CONTRIBUTION OF PATHOLOGY TO THE DIAGNOSIS OF FUNGAL INFECTIONS

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PATHOLOGY – RELEVANCE IN FUNGAL DISEASE

1. Rapid & cost-effective means of arriving at a presumptive diagnosis
   ...Culture may take wks
   ...Organism may not grow at all.

2. Allows definitive interpretation in cases with specific findings
   - unique fruiting head of *Aspergillus* spp.
   - at least 1 unequivocal, intact, endospore-filled spherule of *Coccidioides*.
3. Provides insight into the diagnostic significance of culture isolates
   ....gives information on vascular/tissue invasion & host reaction
   ....address the frequently encountered problems of contamination & infection vs colonization.

4. May be the only method of diagnosis for fungal pathogens like Pneumocystis & Lacazia loboi.

5. Sometimes detects fungal disease in clinically unsuspected cases in which no culture has been sent.
Overview of special stains for fungi

HPE:
1. **PAS [red] & GMS [black]**:
   - Both highlight the cell wall - Used as screening tools.
   - GMS more sensitive than PAS...signal to noise issue..stains lysosomes, inflammatory cells & tissue reticulin.
   - PAS gives a better visualization of surrounding tissue compared to GMS.
   - GMS with H&E counterstain - best combination for fungus & host reaction.

3. **Mucicarmine & Alcian Blue [red & blue]**: Capsule of *Cryptococcus*

4. **Fontana-Masson [black]**: *Cryptococcus* & dematiaceous fungi & some species of *Aspergillus, Mucorales & Trichosporon*.

Cytology (sputum, BAL, CSF, FNA):
Calcofluor white, Uvitex, MGG, Pap, PAS, GMS.
Reporting of fungal infections in tissue & cytological preparations

1. Describe the fungal elements.
   - Yeast / hyphae / Both / endosporulating structures.
   - Yeast forms – Diameter, Budding or Fission / Type of budding (narrow based or broad based).
   - Hyphae - Width, septations, branching angle, dilated bizarre forms (Mucorales), Brown pigmentation (Dematiaceous fungi).
   - Also comment on quantity of fungal elements & viability.

2. Clearly state the fungus which is most frequently associated with the described morphology & enlist the fungi that can display a similar morphology.

3. Identify the inflammatory reaction, comment on presence of vascular or tissue invasion, necrosis, or hemorrhage.

4. Correlate all pathological findings with clinical features, epidemiology & results of alternative testing if available.

MORPHOLOGICAL CLASSIFICATION OF FUNGAL DISEASES

- Diseases Where Yeasts or Yeast-Like Structures Are Usually Seen
- Diseases Where Hyphae Are Usually Seen
- Diseases caused by *Fusarium*, *Scedosporium*, and other hyaline septated molds
- Diseases caused by *Bipolaris/Curvularia* and other *Dematiaceous* fungi
- Dermatophyte disease
- Diseases caused by other molds (coelomycetes)

*The “clinical spectrum & host tissue response “ to every fungus is as important as the “morphology of the fungus” & must be kept in mind while making a diagnosis.*
DISEASES WHERE YEASTS OR YEAST-LIKE STRUCTURES ARE USUALLY SEEN IN TISSUES

- Blastomycosis
- Cryptococcosis
- Histoplasmosis
- Coccidioidomycosis
- Candidiasis
<table>
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<tr>
<th>Morphology of fungus</th>
<th>Clinical spectrum &amp; host tissue response</th>
<th>D/Ds</th>
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<tbody>
<tr>
<td>• Yeasts meas. 8 - 15 μm.</td>
<td>• Acute pneumonia: Mixed suppurative response.</td>
<td>1. Larger forms to be differentiated from a) Immature spherules of C. immitis. b) Yeast forms of P. Brasiliensis. c) Conidia of aspergillus.</td>
</tr>
<tr>
<td>• Show single, broad-based budding.</td>
<td>• Chronic pneumonia: Pyogranulomatous inflammation with numerous multinucleate cells.</td>
<td>2. “Microforms” to be differentiated from the Yeast forms of Candida spp., Histoplasma, Cryptococcus.</td>
</tr>
<tr>
<td>• Clear space around the fungal cell on H&amp;E staining (corresponds to a thick refractile cell wall); stains with silver stains.</td>
<td>• Cutaneous/mucosal lesions: - Marked epithelial hyperplasia. - Neutrophilic microabscesses in epithelium. - Chronic inflammation in the dermis/submucosal tissue.</td>
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<td>• Larger forms may occasionally be accompanied by the “micro forms”.</td>
<td>• Disseminated lesions: Various inflammatory responses depending on immune status.</td>
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<td>*Tuberculosis or neoplasia can be present concomitantly</td>
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**Blastomyces dermatitidis**
<table>
<thead>
<tr>
<th>Morphology of yeasts</th>
<th>Description, diagnosis and comment</th>
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<tbody>
<tr>
<td></td>
<td><strong>Description:</strong> Broad-based budding yeasts (10-15 microns in size).</td>
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<td><strong>Diagnosis:</strong> Broad-based budding yeasts.</td>
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<td></td>
<td><strong>Comment:</strong> The morphology is consistent with <em>Blastomyces dermatitidis</em>; however, endospores of <em>Coccidioides</em> spp., <em>Candida</em> spp, <em>Histoplasma</em>, <em>Cryptococcus</em>, and <em>Aspergillus</em> conidia can be confused histologically.</td>
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<tr>
<td>Cryptococcus spp.</td>
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<tr>
<td><strong>Morphology of fungus</strong></td>
<td><strong>Clinical spectrum &amp; host tissue response</strong></td>
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</table>
| • Spherical-oval yeasts.  
• Marked variation in size.  
• Narrow-based budding.  
• Thick polysaccharide capsule.  
• Pseudohyphae (with massive yeast proliferation). | • Pneumonia & Cryptococcomas: Granulomatous reaction with fibrosis. Occasionally *pseudotumour* formation.  
• Neutrophils unusual – indicate bacterial superinfection/response to massive necrosis.  
• Disseminated disease: Varying infl. response depending on immune status.  
• Immunocompetent individuals: Well formed granulomas with few organisms.  
• Severe T cell deficiency: Minimal inflammation with abundant intra/extracellular organisms (sheets of yeast filled lacunae - “soap bubble lesion”). | **Candida & Histoplasma** - similar in size & can be confused histologically.  
**Well encapsulated organisms** easily differentiated using capsular stains.  
**Organisms with poorly formed capsule** pose some problem and need to be differentiated by melanin stains. |

| India ink: Negative stain - highlights the capsule.  
Mucicarmine & Alcian blue: Capsule.  
PAS & GMS: Cell wall.  
Masons-Fontana: Yeast cell (permits differentiation from most other pathogenic yeasts except Trichosporon beigeli). |  |  |
Description: Narrow-based budding yeasts (4-10 microns in size) with a thick mucicarmine positive capsule and positive with melanin stains (Fontana Masson).

Diagnosis: Narrow-based budding yeasts with mucicarmine positive capsule.

Comment: The morphology is consistent with Cryptococcus spp., however, Candida spp, and Histoplasma can be confused histologically.

CDC public health image library

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<thead>
<tr>
<th><strong>Histoplasma</strong></th>
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<tr>
<td><strong>Morphology of fungus</strong></td>
<td><strong>Clinical spectrum &amp; host tissue response</strong></td>
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</table>
| *H. Capsulatum:*  
• Oval 2-4-μm yeast  
• Narrow-based budding.  
• H&E: Halo corresponding to the cell wall.  
• GMS & PAS: Highlight the cell wall.  
• Intracellular clustering within macrophages - diagnostic hallmark.  
• In African histoplasmosis the yeast size is larger (8-15μm) & yeast may be pigmented. | • Spectrum varies from localized granuloma formation to massive proliferation of MPs containing yeast cells.  
• **Acute pneumonia:** Intra-alveolar aggregates of histiocytes, expand to form nodules of parenchymal/vascular necrosis.  
• **Chronic histoplasmosis:** Multiple small granulomata seen all over, commonly in pleura & spleen...May get hyalinized...Rarely residual necrosis with yeast forms may be seen.  
• **Mediastinitis:** Massive hilar & mediastinal LAP; lamellar fibrosis & calcification (**fibrosing mediastinitis**); organisms rarely found/if found swollen, distorted, no budding..? non-viable. | Include all small sized yeasts which show narrow based budding & Intracellular clustering. |
<table>
<thead>
<tr>
<th>Fungus to be differentiated</th>
<th>Differentiating features</th>
</tr>
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<tbody>
<tr>
<td>“Micro” forms of <em>B. dermatitidis</em></td>
<td>Presence of larger forms &amp; broad based budding</td>
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<tr>
<td>Capsule deficient <em>Cryptococci</em></td>
<td>Show marked size variation &amp; positive melanin staining</td>
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<tr>
<td>Endospores of <em>Coccidioides spp.</em></td>
<td>Presence of remnants of a ruptured spherule or an intact spherule.</td>
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<tr>
<td><em>Pneumocystis jirovecii</em></td>
<td>Does not show budding &amp; has a prominent intracystic focus.</td>
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<tr>
<td><em>Penicillium marneffei</em></td>
<td>Fission, sausage shape, transverse septum.</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>Typically extracellular; no halo, shows greater size variability &amp; pure neutrophilic infiltrate.</td>
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<tr>
<td>Leishmaniasis &amp; toxoplasmosis</td>
<td>H&amp;E stains entire organism; no halo; in leishmaniasis kinetoplast seen by the side of the nucleus; in toxoplasmosis infected cells are cardiomyocytes &amp; neurons rather than histiocytes.</td>
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<tr>
<td>Morphology of yeasts</td>
<td>Description, diagnosis and comment</td>
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<tr>
<td>Description: Small yeasts (2-4 microns in size) with narrow based budding grouped in clusters inside macrophages.</td>
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<tr>
<td>Diagnosis: Small yeasts with narrow based budding.</td>
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<tr>
<td>Comment: The morphology is consistent with <em>Histoplasma capsulatum</em>; however, small variant of <em>B. dermatitidis</em>, capsule deficient <em>Cryptococcus</em>, endospores of <em>Coccidioides</em> spp., <em>Pneumocystis jirovecii</em>, <em>Penicillium marneffei</em>, and <em>Candida glabrata</em> can be confused histologically.</td>
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<tr>
<td><strong>Coccidioides immitis</strong></td>
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<tr>
<td><strong>Morphology of fungus</strong></td>
<td><strong>Clinical spectrum &amp; host tissue response</strong></td>
</tr>
</tbody>
</table>
| • Thick walled **spherules** (10-100 μm) which contain multiple **endospores** (2-5 μm).  
  • Rupture to release the endospores into the surrounding tissues.  
  • H&E stains both the spherules & endospores.  
  • GMS & PAS stain the spherule wall & endospores.  
  • PAS staining fades with the maturity of the organism. | • Pulmonary & cutaneous lesions demonstrate a **mixed suppurative-granulomatous response** with abundant eosinophils.  
  • Eosinophils secrete EBP which induces an intense rim of eosinophilic material around fungal elements. “**Splendore-Hoeplli phenomenon**”.  
  • **Immunosuppressed** - marked necrosis without granulomas formation.  
  • Occ. mycelia may be seen in the cavitary or skin lesions. | **R. seeberi:**  
  • Palatal & nasopharyngeal polyps.  
  • Larger sporangia & endospores.  
  • 50-100mm, can be seen with the naked eye as yellow pin-head sized dots in a polyp.  

**Endospores/Young spherules without endospores** can be confused with **Blastomyces, Emmonsia, Candida & Histoplasma.**  
*Pneumocytis & Coccidioides* can co-exist.
Morphology of yeasts

PAS stain

CDC public health image library collection
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| **C. albicans:** Mats of yeasts measuring 3-5 μm in dm intermingled with pseudohyphae (filaments), which show periodic constrictions but not true septations. | **Superficial & invasive disease.**  
- HPE very important to define invasion.  
- Neutrophilic inflammation with some lymphocytes and macrophages, fibrin, and coagulative necrosis. Rarely few giant cells and granulomas.  
- Invasion of BVs may cause mycotic aneurysms & thrombophlebitis.  
- Necrotizing vasculitis is seen but no demonstrable organism indicating that *Candida* soluble fraction causes the necrotizing lesions. | **C. albicans** - to be differentiated from *Aspergillus* spp. (presence of true septations). & *Trichosporon* spp. (presence of a constriction between the base of the blastospore & the germ tube). |
| **C. glabrata:** Does not produce pseudohyphae. | | **C. glabrata** - to be differentiated from similar sized yeast forms like *Histoplasma*. |
DISEASES CAUSED BY OTHER FUNGI & ORGANISMS RESEMBLING FUNGI THAT DISPLAY YEASTS OR YEAST-LIKE STRUCTURES IN TISSUES

- Pneumocystis
- Sporothrix
- Penicillium
- Paracoccidioides brasiliensis
- R. seeberi.
- Emmonsia crescens

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Morphology &amp; staining</th>
<th>Pathological D/Ds</th>
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<tbody>
<tr>
<td><strong>Pneumocystis jirovecii</strong></td>
<td>Cysts 2-6μm in size with an intracystic thickening called “capsular dot”. Stained with GMS, Gram Weigert &amp; Giemsa</td>
<td>Small yeasts with narrow based budding &amp; intracellular clustering.</td>
</tr>
<tr>
<td><strong>Penicillium marneffei</strong></td>
<td>• Dimorphic fungus –Yeast 2-6μm in size. • Divides by fission (no budding); resemble a sausage with transverse septum.</td>
<td>• Small yeasts with narrow based budding &amp; intracellular clustering. • Most striking clinico-pathological similarity with histoplasmosis.</td>
</tr>
<tr>
<td><strong>Sporothrix schenckii</strong></td>
<td>• Round-oval-cigar shaped yeast measuring 2-6μm • Narrow based or tube like budding. • Can be intra &amp; extracellular. • Splendor-Hoepli phenomenon (40-92% cases).</td>
<td>1. Histoplasmosis (Intracellular clustering) 2. Candida (pseudohyphae) 3. Leishmania (Kinetoplast)</td>
</tr>
<tr>
<td><strong>Paracoccidioides brasiliensis</strong></td>
<td>• Spherical yeasts; double contoured wall; size varying between 4-60μm. • Shows budding with multiple tear drop buds surrounding the parent cell (pilot wheel appearance) better seen with GMS.</td>
<td>Yeast forms showing 1-3 buds with absence the “pilot wheel appearance” need to be differentiated from C.neoformans, S. schenckii &amp; Lacazia loboi.</td>
</tr>
<tr>
<td><strong>Emmonsia crescens</strong></td>
<td>• Dimorphic fungus, inhaled or inoculated in the skin. • Called “Adiaspiromycosis or Haplomycosis” due to presence of adiaspores which are large, double walled, empty structures measuring 20-400μm. GMS stains the wall to demonstrate fenestrations.</td>
<td>Adiaspores need to be differentiated from Coccidioides spherules which contain endospores while adiaspores are empty.</td>
</tr>
<tr>
<td>Morphology of yeasts</td>
<td>Description, diagnosis and comment</td>
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| **Pneumocystis**     | **Description:** Thin-walled spheres (2 to 5 microns in size) with intracystic foci. In lung they appear as foamy intraalveolar eosinophilic exudates. The diagnosis is problematic when they are extrapulmonary and can be confused with *Histoplasma*.  
**Diagnosis:** *Pneumocystis* pneumonia |
| **Penicillium marneffei** | **Description:** Small oval-shaped yeasts (2 to 5 microns in diameter) with transverse septum (due to division by fission).  
**Diagnosis:** *Penicillium marneffei* |
Paracoccidioides brasiliensis

Sporothrix schenckii
Diseases Where Hyphae Are Usually Seen in Tissue

1. Small yeasts intermingled with pseudohyphae & hyphae (morphology consistent with Candida; D/D Aspergillosis, Hyaline septated hyphae).
2. Non-pigmented (hyaline) septated hyphae with acute angled branching (morphology consistent with Aspergillosis; D/D Hyaline septated hyphae).
3. Non-pigmented (hyaline) pauciseptate ribbon like hyphae with right angled branching (morphology consistent with Mucorales; however, Aspergillus spp. & Hyaline septated hyphae can sometimes be confused).
4. Pigmented irregular hyphae & yeast like structures both with septations (morphology consistent with Dematiaceous fungi).
<table>
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<th>Aspergillus spp.</th>
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<td><strong>Morphology &amp; staining</strong></td>
<td><strong>Clinical spectrum &amp; host tissue response</strong></td>
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</table>
| • Thin hyphae (3-12 μm).  
• Septate.  
• Show acute-angle (45°) or dichotomous branching.  
• Vesicles with conidia (when the fungi are present in cavitary lesions or skin).  
• A. Niger infection usually shows calcium oxalate crystals in the HPE specimen. | 1. **Allergic aspergillosis** (ABPA/AFRS): AM with non-invasive hyphae; bronchial wall may show E, N, MPs, granulomas, vasculitis & interstitial fibrosis.  
2. **Chronic colonizing aspergillosis/aspergilloma:** Two types-  
   a) Thin walled aspergillomas (fungal ball surrounded by fibrosis).  
   b) Chronic cavitary/necrotizing aspergilloma (a necrotic layer with abundant hyphae surrounded by granulation tissue & an outer layer of fibrosis).  
3. **Invasive aspergillosis:** Angioinvasion may lead to hemorrhage, infarction & septic embolization to other organs. | 1. Other Hyaline septated molds (Hyalino-hyphomycetes):  
   1. Fusarium spp.  
   2. Scedosporium spp.  
   3. Trichoderma spp.  
   4. Paecilomyces spp.  
2. Sometimes mucorales. |
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<tr>
<td>- Nonpigmented</td>
<td>- Mucorales cause angioinvasive disease in immunosuppressed hosts, wherein the hyphal elements will be found amidst necrosis, hemorrhage, &amp; vascular thrombosis.</td>
<td>Molds which produce nonpigmented hyphae in tissues</td>
</tr>
<tr>
<td>- Broad (5- to 20-µm)</td>
<td>- May present as rhino cerebral, pulmonary or cutaneous disease, any of which may disseminate.</td>
<td>1. Aspergillus spp.</td>
</tr>
<tr>
<td>- Ribbon-like</td>
<td></td>
<td>2. Other hyaline septate molds like Fusarium or Scedosporium.</td>
</tr>
<tr>
<td>- Pauciseptate hyphae showing right-angled branching (Mucorales).</td>
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<td>- The hyphae may vary in width, appear folded or crinkled, and be sparse or fragmented.</td>
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<td>- H&amp;E, GMS &amp; PAS demonstrate only the cell wall with no structure inside.</td>
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<td>- Important to demonstrate invasion in the vessel wall or in the lumen.</td>
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Diseases caused by Bipolaris/ Curvularia & other Dematiaceous fungi

- Naturally pigmented molds whose hyphae and conidia contain melanin.
- Cause skin & S/T infections preceded by trauma.
- Three associated clinical entities: Eumycetoma, Chromoblastomycosis & Phaeohyphomycosis.
- Other associated clinical syndromes - onychomycosis, keratitis, allergic disease, pneumonia, brain abscesses, and disseminated disease.
- Respiratory disease by inhalation of conidia.
- Dematiaceous fungi most frequently associated with eosinophilia & AFRS/ABPA are Bipolaris & Curvularia (clinicopathological features similar to Aspergillosis).
Pathology:
• Biopsy specimens should be obtained from areas with pigment.

• HPE shows pigmented, thin, 2-6 μm, hyphae, some irregularly swollen (toruloid or moniliform) with prominent septations & constrictions & terminal or intercalated vesicular swellings.

• In cases where no pigmentation on H&E, FM staining for demonstration of melanin pigment.

• Pigmented yeast like cells showing septations & budding.

Fusarium species in subcutaneous tissue showing an irregular dilated hyphae

Dematiaceous fungus, cerebral phaeohyphomycosis

20.0 μm
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<th>Fungus</th>
<th>HPE</th>
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</table>
| **Eumycetoma**      | • **Grains** are interwoven mycelia lined by intensely eosinophilic material (Sp. Hoepli phenomenon).  
• **Dematiaceous fungi** produce **black grains** while **Scedosporium, Acremonium** produce **white grains**.  
• **Eumycotic mycetoma** shows septate hyphae, 2-6 μm in dm, staining positive with GMS & PAS.  
• **Actinomycotic mycetoma** shows delicate, branched, gram positive filaments sometimes showing beading & meas. <1μm.. |
| **Chromoblastomycosis** | Pigmented round structures ("copper penny lesions" / "sclerotic bodies" / "muriform cells") with internal septations in multiple planes.  
Intense epidermal HP & HK & pyogranulomatous response. |
| **Phaeohyphomycosis** | • Minimal changes in the epidermis.  
• Cyst wall comprised of dense collagen with granulomatous inflammation.  
• Center has geographic necrosis with FB giant cells & fungal elements (yeasts & septated hyphae). |
Dermatophyte disease

- Hyaline fungi difficult to observe in the keratin layer using H&E.
- GMS or PAS required for identification.
- HPE findings:
  - HK with focal PK.
  - Spongiosis & neutrophilic micro abscesses in acute cases.
  - Varying degrees of PV lymphocytes & plasma cells with prominent papillary dermal edema.
  - Severe inflammation of hair follicles and shafts (neutrophilic – kerion; mononuclear- Majocchi’s granuloma)
Inadequacies of HPE

Reported sensitivity of pathological studies is more than 80%, however, it has some inadequacies:

1. On special staining
   a) Yeasts can be confused with neurosecretory granules & melanin.
   b) Hyphae can be confused with collagen fibers & basement membrane material.
2. Some transversally cut hyphae can look like yeasts that may even appear to be budding.
3. Histopathology usually cannot provide the fungal genus and species.
4. Multiple infections may be difficult to interpret.
Causes for Positive Cultures but Negative HPE

1. The fungus present in the cultures is a colonizer or a contaminant.

2. The tissue is sampled from two different areas, and different samples are sent to microbiology and pathology.

3. The pathologic specimen has not been extensively studied.
Causes for Positive HPE but Negative Cultures

1. When the tissue in the microbiology laboratory is ground too aggressively and the fungal cells are destroyed.

2. When the fungus in the tissue is not viable.

3. When the tissue is sampled from two different areas & different samples are sent for HPE & culture.
To Conclude....

- In developing countries - major role, despite limitations, as improved culture facilities & molecular profiling of fungi, though ideal, are not widely available for routine diagnostic use.

- Microbiologists, Pathologists, and Clinicians need to be aware of the pitfalls of morphological diagnosis, and the alternative tests that can be performed to make organism-specific diagnoses.
References & Resources