Molecular techniques versus MALDI-TOF mass spectrometry in fungal diseases: are they complementary or redundant?

Jean-Pierre GANGNEUX¹, Marie-Elisabeth BOUGNOUX²
1. Rennes Teaching Hospital and INSERM U 1085 – IRSET, Rennes, FR
2. Paris-Necker Teaching Hospital and Institut Pasteur, Paris, FR
21st century: the challenge of fungal biodiversity

Aspergillus fumigatus

Levures
Usual diagnostic tools for fungal infections

- Microscopic examination and culture
- Histology
- Immunology: soluble markers
- Molecular biology
- Mass spectrometry

Are they complementary or redundant?
Limitations of the mycological diagnosis

1. Time to get the final results: Yeasts

- **24 h**: Yeast colonies on a petri dish
- **24-48 h**: Chromogenic media for identification
- **24-48 h**: Agglutination tests
- **3 to 5 days**: Identification of specific yeast species (C. albicans, C. tropicalis, C. krusei, C. kefyr)
Limitations of the mycological diagnosis

1. Time to get the final results: filamentous fungi
   - 5 to 7 days
   - Until 21 days for dermatophytes

2. Mycology and morphology allow only limited discrimination between newly described species and between isolates
3. In tissues, what does mean “hyphae or pseudohyphae”?

Morphology, description, diagnosis, and comment for fungal infections that present with hyphae or pseudohyphae in tissues

Delivering the Empirical Treatment of *Candida* Bloodstream Infection until Positive Blood Culture Results Are Obtained: A Potential Risk Factor for Hospital Mortality

Matthew Morrell,1 Victoria J. Fraser,2 and Marin H. Kollef *

Pulmonary and Critical Care Division1 and Division of Infectious Diseases,2 Washington University School of Medicine, St. Louis, Missouri 63110

Treatment-related risk factors for hospital mortality in *Candida* bloodstream infections

Andrew J. Labelle, MD; Scott T. Micek, PharmD; Nargh Reubinian, MD; Marin H. Kollef, MD

Table 4. Multivariate analysis of risk factors for hospital mortality

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Hospital Cohort</th>
<th>Intensive Care Unit Cohort</th>
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<tbody>
<tr>
<td></td>
<td>Adjusted Odds Ratio</td>
<td>95% Confidence Interval</td>
</tr>
<tr>
<td>APACHE II score (4 point increments)</td>
<td>1.18</td>
<td>1.11-1.25</td>
</tr>
<tr>
<td>Central venous catheter retention</td>
<td>4.85</td>
<td>2.54-9.29</td>
</tr>
<tr>
<td>Corticosteral use</td>
<td>3.41</td>
<td>1.96-5.93</td>
</tr>
<tr>
<td>Inappropriate initial fluconazole dosing</td>
<td>3.31</td>
<td>1.83-6.00</td>
</tr>
</tbody>
</table>
Time of culture
24-------------------------48 h

Identification
24-------------------------48 h

In vitro sensibility

Alternatives:
- Soluble markers of the infection (GM, Mannan, β-glucans)
- DNA detection in samples

Alternatives:
- Molecular identification
- Mass spectrometry
- PNA-FISH
1. Circulating DNA in blood
- Marketed solutions: Septifast and Luminex systems

No amplification:
⇒ Other Candida species, Cryptococcus, Trichosporon, Geotrichum, Saccharomyces, Malassezia
⇒ Filamentous fungi: Fusarium

Candida species identified

6

7
- In-house solutions

- Difficulty to design « pan-fungal » assays

- Targeted PCR: the example of *Aspergillus* PCR
  ⇒ absence of standardisation
  ⇒ total blood? serum? Plasma?
  ⇒ extraction method? Volume?
  ⇒ 1 or 2 determinations/week
  ⇒ infection/colonisation
  ⇒ criteria not validated by the EORTC/MSG 2008 guidelines

⇒ However, multiple initiatives to standardize the assay
2. DNA detection from other fluids and tissues

- BAL, biopsies etc…
- precise molecular dentification
- molecular markers of virulence and antifungal resistance

Comparison of an Aspergillus real-time polymerase chain reaction assay with galactomannan testing of bronchoalvelolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in lung transplant recipients. Luong ML, et al.

=> Sensitivity PCR (100%) > sensitivity GM (85%)
MALDI-TOF mass spectrometry
A fast, simple, robust and flexible access to the identification of numerous fungi

A revolution in the management of fungal infections

ESCMID Online Lecture Library © by Author
C. albicans: 24 h

DNA sequencing
Identification 24 h

MALDI-TOF-MS
Identification ¼ h

C. non albicans: 72 h

MALDI-TOF-MS
Identification ¼ h

Positive blood culture

Biochemistry
Molecular identification 48-72 h

Lamoth, JCM 2010; Lau, JCM 2008; Ferroni, JCM 2010
Phylogenetic tree (ITS1)

Species complex

- **C. parapsilosis**
- **C. orthopsilosis**
- **C. metapsilosis**

- **C. guilliermondii**
- **C. fermentati**

- **C. glabrata**
- **C. nivariensis**
- **C. bracarensis**

- **C. rugosa**
- **C. pseudorugosa**

- **C. haemulonii**
- **C. pseudohaemulonii**

Tsui C. et coll.,
FEMS Yeast res, 2008
Molecular identification and MT-MS are complementary to update databases

⇒ but MT-MS is far more rapid
⇒ cost saving regarding lab technicians
⇒ cost saving regarding treatments
Prospective multicenter study of the epidemiology, molecular identification, and antifungal susceptibility of *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* isolated from patients with candidemia.


Dec 2011, n = 364

*C. glabrata* cryptic species

*C. parapsilosis* cryptic species

Extraction: 1-2 h
Multiplex PCR: 1-2 h
Specific PCR: 1-2 h
Agarose gel: ½ h
Enzymatic digestion: 2 h
Agarose gel: ½ h

March 2011, n = 691

=> Tedious protocol
The particular situation for filamentous fungi:
The examples of Aspergillus and Scedosporium.

- **Genera**: Aspergillus
  - Section
    - Fumigati
    - Flavi
    - Nigri
    - Terrei
    - Nidulantes
  - Species
    - A. fumigatus
    - A. flavus
    - A. niger
    - A. terreus
    - A. nidulans

*Hong et al., 2008*
Molecular identification of *Aspergillus fumigatus*

**Genera**
- Aspergillus
  - Section
    - Fumigati
      - Flavi
      - Nigri
      - Terrei
      - Nidulantes

35 species

*Hong et al., 2008*
Molecular identification of *Aspergillus*

**Genera**
- *Fumigati*
- *Flavi*
- *Nigri*
- *Terrei*
- *Nidulantes*

**Section**
- **35 species**
  - *A. fumigatus*
  - *A. fumigatiaffinis*
  - *A. viridinutans*
  - *N. udagawae*
  - *N. pseudofischeri*

*Aspergillus*

Hong et al., 2008
Molecular identification of *Scedosporium*

Based on the sequencing of 3 loci: ITS, β-tubuline, and calmoduline.

Felix Gilgado et coll. JCM 2005, JCM 2008
Identification of *Aspergillus* and *Scedosporium* by MT-MS

1st quick automated and standardized technique for *Aspergillus* and *Scedosporium* identification:

→ precise identification of species
  – including frequent and rare species
  – discrimination of species with similar morphology
→ simple and quick experimental protocol: few min *versus* few days
→ databases easily and regularly updated

*Aspergillus* = 28 species

- **Common *Aspergillus* species (n=6)**: *A. fumigatus, A. flavus, A. terreus, A. niger, A. nidulans, A. versicolor*


*Scedosporium* = 6 species

*S. prolifigans, S. apiospermum, S. aurantiacum, S. dehoogii, P. boydi, P. minutispora*
Dramatic improvement of the patient management
→ differentiation of environmental and pathogenic species for humans
→ Epidemiological studies on the impact of biodiversity on clinical presentations
→ appropriate antifungal therapy
Concordance between MT-MS and molecular identification for *Aspergillus* and *Scedosporium*

134/136 (98.4%) for *Aspergillus* sp.
61/61 for *Scedosporium* sp.

Alanio A et coll. CMI 2010
Bougnoux ME et coll. 2nd meeting ECMM/ISHAM Fri-CF, 2011
Rapid results = cost savings

High cost of the management of IFI (Hematology settings-France)
- Invasive candidiasis: 35,000
- Invasive aspergillosis: 50,000 €

Rapid adaptation of the antifungal therapy to the species
= Prescription of an efficient drug in case of fluconazole-resistant yeast or voriconazole-resistant Aspergillus

⇒ decreased mortality
⇒ cost-effectiveness strategies: Ex. de-escalation in case of sensitivity
  - Fluconazole ≈ 10 €
  - Echinocandins ≈ 400 €

Michallet, Gangneux, Lafuma et al, J Med Econ 2011
**A significant potential cost saving**

=> empiric treatment: 1 day for 1 patient = 400 €

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CMI 90 (microg/mL)

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<tr>
<th></th>
<th>Fluconazole</th>
<th>Amphotericin B</th>
<th>Caspofungine</th>
<th>Anidulafungine</th>
<th>Micafungine</th>
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<tr>
<td><strong>C. Nivariensis</strong></td>
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<td>1</td>
<td>0.06</td>
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<td><strong>C. Bracarensis (1)</strong></td>
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<td><strong>C. Bracarensis (2)</strong></td>
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<td>1</td>
<td>0.03</td>
<td>0.06</td>
<td>0.015</td>
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=> Biodiversity impacts on antifungal sensitivity

Amarcand study: French prospective multicentric study, 300 episodes of invasive candidiasis in intensive care units.

=> Low sensitivity of *Candida glabrata* isolates to fluconazole, particularly in case of pre-exposure to fluconazole.

![](image)

Leroy, Gangneux, Mira et al, Crit Care Med 2009
Perspectives: biomarkers of antifungal resistance

= Prescription of an efficient drug in case of fluconazole-resistant yeast or voriconazole-resistant Aspergillus
Complementarity of MT-MS and molecular tools: For the management and the Treatment of iFl

-PCR on samples is standardized and helpful, except in blood
-Maldi-Tof MS is powerful for identification: faster than molecular identification and concordant with molecular databases
-DNA sequencing remains the gold standard for taxonomy and epidemiological studies thanks to its high discrimination power

-Maldi-Tof MS: a potential to extend applications?
  - identification of antifungal resistance,
  - identification of virulence markers,
  - discrimination between isolates/amplicons
  - SNP genotyping
  - etc...

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<th>Cost savings</th>
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<td>Lab tech</td>
<td>Treament</td>
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Thank you for your attention

Jean-pierre.gangneux@chu-rennes.fr
mbougnoux6@gmail.com

Necker Enfants Malades Hospital, Assistance Publique-Hôpitaux de Paris