Overview of biomarkers and Invasive Fungal Infections in Haematology

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Fungi

• Eukaryotic organisms

• Ubiquitous
  • Gardens, playgrounds
  • Houses, hotels, hospitals
  • Skin and mucosa of healthy persons and ptt
  • Constructions
  • *Airconds and ventilation*
  • Containers for biodegradable waste
  • *Foods*

• Opportunistic
Autopsy proven IFI in HM

Chamilos, Hematologica 2006
MORTALITY OF INVASIVE ASPERGILLOSIS IN RELATION TO UNDERLYING DISEASE
Lin, Schranz, Teutsch  Clin Infect Dis 2001;32:358
Hospital Ampang Jan-May 2008

- Gm pos 68/152 (44.8%)
  - Staph aureus 32 (2MRSA)
  - Coag neg staph 10
  - Strep haemolyticus 9
- Gm neg 68/152 (44.8%)
  - Acinetobacter baumanii 13
  - E coli 13 (2ESBL)
  - Pseudomonas (2MRO)
  - Klebsiella pneumoniae 9
- Candida 16/152 (6.6%)
  - Candida tropicalis 10
  - Candida glabrata 6
  - Galactomannan assay 7 pos

- Candida spp – 4th most common pathogen from blood cultures (*Pfaller and Diekema 2007*)
Important Pathogens Causing Fungal Infections in Hematology/Oncology Patients

- Aspergillus flavus
- Aspergillus fumigatus
- Aspergillus terreus
- Aspergillus niger
- Aspergillus nidulans

Candida

- C. albicans
- C. dubliniensis
- C. glabrata
- C. guilliermondii
- C. kefyr
- C. krusei
- C. lipolytica
- C. lusitaniae
- C. parapsilosis
- C. rugosa
- C. tropicalis
- C. pseudotropicalis
- C. pseudotropicalis
Fungal infections
Risk factors for IA

- **Prolonged neutropaenia**
- Depressed cellular immunity
  - Viral infections eg. CMV
  - Corticosteroids, CSA, MMF, ATG, anti-TNF, anti-IL2
  - Alemtuzumab
- Mucosal barrier injury
- Stem cell/organ transplant – GVHD, cord blood tx, mismatched and unrelated donors
- Inadequate prophylaxis
- Constructions
  - Park at al 2011
  - Safdar et al 2010
  - Neofytos et al 2009
Risk factors for Candidaemia

- Prolonged use of broadspectrum antibiotics/ steroids
- **Presence of central venous catheters**
- Surgery - GI
- Prolonged ICU stay, mechanical ventilation > 3 d
- Colonisation by Candida of multiple nonsterile sites
- Hyperalimentation
- Haemodialysis
  - Vasquez and Sobell 2011
  - Pappas 2006
GENETIC RISK FACTORS

TOLL-LIKE RECEPTOR 4 POLYMORPHISMS AND ASPERGILLOSIS IN STEM CELL TRANSPLANTATION

BOCHUD ET AL. NEJM 2008;359:17
### Spectrum of fungi in transplant

<table>
<thead>
<tr>
<th>IFI pathogen</th>
<th>HSCT (%)</th>
<th>Kidney (%)</th>
<th>Liver (%)</th>
<th>Lung (%)</th>
<th>Pancreas (%)</th>
<th>Heart (%)</th>
<th>Intestine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus</td>
<td>43–64</td>
<td>11–14</td>
<td>7–11</td>
<td>44–63</td>
<td>5–10</td>
<td>23–25</td>
<td>0</td>
</tr>
<tr>
<td>Mucorales</td>
<td>5–8</td>
<td>1–2</td>
<td>2–3</td>
<td>2–3</td>
<td>0</td>
<td>2–3</td>
<td>0</td>
</tr>
<tr>
<td>Fusarium</td>
<td>2–3</td>
<td>0</td>
<td>0</td>
<td>&lt;1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other mold</td>
<td>3–7</td>
<td>2–3</td>
<td>0–2</td>
<td>9–20</td>
<td>3–5</td>
<td>2–7</td>
<td>0</td>
</tr>
<tr>
<td>Candida</td>
<td>22–28</td>
<td>49–61</td>
<td>68–79</td>
<td>23–24</td>
<td>76</td>
<td>49–65</td>
<td>85</td>
</tr>
</tbody>
</table>

Neofytos et al. [2009]; Kontoyiannis et al. [2010]; Pappas et al. [2010].

IFI, invasive fungal infection; HSCT, hematopoietic stem cell transplantation.
Diagnosis of IFD

- Clinical, microbiological and radiological findings not reliable
- Cultures lack sensitivity/ specificity
- **Tissue sampling and invasive procedures not always feasible**
- **Initiation of appropriate antifungal Rx delayed leading to high mortality rates**
  - *Charmilos 2006*
  - *ECIL-3 BMT 2012*

- Need for reliable biomarkers
CT scans

- Main finding for IA is a peripheral nodule > 1cm
- Halo sign (Georgiadon 2011)
- >90% proven IA have at least 1 nodule
- >33% have positive halo sign
Use of biological markers - Galactomannan

*Wheat and T. Walsh.*

- Antigen component of Aspergillus cell wall
- Commercial sandwich ELISA kit (Platelia)
- Sensitivity 90.6% specificity 94%
- Antigen positive in 64.6% ptt with mean – 8.4d before radiological signs and – 6.9d before clinical sx
- Prolonged neutropaenia, allo SCT
- 2 to 3 times weekly
- Criteria for positivity (Maertens 2009)
  - 2 consecutive samples with GMI >=0.5
  - Single positivity >=0.7
- Considerations
  - Galactomannan Ag does not replace other tests
  - False positivity and false negativity
  - Turn around time should be appropriate
  - Useful in BAL
  - Performance in HM/HSCT different from SOT
  - Prognostic (*Kooet al, 2010*)
Other biological markers – bGlucan

O Marchetti, BMT2012

• 1,3-beta-D-glucan
  • Non-specific
  • Fungitec-G, Fungitell, Wako, Maruha
  • Sensitivity 50% specificity 76-83.8%
  • Different substrates, different reactivities and cut-offs
  • No major differences among assays
  • Diagnostic performance in IA and IC similar
  • Many issues to be resolved
    • Frequency of testing
    • Experience limited
    • Methodological concerns
    • False positives
Mannan Ag(Mn)/anti-mannan Ab (A-Mn)

- Mn is a major component of Candida cell wall
- Detects Candidal Ags, metabolites, Ab
- Platelia Candida Ags; Platelia Candida Abs
- Combined Mn/A-Mn test more useful
  - Sensitivity 83% Specificity 86%
- Sensitivity highest for C. albicans, glabrata and tropicalis
- In hepatosplenic candidiasis, positive at a median of 16d before radiological detection
PCR for IA

• Standardisation remains difficult
• Different gene targets
  • FKS gene, mitochondrial DNA, 18S rRNA, 5.8S rRNA, 28S rRNA
• Different detection techniques
  • Detection systems, clinical specimens, volume of sample
  • DNA extraction, PCR endpoints
• Environmental contamination of reagents
• Negative predictive value may be more useful
Site of Action of Selected Antifungal Agents

Cell membrane
Polyenes
Azoles
Cell wall
Glucan synthesis inhibitors (echinocandins)

Mechanisms of Action of Current Therapies and Implications for Efficacy

<table>
<thead>
<tr>
<th>Agent</th>
<th>Fungal Cell Target</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amphotericin B</strong></td>
<td>Membrane</td>
</tr>
<tr>
<td><strong>Azoles</strong></td>
<td>Membrane</td>
</tr>
<tr>
<td><strong>Echinocandin</strong></td>
<td>Wall</td>
</tr>
</tbody>
</table>

Current available antifungal agents

**Amphotericin B**
- No longer the ‘gold standard’
- Broad spectrum with activity against most yeast and moulds
  **But**
- i-v only
- Toxic; intolerant

**Amphotericin B lipid formulations**
- lipid complex, colloidal dispersion or liposomal amphotericin B
- Not interchangeable, higher doses required
- Similar spectrum of antifungal activity with reduced toxicity
- NOT better than cAmB
  **But**
- COSTLY
- i-v only
- Not devoid of toxicity
Antifungals

• Azoles
  • Fluconazole
  • Itraconazole
  • Voriconazole
  • Posaconazole
    – Activity similar to vori, active vs Mucor

• Echinocandins
  • Caspofungin
  • Anidulafungin
  • Micafungin

• ?combination
Species identification important

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Voriconazole</th>
<th>Amphotericin B</th>
<th>Echinocandins</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>S-DD to R</td>
<td>S-DD to R</td>
<td>S</td>
<td>S-I</td>
<td>S</td>
</tr>
<tr>
<td>C. krusei</td>
<td>R</td>
<td>S-DD to R</td>
<td>S</td>
<td>S-I</td>
<td>S</td>
</tr>
<tr>
<td>C. lusitaniae</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S to R</td>
<td>S</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S-R</td>
<td>S-I</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

Vazquez and Sobel [2011]; S, susceptible; S-DD, susceptible dose-dependent; R, resistant; I, intermediately susceptible.

- Drug of choice for IFI depends on infecting spp and clinical setting
Treatment strategies in IFD

• Strategy employed depends on the different degrees of risks of ptt developing IFI and immune status
Treatment strategies in HIGH RISK neutropenics

Neutropenic patient at risk for IFI*

Asymptomatic
- Fever plus radiologic findings suggestive of fungal infection**, and/or
- Positive screening test***
- No definitive histopathological and/or culture identification of fungal pathogen

Symptomatic
- Fever despite 4-7 days of appropriate antibacterial therapy
- Unknown source of infection
- Cultures remain negative

Documented IFI

Prophylaxis
Preemptive Therapy
Empiric Therapy
Targeted Therapy

References:
* Patient at risk for IFI include prolonged neutropenia after intense chemotherapy for hematologic malignancy, and high-risk HSCT and SCT recipients
** IFI: invasive fungal infections
*** Radiologic findings suggestive of IFI include high-resolution computed tomography scan of the thorax showing new ≥1 cm single or multiple nodules with or without halo sign, lobar consolidation, wedge-shaped consolidative infect.
**** Screening tests include Aspergillus galactomannan, 1,3 beta D-glucan, and for PCR
IDSA 2002 guidelines for empirical therapy

Persistent fever during first 3–5 days of treatment: no etiology
Reassess patient on days 3–5

- Continue initial antibiotics
  - If no change in patient’s condition (consider stopping vancomycin)
- Change antibiotics
  - If progressive disease,
    - if criteria for vancomycin are met
- Antifungal drug, with or without antibiotic change
  - If febrile through days 5–7 and resolution of neutropenia is not imminent

Guide to treatment of patients who have persistent fever after 3–5 days of treatment and for whom the cause of the fever is not found.
# Empirical antifungals

## Empirical Antifungal Therapy of Neutropenic Patients with Prolonged Fever Despite Antibacterial Therapy

<table>
<thead>
<tr>
<th>Empirical therapy</th>
<th>ECIL 2009</th>
<th>IDSA 2008</th>
<th>BSH 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B II</td>
<td>A I/All</td>
<td>Discouraged (A I)</td>
</tr>
<tr>
<td>L-AMB</td>
<td>A I</td>
<td>A I</td>
<td>If given, A I</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>A I</td>
<td>A I</td>
<td>If given, A I</td>
</tr>
<tr>
<td>ABLC / ABCD</td>
<td>B I</td>
<td>A I</td>
<td>-</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>B I</td>
<td>A I</td>
<td>-</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>B I</td>
<td>A I</td>
<td>-</td>
</tr>
<tr>
<td>D-AMB</td>
<td>B / D I</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>C I</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Micafungin</td>
<td>B II</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-AMB or Caspofungin in children</td>
<td>B II</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Rationale for pre-emptive treatment

- Too many patients receive empirical AF therapy unnecessarily
- Targeting the high risk patient (AML, allo HSCT) is a logical response
- Using an individualized approach seems even more logical
- There are new diagnostic advances
  - Galactomannan monitoring
  - Early and repeated lung CT scan
  - Maybe beta-D-glucan
  - PCR in the future?
Common manifestations of IFI

• Unresponsive fever
• Invasive candidiasis
  • Macronodular lesions
  • Muscle tenderness
  • Septic shock; multiorgan failure
  • Candidal endophthalmitis (5%) – late
  • Cholestatic jaundice with hepatosplenomegaly
    (Vasquez and Sobell 2011)
• Invasive aspergillosis
  • Cough; hemoptyis with pleuritic chest pain
  • Nasal bleed; sinus pain
  • Pulmonary infiltrates
  • CNS symptoms; stroke
  • Unusual thrombosis
  • Skin gangrene, digital gangrene
    (Nucci 2003)
Empirical vs Preemptive Antifungal Rx for high risk, febrile, neutropaenic ptt: RCT

*Cordonnier et al. CID 2009;48:1042-51*
Empirical vs Preemptive Rx

![Graph showing cumulative incidence of IFI and antifungal treatments over neutropenia duration]

<table>
<thead>
<tr>
<th>Description</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of antifungal drugs, 2005 €</td>
<td>2252 ± 4050</td>
<td>0–20,726</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Estimated cost of antifungal drugs if liposomal AmB had been used instead of AmB deoxycholate, 2005 €</td>
<td>4261 ± 4760</td>
<td>0–21,727</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>
Tailored diagnostic and therapeutic approach

Aguilar-GM. Haematologica 2012;97:464-71
Posaconazole vs Fluconazole/ Itraconazole prophylaxis in high risk neutropenic ptt
Cornely et al. NEJM 2007;356:348-59

• AML/MDS undergoing chemotherapy – high risk
• 602 ptt – posa 200mg tds(298) vs fluco 400mg (240) & Itra soln 200mg bd(58)
• Proven/probable IFI 2% vs 8% (p<0.001)
• IA 1% vs 7% (p<0.001)
• Survival longer in posa group (p=0.04)
• SAE higher 6% vs 2% (p=0.01)
ECIL 3 guidelines – 2009

J Maertens et al, BMT (2011)46,709-718

• Antifungal primary prophylaxis in leukemia induction
  • Fluconazole 50-400mg/d (C1)
  • Itraconazole soln 2.5mg/kg bid (C1)
  • Posaconazole 200mg tid (A1)
  • Echinocandins iv (insufficient data)
  • Polyenes iv (C1)
### Table 2 Antifungal therapy strategies in ICU patients

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Antifungal agent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prophylaxis</strong></td>
<td>Fluconazole</td>
</tr>
<tr>
<td>No generally recommended. Patients with upper gastrointestinal perforation, heavy <em>Candida</em> colonization or with severe acute pancreatitis might be benefited</td>
<td></td>
</tr>
<tr>
<td><strong>Empirical</strong></td>
<td>De-escalation therapy (*), the choice of antifungal drug must be based on the individual characteristics of the patient</td>
</tr>
<tr>
<td>Use of “<em>Candida</em> score” or the Ostrosky-Zeichner prediction rule</td>
<td></td>
</tr>
<tr>
<td><strong>Pre-emptive</strong></td>
<td>De-escalation therapy (*), the choice of antifungal drug must be based on the individual characteristics of the patient</td>
</tr>
<tr>
<td>Based on detection of galactomannan, (1,3)-β-D-glucan or <em>C. albicans</em> germ tube antibodies</td>
<td></td>
</tr>
<tr>
<td><strong>Targeted</strong></td>
<td>De-escalation therapy (<em>), the choice of antifungal drug must be based on the individual characteristics of the patient (</em>**)</td>
</tr>
<tr>
<td>Based on sterile site culture results</td>
<td></td>
</tr>
<tr>
<td>Strategy</td>
<td>Antifungal agent</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------------</td>
</tr>
<tr>
<td>Prophylaxis</td>
<td>High risk patients*: Itraconazole or Posaconazole or Voriconazole</td>
</tr>
<tr>
<td></td>
<td>Intermediate risk patients*: Fluconazole</td>
</tr>
<tr>
<td>Empirical</td>
<td>Severely neutropenic cancer patients with persistent or relapsing fever despite broad-spectrum antibacterial therapy</td>
</tr>
<tr>
<td>Pre-emptive</td>
<td>Based on GM assays or CT or bronchoscopic cultures</td>
</tr>
<tr>
<td>Targeted</td>
<td>Based on sterile site culture results</td>
</tr>
</tbody>
</table>
# IDSA guidelines for treatment of invasive aspergillosis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Therapy&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Alternative&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive pulmonary aspergillosis</td>
<td>Voriconazole (6 mg/kg IV every 12 h for 1 day, followed by 4 mg/kg IV every 12 h; oral dosage is 200 mg every 12 h)</td>
<td>L-AMB (3–5 mg/kg/day IV), ABLC (5 mg/kg/day IV), caspofungin (70 mg day 1 IV and 50 mg/day IV thereafter), micafungin (IV 100–150 mg/day; dose not established&lt;sup&gt;c&lt;/sup&gt;), posaconazole (200 mg QID initially, then 400 mg BID PO after stabilization of disease&lt;sup&gt;d&lt;/sup&gt;), itraconazole (dosage depends upon formulation)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
IDSA treatment guidelines for invasive candidiasis

<table>
<thead>
<tr>
<th>Condition or treatment group</th>
<th>Therapy</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Candidemia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonneutropenic adults</td>
<td>Fluconazole 800-mg (12-mg/kg) loading dose, then 400 mg (6 mg/kg) daily or an echinocandin® (A-I). For species-specific recommendations, see text.</td>
<td>LFAMb 3–5 mg/kg daily; or AmB-d 0.5–1 mg/kg daily; or voriconazole 400 mg (6 mg/kg) bid for 2 doses, then 200 mg (3 mg/kg) bid (A-I)</td>
</tr>
<tr>
<td>Neutropenic patients</td>
<td>An echinocandin® or LFAMb 3–5 mg/kg daily (A-II). For species-specific recommendations, see text.</td>
<td>Fluconazole 800-mg (12-mg/kg) loading dose, then 400 mg (6 mg/kg) daily; or voriconazole 400 mg (6 mg/kg) bid for 2 doses then 200 mg (3 mg/kg) bid (B-III)</td>
</tr>
</tbody>
</table>
Combination antifungals

• IDSA 2008 – combination has potential but data unclear

• Synnergistic activity
  – Azole + echinocandin
  – Polyene and echinocandin

• Antagonistic interactions
  – Polyene and azoles
  – Combination of voriconazole or posaconazole and AmB did not show antagonism in vitro and enhance efficacy in animal models

• Novel pathways
  • Calcineurin pathway
  • Hsp90
Don’t forget ..

- Handwashing
- Diet
- **Remove indwelling catheters, central lines**
- G-CSF
- Granulocytes
- **Reduce immunesuppression, if possible**
- Surgery
- HEPA filter
  - Reduced PA 6% to 2%
  - TRM in matched sib 0.76
  - TRM in alternate donors 0.65
  - non-HEPA rooms RR 6.5
Conclusions

• Fungal infections underdiagnosed and undiagnosed

• Improved diagnostics available but can be improved; standardisation

• Non-toxic antifungals with good efficacy; costly but more cost effective

• Identification of species, and perhaps drug levels in few situations to be considered