

Histopathology of Invasive Fungal Rhinosinusitis

R.B. Deshpande

Histopathological examination forms the basis of diagnosis, classification, and management of invasive fungal rhinosinusitis [1, 2]. In cases where fungal infection is suspected on clinical grounds, demonstration of fungal elements should be sufficient to start treatment, but histopathological examination is necessary to confirm the diagnosis of “invasive” fungal infection and to properly classify the fungal disease for further management.

Histopathological diagnosis of invasive fungal diseases has three components, namely, (a) demonstration of fungal elements (b) identifying tissue invasiveness, and (c) identifying type of inflammatory reaction – on the basis of which further classification is made.

In majority of the cases, fungal elements can be identified on hematoxylin and eosin (H&E)-stained sections. However, identification becomes easy with special staining techniques like periodic acid-Schiff (PAS) stain and Gomori’s (Grocott–Gomori’s) silver methenamine (GMS) preparation. Gomori methenamine has been described to be the most sensitive of the commonly used stains, and it has been recommended that a negative diagnosis should not be given unless a silver stain has been performed [3].

In both, PAS and GMS techniques, the principle is to demonstrate polysaccharide-rich fungal walls. In PAS staining, periodic acid oxidizes polysaccharides in to aldehydes which on reaction with Schiff’s reagent produces purple or magenta color. Walls of fungal hyphae are stained magenta color highlighting the fungal structure.

In Grocott–Gomori’s (or Gomori’s) methenamine silver technique, the polysaccharides are oxidized in to pair of aldehydes. The aldehydes react with silver nitrate and reduce it in to metallic silver that deposits on the fungal wall making it stand out. In fact, GMS is not a stain in the strictest sense but causes silver deposition that makes the fungal elements more easily demonstrable.

R.B. Deshpande, MD
Department of Pathology, P.D. Hinduja Hospital,
Mahim, Mumbai, Maharashtra 400 016, India
e-mail: atharnas@yahoo.com

Both the PAS stain and GMS techniques are not specific for fungi. They only make the polysaccharide-rich fungal walls easily identifiable. There are many other structures in tissues – including collagen bands and vessel walls. To the uninitiated, these may look like fungal elements. Fungi are identified by their structural characteristics and not on positive or negative staining.

Invasiveness of the disease is determined by the presence of the fungal elements in the submucosa, invasion into the vessel walls and the lumen with consequent vascular thrombosis, and infarction of the tissue. While in the chronic disease, there is predominantly chronic inflammatory cell component or there is granulomatous reaction.

Based on the type of inflammatory reaction and temporal profile of the pathologic process, invasive fungal rhinosinusitis is divided into three groups:

- (a) Acute fulminant invasive rhinosinusitis
- (b) Chronic invasive fungal rhinosinusitis
- (c) Chronic granulomatous rhinosinusitis

Acute Fulminant Invasive Fungal Rhinosinusitis

This occurs mainly in patients with immunosuppression, or ketoacidosis of diabetes mellitus, or uremia of chronic renal disease but is also being reported in immunocompetent patients. The fungi most often involved are *Mucor* species or *Aspergillus*. There is usually little or no appreciable inflammatory cell reaction. Fungal hyphae invade the tissue especially the vessel walls, resulting in thrombosis of the vessels and consequent infarction of the tissue. Both invasive mucormycosis and aspergillosis have an affinity for invading blood vessels [4–7] (Fig. 1). In particular, invasive *Mucor* has a strong affinity for arteries. Histopathological features include growth along the internal elastic lamina that results in dissection away from the media, as well as growth into the blood vessel lumen, producing endothelial damage and initiating thrombosis [8] (Fig. 2).

These fungi, having once invaded the vessels, disseminate rapidly in fulminant fashion, extending to intracranial spaces, carotid artery, etc. and prove fatal in a very short time. Rapid diagnosis and prompt treatment may save at least some of these patients.

Histopathologists involved in diagnosis of such cases should be alerted to the possibility of acute invasive fungal infection. Intraoperative frozen section examination may save considerable time in diagnosis. If intraoperative frozen section examination facility is not available and if the primary physician/surgeon communicates to the pathologist of the clinical suspicion of invasive fungal sinusitis, then routine processing may be done in rapid fashion and the result promptly communicated to the treating physician without delay.

Once the possibility of fungal infection is considered, frozen section diagnosis should not be difficult. Vascular invasion, infarction of the tissue, and presence of fungi are not difficult to identify. Fungi can be identified even on Toluidine blue stain or H&E staining. *Mucor* species which is most often involved in acute

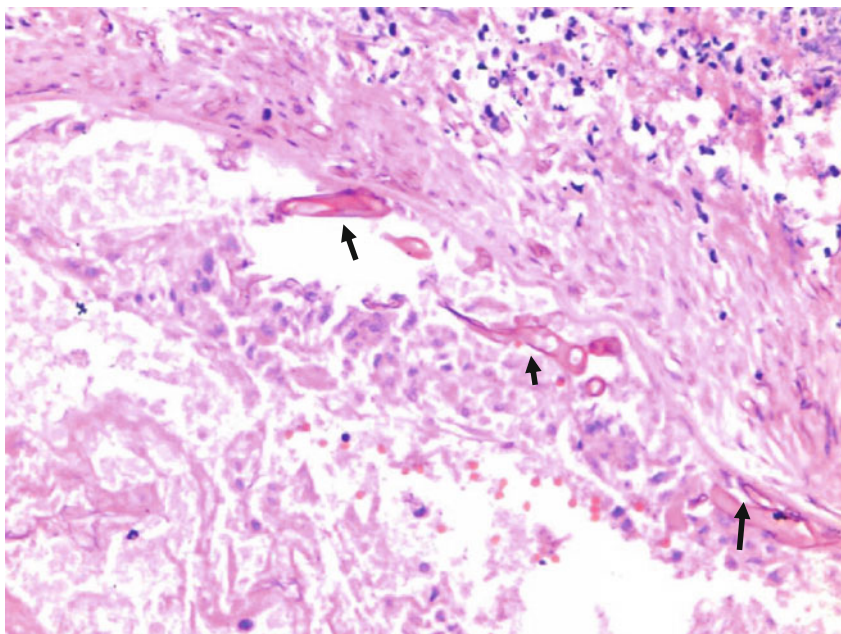


Fig. 1 PAS-stained sections showing broad aseptate fungal hyphae (*arrows*) infiltrating the vessel wall causing a thrombus

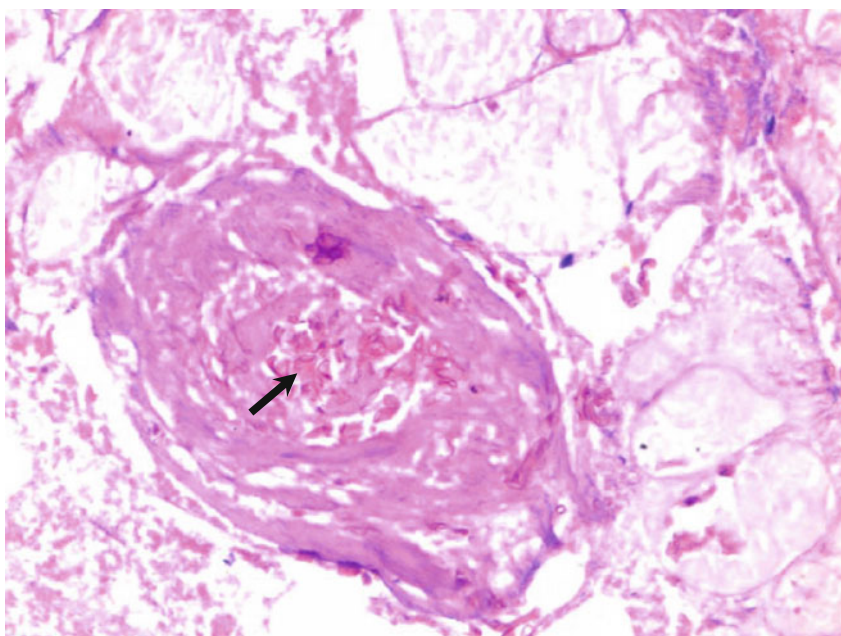


Fig. 2 PAS-stained section showing infarcted adipose tissue with broad aseptate fungal hyphae (*arrow*) infiltrating into the lumen on the vessel causing thrombus

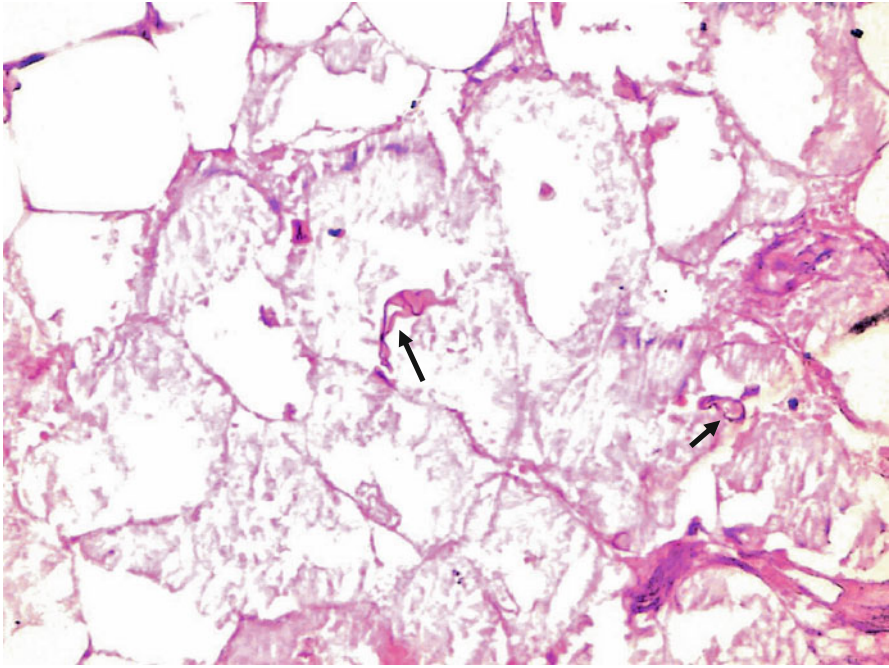


Fig. 3 H&E stain, original magnification 400× showing infarcted fat tissue with broad aseptate fungal hyphae (*arrows*) folding on themselves with characteristic features of mucormycosis

fulminant processes can be identified by broad (10–15 μm wide) ribbon-like aseptate fungal hyphae which fold on themselves and branch at right angles (Figs. 3, 4, and 5), while *Aspergillus* species which also may cause acute invasive fulminant process (Figs. 8 and 9) appears as narrow (2–5 μm wide) septate acutely branching fungal hyphae (Figs. 7 and 8).

Chronic Invasive Fungal Rhinosinusitis (Fig. 6)

In these cases, there is usually destruction of the tissue with chronic abscess-like inflammatory reaction with presence of fungal elements within the exudate. There is usually nothing – either clinically or histologically to alert the pathologist about the possibility of fungal etiology and hence the diagnosis may be missed. Constant alertness and a high level of suspicion of fungal etiology in all such cases irrespective of the underlying disorders helps in identifying such cases. Unlike the neutrophil-rich, highly necrotic, and angiotrophic process seen in acute invasive fungal sinusitis, there is a low-grade mixed cellular infiltrate in affected tissues [9]. Usually this occurs in diabetics, is slowly progressive, and elicits limited inflammation.

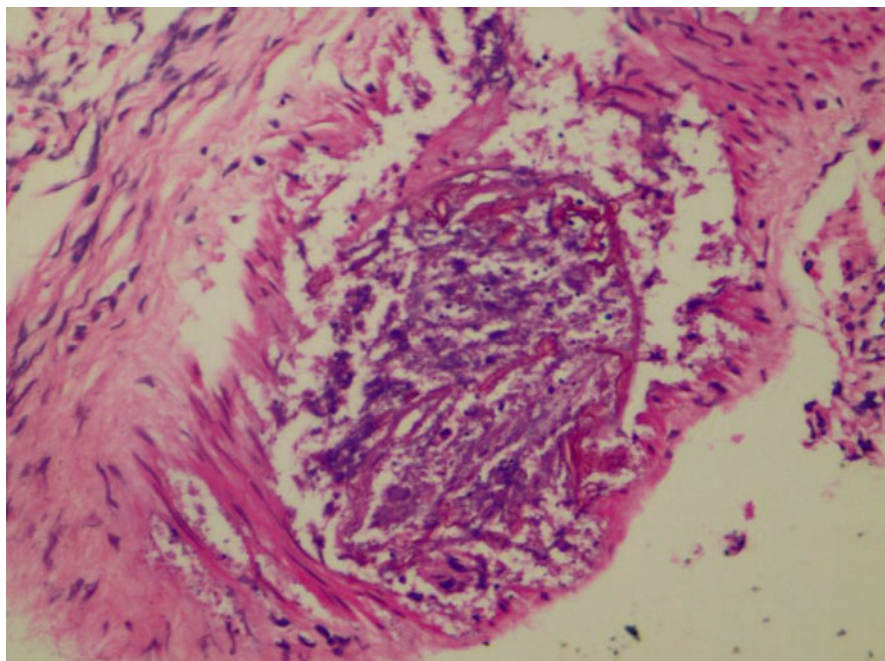


Fig. 4 H&E stain (original magnification 400×) fungal hyphae of Mucor in thrombosed blood vessel

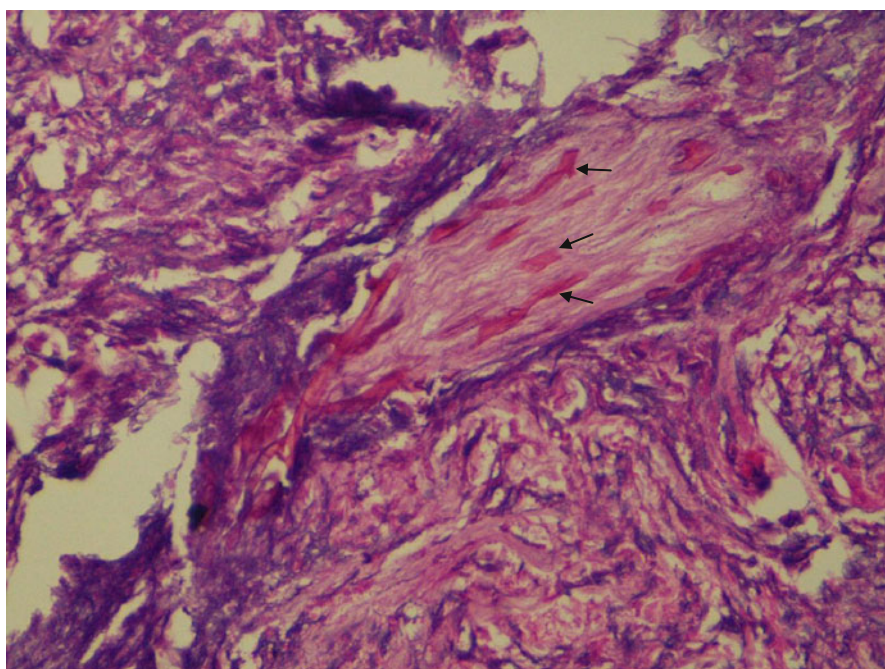


Fig. 5 H&E stain (original magnification 400×) showing fungal hyphae of Mucor (*arrows*) in vascular lumen

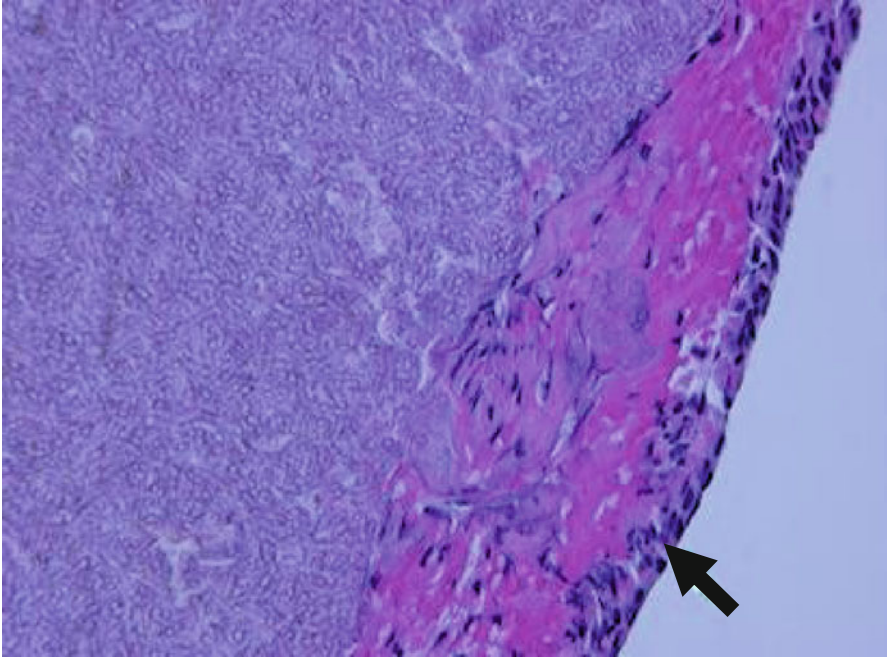


Fig. 6 H&E stain showing chronic inflammatory exudate with fungi (arrow) within the exudate

Chronic Granulomatous Fungal Rhinosinusitis

Other terms for this condition include “indolent fungal sinusitis” and primary paranasal granuloma. Published cases include those from Sudan (due to *Aspergillus flavus*) and St Louis, MO [10, 11]. Patients invariably have no predisposing factors, and the lesion usually presents as a destructive process raising the suspicion of malignancy. There is often destructive growth eroding in to the orbit. Histologically this presents as chronic granulomatous process with non-necrotizing granuloma composed of clusters or sheets of foreign-body-type multinucleated giant cells with fair number of eosinophils in the background. The combination of sheets/clusters of foreign-body-type multinucleated giant cells with eosinophils in the background should be enough to alert the pathologist about possibility of fungal (mainly *Aspergillus flavus*) disease. Close look at the giant cells even in frozen sections or paraffin sections stained with hematoxylin and eosin stain should reveal short well-stained or shadow-like unstained fungal hyphae in the cytoplasm of the giant cells (Fig. 7). GMS stain (Fig. 8) and PAS stain (Fig. 9) also reveal the characteristic features of the fungus within the giant cells. Tuberculosis does not commonly involve the paranasal sinuses. Also concomitant stains for mycobacteria are negative. Hence, any granulomatous disease causing destruction of bone that initially clinically presents as a malignancy in young immunocompetent patients should alert the pathologist to the possibility of chronic invasive granulomatous sinusitis.

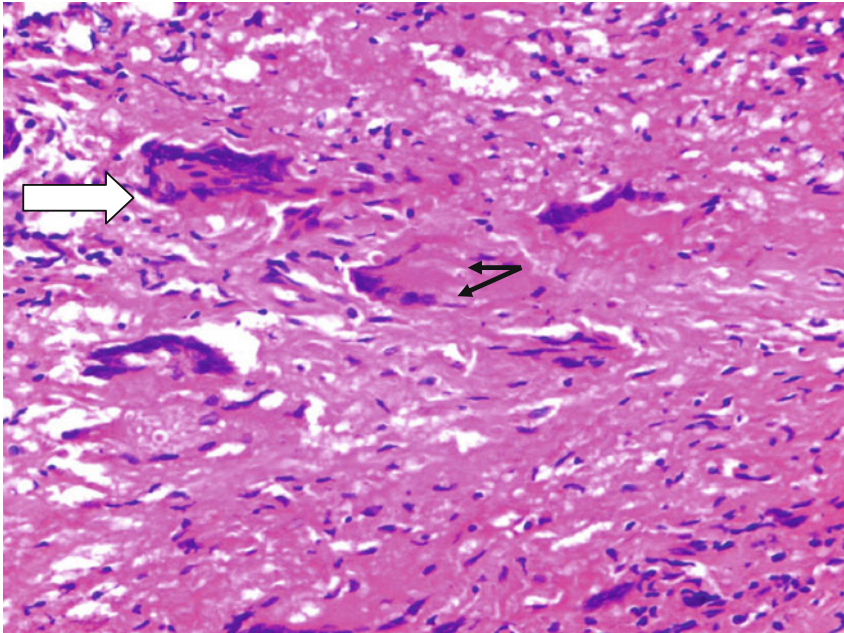


Fig. 7 H&E stain (original magnification 400×) showing clusters of multinucleate foreign-body-type giant cells (*broad white arrow*) with negatively stained fungal hyphae (*thin black arrows*)

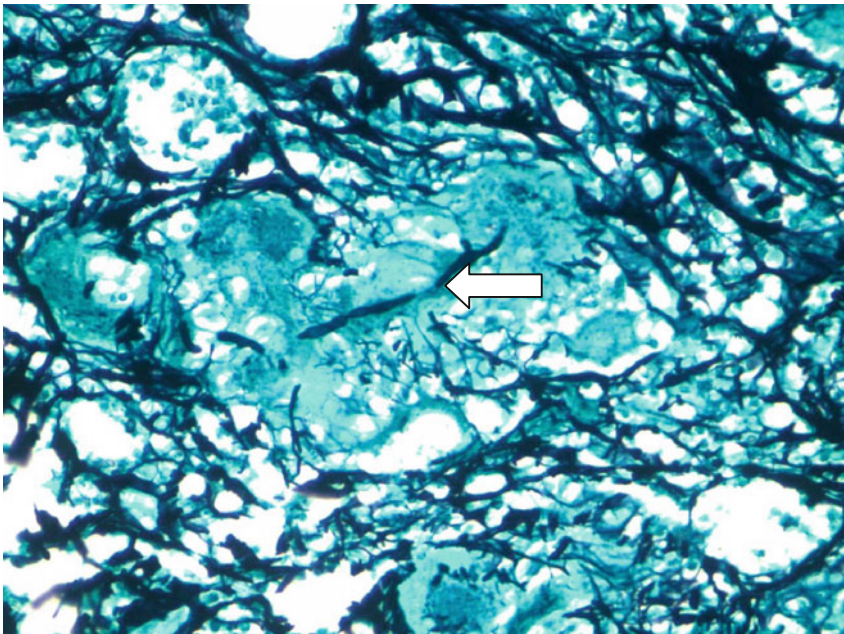


Fig. 8 Gomori's methenamine silver impregnated for fungus showing septate branching fungal hyphae in the giant cells. Note that the collagen surrounding the giant cells are also stained black. Fungi are identified by their septate and branching features (*white arrow*)

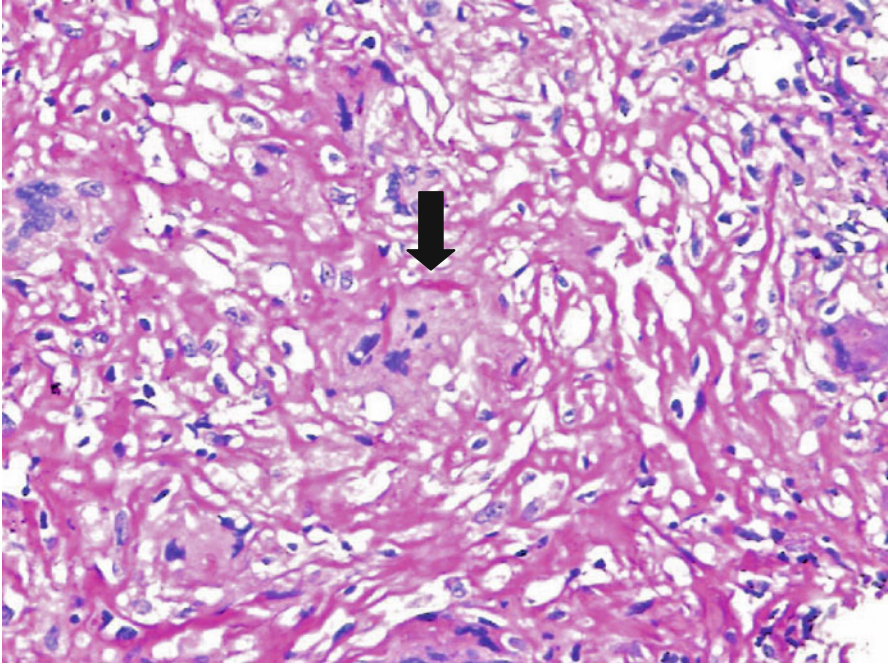


Fig. 9 PAS-stained sections showing septate branching fungal hyphae (*black arrow*) in the giant cell. Surrounding collagen is also stained pink

In all these cases, fungal etiology is missed by the inexperienced pathologist not because of the difficulty in identifying the fungi histologically but because of the lack of awareness of this possibility. Clinicians who suspect fungal etiology should make it a point to discuss the case with the concerned pathologist personally. Acute invasive fungal rhinosinusitis is one of the most critical emergency situations for the pathologists. Diagnosis in such cases should be rapid with prompt communication to the treating physician of the diagnosis.

The pathologist should also be able to identify noninvasive fungal sinusitis and differentiate it from the invasive variety. Noninvasive fungal rhinosinusitis includes “fungal ball” and allergic fungal sinusitis (AFS). These have characteristic histopathological appearance with absence of fungal invasion of the tissues.

Fungal Ball

In these cases, histopathology shows multitude of pale fungal hyphae compressed in the center, with appearance of morphology at the periphery

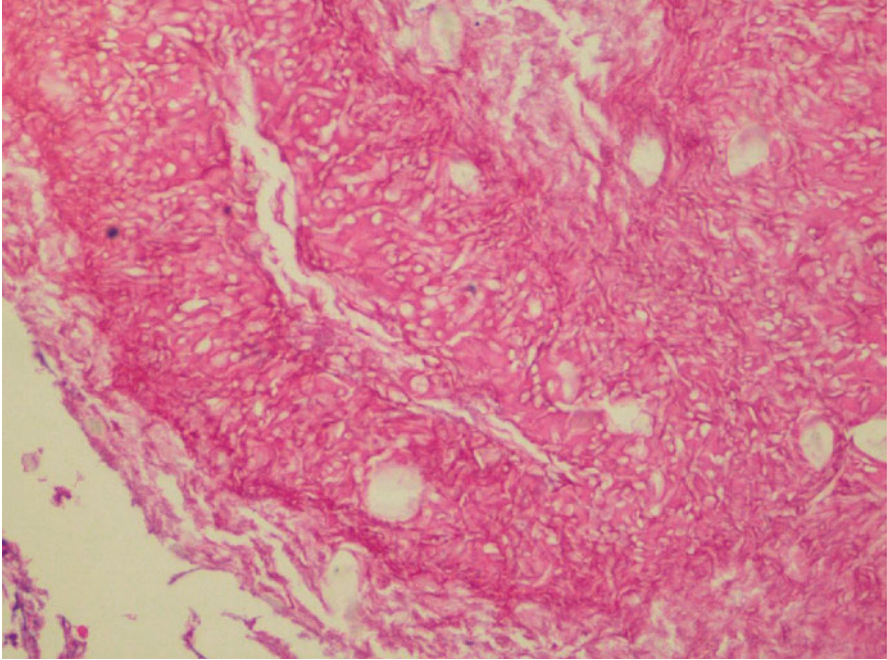


Fig. 10 H&E stain (original magnification 400×) showing compactly packed septate branching fungal hyphae morphologically resembling *Aspergillus* spp. indicative of fungal ball

(Fig. 10). The surrounding mucosa usually shows dense mixed inflammatory infiltrate.

Allergic Fungal Sinusitis (Fig. 11a, b)

The diagnosis of AFS is primarily histopathological although there are clinical and radiological features to distinguish it from invasive fungal sinusitis. Characteristic allergic mucin is seen on H&E stains as strongly staining laminated concretions of pyknotic and degranulated eosinophils surrounded by areas of lightly staining mucin sprinkled with Charcot–Leyden crystals [12–14]. Staining of allergic mucin with fungal stains like Gomori’s (GMS) may show scattered fungal hyphae within the allergic mucin [12–16]. There is no evidence of mucosal fungal invasion, including features of tissue invasion like mucosal necrosis, granuloma formation, or giant cells. An important fact to remember is that allergic mucin without fungal involvement can be seen in patients with the non-AFS “eosinophilic mucin rhinosinusitis” [17, 18].

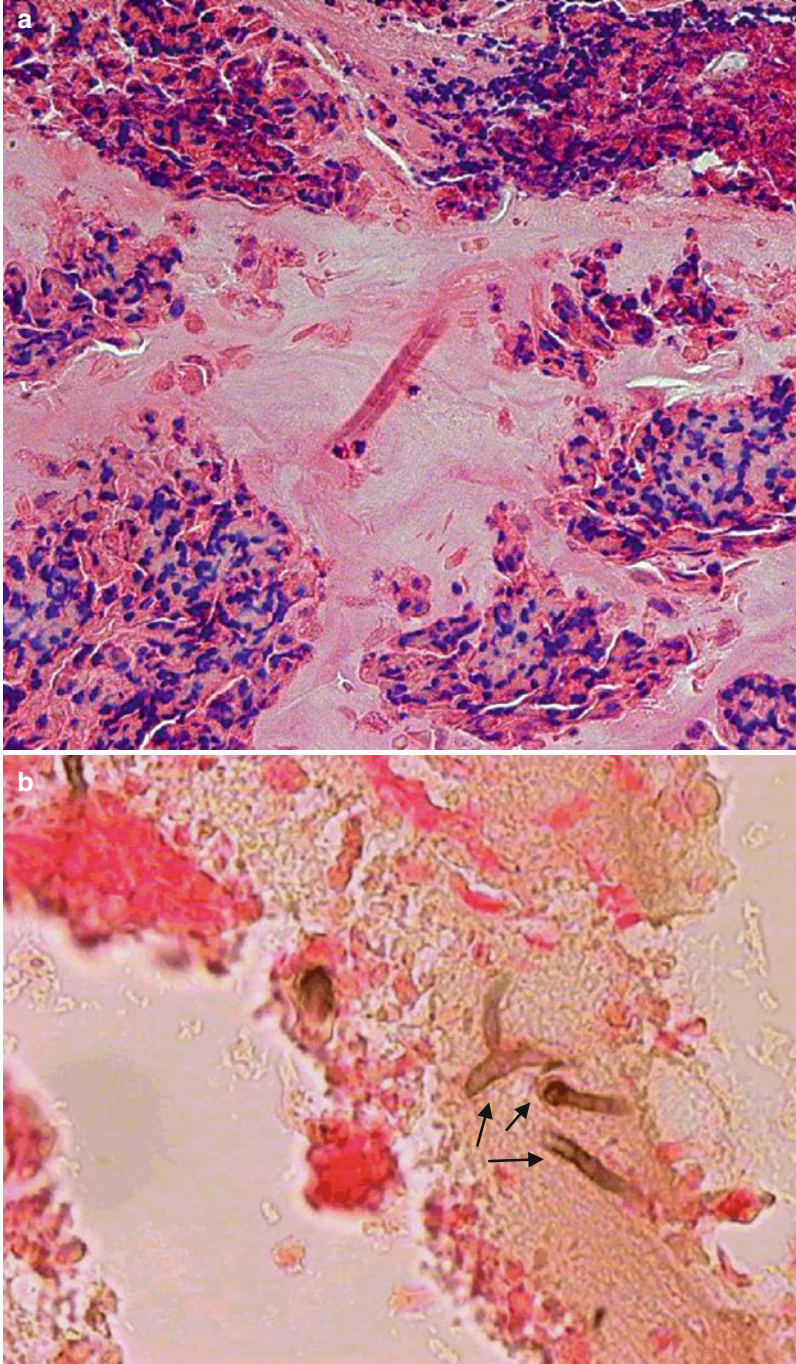


Fig. 11 (a) H&E stain. (Original magnification $\times 400$) showing allergic fungal mucin with Charcot-Leyden crystals. (b) PAS stain (original magnification $\times 400$) showing allergic mucin with septate fungal hyphae (*arrows*)

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