Cytokine expression in temporal arteries: comparative analysis between patients with biopsy-positive giant cell arteritis, biopsy-negative giant cell arteritis and biopsy-negative without arteritis

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ABSTRACT

Objective. *To investigate whether expression of pro-inflammatory cytokines in the temporal artery may aid in differentiating biopsy-negative giant cell arteritis (GCA) patients from those with a negative biopsy without arteritis.*

Methods. *We investigated cytokine expression in temporal artery biopsy (TAB) of 54 consecutive patients: 17 with biopsy-positive GCA, 17 with biopsy-negative GCA, and 20 biopsy-negative without arteritis. We compared the expression rate of the following cytokines among these 3 groups of patients: interleukin-6 (IL-6), osteopontin (OPN), COX-2, and TNF-α.*

Results. *IL-6 was expressed in 13 (76%) patients with biopsy-positive GCA, 0 patients in biopsy-negative GCA, and 1(5%) patient with biopsy-negative without arteritis (p<0.05). OPN was expressed in 17 (100%) patients with biopsy-positive GCA, 2 (12%) patients with biopsy-negative GCA, and 0 patients with biopsy-negative without arteritis (p<0.05). Cox-2 was expressed in 16 (94%) patients with biopsy-positive GCA, 0 patients with biopsy-negative GCA, and 3 (15%) patients with biopsy-negative without arteritis (p<0.05). TNF- α was expressed in 17 (100%) patients with biopsy-positive GCA, 14 (82%) patients with biopsy-negative GCA, and 8 (40%) patients with biopsy-negative without arteritis (p<0.05).*

Conclusion. *IL-6, COX-2 and OPN are significantly more expressed in the presence of a positive TAB compared to a negative TAB. TNF-α is significantly more expressed in GCA patients compared to non-GCA patients. Thus, TNF-α expression may suggest a diagnosis of GCA despite a negative TAB. Further larger studies are needed to confirm these findings.*

Introduction

Giant cell arteritis (GCA) is a large-vessel vasculitis involving large- and medium-sized vessels, particularly the branches of the proximal aorta (1). The diagnosis of GCA is based on clinical grounds (2-4). Temporal artery biopsy (TAB) showing transmural inflammation, often most pronounced in the media, is considered the gold standard for the diagnosis of GCA (5). However, TAB may be negative in up to 40% of the patients (6-10). A TAB may be negative for several reasons: skip lesions missing the characteristic histopathologic changes (11), previous corticosteroid treatment (12), an extracranial disease pattern (13), and an alternative diagnosis other than GCA. When the diagnosis of GCA is still suspected in a patients with a negative TAB, advanced imaging modalities for diagnosing large-vessel GCA, such as computerised tomography with angiography (CTA), magnetic resonance angiography (MRA) or positron emission tomography (PET), may be executed (14). However, these tests are not readily available. In addition, these tests may also be negative in cases of cranial-arteritis without large-vessel involvement. In these cases, TAB may be negative mainly due to skip lesions. Arteries from GCA patients show granulomatous infiltrate consisting mainly of CD4 lymphocytes of Th1 AND Th17 subsets and macrophages with increase production of several cytokines (*e.g.* IFNγ, IL-6, IL-17 and IL-21), growth factors and proteolytic enzymes. These promote a cascade of multiple inflammatory pathways, leading to progression of inflammatory infiltrates through the artery wall (15-16). Several studies investigated temporal artery expression of the cytokines interleukin-6 (IL-6),

osteopontin (OPN), COX-2, and TNF-α and their clinical correlations (17-22). It is unknown whether in GCA, the distribution of pro-inflammatory cytokines in the temporal artery is also segmental. The aim of this study was to investigate whether expression of pro-inflammatory cytokines in the temporal artery may aid in differentiating biopsy-negative GCA patients from those with a negative biopsy without arteritis.

Materials and methods

We retrospectively reviewed cases of patients who underwent a TAB in the Chaim Sheba medical center between the years 2000 and 2017. We identified 54 patients with suspected GCA who underwent a TAB. These patients were enrolled consecutively to the study. We included 3 groups of patients: 17 patients with GCA with inflamed TABs, 17 patients with non-inflamed TABs who were diagnosed clinically with GCA, and 20 patients with non-inflamed TAB who were not diagnosed with GCA. Patients' medical records of those cases were reviewed. Data collected included clinical and laboratory characteristics. The clinical data collected included the presence of constitutional symptoms, headache, jaw claudication, symptoms compatible with polymyalgia rheumatica (PMR), visual manifestations, cerebrovascular manifestations and an abnormal temporal artery on physical examination. The following laboratory data was collected: haemoglobin, leukocytes and platelets levels, erythrocyte sedimentation rate (ESR) and the presence of elevated liver enzymes. Elevated concentration of hepatic enzymes, occurs in approximately 30% of the patients with GCA. Therefore, we included this variable in the baseline clinical laboratory findings of the patients in the study. Additional data included temporal artery specimen length, time interval between initiation of symptoms TAB execution and time interval between corticosteroid treatment initiation and TAB execution. Patients were diagnosed with biopsy-negative GCA based on clinical judgment of the treating physician, provided the patient's symptoms and signs improved within 3 days of corticosteroid treatment (40mg of prednisone or more), no other better alternative diagnosis could be reached after a thorough evaluation and clinical follow-up. A pathologist who had no access to the clinical data reviewed all TAB. Clinical and laboratory parameters were compared between biopsy-positive and biopsy-negative GCA patients. The paraffin blocks of all 54 TAB were retrieved from the pathologic archive. Immunohistochemical analysis for IL-6, OPN, COX-2, and TNF-α was performed on paraffin-embedded sections of the temporal artery in all layers of the artery - media, adventitia and intima.

FFPE blocks were sectioned at 4 μm and a positive control was added on the right edge of the slides. The slides were warmed up to 60ºC for 1 hour and were processed by a fully automated protocol on a Benchmark Ultra staining module (Ventana Medical Systems Inc., USA). Briefly, after sections were dewaxed and rehydrated, a CC1 Standard Benchmark XT pretreatment for heat induced antigen retrieval (HIER) (Ventana Medical Systems Inc., USA) was selected for Interleukin 6 (IL6, 1:75, Abcam, ab6672 , USA) and Cyclooxygenase 2 (COX2, 1:50, Cell Marque , 240R-16 , Germany), a Mild CC1 HIER for Osteopontin (OPN, 1:100, Abcam, ab166709, USA) and an Extended CC1 HIER for Tumour Necrosis Factor-α (TNF-α, 1:50, Abcam, AB1793, USA). IL6, COX2 and OPN were detected with UltraView DAB Detection Kit (Ventana Medical Systems Inc., USA). TNF-α was detected with UltraView Red Detection Kit (Ventana Medical Systems Inc., USA). Sections were counterstained with Haematoxylin II (Ventana Medical Systems Inc., USA). After the run on the automated stainer was completed, the slides were dehydrated in graded ethanols (70%, 96%, and 100%). Before cover-slipping, sections were cleared in Xylene and mounted with Entellan.

Rate of expression of each of the cytokines IL-6, OPN, COX-2, and TNF-α in the temporal artery was calculated and compared between 3 groups: 17 patients with biopsy-positive GCA, 17 patients with biopsy-negative GCA, and 20 patients with biopsy-negative without arteritis. Analysis of the cytokine expression was executed by a pathologist which was blinded to the clinical data of the patients. The study

Table I. Baseline clinical and laboratory findings in 54 patients who underwent a temporal artery biopsy.

Variable	
Males - no. (%)	21 (39)
Age - years, median (IQR)	71 (66-77.8)
Headache - no. (%)	32 (59)
Constitutional syndrome - no. (%)	25 (46)
Abnormal temporal artery on physical examination - no. (%)	14 (26)
Jaw claudication – no. (%)	10 (18.5)
Polymyalgia rheumatica - no. (%)	12 (22.2)
Visual manifestations - no. (%)	17 (31.4)
Cerebrovascular accidents - no. (%)	3 (5.5)
Elevated liver enzymes - no. (%)	11 (20.3)
ESR mm/1st hour, median (IQR)	87.5 (70.8-100)
Haemoglobin (g/Dl), median (IQR)	11.4 (10.3-12.4)
Platelet count /mm^3, median (IQR)	325 (250.8-400.3)
Leukocyte count - cells/microL , median (IQR)	9.2 (7.7-15.5)
Leukocyte count>11000 cells/microL - no. (%)	18 (33)
Anaemia (haemoglobin <12g/dl) - no. (%)	37 (68)
Thrombocytosis (platelets >450x10^3/μl) - no. (%)	10 (18.5)
Length of temporal artery specimen - median (IQR)	0.8 (0.5-1.5)
Temporal artery specimen length <0.5 cm - no. (%)	7 (13)
Fulfillment of ACR criteria - no. (%)	37 (68.5)
Time interval between symptom onset and execution of TAB (weeks), median (IQR)	3 (1-5)
Time interval between steroid initiation and execution of TAB (days), median (IQR)	3 (2-5)

GCA: giant cell arteritis; TAB: temporal artery biopsy; IQR: interquartile range; ESR: erythrocyte sedimentation rate; ACR: American College of Rheumatology.

Table II. Baseline clinical and laboratory findings in 34 patients who underwent a temporal artery biopsy: comparative analysis between biopsy-positve GCA and biopsy-negative GCA patients.

Variable	Biopsy positive GCA n=17	Biopsy-negative GCA n=17	*p*-value
Males - no. (%)	7 (41)	7 (41)	1
Age - years, median (IQR)	73 (67-75)	71 (65-78)	0.89
Headache - no. (%)	13 (76.5)	13 (76.5)	1
Constitutional syndrome - no. (%)	9 (53)	10 (59)	1
Abnormal temporal artery on physical examination - no. (%)	9 (53)	3 (17.6)	0.07
Jaw claudication – no. (%)	8 (47)	2 (12)	0.057
Polymyalgia rheumatica - no. (%)	4 (23.5)	5 (29.4)	1
Visual manifestations - no. (%)	6 (35.3)	4 (23.5)	0.71
Cerebrovascular accients - no. (%)	0	0	1
Elevated liver enzymes - no. (%)	5 (29.4)	6 (35.2)	1
ESR mm/1st hour, median (IQR)	79.9 (26.4)	94.6 (24.8)	0.1
Haemoglobin (g/Dl), median (IQR)	11.8 (10.9-12.5)	10.8 (10.2-11.7)	0.13
Platelet count /mm^3, median (IQR)	395 (298-486)	344 (272-402)	0.57
Leukocyte count - cells/microL , median (IQR)	11.1 (8.2-13.1)	10.8 (8.5-14.4)	0.36
Leukocyte count>11000 cells/microL - no. (%)	7 (41)	8 (47)	1
Anaemia (haemoglobin <12g/dl) - no. (%)	10 (59)	14 (82.3)	0.259
Thrombocytosis (platelets >450x10^3/μl) - no. (%)	5 (29.4)	4 (23.5)	1
Length of temporal artery specimen - cm., median (IQR)	1.05 (0.58)	1.02 (0.52)	0.88
Temporal artery specimen length <1cm - no. (%)	1 (5.9)	3 (17.6)	0.6
Fulfillment of ACR criteria - no. (%)	17 (100)	14 (82.3)	0.2273
Time interval between symptom onset and execution of temporal artery biopsy (weeks), median (IQR)	3 (2-5)	3 (2-4)	1
Time interval between steroid initiation and execution of TAB (days), median (IQR)	2.5 (2-4)	3 (2-5)	1

GCA: giant cell arteritis; TAB: temporal artery biopsy; IQR: interquartile range; ESR: erythrocyte sedimentation rate; ACR: American College of Rheumatology.

Table III. Cytokine expression in temporal artery biopsies of 17 biopsy-positive GCA patients.

Cytokine	IL-6	Osteopontin	COX-2	TNF-α
Patient no. 1	negative -organisation in lumen	positive	positive	positive
Patient no. 2	positive	positive	negative	positive
Patient no. 3	negative -organisation in lumen	positive	negative	positive
Patient no. 4	positive	positive	positive	positive
Patient no. 5	positive	positive	positive	positive
Patient no. 6	positive	positive	positive	positive
Patient no. 7	positive	positive	positive	positive
Patient no. 8	negative -organisation in lumen	positive	positive	positive
Patient no. 9	positive	positive	positive	positive
Patient no. 10	positive	positive	positive	positive
Patient no. 11	positive	positive	positive	positive
Patient no. 12	positive	positive	positive	positive
Patient no. 13	negative -organisation in lumen	positive	positive	positive
Patient no. 14	positive	positive	positive	positive
Patient no. 15	positive	positive	positive	positive
Patient no. 16	positive	positive	positive	positive
Patient no. 17	positive	positive	positive	positive

GCA: giant cell arteritis.

was approved by the local institutional review board. The study was a retrospective pilot study. Therefore, and in agreement with the local institutional review board, informed consent was not required.

Statistical analysis

Data were analysed with SPSS software v. 23.0. (SPSS Inc. Headquarters, 233 S. Wacker Drive, 11th floor Chicago, Illinois 60606, USA). The significance levels were set at 0.05. Baseline clinical and laboratory findings of the patients are presented as medians and interquartile range for continuous variables and as frequencies and percentages for categorical variables. Comparisons between biopsy-positive and biopsy-negative GCA patients in baseline clinical and laboratory findings were performed by Fisher exact tests for categorical variables and independent *t*-test for continuous variables. Rates of each individual cytokine expression amongst the three groups of patients were compared using Fisher exact tests.

Results

Fifty-four patients (61% females) who underwent a TAB, were reviewed. The baseline clinical and laboratory findings of the study population are presented in Table I. The most common presenting symptoms were headache (59%) and a constitutional syndrome (46%). The mean length of the temporal artery specimen was 1.03 (±0.6) cm. Baseline clinical and laboratory findings were similar between biopsy-positive and biopsy-negative GCA patients (Table II). Eventual diagnoses of biopsy-negative non-GCA patients were self limited disease, non-arteritic anterior ischaemic optic neuropathy (AION), central retinal artery occlusion (CRAO), PMR, CNS vasculitis, multiple myeloma, sarcoidosis and cerebrovascular accident (CVA).

The cytokine expression in the temporal artery of the patients with biopsy-

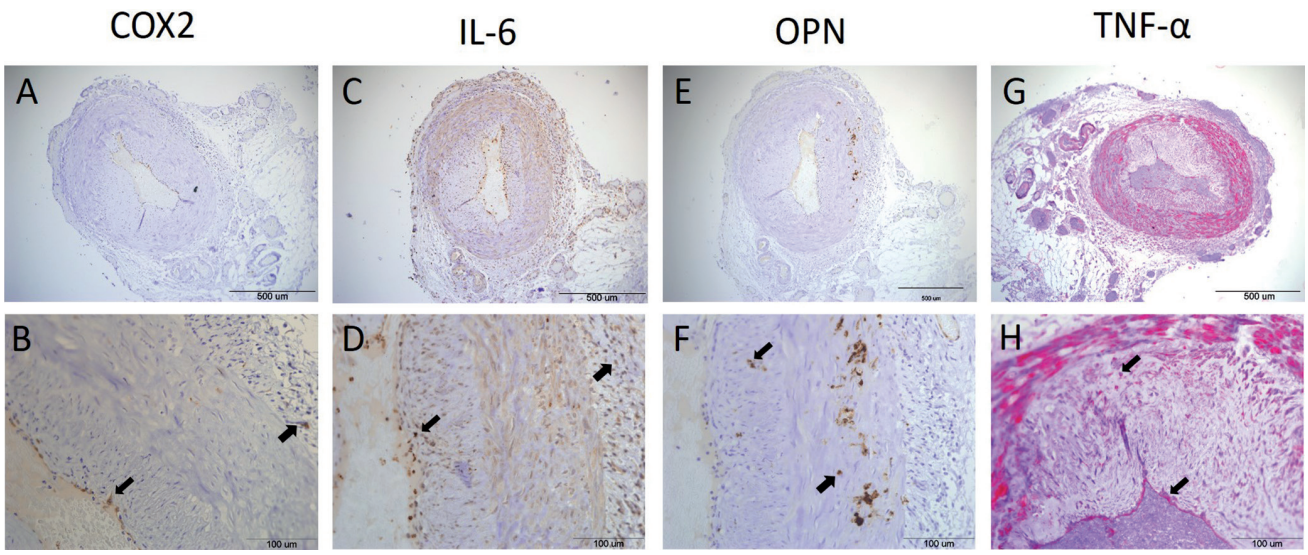

Fig. 1. Cytokine expression in a temporal artery of a biopsy-positive GCA patient. Photomicrograph of cross sections stained with antibodies to COX2 (**A**, **B**), IL-6 (**C**, **D**), OPN (**E**, **F**) and TNF-α (**G**, **H**). **B**, **D**, **F** and **H** are higher magnification views of boxed areas in **A**, **C**, **E** and **G**, respectively. Brown stained COX2+ cells are present in endothelial and few inflammatory cells (arrows in **B**). IL-6 and OPN are expressed mainly by inflammatory cells (arrows in **D** and **F**). TNF-α cells are widespread in endothelial and inflammatory cells (arrows in **H**).

Table IV. Cytokine expression in temporal artery biopsies of 17 biopsy-negative GCA patients.

Cytokine	IL-6	Osteopontin	COX-2	TNF-α
Patient no. 1	negative	Negative	negative	positive
Patient no. 2	negative	Negative	negative	positive-organisation in media and intima
Patient no. 3	negative	Negative	negative	positive
Patient no. 4	negative	Negative	negative	positive
Patient no. 5	negative - few IL6 positive in adventitia	Negative	negative	positive
Patient no. 6	negative	Negative	negative	positive
Patient no. 7	negative	Negative	negative	positive
Patient no. 8	negative	Negative	negative	negative
Patient no. 9	negative	Negative	negative	positive
Patient no. 10	negative	Negative	negative	negative
Patient no. 11	negative - few IL6 positive in adventitia	Negative	negative	positive
Patient no. 12	negative - few IL6 positive in adventitia	few cells positive	negative	positive
Patient no. 13	negative	few cells positive	negative	positive
Patient no. 14	negative	Negative	negative	positive
Patient no. 15	negative	Negative	negative	negative
Patient no. 16	negative	Negative	negative	positive
Patient no. 17	negative	Negative	negative	positive

GCA: giant cell arteritis.

positive GCA is presented in Table III. IL-6 was expressed in 13 (76%) patients. In the remainder of the patients with biopsy-positive GCA, there was neo-organisation of IL-6 in the lumen of the artery. OPN and TNF-α were expressed in all the patients with a positive TAB. Cox-2 was expressed in 16 (94%) patients with a positive TAB. Cytokine expression in the temporal artery of a biopsy-positive GCA patient is illustrated in Figure 1.

The cytokine expression in the temporal artery of the patients with biopsy-negative GCA is presented in Table IV. IL-6 was not expressed in any of the patients. However, in 3 patients few IL-6 were observed in the adventitia. OPN was expressed in 2 (12%) patients. COX-2 was not expressed in any of the patients, and TNF-α was expressed in 14 (82%) patients.

Cytokine expression in the temporal artery of a biopsy-negative GCA patient is illustrated in Figure 2.

The cytokine expression in the temporal artery of the patients with biopsy-negative non-GCA is presented in Table V. IL-6 was expressed in 1 (5%) patient. OPN was not expressed in the temporal artery of any of the patients. COX-2 was expressed in the temporal artery of 3 (15%) patients, and TNF-α was expressed in 8 (40%) patients. Cytokine expression in a temporal artery of a biopsy-negative non-GCA patient is illustrated in Figure 3.

The rate of temporal artery expression of IL-6, OPN and COX-2 was significantly higher among patients with biopsy-positive GCA compared to biopsy-negative GCA and biopsy-neg-

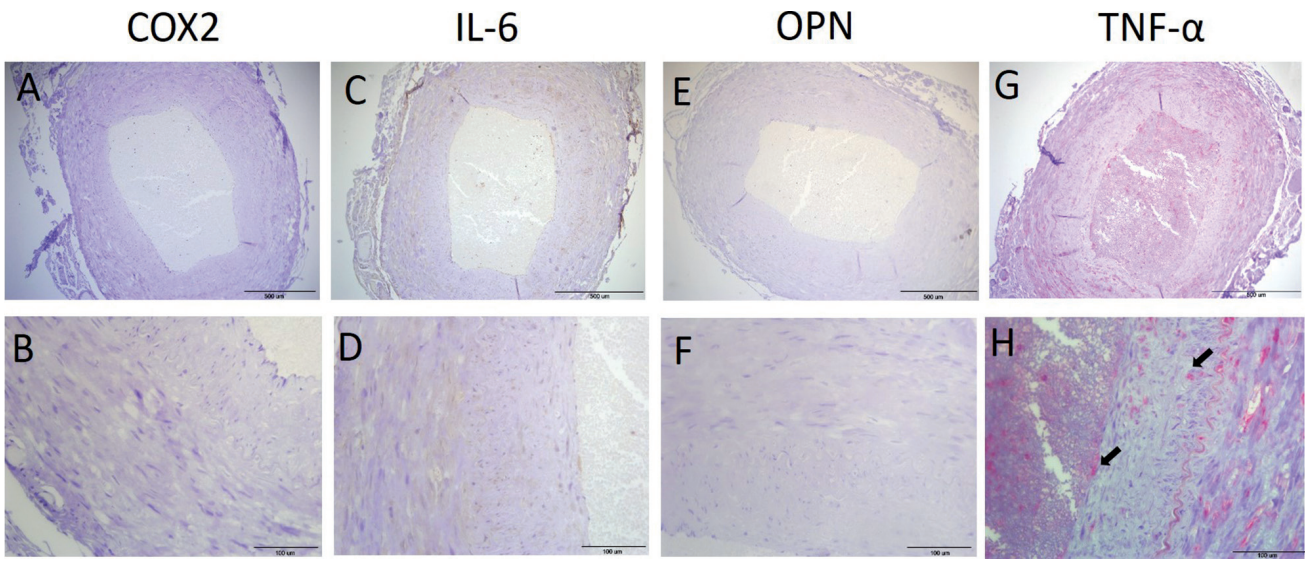

Fig. 2. Cytokine expression in a temporal artery of a biopsy-negative GCA patient. Transverse sections of TABs were stained with antibodies to COX2 (**A**, **B**), IL-6 (**C**, **D**), OPN (**E**, **F**) and TNF-α (**G**, **H**). **B**, **D**, **F** and **H** are higher magnification views of boxed areas in **A**, **C**, **E** and **G**, respectively. The cytokines COX2, IL-6 and OPN are not expressed. TNF-α is widely expressed in endothelial and inflammatory cells (arrows in **H**).

Table V. Cytokine expression in temporal artery biopsies of 20 biopsy-negative non-GCA patients.

Cytokine	IL-6	Osteopontin	COX-2	TNF-α
Patient no. 1	negative	negative	negative	positive
Patient no. 2	negative	negative	negative	positive
Patient no. 3	negative	negative	negative	negative
Patient no. 4	negative	negative	negative	negative
Patient no. 5	negative	negative	negative	positive
Patient no. 6	negative	negative	negative	negative
Patient no. 7	negative	negative	negative	positive
Patient no. 8	negative	negative	negative	positive
Patient no. 9	negative	negative	negative	negative
Patient no. 10	negative	negative	negative	positive
Patient no. 11	negative	negative	negative	negative
Patient no. 12	negative	negative	negative	positive
Patient no. 13	negative	negative	negative	positive
Patient no. 14	negative	negative	negative	negative
Patient no. 15	few cells positive	negative	negative	negative
Patient no. 16	negative	negative	negative	negative
Patient no. 17	negative	negative	negative	negative
Patient no. 18	negative	negative	negative	negative
Patient no. 19	negative	negative	negative	negative
Patient no. 20	negative	negative	negative	negative

GCA: giant cell arteritis.

ative non-GCA patients. No significant difference was found in the expression rate of IL-6, OPN and COX-2 between biopsy-negative GCA and biopsy-negative non-GCA patients. No significant difference was found between the expression rates of TNF-α in the temporal artery of biopsy-positive and biopsy-negative GCA patients. The rate of temporal artery expression of TNF-α was significantly higher among biopsy-negative GCA patients compared to biopsy-negative non-GCA patients (82% *vs*. 40%, $p<0.05$) (Table VI).

Discussion

TAB demonstrating infiltration of mononuclear cells into the arterial wall constitutes the gold standard for diagnosis of GCA (5). However, TAB may be negative in up to 40% of the cases (6-10). This is partially attributed to skip lesions missing the characteristic inflammatory infiltrate in the temporal artery (11). Multiple pro-inflammatory cytokines promote the inflammatory lesions in the temporal artery wall (15-16). In this study, we investigated whether expression of pro-inflammatory cytokines in the temporal artey may aid in differentiating biopsy-negative GCA patients from those with a negative biopsy without arteritis. We have shown that the temporal artery expression rate of IL-6, OPN and COX-2 cannot differentiate biopsy-negative GCA patients from biopsy-negative non-GCA patients, as the expression rate of these cytokines in both groups is quite low. However, we have found that TNF-α is expressed in the temporal artery in a relatively high rate of patients with GCA, both biopsy-positive and biopsy-negative, significantly higher than in patients with a negative TAB not diagnosed with GCA.

Studies assessing whether distinct histopathological findings and cytokine expression in the temporal artery may differentiate biopsy-negative GCA patients from non-GCA patients are scarce. Muratore *et al*. evaluated whether there are histopathological features of negative TAB that allow differentiation between patients with GCA and those without. They assessed the following histopathological features: presence of a local mediointimal scar, medial attenuation, intimal hyper-

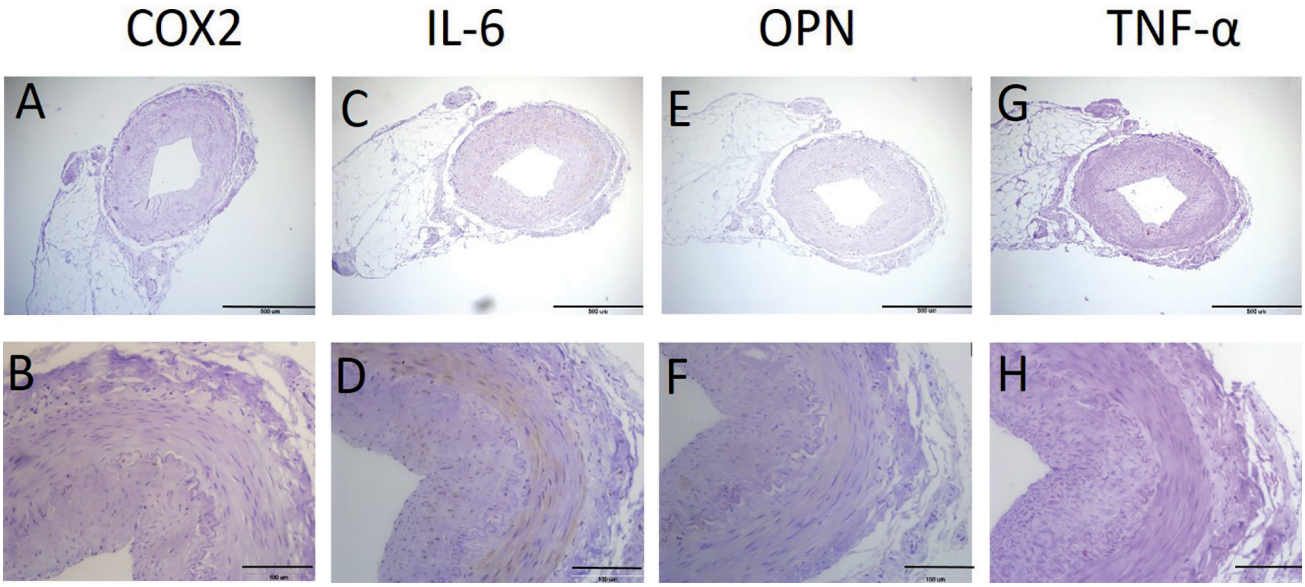

Fig. 3. Cytokine expression in a temporal artery of a biopsy-negative non-GCA patient. Photomicrograph of cross sections stained with antibodies to COX2 (**A**, **B**), IL-6 (**C**, **D**), OPN (**E**, **F**) and TNF-α (**G**, **H**). **B**, **D**, **F** and **H** are higher magnification views of boxed areas in **A**, **C**, **E** and **G**, respectively. None of the cytokines COX2, IL-6, OPN and TNF-α are expressed.

Table VI. Comparative analysis of cytokine expression in temporal arteries of biopsy-positive GCA, biopsy-negative GCA and biopsy negative non-GCA groups.

Cytokine	Biopsy positive GCA n=17	Biopsy-negative GCA n=17	Biopsy-negative without GCA Nn20	*p*-value
IL-6 (%)	13 (76)	0	1 (5)	<0.05*
Osteopontin (%)	17 (100)	2 (12)	0	<0.05*
COX-2 (%)	16 (94)	0	3 (15)	<0.05*
TNF-α (%)	17 (100)	14 (82)	8 (40)	<0.05**

*Statistically significant difference between biopsy positive GCA group and biopsy-negative GCA and biopsy-negative without GCA groups.
**Statistically significant difference between biopsy positive GCA and biopsy-negative GCA groups and biopsy-negative without GCA group.
GCA: giant cell arteritis.

plasia, fragmentation of inner elastic lamina, calcification, adventitial fibrosis, and neoangiogenesis. They found no significant difference between TAB-negative GCA patients and non-GCA patients in the features of all histologic features evaluated (23). Lally *et al*. evaluated the role of rho kinase (ROCK) activity in GCA. Subjects were categorised into 3 groups; TAB-positive GCA, TAB-negative GCA and controls. TAB were stained for phosphorylated ezrin/radixin/moesin (pERM), a surrogate of ROCK activity. They found that subjects with GCA had more intense pERM staining in TAB specimens compared with controls, regardless of whether TAB was positive or negative. Accordingly, they suggested that ROCK pathways should further be investigated in GCA, as it may enhance the sensitivity of TAB (24). Ting *et al*. investigated the association between histological biopsy features and clinical features, such as blindness, in patients with biopsy positive GCA. They found that giant cell are strongly associated with jaw claudication and systemic markers of inflammation. They did not find any histological features that were individually significantly associated with an increased risk of blindness in GCA patients (25).

Several studies investigated cytokine expression in the temporal artery of GCA patients. Hernandez-Rodriguez *et al*. found that expression of the pro-inflammatory cytokines IL-1β, TNF-α and IL-6 in the temporal artery were significantly more abundant in GCA patients with a strong inflammatory response. They also found that TNF-α was associated with longer corticosteroid requirements (17). Manku *et al*. found that IL-6 expression is correlated with increased T-cell proliferation and survival in the temporal artery wall in GCA through mechanisms that are independent of effects on local T reg expansion (18). These finding are in line with the findings in our study, which have demonstrated a high rate of IL-6 expression in the temporal artery of patients with biopsy-positive GCA. These findings underscore the pathological role of IL-6 in GCA, as well as the effects of tocilizumab in GCA. Corbera-Bellalta *et al*. investigated the concentrations of pro-inflammatory mediators of temporal artery section in GCA patients and controls in the presence and absence of dexamethasone (26). They found that protein concentraions of IL-6 of GCA patients was higher than controls, but the difference was not statistically significant. This may be explained by the limited number of patients in their study. Another possible explanation for the inconsistency between Corbera-Bellata's findings and the findings in our study may be explained by the different methods of IL-6 detection.

Several studies have investigated the role of TNF-α in GCA. A previous study, Field *et al*. reported a production of TNF-α by macrophages and giant cells in GCA lesions (19). This study is not compatible with the findings in the study of Seror *et al*., which reported that adalimumab, a humanised anti-TNF-α therapy, was inefficient as a steroid-sparing agent treatment in GCA (20). An additional study by Samson *et al*. found no difference regarding the serum level of TNF-α between untreated GCA patients, treated GCA patients and healthy controls (21). The findings in our study, showing a high rate of TNF-α expression in both biopsy-positive and biopsy-negative GCA patients, corroborate the findings in Field's study (19). However, the finding that TNF-α was expressed in 40% of temporal arteries of controls, argue against a significant pathogenic role of TNF-α in GCA, in accordance with other previous studies (20-21). Since macrophage recruitment is one of the last steps in the inflammatory cascade in GCA, TNF-α is apparently not a key cytokine in the inflammatory process of GCA (27). Among the cytokines investigated in our study, TNF-α was the only one expressed in a high rate of biopsy-negative GCA patients compared to non-GCA patients. Since TNF-α represents a late stage of the inflammatory process in GCA, its expression in the temporal artery may suggest expression of recent inflammatory cytokines upstream the inflammatory cascade. According to the findings of our study, expression of TNF-α in a negative TAB may aid in differentiating GCA and non-GCA patients and suggest a diagnosis of GCA despite a negative TAB. However, larger studies assessing the expression of TNF-α in temporal artery of GCA patients and controls are needed, in an attempt to evaluate the sensitivity and specificity of TNF-α expression in GCA.

OPN is an intracellular glycoprotein that is expressed in a wide range of cells involved in the inflammatory process. It participates in innate and adaptive immune responses, and is up regulated during macrophage differentiation (28). According to his functions, OPN is highly expressed at sites of inflammation and reflects activation of pathways relevant to immune and inflammatory responses that participate in the inflammatory cascade of GCA (29). In a previous study, Prieto-Gonzalez *et al*. explored the role of OPN as a biomarker in patients with GCA. They found that serum OPN was significantly elevated in patients with active GCA compared with controls and in patients with remission, and significantly higher in relapsing vs non-relapsing patients. In addition, in cultured GCA arteries, OPN mRNA expression was not significantly modified by short-term exposure to tocilizumab (22). In our study, we found that OPN was expressed in the temporal artery of 100% of the patients with biopsy-positive GCA, in contrast with only 12% of the patients with biopsy-negative GCA and none of the patients with biopsy-negative non-GCA. These findings reinforce the findings in Prieto-Gonzalez's study, and demonstrate the major role of OPN in the inflammatory process of GCA. The fact that only 76% of the patients with biopsy-positive GCA who expressed OPN also expressed IL-6 may indicate that OPN is not exclusively IL-6 dependent, in accordance with the findings of Prieto-Gonzalez's study. Therefore, OPN may serve as a disease activity marker, particularly in patients treated with tocilizumab. This issue should be explored and validated in larger studies.

Cox-2 is the dominant source of prostaglandin, including prostaglandin E2 (PGE2) (30). Several studies have demonstrated that COX-2/PGET is involved in cardiovascular disease, including neointimal hyperplasia after vascular injury, aortic aneurysm and atherosclerosis (31-32). To date, COX-2 has not been explored in patients with GCA. In this study, we have demonstrated that COX-2 is involved in the inflammatory process of GCA, as it is expressed in the temporal artery of nearly all the patients with biopsy-positive GCA.

Our study has several limitations. First is the study's retrospective design. Another limitation is the relatively small sample size. The findings in our study should be investigated and confirmed in larger studies.

The strength of our study is the investigation of several key pro-inflammatory cytokines involved in the inflammatory process of GCA among GCA patients and controls, and particularly among biopsy-negative GCA patients.

In conclusion, in this study we have shown that IL-6, COX-2 and OPN are significantly more expressed in the presence of a positive TAB compared to a negative TAB. TNF-α is significantly more expressed in patients with GCA, including those with a negative TAB, compared to non-GCA patients. Thus, TNF-α expression may suggest a diagnosis of GCA despite a negative TAB, and may aid in differentiating biopsy-negative GCA patients from non-GCA biopsy-negative patients. Further larger studies are needed to confirm these findings.

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