



Prevalence Of Fungal Isolates In Chronic Rhinosinusitis In Immunocompetent Individuals.

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Abstract

Background: Chronic rhinosinusitis is a common inflammatory disorder of the nose and paranasal sinuses with significant morbidity. Fungal involvement is increasingly recognized not only in immunocompromised individuals but also among immunocompetent patients. Clinical manifestations are often nonspecific, and diagnosis may require integration of radiological, microbiological, intraoperative, and histopathological findings. **Aim:** To determine the prevalence of fungal isolates among immunocompetent individuals with chronic rhinosinusitis using microbiological and histopathological methods. **Materials and Methods:** A hospital-based descriptive observational study was conducted in the Department of Otorhinolaryngology, Head and Neck Surgery, St. John's Medical College and Hospital, Bengaluru, from November 2020 to November 2022. A total of 32 immunocompetent patients aged 18–60 years with chronic rhinosinusitis were included. Clinical symptoms and computed tomography findings of the paranasal sinuses were recorded. Patients undergoing endoscopic sinus surgery were assessed for intraoperative findings, and tissue specimens were subjected to fungal microbiological examination and histopathological examination using appropriate fungal stains. **Results:** Of the 32 patients, 14 (43.8%) were fungal-positive by at least one diagnostic modality. Fungal culture was positive in 11 (34.4%), while histopathology demonstrated fungal elements in 6 (18.8%); only 3 patients were positive by both methods, and the association between culture and histopathology was not statistically significant ($p=0.41$). Nasal obstruction was the most common symptom, followed by nasal discharge; however, none of the clinical symptoms showed a significant association with fungal positivity. Unilateral sinus involvement on CT was significantly associated with fungal positivity ($p=0.044$). Among individual sinuses, fungal positivity was highest with sphenoid sinus involvement, although individual sinus associations were not statistically significant. Intraoperatively, fungal debris was strongly associated with fungal positivity, with 78.6% of patients with fungal debris testing positive compared with 16.7% without fungal debris ($p<0.001$). Histopathology demonstrated acute-angle branching septate fungal hyphae without evidence of tissue, vascular, or bony invasion. **Conclusion:** Fungal involvement was common among immunocompetent patients with chronic rhinosinusitis in the present study. Clinical symptoms alone were insufficient to predict fungal disease, whereas unilateral CT involvement and intraoperative fungal debris were useful indicators. The discordance between fungal culture and histopathology highlights the importance of using both modalities together. A comprehensive diagnostic approach combining clinical evaluation, imaging, intraoperative assessment, microbiology, and histopathology may improve identification of fungal rhinosinusitis in immunocompetent individuals.

Keywords: Chronic rhinosinusitis; fungal rhinosinusitis; immunocompetent; fungal culture.



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INTRODUCTION

Chronic rhinosinusitis (CRS) is a heterogeneous inflammatory disorder of the nose and paranasal sinuses characterized by persistent sinonasal symptoms accompanied by objective endoscopic or radiological evidence of mucosal disease.¹ It represents an important global health problem because of its chronicity, recurrent exacerbations, adverse effects on quality of life, loss of productivity, and considerable healthcare utilization.¹ Epidemiological studies have demonstrated substantial geographical variation in the prevalence of CRS, reflecting differences in diagnostic criteria, environmental exposures, host factors, and population characteristics.²

The pathogenesis of CRS is multifactorial and involves complex interactions among host immunity, mucosal barrier dysfunction, environmental exposures, microorganisms, and inflammatory pathways.¹ Fungi have received particular attention because fungal organisms are ubiquitous in the environment and their spores are continuously deposited within the upper respiratory tract. Depending upon the host immune response, local sinonasal environment, and fungal characteristics, their presence may represent simple colonization, an allergic inflammatory response, or true tissue-invasive disease.³

Fungal rhinosinusitis (FRS) encompasses a broad spectrum of sinonasal disorders and is conventionally classified into invasive and non-invasive forms according to the presence or absence of fungal invasion of sinonasal tissues.³ Non-invasive forms include saprophytic fungal infestation, fungal ball, and allergic fungal rhinosinusitis (AFRS), whereas invasive disease comprises acute invasive, chronic invasive, and granulomatous invasive fungal rhinosinusitis.^{3,4} Although invasive fungal disease is traditionally associated with immunocompromised states, clinically significant fungal rhinosinusitis is also well recognized among immunocompetent individuals.⁴

This distinction is clinically important because fungal ball and AFRS predominantly occur in patients without major immune deficiency, while certain chronic and granulomatous invasive forms may also develop in apparently immunocompetent hosts.^{3,4} The clinical manifestations of fungal rhinosinusitis frequently overlap with conventional CRS and include nasal obstruction, nasal discharge, postnasal drip, facial pain or pressure, headache, and olfactory dysfunction. Consequently, clinical manifestations alone may be insufficient to reliably distinguish fungal from non-fungal CRS.

Radiological features such as unilateral sinus involvement, heterogeneous opacification, hyperattenuating material, sinus expansion, bony remodelling, or erosion may raise suspicion of fungal disease; however, definitive characterization generally requires microbiological and histopathological assessment.³ Fungal culture permits identification of viable fungal organisms, whereas histopathology demonstrates fungal elements, associated inflammatory responses, and, importantly, the presence or absence of tissue invasion. The two methods are therefore complementary, and discordance between fungal culture and histopathological findings may occur.⁵

In the Indian context, fungal rhinosinusitis assumes particular importance because environmental and climatic conditions may facilitate substantial exposure to airborne fungal spores. Indian studies have demonstrated fungal involvement across different clinical forms of CRS and have highlighted considerable variation in culture and histopathological positivity.^{5,7} Kaur et al. demonstrated the diagnostic importance of combining clinical, radiological, microbiological, and histopathological findings in Indian patients with AFRS.⁵ Such observations indicate that reliance on a single diagnostic modality may underestimate or misclassify fungal involvement in CRS.

Despite increasing recognition of FRS, the actual frequency of fungal isolation among immunocompetent patients with CRS remains variable across geographical regions and study populations. Differences in patient selection, specimen collection, fungal culture techniques, histopathological processing, environmental exposure, and diagnostic definitions may partly explain this variation.^{3,7} Determining the local prevalence of fungal isolates is therefore important for understanding the contribution of fungi to CRS and for improving diagnostic strategies.

The uploaded study similarly addresses this clinically relevant question by evaluating fungal isolates in immunocompetent individuals diagnosed with CRS. In the study, microbiological examination and histopathology were used as complementary methods for detecting fungal involvement, which is particularly relevant because fungal culture and tissue demonstration may not always yield concordant results.

Therefore, the present study was undertaken to determine the prevalence of fungal isolates among immunocompetent individuals with chronic rhinosinusitis and to evaluate fungal involvement using microbiological and histopathological methods. The findings may provide institution-specific Indian data regarding the burden of fungal involvement in CRS and support a more systematic diagnostic approach to suspected fungal rhinosinusitis.

AIM

To determine the prevalence of fungal isolates among immunocompetent individuals with chronic rhinosinusitis (CRS) using microbiological and histopathological methods.

OBJECTIVES

Primary Objective

1. To estimate the prevalence of fungal isolates among immunocompetent individuals with chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP).

Secondary Objectives

2. To evaluate the clinical presentation, preoperative computed tomography findings, and intraoperative findings among immunocompetent individuals with CRS and correlate these findings with the presence or absence of fungal isolates.
3. To compare the detection of fungal elements by microbiological examination and histopathological examination among patients with CRS.

MATERIALS AND METHODS

Study Design

A hospital-based descriptive observational study was conducted.

Study Setting

The study was conducted in the Department of Otorhinolaryngology, Head and Neck Surgery, St. John's Medical College and Hospital, Bengaluru, Karnataka, India. The source dissertation describes the study as a descriptive study conducted in this department.

Study Period

The study was conducted over a period of 24 months, from November 2020 to November 2022.

Study Population

Patients aged 18–60 years who presented to the ENT outpatient department with a clinical diagnosis of chronic rhinosinusitis and fulfilled the eligibility criteria were considered for inclusion in the study.

Sample Size

A total of 32 immunocompetent patients with chronic rhinosinusitis were included in the study. The sample size was estimated based on an expected increase in the proportion of fungal isolates compared with the proportion reported in a previous study, considering a 5% Type I error and 80% statistical power.

Sampling Technique

Eligible patients presenting during the study period were recruited using a convenience sampling technique.

Inclusion Criteria

1. Were aged 18–60 years.
2. Had a clinical diagnosis of chronic rhinosinusitis according to the EPOS 2020 criteria.
3. Had two or more symptoms, with at least one being nasal blockage/obstruction/congestion or nasal discharge, with or without facial pain/pressure and reduction or loss of smell, persisting for ≥ 12 weeks.
4. Were immunocompetent and provided written informed consent for participation.

Exclusion Criteria

1. Did not provide written informed consent.
2. Had conditions compromising immune function, including HIV infection or diabetes mellitus.
3. Had a history of prolonged systemic corticosteroid or immunosuppressive therapy.
4. Had an autoimmune disease.

Data Collection and Clinical Evaluation

After obtaining Institutional Ethics Committee approval, all potentially eligible patients presenting to the ENT outpatient department were screened. Written informed consent was obtained from eligible participants after explaining the nature and purpose of the study.

Demographic characteristics and detailed clinical history were recorded using a predesigned and pretested proforma. Presenting symptoms, including nasal obstruction, nasal discharge, postnasal discharge, facial pain, olfactory dysfunction, epistaxis, and facial swelling, were documented.

Radiological Evaluation

All included patients underwent computed tomography (CT) of the paranasal sinuses as part of their clinical evaluation. CT findings were assessed for laterality of disease, presence of nasal polyps, involvement of individual paranasal sinuses, and other relevant radiological abnormalities. The original methodology specifically recorded laterality and the sinuses involved on CT.

Intraoperative Assessment and Specimen Collection

Patients who underwent endoscopic sinus surgery were evaluated intraoperatively. Findings including the presence of allergic mucin, fungal debris, polypoidal changes, and the involved paranasal sinuses were documented.

Adequate tissue specimens were obtained during surgery and were submitted separately for fungal microbiological examination and histopathological examination.

Microbiological and Histopathological Examination

The collected specimens were evaluated microbiologically for the presence or absence of fungal elements and fungal growth. Histopathological examination was performed to identify fungal elements and associated tissue changes. Special stains, including Periodic acid–Schiff (PAS) and Grocott's methenamine silver (GMS), were used for demonstrating fungal elements.

Outcome Measures

The primary outcome was the prevalence of fungal isolates among immunocompetent patients with CRS. Secondary assessments included the association of fungal positivity with clinical presentation, CT findings, intraoperative findings, and concordance between microbiological and histopathological detection.

Statistical Analysis

The collected data were entered and organized using Microsoft Excel 2019 and analysed using R software version 4.2.1. Categorical variables were expressed as frequencies and percentages. The Chi-square test was used to assess associations between categorical variables, while Fisher's exact test was applied when expected cell frequencies were less than 5. A p-value <0.05 was considered statistically significant.

Ethical Considerations

The study was initiated after obtaining approval from the Institutional Ethics Committee of St. John's Medical College and Hospital, Bengaluru. Written informed consent was obtained from all participants, and confidentiality and anonymity of participant information were maintained throughout the study.

RESULTS

Table 1. Microbiological and histopathological detection of fungal elements among patients with CRS (n=32)

Histopathology	Fungal culture positive	Fungal culture negative	Total	p-value
Fungal elements present	3 (9.4%)	3 (9.4%)	6 (18.8%)	0.41
Fungal elements absent	8 (25.0%)	18 (56.3%)	26 (81.3%)	
Total	11 (34.4%)	21 (65.6%)	32 (100%)	

Interpretation: Fungal culture was positive in 11/32 (34.4%) patients, whereas histopathology demonstrated fungal elements in 6/32 (18.8%). Only 3 patients were positive by both modalities. The association between fungal culture and histopathological positivity was not statistically significant (p=0.41), indicating that the two diagnostic methods did not show significant concordance and may provide complementary diagnostic information.

Table 2. Association of clinical symptoms with fungal positivity (n=32)

Clinical symptom	Symptom present n (%)	Fungal positive among symptom-positive n (%)	Fungal negative n (%)	p-value
Nasal obstruction	18 (56.3)	7 (38.9)	11 (61.1)	0.50
Nasal discharge	14 (43.8)	6 (42.9)	8 (57.1)	>0.90
Postnasal discharge	1 (3.1)	1 (100)	0	0.40
Facial pain	6 (18.8)	2 (33.3)	4 (66.7)	0.70
Olfactory dysfunction	1 (3.1)	0	1 (100)	>0.90
Epistaxis	1 (3.1)	0	1 (100)	>0.90
Facial swelling	2 (6.3)	0	2 (100)	0.50

Interpretation: Nasal obstruction was the most frequent symptom, occurring in 56.3%, followed by nasal discharge in 43.8% and facial pain in 18.8%. None of the evaluated clinical symptoms showed a statistically significant association with fungal positivity (all p>0.05). Thus, clinical symptomatology alone was not useful for reliably differentiating fungal from non-fungal CRS.

Table 3. Association between CT laterality and fungal positivity (n=32)

CT laterality	Fungal positive n (%)	Fungal negative n (%)	Total n (%)	p-value
Unilateral involvement	13 (56.5)	10 (43.5)	23 (71.9)	0.044
Bilateral involvement	1 (11.1)	8 (88.9)	9 (28.1)	
Total	14 (43.8)	18 (56.3)	32 (100)	

Interpretation: Among the 23 patients with unilateral sinus disease on CT, 13 (56.5%) were fungal-positive, compared with only 1/9 (11.1%) patients with bilateral involvement. The association between unilateral CT involvement and fungal positivity was statistically significant ($p=0.044$), suggesting that unilateral sinus disease should raise clinical suspicion of fungal involvement.

Table 4. Association of individual sinus involvement on CT with fungal positivity

Sinus involved on CT	Fungal positive n (%)	Fungal negative n (%)	p-value
Maxillary sinus	9/26 (34.6)	17/26 (65.4)	0.064
Ethmoid sinus	8/22 (36.4)	14/22 (63.6)	0.30
Sphenoid sinus	9/16 (56.3)	7/16 (43.8)	0.20
Frontal sinus	3/10 (30.0)	7/10 (70.0)	0.40

Interpretation: Among patients with involvement of the respective sinus on CT, fungal positivity was highest with sphenoid sinus involvement (56.3%), followed by ethmoid (36.4%), maxillary (34.6%), and frontal sinus involvement (30.0%). However, none of the individual sinus involvement patterns demonstrated a statistically significant association with fungal positivity ($p>0.05$).

Table 5. Association of key intraoperative findings with fungal positivity (n=32)

Intraoperative finding	Fungal positive	Fungal negative	p-value
Polyps present (n=19)	7 (36.8%)	12 (63.2%)	0.30
Polyps absent (n=13)	7 (53.8%)	6 (46.2%)	
Fungal debris present (n=14)	11 (78.6%)	3 (21.4%)	<0.001
Fungal debris absent (n=18)	3 (16.7%)	15 (83.3%)	

Interpretation: The presence of intraoperative polyps was not significantly associated with fungal positivity ($p=0.30$). In contrast, 11/14 (78.6%) patients in whom characteristic fungal debris was identified intraoperatively were fungal-positive, compared with only 3/18 (16.7%) without fungal debris. This association was highly statistically significant ($p<0.001$), making the presence of fungal debris the strongest intraoperative correlate of fungal positivity in the study.

Overall Results

Overall, fungal involvement was identified in 14/32 (43.8%) patients by at least one of the two diagnostic modalities, while fungal culture alone was positive in 34.4% and histopathology in 18.8%. Clinical symptoms did not significantly predict fungal positivity. In contrast, unilateral disease on CT ($p=0.044$) and particularly intraoperative fungal debris ($p<0.001$) were significantly associated with fungal positivity. The original study also reported *Aspergillus* as the predominant cultured fungus and described the histopathologically identified fungal elements as acute-angle branching septate hyphae without evidence of tissue, vascular, or bony invasion.

DISCUSSION

The present study evaluated fungal involvement among 32 immunocompetent patients with chronic rhinosinusitis (CRS) and demonstrated fungal positivity by either microbiological or histopathological examination in 14/32 (43.8%) patients. Fungal culture was positive in 11 (34.4%), whereas histopathology demonstrated fungal elements in 6 (18.8%) patients; only three patients were positive by both modalities, and the association between culture and histopathology was not statistically significant ($p=0.41$).

The relatively high frequency of fungal detection in the present study reinforces the importance of considering fungi as a potential contributor to CRS even among individuals without underlying immunosuppression. Saravanan et al. studied 70 Indian patients with CRS and categorized 36 (51.4%) as likely allergic fungal rhinosinusitis (AFRS) and four as sinus mycetoma, demonstrating a substantial burden of fungal-associated disease in an Indian tertiary-care setting.⁸ Among the likely AFRS patients in their study, fungal culture was positive in 32/36 (89%), with *Aspergillus flavus* accounting for 26 isolates.

Swain et al. similarly evaluated fungal rhinosinusitis in an Indian tertiary-care hospital and emphasized that FRS occurs not only in diabetic and immunocompromised subjects but also in immunocompetent individuals.⁹ Their clinic mycological

observations further support the need for systematic fungal examination in patients with persistent CRS rather than restricting fungal investigations only to conventionally high-risk patients.

Syiemlieh and Mariraj reported a 26% prevalence of fungal rhinosinusitis among patients with CRS, which was somewhat lower than the overall 43.8% fungal positivity observed in the present study.¹⁰ In their series, 76.9% of fungal rhinosinusitis was caused by *Aspergillus* species, with *A.*

flavus constituting 61.5%, *A. niger* 15.3%, *Mucor* species 15.3%, and *Curvularia* 7.6%; non-invasive AFRS accounted for approximately 85% of FRS cases. The predominance of *Aspergillus* is comparable with the present study, in which *Aspergillus* species constituted the major fungal growth obtained on culture.

Regarding symptomatology, nasal obstruction (56.3%) was the most frequent complaint in the present study, followed by nasal discharge (43.8%), facial pain (18.8%), facial swelling (6.3%), and postnasal discharge, olfactory dysfunction and epistaxis in 3.1% each. However, none of these symptoms showed a statistically significant association with fungal positivity. This indicates considerable clinical overlap between fungal and non-fungal CRS and demonstrates that fungal etiology cannot reliably be predicted from symptoms alone.

This observation is consistent with reports on non-invasive fungal sinus disease, where clinical presentations are frequently nonspecific. Pagella et al., in a study of 81 patients with paranasal sinus fungus ball, demonstrated that fungal disease commonly occurred in immunocompetent patients and emphasized the importance of combining radiological findings, endoscopy, histology and mycological assessment.¹¹ In that study, only 28/81 (34.5%) cases yielded moulds on culture despite histological confirmation of fungal colonization in all cases, illustrating the limited sensitivity of culture when used in isolation.

Nicolai et al. reported their experience with 160 patients with paranasal sinus fungus ball, further establishing fungal ball as an important non-invasive manifestation occurring predominantly in immunocompetent individuals.¹² Their experience supports the concept that definitive assessment of suspected fungal disease requires evaluation of surgically obtained sinus contents rather than reliance on clinical manifestations alone. One of the most important findings in the present study was the association between unilateral sinus involvement on CT and fungal positivity. Among 23 patients with unilateral disease, 13 (56.5%) were fungal-positive compared with only 1/9 (11.1%) patients with bilateral disease, and this difference was statistically significant ($p=0.044$). Thus, unilateral radiological disease emerged as a potentially useful preoperative indicator of fungal involvement.

Dufour et al., in their retrospective analysis of 173 cases of paranasal sinus fungus ball, similarly characterized fungal ball as a predominantly localized sinonasal condition.¹³ Pagella et al. also found that 73 of 81 patients had involvement of a single sinus, whereas only eight had multiple localizations.¹¹ These findings strengthen the observation from the present study that unilateral or localized sinus disease should increase suspicion of a fungal etiology. Nomura et al. evaluated 104 patients with sinus fungus ball and likewise described the condition as a form of non-invasive chronic fungal rhinosinusitis occurring predominantly in immunocompetent patients.¹⁴ Their radiological and clinical observations emphasized the value of CT in identifying characteristic localized fungal disease. Yoon et al., in a much larger retrospective series of 538 sinonasal fungus-ball cases, further demonstrated the substantial clinical burden of this form of fungal disease and the usefulness of imaging and operative assessment in establishing the diagnosis.¹⁵

With respect to individual sinuses, fungal positivity in the present study was highest when the sphenoid sinus was involved on CT (56.3%), followed by ethmoid (36.4%), maxillary (34.6%) and frontal sinus (30.0%); however, these individual sinus associations were not statistically significant. This differs from several fungus-ball series in which the maxillary sinus predominated. For example, Pagella et al. reported the maxillary sinus as the most frequently involved site, followed by the sphenoid and ethmoid sinuses.¹¹ Nevertheless, isolated sphenoid fungal disease is increasingly recognized. Jiang et al. studied 77 histopathologically confirmed sphenoid sinus fungus-ball cases and reported CT findings of sphenoid opacification in 100%, sclerosis in 93.5%, calcification in 76.6%, and bone erosion in 41.6% of patients.¹⁶ Headache was present in 79.2% of their cases. The relatively high sphenoid fungal positivity in the present study therefore has clinical relevance, particularly because sphenoid disease may present with nonspecific symptoms.

The strongest association identified in the present study was the intraoperative presence of characteristic fungal debris. Fungal positivity was observed in 11/14 (78.6%) patients with fungal debris compared with only 3/18 (16.7%) patients without such debris, and the association was highly statistically significant ($p<0.001$). In contrast, the presence of intraoperative polyps was not significantly associated with fungal positivity ($p=0.30$). These findings emphasize the importance of careful intraoperative examination of sinus contents and appropriate sampling of suspicious material. Histopathological examination in the present study demonstrated acute-angle branching septate fungal hyphae on PAS and GMS staining in 19% of CRS cases, without evidence of angioinvasion or invasion of mucosa or bone, supporting a

predominantly non-invasive pattern of fungal disease. Montone emphasized that histopathology plays a central role in classifying fungal rhinosinusitis because demonstration or exclusion of tissue invasion is essential for distinguishing invasive from non-invasive disease.¹⁷

The discordance between fungal culture and histopathology observed in the present study is also clinically important. Culture identified fungi in 34.4% compared with histopathological demonstration in 18.8%, and only three cases were simultaneously positive by both methods. Such discrepancies may arise from sampling variability, low fungal burden, nonviable organisms, prior therapy, specimen handling and the focal distribution of fungal elements within sinus material. Consequently, a negative result by one modality should not automatically exclude fungal disease when radiological and intraoperative findings remain strongly suggestive. Recent evidence further supports this integrated diagnostic strategy. Soundarya et al. evaluated 85 patients with fungal rhinosinusitis and found fungal growth in 36 of 77 patients with available cultures, with *Aspergillus* constituting the majority of isolates.¹⁸ When histopathology was compared with mycology for detection of *Aspergillus*, it demonstrated 90% sensitivity, 96% specificity and a Cohen κ of 0.8, underscoring the complementary value of pathological and microbiological evaluation.

Overall, the present findings demonstrate that fungal involvement constitutes an important proportion of CRS among immunocompetent individuals. Neither demographic characteristics nor individual sinonasal symptoms reliably distinguished fungal from non-fungal CRS, whereas unilateral involvement on CT and particularly characteristic fungal debris at surgery were important predictors of fungal positivity. The observed discordance between culture and histopathology further indicates that diagnosis should be based on an integrated approach combining clinical assessment, CT imaging, intraoperative findings, fungal culture and histopathological examination rather than any single diagnostic modality.

CONCLUSION

Fungal involvement constituted a substantial proportion of chronic rhinosinusitis among immunocompetent individuals in the present study. Overall fungal positivity was identified in 14 of 32 patients (43.8%) by either microbiological or histopathological examination. Fungal culture was positive in 34.4%, whereas histopathology demonstrated fungal elements in 18.8%, with limited concordance between the two modalities.

Clinical symptoms were nonspecific and did not significantly distinguish fungal from non-fungal CRS. In contrast, unilateral sinus involvement on CT was significantly associated with fungal positivity ($p=0.044$), while the presence of characteristic fungal debris intraoperatively showed a strong association with fungal detection ($p<0.001$).

The findings emphasize that fungal rhinosinusitis should be considered even in immunocompetent patients presenting with CRS, particularly when unilateral radiological disease or characteristic intraoperative fungal debris is present. As microbiological culture and histopathology identified different subsets of patients, a combined diagnostic approach incorporating clinical assessment, CT imaging, intraoperative findings, fungal culture, and histopathological examination is preferable to reliance on any single diagnostic modality.

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