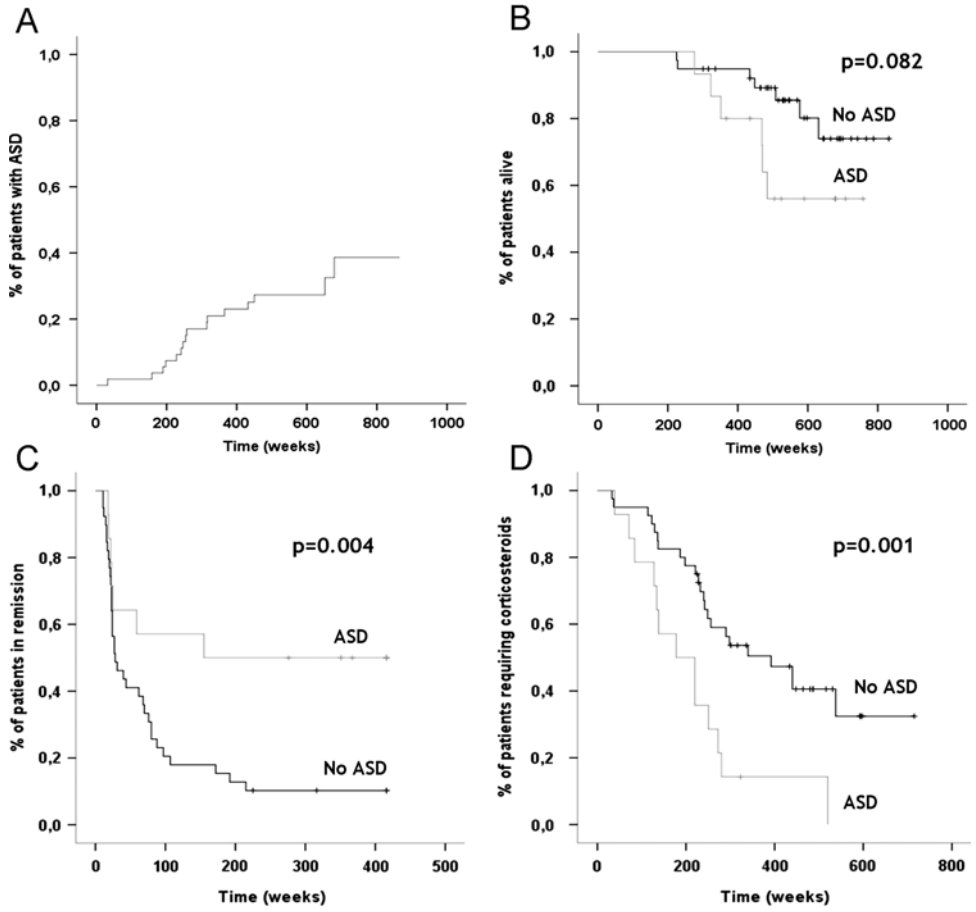

Figure 2 Patient outcome according to the presence or absence of aortic structural damage (ASD). (A) Percentage of patients with ASD over time (weeks). (B) Percentage of patients alive over time (weeks). (C) Relapse free survival. (D) Proportion of patients receiving glucocorticoid therapy over time (weeks).

Table 1 Characteristics of aortic structural damage during follow-up

Patient No	1st screening	2nd screening	3rd screening
1	Dilatation of the ascending aorta. AD 42/30/36 mm	Not done. Death due to lung cancer.	
2	Ascending AA. Surgery declined for advanced age and comorbidities. AD 57/31/38 mm.	Not done. Death due to renal cell carcinoma	
3	Ascending AA repaired (Bentall). AD 60/33/37 mm	Dilatation of the aorta distally to the prosthesis. AD 55/34/49 mm	Pending. Alive at the end of the study
4	Normal chest x-ray	Ascending AA. AD 50/29/29 mm	Not done. Death due to aortic dissection
5	Ascending AA. Surgery declined for advanced age. AD 73/24/28 mm	Ascending AA. AD 90/25/29 mm	Not done. Death due to cerebrovascular accident
6	Ascending AA. AD 47/40/28 mm	Ascending AA. AD 50/32/31 mm.	Pending. Alive at the end of the study
7	Normal chest x-ray	Dilatation of the ascending aorta. AD 41/28/29 mm	Pending. Alive at the end of the study
8	Dilatation of the aortic arch. AD 40/40/30 mm	Dilatation of the ascending aorta and aortic root. AD 42/44/33 mm	Dilatation of the ascending aorta and aortic arch. AD 42/46/34 mm
9	Ascending AA. AD 50/38/31 mm	Not done. Lost to follow-up for 4 years	AA repair 6 years after the first screening
10	Ascending AA. AD 50/42/28 mm.	Ascending AA. AD 55/42/29 mm. The patient declined surgery	Not done. Death due to aspiration pneumonia
11	Dilatation of the ascending aorta. AD 45/38/29 mm	Ascending AA. AD 50/37/29 mm. Surgery declined because of comorbidities	Pending. Alive at the end of the study
12	Ascending AA repaired. AD 55/40/28 mm	Not done. Sudden death 6 months after surgery.	
13	Dilatation of the descending aorta. AD 37/39/40 mm	No significant changes in aortic diameters. AD 39/39/39 mm (CT without contrast)	Not done. Death due to lung cancer
14	No ASD in CT. AD 39/31/25 mm	Ascending AA. AD 51/33/29 mm. The patient declined surgery	Pending. Alive at the end of the study.
15	Normal chest x-ray	No ASD in CT. AD 37/28/26 mm	Dilatation at the ascending aorta. AD 43/28/29
16	Fusiform aneurysm of the abdominal aorta (51×29 mm)	No changes	No changes in abdominal aneurysm by CT

When available, aortic diameters (AD) at different levels of the thoracic aorta are shown (ascending aorta/aortic arch/descending aorta).
AA, aortic aneurysm.

One patient developed an aortic dissection and died shortly after the discovery of an ascending thoracic aneurysm at the second screening which, at that time, was not considered large enough to warrant surgery. At the time of death, the patient was in stable remission and had been able to withdraw corticosteroids 5 years before. This death was considered GCA related. As shown in figure 1, eight additional deaths were unrelated to GCA and three were sudden deaths at the patient's home, including the above mentioned patient who underwent aortic repair 6 months before. Necropsy studies of these three patients were not performed and an acute aortic complication as the cause of death cannot be confirmed or ruled out. Therefore, mortality directly related to aortic complications in our patient cohort was at least 1.9%.

Extended follow-up of the original cohort showed a trend towards an increased mortality (any cause) among patients with ASD although differences did not reach statistical significance (figure 2B).

Development of ASD and disease activity

There were no significant differences in follow-up duration between patients who did or did not develop ASD (9.8 ± 3.1 vs 10.7 ± 3.1 years, NS).

Table 2 Diameters of the thoracic aorta in 17 patients subjected to CT scan at the first and second screenings

	1st screening	2nd screening	p Value
Ascending aorta (mm)	39±11	42±15	0.018
Aortic arch (mm)	32±6	31±6	NS
Descending aorta (mm)	28±5	29±4	0.03

Values are (mean±SD).

At the end of the follow-up period, 11 patients achieved sustained remission with no relapses and 43 patients experienced at least one (median 2, range 0–7). The proportion of patients in sustained remission throughout the study period was significantly higher among patients with ASD (figure 2C). Accordingly, ASD was more frequent among patients without recurrences (73%) compared with those who had experienced at least one relapse (16%) (RR 4.47, 95% CI 2.07 to 9.63, p=0.001).

Patients who developed ASD suffered significantly lower numbers of relapses than patients without ASD (mean 0.9 ± 1.2 vs 2 ± 1.5, p=0.006) during the study period.

At the end of the follow-up period, 36 patients had been able to withdraw therapy and 18 patients were still on prednisone (median 2.5 mg/day, range 1.25–12.5). Only one of the patients who developed ASD was receiving corticosteroids at the end of follow-up (6.2 years). Patients who developed ASD needed shorter periods of time to achieve a maintenance prednisone dose lower than 10 mg/day than patients without ASD (mean 45±32 vs 79 ±65 weeks, p=0.015) and to completely withdraw therapy (mean 213 ± 37 vs 423 ± 41 weeks, p=0.001) (figure 2D). The overall dose of prednisone required at the end of follow-up was also lower among patients who developed ASD (9.5 ± 4 vs 15.4 ± 7.1 g, p<0.001).

Patients who developed ASD exhibited lower levels of the acute phase reactants erythrocyte sedimentation rate and haptoglobin at different time points compared with patients who did not develop aortic dilatation (figure 3). C reactive protein and proinflammatory cytokines (interleukin 6 and tumour necrosis factor α) concentrations did not show significant differences between groups (data not shown).

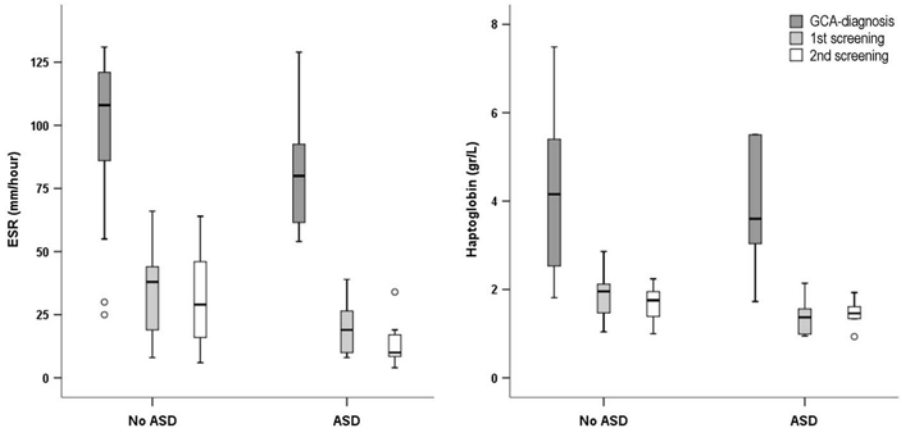

Figure 3 Erythrocyte sedimentation rate (ESR) and haptoglobin concentrations at various time points (diagnosis, first screening and second screening) in patients with or without aortic structural damage (ASD). p<0.05 for the following comparisons: ESR 1st screening, ESR 2nd screening and haptoglobin 2nd screening. GCA, giant cell arteritis.

Histopathological examination of aortic specimens

During the study period, histopathological evaluation of the aortic wall was performed in six patients during surgery or necropsy. Three had undergone reconstructive surgery of an ascending aortic aneurysm. Two patients died from GCA unrelated conditions but one also had an ascending aortic aneurysm. Finally, as mentioned above, one patient developed aortic dissection and died. Aortic specimens were obtained a median of 9.2 years (range 5.1–11.1 years) after GCA diagnosis. All of these patients were in stable remission and had been able to withdraw corticosteroid therapy. Two patients exhibited severe atherosclerosis of the aorta. None of the aortic samples exhibited dense inflammatory infiltrates in the media, suggestive of significant ongoing inflammation. Only two patients exhibited scattered mild inflammatory infiltrates at the media. The most striking finding was the significant loss and disarray of elastic fibres, even in areas devoid of current inflammation (figure 4).

DISCUSSION

Aortic involvement by GCA has received a great deal of attention due to the life threatening nature of its potential complications.[1–3] However, the prevalence, timing and long term outcome of ASD are not well known. In addition, as most patients with GCA are elderly and, consequently, with a limited lifespan and at increased risk for major surgery, the clinical relevance and therapeutic impact of discovering ASD in patients with GCA are unclear. The lack of prospective long term follow-up studies prevents the design of specific recommendations about whether or not GCA patients should be systematically screened, what would be the best cost effective screening method and how often this should be applied.[14]

This is the first prospective study to assess the development and outcome of ASD over a long period of time. Nearly one-third of the initial cohort, and 33.3% of those who completed successive screenings, developed significant ASD during the entire follow-up period, representing a much higher prevalence of aortic aneurysm/dilatation than previously observed in retrospective surveys.[9–11 17]

Similarly to previous studies, clinically significant aortic dilatation predominated at the ascending aorta.[9–11 17] However, CT was able to detect subtle structural abnormalities, such as non-significant dilatation (<4 cm) or thickening of the aortic wall, at other segments (data not shown), suggesting previous inflammatory involvement of larger portions of the aorta, as has been demonstrated in necropsy studies or, more recently, by imaging techniques.[6–8]

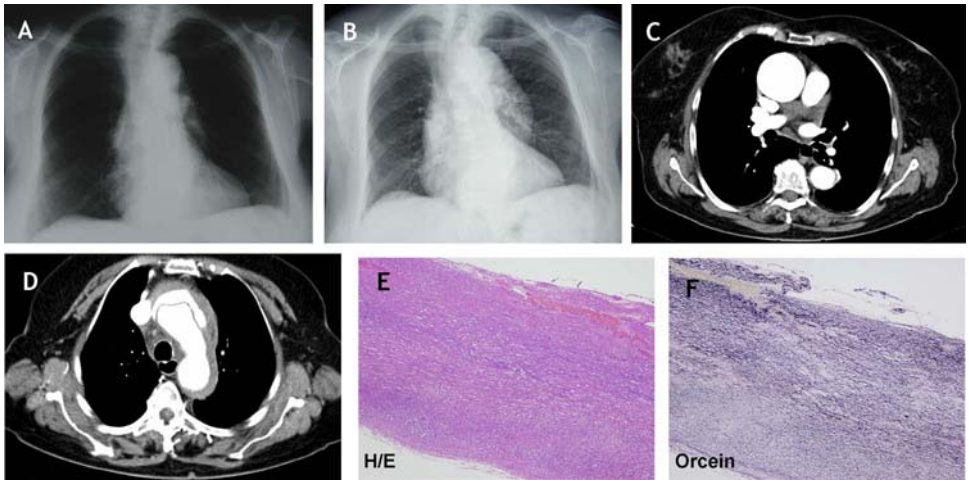

Figure 4 Serial findings in a patient with giant cell arteritis and aortic aneurysm. (A) Chest x-ray at the time of the first screening did not show aortic abnormalities. (B) Chest x-ray at the time of the second screening was suggestive of aortic enlargement. (C) The CT scan demonstrated an aortic aneurysm at the ascending aorta (50 mm). (D) A few months later, the patient developed an aortic dissection and died. (E, F) Histopathological examination of the aortic wall with haematoxylin/eosin (H/E) and orcein staining exhibited loss and disorganisation of elastic fibres in the absence of inflammatory infiltrates.

The chronology of ASD development has not been well defined. A prospective study of aortic involvement in newly diagnosed patients showed that only 15% have some aortic dilatation at the time of diagnosis.[8] This is in contrast with the remarkable prevalence of aortic dilatation during long term follow-up found in the present study and supports the concept that ASD is a delayed complication. As found in a recently published retrospective study,[17] the majority of dilatations occurred during the first 5 years, but continued to occur thereafter. Moreover, existing dilatations progressively increased in size over time. Confirmed dissection occurred in only one patient but aortic complications may have accounted for some of the reported sudden deaths. Overall mortality tended to be higher in patients with ASD but differences were not significant, possibly influenced by the limited size of our cohort. Recently published retrospective studies have also suggested increased mortality among patients with aortitis[18] or aortic dilatation.[17]

In about half of the discovered dilatations, surgical repair was advised, according to current guidelines[16] but this could be applied to only one-third of candidates. The number of patients subjected to repair was too low to draw conclusions about the advantages of elective surgery. Surgery was followed by distal dilatation of the remaining aorta in one patient and sudden death 6 months later in another.

In our series, extended follow-up confirmed that development of significant ASD was not associated with persistence of clinically or analytically detectable disease activity.[12] Late aortic dilatation frequently occurred in patients who had achieved sustained remission. Intriguingly, and as observed in the first screening,[12] patients who developed significant ASD experienced fewer relapses and were able to withdraw therapy earlier than patients who did not develop significant ASD. It has been postulated that smouldering inflammatory activity, not clinically detectable, may progressively destroy the aortic wall. Chronic use of low dose glucocorticoids might then prevent aortic damage. However, patients with ASD exhibited lower concentrations of acute phase reactants, not only at diagnosis but also at various time points during follow-up than patients without aortic dilatation.[12] Moreover, histopathological examination of aortic specimens obtained from six patients disclosed extensive destruction of elastic fibres as the main feature and slight residual inflammatory infiltrates were only observed in two patients. These findings suggest that persistence of inflammatory activity may not be the only or more determinant factor in aortic dilatation and that the characteristics and aggressiveness of the initial inflammation along with deficiencies in subsequent vascular remodelling may have a major role in the development of aortic dilatation.[19–21] As previously suggested, haemodynamic factors may also contribute.[8] Abnormalities in vascular remodelling may turn the aortic wall into a weakened structure that may undergo progressive dilatation, mainly at the ascending portion where mechanical forces are greater.[8 20 21]

In summary, the development of ASD seems to be much more frequent than suggested in retrospective studies. Although the maximal incidence appears to occur within the first 5 years after diagnosis, dilatation may develop subsequently. Moreover, existing dilatations increase in size over time. Therefore, it seems reasonable to periodically screen GCA patients, particularly those in a good general condition who could be eligible for surgery if needed and in whom aortic complications may reduce their life expectancy. The best approach and frequency of performing this surveillance have not been established. Our findings suggest that a simple chest x-ray may be sufficient for a population wide initial screening but this needs to be confirmed in larger studies. Feasibility and outcome of elective surgery needs to be further investigated. It remains to be elucidated whether the duration of low dose glucocorticoid therapy may influence the development of ASD and the risk/benefit of prolonged glucocorticosteroid treatment if that was the case. Moreover, new therapies tested in GCA patients should be evaluated for their ability to reduce the risk of subsequent dilatation as an important outcome measure.[22 23]

Contributors AG-M, SP-G, JH-R and MCC designed the study. PA, GE-F, MAA, MB, IT-B, AG-M, JH-R and MCC generated the data. AG-M and MCC drafted the manuscript. All authors reviewed and commented on the data. All authors read, commented on and approved the manuscript.

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Competing interests None.

Ethics approval The study was approved by the ethics committee of Hospital Clinic.

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CONCISE REPORT

Positron emission tomography assessment of large vessel inflammation in patients with newly diagnosed, biopsy-proven giant cell arteritis: a prospective, case–control study

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Results presented at the European Congress of Rheumatology (EULAR) 2013, Madrid, Spain, June 2013, and at the Annual Congress of European Association of Nuclear Medicine, Lyon, France, October 2013.

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ABSTRACT

Background Positron emission tomography (PET) scan is emerging as a promising imaging technique to detect large-vessel inflammation in giant cell arteritis (GCA). However, the lack of a standardised definition of arteritis based on ^{18}fluorodeoxyglucose (FDG) uptake is an important limitation to the use of PET scan for diagnostic purposes.

Objective To prospectively assess the intensity and distribution of FDG uptake at different vascular territories in patients with newly diagnosed GCA compared with controls.

Methods 32 consecutive, biopsy-proven, GCA patients treated with glucocorticoids for ≤3 days were included. The control group consisted of 20 individuals, who underwent PET/CT for cancer staging. Maximal standardised uptake value (SUVm) was calculated at four aortic segments, supraaortic branches and iliac-femoral territory. Sensitivity and specificity was calculated by receiver–operator characteristic curves (ROC) analysis.

Results Mean SUVm was significantly higher in patients than in controls in all vessels explored and correlated with acute-phase reactants and serum IL-6. Mean of the SUVm at all the vascular territories had an area under the curve (AUC) of 0.830, and a cut-off of 1.89 yielded a sensitivity of 80% and a specificity of 79% for GCA diagnosis. There were no significant differences in AUC among the vascular beds examined.

Conclusions FDG uptake by large vessels has a substantial sensitivity and specificity for GCA diagnosis.

INTRODUCTION

Temporal artery biopsy is the gold standard for the diagnosis of giant cell arteritis (GCA) due to the tropism of GCA for the epicranial arteries.[1 2] With a few exceptions,[3] histopathological demonstration of temporal artery inflammation provides the most definitive evidence of GCA. Doppler ultrasonography (DUS) of temporal arteries has emerged as a useful alternative tool in centres where biopsy is not easily available.[1]

The diagnosis of GCA may be also supported by demonstrating extracranial artery involvement by imaging. Over the past recent years, positron emission tomography/CT (PET/CT), CT angiography, magnetic resonance imaging (MRI) angiography and DUS have revealed that extracranial involvement in GCA is more frequent than previously anticipated, occurring in 30–74% of patients.[4–7]

PET detection of large-vessel involvement in patients with fever of unknown origin, unexplained constitutional symptoms or apparently isolated polymyalgia rheumatica (PMR) has emphasised its diagnostic potential.[8 9] A limitation of PET as a diagnostic tool is the lack of a standardised definition of vascular inflammation based on the intensity of ^{18}fluorodeoxyglucose (FDG) uptake. While visual assessment of intensively positive cases may be clear, there is no consensus about the minimal intensity of FDG uptake necessary to define vascular inflammation. Conversely, atherosclerosis and ageing may increase vascular FDG uptake, potentially leading to vasculitis overdiagnosis.[10]

In this study, we measured FDG uptake by different vascular territories in a cohort of newly diagnosed patients and controls and performed receiver–operator characteristic curves (ROC) analysis to determine sensitivity and specificity of FDG uptake to detect inflammation at different vascular sites. As a secondary endpoint, we analysed potential correlation between FDG uptake and inflammatory biomarkers.

MATERIALS AND METHODS

Patients

Between November 2006 and March 2011, all patients diagnosed with biopsy-proven GCA[2] at our institution were assessed for potential participation in the study. Patients who had received glucocorticoid treatment for >3 days were excluded. Clinical and laboratory data recorded are detailed in the online supplementary methods. The study was approved by the ethics committee (Hospital Clínic, Barcelona).

The control group included 20 patients with no chronic inflammatory diseases, matched for gender, age and cardiovascular risk factors (CVRF), consecutively selected among patients who underwent PET/CT during the same timeframe for early lung cancer staging.

PET/CT protocol

PET scans were performed using a hybrid PET/CT (Biograph, Siemens) with an ECAT EXACT HR+BGO PET and a helicoidal CT scanner (Somatom, Emotion). Patients fasted 4 h before injection of 370 MBq of 18F-FDG. Whole-body PET data were acquired 60 min after in three-dimensional mode and for 5 min per bed position. PET images were reconstructed both with and without CT data for attenuation correction. A region of interest (ROI) in 3-D around the vessel was placed manually in transaxial, sagittal and coronal slices. The standardised uptake value (SUV) was calculated based on the measured activity, decay-corrected injected dose and patient body weight. SUVm=maximal activity (ROI) (mBq/mL)/injected dose (mBq)/weight (g).

Four aortic segments (ascending thoracic aorta, aortic arch, descending thoracic aorta and abdominal aorta) and the main tributaries—carotid, subclavian, axillary, iliac and femoral arteries (each bilaterally)—were evaluated. The control group was subjected to the same PET/CT protocol. Assessment of PET data was carried out by two nuclear medicine specialists (FL and MD), who were blinded to clinical and pathological findings. However, unequivocal masked evaluation could not be guaranteed due to the controls' disease.

Statistical analysis

ROC were applied to each vascular territory to calculate sensitivity and specificity. Area under the curve (AUC) comparison was performed by Hanley and McNeil analysis. Cut-offs with best sensitivity and specificity were selected. Mann–Whitney U test or Student t test, when applicable, were used for quantitative data. Correlations were calculated using Pearson's or Spearman's test. Statistical significance was defined as $p<0.05$. Calculations were performed with the IBM SPSS Statistics (V.20.0, Armonk, New York, USA).

RESULTS

Clinical and laboratory findings of the GCA cohort

Seventy-one GCA patients were diagnosed during the recruitment period. Eight patients refused participation, and 31 had received glucocorticoid treatment for >3 days. The remaining 32 were included. Seventeen of them had been treated for ≤3 days at the time of imaging. Treatment consisted of oral prednisone at 1 mg/kg/day. Two patients received 250 mg intravenous methylprednisolone pulses (1 and 7 pulses, respectively) due to severe cranial ischaemic symptoms.

Online supplementary table S1 shows the clinical and laboratory data of the study group. There were no relevant differences in age, gender or CVRF between patients and controls (see online supplementary table S2).

FDG uptake cut-off for GCA diagnosis

SUVm at any vascular territory explored was significantly higher in GCA patients than in controls (table 1). ROC curves and AUCs are displayed in figure 1 and table 1, respectively. Mean of the SUVm observed at all the vascular territories had an AUC of 0.830 (0.715–0.946). A cut-off of 1.89 had a sensitivity of 80% and a specificity of 79%. Mean of the SUVm at supraaortic vessels showed the highest AUC (0.832). In this site, a cut-off of 1.70 achieved a sensitivity and specificity of 81 and 79%, respectively, for the diagnosis of GCA (95% CI 0.720 to 0.946). FDG uptake at the aorta showed lower AUC (0.738), with a sensitivity and specificity of 90 and 42, respectively, using a cut-off of 2.25, and a sensitivity of 58%, specificity of 90% with a cut-off of 2.65 (95% CI 0.598 to 0.881). However, differences in AUCs among territories did not reach statistical significance.

Vascular/liver uptake ratios were also significantly higher in patients than in controls at the right axillary and carotid arteries,

Table 1 SUVm and AUC at each vascular bed assessed

Territory	GCA patients (mean±SD)	Controls (mean±SD)	p Value	AUC (95% CI)
Ascending aorta	2.63±0.57	2.17±0.26	<0.001	0.778 (0.651 to 0.904)
Aortic arch	2.61±0.50	2.23±0.31	0.002	0.756 (0.621 to 0.891)
Descending thoracic aorta	2.78±0.65	2.39±0.33	0.007	0.739 (0.598 to 0.881)
Abdominal aorta	2.97±0.60	2.56±0.39	0.005	0.748 (0.608 to 0.888)
Right subclavian artery	2.46±0.54	2.14±0.40	0.030	0.763 (0.607 to 0.889)
Left subclavian artery	2.26±0.56	1.89±0.28	0.003	0.764 (0.610 to 0.891)
Right carotid artery	2.33±0.52	1.83±0.25	<0.001	0.812 (0.695 to 0.930)
Left carotid artery	2.32±0.51	1.97±0.30	0.004	0.733 (0.594 to 0.872)
Right axillary artery	1.21±0.31	0.88±0.17	<0.001	0.830 (0.725 to 0.940)
Left axillary artery	1.09±0.34	0.88±0.18	0.001	0.780 (0.627 to 0.886)
Right iliac artery	2.41±0.67	2.01±0.38	0.009	0.747 (0.606 to 0.888)
Left iliac artery	2.46±0.47	2.00±0.41	0.002	0.767 (0.628 to 0.905)
Right femoral artery	1.68±0.39	1.24±0.22	<0.001	0.817 (0.715 to 0.928)
Left femoral artery	1.50±0.37	1.14±0.18	<0.001	0.801 (0.679 to 0.922)
All territories*	2.15±0.37	1.79±0.17	<0.001	0.830 (0.715 to 0.946)
Aorta**	2.75±0.54	2.34±0.23	0.001	0.738 (0.612 to 0.874)
Supraaortic branches**	1.95±0.35	1.59±0.15	<0.001	0.832 (0.732 to 0.968)
Iliofemoral territory**	1.97±0.36	1.62±0.23	<0.001	0.802 (0.679 to 0.925)
Liver	2.76±0.57	2.52±0.42	0.119	0.635 (0.480 to 0.790)

Removal of the two patients who had received intravenous methylprednisolone pulses at the time of PET performance did not significantly modify the results (data not shown).
Differences in AUCs among different vascular territories did not reach statistical significance.
AUC, area under the curve; GCA, giant cell arteritis; PET, positron emission tomography.
*Values represent the mean of the SUVm observed at all the vascular beds assessed.
**Aorta, Supraaortic branches and Iliofemoral territory represents the mean of the SUVm observed at the different vessels of these areas.

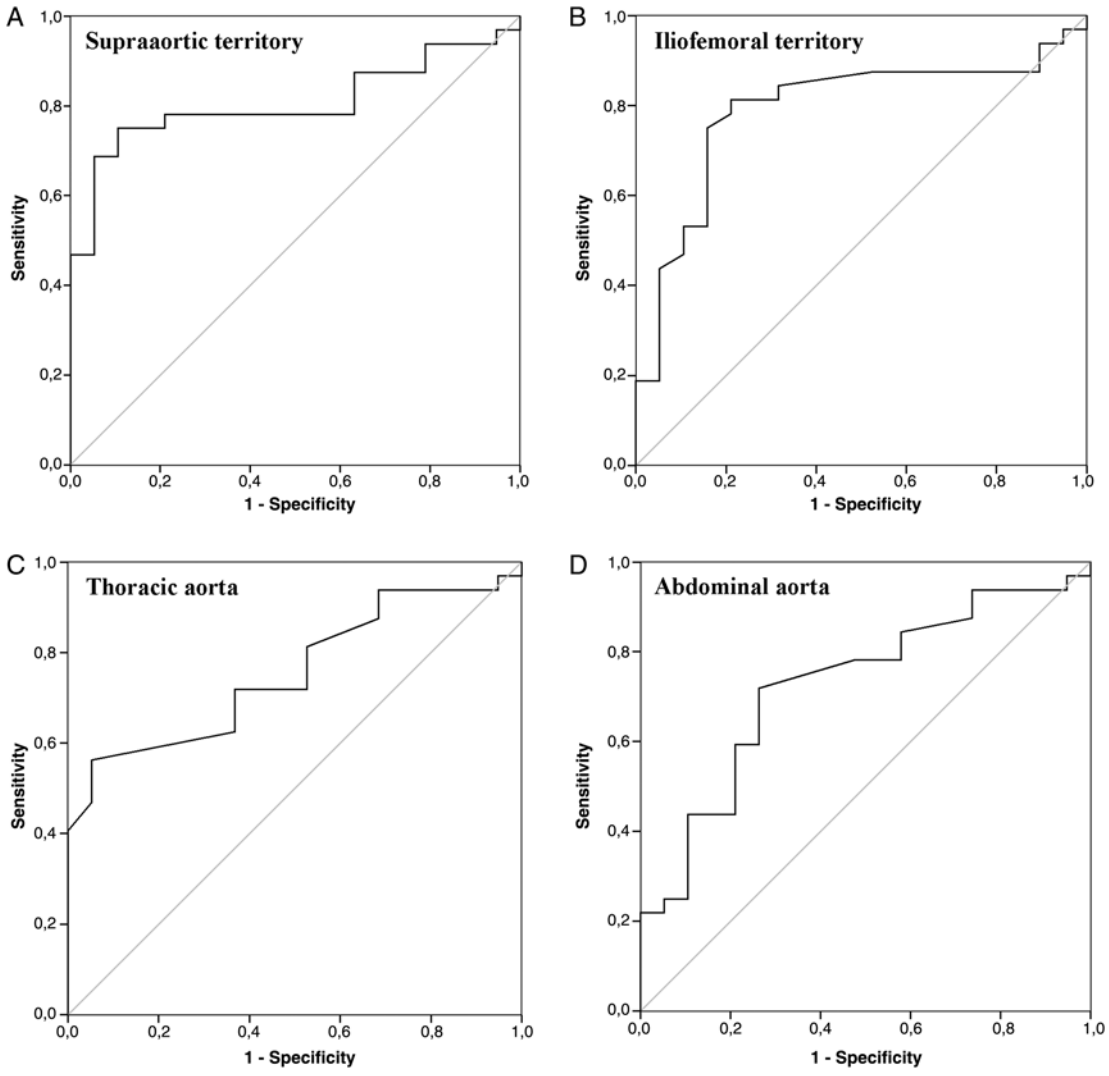

Figure 1 Receiver–operator characteristic curves of standardised uptake value at different vascular regions.

but the overall discriminatory performance was much lower (see online supplementary table S3).

Relationship between FDG uptake and clinical and laboratory findings

Patients with cranial symptoms presented significantly higher values of maximal and mean SUVm (combined average of all vascular territories) than patients lacking cranial manifestations. No relationship between the intensity of FDG uptake and other clinical findings was observed (table 2). No differences in maximal or mean SUVm were observed between treatment-naïve patients and those who had received glucocorticoids. The maximal and mean SUVm correlated with acute-phase reactants and serum IL-6 concentrations (table 2).

DISCUSSION

The present prospective study, performed in an unselected patient cohort with unequivocal GCA, shows that FDG uptake is significantly stronger in patients than in controls in all vascular territories tested, confirming the diagnostic potential of PET/CT.[4 8 9 11] PET/CT allows rapid, reproducible and broad vascular evaluation. Nevertheless, there is no standardised definition of vasculitis based on an objective FDG uptake measure, and strategies employed to establish a PET-based diagnosis of GCA has been heterogeneous. Most studies have used qualitative visual assessment or a semiquantitative score using liver uptake as a reference. Visual scoring has a remarkable investigator dependency and interobserver variability. Liver uptake is influenced by individual metabolic activity, glucocorticoid treatment and the time lapse between injection and scanning.[12]

We tried to overcome this limitation by objectively quantifying FDG uptake by different vascular beds in patients and controls and performing ROC analysis to determine the optimal cut-off for GCA diagnosis at different vascular territories. FDG uptake by supraaortic branches had the highest AUC, in accordance with a pioneer study showing that supraaortic branches were the most frequently involved when assessed by PET.[4] In this area, an FDG uptake cut-off value of 1.70 had the best sensitivity and specificity. A similar value, in the same territory but with lower performance (AUC=0.72), was reported in a retrospective study of 17 patients with GCA and 3 Takayasu arteritis patients.[13] This observation may be useful to differentiate GCA from other inflammatory aortic diseases that may produce systemic complaints and active aortic FDG uptake, including idiopathic aortitis, periaortitis, IgG4 disease and severe atherosclerosis.[10 14–17] This is crucial since a positive PET/CT may be accepted in the near future as a diagnostic criterion and is currently accepted as such in an ongoing clinical trial with tocilizumab in GCA.[18]

Table 2 Relationship between clinical and laboratory data and maximal SUV at any vascular territory (SUVm) and mean of the SUVm obtained at every vascular bed assessed (mean SUVm).

	Maximal SUVm		Mean SUVm	
		p Value		p Value
Cranial symptoms (P/A)	3.21±0.65/2.50±0.52	0.021	2.24±0.32/1.77±0.36	0.004
Systemic symptoms (P/A)	3.12±0.61/2.98±0.82	0.589	2.20±0.35/2.07±0.43	0.354
Ischaemic symptoms (P/A)	2.91±0.61/3.14±0.71	0.402	2.11±0.39/2.17±0.37	0.708
PMR (P/A)	3.11±0.62/3.06±0.71	0.886	2.27±0.35/2.12±0.38	0.321
GC treatment (Y/N)	2.97±0.44/3.20±0.81	0.385	2.14±0.36/2.17±0.40	0.858
CRP, mg/dL	r=0.551	0.001	r=0.476	0.034
ESR, mm/h	r=0.442	0.011	r=0.335	0.050
Haptoglobin, mg/dL	r=0.585	0.008	r=0.358	0.050
IL-6, pg/mL	r=0.616	0.002	r=0.544	0.007

Values are mean±SD.
Removal of the two patients who had received intravenous methylprednisolone pulses at the time of PET performance did not substantially modify the results (data not shown).
A, absence; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; F, female; GC, glucocorticoid; IL-6, interleukin 6; M, male; N, no; NS, not significant; P, presence; PET, positron emission tomography; PMR, polymyalgia rheumatica; SUV, standardized uptake value; Y, yes.

The sensitivity and specificity of PET/CT obtained in this study is close to that calculated in a recent systematic review/meta-analysis of heterogeneous, mostly retrospective studies, and those reported in a retrospective analysis evaluating the impact of PET on the management of patients with suspected large-vessel vasculitis.[19]

Interestingly, FDG uptake by the aorta showed a lower AUC, being worse in the abdominal segment where atherosclerosis is more prevalent in the general population. This fact highlights the diagnostic limitation of PET in this territory since aortic FDG uptake may be markedly influenced by ageing or atheroma plaques. Hautzel *et al* reported a higher sensitivity and specificity of thoracic aorta FDG uptake to detect large-vessel inflammation in a cohort of 18 patients with GCA.[20] A thoracic aorta/liver ratio of 1.0 had a sensitivity and specificity of 88% and 93%, respectively (AUC = 0.932). However, a substantial proportion of the patients assembled in this cohort were selected on the basis of previously known large-vessel involvement demonstrated by other techniques. In our study, direct, territory-focused comparison of SUVm between patients and controls discriminated better than vascular/liver ratios.

A retrospective study evaluating how PET/CT results influenced management of patients with suspected GCA suggested that previous glucocorticoid (GC) treatment decreased the diagnostic yield of PET/CT.[19] Sequential assessments have demonstrated, indeed, that FDG uptake decreases after 3 months of treatment.[4] The present study suggests that a short course of therapy (≤3 days) may not substantially reduce the diagnostic accuracy of PET/CT.

In conclusion, this study provides sensitive and specific, territory-focused cut-off values to detect vascular inflammation by PET/CT. A limitation of the study is that while patients were prospectively recruited, controls were retrospectively selected. Another limitation is the relatively small number of patients analysed, although our cohort is among the largest investigated. Further prospective studies using objective cut-offs are necessary to confirm their diagnostic performance in patients with suspected GCA.

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Contributors MCC had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study design: SP-G, FL and MCC. Acquisition of data: SP-G, MD, AG-M, GE-F, IT-B, FL, MC-B, EP-R, MAA, JMG, JH-R and MCC. Analysis and interpretation of data: SP-G, MD, GE-F, FL, JH-R and MCC. Manuscript preparation: SP-G, FL, JH-R, JMG and MCC. Statistical analysis: SP-G, MAA, MCC.

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Authors' response to the eLetter by Moiseev *et al*

We thank Moiseev and colleagues for their interest in our manuscript addressing the sensitivity and specificity of positron emission tomography (PET)/CT with ^{18}fluorodeoxyglucose (FDG) for detecting large-vessel inflammation in patients with giant-cell arteritis (GCA)[1] and for sharing their own data.[2] We totally agree with the authors that PET-CT may be a useful diagnostic tool for GCA although it may be still premature to state that PET/CT *should be* an independent diagnostic procedure. As mentioned in our article,[1] one of the most important limitations in considering PET/CT as a routine diagnostic procedure is the lack of a precise definition of what is a positive or negative PET/CT. While cases with strong or no FDG uptake may be respectively considered unequivocally positive or negative, there is no validated maximal standardised uptake value (SUVm) threshold to define large-vessel inflammation. Inter-observer variability may be, then, an important limitation. In our study, we tried to contribute some evidence to the definition of vessel inflammation by PET/CT providing sensitivity and specificity for SUVm at different vascular territories but this needs to be confirmed in larger studies. Moreover, the diagnostic potential of PET/CT has not been formally validated against a referral standard. The identification of a referral standard is in itself difficult. As Moiseev and colleagues point out,[2] PET/CT may detect large-vessel involvement in patients with a negative temporal artery biopsy and, therefore, a positive biopsy may not be the only comparator when assessing the diagnostic performance of PET/CT. Regarding existing classification criteria, many investigators are currently dissatisfied with the 1990 American College of Rheumatology (ACR) classification criteria[3] which, in fact, are not diagnostic criteria and do not perform appropriately as such.[4] Current international collaborative efforts are ongoing to establish updated classification and diagnostic criteria and the contribution of the various imaging procedures to diagnosis.[5]

In addition to the intensity of FDG uptake, another important clue is the anatomic distribution of abnormal uptake. Involvement of the supra-aortic branches, as also confirmed by Moiseev *et al*,[2] seems to be relevant. Increased uptake restricted to the aorta may have more limited specificity since it may occur in the setting of severe atherosclerosis, periaortitis, isolated idiopathic aortitis or IgG4-related disease.[6 7]

The authors raise another relevant issue regarding the potential use of PET/CT for evaluating disease activity and guiding therapeutic decisions. Although appealing, there is even more uncertainty surrounding this concept. On the one hand, there is not enough evidence at present to support the use of PET/CT for this purpose and studies exploring correlation between persistence of FDG uptake and clinical outcomes are necessary, particularly considering that currently available treatments are not harmless. Blockmans *et al*[8] performed serial PET in 35 patients with GCA and found that FDG uptake, visually measured with a semiquantitative score, decreased after 3 months of glucocorticoid treatment with no further decrease at 6 months. It is unclear to which extent the remaining FDG uptake represents persistent inflammatory activity or vascular remodelling, a process also requiring metabolic activity. In fact, increased expression of myointimal cell growth factors is observed in vascular tissue from treated patients.[9]

In conclusion, PET-CT emerges as a relevant tool for the diagnosis of GCA that needs to be validated. Moreover, its role in the evaluation of disease activity and response to treatment needs to be explored. Prospective multi-centre studies with large patient cohorts may help to provide answers to these interesting questions.

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Central Nervous System Vasculitis: Still More Questions than Answers

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Abstract: The central nervous system (CNS) may be involved by a variety of inflammatory diseases of blood vessels. These include primary angiitis of the central nervous system (PACNS), a rare disorder specifically targeting the CNS vasculature, and the systemic vasculitides which may affect the CNS among other organs and systems. Both situations are severe and convey a guarded prognosis. PACNS usually presents with headache and cognitive impairment. Focal symptoms are infrequent at disease onset but are common in more advanced stages. The diagnosis of PACNS is difficult because, although magnetic resonance imaging is almost invariably abnormal, findings are non specific. Angiography has limited sensitivity and specificity. Brain and leptomeningeal biopsy may provide a definitive diagnosis when disclosing blood vessel inflammation and are also useful to exclude other conditions presenting with similar findings. However, since lesions are segmental, a normal biopsy does not completely exclude PACNS. Secondary CNS involvement by systemic vasculitis occurs in less than one fifth of patients but may be devastating. A prompt recognition and aggressive treatment is crucial to avoid permanent damage and dysfunction. Glucocorticoids and cyclophosphamide are recommended for patients with PACNS and for patients with secondary CNS involvement by small-medium-sized systemic vasculitis. CNS involvement in large-vessel vasculitis is usually managed with high-dose glucocorticoids (giant-cell arteritis) or glucocorticoids and immunosuppressive agents (Takayasu's disease). However, in large vessel vasculitis, where CNS symptoms are usually due to involvement of extracranial arteries (Takayasu's disease) or proximal portions of intracranial arteries (giant-cell arteritis), revascularization procedures may also have an important role.

Keywords: Vasculitis, Central nervous system.

1. INTRODUCTION

The central nervous system (CNS) vasculature may be targeted by an heterogeneous group of inflammatory diseases. In its isolated, primary form, angiitis of the CNS (PACNS) is a rare form of vasculitis of unknown etiology primarily affecting small and medium sized vessels supplying the brain parenchyma, spinal cord and leptomeninges [1-3]. PACNS results in signs and symptoms of CNS dysfunction with no clinically apparent participation of other organs. The CNS may also be targeted, among other territories, by systemic vasculitides [4, 5]. This review will focus on diagnostic and therapeutic aspects of PACNS and secondary CNS involvement by systemic vasculitides in adulthood. Primary and secondary CNS vasculitis in childhood have been addressed in excellent recent reviews [6-8].

2. PRIMARY CNS VASCULITIS

2.1. Epidemiology

Because of the rarity of PACNS and the absence of definitive diagnostic tests, epidemiologic studies are virtually inexistent. An annual incidence of 2.4 per million people has been recently estimated in North America [9]. PACNS has been reported in children [6-8] and in the elderly. However, it appears to be more frequent in males in their fourth and fifth decades of life [2, 9]. PACNS may represent 1.2% of vasculitis involving the CNS [3].

2.2. Pathogenesis

The pathogenesis of PACNS is unknown. Similar to other chronic inflammatory or autoimmune diseases, PACNS is thought to be triggered by infection. Cytomegalovirus, Ebstein-Barr virus, varicella-zoster virus, human immunodeficiency virus, mycoplasma and chlamydia have been considered given the ability of these agents to produce vasculitic lesions [10-15]. However, in the majority of patients with PACNS a potential relationship with these or other infectious agents cannot be demonstrated.

The granulomatous nature of the vascular inflammatory lesions in most cases suggests a Th1-mediated response [3, 16]. Th1-related cytokines may promote vascular inflammation in PACNS as suggested by several experimental models. Intracerebral injections of interferon-gamma have been shown to trigger inflammatory lesions and vasculitis in rats. [17]. Tumor necrosis factor (TNF) and interleukin-6 pro-inflammatory functions may also contribute to vascular inflammation in PACNS [18, 19]. TNF/TNF receptor p75 transgenic mice develop multifocal CNS ischemic injury secondary to vasculitis [18]. Elevated CSF IL-6 has been found in 3 patients with different types of vasculitis (polyarteritis nodosa, temporal arteritis and Behcet's disease) involving the CNS [19]. Current knowledge of the pathophysi-

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ology of PACNS is very limited delaying progress in the diagnosis and management of affected patients.

2.3. Pathology

PACNS typically involves small-medium sized arteries and veins, especially those located in leptomeninges and subcortical areas. The characteristic histopathologic findings consist of inflammatory infiltration of vessel walls by T lymphocytes and activated macrophages which undergo granulomatous differentiation with giant-cell formation [3, 16]. Inflammatory cells infiltrate the adventitia and subsequently progress through the artery wall causing fragmentation of the internal elastic lamina. Intimal proliferation and fibrosis leading to vascular occlusion is frequently observed [3, 16] (Fig. **1**). This granulomatous pattern is the most commonly seen and led to the previously used term granulomatous angiitis of the CNS [3, 16, 20]. However, granulomatous features may not be always observed and some specimens disclose the so-called atypical CNS angiitis patterns consisting in predominantly lymphocytic infiltrates (lymphocytic pattern), necrotizing vasculitis with fibrinoid necrosis (necrotizing pattern) or mixed patterns [20]. In some cases, B lymphocytes and plasma cells can also be observed [21]. Vascular β amyloid deposits may be found in a subset of patients [20].

Although most patients with PACNS present primarily with CNS dysfunction, necropsy studies may disclose clinically asymptomatic vasculitis in additional locations including lungs, kidneys and gastrointestinal tract [3, 5, 16]. Distinction from systemic vasculitis with prominent CNS involvement may be sometimes difficult to establish.

2.4. Clinical Manifestations

Depending on the areas of the brain involved, PACNS may convey a wide variety of clinical findings. Moreover, disease severity and rapidity of progression may be highly variable among patients, increasing heterogeneity in clinical presentation.

In the largest series reported including 101 patients [9], the median age at diagnosis was 47 years (range 17-84 years). The majority of patients presented with subacute manifestations of diffuse CNS dysfunction. Acute presentation was highly unusual. The most common initial symptoms were headache (63%) and cognitive impairment (50%). Headaches were initially of low intensity and progressively worsened. Cognitive impairment was also insidious. Focal symptoms usually appeared later in the course of the disease and included hemiparesis (44%), stroke (40%), aphasia (28%), transient ischemic attack (28%), ataxia (19%), seizures (16%), dysarthria (15%) and blurred vision or decreased visual acuity (11%). Infrequent manifestations, occurring in less than 10% of patients, included intracranial hemorrhage, amnesic syndrome, spinal cord manifestations such as paraparesis o quadriparesis, parkinsonism, vertigo, dizziness or cranial nerve palsy. Most patients had multiple manifestations. Other published series report similar findings [22, 23].

In order to facilitate clinical recognition and early diagnosis, clinical manifestations have been grouped in three major phenotypes: 1) Acute or more commonly subacute encephalopathy, presenting as a confusional syndrome with progression to stupor and coma; 2) Disease presentation resembling atypical multiple sclerosis with a variety of focal symptoms such as optic neuropathy, brain stem episodes, seizures, headaches, encephalopathic episodes or hemispheric stroke-like events and 3) Intracranial mass lesions, with headache, drowsiness, focal signs and elevated intracranial pressure [24, 25].

It has also been suggested that predominant involvement of small versus medium-sized vessel may influence disease presentation. Small-vessel PACNS manifests as a subacute or acute encephalopathy with persistent headaches, cognitive impairment, confusion, and seizures. MRI usually discloses marked meningeal contrast enhancement whereas angiography may not reveal changes because the affected vessels are small, beyond the detection threshold [26, 27]. This form of PACNS may respond to glucocorticoid monotherapy but 25% of patients relapse. In contrast, when medium-size vessels are involved, in addition to headaches and general CNS dysfunction, focal neurologic deficits and stroke are more common and angiography is more likely to reveal vascular abnormalities [9, 26, 27]. Four clinical features are associated with an increased mortality in patients with PACNS: focal neurological deficit, cognitive impairment, cerebral infarction and involvement of larger vessels [9].

General symptoms and findings suggesting some extent of systemic involvement may occur. Fever, weight loss, *livedo reticularis*, rash, peripheral neuropathy, arthritis and night sweats may be recorded in 20% of patients [2, 9].

2. 5. Diagnosis

The diagnosis of PACNS is a challenge because of the lack of highly sensitive and specific diagnostic tests. Clinical, analytical, neuroimaging, and histopathologic data are important, both in supporting the diagnostic suspicion and in excluding other conditions which may present with similar features.

2.5.1. Laboratory Test Abnormalities

Routine laboratory tests are frequently within the normal range [2, 9, 28]. In some patients features of systemic inflammatory response including anemia, leukocytosis and moderately increased acute phase reactants (ESR, C-reactive protein and platelet counts) can be observed [2, 9]. Laboratory tests are useful to rule out other diseases which may present with similar symptoms such as infection, systemic vasculitis, malignancy, drug abuse and hypercoagulability states [5, 28, 29].

Cerebrospinal fluid (CSF) is abnormal in 80-90% of patients [9]. Increased protein concentration is the most common finding. In a series of 101 patients, mean CSF protein concentration was 7 gr/L (range 1.5-10.3 gr/L) [9]. Pressure is increased in 50% of patients and elevated lymphocyte counts may be observed in 50-80%. CSF oligoclonal immunoglobulins may be found in up to 50% of individuals with PACNS [5, 23]. CSF pleocytosis is modest, rarely exceeding 250 cells/μL. Higher leukocyte counts and the presence of neutrophils are uncommon and, when present, should alert

for possible infection [2]. CSF analysis is useful to exclude infection and malignancy and appropriate bacterial and fungal stains, viral polymerase chain reactions, and flow cytometry studies should be performed.

2.5.2. Imaging

2.5.2.1. Magnetic Resonance Imaging (MRI) and Magnetic Resonance Angiography (MRA)

MRI is sensitive but not specific in revealing changes associated with PACNS [30]. Lesions are frequently multiple and bilateral and include parenchymal or meningeal enhancing areas, ischemic areas or infarcts in the cortex, deep white matter, or periventricular white matter (Fig. **1A**). It may also disclose hemorrhagic lesions [31, 32]. The sensitivity of MRI in biopsy-proven PACNS is very high, disclosing abnormalities in 97% of cases [22, 32-34] but abnormal findings are non specific. Diffusion weighted imaging is highly sensitive in detecting diffusion abnormalities and may be useful in patients with normal MRI [35]. MRA has limited sensitivity and is only able to disclose abnormalities in the largest intracranial vessels. The same limitations apply to CT-angiography [33, 34].

2.5.2.2. Conventional Angiography

Conventional angiography is the most specific imaging technique for the diagnosis of PACNS and, compared to MRA is able to detect abnormalities in smaller vessels. Typical angiographic features of PACNS include multiple "beading" or segmental narrowing in large, intermediate, or small arteries with interposed regions of ectasia or normal luminal architecture [31-33] (Fig. **1D**). Beading may be smooth or irregular and typically occurs bilaterally. Additional changes include aneurysms, collateral flow, isolated areas of vessel narrowing in multiple branches, circumferential or eccentric vessel irregularities, multiple occlusions with sharp cutoffs, and apparently avascular mass lesions [31-33].

Although findings from CNS conventional angiograms may support the diagnosis of PACNS and can be used to direct the site of biopsy, none of these findings alone is diagnostic because similar images can be present in other diseases (Tables **1** and **2**) [2, 5, 22, 28, 36-38].

Although essential for diagnosis, angiography has limited sensitivity and specificity. Patients with biopsy-proven PACNS may have normal appearing angiograms and, conversely, biopsies of angiographically abnormal vessels have been reported as normal [2, 5, 28]. The sensitivity of angiography in detecting PACNS ranges from 20% to 90% [1, 9, 31, 35, 37, 38] and specificity from 20 to 60% [1, 9, 31, 34]. The sensitivity of cerebral angiography decreases along with the caliber of the involved vessels, being most sensitive for involvement of large-medium sized vessels. Angiography is not exempt of side effects. About 0.8% of patients subjected to angiography experience additional neurologic deficits as an adverse event related to the procedure [32]. However, given the severity of PACNS and the difficulties in achieving an accurate diagnosis, the risk/ benefit is acceptable and conventional angiography is recommended as a key diagnostic procedure.

2.5.2.3. Histopathologic Examination

Brain biopsy is considered the gold standard for the diagnosis of PACNS but reveals diagnostic histopathologic abnormalities in only 50% to 75% of cases [1] (Fig. **1B** and **C**). The role of brain biopsy in PACNS is not limited to proving inflammation of blood vessels: it is also important to excluding other conditions such as infection, malignancy, or degenerative diseases for which completely different treatment approaches are required (Table **1**) [5, 27].

In the largest series of PACNS patients undergoing surgical biopsy, including 43 patients, diagnostic sensitivity of brain biopsy was 63% [20]. In this series, the distribution of the various morphologic patterns was as follows: acute necrotizing (14%), purely lymphocytic (28%) and granulomatous (58%), with no statistically significant differences in disease aggressiveness or response to treatment among them. Interestingly, 78% of the biopsies directed to an imaging abnormality were diagnostic, whereas none of the blind biopsies demonstrated vasculitis. Biopsies including leptomeninges were slightly more sensitive in detecting vasculitis than those not including it (58% vs. 40%). In accordance with these results other authors have reported a sensitivity of brain biopsy around 50% [2, 16]. The high proportion of negative biopsies in patients with clinical and radiographic features

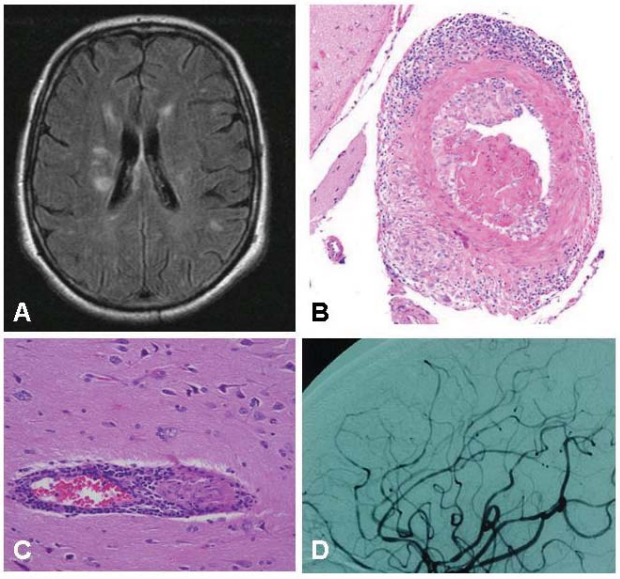

Fig. (1A). Multiple, non-specific, T2 hyperintense lesions in a 63-year old patient with suspected primary angiitis of the CNS who presented with headache and cognitive impairment. **B**) Granulomatous pattern of primary angiitis of the central nervous system. Transmural inflammation involves a muscular artery of the leptomeninges with prominent mononuclear (upper) and granulomatous (lower) adventitial inflammation as well as intimal injury with focal fibrin thrombus formation (hematoxylin and eosin 20×). Courtesy of Dr Carlo Salvarani. **C**) Inflammatory involvement of a small vessel. Courtesy of Dr Leonard H Calabrese. **D**) Multiple areas of irregular stenosis and ectasia in a 44year-old patient with biopsy-proven PACNS. Courtesy of Dr Leonard H Calabrese.

Table 1. Mimics of Primary Angiitis of the Central Nervous System

INFECTIOUS VASCULITIS
Viral (HIV, varicella zoster, progressive multifocal leukoencephalopathy)
Borreliosis
Tuberculosis
Syphilis
Whipple's disease
Endocarditis
INFLAMMATORY DISEASES
Systemic Vasculitis
Behçet's disease
Neurosarcoidosis
Systemic lupus erythematosus
NON-INFLAMMATORY VASCULOPATHIES
Reversible vasoconstriction syndromes (RVCS)
Atherosclerosis
Susac's syndrome
Radiation vasculopathy
Ehlers-Danlos disease
Kohlmeyer- Degos disease
Fibromuscular dysplasia
Fabry's disease
Moya-moya disease
Amyloid angiopathy
CADASIL
Pseudoxanthoma elasticum
Mitochondrial diseases (MELAS)
DEMYELINATING DISEASES
Multiple sclerosis
Acute disseminated encephalomyelitis
THROMBOEMBOLIC DISEASES
Antiphospholipid syndrome
Hypercoagulability states
Cholesterol embolisms
Cardiac myxoma
Nonbacterial thrombotic endocarditis
MALIGNANCIES
Multifocal glioma
CNS lymphoma
Angiocentric lymphoma
Intravascular lymphoma (malignant angioendotheliomatosis)

highly suggestive of PACNS may be explained by the segmental nature of lesions. Moreover biopsies are usually taken from the superficial parenchyma and leptomeninges and, in some instances, involved vessels are of greater size and are located deeper from these areas [20]. To maximize the diagnostic sensitivity of the procedure it is recommended that biopsies are performed in abnormal areas detected by previous imaging and include leptomeninges. Stereotactic biopsy is recommended for mass lesions only [20, 25].

Occasionally, amyloid deposits can be observed [20, 25]. These are more frequently found in samples with a granulomatous pattern and those presenting as mass lesions [20, 25]. Clinically, patients with amyloid deposits are older and more frequently presenting with acute onset and cognitive impairment [39]. Clinical outcome and response to treatment seems to be similar to that of patients with no amyloid deposits [39].

2.5.2.4. Diagnostic Criteria

Since histopathologic confirmation of PACNS is not always feasible, Calabrese and Mallek proposed a series of diagnostic criteria combining, clinical, imaging and histopathologic findings [1]. These include: 1) neurologic deficit that remains unexplained after a vigorous diagnostic workup, including lumbar puncture and neuroimaging studies, 2) angiographic abnormalities highly suggestive of vasculitis or histopathologic evidence of vasculitis within the CNS and 3) no evidence of systemic vasculitis or any other condition to which the angiographic or pathologic findings can be attributed. These conditions are listed in Table **1** (Fig. **2**).

2.5.2.5. Treatment

No randomized controlled trials or prospective studies have been performed with patients with PACNS. Therefore, therapeutic recommendations are based on extrapolation of data obtained from trials performed in other severe systemic vasculitides, retrospective studies, small case series and expert opinion [2, 5, 40]. In a retrospective review of treatments received by 101 patients diagnosed with PACNS (70 by angiography, 31 by biopsy) Salvarani *et al.* found that 97 patients were treated with glucocorticosteroids, 25 of them with 1gr intravenous methyl-prednisolone pulses and the remaining with oral prednisone at a median dose of 60 mg/day [9]. Forty-nine patients received an immunosuppressive agent: 46 cyclophosphamide (oral at 150 mg/day or intravenous at around 1 gr/month) and 3 azathioprine. A favorable response was observed in 81% of the patients treated with glucocorticoids alone and in 81% of those receiving both prednisone and cyclophosphamide. Given the retrospective nature of the survey it is not possible to conclude that immunosuppressive agents are not necessary since the group receiving cyclophosphamide may have been considered more severe by treating physicians.

Treatment with glucocorticoids (oral prednisone or equivalent at 60 mg/day preceded by three 1 gr intravenous pulses in severe cases) should, then, be started as soon as CNS vasculitis (primary or secondary) is clinically suspected and infectious diseases reasonably excluded. Prednisone can be quickly tapered if the diagnosis is eventually ruled out. When the diagnosis of CNS vasculitis is also supported by angiography or biopsy and mimics are convincingly excluded, cyclophosphamide (oral at 150 mg/day or 1gr monthly pulse) is recommended. Pulse intravenous cyclo-

Table 2. Clinical, Laboratory, Imaging and Histopathologic Characteristics Useful to Distinguish RVCS from PACNS

	RVCS	**PACNS**
Clinical data		
age	20-40 years	40-60 years
gender	primarily women	more frequent in men
trigger (drugs, postpartum etc)	frequently identified	absent
headache	acute and severe	insidious
cognitive impairment	unusual	frequent
CSF	Normal or minimal protein increase	Abnormal (increased protein concentration and mild pleocytosis)
MRI	Normal (>70%) *	Abnormal in 90%. Small infarcts in grey and white matter in multiple vascular territories, diffuse white matter lesions, mass lesions
Angiography	Abnormal:diffuse areas of multiple stenoses and dilatations **	May be normal Single or multiple abnormalities (cut-offs, lumen irregularities, avascular mass lesion)
CNS / leptomeningeal biopsy	Normal	Vasculitis

* Except when complicated by stroke, intraparenchymal or cortical subarachnoid hemorrhage or posterior reversible leukoencephalopathy.
** Angiographic abnormalities are required for diagnosis but must be reversible in 6-12 weeks.

phosphamide has equivalent efficacy in inducing remission but it is less toxic than daily oral cyclophosphamide in systemic vasculitis [40]. By analogy to severe systemic vasculitis, switch to a safer immunosuppressive agent (azathioprine, methotrexate or mycophenolate) may be considered after 4-6 months of cyclophosphamide treatment [40-43]. All patients should be given calcium and vitamin D, bone protection agents and *Pneumocystis* infection prophylaxis [5].

Recently it has been shown that rituximab is equally effective than cyclophosphamide in inducing remission in severe ANCA-associated systemic vasculitis [44, 45]. Rituximab has also been successful in treating SLE patients with CNS involvement [46], but there is no experience with rituximab in PACNS. Two glucocorticoid and cyclophosphamide refractory cases responding to TNF blockade have been reported [47].

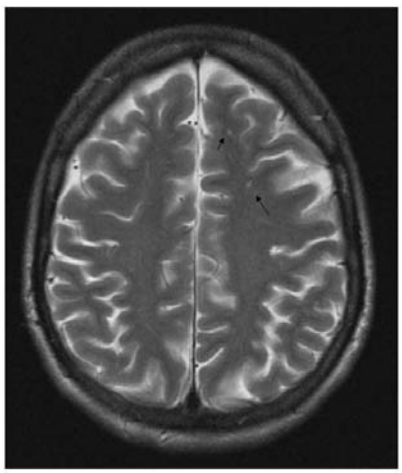

Fig. (2). Puntiform T2 hyperintense white matter lesions in a 40-year old woman with Susac's syndrome. This patient also had sensorineural hypoacusia and bilateral retinal artery branch occlusions as part of the syndrome.

Immunossuppressive treatment should be maintained for 2-3 years [2, 5]. It is important to keep in mind that about 25% of patients may relapse [9]. Response to treatment must be monitored by periodic neurologic evaluation and serial MRI examination every 3-4 months [2, 28].

3. REVERSIBLE CEREBRAL VASOCONSTRICTION SYNDROME (RCSV)

RCVS is a recently proposed term to describe the physiopathologic substrate of a group of conditions characterized by prolonged but reversible vasoconstriction of the cerebral arteries [48]. Previously, these syndromes were referred as benign angiopathy of the central nervous system and, for many years, there has not been a clear distinction between RCVS and true primary angiitis of the CNS. RCVS has received a variety of names: Call-Fleming syndrome, thunderclap headache with reversible vasospasm, migrainous vasospasm or migraine angiitis, postpartum angiopathy, or drug-induced cerebral arteritis or angiopathy [48].

RCVS may occur spontaneously but in most instances is associated with precipitating factors including the use of vasoactive substances (i.e. ergotamine derivatives, amphetamines and nasal decongestants) other drugs (i.e selective serotonin-reuptake inhibitors, contraceptives), recreational drugs (cannabis, ecstasy, LSD, cocaine, alcohol), late pregnancy or puerperium, sexual intercourse, and catecholamine producing tumors [48-50]. The most characteristic initial clinical manifestation include hyperacute severe and recurrent headache that can be associated with neurologic symptoms and signs [48]. Headache is usually diffuse although may be also localized, preferentially in the occipital

area, and may be associated with nausea, vomiting and photosensitivity. Other clinical manifestations include visual dysfunction, transient ischemic attacks and seizures [48]. The major complication of RCVS is stroke that can eventually lead to permanent sequelae and even death [48, 49]. Although the pathophysiology of RCVS is not known, the prevailing hypothesis considers that there is a transient disturbance in the control of cerebral vascular tone [48].

In the largest series reported including 67 patients [49], there was a female predominance (67%) with a mean age at diagnosis of 42.5±11.8 years (range 19-70 years). Precipitating factors were identified in 63%, being the use of vasoactive substances the most frequent (55%). The presenting symptom in all cases was recent severe headache, and this was the only symptom in 76%. Among the 67 patients, 94% had multiple thunderclap headaches (mean of 4.5 episodes) that recurred over a mean period of 1 week. In this series, early complications (within the first week) included cortical subarachnoid hemorrhage (22%), reversible posterior leukoencephalopathy (9%), intrecerebral bleeding (6%) and seizures (3%). Delayed complications (after the first week) included transient ischemic attack in 16% and cerebral infarcts in 4%. The overall outcome in this series was good, with no relapses during a 16±12.4 month follow-up period and only 4% of patients had persistent neurological deficits.

In the absence of validated diagnostic criteria, Calabrese *et al.* [48] proposed a set of key elements required for the diagnosis of RCVS. These include severe, acute headaches, with or without additional neurologic signs or symptoms, normal or near to normal cerebrospinal fluid analysis, neuroimaging tests (transfemoral angiography, CT angiography or MRA) documenting multifocal segmental cerebral artery vasoconstriction, with no evidence for aneurysmal subarachnoid hemorrhage, and reversibility of angiographic abnormalities within 12 weeks [47-49]. Treatment usually consists of calcium-channel blockers [48-51] and brief glucocorticoid courses [50, 52].

The distinction of PACNS and RVCS is important because of the different prognosis and treatment requirements. Key elements for distinction have been proposed [2, 48] and are summarized in Table **2**. PACNS typically affects middle-aged men whereas RVCS is primarily a disease of women between 20-40 years. In the latter almost 60% of patients report a precipitating event [48], usually exposure to vasoactive substances. Headache in PACNS is indolent and progressive [9] whereas headache in RVCS is acute and severe [2, 48, 49]. Unless complicated by bleeding or infarct, MRI does not disclose major changes in RVCS whereas MRI is abnormal in 97% of cases with PACNS [9, 50]. By definition, angiographic abnormalities substantially or completely reverse within approximately 3 months.

4. SYSTEMIC VASCULITIDES INVOLVING THE CNS

The CNS vasculature can be targeted by systemic vasculitis (Table **3**). Usually CNS involvement coexists with other clearly apparent systemic manifestations but some patients may present primarily with prominent symptoms of CNS dysfunction [4, 5, 53]. In systemic vasculitis targeting small-medium sized vessels, CNS involvement is a predictor of poor/guarded prognosis [54, 55] and is one of the factors considered to recommend aggressive treatment with cyclophosphamide in addition to high-dose steroids [40, 54, 55]. However, in large-vessel vasculitis, CNS involvement may benefit from vascular intervention procedures (angioplasty, derivative surgery), antiplatelet or anticoagulation treatment in addition to high dose glucocorticoids rather than intensification of immunossupressive therapy [56-58].

Table 3. Primary Systemic Vasculitis Most Frequently Involving the CNS in Adults

SMALL-MEDIUM VESSEL VASCULITIS(*)
Wegener's granulomatosis
Microscopic polyangiitis
Churg-Strauss syndrome
Cryoglobulinemic vasculitis
Behçet's disease
MEDIUM VESSEL VASCULITIS (**)
Polyarteritis nodosa
LARGE-VESSEL VASCULITIS
Giant-cell arteritis
Takayasu's arteritis(***)

In children, Henoch-Shönlein purpura (*) and Kawasaki disease (**) may occasionally involve the CNS

*** Neurologic complications in Takayasu's arteritis are mainly due to involvement of extacranial vessels.

4.1. CNS Involvement by Small and Medium Sized Vessel Vasculitis

Globally, cerebrospinal involvement is infrequent in small-medium size vessel vasculitis, including Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, polyarteritis nodosa, cryoglobulinemic vasculitis, and Behçet's disease. CNS involvement occurs in less than 15% of patients in most series.

4.1.1. Wegener Granulomatosis (WG)

The prevalence of CNS manifestations in WG ranges from 2.7% to 9% in large series of patients [59-61]. Neurological involvement may account through 3 major mechanisms: vasculitis involving CNS vessels, granulomatous lesions located in the brain, meninges or cranial nerves and direct extension of destructive granulomatous tissue from nasal or paranasal structures [59-62].

Cerebral vasculitis is the most frequent CNS lesion and may present with headache, visual disturbances, seizures, confusion, ischemic stroke, intracerebral or subarachnoid haemorrhage, venous thrombosis or dementia [62, 63]. Granulomatous inflammation and thickening of the duramater, pachymeningitis, may present with chronic headache, multiple cranial nerve palsies, seizures, meningeal signs, encephalopathy, proptosis, limb palsy or ataxia [62-65]. Pituitary involvement leads to central diabetes insipidus, panhypopituitarism or a combination of hormone deficien-

cies [66]. In these patients, MRI is the image technique of choice because it can reveal ischemic or hemorrhagic lesions, dural thickening, pituitary involvement or enhancement of inflamed orbital and paranasal mucosa [63]. In the case of dural involvement, tissue biopsy may disclose granulomatous pachymeningitis [66].

4.1.2. Microscopic Polyangiitis (MPA)

In a series of 85 patients, CNS involvement was present in 10 cases (11.8%) and CNS vasculitis was the cause of death of one of them [67].

There are only scattered case reports of CNS manifestations related to MPA in the literature. Multiple bilateral cerebral infarctions [68], multiple hemorrhagic infarction of the cerebral cortex caused by CNS vasculitis [69], capsular warning syndrome and subsequent stroke [70] and pachymeningitis have been occasionally reported [71, 72].

4.1.3. Churg-Strauss Syndrome (CSS)

In the largest published series of CSS patients the CNS is reported to be involved in 8% to 14% of patients [73-77].

Cerebral infarction is the most frequently reported manifestation of CNS involvement [75, 77], probably as result of cerebral vasculitis (Fig. **3**). Additional less commonly reported CNS events include intracerebral haemorrhage [78, 79] and pachymeningitis [80, 81].

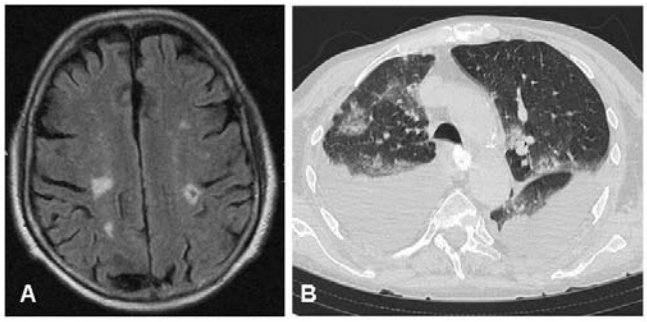

Fig. (3A). Multiple brain infarcts in a patient with Churg-Strauss syndrome. **B)** CT scan from the same patient disclosing pulmonary infiltrates and bilateral pleural effusion. Toracocentesis disclosed predominance of eosinophils in pleural fluid exudate.

4.1.4. Polyarteritis Nodosa (PAN)

In a recent series of 348 patients diagnosed with PAN over a 42-year period, 4.6% presented with central nervous system-related abnormalities [82]. Earlier studies reported a higher prevalence, between 15 and 65% [83]. Perhaps in present days, earlier recognition of the disease with prompt treatment prevents development of severe complications. It is important to remark that, widespread ANCA and cryoglobulin testing has led to re-classification of a substantial proportion of patients with necrotizing vasculitis previously diagnosed with PAN, which, in fact, has become a much more infrequent disease [84].

In an extensive literature review, three major clinical presentations related to CNS involvement have been recognized in PAN: 1) diffuse encephalopathy characterized by cognitive impairment, disorientation or psychosis (8% to 20%), 2) seizures (focal or generalized) and 3) focal neurologic deficits [83]. Accelerated hypertension may also contribute to diffuse encephalopathy in some patients [83]. Abnormal findings reported in neuroimaging studies (MRI and CTscan) include cerebral infarctions located in the brain (cortical or subcortical), cerebellum or brainstem and cerebral hemorrhages [85, 86] (Fig. **4**).

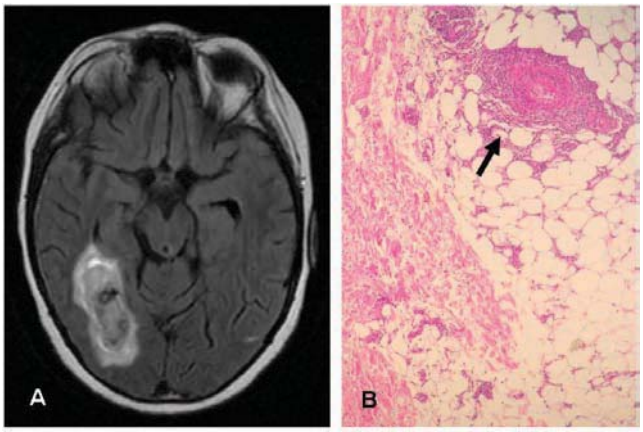

Fig. (4A). Hemorrhagic brain infarct in a patient with systemic poyarteritis nodosa. This patient also had hypertension, postprandial abdominal pain, multineuritis and livedo reticularis. **B)** Skin biopsy of the same patient disclosing necrotizing arteritis in the subcutaneous tissue.

4.1.5. Cryoglobulinemia

CNS involvement is uncommon in cryoglobulinemic vasculitis. In a retrospective series of 209 patients [87], CNS involvement was detected in 3. In a prospective study of 40 patients with mixed type II cryoglobulinemia vasculitis [88] specifically investigating signs of CNS dysfunction, 89% of the patients had some cognitive impairment, being attention the aspect most commonly altered (70.3%), followed by alterations in executive functions and visual construction. Whether these abnormalities are due to CNS vasculitis, co-morbidities, glucocorticoid, immunosuppressive or antiviral treatments or a combination of factors is unclear.

Clinical features of CNS involvement in cryoglobulinemia include encephalopathy, stroke, transient ischemic attacks, lacunar infarctions and hemorrhage [89, 90]. Most of the cases reported are associated to hepatitis C virus infection.

4.1.6. Behçet's Disease

The frequency of neurological involvement in Behçet's disease ranges from 5.3% to 14.3% in prospective studies [91, 92]. Neuro-Behçet occurs more frequently in patients aged 20 to 40 years and is 2-8 times more frequent in men than in women. Neurological manifestations commonly appear when other systemic features are present. CNS involvement is the first disease manifestation in less than 6% of patients with neuro-Behçet [93]. CNS involvement in Behçet's disease may occur through 2 major mechanisms: meningoencephalitis and vascular disease.

Meningoencephalitis is usually subacute and predominantly involves the brainstem but may extend to basal ganglia, thalamus, cortex and white matter [93, 94]. The spinal cord and cranial nerves may also be affected. In the largest series of patients with neuro-Behçet [92] the most common clinical symptoms were pyramidal signs (96%), hemiparesis (60%), behavioural changes, headache and sphincter disturbance or impotence. Less common manifestations were paraparesis, meningeal signs, movement disorders, brainstem signs, seizures, hemianopsia, aphasia, psyachiatric disturbances or cerebellar syndrome. CSF analysis was abnormal 70–80% disclosing moderately elevated protein concentration and pleocytosis with neutrophilia at early stages [89]. MRI discloses hyperintense T2 lesions with contrast enhancement and edema. Lesions are usually unilateral and are located in the upper brainstem extending towards the thalamus and basal ganglia [95]. Tumor-like lesions may occasionally occur [93].

The most common manifestation of vascular neuro-Behçet is central venous thrombosis with signs and symptoms of intracranial hypertension, including papilledema. Intracranial aneurysms and ischemic stroke may also occur but are infrequent complications. Combined parenchymal and vascular involvement may be seen in 20% of patients with neuro-Behçet [93]. Patients with neuro-Behçet are treated with high-dose glucocorticoids and cyclophosphamide. Blocking TNFα with infliximab may be useful in refractory patients.

4.2. Large Vessel Vasculitis

Both giant-cell arteritis of the elderly and Takayasu disease may convey CNS involvement.

4.2.1. Giant Cell Arteritis

GCA preferentially targets the cranial vessels. Consequently the most common ischemic complications occur in territories supplied by the carotid and vertebral arteries. Although GCA is considered a large to medium sized vessel vasculitis, small cranial vessels are frequently affected [96] and the most frequent ischemic complication, visual loss, derives from involvement of the small arteries supplying the optic nerve [97-100]. Visual loss occurs in 15-20% of patients [97-100]. In 80-90% of cases visual impairment is due to anterior ischemic optic neuritis secondary to involvement of the posterior cilliary arteries supplying the optic nerve [101, 102]. Occlusion of the retinal artery is less frequent and underlies visual loss in 10% of cases [99, 100].

Ischemic stroke or multiinfarct dementia occurs in 3-6% of patients and is due to inflammatory involvement of the intracranial branches of the carotid and vertebral arteries. [97, 100, 103, 104]. When explored, ultrasonography of the supraaortic branches are frequently normal [103, 104]. Usually, inflammation is limited to the most proximal, extradural part of these arteries. In some series, strokes are more frequent in the vertebrobasilar territories contrarily to atherosclerotic occlusions which are more frequent in the carotid branches [103]. Brain infarcts are frequently multiple, indicating involvement of various branches, reduced flow from proximal stenosis, distant embolization of proximal thrombi, or a combination of these [97, 103, 104] (Fig. **5A**). Although thrombosis is uncommonly seen in temporal artery biopsies, necropsy studies from patients dying from GCA-related stroke, frequently disclose thrombosis as a precipitating event [100]. Mortality of GCA-related stroke is about 30% [103, 104].

Stroke is more frequent among individuals with visual loss indicating that some individuals may be more prone to develop intracranial involvement and related complications [97, 98]. Several studies indicate that individuals with prominent extracranial large- vessel involvement are less prone to develop cranial ischemic complications, suggesting heterogeneity in patterns of vascular targeting by GCA [105-107]. Several studies indicate that traditional vascular risk factors are more frequent and the systemic inflammatory response is weaker in patients with GCA-related ophthalmic and neurologic ischemic complications, making early diagnosis and follow up more difficult [97-100, 108]. High dose glucocorticoids usually prevent progression of visual impairment. Intravenous methylprednisolone pulses are usually administered in this setting but there is no proof that this approach is more effective than the standard daily 60 mg dose. In 10-27% of patients presenting with visual symptoms, vision may continue to deteriorate during the first 1-2 weeks after the beginning of glucocorticoid treatment [99]. Antiplatelet or anticoagulant therapy is usually given in these circumstances with variable results [99, 101, 102]. After this initial period, the risk of developing subsequent disease-related visual loss is low, about 1% in 5 years [109].

Stroke frequently occurs during the first weeks after the initiation of glucocorticoid treatment. Besides adding antiaggregants, anticoagulants, or both, the classical approach to this situation has been intensifying glucocorticoid and immunosuppressive therapy. However, a recent report indicate that some patients with proximal lesions may better benefit from intracerebral percutaneous angioplasty [57] (Fig **5B**).

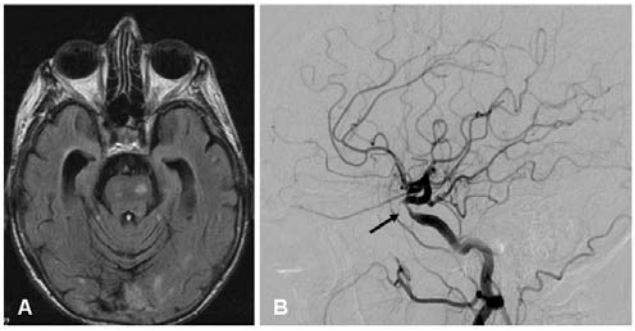

Fig. (5A). Multiple infarcts in the cerebral pons, cerebellum, and occipital lobes in a patient with biopsy-proven giant-cell arteritis who developed ataxia and cognitive impairment after the initiation of glucocorticoid therapy. **B)** Cerebral angiography displaying carotid siphon stenosis in a patient with biopsy-proven giant-cell arteritis who developed recurrent transient ischemic attacks (aphasia and hemiparesis) in spite of high-dose glucocorticoids, antiplatelet and anticoagulant therapy. This lesion was successfully treated with percutaneous transluminal angioplasty (57).

4.2.2. Takayasu Arteritis

Non specific neurologic manifestations such as headache, dizziness of variable intensity, and lightheadedness are highly frequent in patients with Takayasu's arteritis, occurring in 57-90% in most series [58, 110, 111] (Fig. **6**). More severe complications include visual disturbances or visual loss, syncope, transient ischemic attacks and stroke. Most of these symptoms/complications can be related to extracranial steno-occlusive lesions in the subclavian (with subsequent arm-steal syndrome), carotid and vertebral arteries which results in decrease brain flow [112, 113]. Stroke occurs in less than 10% of cases in large cohorts but it is among the leading causes of premature death in these patients [58]. Strokes are usually ischemic and secondary thrombosis of stenotic vessels with subsequent embolization may be precipitating events. It is important to remark that cardiomyopathy secondary to aortic valve insufficiency due to aortic root dilatation or hypertension occurs in about 10% of patients with Takayasu's disease and may also result in thromboembolic strokes [58]. Hemorrhagic stroke related to hypertension has also been reported [112].

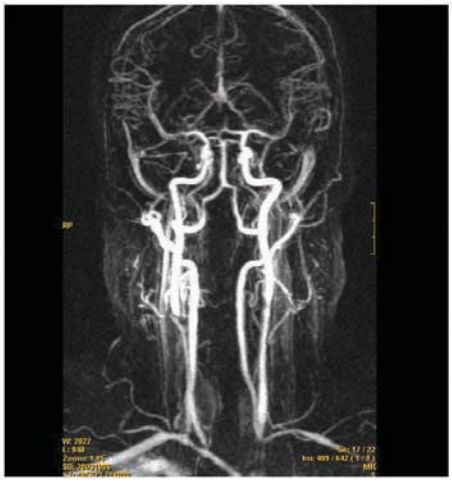

Fig. (6). Multiple stenoses in the carotid and vertebral arteries in a 38-year old patient with Takayasu disease complaining from lightheadedness and slight dizziness.

Intracranial artery involvement seems to be uncommon. A recent prospective study using ultrasonography and MRI in 17 patients with neurologic symptoms, disclosed signs of intracranial involvement in 7 patients [113]. However, no angiography was performed and it was not possible to discern whether these findings were related to vasculitis or previous embolization. Autopsy studies including the brain are scarce in Takayasu's disease, but intracranial involvement seems to be unusual. However, at least one patient with vasculitis of intracranial arteries has been reported [114].

Glucocorticoids and in most instances immunossupressive agents are mandatory to induce and maintain remission in patients with Takayasu disease. Cyclophosphamide and methotrexate have been useful in open-label studies and mycophenolate has been also tried in small case series [58, 110, 115]. Because of its side effects cyclophosphamide is usually avoided and other immunosuppressive agents are preferred, since Takayasu disease is a relapsing condition usually targeting young women [58, 110, 115]. TNF blockade has provided benefit to patients refractory to other therapies [116]. Angioplasty, stenting and by-pass surgery are very important in the management of severe neurological involvement [56, 58]. For better results, revascularization procedures, should be avoided, when possible, during periods of active disease and be performed to patients in remission [115].

CONCLUSIONS

CNS vasculitis, either primary or complicating systemic vasculitis is uncommon. However CNS involvement is a major determinant of severity, morbidity and mortality in patients with vasculitis. Diagnosis of PACNS is a challenge and requires high index of clinical suspicion. Diagnosis is supported by neuroimaging and histologic data but requires exclusion of other conditions with the appropriate work-up. Neuroimaging techniques are pivotal not only to support the diagnosis but also in the follow up of affected patients. PACNS or CNS involvement by systemic vasculitis requires prompt recognition and aggressive treatment in order to reduce mortality and preserve function.

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LETTERS

Giant cell arteritis and disseminated tuberculosis: presentation of two cases

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We report the first two cases in the English literature of giant cell arteritis (GCA) and disseminated tuberculosis (Tb). Case 1 was a 75-year-old man who presented with painful cervical masses that, on biopsy, showed chronic inflammation, granulomas, and giant cells, compatible with tuberculous lymphadenitis. A tuberculin skin test was positive. Treatment with four drugs was initiated, but discontinued because of hepatic toxicity. He did not attend our clinic until 1 year later when malaise and chronic fever had developed. An abdominal computed tomography (CT) scan showed hepatic nodular images that, on biopsy, disclosed caseum necrosis and granulomatous hepatitis with a positive polymerase chain reaction (PCR) for *Mycobacterium tuberculosis*. Treatment with gatifloxacin, clarithromycin, amikacin, and rifampicin was initiated. One month later, fever resumed, bilateral panuveitis with severe papillitis developed, and new hepatic lesions appeared. Treatment was adjusted with rifampicin, gatifloxacin, pyrazinamide, and ethambutol. All symptoms resolved and 6 months of maintenance treatment was completed. Thirteen months later, new-onset occipital headache, incoercible vomiting, fever, and polymyalgia rheumatica (PMR) developed. Disseminated central nervous system (CNS) Tb was suspected. Cerebrospinal fluid examination and cultures and a cerebral magnetic resonance imaging (MRI) scan were normal, as were blood cultures. Erythrocyte sedimentation rate (ESR) performed in triplicate was > 100 mm/h. After extensive diagnostic work-up, a temporal artery (TA) biopsy was performed showing arteritis. Prednisone (PDN) treatment rapidly improved his condition and remission was achieved. He died 3 years later because of acute myelogenous leukaemia.

Case 2 was a 72-year-old woman who presented with a 3-month history of headache, intermittent left eye pain, and scalp tenderness. Sudden left hypoacusia developed 1 month prior to acute loss of left-eye vision due to anterior ischaemic optic neuropathy. Decreased TA pulse was found in addition to an ESR of 34 mm/h. She was diagnosed as having GCA based on three American College of Rheumatology (ACR) criteria; she refused TA biopsy. High-dose intravenous methylprednisolone was initiated, followed by PDN 1 mg/kg/day. PDN treatment led to improvement. One year later, methotrexate was added due to PMR relapse, with remission achieved again. Three years later, fever, 3-kg weight loss, arthralgias, myalgias, and red-brown vaginal discharge developed. On colposcopy, an exophytic bleeding mass was seen. Biopsy revealed granulomatous cervicitis with giant cells compatible with Tb. Cultures and sputum smears were negative. A thoracic CT scan showed several mediastinal calcified lymph nodes. Anaemia, leucocytosis, and an ESR of 92 mm/h were documented. Treatment with isoniazid, rifampicin, pyrazinamide, and ethambutol was started. Because of persistent fever after 4 weeks, another CT scan was performed, showing apical fibrosis and multiple lung nodules (Figure 1) not seen in the first. After the Tb intensive treatment phase was completed, fever, vaginal discharge, and lung nodules resolved. She completed maintenance therapy uneventfully; GCA remained in remission.

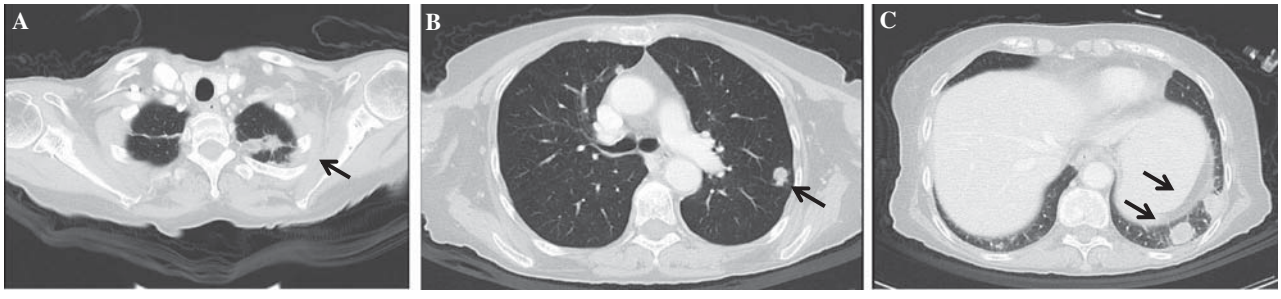

Figure 1. Chest high-resolution computed tomography (HRCT) scan showing (A) apical fibrosis, (B) bilateral lung nodules, and (C) basal bilateral nodules and ground glass opacities.

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Table 1. Differential diagnosis of fever of unknown origin (FUO) in the elderly.

Infections: tuberculosis, endocarditis, abscesses, cytomegalovirus, Epstein-Barr virus, human immunodeficiency virus, osteomyelitis, Whipple's disease
Autoimmune diseases: giant cell arteritis, polymyalgia rheumatica, rheumatoid arthritis, systemic lupus erythematosus, polyangiitis (Wegener's), polyartheritis nodosa, sarcoidosis, Crohn's disease
Malignancy: leukaemia, Hodgkin's disease, multiple myeloma, colon cancer
Miscellaneous: pulmonary embolism, thyrotoxicosis, drug-related fever, factitious

From reference (9).

These cases highlight two important teaching points: first, the influence of GCA treatment in Tb development. In GCA patients, long-term glucocorticoids (GCs) are associated with infections in 15–50% of cases (5–20% severe) (1, 2). Urinary and lower respiratory tract infections are the most frequent, occurring mainly during the first 6 months, coincidental with high-dose PDN (2). Although GCA therapy can increase the risk of serious infections, development of opportunistic infections such as *Pneumocystis jiroveci* (3) and disseminated Tb is rare (4, 5), as observed in a study of 100 GCA patients, where only one case of miliary Tb was documented (5). As for Tb, the incidence of active infection and extra-pulmonary locations is increased in rheumatic patients on moderate- to high-dose GCs treatment (6). Although screening for latent Tb in high-risk patients who will receive steroids may be desirable, this practice is not routine (7). The second point relates to the diagnostic puzzle between GCA and Tb, especially among the elderly. Both diseases can present with headache, visual and constitutional symptoms, and elevated ESR (8). Tb diagnosis is difficult because of its broad, unspecific clinical manifestations, accounting for 12–20% of cases of fever of unknown origin (FUO) in the elderly (9), the target population of GCA. GCA can also present as FUO in this group (Table 1), and Tb recurrence (especially if resistant, as in case 1) and GCA can mimic each other. Worldwide resurgence of Tb indicates that, even in developed countries, where GCA is most prevalent, this infection needs consideration either as an isolated disease or coexisting with GCA.

In summary, GCA and Tb may present with the same clinical manifestations, especially in elderly people. A high index of suspicion and an extensive diagnostic approach are necessary to exclude either of them.

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Clinical features of a new disease concept, IgG4-related thyroiditis: comments on the article by Watanabe et al

We read with great interest the recent report by Watanabe et al (1) entitled 'Clinical features of a new disease concept, IgG4-related thyroiditis'. The authors stated that thyroid lesions associated with hypothyroidism in IgG4-related disease (IgG4-RD) could be considered as a new disease, which they termed IgG4-related thyroiditis. We would like to make some comments about hypothyroidism associated with IgG4-RD.

Watanabe et al reported that the thyroid volume of patients with clinical hypothyroidism is significantly larger than that of euthyroid patients or patients with subclinical hypothyroidism (1). In one patient who

Scalp Necrosis in Giant Cell Arteritis

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An 80-year-old man presented with a 2-week history of new onset headache, jaw claudication, and scalp tenderness. Physical examination revealed scalp necrosis with cyanosis of the surrounding skin (Figure) and a pulseless, thickened left temporal artery.

Laboratory tests disclosed an elevated erythrocyte sedimentation rate and C-reactive protein concentration. Microscopic examination of the temporal artery showed characteristic findings of giant cell arteritis (Supplemental Figures 1 and 2): intimal hyperplasia with complete occlusion of the lumen, rupture of the internal elastic lamina, giant cells, and dense inflammatory infiltration of the artery wall.

Treatment with high-dose glucocorticoids resulted in prompt resolution of symptoms. Healed scalp necrosis with a residual crust was observed after 10 days of treatment (Supplemental Figure 3). Differential diagnosis of scalp necrosis includes herpes zoster, contact dermatitis, pyoderma gangrenosum, postradiation ulcers, bacterial infections, ulcerated skin tumors, and giant cell arteritis.

FIGURE. Left-side scalp necrosis.

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SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at http://www.mayoclinicproceedings.org.

B lymphocytes may play a significant role in large-vessel vasculitis

Evaluation of: Hoyer BF, Mumtaz IM, Loddenkemper K *et al.* Takayasu arteritis is characterised by disturbances of B cell homeostasis and responds to B cell depletion therapy with rituximab. *Ann. Rheum. Dis.* 71, 75–79 (2012). The novel finding of increased number of circulating, newly differentiated, plasmablasts in Takayasu arteritis and their relationship to disease activity raises interesting questions about the role of B lymphocytes in large-vessel vasculitis. Whether this observation is relevant to the pathogenesis of vascular inflammation in Takayasu arteritis or is only a biomarker of immune activation needs to be investigated. Response of a few patients to B-cell depletion therapy supports an important role of B lymphocytes in vascular inflammation, but needs to be confirmed in clinical trials.

KEYWORDS: B lymphocytes ■ giant-cell arteritis ■ inflammation ■ plasmablasts ■ Takayasu arteritis ■ treatment ■ vasculitis

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Takayasu arteritis (TAK) is a chronic granulomatous vasculitis involving the aorta and its major branches. Due to the tropism of TAK for large arteries, tissue samples are not readily available. Vascular specimens are usually obtained at the time of bypass surgery or death from chronic complications and show predominantly fibrotic changes with various degrees of chronic inflammation. However, during earlier stages of the disease, that the vessel wall is infiltrated by lymphocytes and macrophages that undergo granulomatous differentiation with typical formation of multinucleated giant cells [1]. This pattern, characteristic of a delayed-type hypersensitivity reaction, suggests the predominant participation of Th1-mediated mechanisms in the pathogenesis of vascular inflammation in TAK.

Immunopathology studies have shown that inflammatory infiltrates are mainly constituted by $CD4^+$ T cells and activated macrophages. Adaptive immune response against unknown antigens is thought to play a major role in the pathogenesis of TAK [2,3]. This concept is supported by several observations. Although some heterogeneity exists among studies performed in different geographic areas and ethnicities, genetic risk is associated with polymorphisms in the major histocompatibility complex (MHC) region [2]. Analysis of T-cell receptor Vα–Vβ gene usage shows that infiltrating lymphocytes are oligoclonal, suggesting an antigen-driven immune response [3]. Smooth muscle cells undergo apoptosis and this is thought to be driven by cytotoxic T cells [2,4]. There is evidence that γ/δ T lymphocytes and natural killer cells also contribute to cytotoxicity and several apoptosis-triggering molecules are upregulated in inflammatory infiltrates [4].

More recently, the potential participation of B cells in TAK has attracted some interest. B lymphocytes and plasma cells may be present in TAK lesions, particularly in the adventitia [1]. Antiendothelial cell antibodies can be detected in active patients. Some are addressed to annexin V and may promote endothelial cell apoptosis. The specificities of antiendothelial cell antibodies in patients with TAK appear to be heterogeneous [2]. Recently, antibodies recognizing a 62-kD protein in aortic endothelial cells have been detected in patients with TAK and are able to elicit endothelial cell proinflammatory responses and apoptosis [5]. Although endothelial injury does not seem to be the major pathogenic event in a large-vessel vasculitis,increased endothelial cell proinflammatoy activity may participate in the recruitment of inflammatory cells [6] and endothelial damage may contribute to endothelial dysfunction and premature atherosclerosis observed in TAK patients. Regardless of their precise role in vascular inflammation and injury, the presence of antiendothelial antibodies, observed by several investigators, indicates autoreactive B-cell activation.

Underlining the pathogenic potential of B cells, Hoyer *et al.* have recently reported abnormalities in circulating B-derived cell subsets in patients with TAK [7]. The authors not only found increased numbers of memory ($CD19^+$, $CD20^+$ and $CD27^+$) B cells and decreased numbers of naive ($CD19^+$, $CD20^+$ and $CD27^-$)

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B cells, but also demonstrated increased numbers of plasmablasts ($CD19^+$, $CD20^-$ and $CD27^+$) and, more specifically, activated, newly differentiated plasmablasts identified by strong expression of class II MHC antigens. The presence of newly formed plasmablasts correlated with disease activity. Although less striking, these changes resemble what the same group of investigators has previously found in patients with systemic lupus erythematosus [7]. The authors conclude that disturbance of B-cell homeostasis has a seminal role in the pathogenesis of TAK and suggest that this finding provides a strong conceptual basis for B-cell depletion or B-cell modulation therapy in TAK. The authors prove this concept by successfully treating three patients refractory to standard therapy with the anti-CD20 chimeric monoclonal antibody rituximab. Additional case reports have also shown efficacy of rituximab in inducing remission in patients with TAK [7].

The results generated by Hoyer *et al.* need to be confirmed in larger series but are challenging and intriguing. If this abnormality is seminal for disease pathogenesis, as suggested, it is noteworthy that abnormalities found in TAK are similar to those found in systemic lupus erythematosus patients, since these diseases have little in common except that they both occur primarily in young females. Perhaps these cells only reflect hyperstimulation of the immune system rather than provide clues about the pathogenic pathways specifically involved in the development of particular diseases. It would have been interesting to test other vasculitis such as ANCA-associated vasculitis, where B-cell disturbances clearly play a role, or giant-cell arteritis (GCA), a closely related large-vessel vasculitis.

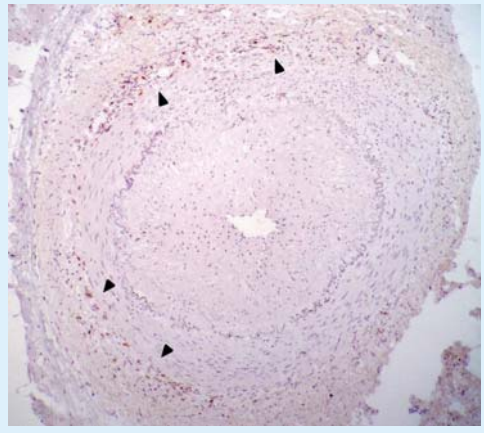

Figure 1. Temporal artery biopsy from a patient with giant-cell arteritis taken after 1 year of corticosteroid treatment.
B lymphocytes, identified by immunohistochemistry using a monoclonal antibody against CD20, can be observed in the adventitia (arrowheads).

Rituximab has no direct effect on antibody-producing plasmablasts since they do not express CD20. However, in other diseases such as systemic lupus erythematosus or rheumatoid arthritis, treatment with rituximab results in decreased circulating plasmablasts by targeting their $CD20^+$ precursors [7,8]. While changes in circulating plasmablasts, if confirmed in large series, may indeed be a biomarker of disease activity, response to rituximab and reduction in circulating plasmablasts upon rituximab treatment cannot be strictly considered a proof of concept of the relevance of circulating plasmablasts to disease pathogenesis. On the one hand, it is not clear that rituximab influences long-lived autoreactive plasma cells retained in inflammatory lesions unless other therapeutic effects reduce the inflammatory microenvironment that creates a favorable niche [8]. On the other hand, and particularly in a disease where T cells undoubtedly play a significant role, rituximab may be affecting B-cell-dependent T-cell activation. Despite limited feasibility given the nature of the vessels involved in TAK, it would have been very interesting to explore changes induced in tissue plasma cells and T-cell activation in tissue.

Another interesting question arising from this study is whether a similar abnormality may be found in patients with GCA, a disease closely related to TAK. GCA and TAK have important similarities. Both involve large vessels and lesions can be indistinguishable. It has even been hypothesized that they are the same disease and differences in phenotype are due to immunosenescence and senescence of the targeted vascular system [9]. However, there are also relevant dissimilarities between these two conditions. In addition to demographic differences, anatomical distribution is not completely alike, although it can be indistinguishable in a subset of patients. In general, GCA frequently involves small vessels in the scalp and distal branches of the ophthalmic artery, whereas TAK invariably involves the main tributaries and the aorta itself and rarely involves small vessels. TAK is primarily a stenosing disease, whereas GCA infrequently leads to symptomatic stenoses requiring re-vascularization procedures [10]. The majority of patients with TAK require adjuvant therapy, whereas most patients with GCA can be treated with glucocorticoids alone. Moreover, while open-label studies suggest efficacy of infliximab

in TAK, a randomized controlled trial did not show benefit over placebo in sustaining glucocorticoid-induced remission in GCA [11]. Although the role of B lymphocytes in GCA has been neglected [12], B cells are indeed present in lesions [13] and, more importantly, may persist after glucocorticoid treatment (Figure 1).

The results generated by Hoyer *et al.*, although preliminary, are challenging and open interesting questions about the pathogenesis of TAK and its related disease GCA. They raise the question of whether B-cell modulators such as the anti-BAFF antibody belimumab may also be useful and whether response to the IL-6 receptor blocking monoclonal antibody tocilizumab reported in case reports of recurrent/refractory TAK and GCA may be, at least partially, a consequence of B-cell modulation.

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Executive summary

- Increased numbers of circulating, newly formed, plasmablasts can be detected in patients with Takayasu arteritis and correlate with disease activity.
- Along with the presence of B cells and plasma cells in lesions observed in immunopathology studies, this finding suggests a previously under-recognized role of B cells in the pathogenesis of Takayasu arteritis.
- The potential relevance of B cells in Takayasu arteritis is supported by several case reports indicating successful treatment of refractory patients with rituximab.
- If confirmed in clinical trials, B-cell-depletion therapy might be a therapeutic option for patients with Takayasu disease refractory to standard therapies.
- An important question raised by this study is whether these findings may also be relevant to giant-cell arteritis, a closely related dirsorder.

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Capítulo 39

Biopsia de la arteria temporal

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DESCRIPCIÓN

La arteritis de células gigantes (ACG) es una vasculitis sistémica primaria que afecta a arterias de mediano y gran calibre. Es la vasculitis más frecuente en individuos mayores de 50 años.

La biopsia de la arteria temporal (BAT) es el procedimiento más eficaz (estándar de oro) para obtener el diagnóstico de la ACG, debido a su fácil acceso y a la predilección de la enfermedad por las ramas de la carótida. La intervención es sencilla, inocua, con un importante valor predictivo, mucho más eficaz y menos peligrosa que una corticoterapia prolongada a ciegas.

Es importante destacar que existen técnicas de imagen capaces de detectar alteraciones muy específicas y que pueden utilizarse como alternativa a la BAT en algunos casos. Por ejemplo el hallazgo de un halo hipoecoico concéntrico en la arteria temporal alrededor de la zona de flujo mediante ecografía doppler tiene una sensibilidad del 69% y especificidad del 82% para el diagnóstico de ACG. La sensibilidad y la especificidad de este procedimiento son inferiores a las de la BAT. Una ventaja de la ecografía es que puede explorar varios territorios arteriales ya que la arteria temporal no se afecta en el 100% de los casos.

Es importante considerar que la arteria temporal no solo se inflama en la ACG sino que ocasionalmente puede resultar afectada en otras vasculitis como la poliangiítis microscópica, la granulomatosis con poliangiítis (Wegener), la poliarteritis nudosa y la tromboangiítis obliterante, entre otras. Para la correcta clasificación de las vasculitis no solo hay que considerar datos histológicos sino también datos demográficos, clínicos y serológicos.

Tradicionalmente, han realizado la BAT especialistas de disciplinas quirúrgicas variadas (oftalmólogos, cirujanos vasculares, cirujanos plásticos, cirujanos generales, cirujanos de cabeza y cuello, neurocirujanos y dermatólogos). Sin embargo, como acto quirúrgico tiene un interés limitado y al no estar estos profesionales directamente involucrados en el diagnóstico, tratamiento y seguimiento de los pacientes con ACG pueden subestimar su importante valor diagnóstico y minimizar datos importantes como la selección cuidadosa de la zona y la longitud de la biopsia. Por ello, es importante enfatizar la importancia que supone para el médico internista saber realizar adecuadamente una BAT.

INDICACIONES

La BAT debe realizarse en todos aquellos pacientes en quienes se sospeche una ACG. El procedimiento tiene una sensibilidad del 81-85%, especificidad cercana al 100% y valor predictivo positivo de 90-94%. Sin embargo, hay un porcentaje de resultados falsos negativos en 5-40%, debido en parte, a la afección segmentaria del vaso.

El rendimiento diagnóstico de la biopsia es muy elevado en presencia de cefalea de aparición reciente, claudicación mandibular y anomalías a la exploración física de las arterias temporales aunque sean sutiles (engrosamiento, signos inflamatorios, debilidad, asimetría o ausencia de pulso). Cuando se sospecha la enfermedad pero los pacientes no presentan estos datos la probabilidad de un diagnóstico alternativo es alta y deben investigarse otras posibilidades diagnósticas antes de realizar una BAT. También puede considerarse la exploración de otros territorios vasculares con técnicas de imagen.

Es importante mencionar que la realización de la BAT no debe retrasar el inicio del tratamiento con corticosteroides en los pacientes con alta sospecha de ACG, especialmente si existen complicaciones isquémicas ya que las lesiones histológicas persisten al menos varias semanas tras el inicio del tratamiento.

CONTRAINDICACIONES

Teóricamente no existen contraindicaciones específicas para realizar una BAT. Sin embargo puede posponerse el procedimiento en caso de:

- Problemas intrínsecos o extrínsecos de la coagulación (anticoagulantes orales, deficiencia de factores de coagulación, trombocitopenia acusada).
- Incapacidad del paciente para tolerar la posición por disnea, inestabilidad hemodinámica, deterioro cognitivo o agitación. La supresión de antiagregantes en pacientes que los reciben no es imprescindible, aunque la hemostasia de la incisión cutánea suele resultar más dificultosa y requerir más tiempo.

DETALLES TÉCNICOS

Existen dos zonas donde puede realizarse una BAT: a nivel fronto-temporal y a nivel preauricular. Ésta última es útil cuando se requiere una muestra amplia, por ejemplo cuando se ha realizado una biopsia previa a nivel fronto-temporal y no ha sido diagnóstica. El procedimiento se realiza de manera ambulatoria en una sala quirúrgica o en un consultorio de intervenciones menores. El material necesario para realizar la BAT se especifica en la tabla 1.

Anatomía quirúrgica de la región temporal. Para realizar la biopsia es necesario tener un conocimiento preciso de la anatomía de la arteria temporal (AT) y sus ramas, su relación con las fascias, la vena temporal y el nervio facial.

La AT es una rama de la carótida externa cuyo origen se localiza a nivel de la parótida, posterior al cuello de la mandíbula y en relación a la raíz posterior del

TABLA 1. Material necesario para una biopsia de arteria temporal

- Mascarilla quirúrgica
- Gorros
- Batas estériles
- Guantes estériles
- Campos estériles
- Gasas estériles
- Esparadrapo o apósito adhesivo
- Rasurador
- Solución antiséptica cutánea (povidona yodada)
- Solución salina al 0,9% (50 ml)
- Anestésico local (1 ampolleta de 10 ml de mepivacaína o lidocaína al 2%)
- Una jeringa de 10 ml
- Selección de agujas:
  - Una de 20 G de 0,9 x 40 mm
  - Una de 23 G de 0,6 x 25 mm
- Hoja de bisturí número 15
- Instrumental quirúrgico:
  - Mango de bisturí
  - Cinco pinzas Kelly curvas
  - Una pinza de disección con dientes
  - Una pinza de disección sin dientes
  - Portagujas
  - Una tijera de punta aguda
  - Una tijera de punta roma
  - Sutura absorbible sintética de poliéster 2-0, con aguja curva
  - Seda negra de 2-0 con aguja curva

proceso cigomático del hueso temporal. Desde la región preauricular en dirección cefálica, la arteria se bifurca en una rama frontal y una parietal, aproximadamente 3 cm por encima del arco cigomático (figura 1). La AT se encuentra contenida en la fascia temporal superficial y se relaciona espacialmente con la rama temporal del nervio facial (caudal a la arteria) y la vena temporal (anterior a la arteria). Dada la proximidad de la arteria al nervio facial se ha delimitado una zona donde existe mayor probabilidad de lesión de este último. Los puntos de referencia de esta región de forma triangular son a) el trago del oído, b) la unión del arco cigomático y la pared lateral de la órbita y c) 2 cm por encima de la pared superior de la órbita (figura 1).

Preparación

El paso inicial consiste en localizar la arteria por medio de una exploración física detallada. Los signos de una arteria temporal patológica incluyen consistencia aumentada, disminución o ausencia del pulso y, con menor frecuencia, dolor a la palpación y aumento de la temperatura local.

En caso de que se presente alguno de estos datos debe elegirse ese lado para realizar la biopsia ya que las anomalías en la exploración física se correlacionan con un mayor índice de positividad histológica. Como se ha mencionado, puede utilizarse la ecografía doppler para guiar la biopsia.

Extracción a nivel temporo-frontal. Una vez identificada la arteria que se ha de biopsiar, se coloca al paciente en decúbito supino con la cabeza ligeramente rotada, se rasura el área y se marca el trayecto del vaso con un rotulador quirúrgico. Recomendamos trazar una línea de por lo menos 5 cm para realizar una incisión de 3-4 cm y extraer una muestra de 2-3 cm.

Posteriormente el médico se viste con bata y guantes estériles, se realiza antisepsia de la región temporal con povidona yodada o clorhexidina, y se cubre la zona alrededor del campo quirúrgico con tallas estériles. El siguiente paso consiste en infiltrar superficialmente un anestésico aproximadamente a 1 cm lateral de la arteria en ambos lados. Una vez realizado lo anterior, mediante una tracción cuidadosa se realiza una incisión con una hoja de bisturí directamente sobre la marca de la arteria. Esta incisión ha de ser superficial sin penetrar completamente en la grasa subcutánea ya que la arteria puede encontrarse justamente debajo. Es imperativo ser cuidadoso en este punto.

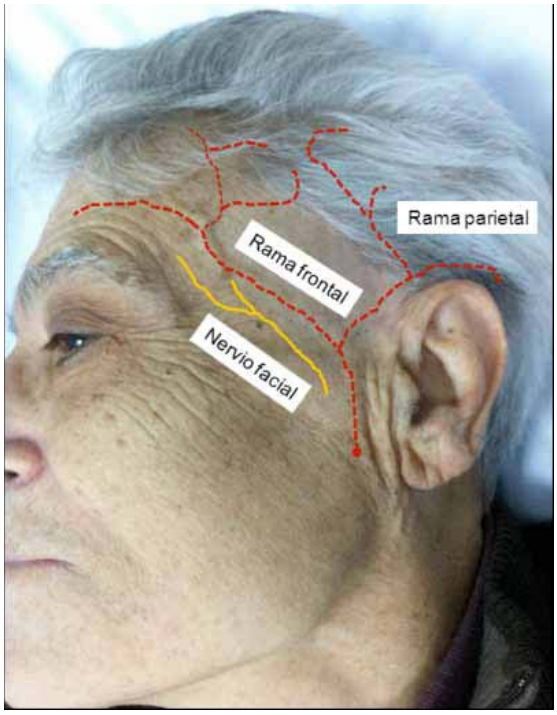

FIGURA 1. Anatomía de superficie de la arteria temporal.

Los bordes de la incisión se amplían mediante pinzas Kelly curvas, profundizando a través de la delgada capa de grasa subcutánea hasta llegar a la fascia temporal superficial (figura 2). La arteria cursa en este plano y debe disecarse delicadamente (figura 3). Dado que la arteria puede variar mucho en tortuosidad y tamaño, con diámetros entre los 1-5 mm, la palpación del suelo de la herida puede identificar el pulso de la arteria y ayudar a localizarla.

Una vez se ha disecado un segmento de 3 cm aproximadamente, los extremos de la arteria y las posibles ramas colaterales se ligan con sutura absorbible (figura 4). La arteria se corta con unas tijeras de punta aguda (figura 5) y se fija en formol al 10% para su procesamiento anatomo-patológico. El manejo cuidadoso de la arteria es esencial para evitar lesionarla y también para prevenir artefactos histológicos.

Finalmente se corrobora la hemostasia y se realiza una sutura subdérmica con seda de 2-0 (figura 6). La región manipulada se limpia con suero fisiológico y se coloca una gasa estéril con un antiséptico. La retirada de los puntos se realiza a los 7-10 días. Los pacientes deben de asear la zona diariamente con agua y jabón colocando una nueva gasa estéril. El tiempo aproximado de realización de la BAT es de 30-60 minutos.

FIGURA 2. Disección de las capas superficiales y exposición de la fascia temporal.

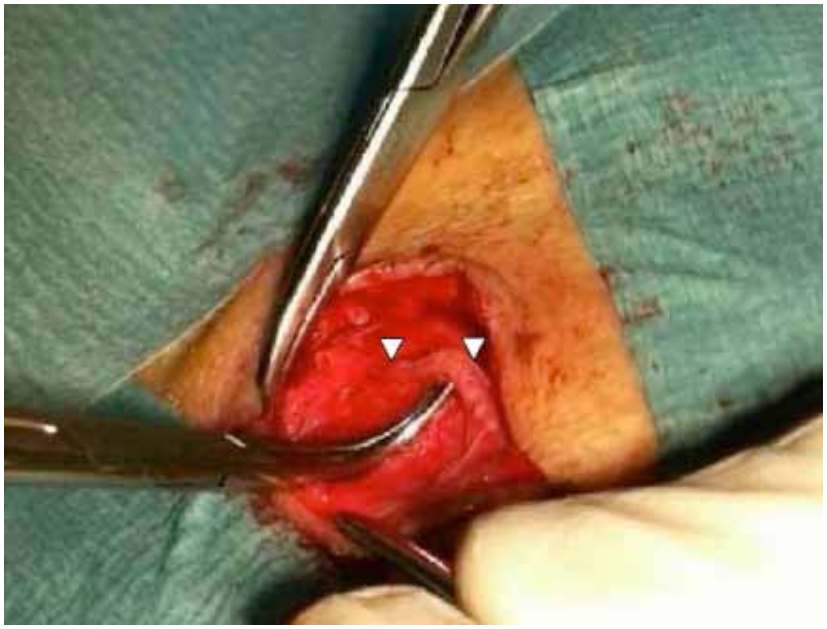

FIGURA 3. Disección de la arteria temporal (puntas de flecha) mediante pinzas Kelly.

Extracción a nivel preauricular

La preparación inicial es la misma que en el caso de la biopsia a nivel fronto-temporal. En esta técnica la región preauricular se infiltra con anestesia y se realiza una incisión de 3 cm céfalo-caudal y anterior al trago.

Posteriormente se diseca la fascia que cubre la porción preauricular identificándose la arteria temporal a nivel del trago. La AT es anterior y más profunda que la vena temporal y el nervio aurículo-temporal. Una vez identificada, la arteria se diseca, liga y extrae como se describió anteriormente.

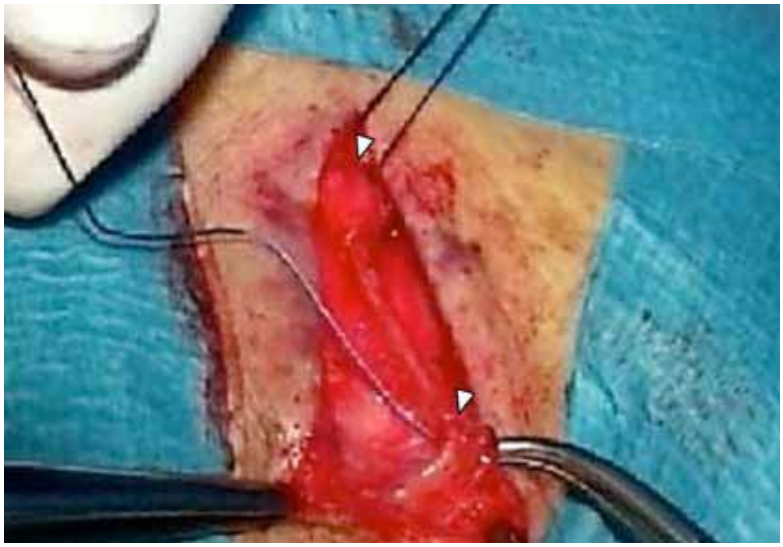

FIGURA 4. Ligadura de los extremos de la arteria (puntas de flecha) con sutura absorbible sintética de poliéster.

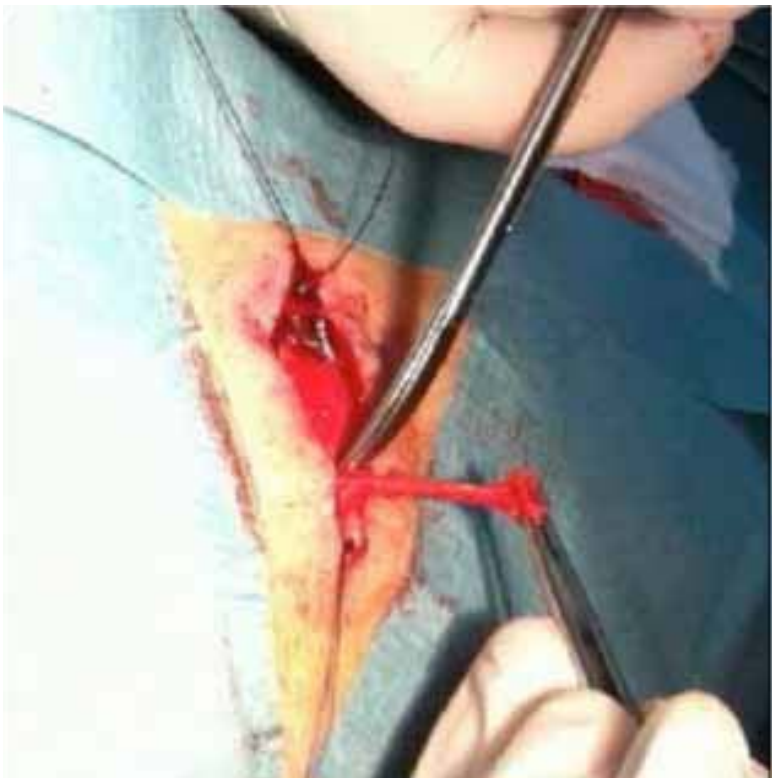

FIGURA 5. Corte de un segmento de 2-3 cm de la arteria temporal.

RECOMENDACIONES ESPECÍFICAS

Longitud

La longitud mínima necesaria para poder realizar un análisis histológico adecuado es cuestionable. Algunos autores sostienen que el tamaño no influye sobre el rendimiento de la biopsia mientras que otros estudios demuestran una mayor probabilidad de resultado positivo cuando la biopsia es amplia.

Nuestra recomendación es extraer al menos un fragmento de 2 a 3 cm de longitud, recordando que tras la extirpación la arteria se contrae y suele existir una contracción adicional de un 8% tras su fijación en formol.

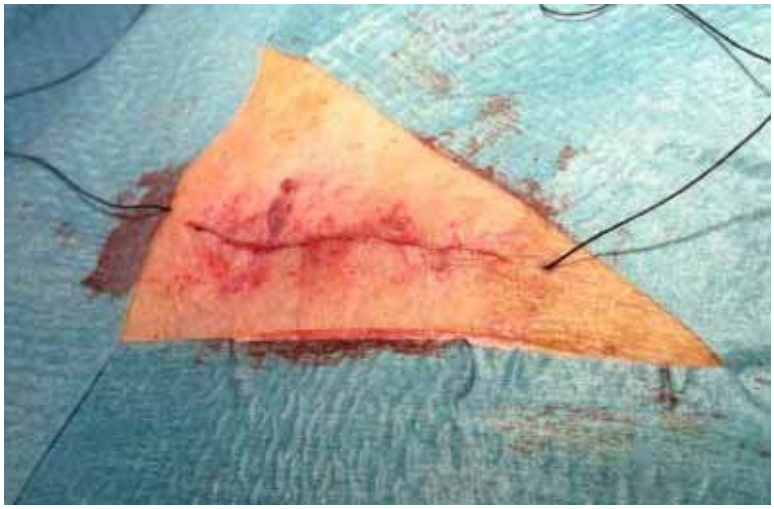

FIGURA 6. Cierre subdérmico de la herida quirúrgica.

Biopsia unilateral frente a bilateral

En algunos hospitales se realiza una biopsia simultánea de ambas arterias temporales en un mismo tiempo quirúrgico. Primero se biopsia una arteria que se analiza de manera peroperatoria y si el resultado es negativo se realiza la biopsia contralateral.

Ya que solo un 5-9% de las muestras contralaterales serán diagnósticas para ACG en caso de negatividad de la primera, en nuestro centro se realiza la biopsia de una sola arteria temporal de la que se examinan múltiples secciones a distintos niveles ya que raramente las lesiones no son segmentarias. Se reserva la biopsia contralateral para aquellos pacientes con alta sospecha clínica a pesar de tener un resultado histológico negativo para intentar evitar un tratamiento a ciegas con esteroides sistémicos.

COMPLICACIONES

En la literatura médica se han reportado pocas complicaciones relacionadas con la BAT, en su mayor parte menores. Como todo procedimiento quirúrgico existe el riesgo de reacciones alérgicas a los anestésicos locales o antisépticos tópicos, además de infección, dehiscencia o cicatrización alterada (queloide) de la herida quirúrgica.

Las complicaciones propias de este tipo de biopsia incluyen formación de hematomas en la región temporal, disestesias en la zona quirúrgica que pueden persistir varias semanas, y paresia de ramas terminales frontales que remiten con el tiempo. Raramente puede producirse lesión del nervio facial (tres casos publicados). De manera anecdótica se ha publicado algún caso de necrosis del cuero cabelludo, necrosis de lengua e infarto de hemisferio cerebral (un caso cada uno) tras la biopsia. Es importante considerar que estos eventos son complicaciones infrecuentes pero propias de la ACG y su coincidencia con la biopsia probablemente sea casual.

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