

Diagnostic Accuracy of Frozen Section in Post COVID Fungal Infections

Anusha Thangaraja¹, Veena Ramaswamy², Tejaswini B N³, Sowmya B Uthaiyah⁴, Gaurav Medikeri⁵

¹Resident Pathologist, Department of Pathology, Triesta Sciences, HCG Hospital, Bangalore, India
Email: anu.vadhana@gmail.com

²Consultant Pathologist, Department of Pathology, Triesta Sciences, HCG Hospital, Bangalore, India
Email: [drveena.r\[at\]hcgel.com](mailto:drveena.r[at]hcgel.com)

³Consultant Pathologist, Department of Pathology, Triesta Sciences, HCG Hospital, Bangalore, India
Email: [bntejaswini\[at\]yahoo.com](mailto:bntejaswini[at]yahoo.com)

⁴Consultant Pathologist, Department of Pathology, Triesta Sciences, HCG Hospital, Bangalore, India
Email: [sowmya.uthaiyah\[at\]gmail.com](mailto:sowmya.uthaiyah[at]gmail.com)

⁵Program Director, Endoscopic Skull base Surgeon, HCG Hospital, Bangalore, India
Email: [gauravsmedikeri\[at\]gmail.com](mailto:gauravsmedikeri[at]gmail.com)

Abstract: *The emergence of fatal fungal infections during the second wave of COVID-19 in 2021 devastated human health worldwide. India had a sudden surge in the incidence of Mucormycosis from April to July 2021 which necessitated rapid diagnosis for a prompt surgical intervention to decrease morbidity and mortality. Intraoperative frozen section diagnosis with permanent histopathology confirmation is one of the standard diagnostic tools for the diagnosis of Acute invasive fungal infections. A single institutional prospective study was conducted on the suspected cases of Mucormycosis in COVID patients who had intraoperative frozen section consultation at HCG Cancer Centre, Bengaluru from April 8th to July 27th 2021. 61 fresh tissue samples from 55 patients were received for frozen section evaluation. Of the 61 samples evaluated in the frozen section, 47 were positive and 14 were negative for mucormycosis. On the evaluation of permanent H&E sections of the 14 negative cases, 3 were positive for mucormycosis, which showed fungal elements on special stains. In our study, the frozen section showed a 94% concordance rate with permanent histopathology sections with 94 % sensitivity and 100% specificity and hence proves to be a diagnostic tool for acute fulminant fungal infections.*

Keywords: Mucormycosis, COVID 19, Frozen Section, AIFRS

1. Introduction

The emergence of the second wave of COVID-19 in 2021 devastated human health worldwide affecting over 220 countries.¹ The second wave of COVID-19 had myriads of complications with an increase in fatal fungal infections which has put the lives of infected patients at further risk.² In these patients, *Zygomycetes* and *Aspergillus*, which are transmitted by airborne asexual spores and conidia respectively, can invade the nasal mucosa and blood vessels, leading to rapid dissemination into the orbits, palate and the brain.³⁻⁴ India had a sudden surge in the incidence of Mucormycosis from April to July of 2021 with more than 45, 432 cases and 4252 deaths as of July 15th, 2021 with Rhino cerebral Mucormycosis (77.6%) being the most common type of presentation.⁵

COVID-19 induces NF- κ B while blocking INF-1, allowing COVID-19 uninhibited replication, leading to inflammatory infiltration. Blockage of INF-1 pathways and direct damage to airway epithelial cells increases susceptibility to fungal invasion. Corticosteroids, administered to control inflammation, are added risk factors for mucormycosis.⁶ Hyperglycaemia is a known side effect of the treatment, which in turn is an immunosuppressive state adding to the infection. In the existence of an underlying diabetic condition, the risk greatly intensifies.⁶

Acute invasive fungal rhinosinusitis (AIFRS) is a fulminant infection in these immunocompromised patients. This requires rapid diagnosis with prompt surgical intervention.⁷ KOH preparation has its own limitation which requires microbiologist expertise and fungal cultures take a longer time. Intraoperative frozen section diagnosis with permanent histopathology confirmation remains a gold standard in the diagnosis and treatment of Acute invasive fungal infections. Frozen section-guided surgical debridement in rhino-orbital Mucor mycosis may cause less morbidity and also provide an alternative to traditional radical surgical excision.⁸ Here, we discuss the diagnostic accuracy of frozen section in post COVID fungal infections, in a tertiary care setup. To the best of my knowledge, this is the 1st study to assess the accuracy of frozen section diagnosis in post-COVID fungal infections.

2. Materials and Methods

A single institutional prospective study was conducted on the suspected cases of Mucor mycosis in COVID patients who had intraoperative frozen section consultation at HCG Cancer Centre, Bengaluru between April 8th to July 27th 2021. The suspected cases of Mucormycosis who did not have a frozen section consultation and the non-COVID cases were excluded from the study. 61 fresh tissue samples from 55 patients were received for frozen section evaluation. 03 samples from the eye, 02 from the brain, 01 from the lung and the rest from the sinonasal region. The fresh tissue from

the operation room was embedded entirely in a Leica biosystems cryostat at a temperature of -18 to -20 degrees Celsius. Thin sections were cut at 4 to 5 micrometres, stained with rapid haematoxylin and eosin (H&E) stain and viewed under a light microscope. These tissues are then taken for permanent sections, stained with H&E and special fungal stains (Gomorimethenamine silver stain and Periodic acid Schiff stain). Mucor was identified as broad-based, aseptate, right angle branching fungal hyphae and Aspergillus as narrow slender septate acute angle branching fungal hyphae. Thin filamentous fungal hyphae were identified as Candida. These fungal hyphae stain black in GMS stain and Magenta pink in PAS stain (Figure: 1). All the cases had final confirmation with permanent histopathology sections. In 23 (42%) cases intra-operative tissue samples were also sent for KOH preparation and fungal culture and were correlated with the frozen section report. The parameters such as age, sex, use of steroids, oxygen support, diabetes and vaccination status were noted. 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. The turnaround time of 20min for the frozen section was maintained in all cases.

3. Results

Of the 55 suspected cases of Mucormycosis, 41 were male and 14 were female, with a mean age group of 51 to 60 years (Table 1). Of these 55 post-COVID patients, 52 patients had a hospitalization history during COVID and 3 patients received home treatment. 50 (90%) patients had a history of steroid treatment during COVID and 21 (38%) had a history of additional oxygen support. 47 (85.45%) were known diabetic (7 patients had associated hypertension, 1 patient had ischemic heart disease and 2 patients had both hypertension and ischemic heart disease in addition to diabetes). Of these 47 patients, 1 patient had type I diabetes mellitus and the rest of the patients had type II diabetes mellitus. 5 (9.09%) patients developed hyperglycemia post steroid treatment. 2 patients had a single dose of COVID vaccination, and the rest were not vaccinated.

Of the 55 suspected cases, 50 patients had 1 sample, 4 patients had 2 samples and 1 patient had 3 samples for frozen section evaluation with a total of 61 samples. Of these 61 samples, 3 were from the brain, 3 from orbit, 1 from the lung and the rest (54) from the sinonasal region. Of the 54 samples from the sinonasal region, 29 were from the maxillary sinus, 13 from the lateral nasal wall and turbinates, 6 from the ethmoid sinus, 5 from the sphenoid sinus and 1 from the lower alveolus.

Of the 61 samples evaluated in the frozen section, 47 were positive and 14 were negative for mucor mycosis. On the evaluation of permanent H&E sections, 50 were positive and only 11 were negative for mucor, with discordance of 3 cases in the frozen section. On analysis of the false negative cases, all 3 cases had very tiny foci of fungal elements in the permanent histopathology sections, which were observed only with special stains (GMS and PAS). One case had a

subsequent frozen section, as there was a high clinical suspicion, which was positive for mucor and underwent immediate surgical debridement. The other 2 cases underwent debridement within 24 hours of permanent histopathology examination.

Overall, our study had a sensitivity of 94.3%, specificity of 100%, a positive predictive value of 100% and a negative predictive value of 78.5%. All the cases reported as positive in frozen were positive in permanent sections also. 15 (30%) samples had Aspergillus in addition to mucor (11 were reported in the frozen section) and 02 had candida. Also identified were fruiting bodies, sporangia, spores, angioinvasion, perineural invasion and invasion of bone by mucor (Figure: 2).

Of the 55 patients, 23 (42%) patients also had microbiological examinations (KOH preparation and fungal culture). Of these 23 patients, 12 were confirmed cases of Mucormycosis. Of these 12 patients, 5 patients had a positive KOH and 10 Patients had a positive fungal culture. KOH preparation had 7 false negative cases and fungal culture had 2 false negative cases, adding more value to our study. All the 11 patients reported as negative for Mucormycosis in the frozen section and permanent histopathology section had a negative KOH and fungal culture.

4. Discussion

Early surgical debridement is the ideal treatment modality for acute invasive fungal rhinosinusitis (AIFRS). This intervention is rarely undertaken without a confirmative histopathology diagnosis. However, as an aggressive and destructive disease, AIFRS requires a faster and more accurate diagnostic method. Frozen section is an attractive alternative, being the fastest available option for a histopathology examination. However, a deeper clarity is required regarding the accuracy of frozen section diagnosis in this context and further implementation of this in the treatment protocols. In our study, we have assessed the accuracy of the frozen section in post COVID AIFRS cases. The previous studies related to frozen section diagnosis in AIFRS were retrospective studies, whereas ours is a prospective study.

A recent retrospective study conducted by Alkhateb R et al⁹ included 53 patients with suspected AIFRS who had intraoperative frozen section consultation between 2009 to 2019. Of the 53 patients, the incidence was more in males with a mean age group of 50 to 60 years, which was similar to our study. 59% were diabetic and the maxillary sinus was the most common site involved. In our study population, 85.45% were diabetic and the most common site of involvement was similar to their study. In their study, the frozen section demonstrated 88.5% sensitivity whereas our study had 94.3% sensitivity.

The retrospective study by Silveira M L C et. al¹⁰ demonstrated better outcomes and low mortality rate in patients who underwent immediate treatment with a frozen section positive nasal endoscopic biopsy. They observed a sensitivity of 90.6% and a specificity of 72.7%. The most

common comorbidity was hematological malignancies (58.2%) with only 2% being diabetics, limiting widespread applicability.

Similarly, the study by Papagiannopoulos P et al¹¹ also predominantly included patients with hematological manifestations with only 2% of diabetic patients. In this study, all the cases were examined by head and neck pathologists, which is not a feasible method in all circumstances.

GhadialiM T et al¹² conducted a retrospective review of 20 patients with a final diagnosis of AIFRS and did not include the suspected cases. They revealed an overall sensitivity of 84%. This study did not contain the analysis of the false negative cases, as it was conducted by a retrospective review of pathology reports. Of the 5 false negative cases, 4 were in patients with mucor.

The study design employed in Melanconet al¹³ included all suspected cases of AIFRS and demonstrated an overall sensitivity of 87.5%. Similar to Ghadiali et al, this study is a retrospective review of pathology reports and failed to explore the pathological factors affecting interpretation.

Also notable are the studies by Hoffman et al¹⁴ and Taxey et al¹⁵, who demonstrated the specificity of 85.7% and 62.5% respectively. These studies failed to offer an analysis of the false negative discrepancies.

Clinically, our patient group was substantially similar to those reported previously with comparable patient demographics and underlying medical conditions, as was the average number of frozen sections performed. Our study demonstrated an increased rate of AIFRS among the biopsied patients with an overall increase in sensitivity compared to the previous studies.

5. Conclusion

Frozen section-guided surgical debridement in Rhino-Orbital Mucormycosis is a rapid and effective diagnostic tool in suspected cases of fulminant fungal infections. Our analysis demonstrated overall high accuracy of frozen sections in AIFRS patients in a post-COVID setting, thereby merits prompt surgical intervention and proves to be an alternative to traditional radical excision.

Table 1: Patient demographics

Age group	Male (%)	Female (%)	Total (%)
21-30	5 (9.09)	1 (1.81)	6 (10.09)
31-40	6 (10.9)	1 (1.81)	7 (12.7)
41-50	7 (12.7)	4 (7.2)	11 (20)
51-60	15 (27.27)	3 (5.45)	18 (32.72)
61-70	5 (9.09)	4 (7.2)	9 (16.36)
71-80	3 (5.45)	1 (1.81)	4 (7.2)
Total	41 (74.54)	14 (25.45)	55 (100)

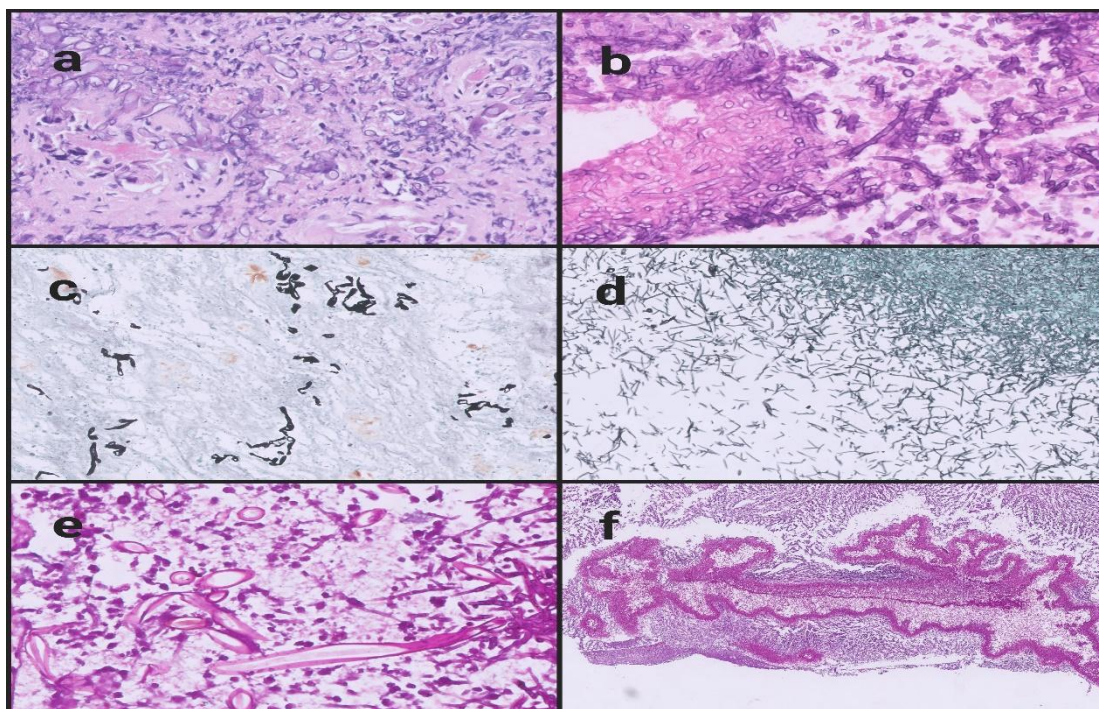


Figure 1: a. H&E 40x. Shows broad based aseptate mucor in frozen section, b. H&E 40x. Shows narrow septate acute angle branching aspergillus in frozen section, c and d. GMS stain 40x. Demonstrates mucor and aspergillus highlighted as black fungal profile in GMS stain, e and f. PAS stain 40x and 10x. Demonstrates mucor and aspergillus highlighted as magenta pink fungal profiles in PAS stain, e. also shows aspergillus forming a wall.

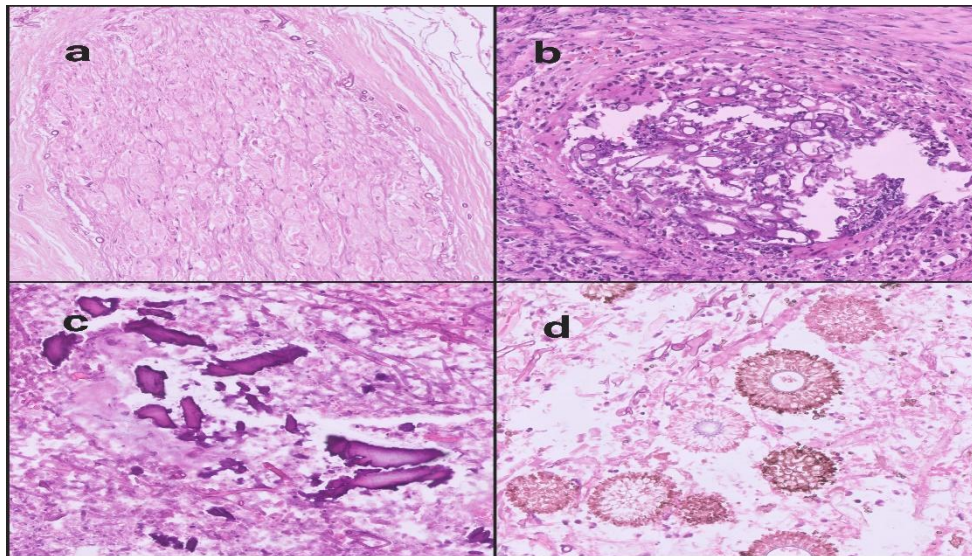


Figure 2: H&E 40x: a. Shows perineural invasion by mucor, b. Shows angioinvasion and destruction of vessel wall by mucor, c. Shows invasion of bone mucor, d. Shows fruiting bodies, sporangia and spores.

Financial support and sponsorship: Nil

Conflicts of interest: There are no conflicts of interest.

Acknowledgments: Nil

Ethics approval: Not applicable

Consent for publication: Not applicable

Availability of data and materials: Not applicable

References

- [1] Worldometers. COVID-19 coronavirus pandemic.2021. <https://www.worldometers.info/coronavirus/> (accessed July 26, 2021).
- [2] Revannavar, S. M., SS, P., Samaga, L., KV, V., 2021. COVID-19 triggering mucormycosis in a susceptible patient: a new phenomenon in the developing world? *BMJ Case Rep.*14 (4).
- [3] 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: 1112–1118. doi: 10.1002/lary.23912. doi: 16.05.2021.
- [4] Chakrabarti A, Denning DW, Ferguson BJ, Ponikau J, Buzina W, Kita H, et al. Fungal rhinosinusitis: a categorization and definitional schema addressing current controversies. *Laryngoscope.*2009; 119: 1809–1818. doi: 10.1002/lary.20520. doi: 16.05.2021.
- [5] Hindustantimes, 2021. India Reported Over 45, 000 Black Fungus Cases So far, Says Mandaviya in RS. JUL 202021
- [6] COVID-19-Associated Mucormycosis: Triple Threat of the Pandemic. American society of microbiology.
- [7] Crist H, Hennessy M, Hodos J, et al., Acute Invasive Fungal Rhinosinusitis: Frozen Section Histomorphology and Diagnosis with PAS Stain. *Head Neck Pathol.*2019 Sep; 13 (3): 318-326. doi: 10.1007/s12105-018-0965-8.
- [8] Langford J D, McCartney D L, Wang R C. Frozen section-- guided surgical debridement for management of rhino-orbital mucormycosis. *Am J Ophthalmol.*1997 Aug; 124 (2): 265-7. doi: 10.1016/s0002-9394 (14) 70804-9.
- [9] Alkhateb R, Menon P D, Tariq H, Accuracy of Intraoperative Frozen Section in Detection of Acute Invasive Fungal Rhinosinusitis. *Arch Pathol Lab Med—Vol 145, June 2021.*
- [10] Silveira, M. L. C., Anselmo-Lima, W. T., Faria, F. M. et al. Impact of early detection of acute invasive fungal rhinosinusitis in immunocompromised patients. *BMC Infect Dis* 19, 310 (2019). <https://doi.org/10.1186/s12879-019-3938-y>
- [11] 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.
- [12] 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.
- [13] 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.
- [14] Hofman V, Castillo L, Betis F, Guevara N, Gari-Toussaint M, Hofman P. Usefulness of frozen section in rhinocerebralmucormycosis diagnosis and management. *Pathology.*2003; 35 (3): 212–216.
- [15] 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.