

**ORIGINAL PAPERS****Particularities of the diagnostic algorithm of non-invasive fungal rhinosinusitis****Claudiu Manea<sup>1</sup>, Codrut Sarafoleanu<sup>1</sup>, Dorin Sarafoleanu<sup>1</sup>, Andreea Pirvan<sup>1</sup>**<sup>1</sup>Sfanta Maria Hospital, ENT&HNS Department**ABSTRACT**

**BACKGROUND:** Fungal rhinosinusitis are more and more present in otorhinolaryngological practice, but still frequently underdiagnosed. This is due to the fact that, until now, there is no diagnostic algorithm widely accepted. Also the therapeutic approach is still controversial.

**OBJECTIVE:** To evaluate the efficiency of various diagnostic methods used for noninvasive fungal rhinosinusitis.

**MATERIAL AND METHODS:** Prospective study on 111 consecutive patients diagnosed with non-invasive allergic or non-allergic fungal rhinosinusitis. All the patients underwent surgery and had histopathologic identification of fungal material. We evaluated the efficiency of mycological and radiological diagnostic methods.

**RESULTS:** Anatomopathologic exam is superior to mycologic culture methods in our experience, and the sensibility of this examination method is influenced by the type of sampling (endoscopic, intraoperative, by sinusoscopy etc.). CT scan is the most valuable radiologic method in the diagnosis of fungal rhinosinusitis, in 80,77% of cases we encountered characteristic radiological findings.

**CONCLUSIONS:** The diagnostic algorithm for fungal rhinosinusitis involves the coordination between several diagnostic tracks: histopathology, mycologic exam, allergy and immunology tests and radiologic examination. In Romania, the histopathological exam remains the most reliable diagnostic test for the detection of fungal infection. In what it concerns the radiological examinations, CT scan is the "golden standard" for this condition.

**KEYWORDS:** fungal rhinosinusitis, histopatologic exam, mycologic exam, fungal allergy

**INTRODUCTION**

Fungal rhinosinusitis (FRS) have raised a lot of controversies regarding the most suitable diagnostic methods needed for these diseases, so similar in terms of symptoms with other forms of chronic rhinosinusitis (CRS), but so different in what it concerns the therapeutic protocol.

The diagnosis of FRS is a complex one, needing clinical examination but also specific radiological findings, allergy tests together with rigorous mycological and histopatologic analysis.

Currently, two major categories of fungal disease affecting the nose and paranasal sinuses are recognized:

1. invasive fungal rhinosinusitis (including acute invasive forms, chronic invasive fungal rhinosinusitis and granulomatous invasive fungal rhinosinusitis), and
2. non-invasive fungal rhinosinusitis (fungal ball and allergic and/or eosinophilic fungal rhinosinusitis).<sup>1,2</sup>

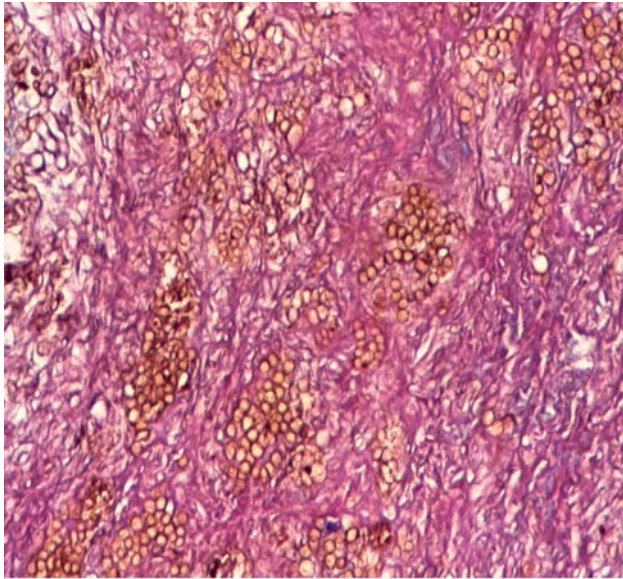
The complexity of the FRS diagnosis, especially in the

case of the allergic and eosinophilic forms, is given by the lack of precise diagnostic criteria. Is not to forget the ubiquitous nature of various types of fungi that can lead to many diagnostic confusions and also the difficulties of sampling, transportation and surveillance of the samples in order to obtain accurate results.<sup>3,4</sup>

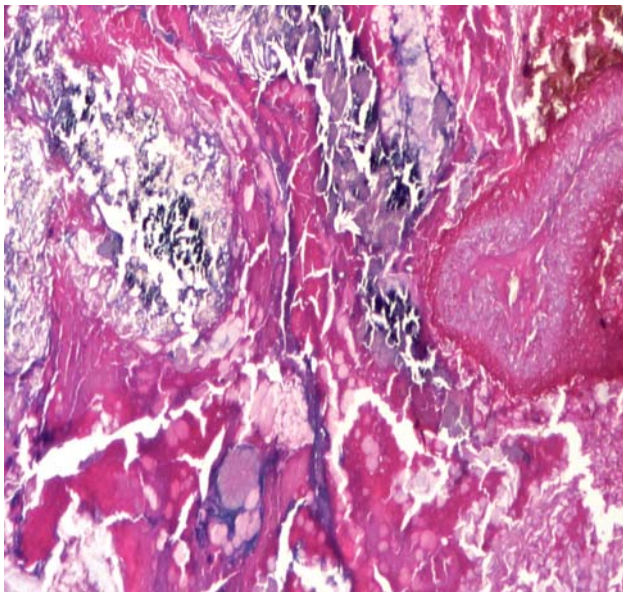
Starting from the premise that in order to have a positive diagnosis of chronic fungal rhinosinusitis must fulfill two criteria – to put the diagnosis of CRS and to find the fungal infection that had produced it, we tried to evaluate the sensitivity and specificity of various types of diagnostic tools used in this particular disease.

**MATERIAL AND METHODS**

A prospective analysis of 912 consecutive patients with the diagnosis of CRS that underwent surgical treatment between



**Figure 1** Histopathologic exam, H-E staining, magnification 10X – nests of *Aspergillus fumigatus* conidiospores



**Figure 2** Histopathologic exam, PAS staining, magnification 10X – eosinophilic mucin – conglomerates of eosinophils and *Aspergillus fumigatus* filaments

January 2005 – December 2008 in the ENT Department, “Sfanta Maria” Hospital, Bucharest, Romania. From all the patients we collected tissue and mucous samples on a saline moistened non-stick sheet that were send for histopathological staining and examination. The standard staining technique used in all patients was Haematoxylin-Eosin (H-E) staining, that allowed the analysis of the tissue response and also of some fungal aspects. This staining allows the visualization of some fungal species (like *Aspergillus* and *Zygomycetes*) (Figure 1), but the majority of fungal agents do not stain or stain poorly with H-E. Even in these cases, the

shadow of non-stained fungal cell can be seen, allowing the diagnosis of fungal presence. In this particular cases, and in those in which the clinical suspicion of fungal rhinosinusitis was high, but unconfirmed histopathological with H-E stain, there were used other special stains, like PAS (Figure 2), Gomori-Methenamine-Silver and Grocott staining. In this way we identified fungal material in 111 patients.

In the group of patients with proved fungal presence, we tried to evaluate the accuracy of the mycological examination, using various sampling techniques. The specimens were collected before, but also during the surgery using a swab for mycological culture. Before surgery the swab was introduced into the middle meatus under endoscopic control, using a 0-degree, 4 mm rigid telescope. To avoid any effects of local anesthetics and vasoconstrictors over the microbiological, cellular count and morphology, sampling procedures were obtained without these medications. Also before surgery, we used sinusoscopy as a method for collecting samples directly from the maxillary sinus.

The collected samples were sent directly to the mycology laboratory, being inoculated on Malt Agar plates containing 0,05% chloramphenicol. The time from sampling to inoculation was under 3 hours. The plates were incubated at 25°C and at 30°C and allowed to grow for 30 days, being examined at 2, 5, 7, 10, 15 and 30 days after incubation. Identification at the species level was performed.

Allergy tests were performed in 42 patients, using intradermal skin tests. Type I (erythema and wheal of any size within 1 hour) and type 4 (induration of more than 5 mm in diameter after 24 hours) hypersensitivity reactions were considered positive only when there was no reaction in the control arm.

The specificity and sensitivity of various radiological examinations were also evaluated, performing before surgery standard Rx examination, CT scan or MRI. The diagnostic criteria that were followed were: presence at CT scan of serpiginous areas of high attenuation especially in ethmoidal and maxillary sinuses, with bone thinning and erosions with dislocation of adjacent structures; presence at Magnetic Resonance images of areas showing decreased signal intensity leading to hypointense T1-weighted and markedly hypointense T2-weighted images with typical void signal; hyperdense areas, simulating the presence of a foreign body, that represents in fact calcium phosphate accumulations in fungal necrotic material are present in standard Rx examination.

The diagnostic criteria used for various type of FRS were as follows:

- For *fungal ball* type:
  - Suggestive radiologic aspect
  - Histopathologic confirmation of fungal presence, with no mucosal invasion
  - Lack of fungal allergy
- For *allergic fungal rhinosinusitis* (AFS)<sup>5</sup>
  - demonstration of fungal elements in nasal discharge or in material obtained at the time of surgery by stain or culture

**Table 1**  
Types of fungi identified by histopatologic and mycological examination (comparative study)

Type of fungi	HP exam		Mycologic exam	
	No.of patients	Percent (%)	No.of patients	Percent (%)
<i>A. fumigatus</i>	93	83,78	79	88,76
<i>A. niger</i>	11	9,91	5	5,61
<i>A. flavus</i>	4	3,60	3	3,37
<i>Cladosporium bantia</i>	2	1,80	1	1,12
<i>Alternaria alternata</i>	1	0,90	1	1,12

- identification of allergic or eosinophilic mucin [thick, tenacious and darkly coloured (peanut butter like) mucus containing aggregates of necrotic eosinophils, nuclear debris, free eosinophil granules, sloughed respiratory tract epithelial cells, and Charcot-Leyden crystals within an otherwise amorphous, pale eosinophilic or basophilic mucinous background] and scattered fungal hyphae
- fungal atopy of the host
- characteristic CT findings (areas of hyperattenuation on CT scan)
- For *eosinophilic fungal rhinosinusitis* (EFS)<sup>6</sup>
  - demonstration of fungal elements in nasal discharge or in material obtained at the time of surgery by stain or culture
  - identification of eosinophilic mucin
  - characteristic CT findings (areas of hyperattenuation on CT scan)

*gillus flavus* (4 patients, 3,60%), 2 patients with *Cladosporium bantia* (1,8%) and 1 patient with *Alternaria alternata* (0,9%) (Table 1).

The mycologic examination gave positive results in 89 patients using various sampling methods, having a sensitivity of 80,18% in comparison with the HP exam. The most frequent fungal specie was *Aspergillus fumigatus* (79 patients), followed by *Aspergillus niger*, *flavus*, *cladosporium bantia* and *alternaria alternata* (Table 1).

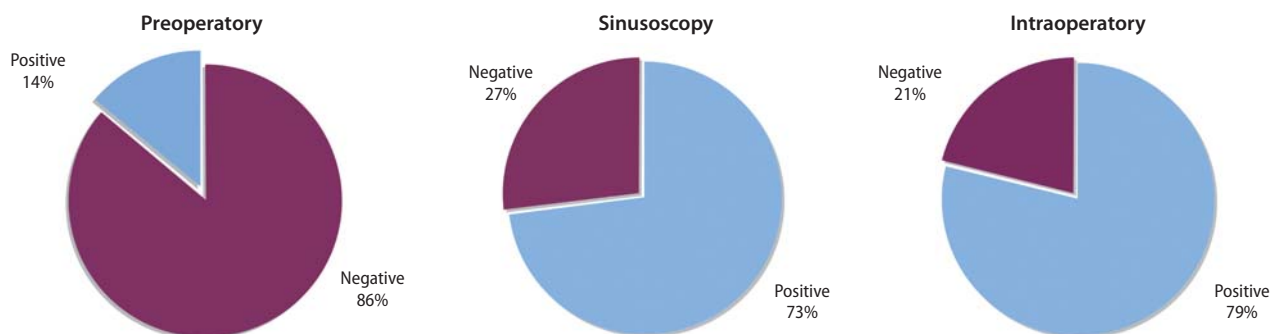
Another important parameter followed in this study was the reliability of different sampling methods for the mycologic exam. There were performed a number of 138 samples that were harvested preoperatory (64 samples), by sinusoscopy (11 samples) and intraoperatory (63 samples). The most reliable method was the intraoperatory sampling method with positive results in 79% of cases, followed by sinusoscopy (73% positive results). Very disappointing results were obtained with the endoscopic from the middle meatus preoperatory sampling method (14% positive results) (Figure 3). The reasons that allowed these poor re-

## RESULTS

From 912 consecutive patients with CRS, 111 of them, representing 12,17%, had the diagnosis of non-invasive FRS. The mean age was 43,63 years, and the male/female ratio was 1,13 (59 males, 52 females). The most frequently identified fungal species by histopatologic exam was *Aspergillus fumigatus* (93 patients, representing 83,78%) followed by *Aspergillus niger* (11 patients, representing 9,91%), *Asper-*

**Table 2**  
Types of fungal rhinosinusitis

Type of FRS	MYC	AFS	EFS
No.of patiens	48	4	59
Percent (%)	43,24	3,60	53,15



**Figure 3** Comparative success rate of various sampling methods for mycologic diagnosis of FRS



**Table 3**  
Patients distribution according to the type of radiologic examination

	CT	MRI	Conventional Rx	Without Rx exam
AFS	2	0	1	1
EFS	42	7	5	8
MYC	34	5	8	5
Total	78	12	14	14

sults will be discussed later.

Regarding the allergy tests, unfortunately in Romania there were not available until the time of the present study fungal extracts for *Aspergillus fumigatus* (the specie most frequently encountered in our study). This is the reason why, from the 42 patients that were tested, only 4 of them were found positive (2 patients with *Cladosporium bantia*, 1 patient with *Alternaria alternata* and 1 patient with *Aspergillus flavus*). We can conclude that in our country the allergy tests for fungi are still to be developed.

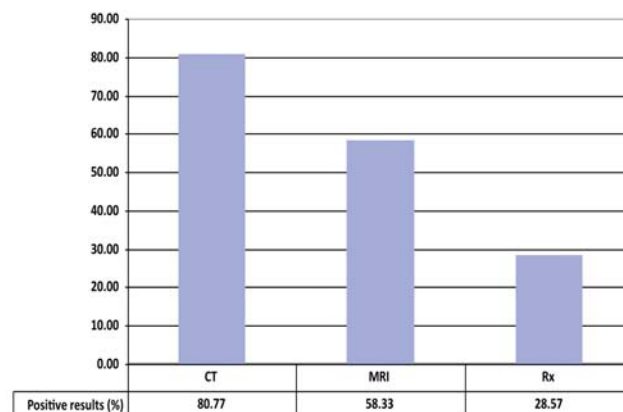
Correlating all the dates described above and according to the diagnostic criteria for the fungal rhinosinusitis, in our series of patients the results were as follows (Table 2): most frequent type of FRS was eosinophilic fungal rhinosinusitis (EFS) – 59 patients (53,15%), followed by mycetoma, or fungal ball (MYC) – 48 patients (43,24%) and allergic fungal rhinosinusitis (AFS) – 4 patients.

Radiologic examination is a key element in the diagnosis of FRS. In our study, we performed the radiologic examination of the nose and paranasal sinuses using conventional Rx, CT scan and MRI. The number of the performed examinations, divided on different types of FRS is shown in Table 3.

We evaluated the accuracy of each individual examination using radiological characteristics previously described, the results being as follows (Figure 4): the most reliable radiologic examination was CT scan, with suggestive radiologic aspects in 63 cases from 78 examinations (80,77% sensitivity), followed by craniofacial MRI – 7 suggestive aspects from 12 examinations (58,33% sensitivity). The classic Rx exam provided the worst results, with characteristic aspects in 28,57% of cases (4 out of 14 cases). We can conclude that the “golden standard” in the diagnosis of FRS is the CT scan.

## DISCUSSIONS

FRS is a controversial clinical entity as far as concerns the diagnosis and also the therapeutic approach<sup>7</sup>. Even if for a long period of time this disease was looked with skepticism, starting with the 90's a lot of debates were raised, especially after the launching of Mayo Clinic's theory<sup>2</sup> regarding the ubiquitous nature of fungi and their role in the chronic rhinosinusities etiology.



**Figure 4** Comparative evaluation of the radiologic investigations sensitivity in the diagnosis of FRS

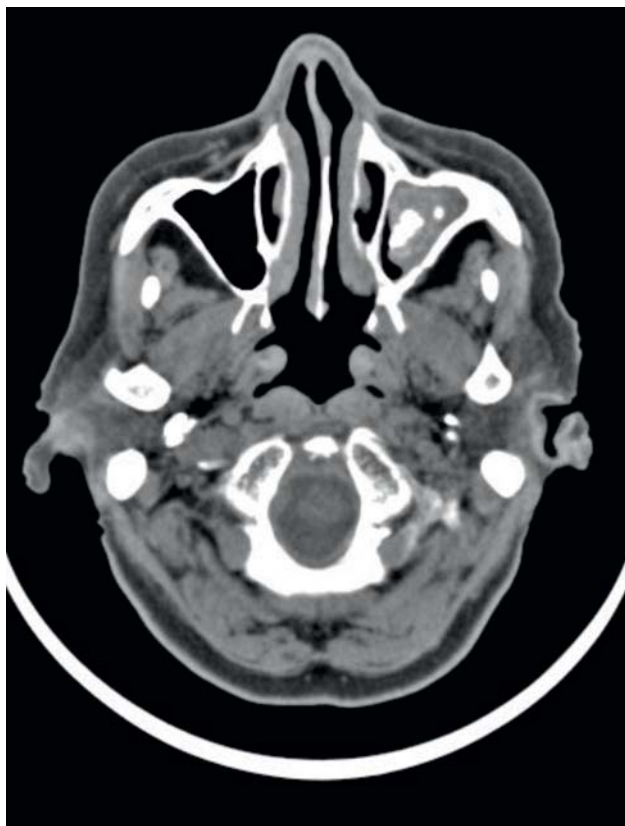
In this period a lot of technologic improvements were made, in terms of endoscopic diagnosis, radiologic examination (CT scan and MRI), and identification of fungal elements by means of mycological analysis (especially by developing new culture media, and standardization of the sampling, transportation and surveillance protocol for the samples) and histopathologic examination, lead to the increasing of the number of positive fungal rhinosinusities diagnosis.<sup>8,9</sup>

Without a heightened awareness of FRS, the diagnosis can be overlooked, resulting in the patient being erroneously treated for bacterial sinusitis. Similarly, a misunderstanding of the boundaries between AFS or EFS and invasive fungal sinusitis, because of the radiographic aspect of bone erosion can result in overaggressive surgical and medical treatment. A greater understanding of what constitutes AFS and EFS is necessary to avoid this scenario. This is the reason we need some clear diagnostic criteria for this disease, and this was the purpose for this study.

To make the diagnosis of fungal rhinosinusitis is necessary to have two conditions met: *diagnosis of rhinosinusitis* (not to forget the ubiquitous nature of fungi) and proving *the existence of fungal infection*. The latter can be achieved by histopathological and / or mycological examination. We considered that the histopathological exam is the benchmark in the study based on data from the literature that give it at least a similar sensitivity with the mycological exam in detecting the fungal sinus infection. Its advantages are that the material removal is achieved by biopsy or intraoperative sinusoscopy



**Figure 5** CT scan – fungal ball sinus of the left maxillary sinus - an image of "metal tone" with aspects of foreign body



**Figure 6** Same patient as in figure 5, axial slice

directly from suspected areas without accidental contamination. Also, rapid results and relatively low cost are other arguments for choosing this option. The necessary conditions that are needed to be met for correct histopathological results are a skilled pathologist, trained in the diagnosis of this condition and also special stains for detection of fungal infection (hematoxylin-eosin, PAS reaction staining, Gomori-Methenamine-Silver and Grocott staining).

Mycological examination is a useful and valuable method, but involving special conditions of harvesting, transportation and processing to obtain positive results. To prevent false positive results - harvesting is essential to be realized at the level of fungal infection source, respectively directly from the affected sinuses (intraoperative sampling) or from the paranasal sinus ostium (sampling from the middle meatus). Other important conditions necessary for obtaining accurate results are the sampling time from sowing to harvest material (to prevent its alteration must be within 4 hours), temperature and incubation medium (Malt Agar plates with chloramphenicol at 25°C and at 30°C).

From the experience of the group of studied patients, the method of harvesting with the best results is the intraoperative or by sinusoscopic sampling (sensitivity 79% and respectively 73%), far superior of endoscopic harvesting from the middle or superior meatus (sensitivity 13%).

From the statistical analysis of this study it result that the mycological examination has a lower sensitivity in comparison with the histopathological exam, it is laborious and requires special conditions of collection, transport, storage and processing, but is a useful examination for diagnosis of fungal rhinosinusitis.

In the Romanian literature have been quoted so far extremely rare cases of FRS, considering for a long period of time that rhinosinusitis is a microbial disease. Given that patient with rhinosinusitis are referred for the diagnosis in particular in the ENT ambulatory, where the possibilities of endoscopic or examination, or sinusoscopy are lower, we recommend radiologic examination as a method of diagnostic orientation. By analyzing data from the study, computer tomography examination is recommended as the best method for radiological diagnosis of FRS, specific aspects of this disease being encountered in 80.77% of cases. The results of this study are consistent with those obtained by literature review.

MRI exams and classic Rx have limited value and can serve as diagnostic tools for guidance, possibly representing a starting point for clinical diagnosis of these entities, most often being needed a CT scan for the disease confirmation. The suggestive CT scan aspects for the diagnosis of noninvasive fungal rhinosinusitis implies, according to the literature, the following aspects (Figure 5, 6): a presence of an image of „metal tone“ at the sinusal level, with aspects of foreign body; one or multiple calcifications at the level of the sinus opacity; a heterogeneous content, unilaterally or in multiple sinuses; lack of areas with osteolysis, eventually bone thinning areas probably due to prolonged pressure exerted by the fungal material on the bony walls.

In the presence of such radiological aspects, the ambulatory ENT specialist should know that it requires a diagnosis of certainty, the histopathological one, requiring the patient guidance to a specialized service, able to make a correct and fast diagnosis, to prevent potential complications and limit recurrences.

## CONCLUSIONS

The role of fungi in CRS remains to be defined FRS being one of the most challenging infections that otolaryngologists diagnose and treat. Favorable outcomes depend on early diagnosis and adequate medical and surgical intervention. The diagnostic algorithm for FRS involves the coordination between several diagnostic tracks: histopathology, mycologic exam, allergy and immunology tests and radiologic examination. In Romania, the histopathological exam remains the most reliable diagnostic test for the detection of fungal infection. In what it concerns the radiological examinations, CT scan is the "golden standard" for this condition. But, first of all, in order to establish the diagnosis of FRS, the clinician must maintain a high index of suspicion and be vigilant.

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