Studies on the Histopathology of Temporal Arteritis

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Key Words

Temporal arteritis · Giant cell arteritis · Granulomatous inflammation · CD68 · Vasculitis

Abstract

Aims: The aim of this paper was to identify the location and to grade the severity of most significant inflammation within positive temporal artery biopsies along with other key clinical and histologic characteristics. **Methods:** Charts and pathology slides for 70 patients diagnosed with temporal arteritis at the University of Wisconsin (UW) Hospital and Clinics from 1989 to 2015 were reviewed. A subset of 48 specimens was immunostained for CD68 and graded on a scale from 0 to +++; the location of staining was recorded. **Results:** The most severe granulomatous inflammation was in the media and adventitia in 13% (9/70) of the biopsies; the remaining had uniform full thickness inflammation. Of the slides that were stained with CD68, 94% (45/48) were positive. In 42% (19/45), the stained cells were found mainly in the muscularis and adventitia. Seven percent (3/45) of the slides had staining solely around the internal elastic lamina, and 2% (1/45) had staining limited to the intima. **Conclusions:** With a few exceptions, granulomatous inflammation in positive temporal artery biopsies is most evident at the media and adventitia or is uniform throughout the layers of the artery.

Our study lends support to the theory that the muscularis and adventitia may play an inciting role in the pathogenesis of temporal arteritis.

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Introduction

Temporal arteritis is an inflammatory vasculitis that is considered an ophthalmic emergency. It is the most common form of systemic vasculitis affecting the elderly and can lead to rapidly progressive and permanent vision loss. Prompt diagnosis and treatment with systemic corticosteroids is required to prevent vision loss [1]. Clinical manifestations of temporal arteritis include headache, visual changes, jaw claudication, scalp tenderness, polymyalgia rheumatica, and fatigue [2]. Laboratory testing frequently reveals elevated inflammatory markers, including elevated erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).

The ‘gold standard’ for diagnosis is the temporal artery biopsy [3]. The current literature describes a positive biopsy as revealing chronic granulomatous inflammation – consisting of epithelioid histiocytes, multinucleated giant cells, T lymphocytes, and macrophages – concentrated at the level of the internal elastic lamina [4–6].

Intimal hyperplasia and fragmentation of the internal elastic lamina are also frequently seen on histopathology. Recently, CD68 immunostaining for the presence of macrophages has been shown to be useful in differentiating between indeterminate and positive temporal artery biopsy specimens [7].

The internal elastic lamina is frequently assumed to be the initial and possible inciting site of temporal arteritis based on (1) the accumulation of giant cells and histiocytes along the zone of the previous elastic lamina; (2) elastic fragments found with the giant cells, and (3) the lack of involvement of arteries lacking an internal elastic lamina (e.g. the intracranial arteries) [4, 8, 9]. Other experimental data suggests that the disease is initiated in the outer layer of the arterial wall, with inflammatory cells entering through the vasa vasorum and subsequently infiltrating into all layers of the artery wall [10].

The purpose of this study is to verify the location and severity of granulomatous inflammation in positive temporal arteries along with other clinical and histologic characteristics and to compare these findings to previous reports.

Methods

Patients and Specimens

The list of patients with biopsy-proven temporal arteritis was generated by searching the University of Wisconsin (UW) Eye Pathology Laboratory and UW Surgical Pathology Laboratory databases for the terms 'giant cell arteritis' and 'temporal arteritis.' Charts for patients with positive temporal artery biopsies from a 25-year period, 1989–2014, were reviewed for demographic information, clinical signs and symptoms, laboratory results, and treatment course. Cases with indeterminate diagnoses were excluded, as were cases where clinical information was unavailable. A total of 70 patients were included in the study.

The upper limit of normal for ESR was determined by the formula proposed by Miller et al. [11] in 1983: age/2 in men and (age +10)/2 in women. Elevated CRP was defined as ≥ 2 , and elevated platelets were defined as ≥ 450 .

All biopsies were submitted in 10% formalin. Upon grossing, the tissues were examined for length and color, then subsequently cut into 2-mm pieces and routinely processed. They were then embedded on end and serially sectioned in 25- μ m intervals with a thickness of 5 μ m. Initially, >50% of the paraffin block was sectioned. If the pathologist saw no granulomatous inflammation in the initial examination, then >90% of the paraffin block was sectioned.

All temporal artery biopsy slides for the 70 patients, including H&E and PAS stained slides, were reviewed for the presence of intimal hyperplasia, lumen occlusion, breaks of the internal elastic lamina, skip lesions, and inflammatory cells, and the location of most severe inflammation was also determined by a trained ocular pathologist.

Table 1. Patient clinical and demographic information

Demographics	
Male	20/70
Female	50/70
Mean age (range)	74.5 (52–94)
Laboratory results	
Elevated ESR	55/69
Mean positive ESR (range)	61.8 (46–130)
Mean negative ESR (range)	28.6 (16–44)
Elevated CRP	32/38
Double negative ESR and CRP	6/38
Elevated platelets	8/35
Presenting symptoms	
Headache	46/70
Jaw claudication	44/70
Fatigue	26/70
Vision loss	26/70
Bilateral	7/26 (14 eyes)
Unilateral	19/26 (19 eyes)
Persistent	17/26 (21 eyes)
Eyes with <20/200 visual acuity	14/21
Scalp tenderness	24/70
Proximal myalgia	23/70
Weight loss	18/70
Fevers	12/70
Anorexia	11/70
Diplopia	7/70
Night sweats	4/70
Ophthalmologic examination findings	
Optic disc edema or pallor	19/59
Afferent pupillary defect	14/57
Retinal artery occlusion	9/59
Sixth nerve palsy	3/59
Scleritis or uveitis	2/59

Immunohistochemistry

A subset of 48 patient specimens was immunostained for CD68. Deparaffinization of all slides was done by placing the slides in a 58°C oven for 10 min. They were rehydrated in a stepwise fashion in alcohols of decreasing concentration and then washed in deionized water. The slides were placed in 3% hydrogen peroxide for 10 min and washed several times in deionized water.

Antigen retrieval was performed by placing the slides in Coplin jars with 1 mM ethylenediaminetetraacetic acid at a pH of 8.0 in a 95°C water bath for 20 min. The Coplin jars were removed from the water bath and allowed to sit for 20 min before being in a jar of tap water for 10 min. The sections were washed 2 times in deionized water and then incubated with primary antibody (monoclonal mouse anti-human CD68; Innovex Biosciences, Richmond, Calif., USA) for 1 h at room temperature in a humidified chamber. The slides were washed for 1.5 min in phosphate-buffered saline (PBS) and then incubated with secondary linking antibody (Innovex Biosciences) for 10 min in a humidified chamber.

Next, the slides were washed with PBS and incubated with horseradish peroxidase label for 10 min in a humidified chamber.

Fig. 1. Histopathology of temporal artery biopsy specimens with H&E staining. **a** Negative temporal artery biopsy. **b** Negative temporal artery biopsy with calcific changes. **c** Positive temporal artery biopsy with granulomatous inflammation centered at the media and adventitia. **d** Positive temporal artery biopsy with full thickness granulomatous inflammation.

The slides were again washed with PBS and incubated with DAB (3,3'-diaminobenzidine)/substrate solution for 8 min. Deionized water was used to rinse the sections several times to stop any DAB reaction. Gill's II hematoxylin was used to counterstain the tissue for 2 min; the sections were then rinsed in deionized water. Saturated lithium carbonate solution (1.4% in water) was utilized for bluing the sections for 1 min. The sections were run for several minutes in tap water prior to being dehydrated in alcohols of increasing concentration followed by xylene and then cover slipping.

The staining was graded based on the scale established by Zhou et al. [7], which includes the following: 0 (no staining), + (up to one third of the circumference of ≥ 1 layer containing cells that stain), ++ (up to two thirds of the circumference of ≥ 1 layer containing cells that stain), and +++ (the complete circumference of ≥ 1 layer containing cells that stain).

Results

Of the 70 total patients, 11 were evaluated by internists prior to biopsy and did not have ophthalmologic examinations done. All other patients were evaluated by an ophthalmologist. The average age of diagnosis for temporal arteritis was 74.5 years, with 71% (50/70) of positive cases diagnosed in women. Clinical and demographic data are summarized in table 1.

Patients were treated with high-dose systemic steroids for an average of 3.2 days prior to biopsy. Unilateral temporal artery biopsies were submitted in 81% (57/70) of the cases; the remaining biopsies were bilateral. The mean artery sample length was 19.6 mm.

Histopathologic features of the positive biopsies included lymphocytes (70/70), epithelioid cells (70/70), intimal hyperplasia (69/70), giant cells (57/70), fragmented internal elastic lamina (66/70), lumen narrowing (34/70), and concomitant calcific changes (24/70). Skip areas were noted in 12% (8/68) of the cases. The most severe granulomatous inflammation was located in the media and adventitia in 13% (9/70) of the biopsies; the remaining specimens had full thickness inflammation. On H&E stained slides, no biopsies had inflammation isolated at or centered on the internal elastic lamina. Figure 1 illustrates the histopathological grading of the temporal artery specimens.

Of the slides that were stained with CD68, 94% (45/48) were positive; 40% (18/45) were graded as +, 22% (10/45) were graded as ++, and 38% (17/45) were graded as +++ (fig. 2, table 2). In 49% (22/45) of the positive samples, the macrophage lysosomal CD68 staining-positive cells were found to be in all three layers of the artery. In 42% (19/45), the stained cells were found mainly in the muscularis and

Fig. 2. CD68 immunohistochemistry staining and grading of temporal artery biopsy specimens. **a** CD68 with no staining of histiocytes. **b** + staining (up to one third of the circumference of ≥ 1 layer). **c** ++ staining (up to two thirds of the circumference of ≥ 1 layer). **d** +++ staining (the complete circumference of ≥ 1 layer).

Table 2. Distribution of inflammation with the temporal arteritis biopsy specimens

	H&E	CD68
Intima only	0/70	1/45
Intima and media only	0/70	3/45
Media only	0/70	1/45
Media and adventitia only	8/70	11/45
Adventitia only	1/70	7/45
Full thickness	61/70	22/45

adventitia. Seven percent (3/45) of the slides had staining solely around the internal elastic lamina, and 2% (1/45) had staining limited to the intima. Six percent (3/48) were negative for CD68 staining; all 3 had been stored with their blocks for more than 15 years.

Discussion

The clinical and laboratory findings in our patients are generally consistent with previous reported series [1]. The most common presenting symptom was head-

ache in 66%. Approximately 24% of the patients presented with permanent vision loss, the most common cause of which was arteritic anterior ischemic optic neuropathy in 76%. Two of the 70 patients (3%) had no systemic symptoms; in the literature, silent or occult presentations of temporal arteritis constitute up to 20% of all cases [12, 13].

A low or normal ESR has been reported in 5–34% of the patients with biopsy-proven temporal arteritis [14–17]. In our cohort, 20% of the patients had a normal ESR based on the formula by Miller et al. [11] for calculating the upper limit of normal. About half the patients in our series with a normal ESR also had a normal CRP.

Other studies have suggested that a lower threshold for ESR should be used in the diagnosis of temporal arteritis. A population study of 3,910 healthy Norwegian adults found that the upper reference levels expected to be exceeded by chance 5% of the time were 19 mm h⁻¹ for men and 23 mm h⁻¹ for women over the age of 90 years [18]. Using that criterion, all but 1 patient in our series would have had an elevated ESR.

The ‘classic’ description of a positive temporal artery biopsy has been described as granulomatous inflammation in all layers, primarily centered at the internal elastic lamina, accompanied by intimal hyperplasia and breaks

in the internal elastic lamina [4–6]. The internal elastic lamina has been suggested as a possible target of the inflammatory reaction [1].

Other experimental data suggest that temporal arteritis is initiated in the outer layer of the arterial wall, with CD4 T cells entering the artery wall through the vasa vasorum and subsequently orchestrating macrophage differentiation [10]. The inflammatory cells then infiltrate into all layers of the artery wall, leading to myofibroblast proliferation, luminal stenosis, and tissue ischemia. The gatekeepers of the vasculitic process are thought to be toll-like receptors located on dendritic cells at the adventitia-media junction [10]. T-cells with signs of recent activation have been found in the adventitia of the artery wall [19], and macrophages in the adventitia resemble highly activated macrophages that circulate in the blood of temporal arteritis patients, producing interleukin-6 [20].

Our findings support the theory that the muscularis and/or adventitia play an inciting role in the pathogenesis of temporal arteritis. In our study, biopsies of patients with temporal arteritis reveal that the principal focus of chronic granulomatous inflammation is at the level of the media and adventitia. CD68 staining, which has been shown to be a reliable immunohistochemical stain for identifying epithelioid histiocytes [7], confirmed that inflammatory cells were centered around the muscularis and adventitia rather than the internal elastic lamina. A previous immunohistochemical study has likewise shown that inflammatory cells and other inflammatory markers, including interleukin-2, are concentrated at the border between the media and adventitia [21].

Our results are not consistent with a recent study reporting that the most common pattern of inflammation

after full thickness inflammation was a concentric bilayer pattern with infiltration of the adventitia and intima with preservation of the media [22].

Conclusions

Biopsies of patients with temporal arteritis reveal that the principal focus of chronic granulomatous inflammation is at the level of the media and adventitia. Our findings are consistent with previous experimental data that suggest that the disease originates with activation of inflammatory cells in the outer layer of the arterial wall. We hypothesize that the muscularis and adventitia may play an inciting role in the pathogenesis of temporal arteritis.

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Statement of Ethics

The study protocol was approved by the university's institutional review board. An informed consent waiver was also approved by the institutional review board, as this was a retrospective study.

Disclosure Statement

None of the authors have any propriety interests, financial completing interests, or conflicts of interest related to this submission.

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