Utility of Acid-Fast Staining for Detection of Mycobacteria in Cutaneous Granulomatous Tissue Reactions

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ABSTRACT

Objectives: Ancillary molecular testing on tissue is available for mycobacterial disease; however, judicious use of highly sensitive tests, such as polymerase chain reaction (PCR) and sequencing, should be guided by histologic parameters. We sought to investigate the utility of performing acid-fast stains (AFS) on skin biopsy specimens with granulomatous inflammation without an otherwise obvious histologic or clinical explanation.

Methods: Our retrospective review identified 31 patients with biopsy specimens showing granulomatous inflammation that had simultaneous AFS and mycobacterial culture or PCR performed.

Results: Biopsy specimens from eight (25.8%) patients had AFS interpreted as positive or suspicious for acid-fast bacilli. Eight had positive cultures and one had positive PCR. One biopsy specimen with AFS that showed occasional acid-fast structures that were interpreted as “suspicious” for mycobacteria was associated with a negative culture and negative PCR. Three (9.7%) biopsy specimens with negative AFS had positive cultures, and 19 (61.3%) biopsy specimens with negative AFS also had negative culture results. In our biopsy specimens, sensitivity of AFS was 72.7% and specificity was 95.0%. Positive predictive value of AFS was 88.9%, and negative predictive value was 86.4%.

Conclusions: AFS has good sensitivity and excellent specificity and should be performed on all unexplained granulomatous tissue reactions of skin in conjunction with mycobacterial culture.

Granulomatous skin reactions are a commonly encountered pattern of inflammation in skin biopsy specimens, which brings up a broad histologic differential diagnosis that includes reaction to ruptured cysts and pilosebaceous units, response to foreign antigen, primary granulomatous dermatitides, and infection—especially mycobacterial and fungal. When a specific cause for granulomatous inflammation, such as foreign material or keratin debris, is not identified, acid-fast stains (AFS) are often performed to evaluate for evidence of a mycobacterial infection. Mycobacterial culture or polymerase chain reaction (PCR) is the standard of care for diagnosing a mycobacterial infection from tissue, but despite expertise in determining species and antimicrobial sensitivity, culture methods still require many weeks for final results. Previous studies have shown that the PCR test (with or without...
sequencing) has higher sensitivity than histopathologic examination of AFS. However, some studies have shown only a marginal and not statistically significant difference,\(^1,2\) while others have found a markedly significant difference in sensitivity when comparing tissue AFS with PCR.\(^3,4\) Regardless, the use of a molecular test for a tissue sample that does not demonstrate organisms on special stains has arguable value in the setting of inappropriate clinical history. We sought to evaluate the utility of AFS in skin biopsy specimens showing granulomatous inflammation compared with culture as the gold standard.

### Materials and Methods

This study was a retrospective review conducted with institutional review board approval from Brigham and Women’s Hospital, Boston, MA. We searched for biopsy specimens from patients with unexplained granulomatous tissue reactions for whom AFS was reviewed and who also had tissue culture and/or PCR performed for mycobacteria at Brigham and Women’s Hospital between 2005 and 2013. Multiple biopsy specimens from the same patient on different days were considered distinct single events for the purposes of data analysis. Sensitivity, specificity, and positive and negative predictive value of AFS were calculated. Biopsy specimens taken during antibiotic treatment or from patients who had recently completed a course of antibiotics were not included.

### Results

We identified 31 patients with granulomatous tissue reactions in skin biopsy specimens who also had AFS performed and tissue sent for culture or PCR [Table 1]. Eight (25.8%) patients with AFS interpreted as positive or suspicious for acid-fast bacilli (AFB) had positive cultures, and one patient who did not have a culture performed had a positive PCR. Cultures identified the following species: *Mycobacterium avium* complex (n = 2), *Mycobacterium marinum* (n = 2), *Mycobacterium haemophilum* (n = 2), *Mycobacterium chelonae* (n = 1), and *Mycobacterium abscessus* (n = 1). In the patient with no culture performed, PCR detected *Mycobacterium kansasii*. Only one biopsy specimen with an AFB stain that showed occasional AFS-positive structures that were “suspicious” for mycobacteria was associated with a negative culture and negative PCR. No cases with AFS that were interpreted as unequivocally positive for mycobacteria had negative cultures. Three (9.7%) biopsy specimens with negative AFS had positive cultures. Cultures from two biopsy specimens grew *M marinum* and one grew *Mycobacterium tuberculosis*. Of interest, the latter infection was contracted in a laboratory worker handling mycobacteria. All 19 biopsy specimens with negative AFS also had negative culture results. One patient with a negative AFS and negative culture also had negative PCR. Sensitivity of AFS was 72.7%, and specificity was 95.0%. The positive predictive value was 88.9%, and negative predictive value was 86.4%.

### Discussion

A distinctive feature of bacteria in the genus *Mycobacterium* is a high content of mycolic acid within the cell wall. The presence of mycolic acid confers to mycobacteria the property of retaining red carbol fuchsin, as in the Ziehl-Neelsen technique (the most commonly used AFS), despite a decolorization procedure with alcohol and acid, giving us the designation acid-fast.

Numerous studies have examined the utility of AFS in lung disease; however, many of these studies are of cytology smear and sputum specimens.\(^5,8\) Furthermore, most studies examining the sensitivity and specificity of AFS in the setting of pulmonary disease tend to be from patient populations with a high incidence of tuberculosis. One study of AFS on skin specimens demonstrated that only 13.8% of biopsy specimens from patients with *M tuberculosis* infection and lesions “clinically compatible” with tuberculosis of the skin had a positive AFS, whereas the PCR was positive in 48 (73.8%) of 65 biopsy specimens [Table 2].\(^3\) In another study, *M tuberculosis* DNA was demonstrated by PCR in paraffin-embedded sections in all five patients studied, with *M tuberculosis* demonstrated by routine culture methods in only three of the five

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**Table 1**

<table>
<thead>
<tr>
<th>Tissue AFS</th>
<th>Culture Positive</th>
<th>Culture Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFS positive/ suspicious</td>
<td>8</td>
<td>1* (PCR was also negative)</td>
</tr>
<tr>
<td>AFS negative</td>
<td>3</td>
<td>19 (PCR was also negative in one patient)</td>
</tr>
</tbody>
</table>

*AFS, acid-fast stain; PCR, polymerase chain reaction.

*The single culture-negative, AFS-positive case was read as “suspicious.”

**Table 2**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Specimen Stained</th>
<th>Sensitivity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Skin biopsy specimen</td>
<td>5.8</td>
</tr>
<tr>
<td>3</td>
<td>Smear prepared from skin biopsy specimen</td>
<td>13.8</td>
</tr>
<tr>
<td>9</td>
<td>Skin biopsy specimen</td>
<td>60.0</td>
</tr>
</tbody>
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cases.\textsuperscript{2} AFS was reported in only one patient and was negative, and one patient had a negative Fite stain but an AFS was not reported (this same patient had a positive AFS performed on a lymph node aspirate).

Another comparative study of the utility of PCR vs histopathologic, smear, and culture examination of biopsy specimens for \textit{M tuberculosis} in a high-risk population showed that PCR was the most sensitive test (79.4%). The authors examined AFB stains of smears from biopsy specimens but did not examine AFB stains of the actual biopsy specimens. They found a sensitivity of only 5.8% for smear specimens, a remarkably low number given the samples were from a high-risk population.\textsuperscript{1} In another study, the sensitivity of AFS was 60% (vs 93% by PCR) for \textit{M tuberculosis} but 90% (vs 80% by PCR) for nontuberculous mycobacteria in a recent study,\textsuperscript{4} suggesting that AFS are less sensitive for detecting \textit{M tuberculosis}.

Whereas identification of AFB on histologic sections allows a diagnosis of certitude, identification of mycobacterial DNA by PCR may also amplify DNA fragments in patients with current or prior infection in other organs (nontuberculous). Primers employed for standard PCR of the genus cannot distinguish \textit{M tuberculosis} from \textit{Mycobacterium bovis} or the bacillus Calmette-Guérin strain. In one study, the PCR test result was positive for \textit{M tuberculosis} in a healthy control patient, raising the possibility of false-positive results.\textsuperscript{9}

We were unable to identify any studies examining the sensitivity and specificity of AFS in skin biopsy specimens showing granulomatous inflammation. For many reasons, it seems highly inappropriate to extrapolate from these previous studies to inform us on the utility of AFS of skin biopsy specimens.

As Renshaw and Gould have stated, “One of the more tedious tasks in histology is review of AFS specimens.”\textsuperscript{10} As these authors point out, we are left wondering if the AFS is worth the bother when tissue can be sent for culture or PCR. The results of our study demonstrate that the AFS in the setting of otherwise unexplained granulomatous tissue reactions in skin has good sensitivity and is extremely specific. These results unhesitatingly support the liberal use and careful scrutiny of the AFS in the setting of granulomatous tissue reactions.

Another reason for liberal use of the AFS is the rapid turnaround time and low cost compared with culture and PCR. While the AFS can be reviewed within 24 hours of ordering, the mean waiting time to obtain a culture result in our cases was 58.11 days (range, 13-242 days). PCR with sequencing can range from 1 to 3 weeks, although results for \textit{M tuberculosis} and \textit{M avium} complex are often faster (facilitated by the use of species-specific probes). A positive AFS, therefore, allows the clinician the opportunity of an initiating treatment much earlier while waiting for culture results and speciation of the organism. While we included a case in which the AFS was interpreted as “suspicious” for mycobacteria along with the frankly positive AFS in our calculations of specificity, a clinician who only treated patients with AFS that were interpreted as frankly positive in our study would not have treated a single patient unnecessarily.

We conclude that histologic workup of an unexplained granulomatous tissue reaction of skin should include a carefully scrutinized AFS, which shows good sensitivity and excellent specificity.

\textbf{References}