Cyto-Pathologist’s Perspective in Diagnosis of Fungal Infection

Dhiraj B Nikumbh

Professor, Department of Pathology, ACPM Medical College, Dhule

ABSTRACT

In the light microscopical diagnosis of fungal infections, the role of cytology is limited but very important for rapid diagnosis on morphology and for initiation of early treatment for better patient compliance and prognosis. Pathologist can easily diagnose the common fungal infections on cytology of body fluid, FNAC and on exfoliative cytology with routine and the help of special stains. The aim of this editorial to enlightened the cytopathologists perspective in diagnosis of common fungal infections.

The role of cytopathology in diagnosing common fungal infections in modern era is very much important in view of its rapidity and reduced turnaround time useful for treatment purposes. In this article, the cytological perspective for diagnosing common fungal infections was discussed. Fine needle aspiration cytology (FNAC), exfoliative cytology and body fluids cytology are the common modes for diagnosis of various fungal infections in common practice. These modalities are very much beneficial in view of its outpatient department (OPD), simple, cost-effective, routine and safe procedure for early and prompt diagnosis and to differentiate various etiologies as the treatment of each is different.

The role of FNAC in diagnosing infectious disease by Greig and Grey in 1904. In their study, they diagnosed motile trypanosomes of sleeping sickness. In Europe after World War II, FNAC advanced for diagnosis of cervical and lung cancers. Since then the role and demand of diagnostic cytology have expanded tremendously.

The material for cytological evaluation is from three methods—exfoliative, abrasive and aspiration. The first report suggests a potential role of FNAC in diagnosing infectious disease by Greig and Grey in 1904. In their study, they diagnosed motile trypanosomes of sleeping sickness. In Europe after World War II, FNAC advanced for diagnosis of cervical and lung cancers. Since then the role and demand of diagnostic cytology have expanded tremendously.

The second most common is exfoliative cytology where the cells continuously shed into body fluids as effusions in pleural, pericardial and peritoneal cavities. Urine by virtue of its easy collection remains one of the most common exfoliative specimens. Cerebrospinal fluid (CSF) is also helpful in various conditions mainly in immunocompromised patients.

Candida albicans is the most frequently encountered fungus in cytological specimens like pap smears, whereas seen in urine and sputum also as

KEYWORDS: cytology, FNAC, body fluids, exfoliative
contaminant. It may be seen in esophageal brushing in squamous epithelial cells as pathological agent.

The yeast form of fungi are challenging due to overlap of its clinical, radiological and cytological features. However, cytologist can able to differentiate these four common differentials of yeast fungi as *Histoplasma*, *Cryptococcus*, *Blastomyces* and *Coccidioides* species as in Table 1 as per Powers CN.6

Fungal sinonasal sinusitis constitutes 6–9% of all rhinosinusitis. Aspergillosis and mucor are the most common non-invasive and invasive fungal infection respectively of head and neck region. Both can pose diagnostic and therapeutic challenge in view of mimics as malignancy. We published a report on cytodiagnosis of sinonasal aspergillosis in 2015 and stressed on importance of FNAC in diagnosing subcutaneous mycotic infections.

A 75-year-old male presented with right maxillary swelling and discharge. FNAC diagnostic of aspergillosis in view of its characteristic features of fungal granulomas. In giant cells, branching septate hyphae (Fig. 1e) with intracytoplasmic negative staining were noted. Many conidia and fruiting bodies are also seen in giant cells.

Blastomyces and Coccidioides are rarely seen in cytology though they are larger than candidiasis. They are difficult to diagnose on cytology due to refractile, rigid capsule, not numerous, often out of plane of focus, etc.6

**Table 1** Staining characteristics of common fungi6

<table>
<thead>
<tr>
<th>Fungus</th>
<th>PAP</th>
<th>Romanowsky</th>
<th>H&amp;E</th>
<th>GMS</th>
<th>PAS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida</em> spp.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><em>Aspergillus</em> spp.</td>
<td>+</td>
<td>Variable</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Zygomycetes</td>
<td>+</td>
<td>Poor</td>
<td>+</td>
<td>±</td>
<td>Poor</td>
</tr>
<tr>
<td>Yeasts other than <em>Candida</em> spp</td>
<td>+</td>
<td>±³</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

³PAS, periodic acid-Schiff; +, visible with stain; −, does not stain. Yeasts stain with variable intensity with Romanowsky stains.

Source: By Powers CN.6

Cryptococcosis neoformans is most often seen in lungs and central nervous system as subacute or chronic course.7 We most commonly encountered Cryptococcus in 1–2 cases of CSF in immunocompromised patients and in one case of FNAC of lymph node in 35-year-old male retro patient. Cytologically, the yeast of Cryptococcus are 5–15 microns, round to oval with clear capsule (Fig. 1c) and narrow-based budding, and punched out staining of capsule more highlighted by Romanowsky stain (Fig. 1d). GMS, PAS or mucicarmine is helpful in FNAC smears and India ink preparation is almost diagnostic of Cryptococcus by negative staining of capsule in CSF preparations.

We do not encountered any case of Histoplasma capsulatum. It is one of the smallest dimorphic fungi and found intracellularly, as foamy or vacuolated structures within cytoplasm of histiocytes.8 The yeast of Histoplasma are 2–5 microns, oval endocellular with single and narrow bud.8 Most common site is lung, but may be systemic. In cytology, bone marrow aspiration may sometimes highlight intracellular Histoplasma. Blastomyces and Coccidioides are rarely seen in cytology though they are larger than candidiasis. They are difficult to diagnose on cytology due to refractile, rigid capsule, not numerous, often out of plane of focus, etc.8

Fungal sinonasal sinusitis constitutes 6–9% of all rhinosinusitis. Aspergillosis and mucor are the most common non-invasive and invasive fungal infection respectively of head and neck region. Both can pose diagnostic and therapeutic challenge in view of mimics as malignancy. We published a report on cytodiagnosis of sinonasal aspergillosis in 2015 and stressed on importance of FNAC in diagnosing subcutaneous mycotic infections. A 75-year-old male presented with right maxillary swelling and discharge. FNAC diagnostic of aspergillosis in view of its characteristic features of fungal granulomas. In giant cells, branching septate hyphae (Fig. 1e) with intracytoplasmic negative staining were noted. Many conidia and fruiting bodies are also seen in giant cells. The mode of entry for Aspergillus includes respiratory tract, damaged skin or operated wounds, cornea and ear. The most common

---

**Fig. 1** (a) Pap smear with budding yeast of *Candida* albicans (Pap, x400), (b) Characteristic budding yeast highlighted on toluidine blue (TBS, x400), (c) *Cryptococcus* yeast forms with clear capsule (Pap, x400), (d) Punched out staining pattern of capsule of *Cryptococcus* by Romanowsky (Leish, x400), (e) Branched septate of *Aspergillus* fungus (Pap, x400), (f) Colonies of filamentous bacilli – actinomy- cetes with projections (Pap, x400).
differential for Aspergillus is Mucor. Aspergillus is a filamentous structure, 3–6 microns with septate hyphae and branching at acute angles. Mucor has a broad, asperate hyphae branching at 90 or right angles. Special stains like PAS and Silver (GMS) stain may be helpful for fungal hyphae. Cytology in such patients avoids unnecessary surgical intervention and treated by medical antifungal drugs.

Mycetoma or madura foot is a chronic suppurrative granulomatous disease with subcutaneous swelling and multiple discharging sinuses tract of microcolonies of causative agent. Infection caused by true fungi (eumycetoma) in 40% or filamentous bacteria (actinomyctoma) in 60% cases. We published a case report on cytodiagnosis of primary actinomycotic mycetoma of the foot in view of its rarity and very limited data on FNAC of these lesions. A 65-year-old patient presented with swelling over right foot with multiple nodules and discharging sinuses. FNAC revealed inflammatory exudates, giant cells and many colonies of brownblack filamentous bacilli (Fig. 1f) with some projections (Splendore–Hoeppli). Gram stain was positive and acid fast was negative. We highlight the importance of FNAC in diagnosing mycetoma and categorization in actinomycetoma or eumycetoma.

Pneumocystis carinii has been difficult to classify and is currently considered as fungus. It is the most common cause of opportunistic infections in HIV patients and infants leading to non-bacterial pneumonia. Bronchoalveolar lavage can give high yields of P. carinii and on cytology it revealed ill-defined, amorphous/foamy macrophage casts. Diff-Quick or GMS is used for the identification of cysts. Cysts are 5–8 microns, round, crescentic or cup shaped containing 6–8 small ovoid trophozoites.

The limitations of fungal infections diagnosed on FNAC or exfoliative are lack of cultures due to loss of follow-up. The same is true in our cases. Fungal culture though a gold standard and complementary to morphology and species identification, most of the time not performed due to long turnaround time, non-representative samples on cytology, not all cultures grown on media and lack of follow-up of patients.

CONCLUSION

Fungal infections diagnosed on cytology require high index of suspicion and always helpful in view of its simple, OPD and less invasive procedure with little discomfort to the patient. Hence, compliance of patients is always better in this method as compared to surgical biopsy. Morphological diagnosis of fungal infections by cytology is not intended to replace microbiological confirmation, instead of it will helps in rapid initial diagnosis and safe, cost-effective method of specimen procurement for the patients, clinicians and cytologists and helping hands to microbiologists.

BIBLIOGRAPHY