Conjunctival biopsy to diagnose neurosarcoidosis in patients with inflammatory nervous system disease of unknown etiology

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Summary

Neurosarcoidosis mimics many neurologic diseases and poses a major diagnostic challenge. Blind conjunctival biopsy is often used to help diagnose neurosarcoidosis when biopsy of affected nervous system tissue is not feasible. While this test is relatively inexpensive and well-tolerated, the diagnostic yield in patients with inflammatory nervous system disease of unknown etiology remained uncertain. We evaluated 440 patients who underwent conjunctival biopsy due to concern for neurosarcoidosis. Only a small minority of patients (3%) had positive conjunctival biopsies consistent with sarcoidosis, and some patients (1%) with positive biopsies were found to have a cause for their neurologic disease other than neurosarcoidosis. Many patients (14%) had negative conjunctival biopsies but were later confirmed to have neurosarcoidosis after additional evaluations. This study demonstrates that conjunctival biopsy has a low diagnostic yield for neurosarcoidosis in patients with inflammatory nervous system disease and suggests that alternative diagnostic means should be pursued.

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Sarcoidosis is an idiopathic multisystem disease characterized by chronic granulomatous inflammation of the lungs, skin, and other internal organs. Up to 15% of patients with sarcoidosis have neurologic involvement, known as neurosarcoidosis. Although neurosarcoidosis is most often seen in the setting of systemic sarcoidosis, it may occur with isolated neurologic involvement.¹ Any part of the nervous system can be involved, but cranial neuropathies, meningeal disease, spinal cord lesions, and parenchymal brain lesions are the most common manifestations.²

Neurosarcoidosis mimics numerous other neurologic diseases, making diagnosis particularly challenging.³ A tissue-based diagnosis is critical in order to direct treatment and clarify prognosis. A definite diagnosis of neurosarcoidosis requires biopsy of affected neural tissue with pathologic evidence of non-necrotizing granulomatous inflammation. Because of the inherent risk of the procedure and occasional eloquent location of the pathology, biopsy of extraneural tissue is often preferred.^{1,2}

Ocular involvement has been described in up to 30% of confirmed cases of neurosarcoidosis,⁴ and ophthalmologic evaluation is often performed in patients with suspected neurosarcoidosis. Conjunctival biopsy has been proposed as a safe, inexpensive, and effective method for diagnosing ocular sarcoidosis.^{5,6} A prior Mayo Clinic series showed that 38% (10 of 26) of patients with known neurosarcoidosis had a positive conjunctival biopsy diagnostic for ocular sarcoidosis despite minimal or no ocular findings prior to biopsy.⁷ This procedure is often performed in patients with presumed inflammatory nervous system pathology to determine whether neurosarcoidosis is a likely cause. We performed a retrospective chart review of patients who underwent conjunctival biopsy for evaluation of inflammatory nervous system disease possibly due to neurosarcoidosis to determine the diagnostic utility of the procedure in this setting.

METHODS

A retrospective chart review was performed for all patients with presumed inflammatory neurologic disease of uncertain etiology who underwent conjunctival biopsy to evaluate for the possibility of neurosarcoidosis. In attempting to ensure complete ascertainment, Mayo Clinic's ophthalmologic, surgical, and clinical pathology databases as well as the electronic medical record between January 1, 1997 and February 14, 2013 were searched for: (1) surgical procedural reports of conjunctival biopsy, (2) pathology reports of specimens containing conjunctival tissue, and (3) neurology clinical notes mentioning conjunctival biopsy.

Patients were included if they underwent conjunctival biopsy to evaluate for the possibility of neurosarcoidosis (figure 1). Patients were excluded if there was no mention of concern for neurosarcoidosis in either the neurology notes or the surgical indication for the conjunctival biopsy. A total of 445 patient records were reviewed. Five patients were discovered to have had conjunctival biopsy for indications other than neurosarcoidosis and were excluded (metabolic disorders 2, primary acquired melanosis 1, conjunctival melanoma 1, and conjunctival lymphoma 1). A total of 440 patients met final inclusion criteria. Demographic data including age, race, sex, and ethnicity were documented. Presenting neurologic symptoms, prior history of systemic sarcoidosis, presence of cranial nerve abnormalities, conjunctival lesions, and corticosteroid or other immunosuppressive medication use within 1 week prior to biopsy were documented. The conjunctival biopsy operative report and pathology report were reviewed for each patient, and mention of ophthalmologic findings was noted. The pathology report was reviewed and results were classified as normal, abnormal with non-necrotizing granulomatous inflammation consistent with conjunctival sarcoidosis, or abnormal with nonspecific changes or diagnoses inconsistent with conjunctival sarcoidosis.

The final diagnosis was determined by review of the neurologic clinical notes documented at last follow-up. Neurosarcoidosis was defined as definite, probable, or possible neurosarcoidosis

Figure 1 Algorithm of patient ascertainment

based on the following criteria adapted from Zajicek et al.⁸ Definite neurosarcoidosis: clinical presentation suggestive of neurosarcoidosis with positive nervous system pathology and exclusion of other possible diagnoses. Probable neurosarcoidosis: clinical presentation suggestive of neurosarcoidosis with laboratory or imaging support for nervous system inflammation and exclusion of alternative diagnoses in addition to positive non-neurologic pathology showing evidence of sarcoidosis. Possible neurosarcoidosis: clinical presentation suggestive of neurosarcoidosis with exclusion of alternative diagnoses but without pathologic evidence of either neurologic or systemic sarcoidosis.

Additional diagnoses were broadly categorized as follows: neoplasia, demyelinating disease, neurodegenerative, vascular/ischemic, idiopathic chronic meningitis/meningoencephalitis, cranial neuropathies of uncertain etiology, parenchymal brain inflammatory lesion(s) of uncertain etiology, inflammatory myelopathy of uncertain etiology, inflammatory multifocal CNS disease (affecting both brain and spinal cord) of uncertain etiology, or other specific diagnosis (spondylosis, vasculitis, etc).

To determine whether the conjunctival biopsy was a main diagnostic indicator of neurosarcoidosis, additional scrutiny was given to those patients whose conjunctival biopsies were positive for non-necrotizing granulomatous inflammation consistent with sarcoidosis. Clinical circumstances surrounding the diagnosis were carefully reviewed, and additional investigations

Figure 2 Conjunctival biopsy

Conjunctival nonkeratinizing squamous epithelium overlying substantia propria showing the presence of a non-necrotizing epithelioid granuloma surrounded by a rim of lymphocytes (hematoxylin & eosin stain; original magnification 200×).

such as prior chest and abdominal organ imaging, skin evaluation, and serum or CSF angiotensin-converting enzyme (ACE) level were documented.

Standard protocols approvals, registrations, and patient consents

This study was approved by the Mayo Foundation Institutional Review Board (IRB# 12-009819). All patients in our study had consented to the use of their medical records for future research purposes at the time of their clinical evaluation at Mayo Clinic.

Statistical analysis

Descriptive summaries were reported as median (minimum, maximum) for continuous variables and frequencies and percentages for categorical variables. Sensitivity, specificity, positive predictive value, and negative predictive value were estimated along with 95% exact binomial confidence interval (CI) using SAS version 9.3 (SAS Inc, Cary, NC).

RESULTS

Patient characteristics

A total of 440 patients were included (figure 1), and 13 patients (3%) had biopsy findings consistent with ocular sarcoidosis. Nine patients (2%) had both positive conjunctival biopsy and a final clinical diagnosis of neurosarcoidosis. Of these 9 patients, 8 were defined as probable neurosarcoidosis (no nervous system pathology confirming) and 1 as definite neurosarcoidosis (confirmed on subsequent brain biopsy). Four patients had positive conjunctival biopsy demonstrating non-necrotizing granulomas (figure 2) with an alternative final neurologic diagnosis (multiple sclerosis [MS] 1, paraneoplastic limbic encephalitis 1, compressive cervical spondylotic myelopathy coexisting with pulmonary and ocular sarcoid 1, and ocular sarcoidosis with diplopia and nonspecific MRI abnormalities but no nervous system involvement 1). The diagnosis of MS in one of these patients was supported by multiple peripheral nonenhancing lesions in the spinal cord and highly characteristic periventricular, gadolinium-enhancing, and nonenhancing lesions in the cerebral white matter meeting McDonald revised 2010 criteria for MS, without leptomeningeal or persistently enhancing lesions more characteristic of neurosarcoidosis. Thus, positive conjunctival biopsy of ocular sarcoidosis assisted

Table 1 Immunosuppressant therapy prior to conjunctival biopsy

	Total no. of patients	No. of patients on immunosuppressants within 1 wk prior to biopsy (%)
Negative conjunctival biopsy	427	124 (29)
Negative conjunctival biopsy and final diagnosis of neurosarcoidosis	60	28 (47)
Positive conjunctival biopsy	13	1 (8)

in the diagnosis of neurosarcoidosis in 9 of 440 patients (2%) and diagnosed ocular sarcoidosis in the absence of neurosarcoidosis in 4 of 440 patients (1%). The median length of time from conjunctival biopsy to last follow-up for patients with positive biopsies was 37 months (range 0–188 months).

In the 427 patients with a negative conjunctival biopsy, 60 (14%) were ultimately diagnosed with neurosarcoidosis. Of these 60 patients, 15 had definite neurosarcoidosis (positive neural tissue pathology), 22 had probable neurosarcoidosis (positive non-neural tissue pathology apart from conjunctiva), and 23 had possible neurosarcoidosis (no definitive pathologic evidence of sarcoid but consistent clinical and radiologic picture without competing diagnosis). Of the remaining 367 patients with negative conjunctival biopsies, the final diagnoses were as follows: other specific diagnosis (spondylosis, vasculitis, etc.) (101), demyelinating disease (77), idiopathic chronic meningitis/meningoencephalitis (50), inflammatory myelopathy of uncertain etiology (40), neoplasia (33), parenchymal brain inflammatory lesion(s) of uncertain etiology (21), cranial neuropathies of uncertain etiology (19), vascular/ischemic (12), neurodegenerative (11), and inflammatory multifocal CNS disease (affecting both brain and spinal cord) of uncertain etiology (3). The median length of time from conjunctival biopsy to last follow-up for patients with negative biopsies was 7 months (range 0–197 months).

Most patients in this study did not have the gold standard diagnostic test for neurosarcoidosis (neural biopsy) available. However, when counting only patients with definite or probable diagnoses as true neurosarcoidosis (excluding possible cases), conjunctival biopsy was found to have a sensitivity of 19.6% (95% CI 9.4%–33.9%), a specificity of 99% (95% CI 97.4%–99.8%), a positive predictive value of 69.2% (95% CI 38.6%–90.9%), and a negative predictive value of 91.3% (95% CI 88.3%–93.8%).

Immunosuppressant therapy Concomitant use of immunosuppressive medications at the time of conjunctival biopsy was reviewed (table 1). Twenty-three patients did not have updated medication lists available at the time of biopsy. Of the 440 included patients, 125 (28%) were on immunosuppressive medications within 1 week prior to their conjunctival biopsy. Twenty-eight of the 60 patients (47%) with negative conjunctival biopsy and a final diagnosis of neurosarcoidosis were on an immunosuppressive medication within 1 week prior to their conjunctival biopsies. One of the 13 patients (8%) with positive conjunctival biopsy was on immunosuppressive medication at the time of biopsy.

A subset analysis was performed on the 292 patients with medication lists available who were not on immunosuppressant medications. Ten of these patients (3%) had positive biopsy. Eight patients (3%) had both positive conjunctival biopsy and a final clinical diagnosis of neurosarcoidosis (1 definite, 7 probable). Conjunctival biopsy was negative in 282 of these patients, of which 30 (11%) were ultimately diagnosed with neurosarcoidosis (9 definite, 13 probable, 8 possible).

Patients with positive conjunctival biopsy Regarding the 13 patients with a positive conjunctival biopsy (table 2), 8 were female, 5 were male, and their median age was 51 years

Table 2 Patients with positive conjunctival biopsy

Age, y	Sex	Ethnicity	Prior diagnosis of sarcoidosis	Chest CT	ACE level	Final diagnosis
44	Female	White	No	Consistent with sarcoid	Normal	Definite neurosarcoid ^a
58	Male	White	Yes ^b	Consistent with sarcoid	Normal	Probable neurosarcoid
61	Female	Other	No	Consistent with sarcoid	Normal	Probable neurosarcoid
45	Female	Black or African American	No	Consistent with sarcoid	Normal	Probable neurosarcoid
63	Male	White	No	Numerous calcified granulomas; not typical for sarcoid	Normal	Probable neurosarcoid
51	Female	White	No	Consistent with sarcoid	Normal	Probable neurosarcoid
55	Female	White	No	No CT available	Normal	Probable neurosarcoid
36	Male	Other	No	Consistent with sarcoid	Normal	Probable neurosarcoid
73	Female	White	No	Consistent with sarcoid	Elevated	Probable neurosarcoid
47	Male	White	No	Consistent with sarcoid	Elevated	Ocular sarcoid without CNS involvement
37	Female	White	No	Numerous patchy infiltrates and several slightly enlarged mediastinal lymph nodes; not typical for sarcoid	Elevated	MS
69	Male	White	No	Centrilobular emphysema; small calcified hilar lymph nodes and granuloma; not typical for sarcoid	Elevated	Small cell lung cancer with paraneoplastic limbic encephalitis
40	Female	Other	No	Consistent with sarcoid	Elevated	Cervical spondylotic myelopathy coexisting with pulmonary/ocular sarcoid

Abbreviations: ACE = angiotensin-converting enzyme; MS = multiple sclerosis.
^aConfirmed by brain biopsy 1 week after conjunctival biopsy.
^bPrior mediastinal lymph node biopsy consistent with sarcoidosis.

(range 36–73). Nine patients with positive biopsy were diagnosed with neurosarcoidosis (1 definite, 8 probable). In 7 of these patients, conjunctival biopsy was the “non-neuro” pathologic finding that confirmed probable neurosarcoidosis, and in one case a mediastinal biopsy had been performed that confirmed systemic sarcoidosis (already by definition probable neurosarcoidosis). Brain biopsy was performed in 1 patient and confirmed definite neurosarcoidosis despite the findings of ocular sarcoidosis on conjunctival biopsy.

Chest CT was interpreted to be consistent with pulmonary sarcoidosis in 9 of the 13 patients with positive conjunctival biopsy. None of the patients had conjunctival abnormalities or lesions on gross ocular inspection at the time of biopsy. Serum ACE was elevated in 5 of 13 cases, including all 4 patients found to have ocular sarcoidosis without neurosarcoidosis.

DISCUSSION

This study demonstrates the low utility of conjunctival biopsy as a diagnostic method for neurosarcoidosis in patients with unknown inflammatory nervous system disease. Only a small minority of patients with conjunctival biopsies were found to have ocular sarcoidosis. Of those patients with ocular sarcoidosis, some had other radiologic or pathologic findings of systemic sarcoidosis and some were found to have a cause for their neurologic disease other

Many patients had conjunctival biopsies without pathologic evidence of ocular sarcoidosis but were confirmed later to have definite, probable, or possible neurosarcoidosis.

than neurosarcoidosis. Many patients had conjunctival biopsies without pathologic evidence of ocular sarcoidosis but were confirmed later to have definite, probable, or possible neurosarcoidosis.

Conjunctival biopsy has been proposed as an easy, low-morbidity first procedure in the diagnosis of sarcoidosis, even in the absence of visible conjunctival lesions.^{5,9,10} However, the diagnostic yield of conjunctival biopsy was very low in this consecutive series of patients in whom there was a clinical suspicion of neurosarcoidosis as the etiology of an inflammatory nervous system disease. Conjunctival biopsy may add diagnostic certainty to highly selected cases with a very high pretest probability of ocular sarcoidosis. A limitation of this study is that patients were not prospectively assigned as having high, low, or indeterminate pretest probability of having neurosarcoidosis. Further, it is possible that patients with a very high likelihood of neurosarcoidosis do not have a conjunctival biopsy in attempt to confirm this and therefore have been missed by this study design. The patients in this study did not have evidence of ocular sarcoidosis on gross inspection, and evaluation of this may assist in better patient selection. Although blind biopsy of conjunctival tissue may be of higher yield than alternative blind biopsy sites such as muscle or bone marrow, it is considered inferior to targeted biopsy sites of abnormal tissue.⁷

The use of immunosuppressive therapy at the time of conjunctival biopsy may have reduced the diagnostic yield of ocular sarcoidosis observed in this study. While ongoing neurologic impairment was seen in these biopsied patients despite that concomitant therapy (almost half of the patients with a final diagnosis of neurosarcoidosis were on immunosuppressants at the time of their negative conjunctival biopsy), it is conceivable that ocular sarcoidosis is treatment-responsive and had resolved with the concomitant immunosuppressive therapy. Ocular sarcoidosis may have been identified pathologically in a greater number of patients with neurosarcoidosis if not confounded by the use of immunosuppressive therapy. The doses and types of immunosuppressant medications were very heterogeneous in this series of patients, and the effect of immunosuppressive medications on conjunctival biopsy results is not well-established. Biopsies from patients on immunosuppressant medications may have been altered by long-lasting medication effects, even if medications were stopped within 1 week of biopsy.

As in other tissues, the role of pathology in the diagnosis of ocular sarcoidosis is the identification of epithelioid granulomas with no or minimal necrosis and the performance of studies to exclude a potential infectious etiology. However, the finding of granulomas alone is not diagnostic of sarcoidosis. This diagnosis is best achieved when the clinical and radiologic findings are supported by the demonstration of microorganism-negative non-necrotizing granulomas in a biopsy specimen.¹¹

As approximately 90% of patients with neurosarcoidosis have other systemic features of sarcoidosis, comprehensive physical examination, ophthalmic evaluation, chest CT, and liver function tests should be performed. 18-Fluorodeoxyglucose PET scanning may assist in identifying potential biopsy sites,¹² and spinal cord hypermetabolism in patients with active

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myelopathy due to sarcoidosis has been described.¹³ Neurosarcoidosis is a complex disorder and requires a multifaceted approach to diagnosis. This study suggests that blind conjunctival biopsy has a low diagnostic yield for neurosarcoidosis in patients with inflammatory nervous system disease of unknown etiology, and a search for extraneural locations of active pathology is essential.

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