Educational Workshop

EW06: New and emerging topics in medical mycology
arranged with EFISG
(ESCMID Fungal Infection Study Group)

Convenors: Malcolm Richardson (Helsinki, FI)
Manuel Cuenca-Estrella (Madrid, ES)

Faculty: Cornelia Lass-Flörl (Innsbruck, AT)
Peter Donnelly (Nijmegen, NL)
Manuel Cuenca-Estrella (Madrid, ES)
Andrew Ullmann (Mainz, DE)
David Denning (Manchester, UK)
Aspergillus species, Candida species, Zygomycetes and other molds are responsible for more than 90% of invasive fungal infections in immunocompromised patients (Neofytos D et al., 2009).

Microscopy and culture are the most heavily relied diagnostic techniques of today! (Aydi et al., 2007).

Yet, the conventional diagnostics fail often OR are too slow OR require invasive procedures! (Chandrasekar, Leukemia & Lymphoma 2009)
Lass-Flörl – Conventional diagnosis of invasive mycosis

Diagnosis of an IFI...what could we detect?

<table>
<thead>
<tr>
<th>Fungal Factors:</th>
<th>Host Factors:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Viable fungus</td>
<td>1. Antibodies</td>
</tr>
<tr>
<td>-culture</td>
<td></td>
</tr>
<tr>
<td>2. Fungal elements</td>
<td>2. Elements of host response</td>
</tr>
<tr>
<td>-direct exam</td>
<td></td>
</tr>
<tr>
<td>3. Fungal antigens/secreted molecules</td>
<td></td>
</tr>
<tr>
<td>-cell wall fragments</td>
<td></td>
</tr>
<tr>
<td>-secreted proteins</td>
<td></td>
</tr>
<tr>
<td>4. Nucleic Acids</td>
<td></td>
</tr>
<tr>
<td>-DNA/RNA</td>
<td></td>
</tr>
</tbody>
</table>

Culture—the conventional diagnostic tool

1. Low Sensitivity for IFIs
   -Candidiasis = 50-60% organisms cleared from blood through both antibody and non-antibody dependent receptor-ligand interactions
   Rand, K.H. et al Mol cell probes 1994: ~25% of Candida positive blood cultures have <1 cfu/mL and ~50% have < 10 cfu/mL
   -Aspergillosis ≤ 1% (blood cultures)
   -Aspergillosis 60% (respiratory tract cultures)
   -Patient on anti-fungal prophylaxis or antifungal treatment effect on blood samples...
2. Optimal sample often not obtainable
3. Grow slowly

Secreted/shed fungal molecules—an overview

Tests in use that have made a significant difference:
--Cryptococcal Antigen Test (Glucuronoxylomannan)
--Histoplasma Urine Antigen (Galactomannoprotein)

Tests in use that have made a valuable input:
--Galactomannan EIA (for diagnosis of Aspergillus)
--Beta-Glucan Test
--Mannan Test (Candida)

Tests on the horizon...
--Secreted proteins of Aspergillus fumigatus and other fungi

Alexander, 2008
Conventional diagnosis of invasive mycosis.
Anything new on CULTURES?

Candidemia in ICUs
Blood cultures – what can we learn?

- More than two thirds of patients with invasive candidiasis in ICU present with candidemia.
- Non-albicans Candida species reach almost half of the Candida isolates.
- Reduced susceptibility to fluconazole is observed in 17.1% of Candida isolates.
- Mortality of invasive candidiasis in ICU remains high.

Leroy O et al., 2009
Is there a need for anaerobic blood cultures to detect C. glabrata?

22.7% (5 isolates of 22) were detected only by anaerobic bottles!
(Hui et al., JMM, 2009)

<table>
<thead>
<tr>
<th>Bottle type</th>
<th>BacT/ALERT 100</th>
<th>BacT/ALERT 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic bottles (3), without bacterial overlay</td>
<td>22.7±1.9</td>
<td>22.2±1.9</td>
</tr>
<tr>
<td>Anaerobic bottles (3), without bacterial overlay</td>
<td>33.5±4.4</td>
<td>37.7±4.4</td>
</tr>
<tr>
<td>Falsenbroth with bacterial overlay (3)</td>
<td>43.1±5.0</td>
<td>33.2±3.7</td>
</tr>
</tbody>
</table>

Aspergillus alabamensis, a new clinically relevant species in the section Terrei
(Balajee et al., 2009)

...most isolates were recovered as colonizing isolates....

Calcofluor White staining of lung biopsies is helpful in differentiating Aspergillus vs Zygomycetes!
(Biopsies n=70
 Cultures were positive in only 21 samples
 Microscopic examinations in 82 samples
 (Lass-Flörl et al., 2009)
Lass-Flörl – Conventional diagnosis of invasive mycosis

<table>
<thead>
<tr>
<th>Positive predictive value for invasive zygomycoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Solid organ transplant pts</td>
</tr>
<tr>
<td>• Hematological malignancies</td>
</tr>
<tr>
<td>• Neutropenia</td>
</tr>
<tr>
<td>• Malnutrition</td>
</tr>
<tr>
<td>• Surgery</td>
</tr>
<tr>
<td>• Trauma</td>
</tr>
<tr>
<td>• Previous antifungal treatment</td>
</tr>
</tbody>
</table>

Torres-Narbona et al., 2008

Conventional diagnosis of invasive mycosis.

Anything new on SEROLOGY?

Some discrepancies in GM testing........

Despite the regular use of galactomannan antigen detections and imaging, an ante mortem diagnosis of invasive fungal disease could only be established in 4 of 10 autopsy-verified cases.

In the remaining 6 patients, deep mycoses were missed clinically and were revealed only by postmortem histology.

The issues with Galactomannan (GM) Assay (Bio-Rad Platelia®)

- Sensitivity lower in patients on anti-fungal therapy and in less susceptible patients
- Negative test can't rule-out IA (prophylaxis)
- False positive tests with some chemotherapy drugs and cGvHD
- False positive tests in patients on piperacillin-tazobactam
- Other fungal organisms contain an apparent cross-reacting molecules. Examples: Penicillium, Alternaria, and Paecilomyces (Huang et al., 2007)

Good correlation of GM ELISA in BAL and IA

Sensitivity was 81.8% in patients with aspergillosis, and specificity was 95.8% in lung transplant patients who underwent BAL for surveillance. (Husain et al, 2008)

Using a cutoff index of 0.5, the sensitivity and specificity of GM detection in BAL fluid was 88 and 87%, in non-neutropenic patients. (Meersseman, 2008)
Using a cutoff index of > 1, the positive predictive value was 100% (Meerssemann, 2008)

Good correlation of a positive single serum GM ELISA and isolation of Aspergillus species in non-neutropenic patients

When serially monitored (2-3 times/week), GM preceded conventional diagnosis by around 1 week and correlates with survival in neutropenic patients (Maertens, 2009)

Sensitivity may be better if BAL fluid used (Nguyen, 2008)

Galactomannan (GM) Assay (Bio-Rad Platelia®)

Cell Wall Galactomannan and β-1,3-Glucan

- Detects a number of different fungi, but no idea of which one. (Acremonium, Aspergillus, Coccidioides immitis, Fusarium, Histoplasma capsulatum, Pneumocystis jirovecii, Saccharomyces cerevisiae, Sporothrix schenckii, and Trichosporon) (Chandrasekar et al., 2009)
- Does not detect Cryptococcus, Zygomycetes (Pazos 2005)
- In head-to-head with GM, was less sensitive, less specific and detected later (Kawazu, et al. J Clin Micro. 2004)
- Using a cut-off value of 60pg/ml, sens., spec., PPV and NPV were 85%, 95%, 97% and 99% (Obayashi T et al., 2008)
- Big specificity problems, especially in an ICU or surgical setting, many medical products contain glucan (Chandrasekar et al., 2009)
- Assay technically difficult (need glucan free tubes)
Conventional diagnosis of invasive mycosis

Long-term ICU pts: elevated (1,3)-b-D-glucan also due to serious underlying diseases. Persistently high levels may be indicative of IFI (Presterl E et al., 2009).

The future?

Yeast Infections
Self-Test
for a Candida Infection

1. Do you feel tired most of the time, or have muscle aches with a cold?
2. Do you suffer from mouth ulcers, reddening of the vulva, or intertrigo?
3. Do you have vaginal or vulvar itching, pain, or other sexual discomforts?
4. Have you ever found white or yellow, thick or slimy discharge?

Conclusion

- Cultures are necessary tools to specify the fungus (new species) and to perform susceptibility testing.
- Serology is very helpful, especially GM testing from BALs. In neutropenic and non-neutropenic patients, use a cut-off value > 0.5.
- Beta-glucan seems to be of value in cases with persistently high (> 3 samples) levels, cutoff value 40 pg/ml.
PCR: A useful tool for the diagnosis of aspergillosis

J Peter Donnelly BSc FIBMS MIBiol PhD
Department of Haematology & Nijmegen University Centre for Infectious Diseases
University Hospital St Radboud Radboud University Nijmegen The Netherlands

EORTC IFCC & NIAID-MSG
Defining Opportunistic Invasive Fungal Infections in Immunocompromised Patients with Cancer and Hematopoietic Stem Cell Transplants: An International Consensus

Clinical Infections Diseases 2002:34:14

Definitions II - Mycology

- Culture of mould from BAL or sputum
- Mould seen in sinus aspirate
- PCR to detect nucleic acid
- Antigen in blood, BAL, CSF
- Would seen in BAL

Mycology
Donnelly – PCR diagnosis of aspergillosis

**Definitions II - Mycology**

- Culture of mould from BAL or sputum
- Mould seen in BAL
- Mould seen in sinus aspirate
- Antigen in blood, BAL, CSF
- PCR to detect nucleic acid

Not until a PCR system is developed that has been externally validated for blood, tissue, or BAL fluid.

**Proven invasive fungal infective disease**

- Histology
- Blood culture
- Tissue culture
- Mycology
- Blood culture

**Defining invasive fungal disease**

- Host factor
- Clinical feature
- A framework
- Mycology

Proven invasive fungal infective disease

A framework
Revised definitions

Definitions II - invasive fungal disease

Host factor + Clinical features

possible

2008

Definitions II - invasive fungal disease

Host factor + Clinical features + Mycology

probable

2008
Donnelly – PCR diagnosis of aspergillosis

Definitions II - Mycology

- Culture of mould from BAL or sputum
- Antigen in blood, BAL, CSF
- Mould seen in sinus aspirate
- Mould seen in BAL
- Beta-D-glucan in serum
- PCR to detect fungal acid

Not until a PCR system is developed that has been externally validated for blood, tissue, or BAL fluid

How good are biomarkers?

Galactomannan
Donnelly – PCR diagnosis of aspergillosis

### Galactomannan

<table>
<thead>
<tr>
<th>Studies</th>
<th>Pooled sensitivity</th>
<th>Pooled specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematological malignancy</td>
<td>0.58 (0.52-0.63)</td>
<td>0.93 (0.92-0.94)</td>
</tr>
<tr>
<td>HSCT</td>
<td>0.65 (0.60-0.78)</td>
<td>0.65 (0.44-0.83)</td>
</tr>
<tr>
<td>0.5 cut-off</td>
<td>0.79 (0.69-0.87)</td>
<td>0.86 (0.83-0.89)</td>
</tr>
<tr>
<td>1.5 cut-off</td>
<td>0.48 (0.41-0.56)</td>
<td>0.95 (0.93-0.96)</td>
</tr>
</tbody>
</table>

Mengoli et al 2009 Lancet Infect Dis 9: 615-23

… and PCR then? … and PCR then?

Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis

A systematic review and meta-analysis was done on the use of PCR tests for the diagnosis of invasive aspergillosis. Data from 2009 studies were used, with PCR tests employing different markers, including galactomannan, galactose, and β-D-glucan. Sensitivity and specificity of PCR for invasive aspergillosis ranged from 83% to 100% and 77% to 96%, respectively. A single positive PCR result is not sufficient to rule in or out a diagnosis of invasive aspergillosis. However, two positive tests are required to confirm the diagnosis. Sensitivity is higher than that obtained from a single positive test. Populations of patients with haematological malignancies or stem cell transplant recipients are more likely to benefit from the use of PCR for screening, which will help to determine the role of PCR and other tests in patients' care.

Mengoli et al 2009 Lancet Infect Dis 9: 615-23
Aspergillus PCR – Forrest plots

Bivariate analysis

Comparison of PCR results
Donnelly – PCR diagnosis of aspergillosis

Comparison of PCR results

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>PCR pos</th>
<th>High DOR</th>
<th>LR+</th>
<th>LR-</th>
<th>DOR (LR+/LR-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hebart BMJ</td>
<td>2000</td>
<td>1</td>
<td>6.74</td>
<td>0.69</td>
<td>976.6</td>
<td></td>
</tr>
<tr>
<td>Hebart JID</td>
<td>2000</td>
<td>1</td>
<td>4.65</td>
<td>0.51</td>
<td>910.8</td>
<td></td>
</tr>
<tr>
<td>Williamson</td>
<td>2000</td>
<td>2</td>
<td>18.24</td>
<td>0.18</td>
<td>328.4</td>
<td></td>
</tr>
<tr>
<td>Buchheidt</td>
<td>2001</td>
<td>1</td>
<td>4.60</td>
<td>0.30</td>
<td>976.6</td>
<td></td>
</tr>
<tr>
<td>Raad</td>
<td>2001</td>
<td>2</td>
<td>12.62</td>
<td>0.16</td>
<td>766.6</td>
<td></td>
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<tr>
<td>Pinedo</td>
<td>2002</td>
<td>1</td>
<td>2.90</td>
<td>0.51</td>
<td>561.6</td>
<td></td>
</tr>
<tr>
<td>Halliday</td>
<td>2004</td>
<td>2</td>
<td>4.73</td>
<td>0.58</td>
<td>556.8</td>
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<tr>
<td>Hebart JID</td>
<td>2005</td>
<td>1</td>
<td>4.35</td>
<td>0.38</td>
<td>556.8</td>
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<tr>
<td>Kawazu</td>
<td>2004</td>
<td>1</td>
<td>3.74</td>
<td>0.14</td>
<td>561.6</td>
<td></td>
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<tr>
<td>Lass-Floerl</td>
<td>2004</td>
<td>2</td>
<td>6.60</td>
<td>0.90</td>
<td>126.9</td>
<td></td>
</tr>
<tr>
<td>Jordanides</td>
<td>2005</td>
<td>2</td>
<td>6.15</td>
<td>0.03</td>
<td>182.4</td>
<td></td>
</tr>
<tr>
<td>Scotter</td>
<td>2005</td>
<td>1</td>
<td>6.60</td>
<td>0.34</td>
<td>561.6</td>
<td></td>
</tr>
<tr>
<td>ElMahallawy</td>
<td>2006</td>
<td>1</td>
<td>9.19</td>
<td>0.08</td>
<td>33.8</td>
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</tr>
<tr>
<td>Florent</td>
<td>2006</td>
<td>2</td>
<td>6.15</td>
<td>0.03</td>
<td>182.4</td>
<td></td>
</tr>
<tr>
<td>Halliday</td>
<td>2006</td>
<td>2</td>
<td>4.03</td>
<td>0.23</td>
<td>303.6</td>
<td></td>
</tr>
<tr>
<td>Stenghele</td>
<td>2006</td>
<td>2</td>
<td>5.31</td>
<td>0.02</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>2006</td>
<td>2</td>
<td>17.19</td>
<td>0.27</td>
<td>211.5</td>
<td></td>
</tr>
</tbody>
</table>

High DOR: 7/16 (44%)

Likelihood ratios for PCR: 1 test = positive

Likelihood ratios for PCR: 2 tests = positive
Donnelly – PCR diagnosis of aspergillosis

**Differences in patient cohorts?**

- Raad et al. 2002
- Halliday et al. 2005
- White et al. 2006

<table>
<thead>
<tr>
<th>Patient Cohorts</th>
<th>Proven/Probable IFD</th>
<th>Possible IFD</th>
<th>No IFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>54 adults with cancer treated for haematological malignancies who had developed pulmonary infiltrates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65 adults treated for haematological malignancies or recipients of an HSCT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>203 adults treated for haematological malignancies or recipients of an HSCT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Differences in methods?**

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Raad et al.</th>
<th>Halliday et al.</th>
<th>White et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell wall disruption</td>
<td>SDS, proteinase</td>
<td>Lysate</td>
<td>Mechanical, glass beads</td>
</tr>
<tr>
<td>DNA extraction</td>
<td>phenol–chloroform</td>
<td>phenol–chloroform</td>
<td>MagnaPure™</td>
</tr>
<tr>
<td>Target gene</td>
<td>alkaline protease</td>
<td>18S rRNA</td>
<td>28S rRNA</td>
</tr>
<tr>
<td>Nested PCR</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>No sample for result</td>
<td>1 and 2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>References for methodology</td>
<td>Tang et al. (19), Bretagne et al. (30), Skidmore et al. (43)</td>
<td>Williamson et al. (28)</td>
<td></td>
</tr>
</tbody>
</table>
European Aspergillus PCR initiative

http://www.isham.org/Groups.html

Working Group “Towards a standard for Aspergillus PCR”

- Laboratory Working party
- Steering committee
- Clinical Working party

Jurgen Loeffler, J. Peter Donnelly, Rosemary Barnes

Structure of the EAPCRI

- Steering committee
- LAB Working Party
- CLIN Working Party
- STAT Working Party
- Commercial Section

Is high sensitivity what we want?
Is high specificity what we want?
Donnelly – PCR diagnosis of aspergillosis

Specificity, prevalence and positive predictive value

Specificity, prevalence and negative predictive value

PCR positive = infection?
Donnelly – PCR diagnosis of aspergillosis

**Biomarkers – ahead of the game?**

![Diagram](image1)

**PCR – ahead of the game**

![Diagram](image2)

**PCR & GM – a powerful combination?**

![Diagram](image3)
Donnelly – PCR diagnosis of aspergillosis

**Combining tests for detecting infection?**

- Galactomannan
- Beta-D-Glucan
- PCR

**Biomarkers to select early treatment**

- Pre-emptive
- Prophylaxis
Conclusion

- There needs to be a standard for PCR
- Several PCR methods are likely
- No method yields high negative and positive predictive values
- A high negative predictive value is required for screening
- A high positive predictive value is required to confirm IFD
- PCR positive may indicate infections before disease is apparent
Challenges in the Diagnosis of Zygomycosis and Emerging Pathogens

19th ECCMID
EFISG WORKSHOP
Manuel Cuenca-Estrella

Mucormycosis

Agents of mucormycosis

- Ubiquitous and thermotolerant
- Decaying matter, vegetables, fruits, seeds, bread, soil, compost piles and animal excreta
- Sporulate abundantly and spores are easily air-borne
- Most common species: *Rhizopus, Mucor, Absidia, Rhizomucor, and Cunninghamella*
Cuenca-Estrella – Zygomycosis and emerging pathogens

Agents of mucormycosis

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Several reports indicate an increase in their prevalence associated to use of antifungal agents against Aspergillus

Aspergillosis: 0.7 per 1000
Zygomycosis: 0.2 per 1000

Please note difference in scale
Breakthrough Zygomycosis
Trifilio et al
BMT 2007; 39:425-9

13 oncological centers in USA
Cohort study after exposure to voriconazole
Five days or more of treatment and diagnose by culture or histology

56/58 case were treated with voriconazole
Therapy with amphotericin B 73% mortality

Antifungal GM MICs

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>AMB GM</th>
<th>IZ GM</th>
<th>VZ GM</th>
<th>PZ GM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizopus oryzae</td>
<td>25</td>
<td>0.64</td>
<td>3.6</td>
<td>12.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Rhizopus microsporus</td>
<td>7</td>
<td>0.9</td>
<td>5.9</td>
<td>8.8</td>
<td>4</td>
</tr>
<tr>
<td>Mucor spp.</td>
<td>17</td>
<td>0.14</td>
<td>8.6</td>
<td>14.7</td>
<td>3.5</td>
</tr>
<tr>
<td>Rhizomucor spp.</td>
<td>7</td>
<td>0.13</td>
<td>0.6</td>
<td>16</td>
<td>0.19</td>
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<tr>
<td>Absidia corymbifera</td>
<td>19</td>
<td>0.12</td>
<td>1.66</td>
<td>14.3</td>
<td>0.76</td>
</tr>
<tr>
<td>Cunninghamella bertholdiae</td>
<td>7</td>
<td>3.2</td>
<td>4.8</td>
<td>19.5</td>
<td>1.78</td>
</tr>
</tbody>
</table>

Data obtained from Mycology Reference Laboratory, Spain
Cuenca-Estrella – Zygomycosis and emerging pathogens

**Agents of mucormycosis**

- Ubiquitous and thermotolerant
- Decaying matter, vegetables, fruits, seeds, bread, soil, compost piles and animal excreta
- Sporulate abundantly and spores are easily air-borne
- Most common species: *Rhizopus, Mucor, Absidia, Rhizomucor, and Cunninghamella*

**But, are we detecting same trend and significant rise in transplant recipients??**

- 16 transplant centers and three reference laboratories that prospectively included all SOT and HSCT recipients from 2003 to 2005
- A total of 3,487 patients were included (SOT 2,615 and HSCT 872, WITH ALLO-HSCT 372)
- The rate of IFI was 3.8% (133 cases). Only two cases of zygomycosis was described (2/133, 1.5%) and both afflicted allo-HSCT recipients

**Resitras**

**SPANISH NET FOR RESEARCH IN TRANSPLANT RECIPIENTS**

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Candida</strong></td>
<td>29 (9.1%)</td>
<td>25 (8.2%)</td>
<td>1 (0.3%)</td>
<td>0 (0.0%)</td>
<td>3 (0.9%)</td>
<td>1 (0.3%)</td>
<td>30 (4.3%)</td>
</tr>
<tr>
<td><strong>Asperg.</strong></td>
<td>3 (0.9%)</td>
<td>2 (0.6%)</td>
<td>1 (0.3%)</td>
<td>0 (0.0%)</td>
<td>1 (0.3%)</td>
<td>0 (0.0%)</td>
<td>4 (0.6%)</td>
</tr>
<tr>
<td><strong>Zygom.</strong></td>
<td>2 (0.6%)</td>
<td>2 (0.6%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>2 (0.6%)</td>
<td>0 (0.0%)</td>
<td>4 (0.6%)</td>
</tr>
<tr>
<td><strong>S. apiosp.</strong></td>
<td>1 (0.3%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>1 (0.3%)</td>
<td>0 (0.0%)</td>
<td>1 (0.2%)</td>
</tr>
</tbody>
</table>
Cuenca-Estrella – Zygomycosis and emerging pathogens

### RESITRA

**SPANISH NET FOR RESEARCH IN TRANSPLANT RECIPIENTS**

<table>
<thead>
<tr>
<th>Infection</th>
<th>Nb in SOT (2,615)</th>
<th>Nb in HSCT (872)</th>
<th>TOTAL (3,487)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus</td>
<td>18 (0.7%)</td>
<td>50 (5.7%)</td>
<td>68 (1.9%)</td>
</tr>
<tr>
<td>Candida</td>
<td>39 (1.5%)</td>
<td>17 (1.9%)</td>
<td>56 (1.6%)</td>
</tr>
<tr>
<td>Fusarium</td>
<td>0</td>
<td>8 (0.7%)</td>
<td>8 (0.2%)</td>
</tr>
<tr>
<td>Zygomyc.</td>
<td>0</td>
<td>2 (0.2%)</td>
<td>2 (0.06%)</td>
</tr>
<tr>
<td>S. apiosper.</td>
<td>0</td>
<td>1 (0.1%)</td>
<td>1 (0.03%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>57 (2.2%)</td>
<td>76 (8.4%)</td>
<td>133 (3.8%)</td>
</tr>
</tbody>
</table>

### Incidence in other countries

#### 1970-2005

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>SOT</th>
<th>HSCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain (RESITRA)</td>
<td>2003-2005</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>USA (TRANSNET) and single inst.</td>
<td>1995-2003</td>
<td>5.7% (liver/kidney)</td>
<td>0.2-2%</td>
</tr>
<tr>
<td>Belgium (Leuven)</td>
<td>1990-1999</td>
<td>--</td>
<td>1.9%</td>
</tr>
<tr>
<td>Australia (Ipswich)</td>
<td>1993-2005</td>
<td>0.28%</td>
<td>--</td>
</tr>
<tr>
<td>Iran</td>
<td>1993-2003</td>
<td>7.8% (liver)</td>
<td>--</td>
</tr>
<tr>
<td>Brasil</td>
<td>1982-2000</td>
<td>2% (liver)</td>
<td>--</td>
</tr>
</tbody>
</table>

### Reviewing, 1970-2005


Almyroudis et al. Am J Transplant 2006

<table>
<thead>
<tr>
<th>160 cases in transplant recipients (may be more)</th>
<th>106 SOT</th>
<th>73 renal transplant 19 liver 16 heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary 26%</td>
<td>Rhinocerebral 15%</td>
<td>Disseminated 14%</td>
</tr>
<tr>
<td>Rhino-sino-orbital 19%</td>
<td>Cutaneous 17%</td>
<td>Gastrointestinal 12%</td>
</tr>
</tbody>
</table>
Cuenca-Estrella – Zygomycosis and emerging pathogens

Agents of mucormycosis

- Ubiquitous and thermotolerant
- Decaying matter, vegetables, fruits, seeds, bread, soil, compost piles and animal excreta
- Sporulate abundantly and spores are easily air-borne
- Most common species: Rhizopus, Mucor, Absidia, Rhizomucor, and Cunninghamella

And since 2006 to May 2008 Has Incidence Increased????

Resitra

Since 2006 ten more cases (7 in HSCT and 3 SOT) and incidences of 0.62 cases/100,000 hospital admissions have been reported from some RESITRA institutions

JCM 2007;45:2051-53

Breakthrough Zygomycosis
Trifilio et al
BMT 2007; 39:425-9

<table>
<thead>
<tr>
<th>13 oncological centers in USA</th>
<th>Cohort study after exposure to voriconazole</th>
<th>Five days or more of treatment and diagnose by culture or histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>56/58 case were treated with voriconazole</td>
<td>Therapy with amphotericin B</td>
<td>73% mortality</td>
</tr>
</tbody>
</table>

30
Cuenca-Estrella – Zygomycosis and emerging pathogens

**Breakthrough Zygomycosis**
Trifilio et al
BMT 2007; 39:425-9

13 oncological centers

Five days or more

36/58 cases, 62%, were described in HSCT

56/58 treated with voriconazole

Therapy with amphotericin B 73% mortality

Trifilio et al, BMT 2007; 39:425-9

36/58 cases, 62%, were described in HSCT

Roden et al. Clinical Infectious Diseases 2005; 41:634–53

Analysis per predisposing factor of 929 cases

Malignancy

Bone marrow transplantation

Roden et al. Clinical Infectious Diseases 2005; 41:634–53
Cuenca-Estrella – Zygomycosis and emerging pathogens

Roden et al. Clinical Infectious Diseases 2005; 41:634–53 and Review of case reports and congress abstracts

Please, note underestimation, culture is more sensitive than microscopical examination
Cuenca-Estrella – Zygomycosis and emerging pathogens

**Scedosporium spp.**

- Ubiquitous organism
- Subtropical areas
- Soil, vegetation, sewer
- It causes:
  - Colonization (cystic fibrosis and fungus ball)
  - Allergy
  - Subcutaneous infections (mycetoma and hyalohyphomycosis)
  - Deep Infections:
    - Focal
    - Disseminated

**Scedosporium apiospermum**

- 1980s: 50% cases without culture.
- 2000s: 25% cases without culture
- Is there an increase of incidence related to improvements in diagnostic procedures?
**Cuenca-Estrella – Zygomycosis and emerging pathogens**

**Scedosporium apiospermum**

- Ubiquitous organism
- Subtropical areas
- Soil, vegetation, sewer

- It causes:
  - Colonization (cystic fibrosis and fungus ball)
  - Allergy
  - Subcutaneous infections (mycetoma and hyalohyphomycosis)
  - Deep Infections:
    - Focal
    - Disseminated

Inhalation of conidia may cause nosocomial infections clinically similar to sinus, pulmonary or disseminated aspergillosis.

But nosocomial outbreaks have not been described. It appears as an outdoor pathogen.

**AMB** | **ITC** | **VRC** | **POS** | **TBF**
---|---|---|---|---
Range | 1.0-32.0 | 0.5-16.0 | 6.12-16.0 | 0.25-16.0 | 8.0-32.0
GEO Mean | 4.7 | 3.7 | 0.9 | 1.3 | 17.1
**MIC**<sub>50</sub> | 4.0 | 8.0 | 1.0 | 1.0 | 8.0
**MIC**<sub>90</sub> | 16.0 | 16.0 | 8.0 | 8.0 | 32.0

Cuenca-Estrella et al. AAC 2006; 50:917-21
### Scedosporium apiospermum

**N=69 organisms from deep infection**

<table>
<thead>
<tr>
<th></th>
<th>AMB</th>
<th>ITC</th>
<th>VRC</th>
<th>POS</th>
<th>TBF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Range</strong></td>
<td>1.0-32.0</td>
<td>0.5-16.0</td>
<td>0.12-16.0</td>
<td>0.25-16.0</td>
<td>8.0-32.0</td>
</tr>
<tr>
<td><strong>GEO Mean</strong></td>
<td>4.7</td>
<td>3.7</td>
<td>0.9</td>
<td>1.3</td>
<td>7.1</td>
</tr>
<tr>
<td><strong>MIC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>4.0</td>
<td>8.1</td>
<td>1.0</td>
<td>1.0</td>
<td>-0.0</td>
</tr>
<tr>
<td><strong>MIC&lt;sub&gt;90&lt;/sub&gt;</strong></td>
<td>16.0</td>
<td>16.0</td>
<td>8.0</td>
<td>8.0</td>
<td>32.0</td>
</tr>
</tbody>
</table>

*Cuenca-Estrella et al. AAC 2006.50;917-21*

---

### Voriconazole and posaconazole are active in vitro against most of isolates

<table>
<thead>
<tr>
<th></th>
<th>AMB</th>
<th>ITZ</th>
<th>TERBI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Range</strong></td>
<td>0-32.0</td>
<td>6.0</td>
<td>100</td>
</tr>
<tr>
<td><strong>GEO Mean</strong></td>
<td>7.1</td>
<td>100</td>
<td>6.0</td>
</tr>
<tr>
<td><strong>MIC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>8.0</td>
<td>100</td>
<td>6.0</td>
</tr>
<tr>
<td><strong>MIC&lt;sub&gt;90&lt;/sub&gt;</strong></td>
<td>16.0</td>
<td>100</td>
<td>6.0</td>
</tr>
</tbody>
</table>

---

### Scedosporium prolificans

- Uncommon organism
- Temperate countries, South of Europe
- Soil, vegetation, sewage
- It causes:
  - Colonization
  - Subcutaneous infections
  - Deep Infections:
    - Focal
    - Disseminated
  - Nosocomial pathogen in some hospitals
  - Air-borne
  - Related to catheter

*5. prolificans on Sabouraud agar*
Cuenca-Estrella – Zygomycosis and emerging pathogens

**Scedosporium prolificans**
Rodriguez-Tudela, In press

- **59 cases of deep infection in hematological patients**
  - 51/59 86.4% **ACUTE LEUKAEMIA**
  - 43/59 72.9% **DISSEMINATED INFECTIONS**
  - 10/59 16.9% **PULMONARY INFECTIONS**
  - 43/59 72.9% **POSITIVE BC**
  - 54/59 91% **DECEASED**

- **13 deep infections in immunocompromised non-hematological patients, (AIDS, solid organ cancer or transplantation)**
  - 8/13 61.5% **DISSEMINATED INFECTION**
  - 8/13 61.5% **DECEASED**

- **10 therapeutic successes**
  - **Antifungal agent**
  - In some cases combination therapy with AMB plus FC or VRC plus TBF
  - **G-CSF**
  - Surgery (if possible)
Nosocomial outbreaks of *S. prolificans*

- Several outbreaks have been described
- Air-borne infection
- Hematological patients


- Six leukaemic patients
- Disseminated infection
- Organism was detected in air samples
- 100% mortality

Isolates were typed in Spanish Mycology Reference Lab

*Scedosporium prolificans*

<table>
<thead>
<tr>
<th></th>
<th>AMB</th>
<th>ITC</th>
<th>VRC</th>
<th>POS</th>
<th>TBF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>2.0–32.0</td>
<td>8.0–16.0</td>
<td>8.0–16.0</td>
<td>8.0–16.0</td>
<td>8.0–32.0</td>
</tr>
<tr>
<td>GEO Mean</td>
<td>12.8</td>
<td>9.6</td>
<td>9.4</td>
<td>10.5</td>
<td>19.2</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>16.0</td>
<td>8.0</td>
<td>8.0</td>
<td>16.0</td>
<td>16.0</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>32.0</td>
<td>16.0</td>
<td>16.0</td>
<td>16.0</td>
<td>32.0</td>
</tr>
</tbody>
</table>

Cuenca-Estrella et al. AAC 2006; 50:917-21
Scedosporium prolificans

N = 66

<table>
<thead>
<tr>
<th></th>
<th>AMB</th>
<th>ITC</th>
<th>VRC</th>
<th>POS</th>
<th>TBF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Range</strong></td>
<td>2.0-32.0</td>
<td>8.0-16.0</td>
<td>8.0-16.0</td>
<td>8.0-16.0</td>
<td>8.0-32.0</td>
</tr>
<tr>
<td><strong>GEO Mean</strong></td>
<td>12.8</td>
<td>9.6</td>
<td>9.4</td>
<td>10.5</td>
<td>19.2</td>
</tr>
<tr>
<td><strong>MIC&lt;sub&gt;50&lt;/sub&gt;</strong></td>
<td>16.0</td>
<td>8.0</td>
<td>8.0</td>
<td>16.0</td>
<td>16.0</td>
</tr>
<tr>
<td><strong>MIC&lt;sub&gt;90&lt;/sub&gt;</strong></td>
<td>32.0</td>
<td>16.0</td>
<td>16.0</td>
<td>16.0</td>
<td>32.0</td>
</tr>
</tbody>
</table>

Cuenca-Estrella et al. AAC 2006.50;917-21

• *S. prolificans* is a multi-resistant organism

- Early Diagnosis
- Prophylaxis
- Aerial control measures
- Combination therapy

Fusarium spp.
Fusarium spp.

- Ubiquitous, soil saprophytes
- Pathogenic for plants
- Mycotoxins
- Opportunistic mycosis
- Port of acquisition: lung, skin, foreign bodies
- Community-acquired infection but more frequently acquired nosocomially

It causes superficial and deep mycosis in IC patients with hemorrhagic lesions, BC+ and high rates of mortality.

Fusarium is recognized as the second most common nosocomial fungal pathogen after Aspergillus in some tertiary care cancer centers (Walsh et al. Transpl Infect Dis. 1999;1:247-61).

It could be more frequent in future due to its ubiquity and resistance. Nosocomial outbreaks have been described.
Cuenca-Estrella – Zygomycosis and emerging pathogens

**Nosocomial outbreak of *Fusarium solani***

- 8 hematological patients with disseminated infection (7/8 died)
- Fungi were isolated in cutaneous biopsies, BC, catheter and respiratory samples
- Organisms were also detected in moisturizing cream supposedly sterile used to care for hospitalized patients

**Identification of *Fusarium solani***

Organisms colonized skin, respiratory tract and catheter and after caused disseminated infection
Isolates were typed in Spanish Mycology Reference Lab by M-13 and GACA-4.

Three control strains exhibited a band pattern similar to that of outbreak strains.
Cuenca-Estrella – Zygomycesis and emerging pathogens

**Typification of Fusarium spp. by M13**

Fusarium spp. are undergoing extensive taxonomic reevaluation and molecular ID of strains from the outbreak was done.

---

**Sequencing of EF1α**

Rates of similarity:

- 60% similarity
- 90% similarity
- 96% similarity
- 100% similarity

Strains:

- F. solani f sp. phaseoli
- F. solani f sp. cucurbitae
- 3 control strains
- 23 outbreak strains
- F. solani f sp. batatas
- F. oxysporum

PCR fingerprinting by M-13 and GACA4 are not useful for typing the forma specialis.

---

**Typification of Fusarium solani by M13**

Isolates were typed in Spanish Mycology Reference Lab.

Three control strains exhibited a band pattern similar to that of outbreak strain Fusarium spp. are undergoing extensive taxonomic reevaluation and molecular ID of strains from the outbreak was done.
**F. solani f sp cucurbitae** is a plant pathogen mainly for *Cucurbitaceae*. It appears as the most pathogenic for humans among *Fusarium*.

### Fusarium spp.

<table>
<thead>
<tr>
<th>Fusarium spp. (N)</th>
<th>AMB</th>
<th>ITZ</th>
<th>VOR</th>
<th>POS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>CR</td>
<td>Range</td>
<td>CR</td>
<td>Range</td>
</tr>
<tr>
<td>F. solani (23)</td>
<td>0.5-8.0</td>
<td>1.72</td>
<td>8.0-16.0</td>
<td>8.49</td>
</tr>
<tr>
<td><em>F. oxysporum</em> (13)</td>
<td>0.03-8.0</td>
<td>0.69</td>
<td>4.0-16.0</td>
<td>8.43</td>
</tr>
<tr>
<td><em>F. verticillioides</em> (5)</td>
<td>1.0-4.0</td>
<td>2.0</td>
<td>0.50-8.0</td>
<td>3.48</td>
</tr>
<tr>
<td><em>F. proliferatum</em> (2)</td>
<td>0.50-2.0</td>
<td>1.0</td>
<td>8.0-16.0</td>
<td>12.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>%R to AMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. solani</td>
</tr>
<tr>
<td>F. oxysporum</td>
</tr>
<tr>
<td>F. verticillioides</td>
</tr>
<tr>
<td>F. proliferatum</td>
</tr>
</tbody>
</table>

---

Cuenca-Estrella – Zygomycosis and emerging pathogens
### Cuenca-Estrella – Zygomycosis and emerging pathogens

#### Fusarium spp.

<table>
<thead>
<tr>
<th>Species</th>
<th>IC50</th>
<th>Range (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. solani</em></td>
<td></td>
<td>0.5-8.0</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td></td>
<td>0.03-8.0</td>
</tr>
<tr>
<td><em>F. verticillioides</em></td>
<td>1.0-4.0</td>
<td>2.0</td>
</tr>
<tr>
<td><em>F. proliferatum</em></td>
<td>0.50-2.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

VOR is recommended to treat infections due to *Fusarium* spp and some therapeutic success have been reported using total inhibition as endpoint of AST. 90% of *Fusarium* are resistant *in vitro* (also to ITC and POS). They should be considered as multi-resistant species.

#### Other rare emerging nosocomial pathogens (the time flies...)

- *Alternaria* spp.
- Subcutaneous phaeohyphomycosis in legs of transplantation patients
- No predictable susceptibility
Cuenca-Estrella – Zygomycosis and emerging pathogens

Other rare emerging nosocomial pathogens (the time flies...)

- **Alternaria spp.**
- Subcutaneous aphaenohomycosis
- **Paecilomyces lilacinus**
  - Air-borne pathogen
  - Disseminated infection in hematological patients
  - It seems resistant in vitro to AMB and susceptible to VRC and POS

- **Scopulariopsis brevicaulis**
  - Air-borne or colonization of devices and prosthesis
  - Multi-resistant organism (Cuenca-Estrella et al. AAC. 2003;47:2339-41)

Prevalence of emerging moulds such as Mucorales

- Their prevalence is increasing owing to:
  - Rise of susceptible patients
  - New predisposing factors
  - Efficient control of more prevalent species extending the niche of rare and may be more resistant species
Prevalence of emerging moulds

- Their prevalence is increasing owing to:
  - Rise of susceptible patients
  - New predisposing factors
  - Efficient control of more prevalent species extending the niche of rare and may be more resistant species

OR

The prevalence is not really increasing
They were underestimated due to several reasons

OR

Prevalence of emerging moulds

- Short number of experts in Medical Mycology
- Lack of epidemiological surveys
- Lack of interest in identifying pathogens at species level
- No alternatives to treat patient
- No clinical differences in fungal infections caused by distinct species
Cuenca-Estrella – Zygomycosis and emerging pathogens

From my point of view...

Combination of both explanations:
1. More and more frequent predisposing factors
2. Possible extension of rare/emerging pathogens
3. Availability of several antifungal agents with distinct activity profile increases the interest
4. More and more experts (microbiologist, infectious diseases, hematologist...) are interested in identification at species level
Possible, Presumptive, or Proven Invasive Fungal Infection - Any Help?

Andrew J. Ullmann, M.D., FIDSA
Universitätsmedizin
Johannes Gutenberg-Universität
III. Medizinische Klinik und Poliklinik
Mainz, Germany

Potential conflict of interest:
- Consultant: Basilea, Pfizer, Schering-Plough, Astellas, Gilead, Alcuris
- Speaker's bureau: Schering-Plough, Astellas, Gilead, Merck/MSD, Astellas, and Pfizer

Objective:
- Why definitions
- Review of the new definitions of EORTC
- Strengths
- Limitations
- Biomarkers helpful?
**Why were definitions developed?**
- to facilitate the identification of reasonably homogeneous groups of patients for clinical and epidemiologic research
- to help design clinical trials to evaluate new drugs and management strategies

**What are the criteria?**

- Host
- Clinical
- Mycology

**Level of Uncertainty**
*by Webster Dictionary*

- Possible: that can exist or be
- Presumption: guess, hypothesis
- Probable: likely to occur or be
- Proven: to establish as true
“Old” Definition

- Three levels of certainty:
  - Possible (clinical or microbiological evidence)
  - Probable (would need microbiological evidence)
  - Proven

- All needed to have satisfied the host criteria
Strength of the definitions

- Accepted worldwide by
  - Pharmaceutical companies
  - Numerous trials
  - Registration authorities
  - Journals

But...

... Numerous trials utilized so-called modified criteria for including and treating patients.

Basically host factors (hematology) and major criteria (on HRCT) were inclusion criteria

DO THEY REALLY WORK?
Ullmann – Invasive fungal infections

<table>
<thead>
<tr>
<th>Results of the EORTC-Study</th>
<th>Successrate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vori</td>
</tr>
<tr>
<td>Total success rate (ITT)</td>
<td>53</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>55</td>
</tr>
<tr>
<td>Extrapulmonary disease</td>
<td>43</td>
</tr>
<tr>
<td>Allogeneic SCT/BMT</td>
<td>32</td>
</tr>
<tr>
<td>Other hemat. disease</td>
<td>63</td>
</tr>
<tr>
<td>others</td>
<td>50</td>
</tr>
<tr>
<td>neutropenic</td>
<td>51</td>
</tr>
<tr>
<td>non-neutropenic</td>
<td>54</td>
</tr>
<tr>
<td>proven IA</td>
<td>45</td>
</tr>
<tr>
<td>probable IA</td>
<td>60</td>
</tr>
<tr>
<td>ITT</td>
<td>50</td>
</tr>
</tbody>
</table>

2008

Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group

De Pauw B et al. CID 2008

Disease vs Infection
What are the differences

The term “invasive fungal disease” (IFD) was adopted to reflect more accurately the notion that we are dealing with a disease process caused by fungal infection.
What is the difference between the two?

HOST

- Neutropenia (ANC ≤ 1000/μl) on admission or Day 1.

Post-factor:
- Baseline history of neutropenia (ANC ≤ 1000/μl) on admission or Day 1.

Who is missing?

- Leukemia
- Allogeneic SCT
- AIDS/HIV
- SOT
- Primary immunodeficiencies

Yes, they are in the definitions

In new ones these patients are now included
CASE 2

45 year old man diagnosed with ALL. He moved to Germany (from Russia) 5 year prior to his admission. He received chemotherapy for ALL. He had no antifungal prophylaxis. He developed fever during neutropenia and received piperacillin/tazobactam. He did not defervesce and a HRCT was performed. No other symptoms besides cough were reported. The radiologist reported multiple wedge shaped infiltrates with halos.

Which of the following are reasonable differential diagnoses?

1. Pulmonary tuberculosis
2. Nocardiosis
3. Staphylococcus pneumonia
4. Invasive aspergillosis
5. None of the above
Ullmann – Invasive fungal infections

What level of certainty for IFD would you classify this patient?

1. No IFD
2. Possible IFD
3. Probable IFD
4. Proven IFD

What treatment would you offer?

1. Oral/IV itraconazole
2. Oral fluconazole
3. Oral voriconazole
4. IV voriconazole
5. Caspofungin
6. IV amphotericin B
7. None

Specificity of radiographic imaging

Pulmonary nocardiosis
PTLD
Pulmonary toxoplasmosis

[Images of radiographs with text overlay]

[Image of radiographs with text overlay]
What is important for a trial?

- Site of infection
- Additional risk factors
- Work-up by microbiology

What is important?

- Site of infection
- Additional risk factors
- Work-up by microbiology

Survival: Aspergillosis with Amphotericin B

Site of Infection

<table>
<thead>
<tr>
<th>Site of Infection</th>
<th>Cumulative Survival Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinusitis (n=17)</td>
<td>0.00</td>
</tr>
<tr>
<td>Multi-site (n=11)</td>
<td>0.10</td>
</tr>
<tr>
<td>Aspergilloma (n=10)</td>
<td>0.20</td>
</tr>
<tr>
<td>Pulmonary (n=83)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Days

0 30 60 90 120 150 180 210 240 270 300 330 360
What is important?

- Site of infection
- Additional risk factors
- Work-up by microbiology

Caspofungin as first-line therapy of probable and proven invasive aspergillosis

Results in Neutropenia

Results by Karnofsky score
AmBiLoad: Patients with uncontrolled malignancy: lower survival @ 12 wks

- Controlled Malignancy
- Uncontrolled Malignancy

- Lymphomas
- Acute Leukemias
- Leukemias (all)
- Heme Malignancy (all)

% of patients survival
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

P= .004
P= .027
P< .001

Δ 27%; 95% CI 15%- 39%

*P<0.001

What is important?

- Site of infection
- Additional risk factors
- Work-up by microbiology

Mycological criteria

Direct test (cytology, direct microscopy, or culture)
- Mold in sputum, bronchoalveolar lavage fluid, bronchial brush, or sinus aspirate samples, indicated by 1 of the following:
- Presence of fungal elements indicating a mold
- Recovery by culture of a mold (e.g., Aspergillus, Fusarium, Zygomycetes, or Scedosporium species)
**Mycological criteria**

Indirect tests (detection of antigen or cell-wall constituents)
- Galactomannan antigen detected in plasma, serum, bronchoalveolar lavage fluid, or CSF
- Invasive fungal disease other than cryptococcosis and zygomycoses
- β-D-glucan detected in serum

---

**Issues regarding microbiological evidence**

- Galactomannan cut-off not defined
- GM in BAL, CSF; but not approved by manufacturer
- BD-Glucan cut-off not defined
- What if previously received prophylaxis, biomarker useful?
- What about positivities in lung transplantation?

---

**Performance of (1,3)-β-D- Glucan**

Invasive fungal infection by EORTC/MSG criteria in 333 subjects

<table>
<thead>
<tr>
<th>BG cutoff</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Positive predictive value %</th>
<th>Negative predictive value %</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>79.1</td>
<td>79.4</td>
<td>78.7</td>
<td>79.5</td>
</tr>
<tr>
<td>60</td>
<td>73.9</td>
<td>82.0</td>
<td>80.4</td>
<td>76.2</td>
</tr>
<tr>
<td>80</td>
<td>69.9</td>
<td>87.1</td>
<td>80.8</td>
<td>78.1</td>
</tr>
<tr>
<td>100</td>
<td>64.4</td>
<td>92.4</td>
<td>89.0</td>
<td>72.8</td>
</tr>
<tr>
<td>125</td>
<td>62.6</td>
<td>94.7</td>
<td>91.9</td>
<td>72.5</td>
</tr>
<tr>
<td>150</td>
<td>60.1</td>
<td>96.5</td>
<td>94.2</td>
<td>71.6</td>
</tr>
<tr>
<td>180</td>
<td>52.1</td>
<td>97.6</td>
<td>96.9</td>
<td>70.3</td>
</tr>
</tbody>
</table>
Performance of (1,3)-β-D-Glucan

Invasive fungal infection by EORTC/MSG criteria in 333 subjects

<table>
<thead>
<tr>
<th>Cut-off value, pg/mL</th>
<th>No. of subjects with positive result</th>
<th>Sensitivity %</th>
<th>No. of subjects with positive result</th>
<th>Sensitivity %</th>
<th>No. of subjects with positive result</th>
<th>Sensitivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>80</td>
<td>89.2</td>
<td>23</td>
<td>89.5</td>
<td>3</td>
<td>100.0</td>
</tr>
<tr>
<td>50</td>
<td>76</td>
<td>94.8</td>
<td>22</td>
<td>84.6</td>
<td>3</td>
<td>100.0</td>
</tr>
<tr>
<td>65</td>
<td>76</td>
<td>94.5</td>
<td>21</td>
<td>89.8</td>
<td>3</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Performance of (1,3)-β-D-Glucan

- 1, healthy volunteers
- 2, bcx for yeast
- 3A, bx positive gram-negative bacteria
- 3B, bx positive for gram-positive bacteria
- 4A, Histoplasma antigen positive
- 4B, Histoplasma antigen negative
- 5A, galactomannan positive
- 5B, galactomannan negative

CASE 1
35 year female diagnosed with AML received chemotherapy. Her clinical course was unremarkable except for fever during neutropenia.

She received piperacillin/tazobactam and was on posaconazole prophylaxis.

She was monitored twice weekly for galactomannan.

During screening at approximately two weeks after chemotherapy an index value of 0.7 was detected.

Which of the following are reasonable differential diagnoses?

1. False positivity due to piperacillin/tazobactam
2. Breakthrough invasive aspergillosis
3. None of the above
4. False positivity

What level of certainty for IFD would you classify this patient?

1. No IFD
2. Possible IFD
3. Probable IFD
4. Proven IFD
What 2 investigations are most likely to be helpful?
1. Fungal culture of sputum
2. BAL and galactomannan
3. No clinical sign and symptoms: we can wait
4. If HRCT demonstrates any kind of infiltrate then BAL and microbiological culture

What do you intend to do?
1. Change antibiotic treatment
2. Change antifungal regimen
3. Add an additional antifungal agent
4. Change nothing

Are markers useful?
Galactomannan and Computed Tomography-Based Preemptive Antifungal Therapy in Neutropenic Patients at High Risk for Invasive Fungal Infection

- Febrile neutropenia: 117 episodes
- Reduced antifungal usage by 78% (35% down to 7.7%)
- No breakthrough aspergillosis
- BUT: 1x zygomycetes case was not detected

What else is different?

- Possible would include only “host and clinical” criteria
- “Host and mycological” criteria not defined
- No minor or major clinical features: clinical features need to be “fungal typical”
Limitations

How is therapeutic success defined
Organ manifestation is critical for outcome.
What is refractory disease
How to deal with prophylaxis
Aim: homogeneous group of patients but how to handle risk situations:
- Site
- Pathogen
- Additional risk factors

Summary

Consensus definitions any help?

Pro:
- International accepted definitions for invasive fungal diseases
- Results useful in a given trial
- Good definition for proven IFD

Con:
- Not useful in daily patient care
- Probable, possible IFD remain problematic
- No comparison possible between trials
- We need better diagnostic tools!
- And a lot more to do
Chronic respiratory fungal diseases

David W. Denning
Director, National Aspergillosis Centre
University Hospital South Manchester
[Wythenshawe Hospital]
University of Manchester

Interaction of *Aspergillus* with the host
A unique microbial–host interaction

After Casadevall & Pirofski, Infect Immun 1999;67:3703

Aspergillosis
Invasive ‘saprophytic’ /chronic allergic
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**CLASSIFICATION OF ASPERGILLOSIS**

- **Invasive aspergillosis**
  - Acute (<1 month course)
  - Subacute/chronic necrotising (1-3 months)

- **Chronic aspergillosis** (>3 months)
  - Chronic cavitary pulmonary
  - Aspergilloma of lung
  - Chronic fibrosing pulmonary
  - Chronic invasive sinusitis
  - Maxillary (sinus) aspergilloma

- **Allergic**
  - Allergic bronchopulmonary (ABPA)
  - Extrinsic allergic (broncho)alveolitis (EAA)
  - Asthma with fungal sensitisation
  - Allergic Aspergillus sinusitis (eosinophilic fungal rhinosinusitis)

---

**Simple (single) aspergilloma**

Patient RK
2004
Haemoptysis, nil else
Lobectomy

---

**Aspergilloma**

---
Chronic Cavitary Pulmonary Aspergillosis
Normal 30 year female smoker

Chronic Cavitary Pulmonary Aspergillosis

Denning – Chronic respiratory fungal diseases

Aspergillus precipitins

Chronic Cavitary Pulmonary Aspergillosis
Patient JA
Jan 2001

Chronic Cavitary Pulmonary Aspergillosis
Patient JA
Feb 2002
'Multicavity' disease is the hallmark of chronic cavitary pulmonary aspergillosis (CCPA).
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**Chronic cavitary pulmonary aspergillosis (CCPA) – sputum production**

Aspergillus cultures positive in CCPA in 10-40% of cases only

**Chronic cavitary pulmonary aspergillosis**

Patient JP
June 1999

**Chronic Cavitary Pulmonary Aspergillosis, with aspergilloma**

Patient JP
July 2001, untreated
Chronic respiratory fungal diseases

**Chronic Fibrosing Pulmonary Aspergillosis**

*Patient JP April 2002, Untreated*

**Chronic pulmonary aspergillosis - pre-existing disease**

Prior pulmonary disease esp:
- Atypical mycobacteria pulmonary infection
- Sarcoidosis
- Tuberculosis
- Recurrent pneumothorax
- Prior pulmonary surgery
- ABPA

**Frequency of chronic pulmonary aspergillosis after TB**

- 544 patients with proven TB & residual cavities on a 2.4cm diameter
- 544 patients had 1.6cm diameter
- 94 patients with no Aspergillus positive sputum
- 74 patients with no ABPA on sputum
- ~10% of all cases of pulmonary TB get CPA
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Underlying diseases - CCPA

- Classical tuberculosis *
- Atypical tuberculosis *
- Allergic bronchopulmonary aspergillosis *
- Lung cancer survivor *
- Pneumothorax *
- COPD/emphysema *
- Sarcoidosis (stage II/III) *
- Rheumatoid arthritis
- Thoracic surgery
- Asthma
- Chest radiotherapy

* Common

Chronic cavitary pulmonary aspergillosis as a complication of ABPA

Chronic pulmonary aspergillosis - serology

All 18 patients had positive Aspergillus precipitins (1-4+)

All 18 patients had elevated inflammatory markers, CRP, PV and/or ESR

May have elevated total IgE and Aspergillus specific IgE (RAST)

Only 40% have a positive sputum culture
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**CPA and immune defects**

- Mannose binding lectin
- Surfactant A2
- TLR4
- Lymphocyte proliferation
- Th2 dominated cytokine profile
- Others

**CPA treatment - principles**

- Important defects in innate immunity so long term (i.e. life-long) antifungal treatment, if possible
- May fail itraconazole initially, respond to IV amphotericin B, and be successfully maintained on itraconazole
- Itraconazole failures may respond to voriconazole
- Caspofungin not very effective (personal observation)
- Gamma IFN helpful in some cases
- Monitor for azole resistance

**Antifungal therapy**

Table 2: Summary of recommendations for the treatment of monilial yeast.

| Condition | Treatment | Evidence
|-----------|-----------|----------|
| Chronic mucocutaneous candidiasis | Fluconazole in maintenance | Level 1a evidence
| Chronic mucocutaneous candidiasis | Amphotericin B | Expert opinion

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### CPA prognosis

![Graph showing CPA prognosis](image)

**Parameters of response in CPA (with voriconazole)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline value (mean ± SD)</th>
<th>1 month change (mean ± SD)</th>
<th>3 months improved (%)</th>
<th>6 months improved (%)</th>
<th>9 months improved (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>75.6 ± 11.1</td>
<td>-1.1 ± 4.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>FRA (inL/min)</td>
<td>45.3 ± 9.8</td>
<td>-0.04 ± 0.9</td>
<td>0.9 (0.6)</td>
<td>-12.8</td>
<td>-12.8</td>
</tr>
<tr>
<td>FRC (inL)</td>
<td>68.2 ± 14.5</td>
<td>-0.01 ± 0.6</td>
<td>0.1 (0.3)</td>
<td>-9.8</td>
<td>-9.8</td>
</tr>
<tr>
<td>RvL (s)</td>
<td>44.1 ± 11.4</td>
<td>-0.1 ± 0.1</td>
<td>0.1 (0.0)</td>
<td>-10</td>
<td>-10</td>
</tr>
<tr>
<td>Aspergillosis precipitate (lines)</td>
<td>18.1 ± 0.2</td>
<td>-0.2 ± 0.1</td>
<td>7.7 (0.3)</td>
<td>-1.0</td>
<td>-1.0</td>
</tr>
<tr>
<td>Aspergillus precipitins in serum (U/mL)</td>
<td>3.0 ± 0.4</td>
<td>-0.1 ± 0.6</td>
<td>4.6 (0.1)</td>
<td>-1.4</td>
<td>-1.4</td>
</tr>
</tbody>
</table>

**Chronic fibrosing pulmonary aspergillosis, with bilateral aspergillomas and azole resistance**

**Patient SM**
**June 2004**
**After treatment with Itraconazole 200mg daily and later Voriconazole**

<table>
<thead>
<tr>
<th>MICs</th>
<th>02/04</th>
<th>06/04</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itra</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Vor</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Posa</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
Aspergillus fumigatus

Aspergilloma in context of chronic cavitary pulmonary aspergillosis

MICs A. fumigatus

- Itraconazole = 8.0 mg/mL
- Voriconazole = 2.0 mg/mL
- Posaconazole = 0.25 mg/mL

In association with worse cough, marked fatigue and rising Aspergillus precipitins, after treatment for 2 years with itraconazole.

Chronic cavitary pulmonary aspergillosis

MICs A. fumigatus

- Feb 2004 (x2)
  - Itraconazole = 0.25 mg/mL
  - Voriconazole = 0.25 mg/mL
  - Posaconazole = 0.13 mg/mL

- June 2004 (x2)
  - Itraconazole = >8.0 mg/mL
  - Voriconazole = 0.25 mg/mL
  - Posaconazole = 1.0 mg/mL

In association with enlargement of his fungal ball and continuing cough.

Consistently low levels of itraconazole (rifabutin interaction).
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**Chronic cavitary pulmonary aspergillosis**

**MICs A. fumigatus**  
Feb 2004 (x2)  
- Itraconazole = 0.25 mg/mL  
- Voriconazole = 0.25 mg/mL  
- Posaconazole = 0.13 mg/mL  

June 2004 (x2)  
- Itraconazole = >8.0 mg/mL  
- Voriconazole = 0.25 mg/mL  
- Posaconazole = 1.0 mg/mL  

Treated with posaconazole with response

**Chronic cavitary pulmonary aspergillosis (CCPA) in HIV**  
February 2005  
32 yr old from Malawi, on HAART Rx  
- Haemoptysis  
- Aspergillus precipitin titre 1/16  

CT scan shows 2 large cavities with aspergillomas, with additional lesions (October 2005)  

Surgical removal would require a pneumonectomy  
So treated with itraconazole

**CCPA in HIV**  
February 2007  
On HAART Rx, with low viral load, CD4 count >200  
- New haemoptysis  
- Aspergillus precipitin titre 1/32  

CXR & CT scan showed  

**MICs A. fumigatus**  
Feb 2007  
- Itraconazole = >8.0 mg/mL  
- Voriconazole = 0.5 mg/mL  
- Posaconazole = 1.0 mg/mL
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CCPA in HIV – CT scan change

October 2005

March 2007

CCPA in HIV - low itraconazole concentrations

<table>
<thead>
<tr>
<th>Date</th>
<th>Itraconazole concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov 05</td>
<td>2.5 mg/L</td>
</tr>
<tr>
<td>Dec 05</td>
<td>3.4 mg/L</td>
</tr>
<tr>
<td>March 06</td>
<td>4.5 mg/L</td>
</tr>
<tr>
<td>July 06</td>
<td>6.7 mg/L</td>
</tr>
<tr>
<td>Feb 07</td>
<td>8.4 mg/L</td>
</tr>
</tbody>
</table>

Do low concentrations of antifungal predispose to the development of resistance?

Resistance in context of invasive aspergillosis
Denning – Chronic respiratory fungal diseases

**Mechanisms of resistance**
- Target mutations (CYP 51A only)
  - ie G54 x5, M220 x3, 6138, Y431
- Promoter insertion/repeat
- Cyp51A upregulation/over expression
- Efflux pump upregulation (?)
- Permease downregulation (?)
- Multiple mechanisms simultaneously

**CLASSIFICATION OF ASPERGILLOSIS**

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  - Acute (<1 month course)
  - Subacute/chronic necrotising (1-3 months)
- **Chronic aspergillosis** (1-3 months)
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  - Aspergilloma of lung
  - Chronic fibrosing pulmonary
  - Chronic invasive sinusitis
  - Maxillary (sinus) aspergilloma
- **Allergic**
  - Allergic bronchopulmonary (ABPA)
  - Extrinsic allergic (broncho)alveolitis (EAA)
  - Asthma with fungal sensitisation (SAFS)
  - Allergic Aspergillus sinusitis (eosinophilic fungal sinusitis)

**Allergic bronchopulmonary aspergillosis**

ABPA
Denning – Chronic respiratory fungal diseases

ABPA - Diagnostic clues

• Asthma/CF not well controlled
• History of ‘pneumonia’
• History of coughing up plugs, or paroxysms of coughing that clear when chest clears
• Central bronchiectasis on CT scan, or mucoid impaction
• Eosinophilia

Rare cases in non-asthmatics, non-CF patients

31/03/99

FEV1 = 3.00
ABPA RAST = 31
IgE = 1900
No Rx

29/09/99

FEV1 = 1.6,
IgE = 3000
RAST = 52.5
Rx Atypical Pneumonia
FEV1 = 3.3 (post antibiotics)
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ABPA – bronchoscopy views showing mucous plugging

Mucoid impaction due to ABPA

Mucoid impaction due to ABPA
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Sputum in ABPA

A. fumigatus in BAL and in Bronchial Tissue in ABPA

Charcot Leyden crystals
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Eosinophils in bronchial tissue – ABPA is eosinophil/IgE mediated

Effect of A. fumigatus proteases on bronchial epithelium – H. Kauffmann

Colonisation in ‘normal’ lungs

<table>
<thead>
<tr>
<th>Study group</th>
<th>Patients</th>
<th>Fungal growth</th>
<th>No fungal growth</th>
<th>Total patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30</td>
<td>22 (73%)</td>
<td>8 (27%)</td>
<td>30</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>30</td>
<td>10 (33%)</td>
<td>20 (67%)</td>
<td>30</td>
</tr>
</tbody>
</table>

22 of 30 (73%) grew a fungus in both lung samples taken
10/30 (33%) grew >1 species

Lass-Florl et al, Br J Haematol 1999;104:745
Airborne fungal fragments

Summary - Immunopathogenesis of ABPA

- HLA-DR2/DR5 restriction
  - DRB1*1501, 1502, 1503, 1601
  - HLA-DR5: DRB1*1101, 1103, 1104, 1202
  - HLA-DQ2 is protective (DQB1*0201)
- IL-4Ra polymorphism
- IL-13 polymorphism
- IL-10 polymorphism
- SP-A2 polymorphism
- CFTR gene mutation

Central bronchiectasis as a complication of ABPA
Denning – Chronic respiratory fungal diseases

Retrospective comparison of antifungal treatment of SAFS with ABPA

Pasquallotto et al, unpublished data

22 patients with SAFS were compared with 11 with ABPA

Randomised studies of antifungals and ABPA and/or asthma

<table>
<thead>
<tr>
<th>Disease</th>
<th>Antifungal, duration</th>
<th>Benefit?</th>
<th>Author, year</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABPA</td>
<td>Natamycin inh, 52 wks</td>
<td>No</td>
<td>Currie, 1990</td>
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<tr>
<td>ABPA</td>
<td>Itraconazole, 32 wks</td>
<td>Yes</td>
<td>Stevens, 2000</td>
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<tr>
<td>ABPA</td>
<td>Itraconazole, 16 wks</td>
<td>Yes</td>
<td>Work, 2003</td>
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<tr>
<td>&quot;Trichophyton&quot; asthma</td>
<td>Fluconazole, 20 wks</td>
<td>Yes</td>
<td>Ward, 1999</td>
</tr>
<tr>
<td>SAFS</td>
<td>Itraconazole, 32 wks</td>
<td>Yes</td>
<td>Denning, 2009</td>
</tr>
</tbody>
</table>

Mystery cases
Denning – Chroni c respiratory fungal diseases

Mystery case 1

__________________________

__________________________

__________________________

__________________________

Mystery case 2

__________________________

__________________________

__________________________

__________________________

Mystery case 3

__________________________

__________________________

__________________________

__________________________
Denning – Chronic respiratory fungal diseases

Mystery case 4

The National Aspergillosis Centre

- Funded by the DoH National Commissioning Group
- Provision of care for all patients in England and Scotland with chronic pulmonary aspergillosis
- Strengthening of Regional Mycology Laboratory, Manchester
- Focus on outpatient care, with in patient, thoracic surgery and interventional radiology available
- Strong multi-disciplinary ethos, including immunology, mycology and respiratory medicine
- Support for clinical research in CPA and related issues (ie ABPA and diagnostics)
- All antifungal costs, including inpatient and outpatient IV therapy, payable through the NAC

www.aspergillus.org.uk
Denning – Chronic respiratory fungal diseases