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Concise report

CD3 immunohistochemistry is helpful in the diagnosis of giant cell arteritis

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Abstract

Objective. To evaluate whether CD3 staining performed routinely on temporal artery biopsy specimens might improve the sensitivity of temporal artery biopsy in patients with biopsy-negative GCA.

Methods. Two hundred and seventy biopsies were considered for this study, stained with haematoxylin and eosin and with an anti-CD3 antibody.

Results. The addition of CD3 staining modified the sensibility and the specificity of the histologic examination in 89.47 and 95.00%, respectively, with a positive and negative predictive values of 97.00 and 79.78% .

Conclusion. The addition of CD3 immunostaining to the classic histologic evaluation is accompanied by a significant increase in the sensibility with a comparable specificity.

Key words: giant cell arteritis, CD3, immunohistochemistry, diagnosis

Rheumatology key messages

- CD3 immunohistochemistry may be useful in histological-negative arteries of patients with suspected GCA.
- The addition of CD3 staining significantly increased sensibility and specificity of classic histologic examination.

Introduction

GCA is a large- and medium-sized arteries systemic vasculitis [1] resulting in a wide variety of systemic, neurologic and ophthalmologic complications. GCA is histopathologically characterized by transmural inflammation of the all the layers of affected arteries by monocyte-derived macrophages, activated T cells and multinucleated giant cells. In particular, a massive infiltration by Th1, Th9 and Th17 $CD3^+$ effector T cells is observed in temporal artery biopsy (TAB) specimens obtained from patients affected by GCA with positive biopsy [2, 3]. GCA is diagnosed by combining symptoms, clinical findings, laboratory results and diagnostic imaging. The gold standard for the diagnosis of GCA is the demonstration of artery wall inflammation on TAB [1]. Frequently, TAB results in false-negative biopsies in a relevant number of patients, leading to a significant delay in treatment and possible irreversible complications [4]. The need to improve the diagnosis of GCA requires the developement of new staining techniques of TAB specimens. Due to the important role of $CD3^+$ T cells in the pathogenesis of the disease we aimed to investigate whether CD3 staining performed routinely on TAB specimens may improve the sensitivity of TAB in patients with biopsy-negative GCA.

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Methods

Patients and immunohistochemistry

For this study, we retrospectively evaluated all the biopsies performed in our centres from January 2003 to June 2016 in patients with suspected GCA. Two hundred and seventy biopsies obtained from as many patients were considered. At the time of diagnosis, 28 patients had vision loss and PMR was associated in 45 patients. At the time of artery biopsy all the patients were treatment-naïve or had received glucocorticoids for $\leqslant$1 week. Prior or current diagnosis of cancer, autoimmune disease other than GCA or chronic infection was carefully excluded. All the paraffin-embedded TAB specimens were retrieved, the tissue cut into 4-μm sections (8–10 per patient) and stained with haematoxylin and eosin (H&E). Immunohistochemical analysis for CD3 was also

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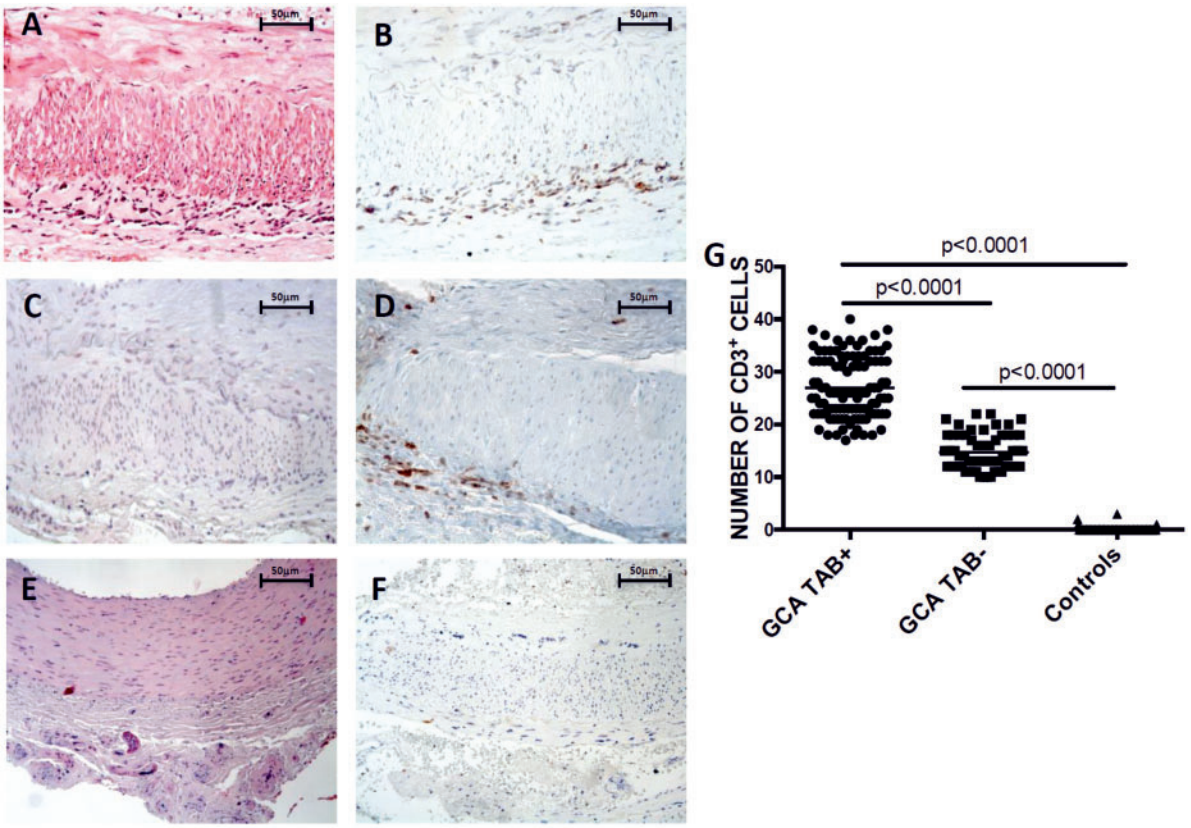
Fig. 1 CD3 immunostaining in histology positive and negative GCA arteries



(**A**–**B**): representative microphotographs showing hematoxylin and eosin staining (**A**) and CD3 immunostaining of GCA artery showing transmural inflammation. (**C**–**D**): representative microphotographs showing hematoxylin and eosin staining (**C**) and CD3 immunostaining in GCA artery without apparent inflammatory infiltrates (**D**). (**E**–**F**): representative microphotographs showing hematoxylin and eosin staining (**E**) and CD3 immunostaining in control artery (**F**). G: number of CD3+ cells in GCA TAB+, GCA TAB− and control arteries.

performed on 5-μm-thick paraffin-embedded sections from artery biopsies and from tonsils (used as positive controls) as previously described [5]. Briefly, following rehydration, antigen was unmasked for 45 min at 95°C using Dako Target retrieval solution (pH 6; Dako, Carpinteria, CA, USA). Endogenous peroxidase was blocked for 10 min with Dako peroxidase blocking reagent, and non-specific binding was blocked for 20 min with Dako protein block. The primary antibodies mouse monoclonal anti-human CD3 (ab5690, AbCam, Cambridge, UK) was added and incubated for 1 h at room temperature. An isotype-matched irrelevant antibody was used as a negative control. Following three washes with Tris-buffered saline, slides were incubated for 30 min with peroxidase-conjugated Dako EnVision polymer. After three further washings, peroxidase activity was visualized using di-aminobenzidine chromogen (Dako, Carpinteria, CA, USA) and slides were lightly counterstained with haematoxylin before dehydration and mounting in DePex (VWR International, Oslo, Norway). The number of immune-reactive cells was determined by counting positively stained cells on photomicrographs obtained from three random high-power microscopic fields (400× magnification) under a Leica DM2000 optical microscope, using a Leica DFC320 digital camera (Leica, Rijswijk, The Netherlands). Two experienced pathologists (A.R. and A.C.) with an expertise in vasculitides and with no access to clinical data evaluated the arteries. The intra-rater agreement and the inter-rater agreement calculated by the Cohen's κ coefficient for the two pathologists were 0.81 and 0.72, respectively. In order to study the presence of atherosclerosis, artery sections were stained with Masson's trichrome (Sigma-Aldrich, Sigma, Saint Louis, Missouri, USA) for cellular components [smooth muscle cells (SMCs): pink, and red blood cells: red], fibrous tissue (blue), and fibrin and platelet-rich thrombus (purple). The study was conducted according to the Declaration of Helsinki and Italian legislation. Consent was obtained from all enrolled subjects after the nature of the investigation was explained and in accordance with

Table 1 Summary of the patients enrolled in the study

Artery characterization	$CD3^+$, *n* (%)	$CD3^-$, *n* (%)	Total, *n*
GCA TAB^+	110 (100)	0	110
GCA TAB^-	60 (75)	20 (25)	80
Control arteries	4 (5)	76 (95)	80

the approved protocol from the Institutional Review Board at the University of Palermo and the Arcispedale Santa Maria Nuova, Reggio Emilia, Italy.

Statistical analysis

Statistical analysis of quantitative variables was performed using the Mann–Whitney rank-sum test. $P < 0.05$ were considered significant.

Results

One hundred and ten arteries displayed typical histological findings of GCA (90 women and 20 men, median age 77 years, range 62–80 years). Eighty patients did not display any histological feature of GCA but were classified to be affected by GCA (65 women and 15 men, median age 77 years, range 57–83 years) according to the ACR classification criteria [6]. Finally, 80 were histologically normal TAB (62 women, 18 men; median age 68 years, range 57–83 years) from as many patients who did not fulfil the ACR classification criteria for GCA. In these patients, the absence of GCA was confirmed by a retrospective evaluation of all medical records from symptoms' onset to the last visit. We calculated that in our cohort the sensibility and the specificity of H&E staining were 57 and 100%, respectively (positive predictive value 100% and the negative predictive value 50%).

CD3 immunohistochemistry was performed in all the arteries and the results were considered only in arterial areas not displaying evidence of atherosclerosis. According to H&E results, anti-CD3 staining was positive in all the biopsy-proven GCA patients (Fig. 1A and B; Table 1). Interestingly, $CD3^+$ staining was also observed in 60 out of 80 patients with TAB-negative GCA (in the context of the adventia layer in 54 out of 60 patients and in the context of media layer in the remaining six) (Fig. 1C and D; Table 1). Quantitative evaluation of $CD3^+$ cells demonstrated a significantly higher number of $CD3^+$ cells in TAB-positive and -negative GCA arteries compared with controls (Fig. 1G). No significant differences were observed regarding ACR criteria between between biopsy-negative GCA patients with or without T cells in their biopsies. Among control arteries only 4 out of 80 displayed CD3 positivity (<10 positive cells) (Fig. 1E and F; Table 1). The addition of CD3 staining to H&E evaluation modified the sensibility and the specificity of the histologic examination in 89.47 and 95.00%, respectively. The positive and negative predictive values were 97.00 and 79.78%.

Discussion

The histologic diagnosis of GCA does not require any immunohistochemical study of the affected arteries. This approach might be considered adequate in the presence of classic transmural inflammation. However, different subsets of non-classic transmural inflammation have been described in GCA patients and many other GCA patients display no histologic inflammation [1]. GCA has long been considered as a T cell-mediated disease. The massive infiltration of GCA-positive arteries by activated T effector cells such as Th1, Th17 and Th9 highlights the important role of these cells in the pathogenesis of the disease and makes T lymphocytes an interesting subject for diagnostic purposes [2, 3]. The presence of $CD3^+$ infiltrating T cells has been demonstrated in TAB-negative GCA arteries in a series of studies without, however, evaluation in a large series of GCA patients [7–9].

In this study, we demonstrated that $CD3^+$ staining was present in the majority of arteries obtained from GCA patients with a negative TAB. Since arteries are immune-privileged sites, the presence of $CD3^+$ cells in the context of apparently histologically uninflamed non-atherosclerotic GCA arteries may suggest the occurrence of tissue inflammation. In particular, CD3 immunostaining was mainly observed in the context of the adventia layer where vasa vasorum are located. Activation of adventitial vasa vasorum has been suggested to occur in the early stage of the disease, leading to the active recruitment of inflammatory mononuclear cells in the inflamed arteries [1, 2].

The addition of CD3 immunostaining to the classic histologic evaluation was accompanied by a significant increase in the sensibility with a comparable specificity. Although biopsy-negative GCA characterizes a subset of patients who often have a different clinical spectrum of the disease with less severe ischaemic complications than that of biopsy-proven GCA [10], these results seem to suggest that CD3 immunostaining should be added to the routine evaluation of arteries when GCA is strongly suspected. The main limitation of the present study is the retrospective design of the study. However, the perfect concordance between the presence of $CD3^+$ cells and the classic histologic signs of GCA and the substantial absence of $CD3^+$ cells in control arteries seem to confirm that the presence of abundant $CD3^+$ infiltrating cells specifically marks inflamed arteries.

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