

Microbiological diagnostics in pulmonary fungal infection

NSMM 21.05.2014

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Diagnosics from a microbiological perspective

Fungal pulmonary infection

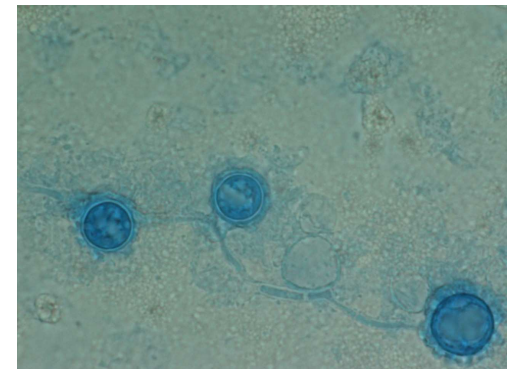
- Opportunistic
 - Aspergillosis
 - PCP
 - Mucormucosis
- Endemic mycosis
- Chronic pulmonary aspergillosis
 - In underlying lung disease

Undiagnosed problem?

- In many groups of immunosuppressed patients
- critically ill patients
 - Particularly intensive care patients
 - End stage lung and liver disease
 - Near drowning
 - Asphyxia
 - Diabetes mellitus

Emerging endemic mycosis?

- Patients from endemic areas or patients returning from travel to such areas challenge our standard diagnostic workup
- With or without immunosuppression



Histoplasma capsulatum

Do we perform?

- Knowing which fungus is involved guides appropriate antifungal choice, dose and duration of therapy
- Antifungal resistance present?
- Alternative antifungal agent required?
- Dual infections?

Diagnosis of invasive fungal infections

- A high level of clinical suspicion
- Multi-disciplinary approach
 - Clinical -
 - Radiological-
 - **Microbiological-**
 - Histopathological- or cytopathological examination



Fungal pulmonary infection

- Possible tests to perform
 - Antigen testing
 - Beta 1,3-D-glucan
 - Antibody testing
 - Microscopy of fungi
 - Fungal Culture
 - Molecular mycology

Galaktomannan

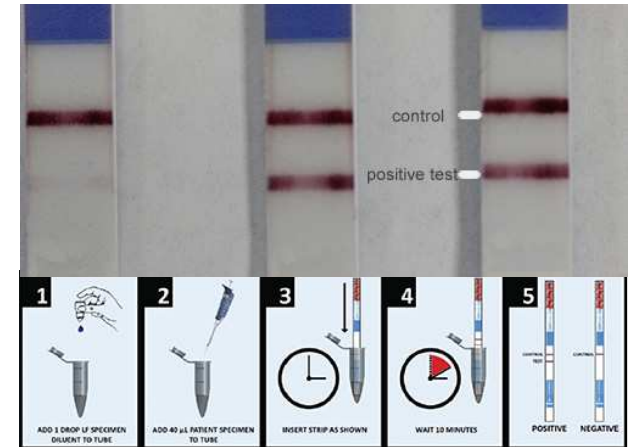
- Serial testing of serum of high risk patients
 - followed by diagnostic work up inclusive HRCT when positive
- Possible in BAL (and other body fluids) when fungal lung infection is suspected

Beta 1,3-D-glucan

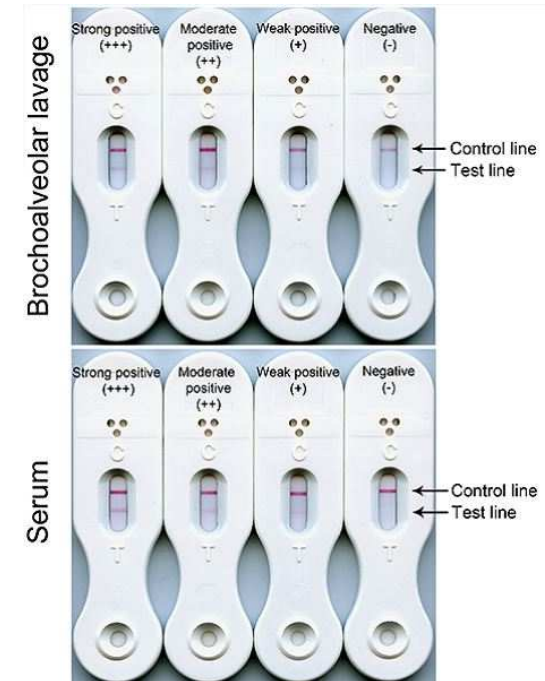
- More panfungal
- **14.10 : screening in adult high risk hematological patients**
- Helena S. Hammarström SE

Antigen testing

- Lateral flow
 - Cryptococcus
 - Aspergillus



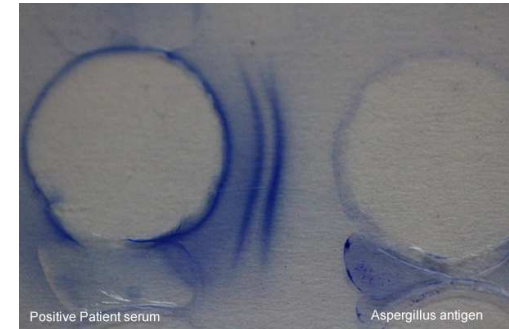
Clin Infect Dis. 2011;53:321–5



J Clin Microbiol. 2013; 51(2): 459–465.

Antibody testing

- Aspergillus
 - IgG
 - Precipitins
 - The best IgG assays have a 90-95% sensitivity for chronic pulmonary aspergillosis and aspergilloma caused by *A. fumigatus*,
 - allergic bronchopulmonary aspergillosis (ABPA)
- Coccidioidomycosis
- Histoplasmosis
- Blastomycosis
- Paracoccidioidomycosis



Aspergillus precipitins

www.life-worldwide.org

Molecular mycology

- PCP
- Aspergillus
- Candida
- Mucormucosis
- ITS and sequencing

Fungal culture

- Some samples are easy to take and process
- Sputum, induced sputum, BAL
- Low sensitivity
- Isolation and identification can take several weeks
 - results may be too late to guide treatment
- Specificity depends on specimen obtained and patient population
- Contamination, colonization or infection?
- Blood cultures usually negative

Respiratory samples

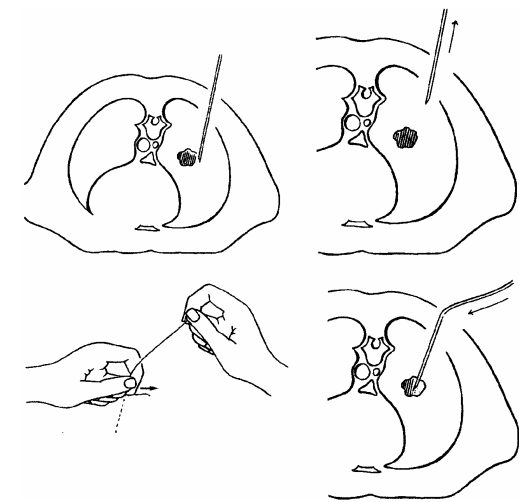
- Do we need selective medium for slow growing fungi ?
 - benomyl, cycloheximide or amphotericin B
 - birdseed agar
 - might be needed to avoid growth of rapidly growing *Aspergillus* spp. and *Candida* spp
 - To enable of *Scedosporium* spp, *Cryptococcus neoformans* and other slow growing species.

Do we get the best specimens?

- And do we handle them the best way possible in the lab?

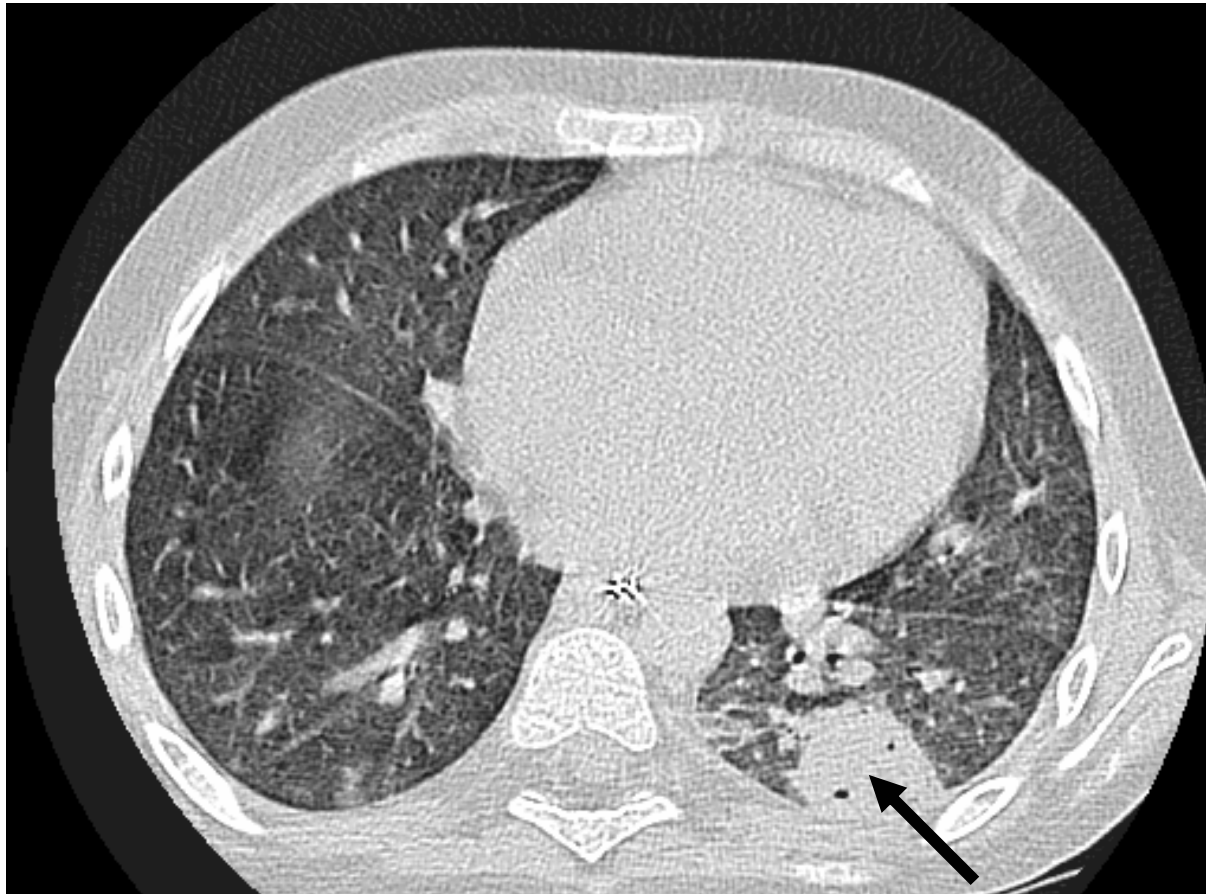


Cancer?



CT guided intervention performed

What about cavities and nodules of presumed infectious origin?



No invasive samples performed

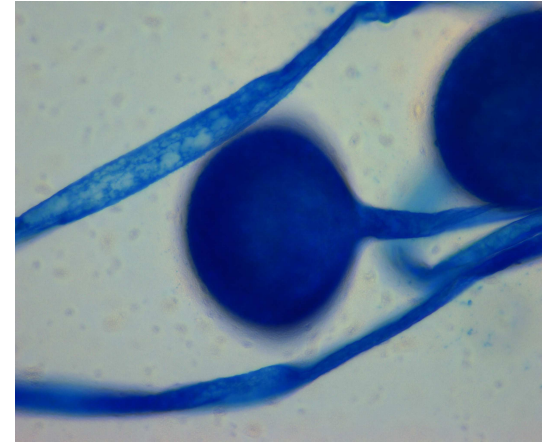
- *A.flavus* in sputum
 - indicative of infection in an immunocompromised patient with pulmonary infiltrate
- Voriconazol
- Cure

Antifungal treatment

- Knowing which fungus is involved guides appropriate antifungal choice, dose and duration of therapy
 - No longer empirical Amphotericin B for all
- Species specific guidelines
- Need for surgical intervention?

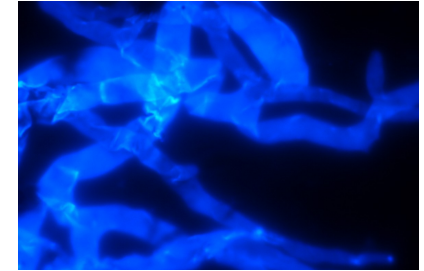
Mucormycosis

- The diagnosis is challenging
 - No circulating antigen detection is available
 - No standardized blood PCR test is available
- Specimens from clinically involved sites is mandatory for diagnosis.
- Tissue biopsies for histopathology and culture when possible



From: Diagnosis and treatment of mucormycosis in patients with hematological malignancies: guidelines from the 3rd European Conference on Infections in Leukemia (ECIL 3)

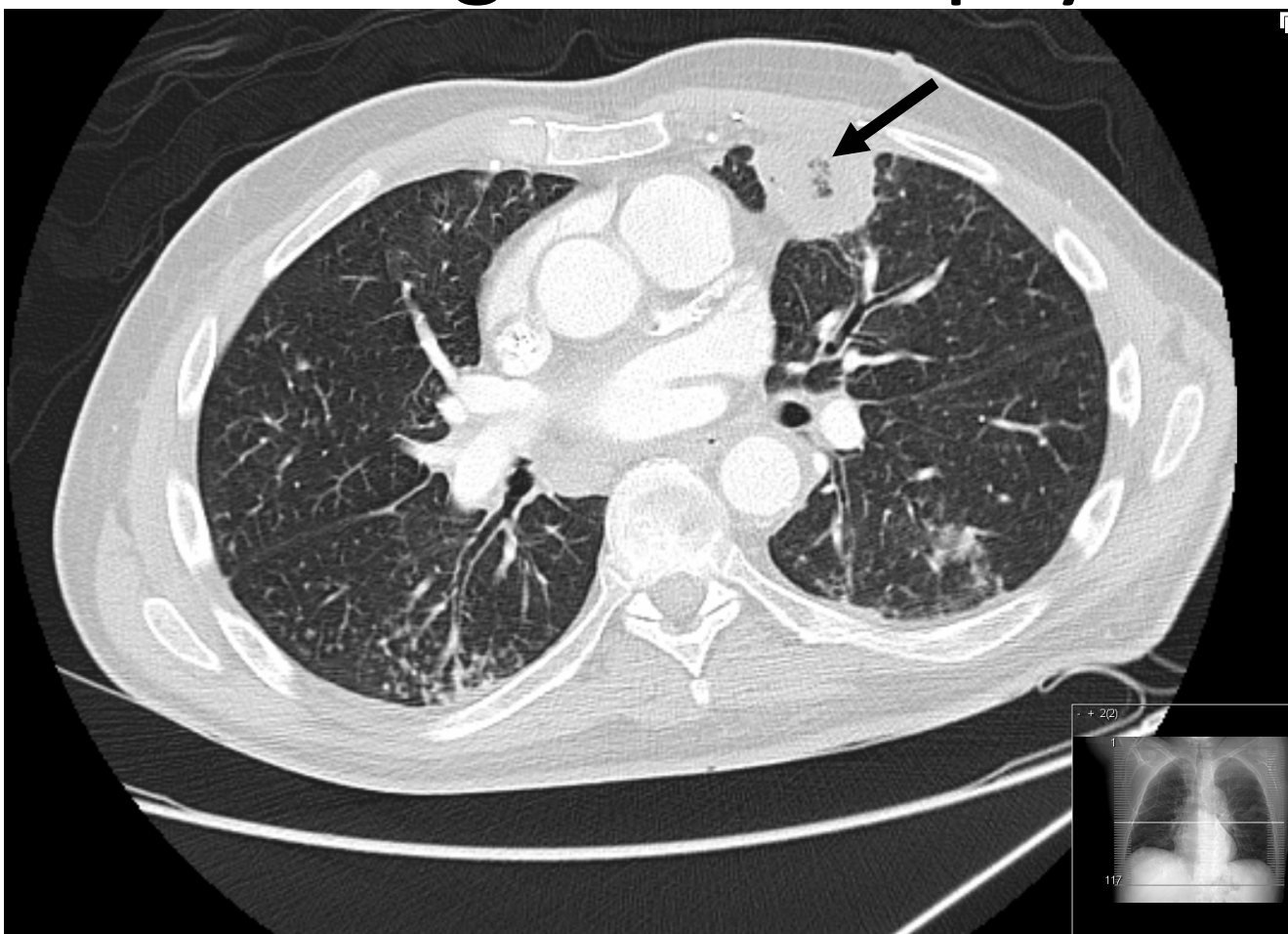
Mucormycosis



- All specimens: direct examination and culture. (Eg sputum)
- In case of pulmonary involvement
 - If sputum smear analysis is negative:
 - broncho-alveolar lavage or
 - pulmonary biopsies

Clinical Microbiology and Infection, Volume 20 Supplement 3, April 2014

Ultrasound guided biopsy



Biopsy- gold standard

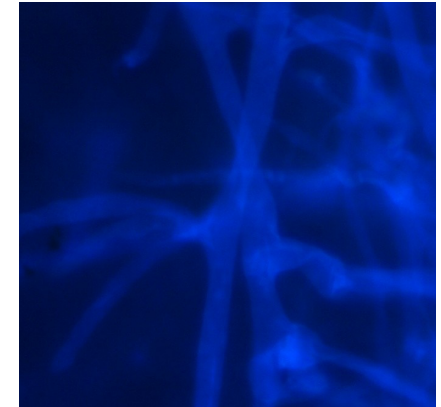
Specimen in NaCl:

- Microscopy (Blankophor or Calcofluor)
 - Mucorales hyphae
 - variable width, non-septate or pauci-septate irregular, ribbon-like appearance.
 - Variable angle of branching, includes wide-angle (90°) bifurcations
 - *Aspergillus* hyphae
 - Septate, angle 45°
 - impossible to distinguish from those of *Scedosporium spp* and *Fusarium spp*
- Culture
- PCR
 - ITS+ sequencing
 - *Aspergillus* PCR

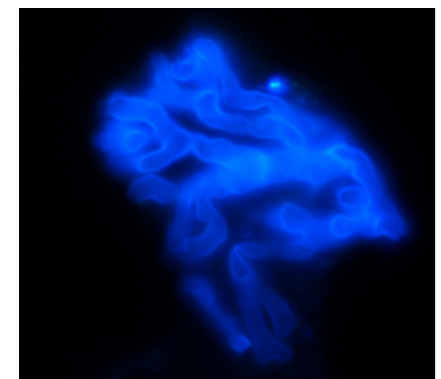
Specimen in formalin:

- histopathology

Rhizopus



A. fumigatus

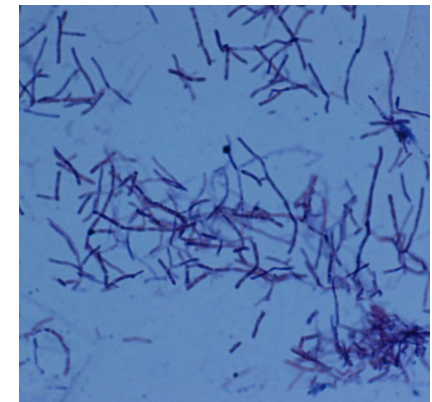


Often small samples *

But still we want to perform

- Microscopy
- Culture
- Molecular diagnostics
- And rule out
 - Bacteria
 - Mould
 - Cryptococcus
 - PCP
 - Nocardia
 - Legionella
 - TBC

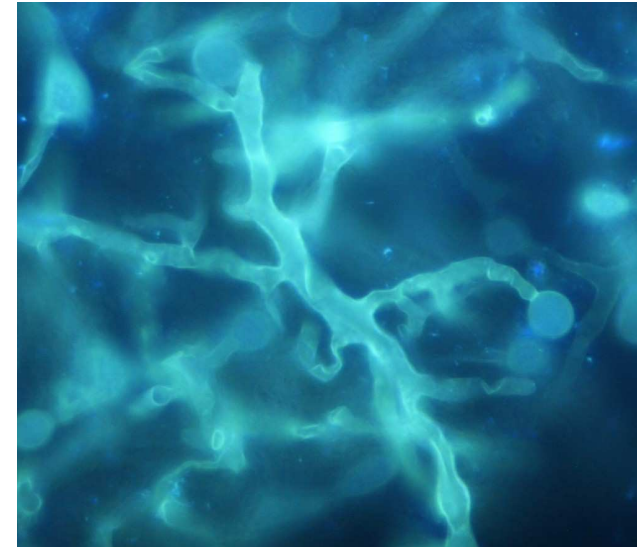
*endoscopic, computed tomography (CT)-guided: fine needle



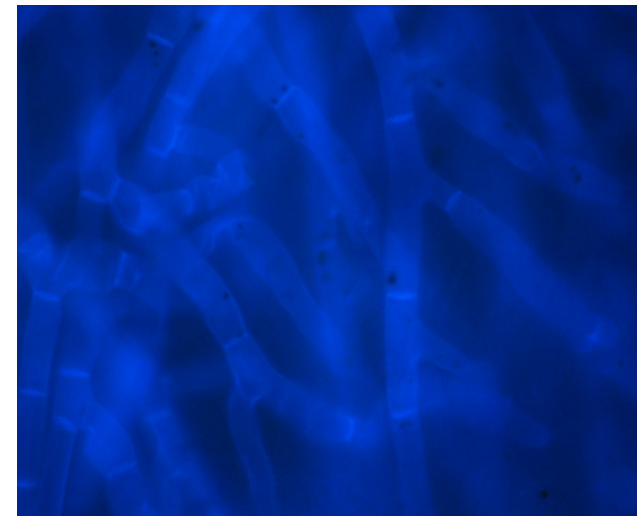
Nocardia sp

BAL fluid

- Microscopy
- Culture
 - may be falsely negative
- PCR
- PCP
 - Aspergillus
 - ITS
- TBC/Nocardia/Legionella



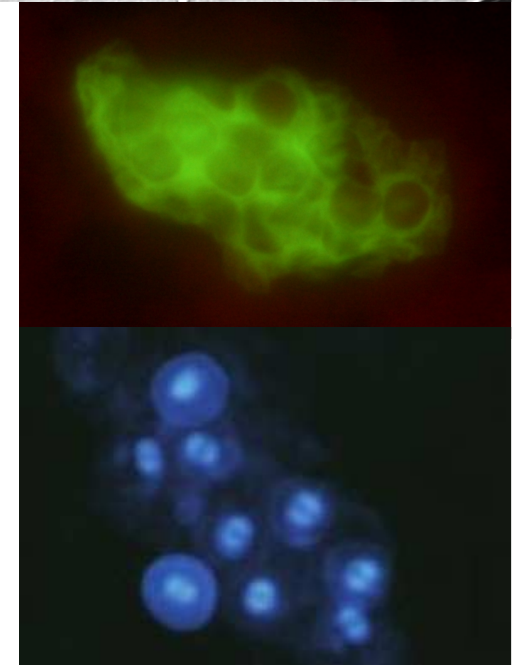
Rhizopus microsporus



Aspergillus fumigatus


Pneumocystis jirovecii

- PCR
 - Semi-quantitative
 - Induced sputum
 - BAL
 - Lung biopsy
- IF microscopy
- Calcofluorwhite microscopy
- Toluidin blue
- Gomori's methenamin-silver
- Beta 1,3-D-glucan is usually raised in blood and can assist with diagnosis



Microbiological tests

- Essential role in the investigation of infectious respiratory diseases
 - caused by viruses, bacteria, fungi or parasites.
- Knowing which fungus is involved guides appropriate antifungal choice, dose and duration of therapy



Thank you for your attention!