

Infectious Disease Cytopathology

Mycology

Heather Ruff October 26, 2019





Assessing Fungal Hyphae

- True hyphae or pseudohyphae
- Pigmented or non-pigmented (hyaline)?
- Septate or non-septate?
- Branching -
 - Acute angle, right angle, variable?
 - Dichotomous (equal) vs. non-dichotomous (variable)?
- Arrangement uniform/parallel vs. disorganized
- Other structures
 - Fruiting Bodies (conidia, phialides, vesicle)
 - Pseudohyphae
 - Yeast

Hyphal Forms



Hyphae

True Hyphae with Constricted septations

Cervical Pap ThinPrep 29 Female



What is this?



Candida

- Oval budding yeast (3-6 μm)
- Pseudohyphae
- True hyphae (less common)
- Blastoconidia (rare)

Dematiaceous (Pigmented) Fungi

- Exophiala, Phialophora, Cladophialophora, Bipolaris, and others
- Pigmented Usually visible (brown) unstained
 - If not visible, may be demonstrated with Fontana-Masson
- Narrow (2-12 μm diameter, usually 2-6 μm)
- Septate (constricted septations common)
- Branching Irregular
- Moniliform (beaded) appearance
- May have yeast forms and secondary structures (e.g. chlamydoconidia)



Picture from https://www.sciencedirect.com/topics/agricultural-andbiological-sciences/phaeohyphomycosis



Zygomycetes

- Mucor spp., Rhizomucor sp., Rhisopuz spp., and others broad non-pigmented hyphae
- Non-pigmented
- Non-septate (really Pauci-septate)
- **Broad** (5-25 μm diameter, avaerage 12 μm)
- Branching Irregularly-spaced, nondichotomous, variable angles (often 90°)
- Twisted, folded, ribbon-like morphology



Hyaline Fungi

- <u>Aspergillus</u>, Fusarium, Paecilomyces, Acremonium, Scedosporium, Pseudallescheria, and many, many others)
- Non-pigmented
- Septate
- <u>Narrow</u> (2-12 μm diameter, usually 2-8 μm)







Aspergillus

- Organized
- Branching
 - \circ Parallel
 - ODichotomous (equal)
 - ○<u>Acute angle</u> (45°)
- May have fruiting bodies



Other Hyaline Fungi

- Disorganized
- Branching
 - \circ Variable orientation
 - ○Non-dichotomous (unequal)
 - ○<u>Variable</u> angles (<u>45° & 90°)</u>
- May see fruiting bodies



Esophageal Brush 56 F w/ SLE on prednisone.

Diagnosis?

BAL 54 M s/p lung transplant. Diagnosis?



BAL 54 M s/p lung transplant. Diagnosis?





Assessing Yeast

- Size Small, large, variable
- Shape Round, oval, irregular
- Other morphology
 - Wall Thin, thick, refractile
 - Capsule, pseudocapsule, none
 - Division Budding (Broad-based, narrow-based), Fission
 - Intracellular, extracellular, both
 - Internal structures
- Stains:
 - Mucin (Mucicarmine)
 - Pigment (Fontana-Masson)











Blastomyces sp.

• Round

- Large (range 3-40 μm diameter, usually 8-15 μm)
- Thick-walled, refractile
 (double-contour)
- Broad-based budding (4-5 μ m)
- No capsule



Cryptococcus sp.



- Variable shapes (Round, oval, collapsed crescents)
- Variable sizes (range 2-20 μm diameter, usually 4-10)
- Thin-walled, Narrow-based budding
- Encapsulated and unencapsulated forms
 - Capsule is clear on Romanowsky, Pap
 - Capsule stains pink on Mucicarmine
- Contains melanin-like pigment
 - Stains brown with <u>Fontana-Masson</u>
 - Especially good for unencapsulated small forms



Histoplasma sp.

- Ovoid
- Small (2-4 µm diameter)
 - H. capsulatum v. suboisii, 8-15 μm, West Africa
- Thin-walled
- Narrow-based budding
- <u>Pseudocapsule</u>
- Predominantly intra-cellular
- Crescent-shaped chromatin stains dark violet (purple cap)





Pneumocystis jiroveci

- Round to ovoid cysts
 - Collapsed crescent, <u>cup</u> appearance
- Small (3-7 µm diameter)
- Thin membrane
- No capsule, pseudocapsule
- <u>No budding</u>!
- In "foamy exudate" (collection of cysts)
- Romanowsky, Pap clear cyst with dark dots (nuclei, intra- and extra-cystic bodies)
- GMS <u>Dot</u>, comma, or parenthesis-shaped (thickening in membrane)



BAL 73 M Hx rectal ca, carcinoid, immunosuppressed. Diagnosis?



BAL 55 M w/ HIV and diffuse pneumonitis. Diagnosis?



BAL 49 M w/ HIV, immunosuppressed. Diagnosis?



BAL 58 M w/ AML, neutropenia. Diagnosis?



Additional Reference:

TABLE

(from Diagnostic Pathology of Infectious Disease 2nd Ed. Kradin)

Inflammation	Organisms	Ideal Stains for Microorganism in Cytology	Other Useful Special Stains	Comments
Purulent	 Pyogenic Actinomycetes Aspergillus/Zygomycetes 	1. Romanowsky or Gram 2. Gram-Weigert/Gram 3. Pap	1. None 2. Modified Kinyoun 3. GMS <i>(Aspergillus)</i>	 Modified Kinyoun is useful for distinguishing between Nocardia spp. or Mycobacterium fortuitum and Actinomyces spp.
Allergic mucin	1. Aspergillus/ dematiaceous fungi	1. Romanowsky or Pap	 GMS Fontana-Masson (melanin) 	
Granuloma	 Mycobacteria Histoplasma Cryptococcus 	1. Kinyoun or Ziehl-Neelsen 2. GMS 3. GMS	1. Modified Kinyoun —- —-	2 & 3. In well-formed granulomas, Histoplasma and Cryptococcus tend to be scant in number and nonviable (Histoplasma) or paucicapsulated (Cryptococcus); GMS is the preferred stain in this situation.
Mixed granuloma/ purulent	 Blastomyces Coccidioides Paracoccidioides Chromoblastomycosis and phaeohyphomycosis agents Sporotrichosis 	1. Pap 2. Pap 3. Pap 4. Pap 5. GMS	 4. Fontana-Masson (melanin)	 Sporothrix is usually very scant in cytologic and histologic samples and poorly stained by Pap unless a SHP is seen; in GMS, the yeast forms have elongated, cigar-shaped blastoconidia.
Diffuse macrophage	 Mycobacteria, especially MAC Histoplasma Leishmania 	 Romanowsky and Kinyoun/Ziehl-Neelsen Romanowsky Romanowsky 	 2. GMS	 MAC and other mycobacteria often have a negative stain/refractile red appearance with Romanowsky stain.
Scant to none	 Pneumocystis Cryptococcus Aspergillus/Zygomycetes (neutropenic patients) 	1. Romanowsky/GMS 2. Romanowsky 3. Pap	2. Mucin 3. GMS <i>(Aspergillus)</i>	 Pneumocystis can usually be presumptively diagnosed on Pap-stained slides.
	 Candida (neutropenic patients) 	4. Romanowsky	4. Gram	

Summary of Inflammatory Patterns, Likely Organisms, and Stains of Value in Diagnosis

GMS, Grocott-Gomori methenamine silver stain; MAC, Mycobacterium avlum complex; Pap, Papanicolaou stain; SHP, Splendore-Hoeppli phenomenon.

Differentiating Hyphal Fungal Organisms

Zygomycetes	Aspergillus	Other Hyalohyphomycetes	Phaeohyphomycetes
Non-pigmented	Non-pigmented	Non-pigmented	Pigmented
Broad	Narrow	Narrow	Narrow
Non/Pauci-septate	Septate	Septate	Septate (constricted)
Irregular, uneven, 90°	Regular, even, 45°	Irregular, uneven	Irregular, uneven
Twisted, folded, ribbon-like	Parallel, radiating branches	Haphazard arrangement	Haphazard arrangemet
	+/- fruiting bodies	+/- fruiting bodies	+/- Yeast-like forms and chlamydoconidia

Coccidioides sp.

- Round, large thick-walled spherules (5-100 μm, usually 10-100 μm)
- Round small endospores (2-5 μm)
- Ruptured, fragmented spherules
- Filamentous forms (septate hyphae, barrel-shaped arthroconidia rarely seen



Paracoccidioides sp.

- Round to oval
- Large yeast (3-30 μm)
- Narrow based budding
- Multiple bud attached to single parent cell (ships wheel)



Penicillium marneffei

• Round to oval

- Small (3 μm diameter, 2.5-5 μm long)
- Transverse septum (fission, not bud)
- Pseudocapsule
- Predominantly intracellular
 - If extracellular elongate up to 8 μm, have septae, become curved

Penicillium marneffei (E) - Courtesy of Vicki Schnadig at UTMB Paracoccidiioides (*) - Courtesy of Diane Dziedzic at microworld.org



Differentiating Yeast - Large Forms

Blastomyces	Cryptococcus	Coccicdioides	Candida
 All large 	 Variable sizes 	 Large spherules, Small 	 Small yeast, Large conidia
 Round 	 Variable shapes 	endospores	Oval
 Refractile, 	 Thin walled 	Round	 Thin walled
Double contour		 Thick walled 	
 Broad budding 	 Narrow 		 Buddings
	budding	 No budding 	
 No capsule 	 Capsule 		 No capsule
	 Melanin pigment 	 No capsule 	

Differentiating Yeast - Small Forms

Pneumoncystis	Cryptococcus	Histoplasma	Candida
 All small 	 Variable sizes 	All small	 Small yeast, Large conidia
• Round, cups	 Variable shapes 	Oval	Oval
 No budding 	 Narrow budding 	Narrow budding	 Budding
 No capsule 	 Capsule (mucicarmine) 	 Pseudocapsule 	 No capsule
 "Foamy exudate" GMS → . , " 	 Pigment (Fontana Masson) 	 Purple cap on Romanowsky Mostly Intracellular 	 Pseudohyphae, rare true hyphae
		macendia	

Answers To Cases

Esophageal Brush 56 F w/ SLE on prednisone.

Diagnosis:

Fungal organisms consistent with Candida

BAL 54 M s/p lung transplant. Diagnosis:



Fungal hyphae present Hyaline (non-pigmented) and pigmented hyphae

Culture grew Aspergillus fumigatus

BAL 54 M s/p lung transplant. Diagnosis:

Fungal hyphae present Non-pigmented (hyaline) hyphae

Culture grew Aspergillus fumigatus

BAL 73 M Hx rectal ca, carcinoid, immunosuppressed. Diagnosis:



BAL 55M w/ HIV and diffuse pneumonitis. Diagnosis:



BAL 49 M w/ HIV, immunosuppressed. Diagnosis:



BAL 58 M w/ AML, neutropenia. Diagnosis:

Don't forget to look for the second organism!

Pneumocystis & CMV