The Impact of Histopathology on Antifungal Treatment

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Accurate diagnosis of mycoses

Essential for:

- Appropriate therapy
- Reliable prognosis
- Study/understanding of pathogenesis
- Elucidation of pathology
- Epidemiological studies
- Knowledge of fungal biology

Mortality in invasive mycoses

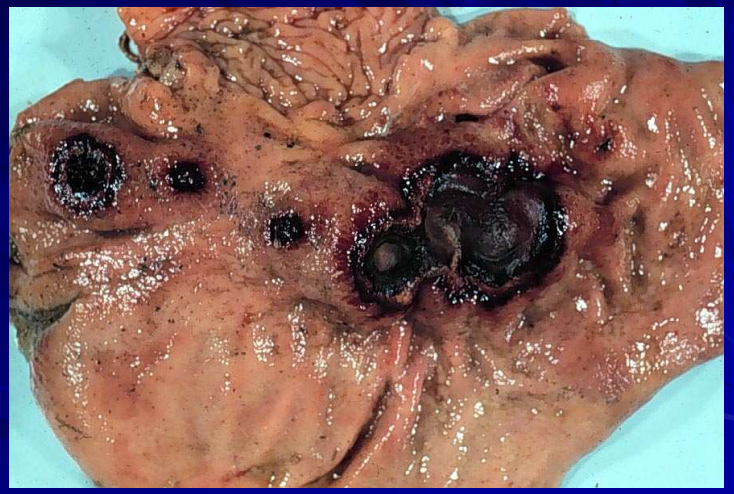
1. Lack of a reliable and timely diagnosis.
2. Insignificant signs and symptoms – first present at an advanced stage.
3. Antifungal therapies are not optimal, and often depending on correction of underlying diseases.

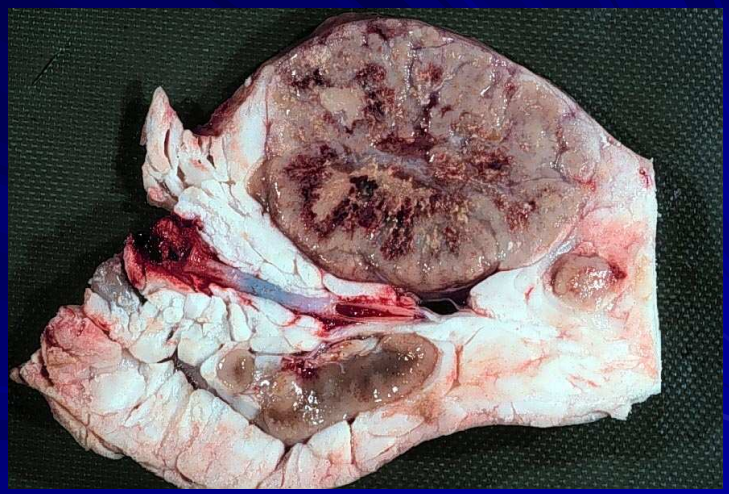
Diagnosis of invasive fungal infections

1. Imagining diagnostic techniques (including gross pathology)
2. Conventional mycology
3. Non-cultural based procedures

Imagining techniques and gross pathology

- Often impossible
- Acute contra chronic lesions
- Neoplasms
- Other non-mycotic infections





Cultivation of fungi in tissues

- Impossible (no tissue left/in formalin)
- Negative
- Contamination problems
- Recovery of a different fungus
- No isolation techniques

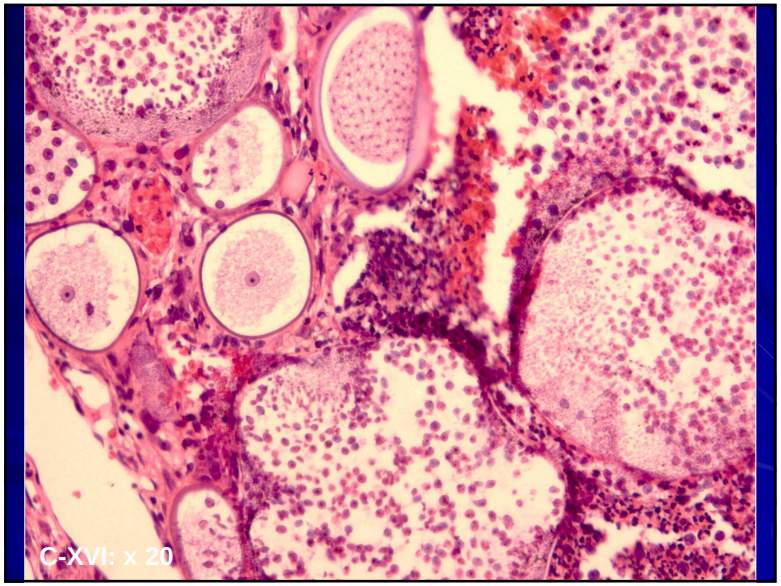
III. MYCOSES BY OBLIGATORILY PATHOGENIC FUNGI

b) Predominantly skin and mucosae

Mycosis	*Agent*	*Fungus*		*Particularities*
		Tissue	*Culture*	
Sporotrichosis	*Sporothrix schenckii*			Lymphogenous dissemination; chains of satelliite ulcers. Gardeners, farmers, fishermen. Fungus cells visible only in recent lesions and in immunocompromized patients. Pulmonary systemic myc. rare
Rhinosporidiosis	*Rhinosporidium seeberi*		Does not grow	Habitat in water? Large cystic fungus cells with endospores (sporangia)
Lobomycosis (queloidblasto-mycosis)	*Loboa loboi*		Does not grow	Only in the north of south and in central America
Chromoblasto-mycosis	Spec. of Fonsecaea and Phialophora; *Cladosporium carrionii*			Brown spherical fungi of the group of Dematiacetes
Mycetomas				
Eumycetomas	True fungi 16 species		Hyphae	In both, the grains in abscesses; later with granulomatous reaction. Only cutaneous lesions.
Actinomycetomas	Actinomycetes 6–8 species		Filamentous bacteria	Botryomycosis (Actinophytosis or bacterial pseudomycosis: grains consisting of bacteria).

IV. RARE, EXCEPTIONAL OR LESS IMPORTANT MYCOSES

1. Adiaspiromycosis (Haplomycosis)	*Chrysosporium parvum* var. *parvum* (Emmonsia)
	Chrysosporium parvum var. *crescens* (Emmonsia)
2. Penicilliosis marneffei	*Penicillium marneffei*
3. Pseudoallescheriasis (Monosporiosis)	*Pseudoallescheria boydii* (Scedosporium apiospermum)
4. Bagassosis	*Hyalohyphomycetes* (?), *Aspergillus* (?)
5. Basidiomycoses	*Basidiomycetes*
6. Hyalohyphomycoses	Numerous species of many genera. Hyalohyphomycoses show non-pigmented hyphae in tissues, in contrast to the phaeohyphomycoses with pigmented (brown) hyphae in tissues, i.e. hyphae from the group of *Dematiacetes*



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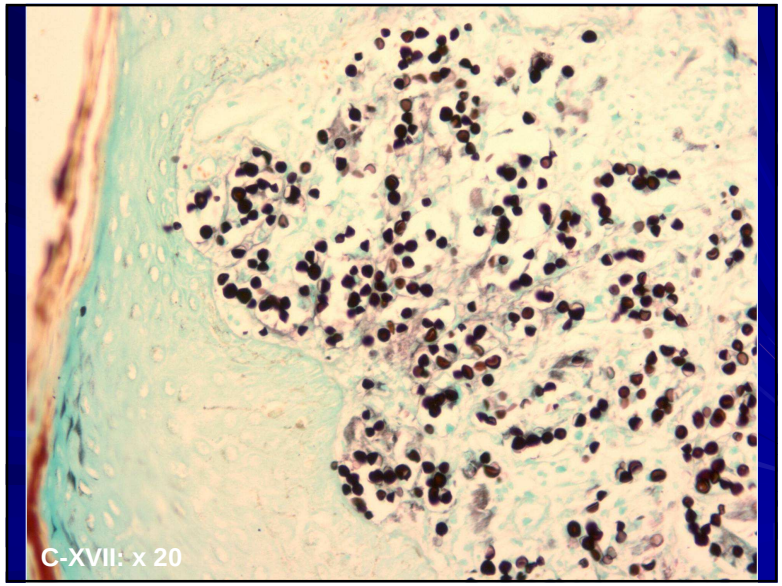

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Non-cultural based techniques

- Histopathology
- Immunohistochemistry / in situ hybridization
- Fungal antibodies
- Antigen extraction from tissue
- Antigen detection in fluids
- Metabolites in fluids
- PCR-techniques (fluids and tissues)

Tentative diagnosis based on histopathology

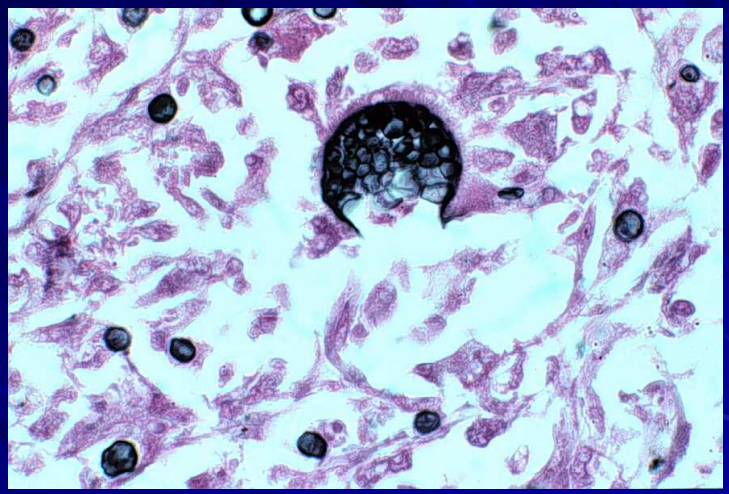
- Area of the world
- Conidial heads
- Calcium oxalate crystals
- Asteroid bodies (Splendor-Hoplii)

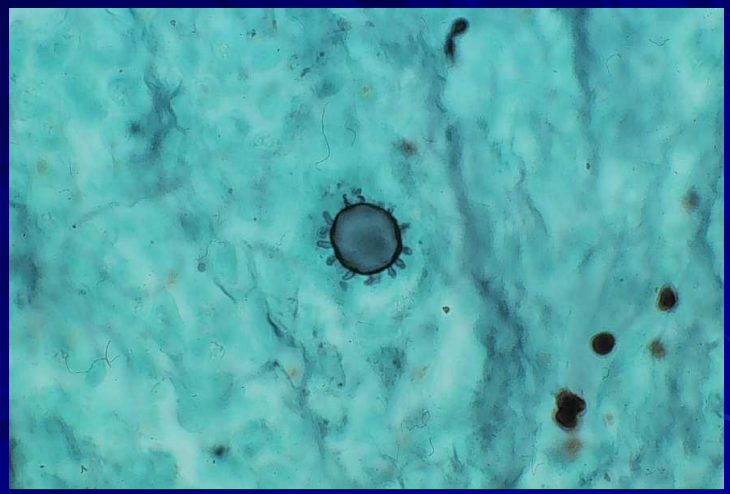
- Typical morphology, staining properties, and propensity for tissues may allow differentiation to a sudden level

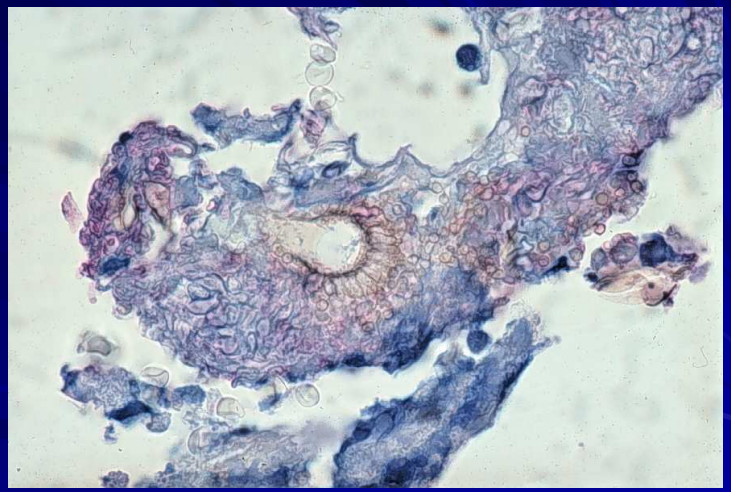
III. MYCOSES WITH OBLIGATORILY PATHOGENIC FUNGI
(Primary, exogenous, generally endemic)

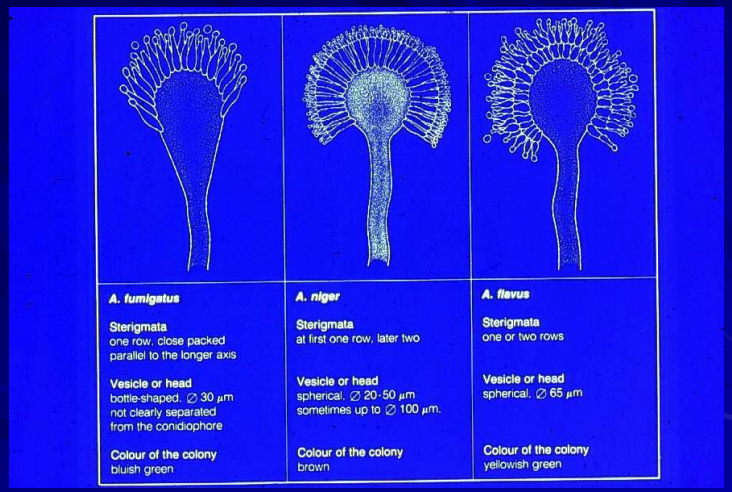
a) Predominantly systemic[1*]

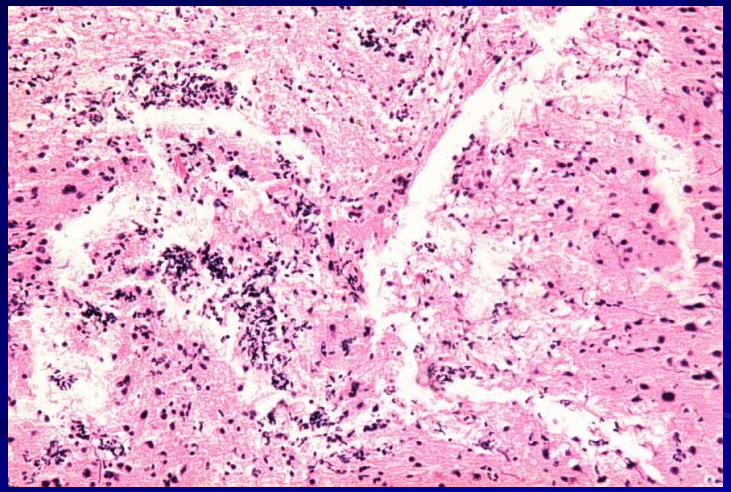
Disease	*Agent*	*Fungus*		*Geogr. distrib.; endem. reg.*	*Portal of entry; tropism*
		Tissue	*Culture*		
Histoplasmosis capsulati	*Histoplasma*[3] *capsulatum* var. *capsulatum*			North, central and south America, Asia, Africa, Europe, caves, soil, chicken coops, bats	Lungs,[2] PMS system, adrenals
Histoplasmosis duboisii	*Histoplasma*[4] *capsulatum* var. *duboisii*		Same as *H. caps.* var. *caps.*	Africa	Lungs (?), skin, bones
Coccidioido-mycosis	*Coccidioides immitis*			North, central and south America; deserts with sand soil	Lungs[2], meninges, erythema nodosum
Blastomycosis	*Blastomyces*[4] *dermatitidis*			North and parts of south America, Africa, Israel, Saudi, India	Lungs[2], skin, intern. masc. genital organs
Paracoccidioido-mycosis	*Paracoccidioides brasiliensis*			South and central America and Mexico	Lungs[2], face (skin and mucosae), adrenals

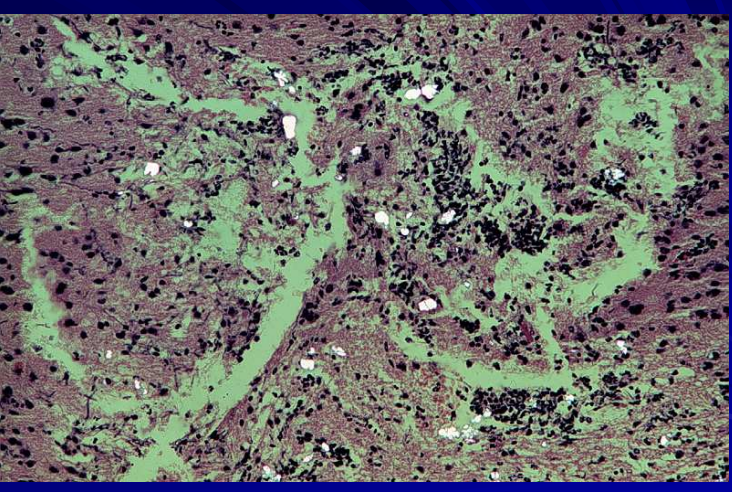


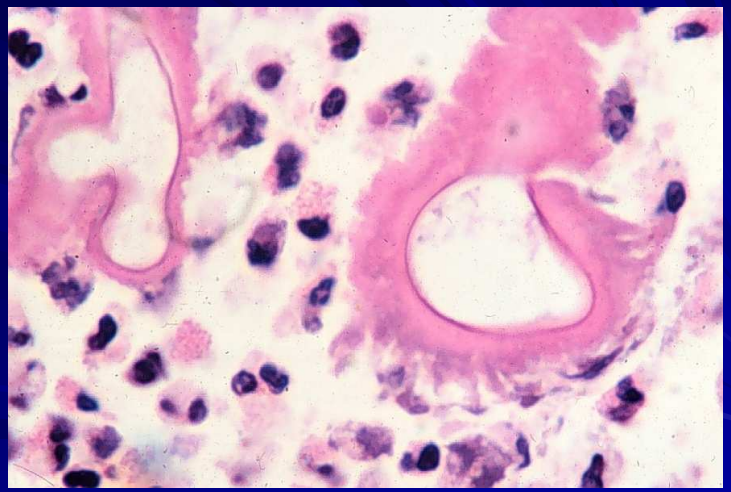





A. fumigatus
Sterigmata
one row, close packed
parallel to the longer axis
Vesicle or head
bottle-shaped, ∅ 30 µm
not clearly separated
from the conidiophore
Colour of the colony
bluish green
A. niger
Sterigmata
at first one row, later two
Vesicle or head
spherical, ∅ 20-50 µm
sometimes up to ∅ 100 µm.
Colour of the colony
brown
A. flavus
Sterigmata
one or two rows
Vesicle or head
spherical, ∅ 65 µm
Colour of the colony
yellowish green





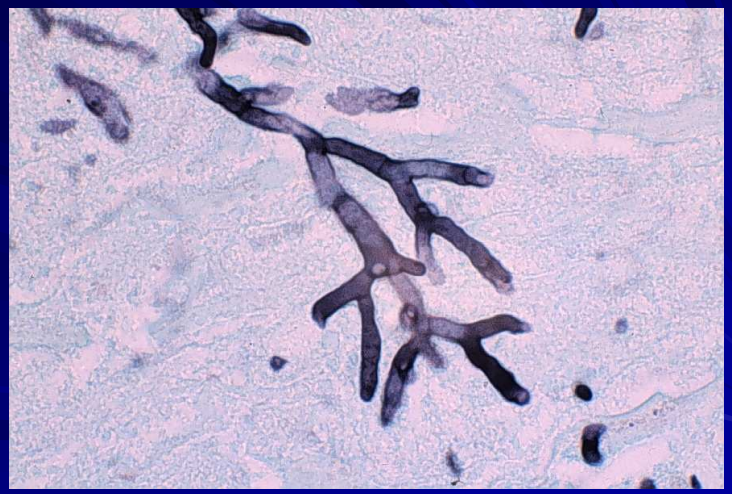


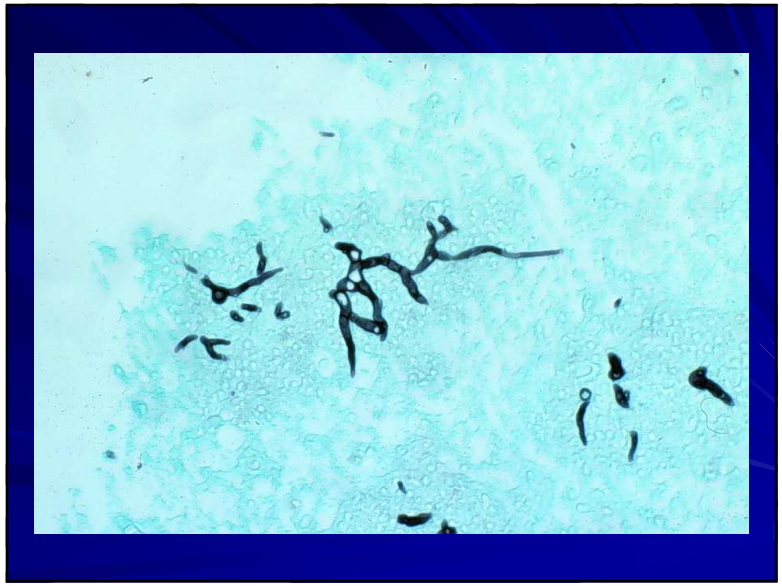
Tentative diagnosis by morphology

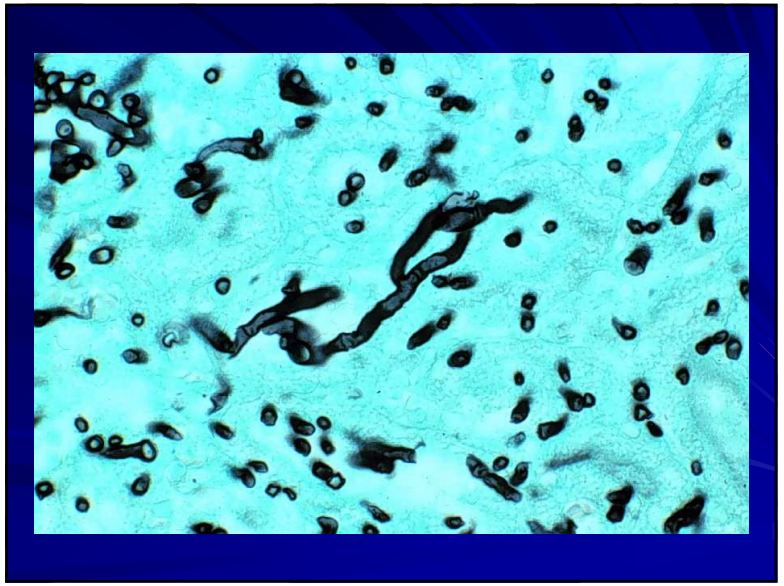
- Atypical/bizarre structures
- Morphological similarities
- Scanty elements present
- Steric orientation
- Age/viability of the fungus
- Type of tissue
- Host response

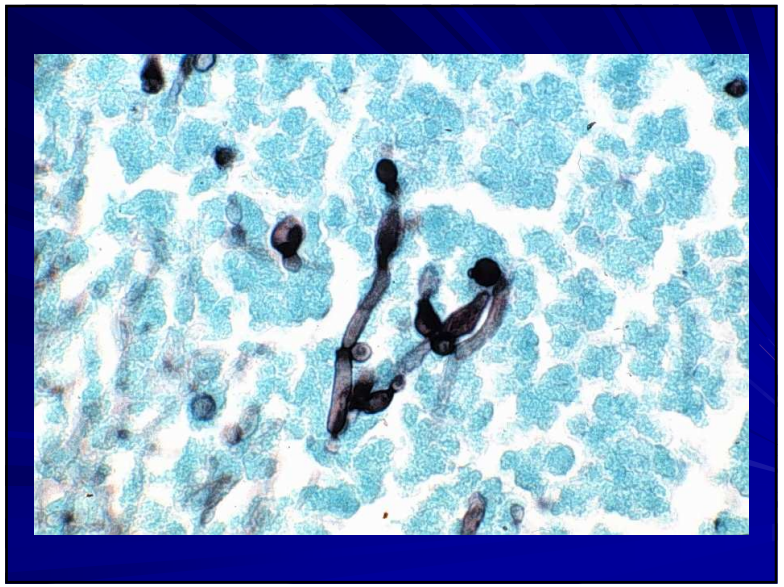
Morphology of hyaline hyphae in tissues

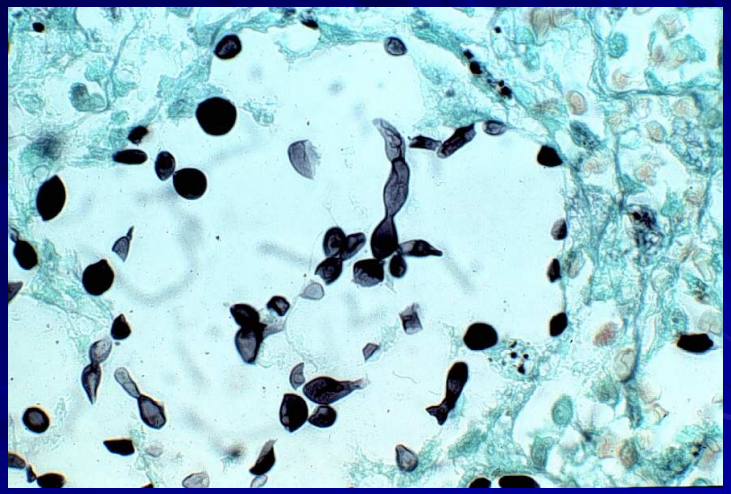
Feature	Aspergillus spp	Fusarium spp.	Scedosporium spp.	Zygomycetes
Width (μm)	3-6	3-8	2-5	6-25
Contours	Parallel	Parallel	Parallel	Irregular
Pattern of branching	Dichotomous	Dichotomous Right angle	Dichotomous Right angle	Haphazard
Orientation of branching	Parallel Radial	Parallel Random	Parallel Random	Random
Septation	Frequent	Frequent	Frequent	Absent
Angio-invasiveness	Yes	Yes	Yes	Yes

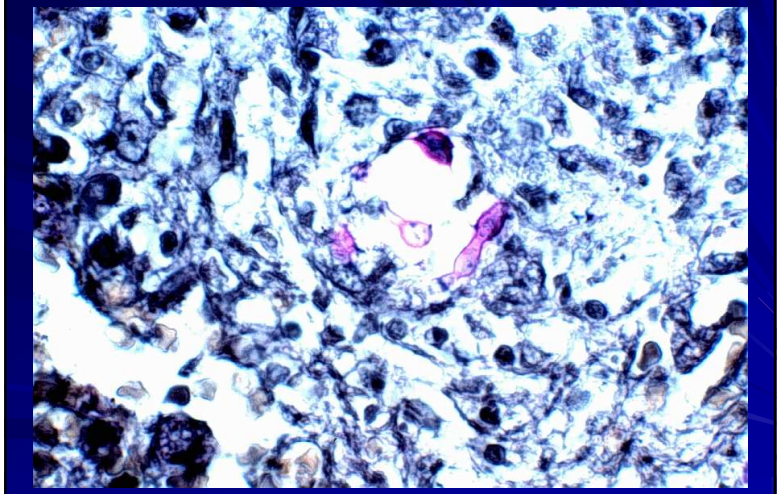












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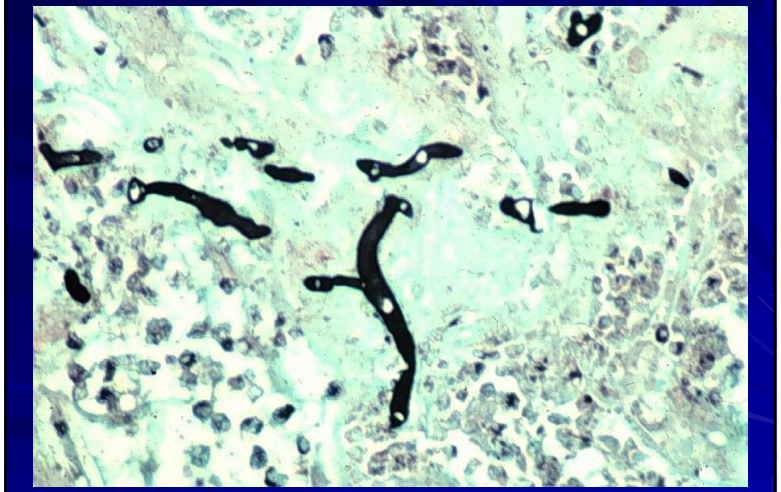

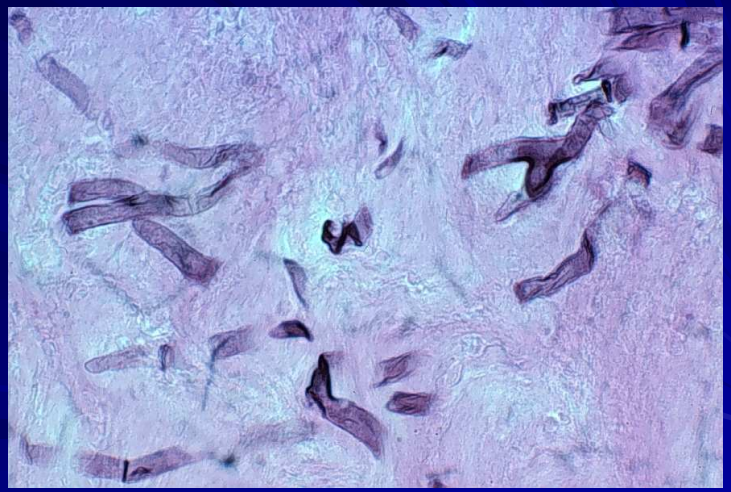

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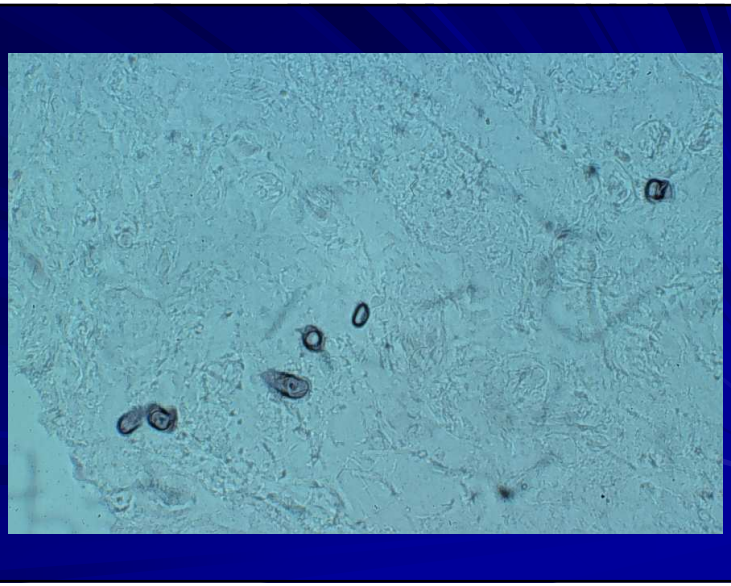

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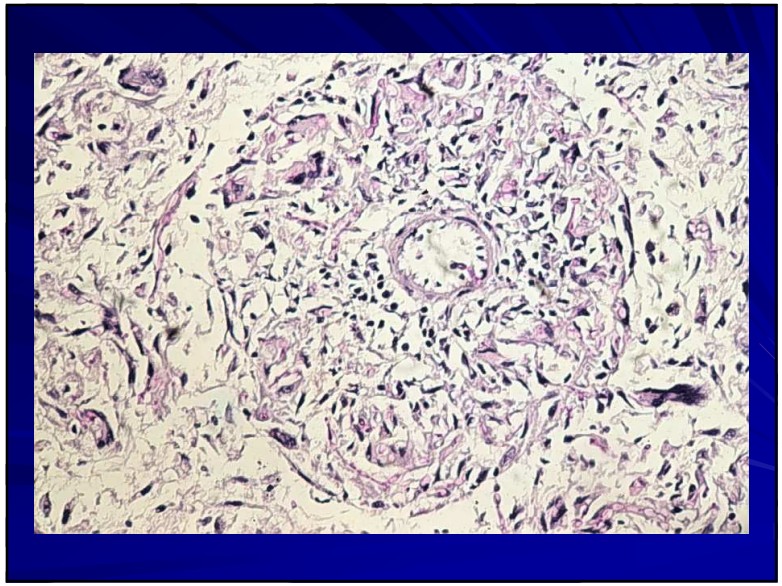

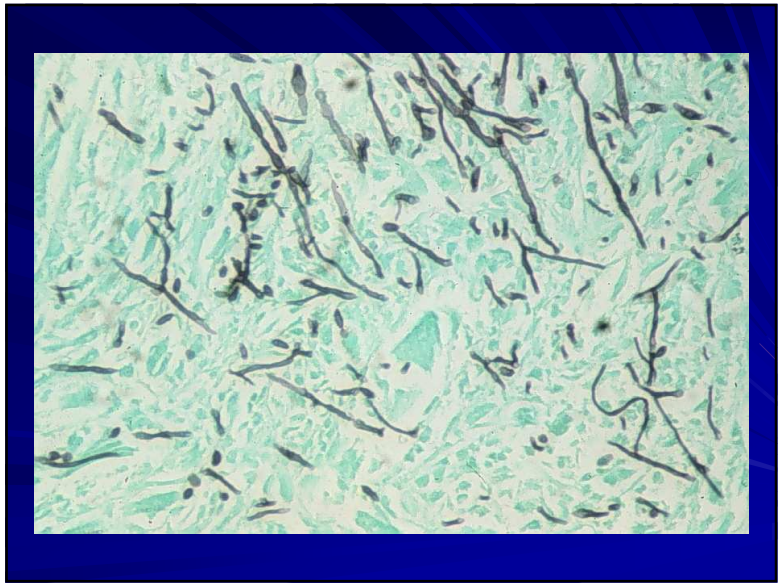

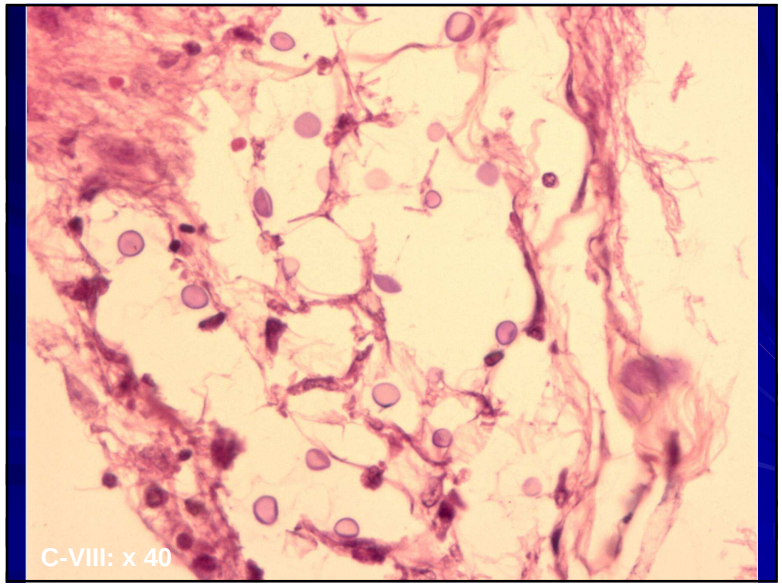
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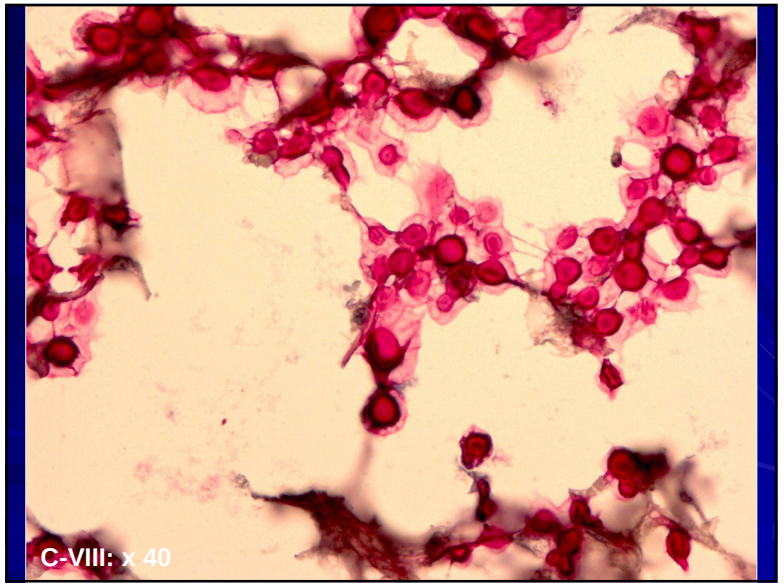
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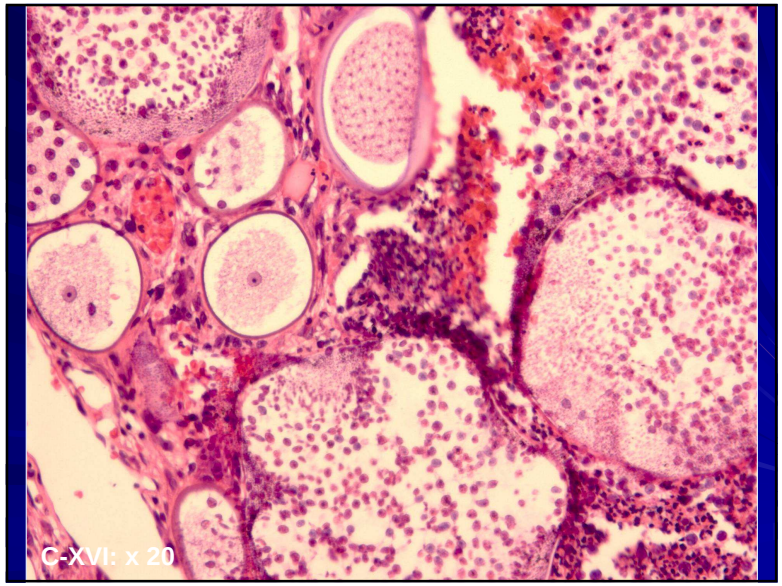
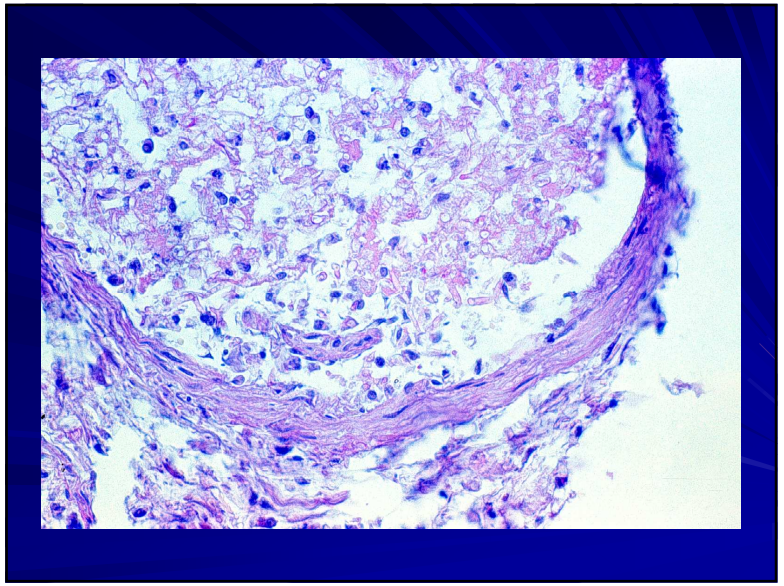




Primary antibodies used in immunohistochemical techniques

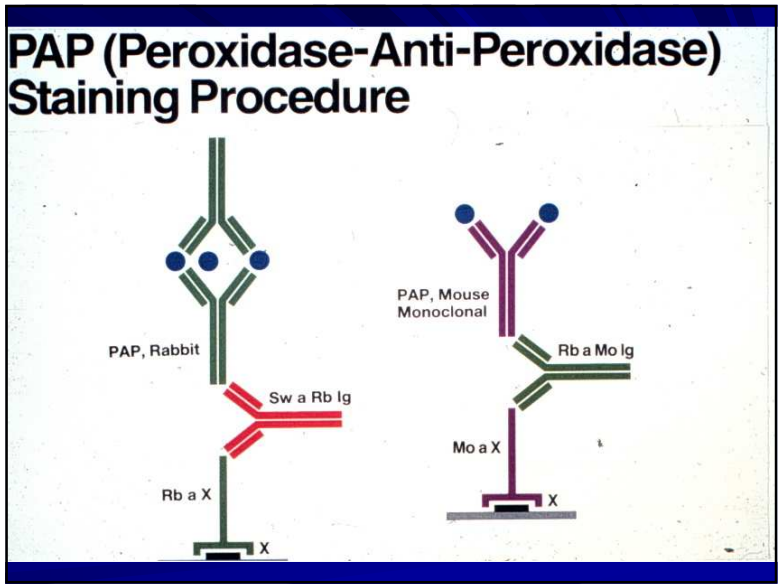
Monoclonal antibodies:

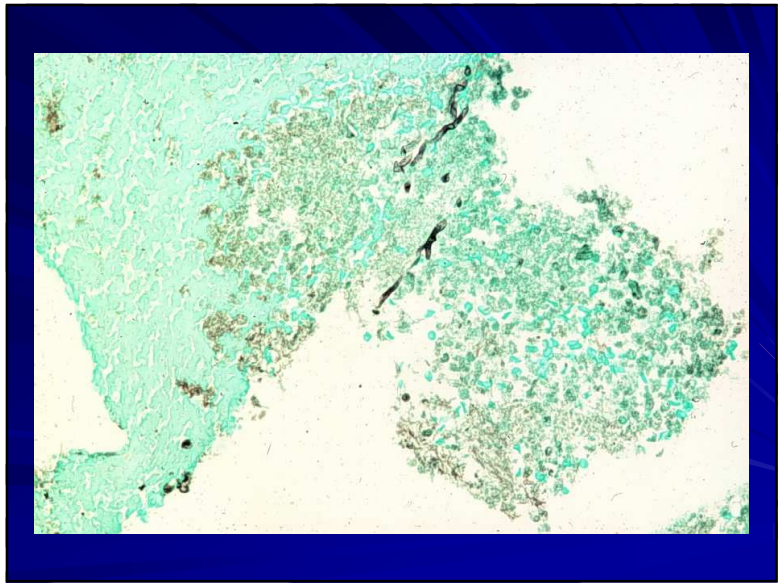
Aspergillosis and zygomycosis

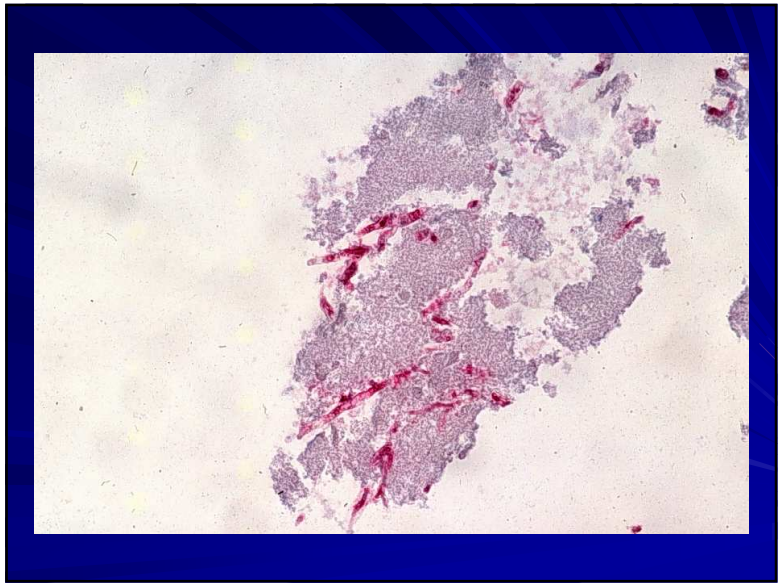
Heterologously absorbed polyclonal antibodies:

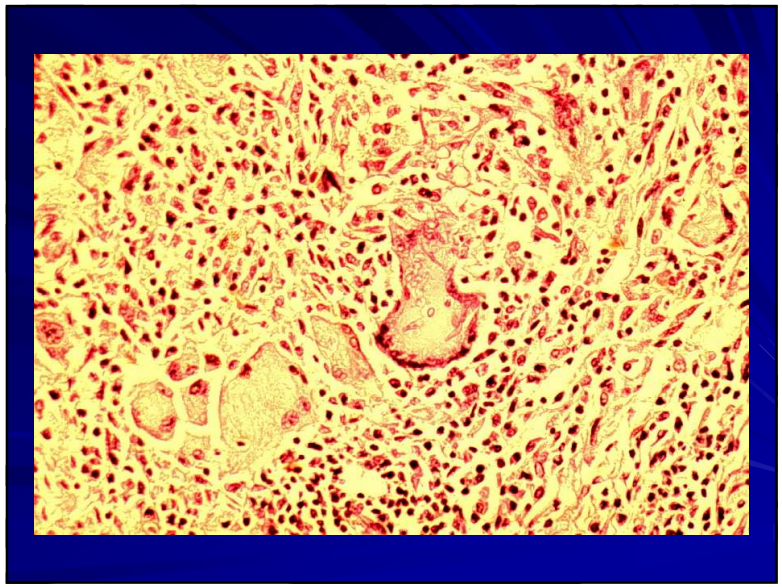
Aspergillosis (spp. level), candidosis (spp. level), fusariosis (spp. level), geotrichosis, scedosporiosis (spp. level), zygomycosis (genus level), cryptococcosis (type level), S. schenckii, C. immitis. H. capsulatum sp. capsulatum, P. carinii, B. dermatitidis, Prototheca (spp. level)

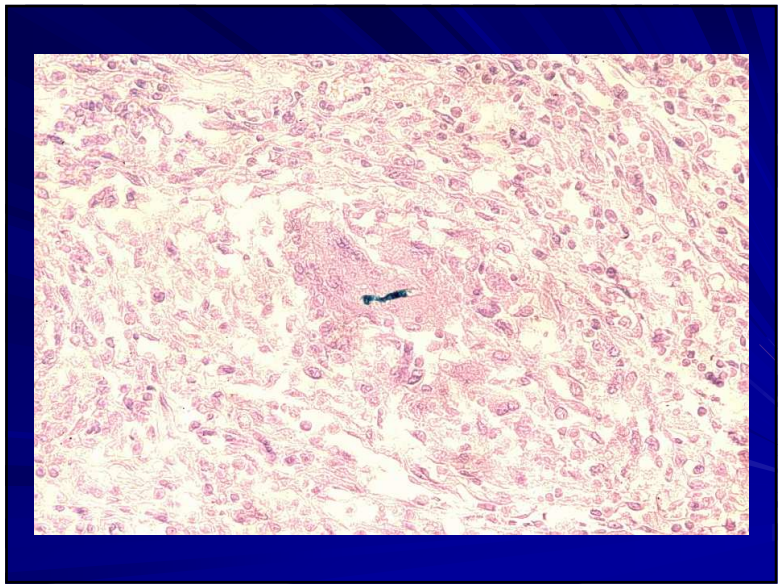
All antibodies were used on formalin-fixed, paraffin-embedded tissue sections

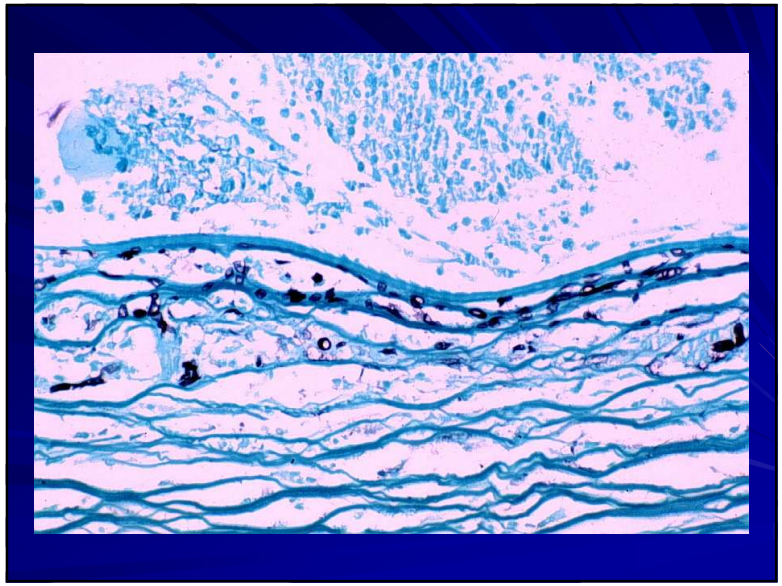


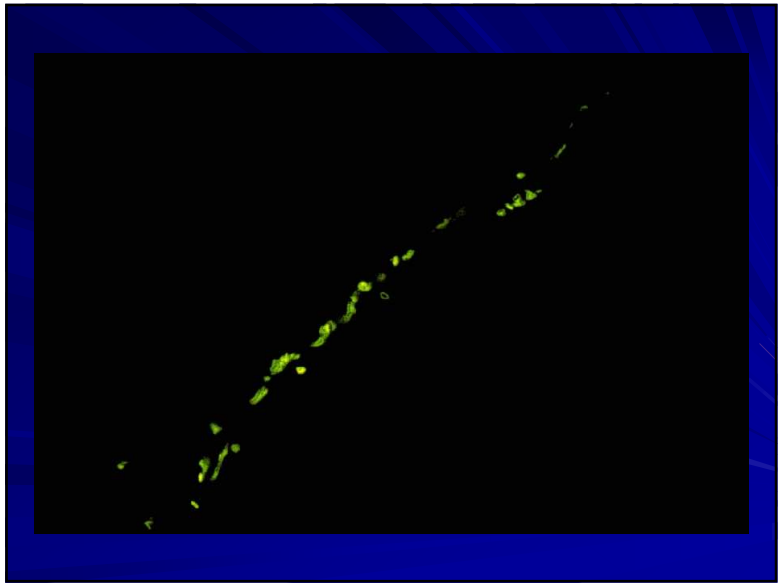


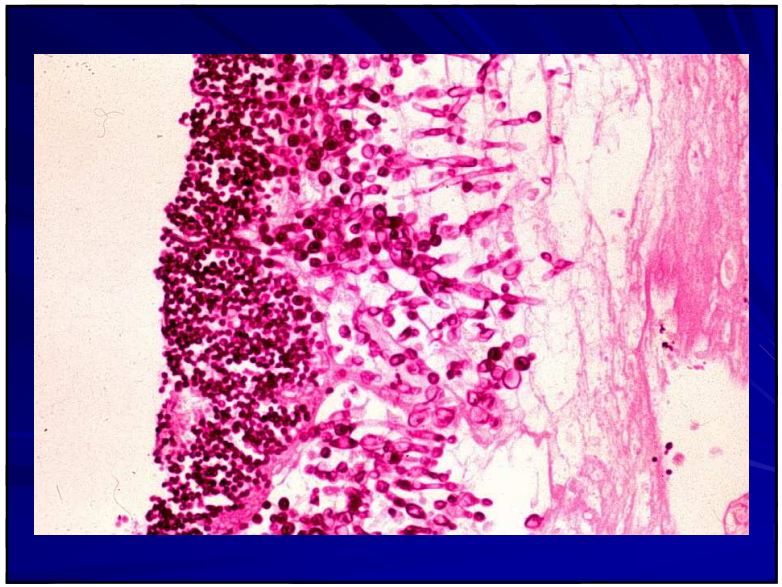


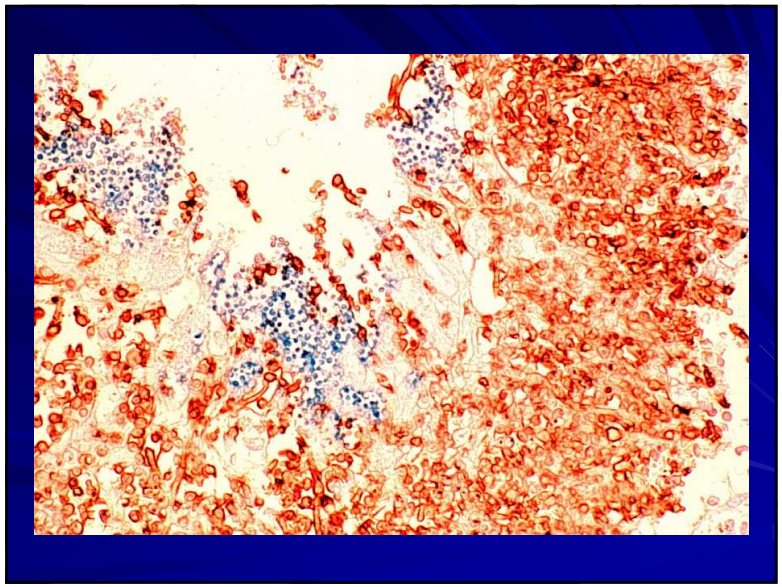












Diagnosis of mycoses in 109 lesions - I

Histopathologically	Immunohistochemically
Aspergillosis (n = 64)	41 = Aspergillosis
	3 = Aspergillosis + candidosis
	5 = Candidosis
	6 = Zygomycosis
	2 = Fusariosis
	4 = Scedosporiosis
	3 = Not identified
Candidosis (n = 8)	5 = Candidosis
	2 = Aspergillosis + candidosis
	1 = Aspergillosis

Diagnosis of mycoses in 109 lesions - II

Histopathologically	Immunohistochemically
Zygomycosis (n = 18)	10 = Zygomycosis
	2 = Zygomycosis + aspergillosis
	6 = Aspergillosis
Aspergillosis + candidosis (n=5)	1 = Aspergillosis + candidosis
	4 = Aspergillosis
Zygomycosis + candidosis (n=1)	1 = Zygomycosis
Not identified (n=13)	3 = Aspergillosis
	3 = Candidosis
	2 = Zygomycosis
	2 = Scedosporiosis
	3 = Not identified

Diagnosis of mycoses: Histology versus immunohistochemistry

Correct diagnosis of aspergillosis	= 69%
Correct diagnosis of candidosis	= 88%
Correct diagnosis of zygomycosis	= 67%
Correct diagnosis of dual infections	= 17%
Actual occurrence of dual infections	= 4%
Overall correct diagnoses of mycoses	= 60%

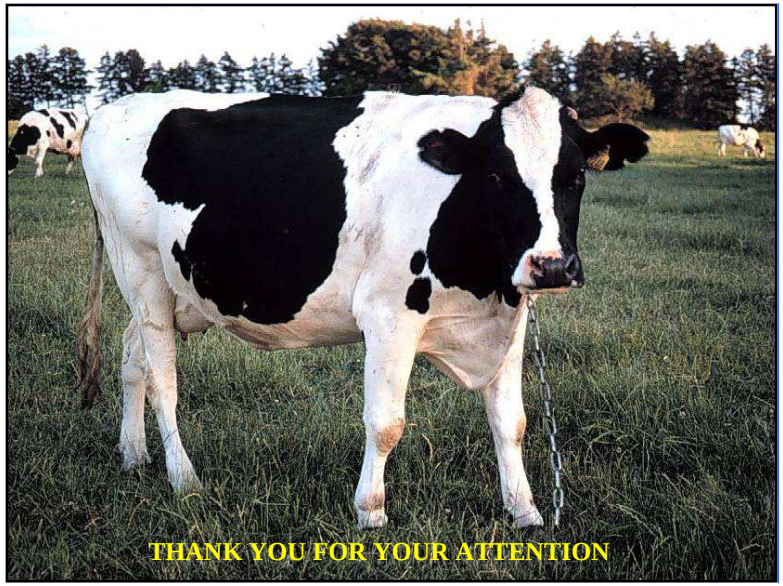
The Impact of Histopathology on Antifungal Treatment

1) Histomorphology is reliable for the diagnosis “mycosis”.

2) A tentative diagnosis may be obtained with presence of typical morphology, staining properties, and propensity for tissues.

3) Especially challenged in lesions with chronic reactions, necrosis, and few fungal elements.

4) Application of immunohistochemistry is enhancing the diagnostic sensitivity and specificity


THANK YOU FOR YOUR ATTENTION