Invasive Fungal Infections in Critically Ill Patients

Dr Ravinder Kaur
Director Professor&HOD
Department of Microbiology,LHMC
Invasive fungal infections (IFIs)

- Major causes of morbidity and mortality in seriously ill hospitalized patients / I/C pts

- Candidiasis and Aspergillosis are the most common etiological agents in pts receiving Immunosuppressive t/t for C/A / organ transplants

- No. of cases of IFIs \(\uparrow\) ed in last decades

- Increasing mortality
INCREASE IN FUNGAL INFECTIONS

- less mortality from other causes
  - underlying disease
  - better antibacterial therapy
- Increasing age of pts.
- Better diagnostic tools
- More complex interventions
The no. of **fungal spp** → considered as **potential fungal pathogens** has increased during last few decades
Review of our Fungal “Players”

• Oppportunistic fungi
  – Normal flora
    • Candida spp.
  – Ubiquitous in our environment
    • Aspergillus spp.
    • Cryptococcus spp.
    • Mucor spp.

• Endemic geographically restricted
  • Blastomyces sp.
  • Coccidioides sp.
  • Histoplasma sp.

• Newly emerging fungi
  • Fusarium spp
  • Scedosporium spp
  • Trichosporum spp
Increasing rate of candidiasis in the US

Martin et al, NEJM 2003;348:1546
Aspergillus –
38 species have caused disease
Common in the environment

A. nidulans – may be amphotericin B resistant
A. terreus – resistant to AmB

Aspergillus – 38 species have caused disease
Common in the environment
Aspergillosis

Aspergillus species are found in:

- Soil
- Air; spores may be inhaled
- Water / storage tanks in hospitals etc
- Food
- Compost and decaying vegetation
- Fire proofing materials
- Bedding, pillows
- Ventilation and air conditioning systems
- Computer fans

Aspergillus spores
Development of Aspergillosis

- INHALATION
  - Environmental exposure
  - Construction

- COLONIZATION
  - Pulse steroid
  - OKT3/Antilymphocyte therapy
  - Antibiotic use
  - Organ failure
  - Re-transplantation
  - Thrombocytopenia

- INFECTION

- DISSEMINATION
Zygomycosis

- Vulnerable populations
  - Malignancy
  - Bone marrow transplantation
  - Solid organ transplantation
    - Corticosteroid exposure
    - GvHD
    - CMV reactivity
    - Neutropenia
    - Uncontrolled Diabetes mellitus
  - Initial presentation with sinusitis
  - Occurrence as breakthrough prior Voriconazole prophylaxis
- 56% mortality

Others...
Fusarium sp.
Penicillium sp.
Trichosporon sp.

Marr, CID 2002
Steinbach, J Infect 2004

Kontoyiannis et al. JID, 2005; 191:1350-60.
Siwek et al. CID 2004; 39:584-87.
STRANGE DUCKS IN THE IMMUNOSUPPRESSED POND

Fusarium

Pseudallescheria boydii

Scedosporium

Mucor/Rhizopus

Alternaria
DEVELOPMENT OF FUNGAL INFECTIONS OVER TIME

- Aspergillus
- Candida
- Other yeasts
- Other moulds
Incidence of Invasive Fungal Infections

- **Solid Organ Transplant**: 5 - 42%
  - Kidney: 5 – 14%
  - Heart: 5 – 32%
  - Heart-Lung/Lung: 15 – 36%
  - Pancreas: 18 – 38%
  - Liver: 7 – 42%

- **Bone Marrow Transplant**: 15 - 25%

- **Intensive Care Unit**: 17%
Distribution of fungal pathogens causing invasive fungal infections in transplant recipients

<table>
<thead>
<tr>
<th>IFI pathogen</th>
<th>Type of transplantation</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>HSCT (%)</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.
Invasive Candida infections in the USA The NEMIS study

• 6 Surgical **Intensive Care Units** in USA
• Overall rate 9.82/1000 admissions or 0.98/1000 patient days (range 0.28-1.78)
• **48% C. albicans**
• **Mortality of Candida bloodstream infections** 41% vs 8% in those without

Blumberg HM et al, Clin Infect Dis 2001:33 177-86
Prospective study of candidaemia in European cancer centres

- 289 episodes
- C. albicans in 70% of cancer and 36% of leukaemia patients
- Other species – C. parapsilosis (27)
  - C. tropicalis (23)
  - C. glabrata (21)
  - C. krusei (21)
  - C. guilliermondii (11)
  - other Candida spp. (7)

Mortality Rates

- **Candidemia** has a mortality rate of ~40%.

- **Invasive aspergillosis** continues to be a highly lethal opportunistic infection:
  - 375% increase in mortality due to *Aspergillus* species from 1980 to 1997.
  - Overall mortality rate in patients with invasive aspergillosis is reported to be 58%.

- Mortality continues to be high regardless of the antifungal therapy used.
Adjusted* odds ratio for difference:
2.09 (95% CI, 1.53-2.84; P=0.018)

FIGURE 2. RISK FOR HOSPITAL MORTALITY INCREASES SIGNIFICANTLY WITH DELAYED ANTIFUNGAL THERAPY
LETHALITY OF THE VARIOUS INVASIVE FUNGAL INFECTIONS

- Aspergillus: 42% casualties
- Zygomycetes: 61% casualties
- Fusarium: 53% casualties
- Candida: 33% casualties
- Cryptococcus: 50% casualties
- Trichosporon: 29% casualties

Number of cases:
- 0
- 100
- 200
- 300
- 400

Legend:
- Gray: cases
- Red: casualties
# Invasive fungal infection – current mortality rates

<table>
<thead>
<tr>
<th>Infection Type</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillosis</td>
<td></td>
</tr>
<tr>
<td>Pulmonary aspergillosis</td>
<td>50-75%</td>
</tr>
<tr>
<td>Cerebral aspergillosis</td>
<td>95%</td>
</tr>
<tr>
<td>Candidiasis</td>
<td></td>
</tr>
<tr>
<td>Candidaemia</td>
<td>40%</td>
</tr>
</tbody>
</table>
Indian Perspective

- Recipients of solid organ transplants
- have 6–10% incidence of opportunistic fungal infections
- very high mortality of 70–100% in the Indian subcontinent.
Trends in fungal diseases

- Increasing cases of invasive fungal infections
- Replacement of sensitive species by resistant ones
# Antifungal susceptibility in *Candida* spp.

<table>
<thead>
<tr>
<th>Usually susceptible</th>
<th>Less susceptible</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fluconazole</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td><em>C. tropicalis</em></td>
<td><em>C. glabrata</em></td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td></td>
<td><em>C. krusei</em></td>
</tr>
<tr>
<td>All others</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Amphotericin B</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td><em>C. lusitaniae</em></td>
<td><em>C. krusei</em></td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td><em>C. glabrata</em></td>
<td></td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Caspofungin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td><em>C. parapsilosis</em></td>
<td></td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td><em>C. guilliermondii</em></td>
<td></td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td><em>C. lusitaniae</em></td>
<td></td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Candida glabrata and Candida krusei

- Fluconazole intermediate or resistant
- Respond poorly to amphotericin B treatment
- Increasingly common
Biofilms and *Candida parapsilosis*

- 2nd most common species in blood, related to catheters and glucose solutions
- Causes biofilms which usually require removal of catheters etc, as antifungal drugs are ineffective in eradicating biofilms

Infected pacemaker and heart valve, after death
**Candida auris**

- **serious infections** -- bloodstream infections
  - death -- hospitalised pts
- **resistant to medicines** -- all three types of antifungals
- **more common** -- discovered in 2009
  - spread quickly -- infns > a dozen countries
- **difficult to identify...??** *Candida haemolouni*
- **spread in hospitals** -- causes outbreaks
  - spread through contact -- pts & contaminated surfaces or equipment
Trends in fungal diseases

- Increasing cases of invasive fungal infections

- Poor diagnostic tools---Diagnostic limitations

**Challenges**

Delaying antifungal therapy until blood cultures are positive is associated with increased mortality
Prevalence of invasive aspergillosis at autopsy

In 1992, 60% of the patients were undiagnosed and untreated.

ESTIMATING TIME FOR INTERVENTION

Aspergillus

Persisting fever +

• very high risk
or
• a suggestive symptom
or
• a suspected sign
or
• any positive test

day 1  5  7  12  //  28  > 42
• Diagnosis of fungal infection still **problematic**

  - Cl symps non specific
  - Conventional assays may take many days

• Direct Microscopic examinations

• Culture Results

• Biomarkers
  - 1,3 b-D-glucan (BG)
  - Galactomannan (GM)a

• Serological Diagnosis (Antigen Detection Assay)

• Molecular Methods (PCR)
Diagnosis

• Early Diagnosis is desirable
  – Models
    • Candida score for IC
    • EORTC/MSG criteria for IA
  – Limitations
    • Predictive value (PPV/NPV) depends on prevalence
      – Sensitivity and specificity
    • Active surveillance is required
      – Cost effectiveness
Revised Definitions of Invasive Fungal Disease
An International Consensus (EORTC/MSG)

Invasive Fungal Infections Cooperative Group:
European Organization for Research and Treatment of Cancer
Mycoses Study Group:
National Institute of Allergy and Infectious Diseases
Clin Infect Dis 2008 Jun; 46:1813-21
Audit definitions of IFI

| Proven          | Probable                                                       | Possible                                         |
|-----------------|                                                               |                                                 |
| Fungi in histology, micro from a sterile site or BC       | Host factors & Clinical/radiology (eg HRCT – dense lesions +/- halo) & Micro criteria (eg fungi from sputum/BAL) | Host factors & Clinical/radiology (eg HRCT – dense lesions +/- halo) (ie no micro results) |
|                 |                                                               |                                                 |
| (ie need biopsy) |                                                               |                                                 |

Overall IFI = proven + probable

Clinical IFI = proven + probable + possible

Ascioglu et al CID 2002;34 p7-13
Pauw et al 2008 CID 2008: 46 p1813
Clinical approaches to assess risk

• **Fungal colonizing index**: the greater the number of positive sites, the greater the increased risk for invasive infection

• **Combine colonization with other risk factors**: surgery on admission, TPN, and sepsis

• **No colonisation index but include variables**: ≥ 4 days in ICU, CVC, DM, new hemodialysis, TPN, and broad-spectrum antibiotics

Diagnostic Tools

Colonization Index

Candida Score

Prediction Rule
The Colonization Index (CI) & CCI

\[
\text{CI} = \frac{\text{Number of colonized sites}}{\text{Number of tested sites}}
\]

\[
\text{CCI} = \text{CI} \times \frac{\text{Number of site with heavy colonization}}{\text{Number of tested sites}}
\]
<table>
<thead>
<tr>
<th>Colonization Parameters</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Negative Predictive Value, %</th>
<th>Positive Predictive Value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonization at ≥2 body sites</td>
<td>100</td>
<td>22</td>
<td>100</td>
<td>44</td>
</tr>
<tr>
<td>Colonization at &gt;2 body sites</td>
<td>73</td>
<td>56</td>
<td>77</td>
<td>50</td>
</tr>
<tr>
<td>Colonization at ≥3 body sites</td>
<td>45</td>
<td>72</td>
<td>68</td>
<td>50</td>
</tr>
<tr>
<td>Colonization index*</td>
<td>100</td>
<td>69</td>
<td>100</td>
<td>66</td>
</tr>
<tr>
<td>Corrected colonization index*</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
The Candida Score

Calculation of the Candida score:

<table>
<thead>
<tr>
<th></th>
<th>Coefficient (β)</th>
<th>Rounded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multifocal <em>Candida species colonization</em></td>
<td>1.112</td>
<td>1</td>
</tr>
<tr>
<td>Surgery on ICU admission</td>
<td>0.997</td>
<td>1</td>
</tr>
<tr>
<td>Severe sepsis</td>
<td>2.038</td>
<td>2</td>
</tr>
<tr>
<td>Total parenteral nutrition</td>
<td>0.908</td>
<td>1</td>
</tr>
</tbody>
</table>
The Candida Score

With a cut-off value of 2.5: sensitivity of 81% and a specificity of 74%, we shall only need the presence of sepsis and any one of the three other remaining risk factors or the presence of all of them together except sepsis in order to consider starting antifungal treatment for one particular patient.
Clinical Prediction Rule

<table>
<thead>
<tr>
<th>One of the following factors:</th>
<th>Systemic Antibiotic</th>
<th>Presence of CVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ at least two other risk factors</td>
<td>Total parenteral nutrition</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Major surgery</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pancreatitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Any use of steroids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Use of immunosuppressive agents</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity of 34% and specificity of 90%, a positive predictive value of 10% and a negative predictive value of 97%.

Mainly helps in ruling out invasive candidiasis.
Clinical Diagnosis: Candida

The clinical manifestations of IC are nonspecific, but may include:

- **Fever and progressive sepsis** with multi-organ failure despite antibiotics.
- **Invasive candidiasis (IC) related cutaneous lesions.**
  - Macronodular rash frequently confused with drug allergies. A biopsy of the deeper layers of skin particularly the vascularized areas and the dermis is important.
- **Ophthalmic lesions (Candida endophthalmitis).**
  - A fundoscopic evaluation for the presence of *Candida* endophthalmitis should be performed in patients with candidemia.
Microbiological Criteria (I):

- Collecting appropriate tissues and other sterile specimens
- Difficulties
  
  Patient’s condition unstable
  Neutropenia / platelet count is low,
  May require invasive procedures.
  Deep tissue sampling-NA/ Impossible
Microscopy-Histopathology

• Gomori methenamine silver or periodic acid-Schiff (PAS) staining.
• Fluorescent staining
• Most tissue stains are inexpensive.
• Performed easily in various specimens, such as sputum, BAL fluid, aspirates from lesions, CSF and other tissues.
• Sensitivity – 100%
• Specificity – 100%

• Most accurate tissue test is tissue biopsy/deep tissue sampling
• Used as a last resort in undiagnosed cases.
Histopathology

- Detects both the invasion of various tissues by fungi and the host response or tissue necrosis.
- Clarify if a positive culture is the result of infection, colonization, or contamination.
- Have the potential to inform clinicians if a fungal biofilm has formed, a condition that is known for its resistance to commonly used antifungal regimens.

False positive results:
- accurate identification to the species may not be possible
- *Fusarium* and *Scedosporidium* spp. have similar macroscopic appearances

False negative results: Formation of pseudoseptations by the organism
Fluorescence in situ hybridization (FISH)

• Uses fluorescent probes to identify target areas on genomes of microbial pathogens in human samples, which can then be detected by fluorescence microscopy.

• High accuracy for the identification of Candida sp. infections from blood culture bottles.

• Data from two studies, on coccidioidomycosis and invasive fungal rhinosinusitis, show that the method has a promising performance on frozen tissue sections, even in cases where cultures are not available or have not been performed.
Fluorescence in situ hybridization (FISH)

Fluorescent DNA probes against target DNA sequence → Add tested sample → Probes bind to target sequence → Fluorescence detected using fluorescent microscope
Blood cultures

- Gold standard
- Initial diagnostic test when candidemia suspected
- Cultures take 1 to 3 days to grow
- Additional 1 to 2 days for identification of organism
- Sensitivity: 50% - 60%
- Specificity: 95%
- Delay in initiation of targeted treatment
- Cause daily increases in mortality
- Increased hospitalization costs
False negative results: if clinical specimens are obtained after treatment with antifungal agents

Multiple or repeated blood cultures --- to increase the likelihood of detecting candidemia, and filamentous fungi.

Blood cultures (BC) may be negative in the face of disseminated disease.

In zygomycosis, aetiologic agent loses its viability during tissue homogenization before culture.
Blood culture sensitivity – A controversy

• Culture can be useful and are thus needed to optimize patient management.

• Negative result on direct or pathologic smears and cultures do not rule out infection, so it is essential to use other suggested methods.
Bactec 9240 and Bac/T Alert

- Most commonly used
- Average detection time ranges from 14 to 38 h
- May take up to 72 h
- Varies depending on the culture conditions used
- And on the number of circulating cells
- Still unclear whether such collection system can improve the diagnostic yield in cases of fungemia
Current limitations of classical and new Diagnostic test for IFI- Issues

Conventional methods of Microscopy and culture rarely positive because:

Invasive Candidiasis (IC)
- Patients on antifungal prophylaxis
- Imaging not helpful
- Diagnosis is mainly clinical

Invasive Aspergillosis (IA);
- Initially affects the lungs, easily go unnoticed because no clinical symptoms
- Even when recognized early, suitable specimens can be difficult to obtain
- In LTR pts with IA - 50% who had Aspergillus in BAL, 22 fold at risk of IA (Singh et al 1997)

Lack of adequate diagnoses makes estimating the prevalence and incidence of IFI unreliable
B-glucan assay

- Major component of fungal cell wall -- Presumptive diagnosis of IFI
- Optimal specimen type – Serum
- A cutoff of 80pg/ml is associated with higher accuracy
- Excellent specificity (98.9%)
- Low sensitivity (49.6%)
- used as screening test for various fungal infections
- Useful for patients with intra-abdominal infections
- Sensitive with a good negative predictive value i.e. good for excluding infection
• BG testing in adults is considered as having good diagnostic accuracy for early diagnosis of IFD;
• In children, data are too limited to make any recommendations
• Found in sera of patients suffering from invasive candidiasis, invasive aspergillusosis (IA), invasive fusariosis, and Pneumocystis jirovecii infection---- not specific to a fungus
B-glucan assay

- When measured before starting antifungal therapy empirically on postoperative patients, colonized with candida infections.
  - 47% of those with positive test responded to Rx
  - Number of sites colonized with candida also predicted response. Colonization at \( \geq 3 \) sites vs. 1 site (p=0.03) (OR=7.57).

- In postoperative patients colonized with candida & with fever despite antibiotics
  
  useful for deciding whether to start empiric therapy.

In patients with leukemia ---- who were receiving antifungal prophylaxis ---- Obtaining multiple samples increased the sensitivity, PPV, and NPV of the BDG assay to \( >98\% \).
B-glucan assay

**False positive results**
- Dialysis filters made from cellulose
- Bacteremia
- Antibiotics such as cefepime, piperacillin/tazobactam or meropenem may cause positive BG levels....False positive

**False negative results**
- Hemolyzed samples
- Higher cutoff values
Galactomannan (GM) assay

- Detection of component of Aspergillus cell wall, **Galactomannan**
- Suggested as **predictor of all-cause mortality**
- Optimal diagnostic cutoff is not yet established.
- **Serum value of >1** is considered a sign of **therapeutic failure** in adults and paediatric patients.
- Quantification in BAL (cut-off >1) and CSF (cut-off >0.5) samples may be useful in neutropenic and non-neutropenic patients.
- Studies have shown that using an **index cutoff for positivity of 0.5 versus greater indices** substantially increases sensitivity, with only minimal loss in specificity.
- GM levels could be used to **monitor the response to treatment**
- **IDSA guidelines** currently recommend using GM EIA in conjunction with CT scans for **early, noninvasive diagnosis** of invasive aspergillosis in high-risk patients
Galactomannan (GM) assay

• Specificity and sensitivity vary from 40 to 100%.
• Serum serial testing is also useful in neutropenic paediatric patients.

• Comparison of 5 studies and meta-analysis, indicate sensitivity and specificity of 76% to 73% and 86% to 90% in children and adults, respectively.
• Sensitivity is higher
  non-fumigatus aspergillosis
  hematological malignancies > I/C patients.
  performed with BAL fluid > serum
  Caspofungin t/t
Galactomannan (GM) assay

- Factors, which increase false positivity and influence the specificity of the assay, include
  - A low level of cut-off (<0.5)
  - Colonization with Bifidobacterium bifidum in the intestinal flora, which mimics the epitope recognized by the EB-A2 in ELISA kit
  - Invasive infections with other fungi, such as spp., histoplasmosis, and blastomycosis. *Penicillium*
  - Cross-reactivity of the assay has been shown with the use of piperacillin/ tazobactam or amoxicillin/ clavulanate antibiotic therapy
  - in infants with the nutrition of milk-based formulas.
Galactomannan (GM) assay

- Severe mucositis and gastrointestinal GHVD following HSCT - can occasionally lead to false positive results, due to translocation of GM across the intestinal mucosa during periods of reduced mucosal integrity.

- Younger age associated with lower specificity rates – due to high concentration of GM in children’s food (e.g., cereals).
• **PCR offers the promising potential of being able to identify the**
  - presence of fungal pathogens within human fluids
  - define the species
  - quantify the infection (Real time)
  - detect antimicrobial resistance markers

**Real-time PCR**
• Real-time PCR and in situ hybridisation with commercial probes have shown the best results (i.e., high sensitivity, specificity, PPV and NPVs) for the diagnosis of IC using blood specimens.
• PCR-based assay had a **sensitivity of 90.9%** versus 45.4% for blood culture.
  - Invasive Candidiasis (80% versus 56%)
  - Deep-seated Candidiasis (89% versus 53%)

**Multiplex PCR**
• Detect a wide variety of fungi at once in the same specimen.
• At the forefront of conditions that need to be optimized are
  ➢ nucleic acid isolation methods
  ➢ primer selection
  ➢ fluid sampling

• Eventual total automation will help with enhancing the reproducibility of this technique.
MALDI-TOF-MS

• Matrix Assisted Laser Desorption Ionisation – Time of Flight – Mass Spectroscopy
• Identify the protein fingerprints of different microorganisms
• Accurately and rapidly identify Candida spp. and Aspergillus spp. from positive cultures, with a high concordance (consistently 90%).
• Much faster (3 h versus 24 h)
• Outperform traditional identification techniques
• It is possible to identify the detected microbe at the genus, species, and even strain levels
MALDI-TOF-MS

B  MALDI-TOF (Matrix assisted laser desorption ionization time-of-flight mass spectrometry)
Future trends......

• Nucleic acid testing will continue to be one of the leading growth areas in laboratory medicine with increasing applications.

• Less complex and more accessible techniques.

• Increased automation with simple automated sample processing.

• Use of multiplex / broad range assays.

• DNA Microarray / Microchip based systems will move from research to clinical labs.
Take Home Messages (I)

• Diagnosis
  – A challenging issue for clinicians
  – Risk Factors
    • IA and IC or other fungi
  – Usefulness
    • CT image
    • Biomarkers
      – Galactomannan test
Take Home Messages (II)

• Selection of appropriate antifungal agents
  – Disease severity
  – Risk category
  – Prior antifungal exposure
  – Fungal species
    • Candida vs. Aspergillus
    • Less susceptible for azoles (fluconazole)
      – C. krusei, C. glabrata
Take Home Messages (III)

• Antifungal agents
  – Azoles: best in terms of tissue penetration and bioavailability; but problematic with drug-drug interactions
  – Echinocandins: good in toxicity profiles; few drug-drug interactions; fungicidal against *Candida* species and generally fungistatic against *Aspergillus* species; **not active against other fungi**
  – Amphotericin-B: broad spectrum in antifungal activity but toxicity is of concern; active against various fungi
Take Home Messages (IV)

• Antifungal prophylaxis
  – Not favored in general ICU patients
  – Roles in high risk groups such as transplant recipients or cancer patients
The utility of standard blood cultures is limited because of a high percentage of false-negative results, particularly in patients with disseminated aspergillosis.

Antifungal strategy in Acute Lymphoblastic Leukemia (ALL) patients
Clinical Management of Invasive Fungal Infections:
An Evidence-Based Approach
Diagnostic Dilemma

- Clinical Setting: with other risk factors
- Radiology: applicable more for Aspergillus
- Cultures: Low yield and longer time
- Staining: GMS and Calcofluor white
- PCR Assay: not widely available
- 1-3 Beta Glucan Assay:
- Galactomannan Assay: For Aspergillus
- PNA FISH:
Challenges in Management of Invasive Fungal Infections (IFI) in Immunocompromised (IC) Patients

New mycologic challenges in IC patients

- Know the Changing Epidemiology of IFI
- Nonspecific presentation of IFI
- Inadequate diagnostic methods
- Antifungal prophylaxis – Does it work?
- Breakthrough Infections while on antifungal therapy
- Refractory to antifungal treatment
Lessons learned

• Fungal infections have significant consequences, both clinical and economic

• Measurement is critical – if you don’t measure you don’t know!

• For complex problems, solutions often cross Departments e.g. Haematology, Pharmacy, Ward, Engineering, Pathology etc

• Executive support is essential to facilitate this.

• Big gains can be made where you may least expect them, both in terms of patient safety and financial
"I suppose this is goodbye."