Accuracy of Intraoperative Frozen Section in Detection of Acute Invasive Fungal Rhinosinusitis

Rahaf Alkhateb, MD, MSc; Preethi Dileep Menon, MD; Hamza Tariq, MB, BS; Sarah Hackman, MD; Alia Nazarullah, MD; Daniel D. Mais, MD

• ***Context.***—**Acute invasive fungal rhinosinusitis (AIFRS) is an aggressive form of fungal sinusitis, which remains a significant cause of morbidity and mortality. Early diagnosis and intervention are keys to improving patient outcomes. Intraoperative consultation has shown promise in facilitating early surgical intervention, but the accuracy of frozen section has not been clarified in this setting.**

Objectives.—**To assess the accuracy of frozen-section diagnosis in patients with clinically suspected AIFRS.**

Design.—**All cases of clinically suspected AIFRS during a 10-year period (2009–2019) were retrospectively reviewed. The frozen-section results were compared with the final permanent sections as well as the tissue fungal culture results, following which the accuracy of frozen section was determined.**

Results.—**Forty-eight patients with 133 frozen-section evaluations for AIFRS were included in the study. Thirty of 48 patients and 61 of 133 specimens were positive for AIFRS on final pathology. Of 30 positive patients, 27 (90%) had at least 1 specimen diagnosed as positive during intraoperative consultation; among the 61 positive specimens, 54 (88.5%) were diagnosed as positive during intraoperative consultation. Of 72 negative specimens, all were interpreted as negative on frozen section. Thus, frozen sections had a sensitivity of 88.5% (95% CI, 0.78–0.97), specificity of 100% (95% CI, 0.94–1), positive predictive value of 100% (95% CI, 0.92–1), and negative predictive value of 90.6% (95% CI, 0.82–0.97).**

Conclusions.—**This study represents the largest series assessing the diagnostic accuracy of frozen section analysis in AIFRS. These findings are useful in frozen section–informed intraoperative decision making.**

(*Arch Pathol Lab Med.* 2021;145:736–743; doi: 10.5858/arpa.2019-0696-OA)

Acute invasive fungal rhinosinusitis (AIFRS) is a potentially lethal and rapidly progressive disease. It primarily affects immunocompromised or diabetic patients, in whom organisms that are ubiquitous but usually harmless can invade mucosa and rapidly disseminate to the orbit and cranial cavity. The incidence of AIFRS has been on the rise during several decades, a trend that is attributed to the increasing incidence of these underlying predisposing conditions.[1–3]

At the same time, the reported mortality rate in AIFRS has remained essentially unchanged, ranging from 33% to 80%.[2–7] Diagnostic delay is a factor that has consistently been associated with poor outcome in AIFRS,[2,8–11] and this, in part, is related to the fact that AIFRS remains a histopathologic diagnosis. The clinical features are nonspecific, and sinus computed tomography is limited by lack of sensitivity for early AIFRS and poor specificity thereafter.[4,5,8,10–12] Aggressive surgical debridement is a key component of effective treatment, but one that may lead to profound dysfunction and disfigurement and is preferentially undertaken only after the diagnosis has been established definitively.

To avoid the delay inherent in tissue processing, evaluation of biopsies with intraoperative frozen section has tremendous appeal.[13–15] In some reports, frozen section was considered useful for providing accurate and timely diagnosis, while in others it was deemed unreliable.[15–17] To bring clarity to this question, we retrospectively assessed the accuracy of frozen-section diagnosis in patients with clinically suspected AIFRS during a 10-year period in our institution.

METHODS

After institutional review board approval was obtained, a retrospective search was conducted of the anatomic pathology information system at University Hospital to identify cases that were clinically suspicious for AIFRS from January 2009 to January 2019. Separate keyword searches were conducted, querying all report fields, using any of the terms "fungus," "fungal," "fungi," "hyphae," "ulcer," "necrotic," "Grocott methenamine silver stain," and "Periodic acid–Schiff stain" in conjunction with any of the terms "sinonasal," "sinus," "nasal," "sinusitis," "palate," or "orbit." The resulting lists were manually merged and then culled to remove duplicates. After review of the preoperative clinical documentation, cases were excluded if AIFRS was not clinically suspected or if no intraoperative consultation was obtained.

Accepted for publication July 10, 2020.
Published online October 22, 2020.
Supplemental digital content is available for this article at https://meridian.allenpress.com/aplm in the June 2021 table of contents.

From the Department of Pathology and Laboratory Medicine, UT Health San Antonio, San Antonio, Texas.

The authors have no relevant financial interest in the products or companies described in this article.

Corresponding author: Rahaf Alkhateb, MD, MSc, Department of Pathology and Laboratory Medicine, UT Health San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900 (email: Alkhateb@uthscsa.edu).

Per procedure, frozen-section samples were received fresh from the operating room, embedded in optimal cutting temperature medium, and allowed to freeze within a cryostat at an approximate temperature of –20°C. Biopsies were entirely embedded when size permitted. Sections were prepared using a Leica Biosystems cryostat, at a thickness of approximately 5 μm, across 2 different levels. These were stained with hematoxylin and eosin (H&E) and interpreted by the general surgical pathologist on-call for frozen sections. After this, the frozen tissue was thawed, fixed in formalin, and processed for permanent sections. Permanent tissue sections were stained with H&E special stains for fungi were performed routinely if H&E-stained sections revealed no fungal organisms. Additional sterile specimens were sent for fungal culture based on the clinical team decision. All the specimens collect for fungal culture are handled aseptically by the microbiology lab. At the microbiology lab the tissue specimens are handled based on their size. If the tissue sample received larger than a pea size, aseptically transferred to a sterile, disposable Petrie dish, and by using a sterile scalpel, it is cut into 5 pea-sized portions. The portion that is selected for culture usually that is bordering and within the area of infection (ie, necrotic tissue, red, and purulent areas). Four portions usually used to inoculate the fungal culture media, whenever possible, whole pieces of tissue are used to inoculate fungal cultures.

For the purposes of calculating performance characteristics, only the original interpretations were used. We calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with the frozen section as the test method and the permanent section as the gold standard. For proportions, 95% CI were calculated according to the efficient score method and corrected for continuity. The sensitivity, specificity, PPV, and NPV were estimated with 95% CI by using descriptive measures from contingency table by using R-3.5.1 and R Studio-1.1.463.[18]

We then retrospectively reviewed frozen-section and permanent slides from all cases to identify background histopathologic features. In particular, we noted the presence or absence of neutrophil-mediated inflammation, fibrinopurulent debris, and tissue necrosis. Last, discrepant cases were reviewed to determine contributing factors.

RESULTS

Our search identified 53 cases of suspected AIFRS, 48 of which (90.6%) had at least 1 specimen sent for intraoperative consultation, resulting in 133 frozen-section specimens. Of 48 patients (Table), 30 (62.5%) had a final diagnosis of AIFRS on permanent section in at least 1 biopsied site (Figure 1), and 18 (37.5%) were negative for AIFRS. Of note, none of the 18 negative patients were diagnosed with AIFRS in the following 90 days. Of 30 patients with a final diagnosis of AIFRS, there were 12 (40%) females and 18 (60%) males, and the mean age was 56 years (range, 19–77). Of 18 patients whose final diagnosis was negative for AIFRS, 8 (44.4%) were females and 10 (55.6%) were males, with mean age of 57 years (range, 34–79).

Of 30 patients who had a final diagnosis of AIFRS, 28 (87.5%) had known predisposing conditions. One of the remaining 2 patients had a history of asthma with no immunosuppressive therapy, while the other had a history of rheumatoid arthritis with no immunosuppressive therapy. The most common underlying condition was diabetes mellitus, present in 19 patients (59%); others included myelodysplastic syndrome, acute leukemia, HIV/AIDS, iatrogenic immunosuppression, and cirrhosis with leukopenia.

The total number of frozen sections sent for pathologic interpretation per patient ranged from 1 to 8 (mean, 2.4). The mean number of frozen-section specimens in positive patients was 2.6 (range, 1–8), and the mean number in negative patients was 2.33 (range, 1–4). The most common subsites biopsied were the middle turbinates (25 specimens, 19%) and the maxillary sinus (24 specimens, 18%). The most commonly positive AIFRS subsite was the maxillary sinus, comprising 21% (13 specimens) of all positive samples, followed by the lateral nasal wall, comprising 18% (11 specimens).

Microbiologic findings included *Rhizopus* species in 12 of 30 positive cases, *Aspergillus* species in 5, *Mucor* species in 2, *Candida* species in 2, *Bipolaris* species in 1, *Alternaria* species in 1, and 7 showed no growth (Figure 2, A through D). *Bipolaris* and *Alternaria* species are commonly encountered as colonizers; however, each was isolated in association with concomitant histologic evidence of tissue invasion. It is possible that these represent culture contamination with colonizing agents, but invasive infection has been reported rarely in association with both agents.[19–26]

There were no cases in which fungal culture was positive after a negative histopathologic diagnosis. All Mucormycetes (formerly known as the Zygomycetes) were accurately classified histopathologically, as were all non-Mucormycetes. The cases of *Candida* were accurately classified as "morphologically consistent with *Candida* species."

Of 18 patients who were negative for AIFRS, 13 (72.2%) had known predisposing conditions, including diabetes mellitus in 6 (33%) patients, iatrogenic immunosuppression, chronic myeloid leukemia, and acute leukemia. Of 18 patients, none were diagnosed with AIFRS in the next 90 days, while 2 were diagnosed as granulomatosis with polyangiitis (Wegener), 1 as sinonasal squamous cell carcinoma, and 1 with natural killer/T-cell lymphoma.

Among 30 patients who had a final diagnosis of AIFRS, 7 (23%) patients had at least 1 false negative at the time of intraoperative consultation. Four of 7 patients (patients #: 15, 16, 20, and 23) had multiple specimens and the diagnosis of AIFRS was ultimately made intraoperatively as a result of multiple samples from various sites for each patient. In the other 3 patients (patients #: 31, 47, and 48) with false-negative diagnoses, 1 underwent extensive debridement during the initial surgical procedure because of strong surgical suspicion for AIFRS; the other 2 underwent extensive debridement within 24 hours based upon final pathologic examination. All survived until discharge from the hospital, 20, 36, and 60 days later, respectively. Among 18 patients whose final diagnosis was negative for AIFRS, none had a frozen-section specimen interpreted as positive during intraoperative consultation.

Among the 133 individual specimens (see table in supplemental digital content at https://meridian.allenpress.com/aplm in the June 2021 table of contents), the final pathologic diagnosis was positive for AIFRS in 61 (45.8%) specimens and negative for AIFRS in 72 (54.2%). No false-positive frozen-section diagnoses were made (Figure 1), as all of the specimens receiving a positive diagnosis on frozen section had a positive diagnosis confirmed on permanent section. Of 61 positive cases on permanent sections, 54 (88.5%) were diagnosed with AIFRS on frozen section. Thus, individual frozen sections had a sensitivity of 88.5% (95% CI, 0.78–0.97) and specificity of 100% (95% CI, 0.94–1), with a 100% PPV (95% CI, 0.92–1), and 90.6% NPV (95% CI, 0.82–0.97).

Of 61 specimens that demonstrated AIFRS on permanent sections, 57 (93.4%) demonstrated tissue infiltration by neutrophils, 53 (86.6%) contained regions of necrosis, and fibrinopurulent debris was present in 37 (60.1%). At least 1

Patient Data									
Patient	Age	Sex	Race	Frozen Section Diagnosis in at Least 1 Specimen	Final Diagnosis	Fungal Classification (Morphology)	Fungal Isolate	Comorbidities	Number of Specimens
1	38	F	White	NG	NG	NA	NG	RA, pulmonary fibrosis	2
2	54	M	White	NG	NG	NA	NG	RA on methotrexate and prednisone	5
3	61	M	Hispanic	NG	NG	NA	NG	None	1
4	54	M	White	POS	POS	Zygomycetes	Mucor species	DM 2, chronic lymphoid leukemia	4
5	55	M	White	POS	POS	*Candida* species	*Candida tropicalis*	HIV/AIDS, hemophagocytic lymphohistiocytosis,	2
6	34	M	White	NG	NG	NA	NG	DM II	1
7	65	F	Hispanic	NG	NG	NA	NG	ESLD due to hepatitis C, ESRD	1
8	57	F	Hispanic	NG	NG	NA	NG	ESRD	1
9	63	M	White	NG	NG	NA	NG	NK/T-cell lymphoma	2
10	36	M	White	POS	POS	*Candida* species	*Candida tropicalis*	HIV/AIDS, kaposi sarcoma	1
11	56	F	African American	NG	NG	NA	NG	DM 2	1
12	61	M	Hispanic	NG	NG	NA	NG	DM 2	1
13	51	F	African American	NG	NG	NA	NG	Chronic myeloid leukemia	4
14	72	F	Hispanic	POS	POS	Zygomycetes	*Rhizopus* species	DM 2	1
15	58	M	White	POS	POS	Zygomycetes	NG	DM 2	2
16	62	F	White	POS	POS	Zygomycetes	*Rhizopus* species	DM 2	3
17	61	F	African American	POS	POS	*Aspergillus*	*Aspergillus flavus*	DM 2	2
18	76	F	Hispanic	POS	POS	Zygomycetes	NG	DM 2	1
19	63	F	Hispanic	POS	POS	Zygomycetes	*Mucor* species	DM 2	1
20	66	F	Hispanic	POS	POS	Zygomycetes	*Rhizopus* species	DM 2	3
21	41	F	Hispanic	POS	POS	Non-zygomycetes	NG	RA	2
22	58	F	Hispanic	NG	NG	NA	NG	Squamous cell carcinoma head and neck	2
23	52	F	White	POS	POS	Zygomycetes	*Rhizopus* species	DM 2	2
24	45	F	Hispanic	POS	POS	Zygomycetes	*Rhizopus* species	DM 2	5
25	44	F	Hispanic	POS	POS	*Candida* species	*Candida albicans*	Myelodysplastic syndrome	5
26	41	M	White	POS	POS	Zygomycetes	*Rhizopus* species	DM 2	8
27	63	M	White	POS	POS	Zygomycetes	*Rhizopus* species	B-acute lymphoid leukemia	1
28	59	M	African American	POS	POS	Zygomycetes	NG	DM II	2
29	63	F	Not available	POS	POS	Zygomycetes	*Rhizopus* species	DM II	1
30	66	F	Hispanic	NG	NG	NA	NG	DM II	2
31	50	M	Hispanic	NG	POS	Zygomycetes	*Rhizopus* species	DM II, ESLD due to hepatitis C	2
32	19	M	African American	POS	POS	Aspergillus	NG	Asthma	5
33	63	M	Hispanic	POS	POS	Zygomycetes	*Rhizopus* species	DM II	7
34	60	M	White	POS	POS	Non-zygomycetes	*Aspergillus fumigatus*	DM II, lung transplant	1
35	77	M	Hispanic	POS	POS	Zygomycetes	NG	Cirrhosis with chronic leukopenia	1
36	52	M	White	POS	POS	Zygomycetes	*Rhizopus* species	DM II	1
37	45	M	Hispanic	NG	NG	NA	NG	Acute myeloid leukemia on chemotherapy	1
38	79	M	Hispanic	NG	NG	NA	NG	None	4
39	54	M	Hispanic	POS	POS	Zygomycetes	NG	None	2
40	49	M	Hispanic	NG	NG	NA	NG	DM 2	4

Continued									
Patient	Age	Sex	Race	Frozen Section Diagnosis in at Least 1 Specimen	Final Diagnosis	Fungal Classification (Morphology)	Fungal Isolate	Comorbidities	Number of Specimens
41	62	F	Hispanic	NG	NG	NA	NG	None	1
42	61	M	Hispanic	NG	NG	NA	NG	DM 2	1
43	61	M	Hispanic	POS	POS	Non-zygomycetes	*Alternaria* species	DM 2	1
44	54	M	Hispanic	POS	POS	Zygomycetes	*Rhizopus* species	DM 2	6
45	68	M	White	NG	NG	NA	NG	Chronic sinusitis	4
46	72	M	White	POS	POS	Non-zygomycetes	*Bipolaris* species	Lung transplant	2
47	29	M	White	NG	NG	Non-zygomycetes	*Aspergillus flavus*	Asthma, chronic sinusitis, steroids	1
48	66	F	Hispanic	NG	POS	Non-zygomycetes	*Aspergillus flavus*	DM 2	2

Abbreviations: DM 2, diabetes mellitus type; ESLD, end-stage liver disease; ESRD, end-stage renal disease; NA, not applicable; NG, negative; NK, natural killer; POS, positive; RA, rheumatoid arthritis.

of these features was present in 58 positive specimens (95%), but in 3 (5%) positive specimens none of these features were present. These specimens demonstrated only mild chronic inflammation (Figure 3, A through F). Of 72 specimens that were negative for AIFRS on permanent sections, 27 (37.5%) had neutrophilic infiltration, 12 (16.7%) demonstrated regions of necrosis, 22 (30.5%) had fibrinopurulent debris, and 33 (45.8%) had at least 1 of these features.

The 7 false-negative specimens in our study included 5 with features of Mucormycetes and 2 with features of non-Mucormycetes (Figure 4, A through D, and Figure 5, A and B). In 1 of 5 false-negative Mucormycetes specimens, fungal hyphae were retrospectively identified on the frozen-section slide; in this case they were present only near the cauterized edge and somewhat obscured. In the other 4 of 5 false-negative Mucormycetes specimens, no fungi were identified in retrospective review of the frozen section slides. In 1 of 2 false-negative specimens with features of non-Mucormycetes, rare organisms were found after retrospective review. Thus, 2 of 7 false negatives could be classified as interpretive errors, while the other 5 reflected sampling variation. All of the false-negative specimens were submitted entirely for frozen section, so the number of frozen blocks was not a contributing factor.

DISCUSSION

The reduction in mortality associated with early diagnosis of AIFRS is thought to be related to early aggressive surgical debridement.[27–29] Such an intervention is rarely undertaken without definitive histopathologic diagnosis. Frozen section, as the fastest of the available options for histopathologic confirmation, is an attractive alternative. However, without clarity regarding the accuracy of frozen sections in this context, it is difficult to know how best to incorporate them into treatment protocols.

A few small studies have been published on this question. Ghadiali et al[13] conducted a retrospective review of 20 patients with AIFRS at the University of Miami between 1993 and 2005. The study revealed overall sensitivity of 84%, specificity of 100%, PPV of 100%, and NPV of 72%. Of note, most of the false negatives (4 of 5) were in patients with *Mucor*. However, the study was designed to include only patients with a final pathologic diagnosis of AIFRS due to *Mucor* or *Aspergillus* species and did not include patients in whom the diagnosis was suspected but subsequently excluded or patients with other genera/organisms. Furthermore, the study was conducted by retrospective review of pathology reports and did not contain analysis of the false-negative specimens.

Papagiannopoulos et al[14] retrospectively reviewed 18 patients and reported a sensitivity of 72.7%, specificity of 100%, PPV of 100%, and NPV of 64.7%. However, like the study from Ghadiali et al,[13] this cohort consisted only of patients with a final pathologic diagnosis positive for AIFRS. Furthermore, all cases were examined by a head and neck pathologist, a circumstance that may not be feasible in most practices. Last, the study included predominantly patients with profound neutropenia secondary to underlying hematologic malignancies (14 of 18) and very few patients with diabetes mellitus (2 of 18), a context that may limit widespread applicability.

The study design employed in Melancon et al[30] was similar to ours in that it sought to include all patients with clinically suspected AIFRS. It included 28 patients in whom 21 had a final diagnosis of AIFRS. Frozen section demonstrated 87.5% sensitivity, 100% specificity, 100% PPV, and 70% NPV. Search terms used in this study may have significantly limited inclusion of negative cases. Moreover, like the Ghadiali et al[13] study, this was a retrospective review of pathology reports in which pathologic factors affecting interpretation were not explored.

Last, a few very small studies are notable[17,31,32] primarily for the frequency with which they are cited in regard to the question of frozen section sensitivity. Adelson et al[17] report data related to a single patient with invasive *Mucor* in whom 6 of 6 frozen sections were false negatives. This, together with the Ghadiali et al[13] study, is sometimes touted as selectively low frozen-section sensitivity for *Mucor*. Taxy et al[32] identified 8 patients with confirmed cases of AIFRS in whom frozen sections were positive in only 5 (62.5%). The frozen sections were interpreted by rotating general pathologists; however, there was selective use of Romanowsky-stained touch imprints in these cases, and frozen section was performed in only 8 of 12 cases of AIFRS identified during the study period, indicating a possible patient selection bias. Likewise, Hofman et al[31] studied only patients with final pathologic diagnosis of invasive mucormycosis in whom 6 of 7 (85.7%) had a positive frozen-section diagnosis. As in the study by Taxy et al,[32] frozen sections were performed in only 7 of 12 cases identified during the study period, and there was selective use of adjunctive stains intraoperatively, including Toluidine blue, Gomori Grocott's methenamine silver, and periodic acid-

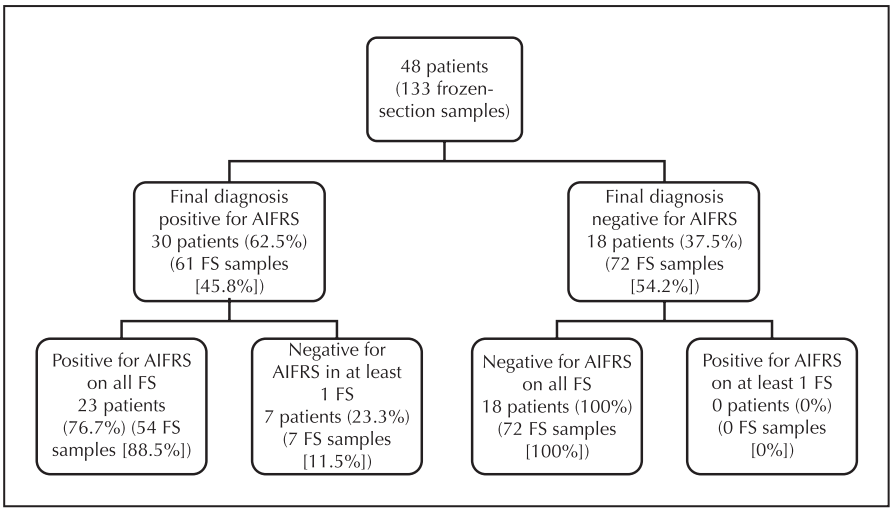

Figure 1. *Schematic chart illustrating patients and frozen section results in acute invasive fungal rhinosinusitis. Abbreviations: AIFRS, acute invasive fungal rhinosinusitis; FS, frozen section.*

Schiff. Analysis of the false-negative frozen-section discrepancies is not offered in any of the above studies, including the 2—Hofman et al[31] and Taxy et al[32]—that were conducted by pathologists.

Our search identified 53 patients in whom tissue was obtained because of clinical suspicion for AIFRS, and among these 48 (90.5%) had frozen sections performed. We believe that this reflects a practice pattern in our hospital in favor of using frozen sections for this purpose and that their use was not substantially influenced by clinical parameters over and above those informing the decision whether to perform a biopsy at all.

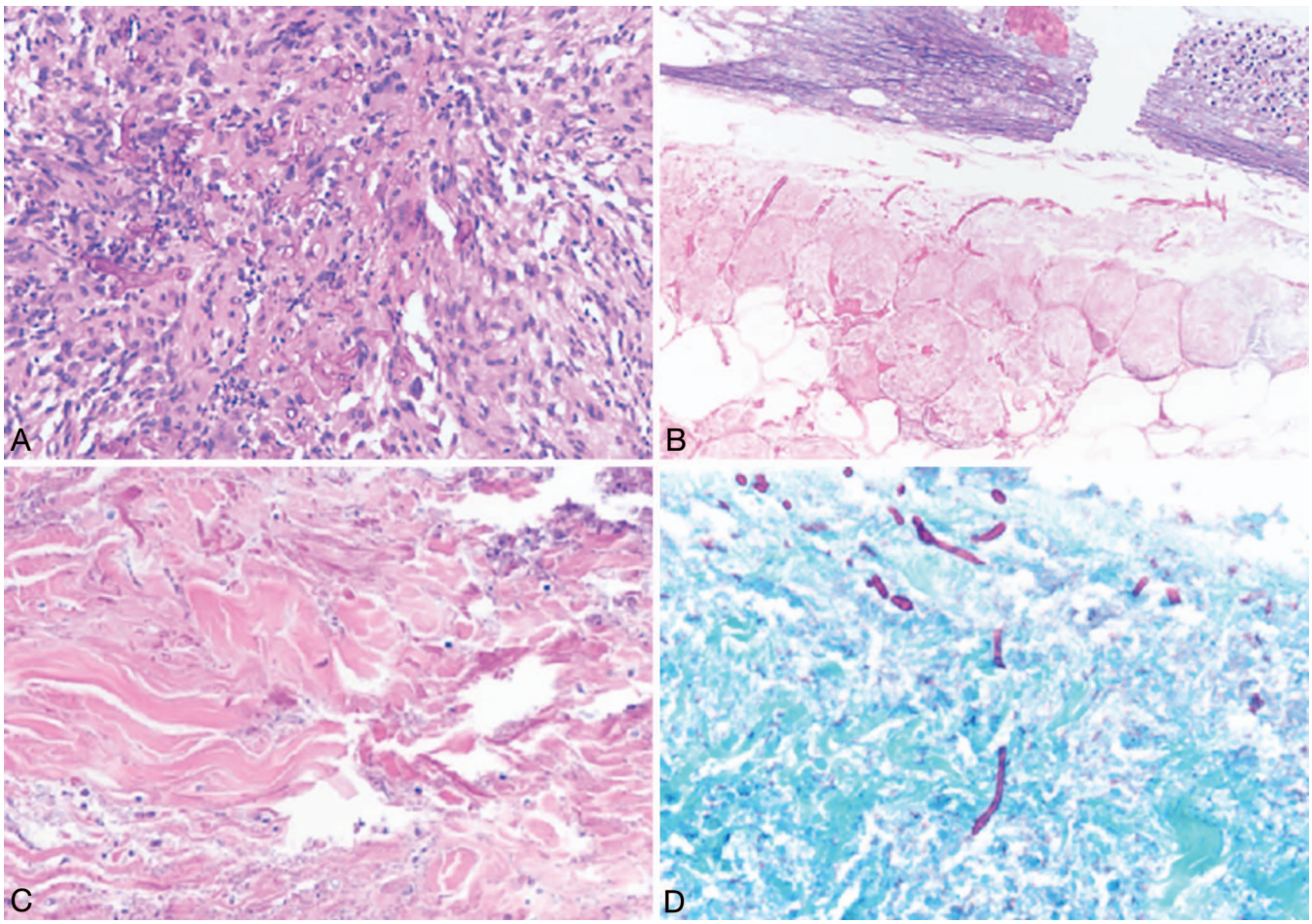

Figure 2. *Most common agents. Mucormycetes demonstrated broad hyphae with wavy, nonparallel walls and few septa (A). Nonmucormycetes, which were predominantly* Aspergillus *species by culture, demonstrated thin delicate hyphae with parallel walls and frequent septa (B).* Candida *species were distinctive for a mixture of yeasts and pseudohyphae (C and D) (hematoxylin-eosin, ×400 [A and C], ×200 [B]; periodic acid–Schiff stain, ×400 [D]).*

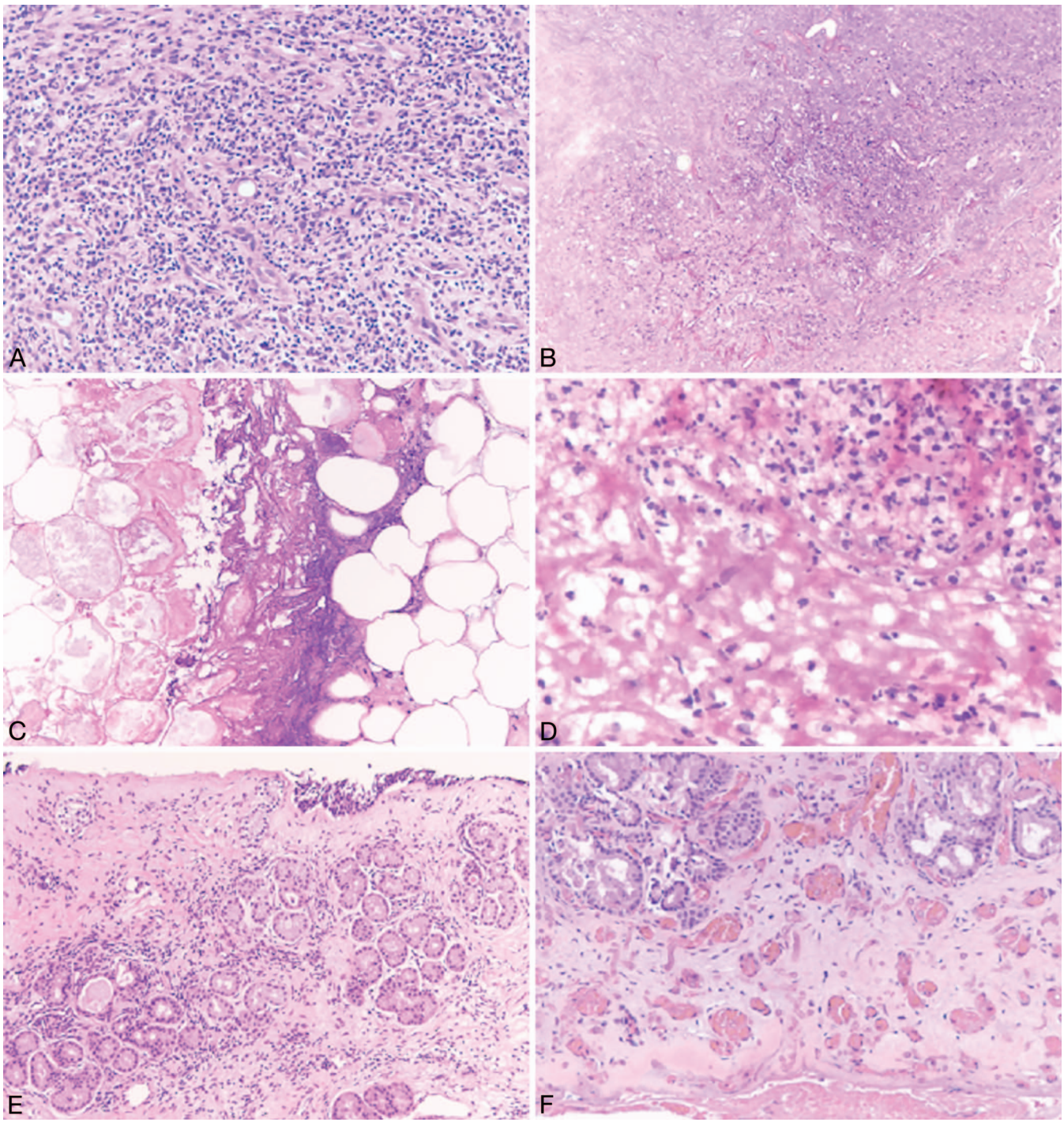

Figure 3. *Tissue reactions in acute invasive fungal rhinosinusitis. Acute inflammation was noted when neutrophils were present within intact tissue fragments (A). Tissue necrosis was characterized by abrupt coagulative-type necrosis (B) as well as fat necrosis (C). Fibrinopurulent debris consisted of admixed neutrophils and fibrin, usually found on the surface of tissue fragments or in detached fragments, composed of neutrophils and fibrin (D). Some cases that were positive for acute invasive fungal rhinosinusitis demonstrated only chronic inflammation (E and F) (hematoxylin-eosin, ×400 [A, C, and D], ×200 [B and F], ×100 [E]).*

Clinically, our patient group was substantially similar to those reported previously,[13,14,17,30–32] that is, patient demographics and underlying medical conditions were comparable, as was the average number of frozen sections performed and rate of AIFRS among the biopsied patients. As in prior studies, we found a preponderance of Mucormycetes and *Aspergillus* species, but these were included in our study cases of invasive *Candida*, *Alternaria*, and *Bipolaris* species.

As in prior studies, diabetes mellitus was the most common underlying medical condition in our patients with AIFRS.[2] The most commonly implicated agents, based upon histomorphology and/or culture, was *Mucormycete* species. The most commonly biopsied sites in our study were the

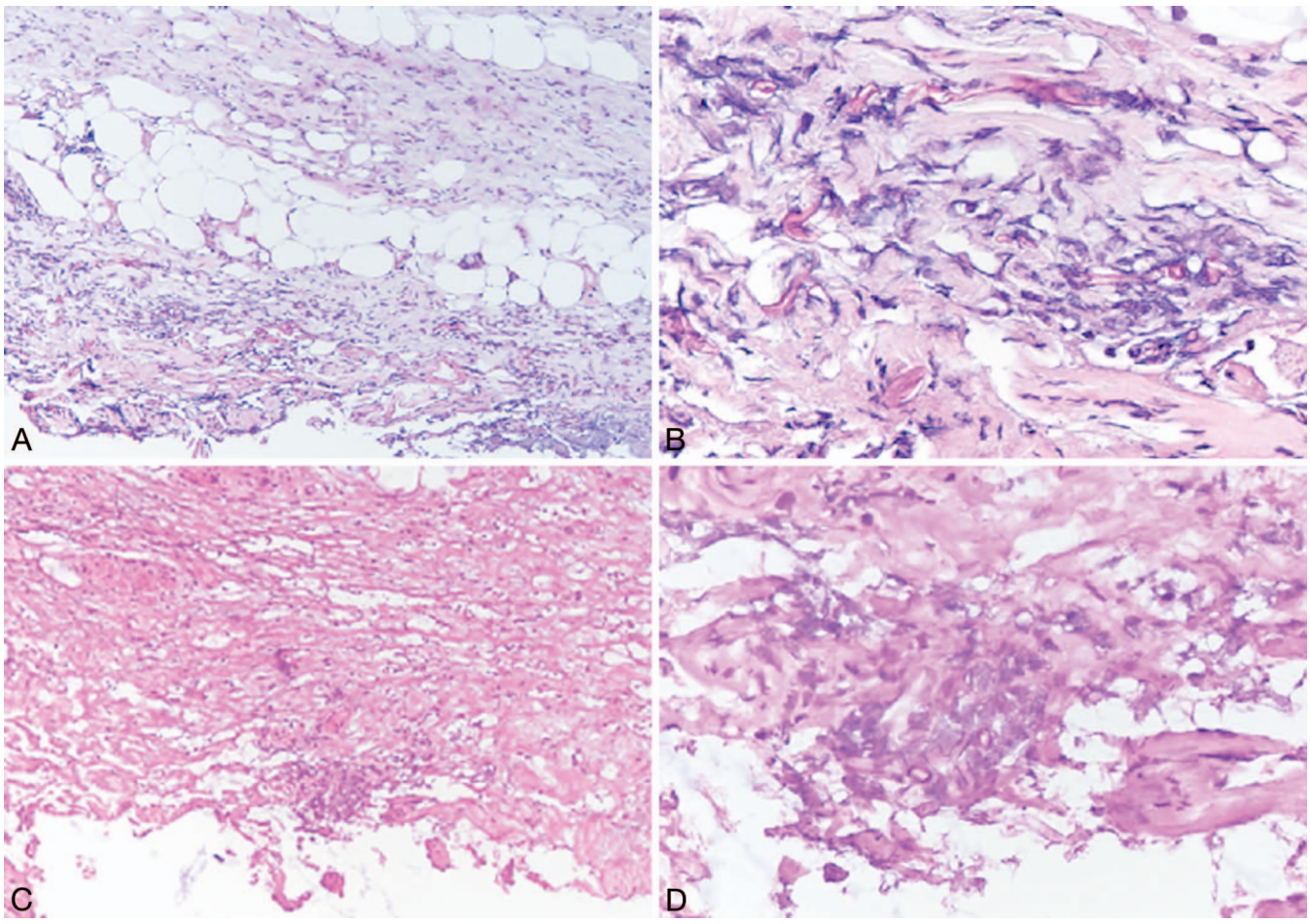

Figure 4. *False-negative case of mucormycete. Fungal hyphae are seen on permanent section (A and B). These were identified retrospectively in frozen-section slides, near the deep aspect of the tissue where cautery artifact was present (C and D) (periodic acid–Schiff stain, ×100 [A], ×400 [B]; hematoxylin-eosin, ×200 [C], ×400 [D]).*

turbinates, especially the middle turbinate (19% of biopsies), and the most commonly involved (positive) site was the maxillary sinus (21%), both findings consistent with those previously reported.[9,33,34]

In our study, the sensitivity of frozen-section diagnosis was 88.5% per specimen and 90% per patient; the per-patient sensitivity was somewhat higher owing to multiple samplings. The specificity was 100% for both individual

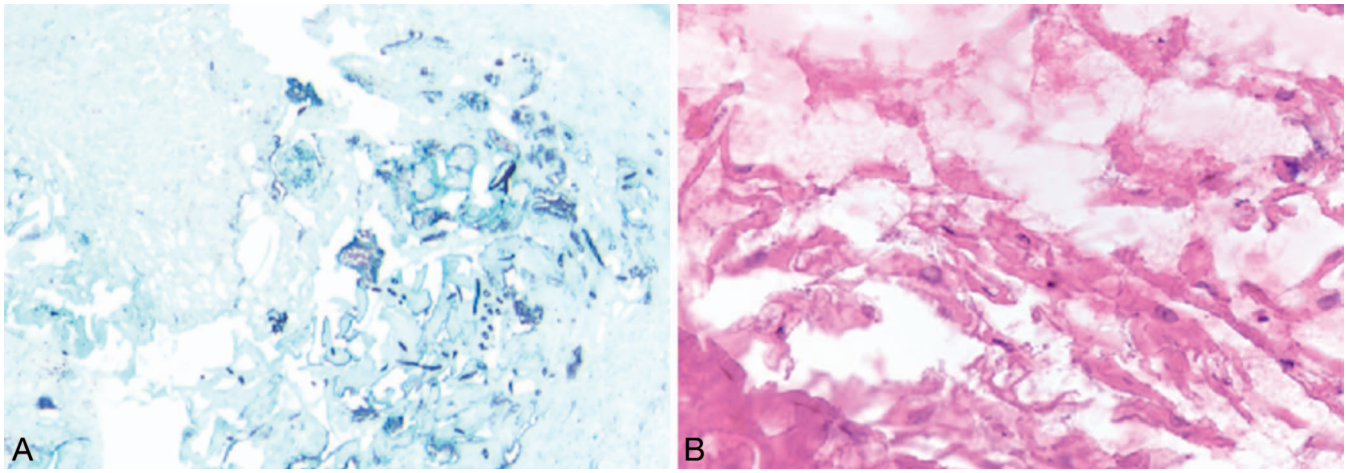

Figure 5. *False-negative case of nonmucormycetes. Fungal hyphae are seen on permanent section (A), which are faintly detectable retrospectively in frozen section slides as areas of attenuated (ghosted) staining (B) (Grocott Methenamine Silver stain, ×200 [A]; hematoxylin-eosin, ×400 [B]).*

samples and patients. These performance characteristics were similar to those reported previously.

The 7 false-negative specimens in our study included 2 that were retrospectively deemed to be interpretive in nature in both cases because of a rarity of fungal hyphae. That is, the fungal structures were visible on H&E-stained frozen sections after thorough retrospective review in only 2 of 7 false-negative specimens. The false-negatives included 5 with Mucormycetes and 2 with *Aspergillus* species, and the 2 interpretive errors included 1 of each.

The final diagnosis in those patients who were negative for AIFRS is not reported in the prior studies. In our cohort, 2 of 18 negative patients were ultimately diagnosed as granulomatosis with polyangiitis (Wegener), 1 as sinonasal squamous cell carcinoma, and 1 as natural killer/T-cell lymphoma; the remaining 14 were classified as nonspecific rhinosinusitis, and none of these was diagnosed with AIFRS in the next 90 days.

Positive specimens were usually characterized by extensive necrosis, fibrinopurulent debris, and rare to abundant fungal hyphae. When we considered the background histopathologic findings, necrosis was frequently present in specimens positive for invasive fungi (86.6%), while fibrinopurulent debris was often present as well (60.1%). In fact, at least 1 of these findings was present in 95% of specimens containing invasive fungus; however, 5% of such specimens lacked both and demonstrated only the expected intensity of positive specimens. These specimens demonstrated viable tissue with only the expected intensity of chronic inflammation. Nevertheless, these features were significantly more common in the specimens with invasive fungus than those without.

CONCLUSIONS

The decision to undertake surgical debridement in patients with AIFRS requires definitive histopathologic diagnosis, for which intraoperative consultation with frozen section may be requested. Our analysis has demonstrated overall high accuracy of frozen sections in this scenario, and this information may be valuable in the surgical management of these patients.

References

1. Kasapoglu F, Coskun H, Ozmen OA, Akalin H, Ener B. Acute invasive fungal rhinosinusitis: Evaluation of 26 patients treated with endonasal or open surgical procedures. *Otolaryngol Head Neck Surg*. 2010;143(5):614–620.
2. Turner JH, Soudry E, Nayak JV, Hwang PH. Survival outcomes in acute invasive fungal sinusitis: a systematic review and quantitative synthesis of published evidence. *Laryngoscope*. 2013;123(5):1112–1118.
3. Callejas CA, Douglas RG. Fungal rhinosinusitis: what every allergist should know. *Clin Exp Allergy*. 2013;43(8):835–849.
4. Süslü AE, Öğretmenoğlu O, Süslü N, Yücel ÖT, Önerci TM. Acute invasive fungal rhinosinusitis: Our experience with 19 patients. *Eur Arch Otorhinolaryngol*. 2009;266(1):77.
5. Parikh SL, Venkatraman G, DelGaudio JM. Invasive fungal sinusitis: a 15-year review from a single institution. *Am J Rhinol*. 2004;18(2):75–81.
6. Butugan O, Sanchez TG, Goncalez F, Venosa AR, Miniti A. *Rhinocerebral mucormycosis*: predisposing factors, diagnosis, therapy, complications and survival. *Rev Laryngol Otol Rhinol (Bord)*. 1996;117(1):53–55.
7. Iwen PC, Rupp ME, Hinrichs SH. Invasive mold sinusitis: 17 cases in immunocompromised patients and review of the literature. *Clin Infect Dis*. 1997; 24(6):1178–1184.
8. Spellberg B, Edwards J Jr, Ibrahim A. Novel perspectives on mucormycosis: pathophysiology, presentation, and management. *Clin Microbiol Rev*. 2005;18(3): 556–569.
9. Sun H, Forrest G, Gupta KL, et al. Rhino-orbital-cerebral zygomycosis in solid organ transplant recipients. *Am J Transplant*. 2010;90(1):85–92.
10. Dhiwakar M, Thakar A, Bahadur S. Improving outcomes in rhinocerebral mucormycosis-early diagnostic pointers and prognostic factors. *J Laryngol Otol*. 2003;117(11):861–865.
11. Silveira ML, Anselmo-Lima WT, Faria FM, et al. Impact of early detection of acute invasive fungal rhinosinusitis in immunocompromised patients. *BMC Infect Dis*. 2019;19(1):310.
12. Zuniga MG, Turner JH. Treatment outcomes in acute invasive fungal rhinosinusitis. *Curr Opin Otolaryngol Head Neck Surg*. 2014;22(3):242–248.
13. Ghadiali MT, Deckard NA, Farooq U, Astor F, Robinson P, Casiano RR. Frozen-section biopsy analysis for acute invasive fungal rhinosinusitis. *Otolaryngol Head Neck Surg*. 2007;136(5):714–719.
14. Papagiannopoulos P, Lin DM, Al-Khudari S, et al. Utility of intraoperative frozen sections in surgical decision making for acute invasive fungal rhinosinusitis. 2017;7(5):502–507.
15. Langford JD, McCartney DL, Wang RC. Frozen section-guided surgical debridement for management of rhino-orbital mucormycosis. *Am J Ophthalmol*. 1997;124(2):265–267.
16. Pelton RW, Peterson EA, Patel BC, Davis K. Successful treatment of rhino-orbital mucormycosis without exenteration: the use of multiple treatment modalities. *Ophthalmic Plast Reconstr Surg*. 2001;17(1):62–66.
17. Adelson RT, Zito J, Romaguera RL. Invasive fungal adenoiditis. *Otolaryngol Head Neck Surg*. 2006;134(4):713–714.
18. Stevenson M, Nunes T, Sanchez J, et al. epiR: an R package for the analysis of epidemiological data. *R package version 0.9-43*. 2013.
19. Adam RD, Paquin ML, Petersen EA, et al. Phaeohyphomycosis caused by the fungal genera bipolaris and exserohilum. A report of 9 cases and review of the literature. *Medicine*. 1986;65(4):203–217.
20. Castelnuovo P, De Bernardi F, Cavanna C, et al. Invasive fungal sinusitis due to bipolaris hawaiiensis. *Mycoses*. 2004;47(1-2):76–81.
21. Levine PA, Antoine GA, Raterink MH. Bipolaris: a serious new fungal pathogen of the paranasal sinus. *Otolaryngol Head Neck Surg*. 1989;100(2):158–162.
22. Zieske LA, Kopke RD, Hamill R. Dematiaceous fungal sinusitis. *Otolaryngol Head Neck Surg*. 1991;105(4):567–577.
23. Kronen R, Liang SY, Bochicchio G, Bochicchio K, Powderly WG, Spec A. Invasive fungal infections secondary to traumatic injury. *Int J Infect Dis*. 2017;62: 102–111.
24. Pastor FJ, Guarro J. Alternaria infections: laboratory diagnosis and relevant clinical features. *Clin Microbiol Infect*. 2008;14(8):734–746.
25. Sharma B, Kamble U, Ghosh G, Prasad A, Chowdhary A. Invasive rhinosinusitis due to alternaria alternata and rhizopus arrhizus mixed infection: a case report and review. *Int J Infect*. 2017;4(4):e42127.
26. de Sousa Ferreira I, Teixeira G, Abecasis M. Alternaria alternata invasive fungal infection in a patient with fanconi's anemia after an unrelated bone marrow transplant. *Clin Drug Investig*. 2013;33(1):33–36.
27. Peterson KL, Wang M, Canalis RF, Abemayor E. *Rhinocerebral mucormycosis*: evolution of the disease and treatment options. *Laryngoscope*. 1997;107(7): 855–862.
28. Strasser MD, Kennedy RJ, Adam RD. Rhinocerebral mucormycosis: therapy with amphotericin B lipid complex. *Arch Intern Med*. 1996;156(3):337–339.
29. Valera FC, do Lago T, Tamashiro E, Yassuda CC, Silveira F, Anselmo-Lima WT. Prognosis of acute invasive fungal rhinosinusitis related to underlying disease. *Int J Infect Dis*. 2011;15(12):e841–e844.
30. Melancon CC, Clinger JD. The use of frozen section in the early diagnosis of acute invasive fungal sinusitis. *Otolaryngol Head Neck Surg*. 2017;157(2):314–319.
31. Hofman V, Castillo L, Bétis F, Guevara N, Gari-Toussaint M, Hofman P. Usefulness of frozen section in rhinocerebral mucormycosis diagnosis and management. *Pathology*. 2003;35(3):212–216.
32. Taxy JB, El-Zayaty S, Langerman A. Acute fungal sinusitis: natural history and the role of frozen section. *Am J Clin Pathol*. 2009;132(1):86–93.
33. Middlebrooks EH, Frost CJ, De Jesus RO, Massini TC, Schmalfuss IM, Mancuso AA. Acute invasive fungal rhinosinusitis: a comprehensive update of CT findings and design of an effective diagnostic imaging model. *AJNR Am J Neuroradiol*. 2015;36(8):1529–1535.
34. Saedi B, Sadeghi M, Seilani P. Endoscopic management of rhinocerebral mucormycosis with topical and intravenous amphotericin B. *J Laryngol Otol*. 2011;125(8):807–810.