Aspergillus
Still the same as before?

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Description of the genus

- *Ubiquitous filamentous fungi largely spread in the environment*
- *Many species described*

The species *Aspergillus fumigatus* was described in 1863 by Johann Baptist Georg Wolfgang Fresenius

1965. Rapper and Fennel. Morphological.

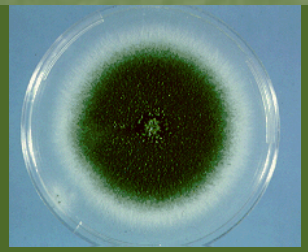
132 species, 18 groups

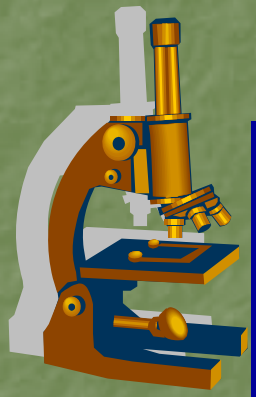
1996-2004 Biochimical+/_morphological: lot of papers, books

Aspergillus

Identification at the species level

- MACROSCOPIE + MICROSCOPIE
  - based on colour, shape, ornementation
  - sexual or asexual stages recognition





- The extend of species responsible of invasive aspergillosis is underestimated
- New species, less susceptible is responsible for invasive aspergillosis

- *1999- molecular identification*

Molecular studies reveal frequent misidentification of *Aspergillus fumigatus* by microscopy.
Balajee SA, Nickle D, Varga J, Marr KA.
Eukaryot Cell. 2006 Oct;5(10):1705-12

- *Study*. Genetic diversity of 50 isolates of *A. fumigatus + in vitro* sensitivity to antifugnal drugs used in IA
- *Method*
  - 50 isolates of *A. fumigatus (phenotypic identification)*
  - RFLP
- *Results*
  - 34 *A. fumigatus,* 16 *A . non fumigatus*
    - 3 distinct species: *A. fumigatus + A. lentulus (T°<50°C), A. udagawae*
  - *In vitro* susceptibilities
    - *A. lentulus*: decreased sensitivity to AmB, VOR, Caspo
    - *A. udagawae*: decreased sensitivity to AmB, VOR
- *Conclusion* differential antifungal susceptibilities may account for some of the reported poor outcome of therapy in clinical studies

- Do we have to test the *in vitro* sensitivity of each clinical *Aspergillus* isolate?

Clinically relevant Aspergilli

- 7 « subgenera » divided into sections (species)
  - Aspergillus _section *Aspergillus* (21), *Restrictus* (3)
  - Fumigati _
    - section *Fumigati (16)*: *A. fumigatus, A. lentulus, N. fischeri, N. pseudofischeri, N. spinosa*
    - *Section Cervini* (5)
  - Ornati _section *Ornati (7)*
  - Clavati _section *Clavati* (4)
  - Nidulantes _section *Nidulantes* (35)_*Versicolores* (24)-, *Usti* (5), *Terrei* (1-3), *Flavipes* (4)
  - Circumdati 7 sections
  - Stilbothamnium 5 sections
  - Ochraceoroseus 2 sections

Fumigati section: other pathogenic species

Osteomyelitis caused by *Neosartorya pseudofischeri*

Padhye AA, Godfrey JH, Chandler FW, Peterson SW..

J Clin Microbiol. 1994 Nov;32(11):2832-6.

First description of the new species. Optical and electronic microscopy and DNA hybridization methods were used as identificaiton methods.

Isolation of *Neosartorya pseudofischeri* from blood: first hint of pulmonary aspergillosis

Järv H, Lehtmaa J, Summerbell RC, Hoekstra ES, Samson RA, Naaber P.

J Clin Microbiol. 2004 Feb;42(2):925-8.

17-year-old male patient with Hodgkin's disease,
Fever of 38.6°C, and a nonproductive cough.
Isolation in blood culture Bactec 9050 (BD) in the fungal blood culture medium

Alternative: MASS-SPECTROMETRY

MALDI-TOF Mass Spectrometry for fast and accurate identification of clinically relevant Aspergillus species.

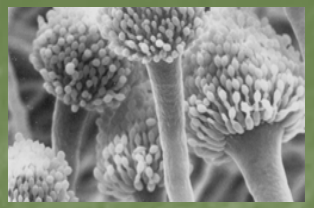
Alanio A. Peretti JL, Dauphin B., Mellado E., Ouesne G., Lacroix C., Amara A., Berche P., Nassit X., Bougnoux ME.

Department of Microbiology, Hôpital Necker-Enfants Malades, Paris, France; Université Paris Descartes, Paris, France.

Clin Microbiol Infect. 2010 Jul 29. [Epub ahead of print]

- Method.
  - 124 clinical
  - 16 environmental isolates
- Characterised by partial sequencing of the beta-tubulin and calmodulin genes
- Methodology: engineering of Maldi-tof MS data base with reference strains
- No extraction: water+ DHB matrix solution (di-hydroxybenzoique acid)
- Results
  - Identification performed in 10 minutes
  - 98.6% correctly identified (2 could not be identified)
  - 100% specificity
- Conclusion: rapid methodology that replaces avantageously phenotypic identification. It is less time consuming and must cheaper than sequencing.

Neosartorya fumigata



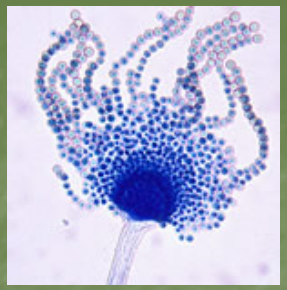
- *Anamorphic filamentous organisms which reproduce by means of asexual spores*
- *Sexual forms of many aspergillus have been described*

« Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus* »

O'Gorman C. et al. Nature. 2009 Jan 22;457(7228):471-4

Sequencing of the whole genome

Nature. 2005 Dec 22;438(7071):1151-6.
Genomic sequence of the pathogenic and allergenic filamentous fungus Aspergillus fumigatus.
Nierman WC et al. , Nature. 2005 Dec 22;438(7071):1151-6.



Clinical aspects

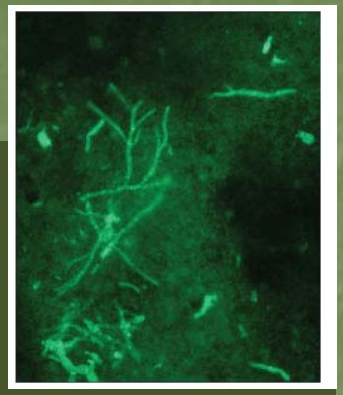
- Main portal of entry: lungs and sinusis
- Disease
  - Allergic (ABPA)+ allergic pulmonary alveolar diseases
  - Colonisation of air spaces such as fungus balls of the lungs and sinusis, endobronchial colonisation or association with EAR pathology.
  - Invasive forms↔ immunosuppression
    - Predisposing factors: neutropenia, long-term steroid therapy, lymphoma, diabetes, burns, alcoholism, the neonatal state, prior tuberculosis, immunosuppressive therapy, trauma, liver failure, operative procedures
    - Recently: NON CLASSICAL TYPE of at risk patients:
      - Particularly in intensive care units ,
        - patients **with chronic lung diseases or influenza**
        - Patients with **short courses of steroids**
      - **AIDS patients**: up to 9% of patients with progressing diesase may develop this complicaton (mortality of 90%)

Aspergillosis in the ICU: the new 21st century problem?

Koenraad H. et al. , Medical mycology, september 2006, 44:S71-S76

- Other category of population without apparent severe immunodeficiency:
  - patients with critical illness
- Data are scarce
- Incidence of IA ranges from 0.3 to 5.8%, mortality rate 80%.
- Predisposing factors: Malnutrition, diabetes mellitus, pulmonary disorder, liver cirrhosis and corticoid use
  - Corticoids are used in persistent septic shock (benefic effect)
  - Patients with underlying disease: corticoids are a risk factor for IA
- Difficulty in establishing diagnostic: which tests can be used?
  - Culture: first clue: disease/colonisation/ marker for disease?
  - IMPORTANT: direct positive examination
  - Tissue sampling: not easy in ICU patients
  - Serological markers:
    - GM, beta glucan : few data
    - Antibodies: no data
  - PCR: Could be applied in the blood. No data in ICU
  - Radiological findings: not specific: atypical infiltrates++
- Mortality high because: delay in the diagnosis.

Still diagnostic problems for invasive aspergillosis?



- Clinical
- Radiological
- Mycological
  - Culture + calcofluor (hyphae)
  - Antigens detection:
    - Galactomannan: released during hyphae growth
    - EIA: Platelia® Aspergillus, Biorad
    - Index: 1.5 $\Rightarrow$0.5 (Maertens J.)
      - sensitivity 92% specificity 95%
      - Meta-analysis: reports less good results 64% sensitivity/93% spcecificity
      - Better results in neutropenic patients
      - GM: « the » IA diagnostic test
    - Where? serum, bronchoalveolar lavage (Meersseman et al.), CSF

Galactomannan in bronchoalveolar lavage fluid: a tool for diagnosing aspergillosis in intensive care unit patients.

Meersseman W, Lagrou K, Maertens J, Wilmer A, Hermans G, Vanderschueren S, Spriet I, Verbeken E, Van Wijngaerden E.

Am J Respir Crit Care Med. 2008 Jan 1;177(1):27-34. Epub 2007 Sep 20.

- **Population**: host factors: Haematologic malignancies+less immunosuppressed patients (short course of steroids during or before admission), HIV, solid organ transplant recipients, immunosuppressive treatment. 78% non neutropenic patients.
- **Method**: 18 months study+clinical features sugestive of fungal infection
  Comparison of GM in BAL (0.5 index), GM in serum, CT-scan.
- **Results: 26 proven IA:**
  - **GM in serum**
    - **Sensitivity: 42%**
    - **Median value of GM in serum : 0,3**
    - **14/26 negative when GM positive in BAL(>50%)**
  - **GM in BAL**
    - **Sensitivity: 88%**
    - **Specificity: 87%**
    - **11/26: GM in BAL unique positive test (culture+GM in serum: NEGATIVE)**
  - False positive tests?
    - 13% of the patients in the truly negative group
  - Any difference between patients with or without neutropenia?
    - GM on BAL performed equally in patietns with or without neutropenia
    - GM in serum performed **better** in patients with neutropenia
  - **Conclusion: very promising. Has to be confirmed in other ICU settings.**

A prospective comparison of galactomannan in bronchoalveolar lavage fluid for the diagnosis of pulmonary invasive aspergillosis in medical patients under intensive care: comparions with the diagnostic performance of galactomannan and of (1→3)β-D-glucan chromogenic assay in serum samples

Acosta J, Catalan M, Del Palacio-Peréz-Medel A, Lora D, Montejo JC, Cuetara MS, Moragues MD, Ponton J, Del Palacio A.

Clin Microbiol Infect. 2010 Sep 3. [Epub ahead of print

- GM on BAL performs better than on serum
- GM performs better than β-D-glucan on BAL and serum
- Conclusion: GM in BAL improved the diagnosis of IA in critical ill patients

BAL fluid GM for the diagnosis of invasive pulmonary aspergillosis inpatients with haematologic diseases *Maertens J. et al., CID, 2009, 49/1688-1694*

Index ≥1 sensitivity 96% specificity 87%

β(1-3) – D glucan detection
Do we have to use this test?

- **Utility of Galactomannan Enzyme Immunoassay and (1,3) β-D-Glucan in Diagnosis of Invasive Fungal Infections: Low Sensitivity for *Aspergillus fumigatus* Infection in Hematologic Malignancy Patients**

  R. Y. Hachem,* D. P. Kontoyiannis, R. F. Chemaly, Y. Jiang, R. Reitzel, and I. Raad

Prospective study in neutropenic patients

Beter sensitivity for BG (67%) vs GM (38) (sampling only once a week)

Better sensitivity for GM for detecting non-*A. fumigatus* infection. No difference for BG between A. fumigatus and non-fumigatus infection.

Conclusion: BG greater sensitivity than GM in detecting IA??

different reactivity in GM production depending on the species

suggests the interest of using both tests for IFI diagnosis?

EORTC/MSG

- Revised definitions of invasive fungal disease from the European Organisation for Research and Treatment of Cancer/invasive fungal infections Cooperative group and the national institue of allergy and infectious diseases mycoses study group (EORTC/MSG) Consensus Group

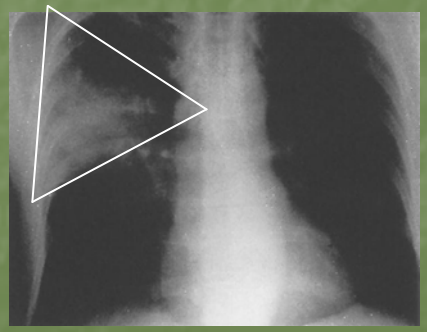
  Ben De Pauw et al., EORTC/MSG consensus group, Clin Infect Dis 2008 46 (12):1813-21

- 2002. EORTC/MSG :standard definitions for invasive fungal infections for clinical and epidemiological research.
  - 3 level of probability to the diagnosis of IFA in immunocompromised patients with cancer and in hematopoietic stem cell transplant patients: possible/probable/proven invasive infection.
- Revision process started in 2003, approved in 2005
- What has been changed?
  - *Definitions possible/probable/proven*
    - *Probable expanded*
    - *Possible diminished*
    - *Proven : can be applied to patients that are not immunocompromised*

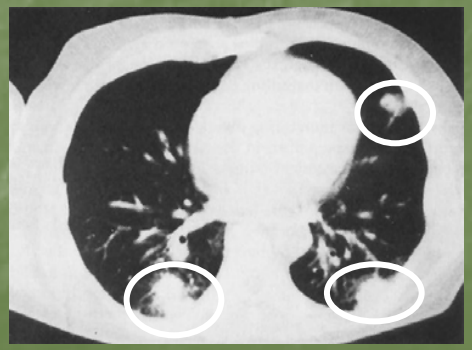
EORTC/MSG: which changes?

- « Invasive fungal infection » disease was adopted to reflect an infectious disease process
- « Possible invasive fungal infection » too many dubious cases were included
  - Only cases that are highly likely to be caused by a fungal etiology even without evidence of mycological evidence
- Populations: too restrictive→ extended to:
  - Solid organ transplant, primary immunodeficiencies, connective tissue disorders, receipt of immunosuppressive agents (corticosteroids, T-Cell immunosuppressants)

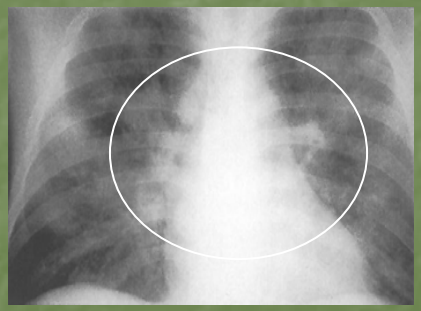
- Host factors: Minor or major clinical criteria: ABANDONNED in favor of more characteristic and objectively verifiable evidence
  - findings of medical imaging with **standardized glossary of definitions**
    - **Ex. Patients with IPA: focal rather than diffuse pulmonary infiltrates**



dense wedge-shaped infiltrate in the right upper lobe.



CT scan of the chest showing **multiple peripheral nodules** in IPA



Diffuse pulmonary infiltrates in a bone marrow transplant recipient due to IPA

EORTC/MSG: which changes?

- « Probable and proven » integration of more indirects tests in the definitions
  - Thresholds recommended by the manufacturer
  - Platelia *Aspergillus* galactomannan EIA can be applied to the CSF, serum or plasma and BAL.
  - β-D-glucan (Fungitell®) included in the mycological tests for diagnosing IFD except cryptococcosis and zygomycosis
  - ~~PCR methods~~

PCR or not?

- DNA comes from breakdown of hyphae
- Specific or panfungal?
- First on BAL: colonisation or infection? Quantification can not resolve the problem
- In serum or plasma or blood? Previously no consensus. Short series. Case reports.
- No commercial valuable *Aspergillus* PCR
- Many scientists do not rely on PCR for *Aspergillus* detection

PCR: international group of standardisation

Aspergillus PCR: one step closer to standardization.

White PL, Bretagne S, Klingspor L, Melchers WJ, McCulloch E, Schulz B, Finnstrom N, Mengoli C, Barnes RA, Donnelly JP, Loeffler J; European Aspergillus PCR Initiative.

J Clin Microbiol. 2010 Apr;48(4):1231-40. Epub 2010 Feb 10.

Sampling: 3 ml

Standardisation of DNA Extraction method

Validation of the different target used

Creation of a « quality control » for laboratories resecting the desigh of the study.

Treatment: evolution…

- AmB⇒lipid formulations
- ⇒new triazole=Voriconazole: first line therapy with AmB or Caspofungin as 2d line treatment
- ⇒ Combination therapy?? Still controversial, no consensus
- Posaconazole: treatment and prophylaxy

Comparative survival and cost of antifungal therapy: posaconazole versus standard antifungals in the treatment of refractory invasive aspergillosis.
Herbrecht R, Rajagopalan S, Danna R, Papadopoulos G.

- Curr Med Res Opin. 2010 Oct;26(10):2457-64.

Conclusion. Survival benefit and reduced total drug cost in treatment of probable or proven refractory IA

« le » site à connaître

- www.aspergillus.man.ac.uk
- Inscription gratuite
- Accès à de nombreux articles

Conclusion

- Still the same? Not really
  - Sophistication of the identification methods
  - New species
  - Niew species involved in pathology
  - *In vitro* sensitivities have to be tested
  - PCR methods : start of standardisation methods
  - See you in 2020…

Thanks to the sponsors

- Pfizer
- Merck Sharp & Dohme
- Biorad

Thanks to Danielle