

Frozen Section as a Rapid and Accurate Method for Diagnosing Acute Invasive Fungal Rhinosinusitis

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Abstract

Objective. Identify methods to improve the frozen-section diagnosis of acute invasive fungal rhinosinusitis.

Study Design. Biopsies with frozen section for suspected acute invasive fungal rhinosinusitis were reviewed to identify causes for missed diagnoses and evaluate methods for potential improvement.

Setting. All aspects of the study were performed at the Penn State Milton S. Hershey Medical Center.

Subjects and Methods. All frozen sections performed for suspected acute invasive fungal rhinosinusitis between 2006 through 2017 were reviewed with their diagnoses compared to the final diagnoses. Sensitivity and specificity were determined for each biopsy specimen to evaluate the diagnostic method and for each patient for its effectiveness on outcome. Causes for frozen-section failures in diagnosis were identified. A periodic acid–Schiff stain for fungus (PASf) was modified for use on frozen tissue (PASf-fs) and applied both retrospectively and prospectively to frozen sections to determine its ability to identify undetected fungus and improve diagnostic sensitivity.

Results. Of 63 biopsies positive for acute invasive fungal rhinosinusitis, 51 were diagnosed on frozen section, while 61 were identified by including the novel PASf-fs stain, reducing the failure rate from 19% to 3%. Of 41 cases that were positive, 34 were diagnosed on frozen section. Of the 7 that were not, 5 were identified by including the PASf-fs, reducing the failure rate from 17% to 5%.

Conclusions. Frozen section interpretation of biopsies for suspected acute invasive fungal rhinosinusitis using a PASf-fs stain should enable a rapid and accurate diagnosis with improved outcomes by shortening the time to surgery.

Keywords

acute invasive fungal rhinosinusitis, frozen section, early diagnosis, PASf, fungal sinusitis, retrospective, biopsy

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Acute invasive fungal rhinosinusitis (AIFRS) is a rare but aggressive fulminant fungal infection involving the sinonasal passages and sometimes extending to surrounding structures. The disease occurs almost exclusively in the immunocompromised host, particularly those with hematologic malignancies, including patients undergoing chemotherapy or bone marrow transplantation.¹ Other groups susceptible to AIFRS include iatrogenic immunosuppressed individuals, patients with AIDS, and diabetics.² Despite advancements in care, AIFRS continues to exhibit a mortality rate, reported as high as 50.3%.^{2,3} The angioinvasive nature of the fungus causes tissue ischemia and necrosis and can quickly spread from the nasal mucosa and sinuses into the orbit and brain. Morbidity and mortality can be reduced by early diagnosis followed by immediate treatment with aggressive surgical debridement, antifungal therapy, and immune system support.^{1,4-7} However, establishing a rapid diagnosis can be quite challenging as the presenting symptoms and early physical findings in patients with AIFRS are often nonspecific.^{1,4-7} Furthermore, sinus computed tomography (CT), the preferred imaging modality for diagnosis, does not provide pathognomonic findings or definitive evidence of bone invasion until late in the disease

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Table 1. Case Data: Accuracy of H&E and PASF-fs in the Diagnosis of AIFRS on Frozen Section following Biopsy for the Diagnosis of AIFRS in Individual Cases^a

Characteristic	No. of Cases (N = 124)	Final Diagnosis, No.	H&E Frozen-Section Diagnosis, No. (%)	PASF Frozen-Section Diagnosis, No. (%)
Positive diagnosis	41	(+) 41 (-) 0	(+) 34 (83) (-) 7 (17)	(+) 39 (95) (-) 2 (5)
Negative diagnosis	83	(+) 0 (-) 83	(+) 0 (0) (-) 83 (100)	NA NA

^aEach case contains multiple specimens.

Abbreviations: AIFRS, acute invasive fungal rhinosinusitis; H&E, hematoxylin and eosin; NA, not applicable; PASF, periodic acid–Schiff stain for fungus; PASF-fs, periodic acid–Schiff stain for fungus modified for use on frozen tissue; +, positive; –, negative.

course. Thus, diagnosis may be delayed, and maintaining a high level of concern in at-risk patients coupled with a low threshold for prompt biopsy is critical to obtaining an early diagnosis and providing timely and effective treatment. Classic histologic evaluation with special staining for fungus is the gold standard for confirming AIFRS, but it is a time-consuming process, and reliance upon it delays initiation of treatment. Therefore, evaluation of biopsies on frozen section has become the preferred method of diagnosis.^{7–11} However, historically, frozen sections have not been as sensitive for AIFRS as routine histologic processing.^{8,9} Reasons for this have been attributed to pathologic changes in tissues in AIFRS and the difficulty in identifying fungus on frozen sections. This study introduces the novel use of a stain modification to address the difficulty in frozen-section fungus identification, as well as assess its effectiveness through retrospective examination and through prospective use in a new approach to diagnosis.

Methods

Approval for the study was obtained from the Pennsylvania State College of Medicine Institutional Review Board. All sinonasal biopsy specimens sent for frozen section with a clinical suspicion for AIFRS at our institution were collected retrospectively from 2004 through 2016 and prospectively through the entire calendar year 2017. Biopsy specimens were reviewed for frozen section and final diagnoses, histopathologic changes, and the presence or absence of fungus on both hematoxylin and eosin (H&E) and Gomori Methenamine Silver (GMS) stains and re-reviewed in comparison with the Periodic acid–Schiff stain for fungus (PASF) that was developed for use on frozen sections (PASF-fs) by the study pathologists (H.C. and J.I.W.). Both pathologists have special interest in head and neck pathology and provide much of the head and neck pathology services at the institution.

The review pathologists were blinded to the frozen section and final diagnoses and the presence of fungus through all phases from the initial review and the PASF-fs re-review. Of note, there is no difference between head and neck and other surgical pathologists in the ability to identify fungus on special stains as it is a common skill required equally in all areas of surgical pathology. The morphologic features of AIFRS were used to define its pathology with characteristics

that could assist in diagnosis. A modification of the PASF was developed for use on frozen sections (PASF-fs) and was applied over the top of an original H&E frozen section slide from all available cases to determine its ability to stain fungus and enable its identification. The stain was subsequently introduced for evaluation and used prospectively in the frozen-section diagnosis of AIFRS. Sensitivity and specificity for the frozen-section diagnosis were calculated and recorded using each diagnostic procedure (patient case) as a subject case for clinical outcome. Final pathologic diagnosis was considered the true diagnosis for all calculations.

Results

The study cohort consisted of 124 diagnostic procedures (patient cases) where frozen section was performed on 271 individual biopsy specimens for the diagnosis of AIFRS. On final diagnosis with permanent section, 41 patient cases were positive and 83 were negative. No false-positive diagnoses were made, as all of the cases receiving a false-positive biopsy specimen diagnosis had a true positive diagnosis on another biopsy. Of the 41 positive patient cases, 34 (83%) were diagnosed with AIFRS on H&E frozen section (**Table 1**) and 7 (17%) were called negative (false negative). Of these false-negative diagnoses, 5 of 7 had invasive fungus on the PASF-fs frozen-section slides stained on review for this study. For the remaining 2 patient cases, there was no fungus on the frozen-section slides. In summary, with the addition of PASF-fs staining, the sensitivity of frozen section in the diagnosis of AIFRS increased from 85.4% to 95.2%. As no false-positive diagnoses were rendered on H&E or PASF-fs, the specificity of frozen section in diagnosing AIFRS remained 100% in our study. The advantage of this staining is evident by fungus, which cannot be seen on H&E being made visible on the PASF-fs stain (**Figure 1**). The utility of PASF-fs frozen section on a biopsy specimen basis is shown by its identifying fungus in 61 of 63 (97%) positive biopsy specimens. Of the 12 false-negative specimens on H&E, PASF-fs enabled the fungus to be visible in 10 of 12.

In evaluating the effects of the false-negative diagnoses, surgical debridement was delayed in all 7 patient cases that received a false-negative diagnosis (**Table 2**). In 1 patient case, the patient underwent additional debridement the

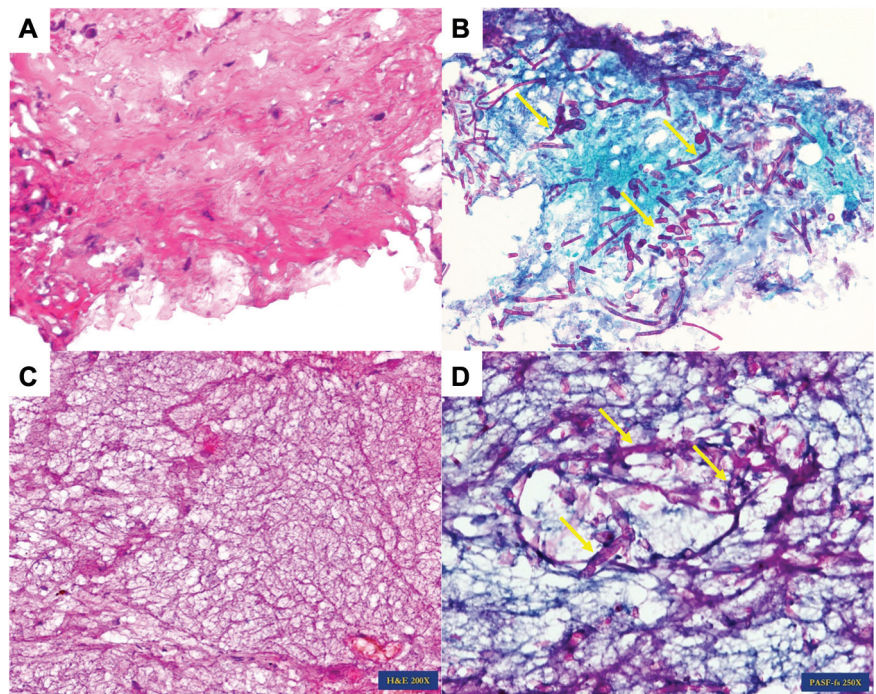


Figure 1. PASF-fs identifying fungus not seen on classic H&E staining. (A, C) H&E: complete necrosis with fungus not visible. (B, D) PASF-fs: same fields from both cases with purple staining fungus clearly visible (arrows). H&E, hematoxylin and eosin; PASF-fs, periodic acid–Schiff stain for fungus modified for use on frozen tissue.

Table 2. Specimen Data: Study Makeup and Results of Frozen-Section Re-Review and PASF-fs Staining for Each Individual Specimen.

Characteristic	Specimens (n = 271)	Final Diagnosis, No.	H&E Frozen-Section Diagnosis, No. (%)	PASF Frozen-Section Diagnosis, No. (%)
Positive biopsies	63	(+) 63 (–) 0	(+) 51 (81) (–) 12 (19)	(+) 61 (97) (–) 2 (3)
Negative biopsies	208	(+) 0 (–) 208	(+) 8 (4) (–) 200 (96)	NA NA

Abbreviations: H&E, hematoxylin and eosin; NA, not applicable; PASF, periodic acid–Schiff stain for fungus; PASF-fs, periodic acid–Schiff stain for fungus modified for use on frozen tissue; +, positive; –, negative.

following day after an intraoperative margin showed AIFRS on the subsequent permanent section. In 4 patient cases, surgical resection was delayed for 2 days until final biopsy pathology results returned as positive. The remaining patient case experienced a 5-day delay in further surgical debridement when a surgical margin of the septum was incorrectly read as negative. PASF-fs identified invasive fungus in 5 of 7 false-negative patient cases.

In determining a statistical significance, patient cases with a negative final diagnosis and having no fungus (negative on H&E and GMS) were considered negative for PASF-fs in the calculation. The difference in diagnostic classification between frozen-section H&E alone and with the PASF-fs stain was not found to reach statistical significance ($P = .07$, McNemar test). Given the rare nature of AIFRS, this study’s sample size is small, and statistical difference might be achieved by larger sample sizes.

Discussion

AIFRS is a rare and aggressive fungal infection that occurs almost exclusively in the immunocompromised host with loss of cellular immunity, particularly in those with hematologic malignancies.¹ Mortality is high and progression from initial presentation to death can be rapid, from hours to days.² Thus, when AIFRS is suspected in an at-risk patient, a rapid evaluation and diagnosis is critical. Prompt diagnosis coupled with aggressive surgical debridement and medical therapy has been shown to both improve patient outcomes and decrease the extent of surgical debridement and long-term morbidity.¹² However, an early diagnosis can be quite challenging to obtain because the presenting symptoms and findings in patients are often nonspecific. The initial evaluation of a patient with suspected AIFRS should consist of a detailed history, thorough head and neck examination, and nasal endoscopy. In at-risk patients, the presence of fever

Table 3. Patient Characteristics and Outcomes following False-Negative AIFRS Diagnosis on Frozen Section.

Patient Case No.	Findings on Nasal Endoscopy	Adverse Outcome	Outcome Due to False-Negative FS Result	Fungus on PASF-fs
1	Irregular mucosa with edema and impaired vascularity	Yes	Extensive debridement delayed 2 days until final diagnosis	Yes
2	Irregular mucosa with edema and impaired vascularity	Yes	Treatment delayed 24 hours and AIFRS spread into orbit	No
3	Necrotic changes of middle turbinates bilaterally	Yes	Septectomy was not done following negative FS	Yes
	Slight discoloration of nasal septum		Disease progressed and septectomy + additional debridement done 5 days later	
4	Erythema, crusting, and petechiae bilaterally	Yes	Surgery delayed 2 days until final pathology report showed AIFRS	Yes
5	Mucosa appeared normal	Yes	Surgery was delayed 2 days until final pathology report showed AIFRS	Yes
6	Irregular mucosa with impaired vascularity	Yes	Extensive debridement was delayed 2 days until final pathology report showed AIFRS	Yes
7	Inflamed mucosa in left nasal passage with no evidence of necrosis	Yes	Debridement was stopped with negative margins, but margin returned as positive on permanent section the next day and patient underwent additional debridement	No

Abbreviations: AIFRS, acute invasive fungal rhinosinusitis; FS, frozen section; PASF-fs, periodic acid–Schiff stain for fungus modified for use on frozen tissue.

with sinonasal symptoms, low absolute neutrophil counts, or abnormal sinonasal radiographic findings are important signs. Findings on endoscopic exam can be variable, from nonspecific early in the disease course, such as edematous, dry, or pale mucosa, to frank necrosis when advanced.⁷ Biopsy should be performed of any abnormality present on endoscopic exam. However, at times of high suspicion and sufficient risk factors, biopsy of normal-appearing tissue may be considered. The middle turbinate has been shown to be the most common site of fungal invasion, having a sensitivity of 75% to 86% and a specificity of 100% for the disease and should be considered for biopsy even when definite abnormalities are not found.^{5,6} Given the aggressive nature of AIFRS and the necessity of early diagnosis, we employ a low threshold for biopsy with frozen section.

A definitive diagnosis on pathology requires identifying the presence of fungus invading the tissue causing necrosis, frequently showing the occluding fungal vascular thrombi. In the end stages, the destruction may be so complete as to be unrecognizable as the remnants of residue tissue on the pathology. Also, the very early lesions may not be evident clinically, consisting of mucosal erosions or superficial ulcerations so tiny as to be seen only microscopically.³ A larger biopsy will be more likely to capture such minute foci, while in a very small biopsy, it becomes more fortuitous for a diagnosis to be rendered. Including an edge of mucosa leading into an ischemic-appearing area or some tissue appearing a bit less affected is suggested, but it is the necrosis that is important for the pathologist to examine.

In these 2 extremes, the diagnosis needs to be suspected by both the clinician and the pathologist. Prior to obtaining the biopsy, the pathology department should be alerted that

a frozen section for suspected AIFRS is being performed, with the biopsy then hand-delivered to the laboratory by the otolaryngologist.⁷ It is important for the otolaryngologist to convey to the pathologist the clinical impression, including appearance of mucosa, erosion, ulceration, necrosis, or suspected ischemia, as it can assist in the frozen-section interpretation. Conversely, when the diagnosis is not definitive, the pathologist needs to tell the otolaryngologist about the findings that are suspicious, such as necrosis or fungus that is present in the specimen but not associated with necrosis. Debridement and antifungal therapy are indicated at times in the absence of a definitive pathologic diagnosis.^{8,10,11}

The most significant potential for reduction in false-negative diagnoses was demonstrated by the ability of the PASF-fs to identify fungus when applied to all 61 frozen-section slides that contained fungus. Furthermore, when the PASF-fs stain was applied to the slides from the 7 cases that received a false-negative diagnosis on frozen section, it was able to identify invasive fungus in 5 cases (**Tables 2 and 3**). Had this stain been used at the original time of diagnosis, these patients would have had significantly reduced morbidity as debridement was delayed by at least 24 hours for all 7 patients. For the 2 false-negative cases in which PASF-fs was unable to identify fungus on frozen section, the fungus was missed due to inadequate biopsies in which there was no fungus present in the tissue, rather than the PASF-fs failing to identify the fungus (**Table 3**). The introduction of the PASF-fs as part of the routine frozen-section process for suspected AIFRS during the final 12 months of the study enabled the collection of 30 biopsy specimens from 15 patients using the PASF-fs in real time for the diagnosis. Although a much smaller number, the data showed

this prospective specimen group to be representative of the retrospective group, with the results showing 5 positive and 25 negative biopsy specimens (20% vs 23% retrospective), 1 false negative on H&E (20% vs 21%), and 5 of 5 positive fungal staining on PASF-fs (100% same as retrospective). The 5 positive patient cases included the angioinvasive fungal types most often causing AIFRS, *Aspergillus* species and mucormycosis, in addition to an uncommon *Alternaria* species. These findings support the indication from the retrospectively stained specimens that if PASF-fs is performed at the time of biopsy, the diagnostic accuracy of frozen section for AIFRS can be improved. This new technique is simple and rapid, and it can be performed in parallel with the typical H&E slides in the frozen-section suite in off-hours requiring only 3 additional staining reagents and has become an important part of the routine process in our laboratory (see Appendix 1, available in the online version of the article).

We acknowledge that a limitation of this study is the small number of cases available for analysis, which is a product of the relative rarity of AIFRS.

Conclusion

In this study, frozen section was shown to be an accurate way to diagnose AIFRS, with false-negative interpretations identified as the reason for errors in diagnosis being caused by the inability to identify fungus consistently on H&E. Modification of a stain for fungus on frozen tissue (PASF-fs) was demonstrated as effective in enabling its identification. Including the PASF-fs stain in the routine process for frozen-section diagnosis offers the hope that with prospective validation through use, it will improve the outcome of patients with AIFRS.

Author Contributions

Max Hennessy, wrote article, data collection and analysis; **Johnathan McGinn**, study design, revised paper; **Bartholomew White**, data collection, revised paper; **Sakeena Payne**, data collection, revised paper; **Joshua I. Warrick**, data analysis, revised paper; **Henry Crist**, study design, data collection and analysis, revised paper.

Disclosures

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Supplemental Material

Additional supporting information is available in the online version of the article.

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