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Original Contribution

Evaluation of deeper levels in initially negative temporal artery biopsies and likelihood of a positive result

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ABSTRACT

Giant cell arteritis is a vasculitis that affects large- and medium-sized vessels in patients over the age of 50 years. The demonstration of granulomatous arteritis is the criterion standard to establish a definitive diagnosis. However, temporal arteritis is known to discontinuously involve the artery, and there is no standardization of the number of sections which should be examined in a length of sampled artery. The goal of the study is to determine, if by examining additional sections from temporal artery (TA) biopsy cases initially interpreted as negative, do we uncover cases of vasculitis. We conducted a retrospective review of the clinical and histologic features of 75 consecutive temporal artery biopsy cases. Our findings showed that the vast majority (94%) of cases that were biopsy "proven" to be negative for temporal arteritis on initial examination remained negative after examination of all subsequent deeper levels (median of 337 total levels examined). These cases were less likely to show classical GCA signs and symptoms and typically presented at a younger age than the biopsy-positive cases. However, 4 (6%) of the initially "biopsy-negative" cases did turn out to be positive on deeper levels, with 56, 109, 346, and 590 total levels examined, respectively. At least 2 of these 4 patients did not receive prednisone or were weaned off prednisone treatment and experienced persistent/recurrent GCA symptoms. We conclude that routine sampling may miss the diagnosis in a subset of cases and in some cases, sectioning deeper into the paraffin block may be warranted.

1. Introduction

Giant cell arteritis (GCA) or temporal arteritis is a vasculitis that affects large- and medium-sized vessels in patients over the age of 50 years. The demonstration of granulomatous arteritis on a tissue sample of the temporal artery (TA) is the criterion standard from the American College of Rheumatology (ACR) to establish a definitive diagnosis and it has 100% specificity [1]. However, there is no standard practice regarding the number of sections which should be examined in a length of sampled artery. Due to the patchy or "skip" nature of the disease, the positive diagnostic yield of this test is low with a relatively low sensitivity (15–40%) [2]. Our objective in this study is to determine how many cases that were initially diagnosed as negative when routine sectioning was employed actually harbored vasculitis when the entire block was sectioned and examined.

2. Materials and methods

2.1. Patients

Institutional Review Board (IRB) approval was obtained prior to commencement of the study. The Department of Anatomic Pathology files were searched to identify a series of 75 consecutive TA biopsies performed in our institution in 2004; these biopsied patients form the study group. Patient medical records and pathology reports were reviewed to obtain clinical information on the patients studied as well as information about the biopsy itself (e.g., length of the biopsied artery). All available microscopic slides were also reviewed on each case. The number of patients who had a positive biopsy result on routine evaluation was collected.

2.2. Tissue specimens and processing

In 2004, the protocol for routine evaluation of TA biopsies was to trim the tissue into two to three 3 mm segments, embed as a lumen, then cut two sections for hematoxylin and eosin onto two slides, with

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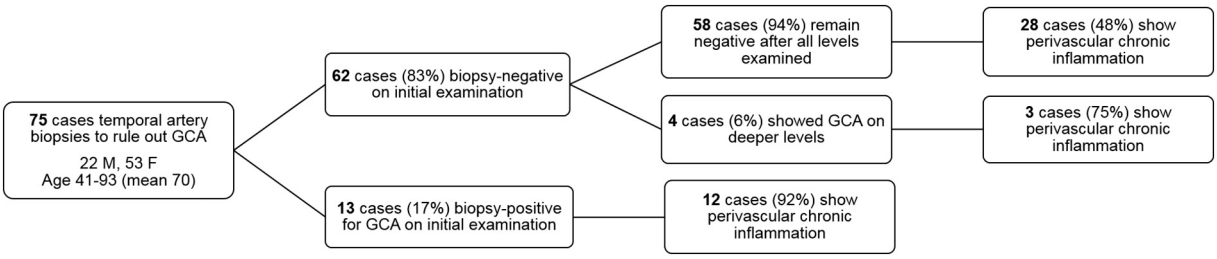

Fig. 1. Flow chart summarizing the study cohort and groupings.

Table 1
Histologic summary of the biopsy-positive versus biopsy-negative groups.

Pathologic data	Biopsy-positive cases on initial examination (n = 13)	Biopsy-negative on initial examination		P-value[a]	Total
		Remain negative after all levels examined (n = 58)	Positive on subsequent levels (n = 4)		
Median & range of arterial segment length (cm)	2.4 (0.8–4.0)	2.4 (0.3–4.5)	1.6 (0.6, 1.0, 2.1, 2.4)	0.4	2.4
Median & range of no. of initial levels examined	8 (3–28)	8 (1–24)	9 (6, 9, 9, 10)	0.2	8 (1–28)
Median & range of no. of additional levels examined	0	332 (56–664)	220 (47, 100, 340, 580)	N/A	332 (47–664)
Median & range of no. of total levels examined	8 (3–28)	337 (70–666)	228 (56, 109, 346, 590)	N/A	337 (3–666)
Perivascular chronic inflammation	12 (92%)	28 (48%)	3 (75%)	0.004	43 (57%)
Calcifications	4 (31%)	30 (52%)	3 (75%)	0.1	36 (48%)
Thrombosis	0	0	1 (25%)	N/A	1 (1.3%)
Intimal fibroplasia	13 (100%)	58 (100%)	4 (100%)	N/A	75 (100%)

[a] Two group comparison between initially biopsy-positive versus all initially biopsy-negative cases.

anywhere from one to nine levels per slide. For patients who had a negative biopsy result (n = 62), the paraffin tissue blocks were sectioned through in their entirety and stained, looking for arteritis, and at what level the arteritis was first noted. TA biopsies were considered positive if there was a mononuclear cell infiltrate in the vessel wall.

2.3. Statistical analysis

Statistical comparison was performed using chi-square analysis for categorical data and student t-test for mean comparison, with a P-value probability threshold of <0.05 considered statistically significant.

3. Results

3.1. Patient cohort

Among our cohort of 75 patients (53 females, 22 males; median age 71 years, age range 41–93 years) who underwent TA biopsies, only 13 had a positive diagnosis for temporal arteritis on routine histologic evaluation (6 males, 7 females, median age 79 years, age range 65–93 years). Among the 62 patients (16 males, 46 females, median age 68 years, age range 41–93 years) who were diagnosed as negative for temporal arteritis on initial biopsy, 4 cases turned out to be positive for temporal arteritis on subsequent levels when we sectioned into the archived paraffin blocks (Fig. 1). Thus, in our cohort, the negative predictive value of an initially negative TA biopsy was 93.5% (58/62).

3.2. Pathologic findings

The pathologic findings are summarized in Table 1 for the 3 study subgroups: 1) the cases that were biopsy-positive on initial examination (N = 13), 2) the cases that were biopsy-negative and remained negative after all subsequent levels were examined (N = 58), and 3) the cases that were initially biopsy-negative but turned out to be positive on deeper levels yielded the following findings (N = 4). The length of the arterial fragment biopsied and the total number of initial levels examined were similar across all groups (median 8–9 levels examined). For the cases in which the paraffin tissue blocks were sectioned in their entirety, a median of 332 additional levels were examined, resulting in a median of 337 total levels examined for the initially biopsy-negative cases. For the 4 cases that revealed temporal arteritis on subsequent levels, the levels that were first noted to be positive were 10, 17, 56, and 97 (median 36.5), with a median of additional 220 slides created in the process. The degree of inflammation within the arterial wall for these 4 cases was noted to be less marked than the cases that were called positive on the initial levels, and multinucleated giant cells were absent (Fig. 2A,B). More than half of all cases showed perivascular chronic inflammation (PVCI), where lymphocytic infiltration was present only in the small blood vessels in the adventitia (vasa vasorum) or in the interstitium surrounding the main temporal arterial wall (Fig. 2C). Interestingly, the frequency of PVCI was significantly higher in the initially biopsy-positive cases compared to the biopsy-negative ones (P < .004). Moreover, half of all cases showed calcifications, and all cases showed intimal fibroplasia, a reflection of atherosclerosis being a universal finding among patients in the sixth or seventh decades of their lives.

3.3. Clinical findings

Table 2 summarizes the salient clinical features of each group. Compared to those who were initially diagnosed as negative, the patients who had initially positive TA biopsies were more likely to be older (median age 79 vs 68 years), have a higher erythrocyte sedimentation rate (median ESR 83 vs 58 mm/h), and show symptoms and signs of temporal arteritis, and all of them received prednisone treatment. All the patients who had an initially positive TA biopsy received a clinical diagnosis of GCA, compared to only 8% (5 out of 62) of the patients who received a negative diagnosis on initial biopsy (P < .001). Patients were diagnosed with biopsy-negative GCA when the ACR clinical criteria were met and their symptoms improved rapidly with

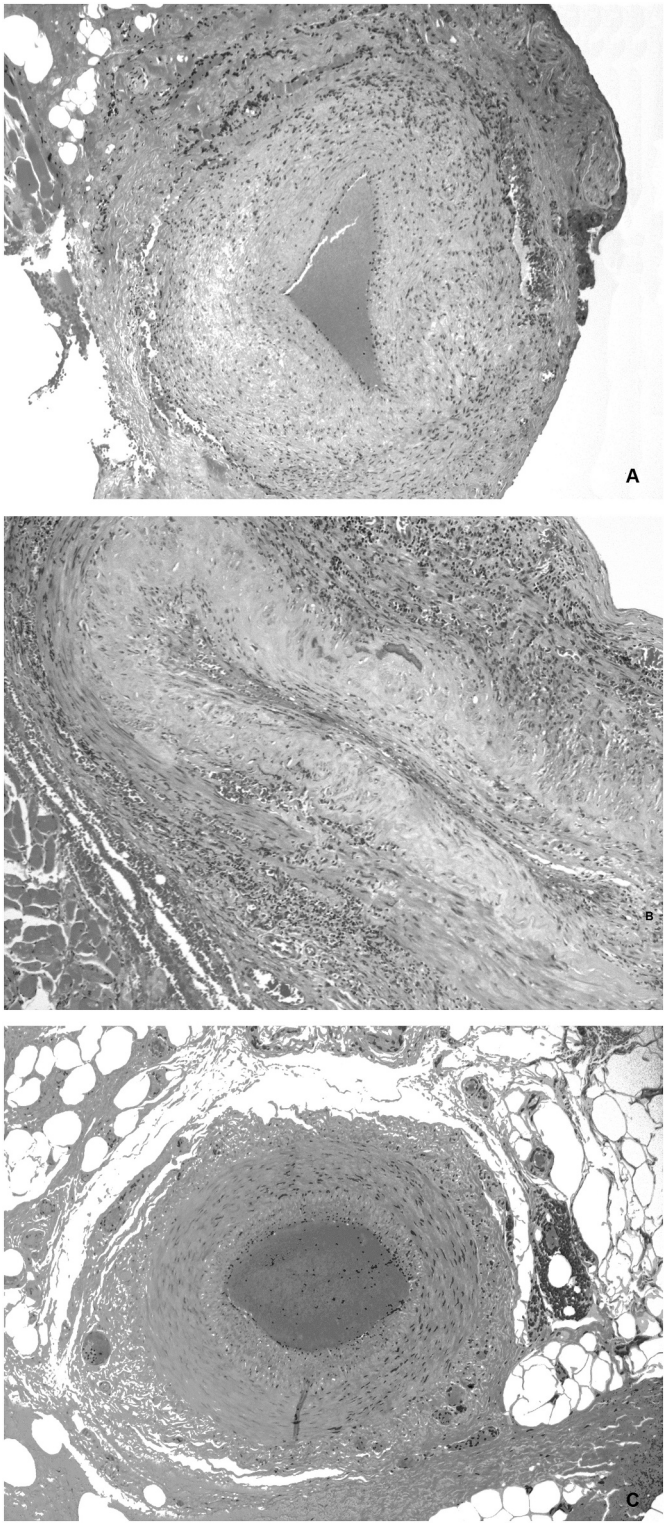

Fig. 2. A. A case of temporal arteritis demonstrating mild vasculitis, as evidenced by lymphocytic infiltration within the temporal arterial wall that was diagnosed as negative for temporal arteritis on initial temporal artery (TA) biopsy, but was discovered on deeper sectioning (hematoxylin and eosin, original magnification 100×).
B. A case of temporal arteritis demonstrating marked lymphocytic infiltration within the arterial wall that was diagnosed as positive for temporal arteritis on initial biopsy on the first level of sectioning (hematoxylin and eosin, original magnification 100×).
C. TA biopsy showing perivascular chronic inflammation but negative for vasculitis, which could potentially lead to a false positive diagnosis of temporal arteritis (hematoxylin and eosin, original magnification 100×).

corticosteroid therapy (see discussion for details).

Interestingly, despite receiving a negative diagnosis on initial biopsy, among the 46 patients whose treatment information was available, 74% still received prednisone treatment, even though only 8% were clinically diagnosed as GCA. One possible reason is that a subset of these patients had a clinical diagnosis of other rheumatologic conditions with overlapping signs and symptoms that warranted treatment with steroids. Among the biopsy-negative patients who received prednisone treatment, 82% experienced improvements of symptoms. Nevertheless, the portion of patients with a negative TA biopsy who received prednisone was still significantly less than those with a positive TA biopsy ($P = .03$) (Table 2).

3.4. Subsequently positive cases

Among the 4 cases that were biopsy-negative initially but were positive upon examination of subsequent levels, all were female (ages 61, 62, 65, and 86 years). Three out of 4 presented with classical GCA symptoms, namely, unilateral temporal headache and/or unilateral vision changes. Two had unilateral TA tenderness and occlusion of the central renal artery, respectively. Their ESR on presentation ranged from 57 to 105 mm/h (median 51 mm/h). Interestingly, even though only one of these 4 patients received a presumed clinical diagnosis of GCA despite a negative biopsy, 3 of them received prednisone, and experienced improvement of symptoms and ESR. One of the patients, nonetheless, was weaned off prednisone since she was deemed to be negative for GCA, and she experienced relapse of her symptoms and rise in ESR. The patient who never received prednisone continued to experience headaches and facial pain, and slightly elevated ESR. These clinical features are summarized in Table 3.

4. Discussion

This is a retrospective study of patients with negative TA biopsies to identify possible temporal arteritis. Since there is no standardization of the number of sections needed to be examined in a length of sampled TA [1] and given that the vasculitis may be segmentally present in an artery resulting in skip lesions, we sectioned through the remainder of the paraffin tissue blocks to see how often temporal arteritis is uncovered on deeper sections. The objective is to determine if, by examining additional sections from TA biopsy cases initially interpreted as negative, could we uncover cases of vasculitis.

In 1990, the ACR developed a set of criteria for the diagnosis of GCA. This include a *traditional format classification*: 1) age of onset ≥50 years, 2) new onset of localized headache, 3) temporal artery tenderness or decreased pulsation, 4) elevated ESR ≥ 50 mm/h, and 5) abnormal artery biopsy characterized by a predominance of mononuclear cell infiltrates or a granulomatous process with multinucleated giant cells [1]. The presence of 3 or more of these 5 criteria was associated with a sensitivity of 93.5% and a specificity of 91.2%. They also proposed a *classification tree* that were the same as the traditional format, except that elevated ESR was excluded, and 1) claudication of jaw, tongue, or on deglutition, and 2) scalp tenderness or nodules were included. The tree format was associated with a sensitivity of 95.3% and specificity of 90.7% [1].

Few studies had explored the yield of examining TA biopsies at multiple levels. Two studies looked at whether the length of the TA biopsies had any effect on the rate of positive temporal arteritis diagnosis [3,4]. The first study reviewed 173 patients with biopsy-positive or biopsy-negative GCA. After formalin fixation, the TA specimen was serially sectioned into 2 mm long slides prior to embedding. Data pertaining to number of levels examined were not reported. Biopsy-negative GCA was diagnosed when patients fulfilled the ACR classification criteria for GCA and responded favorably to steroid therapy. They determined that the rate of positive biopsies increased significantly from 19% to 71–79% when the TA biopsy length increased

Table 2
Clinical features of the biopsy-positive versus biopsy-negative groups.[e]

Clinical Parameters	Biopsy-positive cases on initial examination (n = 13)	Biopsy-negative on initial examination		*P*-value[a]	Total
		Remain negative after all levels examined (n = 58)	Positive on subsequent levels (n = 4)		
Gender	6 M, 7 F	16 M, 42F	0 M, 4 F	0.1	22 M, 53 F
Median & range of age (years)	79 (65–93)	68 (41–93)	64 (61–86)	0.0004	71 (41–93)
Median & range of ESR (mm/h)	83 (25–128)	58 (2–135)	51 (57–105)	0.08	59 (2–135)
TA symptoms[b]	13 (100%)	40 (69%)	3 (75%)	0.03	56 (75%)
TA signs[c]	10 (77%)	22 (36%)	2 (50%)	0.007	34 (45%)
Laterality	8 left, 5 right	27 left, 31 right	3 left, 1 right	0.3	38 left, 37 right
Associated PMR	2 (15%)	12 (21%)	0	0.6	14 (18.7%)
Received prednisone?	13 (100%)	31/43 (72%)[d]	3 (75%)	0.03	47 (78%)
Response to prednisone (if treated)	13 (100%)	20/28 (71%)[e]	3 (100%)	0.03	36 (82%)
Eventual diagnosis of GCA	13 (100%)	4 (7%)	1 (25%)	< 0.001	18 (24%)
Eventual diagnoses of other rheumatologic conditions	0	7 (13%)	1 (25%)	N/A	8 (11%)

ESR: erythrocyte sedimentation rate; NS: not statistical significant; PMR: polymyalgia rheumatica; TA: temporal arteritis.
[a] Two group comparison between biopsy-positive versus all initially biopsy-negative cases.
[b] TA symptoms include headache and vision disturbances.
[c] TA signs include scalp/temporal tenderness, asymmetric TA pulsability, and TA nodularity/induration.
[d] Treatment information unavailable for 15 cases.
[e] Response to treatment unavailable for 3 cases.

from ≤5 mm to 6–20 mm, and to 89% when the biopsy length was >20 mm. Thus the authors proposed that longer TA biopsy lengths are required for accurate histologic diagnosis of GCA [3]. In the second study, 1520 historical TA biopsy reports were reviewed. Data pertaining to the number of segments cut or number of levels examined were not included. The authors found that when the TA biopsy length was ≥0.5 cm, the odds of a positive GCA diagnosis with or without multinucleated giant cells were 5.7 and 4.0 times, respectively, that of a positive GCA diagnosis when the TA biopsy length was <0.5 cm. With this finding, the authors concluded that a TA biopsy length of at least 0.5 cm could be sufficient to make a histological diagnosis of GCA [4].

One study attempted to examine whether there is increase in diagnostic yield in examining multiple levels. The authors sectioned the paraffin block at three levels, each at a quarter of the thickness of the tissue, at both transverse and longitudinal directions. Out of the 101 cases that were biopsy-negative on initial sections, deeper sectioning did not reveal any cases harboring temporal arteritis. Hence the authors concluded that examining TA biopsies at multiple levels did not show increased diagnostic yield [5]. However, the current study is the first study that completely sectioned through the entire paraffin block in an attempt to uncover temporal arteritis on deeper sections.

Our findings showed that the vast majority (94%) of cases that were biopsy "proven" to be negative for temporal arteritis, on examination of <10 levels initially, remained negative, even after examination of all subsequent deeper levels. These cases were less likely to show classical GCA signs and symptoms and typically presented at a younger age than the biopsy-positive cases. They were also less likely to receive an eventual diagnosis of GCA and receive prednisone treatment. Several previous studies looked at the differences in clinical spectrum and features between biopsy "proven" versus biopsy "negative" patients with clinical suspicion for GCA [6-8]. Patients were diagnosed with biopsy-negative GCA when they fulfill the ACR criteria for GCA despite a negative biopsy, and their symptoms improved rapidly with corticosteroid therapy. In one study, compared to patients with biopsy-proven GCA (n = 207), those with biopsy-negative GCA (n = 85) were less likely to present with visual problems, jaw claudication, temporal artery palpation abnormalities, and elevated ESR, and more likely to show less specific symptoms such as headache or associated polymyalgia rhematica (PMR). None of the patients with biopsy-negative GCA developed blindness after treatment, compared to an incidence of 2.97/year/100 patients within the biopsy-positive GCA cohort [6]. These findings were echoed by another study, which showed that patients with biopsy-negative GCA (n = 29) frequently present with headache and polymyalgia rheumatica but less commonly with jaw claudication, abnormal TA on physical examination, visual complications, and constitutional symptoms, and also had lower levels of ESR compared to patients biopsy-proven GCA (n = 161) [7]. A third study found that patients with biopsy-negative GCA (n = 11) were more likely to be of older age, presented with headache and thrombocytosis compared biopsy-negative, non-GCA patients (n = 47) [8].

However, 4 (6%) of the initially "biopsy-negative" cases did turn out to be positive for temporal arteritis when we sectioned through the entire paraffin block, and the levels which were first noted to be positive were highly variable, but significantly deeper than what is commonly examined in routine pathologic practice. At least 2 of these 4 patients did not receive prednisone or were weaned off prednisone treatment and experienced persistent/recurrent GCA symptoms, due to the initially false-negative biopsy result. This indicates that the diagnostic yield may be slightly improved in examining deeper and multiple levels in TA biopsies.

Furthermore, PVCI was a frequent finding in our study, and was more commonly seen in cases diagnosed as positive on initial examination compared to the initially biopsy-negative cases. The significance of PVCI in the absence of arteritis in the diagnosis of GCA is controversial, with studies showing no difference in the frequency of patients meeting ACR criteria of GCA [9], to studies proposing that PVCI surrounding a spared TA is sufficient to establish a diagnosis of arteritis [10,11]. In our study, PVCI alone surrounding an intact, spared TA is considered negative for temporal arteritis, in keeping with the ACR standard histologic definition.

In the 2000s, the protocol for TA biopsies in our laboratory was to trim the tissue into two to three 3 mm segments, embed as a lumen, then cut two sections for hematoxylin and eosin onto two slides, with anywhere from one to nine levels per slide. Current protocol in our laboratory for TA biopsies is to trim the tissue into two to three 3 mm segments, embed as a lumen, then cut three sections for H&E plus one for Movat stain onto four slides, with two to three levels per slide.

We recommend following the histological protocol for that of sentinel lymph node examination in breast cancer [12], where the presence of metastasis carries important prognostic information, in that each lymph node should be serially sectioned every 2 mm. Following embedding as lumens, at least 5 sections, with two to three levels per slide,

Table 3
Cases positive on subsequent levels but were biopsy-negative on initial examination.

Case	1	2	3	4
Age/gender	86 F	61 F	62 F	65 F
Clinical presentation	Fatigue, global headache, facial pain, vision changes (black out), loss of central vision on left eye	Fever, joint pain, skin rash, arthralgia	Bilateral temporal headache (left > right), jaw claudication, eye flashes	Right temporal headache, vision changes in right eye
Clinical Examination	Central renal artery occlusion	Symmetric temporal arterial pulsability, no TA induration or tenderness	Left temporal tenderness	N/A
Initial ESR (mm/h)	57	58	105	59
Laterality	Left	Left	Left	Right
Presumed diagnosis	Tension headache	Possible adult onset Still's Disease	GCA	Headache[b]
Received prednisone?	No	Yes[a]	Yes	Yes (short course[c])
Subsequent course	Continue to feel weak with global headaches and facial pain. ESR: 39–53 mm/h.	Skin rash and fever resolved. ESR: 8 mm/h.	ESR:14 mm/h. Significantly improved symptoms	Vision improved initially with prednisone. Symptoms persisted/ relasped after weaned off prednisone.[d] ESR 113–132 mm/h 4 months later.
Level first noted to be positive	56	97	17	10

[a] Steroids given for presumed autoimmune disease.
[b] Initially with presumed diagnosis of GCA. But no longer considered to be GCA after biopsy came back negative.
[c] Given prednisone initially with presumed GCA diagnosis. Weaned off after biopsy came back negative.
[d] Right temporal headache, visual hallucinations, and weakness.

should be cut. Nonetheless, even with examination of multiple deeper levels, it is important for pathologists to communicate with the clinicians of the possibility of biopsy-negative GCAs, and that clinicians should not base their decision to initiate or continue steroid treatment solely on a negative histologic diagnosis; whereas, a positive TA biopsy is diagnostic of GCA.

In conclusion, we examined additional sections from TA biopsy cases initially interpreted as negative by completely sectioning through the parrafin blocks to see if we uncover cases of vasculitis. We found that the vast majority of cases that were biopsy "proven" to be negative for temporal arteritis on initial examination remained negative after examination of all subsequent deeper levels. However, considering the small group of patients that turned out to be positive for temporal arteritis on deeper sections, our study indicates that there is slightly improved diagnostic yield in examining deeper and multiple levels in TA biopsies, as a false negative histologic diagnosis in a patient who otherwise demonstrates signs and symptoms characteristic of GCA, significantly altered the management of the patient. Nonetheless, due to the relatively low yield of cases that were positive on deeper levels, the exact number of levels and depth of additional sectioning versus the additional costs incurred with obtaining multiple and deeper levels remains to be examined in future, larger scale studies.

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Declaration of competing interest

All authors report no conflict of interests or funding sources related to this study.

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