The Sensitivity and Specificity of Touch Preparation for Rapid Diagnosis of Invasive Fungal Sinusitis: A Pilot Study

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Abstract
Invasive fungal sinusitis is a morbid pathology that typically affects immunocompromised patients and may quickly progress to fulminant disease. The purpose of this study was to measure the sensitivity and specificity of touch preparation of nasal debridement specimens as a rapid diagnostic tool for invasive fungal sinusitis. A retrospective chart review was performed of 22 patients undergoing nasal debridement due to suspicion for invasive fungal sinusitis over a 10-year period. Thirteen patients had touch preparation of nasal specimens followed by routine histologic processing; two of these patients underwent 2, and 1 patient had 3 separate debridements, for a total of 17 touch preparations performed. The sensitivity and specificity of touch preparation were calculated by correlating the initial results with the presence of fungal invasion on final pathologic analysis. The sensitivity of touch preparation was 56% (95% confidence interval [CI]: 0.23-0.85), specificity was 100% (95% CI: 0.60-1.00), positive predictive value was 100% (95% CI: 0.46-1.00), and negative predictive value was 67% (95% CI: 0.35-0.89). This procedure may be a useful adjunct in situations requiring rapid diagnosis of invasive fungal sinusitis but should not be used as the sole criteria for determining the need for surgical intervention.

Keywords
touch preparation, cytologic imprint, invasive fungal sinusitis, rapid diagnosis

Introduction
Invasive fungal sinusitis (IFS) is a rare but serious infection that most commonly presents in immunocompromised individuals and carries a risk of significant morbidity and mortality. A recent review of the literature reported an overall survival rate for IFS of less than 60%.1 Common fungal pathogens implicated in IFS include Rhizopus, Mucor, Rhizomucor, and Aspergillus species. The potential for rapid progression to fulminant disease makes timely diagnosis and management of this entity imperative; early antifungal therapy and surgical debridement have been associated with a survival advantage.1,2

Histopathologic evaluation of tissue for the presence of hyphae is an important adjunct to clinical examination for the diagnosis of IFS. Permanent section with Gomori methenamine silver (GMS) is the most sensitive of the commonly used special stains but requires substantial time for tissue processing and staining when compared to frozen sections or touch preparations (TPs).3 Alternatively, frozen sectioning (FS) of debrided tissue yields a more rapid diagnosis and, if positive, supports the need for appropriate antifungal therapy and surgical debridement to be initiated without delay.4 The FS analysis can be performed on bedside biopsies, such as the middle turbinate,5 or from intraoperative tissue specimens. Previous studies have demonstrated that FS has a high positive predictive value (PPV) for both bedside and intraoperative diagnosis of acute IFS.6

Touch preparation provides an alternative or complimentary histological method to FS for rapid diagnosis of fungal pathology. Touch preparation is performed by touching the margin of submitted tissue onto a glass slide to form an imprint, followed...
by air-drying, Diff-Quik staining, and microscopy. A preliminary diagnosis of the presence of fungal forms can be obtained within 15 minutes using the TP technique. Touch preparation has been shown to have a similar accuracy to single FS for intraoperative evaluation of sentinel lymph nodes in breast cancer.7 Within the dermatologic literature, TP has been reported to be a viable technique for the rapid diagnosis of multiple fungal entities, including cryptococcal skin lesions,8 cutaneous zygomycosis,9 and disseminated candidiasis.10 We are unaware, however, of any previous study that has attempted to evaluate the accuracy of TP for the diagnosis of IFS.

**Patients and Methods**

A retrospective chart review was performed of patients undergoing nasal debridement due to suspicion for IFS between March 2003 and April 2013 at an academic tertiary care medical center. Approval was obtained from the institutional review board. Patients were identified by International Classification of Diseases, Ninth Revision and Common Procedural Terminology code search through the electronic medical record, as well as a separate pathology database. Of 22 patients undergoing surgery for suspected IFS, 13 had initial TP analysis of nasal specimens. Only those patients who underwent both permanent section and TP were included in this retrospective chart review. Data regarding demographics, comorbidities, pathologic results, fungal organisms, and short-term survival outcomes were recorded for each patient. A patient was considered alive if they survived to hospital discharge without expected imminent mortality (eg, hospice care). In several cases, patients recovered from the episode of IFS but ultimately succumbed to unrelated complications of their underlying disease; these patients were considered alive for the sake of this analysis.

Tissue designated for TP was removed from the patient and placed on a saline-moistened nonadhesive Telfa gauze pad (Medtronic—Covidien, Dublin, Ireland) and transferred to a dry specimen container. The TP method consisted of using forceps to lightly touch the margin of submitted tissue onto a glass slide, forming an imprint. Minute amounts of material were transferred to the slide, including fragments of sinus mucosa, inflammatory cells, mucous, and fungal hyphae. The sample was then air-dried, stained using the Diff-Quik method, and examined microscopically. As most samples were small, a single TP was usually adequate to imprint the entire margin of the specimen, although a second imprint was considered for larger specimens. Some TP samples had only rare or scattered hyphal fragments, as illustrated in Figure 1A and B, and hyphae could be missed if tangled in debris or obscured by inflammation. If hyphal elements were identified, the TP was considered positive. Frozen section analysis could then be performed at this point in the workflow, depending on pathologist preference.

All samples were formalin fixed, paraffin embedded, and subsequently stained with hematoxylin and eosin (H&E) and GMS, as displayed in Figures 1C and D. Results from TP of nasal tissue and final pathologic interpretation were compared. Ninety-five percent confidence intervals (CIs) were calculated.
for sensitivity, specificity, PPV, and negative predictive value (NPV) using the efficient score method (corrected for continuity).

**Results**

A total of 22 patients with suspicion for IFS were identified between March 2003 and April 2013. Thirteen patients had TP of nasal specimens followed by routine histologic processing; 2 of these patients underwent 2, and 1 patient had 3 separate debridements, for a total of 17 TPs performed. The remaining patients had only FS or permanent histologic analysis and were excluded. The 8 male and 5 female patients ranged from 13 to 66 years old at the time of diagnosis. Demographic data for each patient, comorbidities, and short-term survival outcomes for the acute illness are listed in Table 1.

Of the 13 patients undergoing TP, 7 were found to have IFS on final pathologic analysis (patients P1-P7), whereas 6 patients had no evidence of fungal invasion (patients N1-N6). Three patients (N1, P4, and P7) had multiple nasal biopsies on separate occasions; each specimen is counted as a separate data point for analysis. One patient (P7) had nasal debridement performed on 3 separate occasions, each preceded by a negative TP; the first 2 procedures produced positive pathologic specimens, whereas the final debridement showed successful clearance of invasive fungal disease. The TP and final pathologic results for each patient are depicted in Table 1. Where available, fungal organism is also reported, although in most cases definitive classification was not possible due to inherent limitations in pathologic processing techniques. For patients P5 and P6, available data from positive fungal cultures taken at the time of debridement are included.

The calculated sensitivity of TP was 56% (95% CI: 0.23-0.85), specificity was 100% (95% CI: 0.60-1.00), PPV was 100% (95% CI: 0.46 -1.00), and NPV was 67% (95% CI: 0.35-0.89).

Where applicable, the results of concurrent FS are also included in Table 1. For the 13 specimens upon which both TP and FS were utilized, there was only one case of disagreement: patient P4, sample #2 had a negative FS but positive TP.

**Discussion**

Acute IFS is an uncommon infection that occurs predominantly in immunocompromised patients, often resulting in rapid progression and death if not diagnosed and treated promptly. Previous studies have identified an overall survival rate for IFS of less than 60%. Our small cohort supports the direness of this condition, with 43% (3/7) of patients with pathology-proven fungal sinonasal invasion succumbing to their acute illness. Previous studies have shown that early antifungal therapy and surgical debridement of necrotic tissue are associated with a survival advantage. Currently, the gold standard for the diagnosis of IFS is pathological examination of permanent sections.
using H&E and GMS stains, but this can be a time-consuming process.\(^3\)

Touch preparation is a histopathologic procedure that offers the potential for rapid diagnosis of fungal forms in debrided tissue. Touch preparation can be performed on biopsy specimens or nasal crusts removed at the bedside of a patient suspected of having IFS. Previous studies have demonstrated that TP has a similar accuracy to FS for intraoperative evaluation of sentinel lymph nodes in breast cancer.\(^7\) Touch preparation also has an established role in dermatopathology for the rapid diagnosis of multiple cutaneous fungal diseases.\(^8\)-\(^10\)

In this retrospective study, a total of 17 TPs were performed on 13 patients over a 10-year period. The accuracy of TP was compared with permanent H&E- and GMS-stained slides. Four TPs were found to represent false negatives, whereas there were no false-positive TPs. Overall, the calculated sensitivity of TP was 56\% (95\% CI: 0.23-0.85), specificity was 100\% (95\% CI: 0.60-1.00), PPV was 100\% (95\% CI: 0.46-1.00), and NPV was 67\% (95\% CI: 0.35-0.89). As a comparison, in a study of 20 patients with IFS over a 22-year period by Ghadiali et al, frozen section was found to have a sensitivity of 84\%, specificity of 100\%, PPV of 100\%, and NPV of 72\% for the rapid diagnosis of IFS.\(^6\)

Our small, retrospective study was not designed to provide a head-to-head comparison between the accuracy of TP and FS, and the decision on whether to perform one or both techniques was nonstandardized. In our institution, the decision to perform TP versus FS as the initial rapid diagnostic tool for IFS is made by the pathologist involved in the consultation. Touch preparation may be selected if the consulting physician is a cytopathologist, and thus more comfortable with the technique, specimen is limited (TP preserves specimen for subsequent permanent sectioning), or high laboratory workload makes FS a more time-consuming endeavor.

This study illustrates that TP has high specificity and PPV as a diagnostic test for IFS, but only moderate sensitivity and NPV. Ghadiali et al reported a similar pattern for FS in the diagnosis of IFS,\(^6\) although sensitivity and NPV were better for FS. Both studies suffer from relatively low patient numbers, however, making a direct comparison of the specificity and sensitivity of these techniques across studies problematic.

For those samples upon which both TP and FS were performed, the results are included in Table 1. In all cases, there was concordance between TP and FS, with the exception of a single specimen, patient P4, sample 2, in which the FS was negative but TP positive. Although this finding might be taken to suggest that at our institution TP is equally if not more sensitive than FS in the diagnosis of IFS, caution should be used in comparing these techniques within our data set, as pathologists were not blinded to the results of each test, and in practice, they may be used complimentarily to optimize rapid diagnostic accuracy. Future prospective studies with rigorous methodology are necessary to reliably compare the utility of TP and FS for the diagnosis of invasive fungal disease.

The high specificity but only moderate sensitivity of both TP and FS in the diagnosis of IFS suggests that these techniques may be useful adjuncts in the preoperative workup of the suspected patient with IFS but should not be relied upon as sole criteria in determining the need for surgical intervention. Data from Ghadiali et al\(^6\) and the current study support the conclusion that any patient with a high pretest probability of IFS and a positive TP or FS from bedside nasal biopsy should be managed aggressively with antifungal therapy and prompt surgical debridement, as IFS is very likely. A negative rapid diagnostic test does not rule out the disease, however, and the overall clinical picture should be considered in determining further management.

The relatively low sensitivity and NPV of TP in this study may be due to the inherent difficulty of identifying the presence of fungal hyphae using TP without the benefit of GMS stains. False negatives can be attributed to the presence of only rare or scattered hyphal fragments, which may be missed if tangled in debris or obscured by inflammation. Figure 1 illustrates such a case, in which the photo depicts the only identifiable hyphal structure present in the entire slide; review of the permanent slides confirmed the presence of IFS in this patient. In the setting of strong clinical suspicion, the presence of necrosis, fragmented vessel, and submucosal elements such as multinucleated giant cells or inflammatory cell populations, even in the absence of hyphae, are suspicious for IFS.\(^11\)

With FS established as a rapid diagnostic test for IFS, the question arises as to whether TP provides additional value in the clinical setting. Different pitfalls complicate both TP and FS. Unlike TP, which demonstrates only the presence of fungal forms, FS can reveal tissue invasion, yet freezing artifact can mimic or obscure hyphae (especially zygomycetes).\(^12\) Touch preparation, in turn, does not have this processing issue. In our laboratory, TP analysis requires approximately 15 minutes and thus is often performed as the initial diagnostic test in nasal specimens from patients at risk for IFS. If TP is inconclusive or the consulting pathologist prefers, proceeding to FS is an option. Frozen and/or permanent section histology is mandatory to confirm fungal invasion of tissue but may not be required prior to formal operative debridement if clinical suspicion for IFS is high.

A significant weakness of this study is the low number of participants, which is a factor of the overall rarity of IFS. A larger, prospective study would allow for more reliable estimation of sensitivity, specificity, PPV, and NPV for TP in the rapid diagnosis of IFS, as well as provide the opportunity for rigorous comparison of TP and FS for each tissue specimen. Further research could also explore whether disease characteristics such as fungal organism or location of disease affect the sensitivity and specificity of TP and FS and furthermore if concurrent use of these techniques results in improvements in rapid diagnosis. To our knowledge, however, this study represents the first attempt to characterize the use of TP as a rapid diagnostic technique for IFS. Given the significant morbidity and mortality associated with IFS, the development of multiple methods for obtaining a timely and accurate diagnosis of this pathology is crucial.
Authors’ Note
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