**Mortality** due to invasive fungal infection
- 97-100% if not treated
- ~50% even after proper treatment
- Why so poor outcome despite antifungals?

**Management 'dictum'** — early diagnosis & prompt therapy

**Dilemma** — in absence of diagnosis, Which patient has fungal infection?

**Clinical symptoms & signs not specific**
- Occult in immunosuppressed patients, attenuated till late
- How to distinguish from bacterial sepsis?

**Imaging**
- Findings subtle
- Halo sign, air-crescent signs are absent in non-neutropenic

---

4 common clinical situations:
1. Inaccurate diagnosis of fungal sepsis - resulting in inappropriate use of broad-spectrum antibacterial drugs
2. Failure to diagnose chronic pulmonary aspergillosis in smear-negative pulmonary tuberculosis – use of second line antitubercular drugs
3. Misdiagnosis of fungal asthma & invasive aspergillosis in COPD - resulting in unnecessary antibacterial drugs
4. Overtreatment and undertreatment of *Pneumocystis pneumonia* in HIV-positive patients.

**Access to advanced fungal diagnostics** would benefit clinical outcome, antimicrobial stewardship, & control of antimicrobial resistance
It is easy to advice - diagnose & then treat!
(Candida sepsis in ICUs)

- Blood culture positivity ~50%
- Candida score, colonization index – sampling for all colonization sites daily, impractical in clinical situation, not cost effective
- Indian study - 97% patients were colonized with Candida species at any point of time during ICU stay
- Ostrosky’s rule – easier to implement, but only 10% of those patients will develop proven or probable IC
- Do you know, which patients to be treated with antifungal when predictive rules, candida score, blood culture fail?

Laboratory diagnosis – some success

- Sample collection –
  - Difficult to avoid colonizers & to collect from deep tissue
  - Improvement in invasive procedure (FNAC/lung biopsy)
- Direct microscopy, culture & Histopathology –
  -Insensitive, time, difficult to distinguish from colonizer
  - Very important (especially PJP), can see mycelial fungi, takes few minutes
- Identification – important, as you can choose the drug
  - Phenotypic method – time consuming & need expertise
  - MALDI & sequencing – revolutionized
- Ab detection – does not help in immunosuppressed hosts
- Ag detection – excellent in Cryptococcus, Histoplasma (urine – 80-90% positive)
Identification of fungus in tissue

- Immunohistochemistry
- Extraction of DNA from tissue & sequencing

Molecular diagnosis of rhino-orbito-cerebral mucormycosis from fresh tissue samples

MALDI

- Identification of bacteria & fungi within few minutes
- Susceptibility testing
- Molecular typing

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for the rapid identification of yeast causing bloodstream infections

- 354 sequence yeast (standardization)
- 367 blind clinical yeast (validation)
- Database updated for Candida auris, C. viswanathii, Kodamaea ohmeri etc.

MALDI-TOF correctly identified 98.9% as compared to PCR-sequencing

Presented at MMTN Conference, 5-6 Aug 2017

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But what would be real success?

Culture independent methods – proteomic vs. genomic approach

- Detection in clinical sample – promising, but success limited
- Limitation
  - Presence of biomarker in pg
  - No scope of prior amplification before detection
- Pre-amplification possible
- Higher sensitivity & specificity
- Low turn around time
- GM released in active growth, PCR better in prophylaxis

But real challenge diagnosis in clinical samples


- 1,017 patients with hematological malignancies autopsied
  - 31% were found to have invasive fungal infections
  - 75% were not diagnosed before death
Improvement in diagnosis (MD Anderson autopsy data on haematological malignancy)

- 84% of IFI were diagnosed post-mortem during 1989-93
- 49% of IFI were diagnosed post-mortem during 2004-08
- Improvement in aspergillosis diagnosis due to pre-emptive approach or introduction of molecular tests

Lewis RE et al. Mycoses 2013; 56: 638

Biomarker tests

Existing benchmark tests
- CRP & Procalcitonin
- Serum galactomannan
- BAL galactomannan
- Serum Beta-D-gulcan
(Caution: may need ‘expert’ interpretation)

New biomarkers
- Aspergillus PCR
- Aspergillus GM + PCR
- Aspergillus Lateral flow
- BAL Beta-D glucan
- Mucorales PCR from blood
- Breath Volatile metabolites
- Many potential POCT

Galactomannan

- Galactomannan (GM) testing has been reviewed in several meta-analyses1,2. Different cut-offs to define
- Better performance in patients undergoing intensive chemotherapy compared to solid-organ transplant patients


Patients

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematological malignancy</td>
<td>58 (52–64)</td>
<td>95 (94–96)</td>
</tr>
<tr>
<td>HSCT</td>
<td>65 (60–78)</td>
<td>65 (44–83)</td>
</tr>
<tr>
<td>Solid organ transplant</td>
<td>41 (21–64)</td>
<td>85 (80–89)</td>
</tr>
</tbody>
</table>

Diagnosis-driven strategy: GM monitoring every 3–4 days combined with clinical and microbiological evaluation and high-resolution CT imaging (A II recommendation)

Galactomannan in BAL

**Cut-off of 0.5**

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>94</td>
<td>Guo, Chest 2010</td>
</tr>
<tr>
<td>90</td>
<td>96.4</td>
<td>Avni, JCM 2011; 4:665-70</td>
</tr>
<tr>
<td>87</td>
<td>94</td>
<td>Zou, PlosOne 2012; 7:e52833</td>
</tr>
<tr>
<td>92</td>
<td>98</td>
<td>Heng, Crit Rev Microbio! 2015;41:124-34 (haematological patients)</td>
</tr>
</tbody>
</table>

**False positive & negative results**

**False positive results**

- **Host-related**
  - Renal failure, mucositis, food intake of galactofuranose, gut colonization & possible translocation of *Bifidobacterium*, gastrointestinal microflora of neonates
- **Iatrogenic**
  - Blood derivatives, intravenous solution containing gluconate, treatment of antibiotics derived from the fermentation of *Penicillium* (piperacillin-tazobactam, amoxicillin-clavulanic acid)

**Sample collection**

- Cotton swab & cardboard

**Environmental**

- Presence of non-Aspergillus fungi, e.g. *Penicillium*, *Aletnaria*, *Paecilomyces*, *Geotrichum*, *Histoplasma*, rarely *Cryptococcus*

**Food**

- Pasta & yoghurt

**False-negative results**

- **Host conditions**
  - Chronic granulomatous disease
- **Iatrogenic**
  - Treatment with antifungals

**Sample collection**

- Long-term storage

---

**Pros & Cons of GM test**

- **Pros**
  - FDA approved GM test in serum & BAL
  - Detectable GM precedes clinical infection
  - BAL GM precedes serum GM
  - Good positive & negative predictive value in Haematology-Oncology

- **Cons**
  - Not yet standardized in ICU patients

**Limitation**

- Cross-reaction with some fungi (Candidiasis, *Penicillium*, *Histoplasma* etc.)
- Variable turnaround time depending of number of specimens
- False positive tests
- Well-equipped laboratories & trained staff to perform the test

Amsden JR, Curr Fung Infect Rep 2015; 9:111
**1,3-β-D-glucan detection**

---

All 21 patients with baseline negative BDG discontinued anidulafungin on day 4. None developed candidaemia until day 30.

**Conclusions:** Early discontinuation of empirical echinocandin therapy in high-risk ICU patients based on consecutive negative BDG tests may be a reasonable strategy, with great potential to reduce the overuse of echinocandins in ICU patients.

---

**The performance of BDG as per meta-analysis**

- Pan-fungal marker except Mucor & possibly Cryptococcosis
  - Positive before clinical symptoms; Helps to monitor therapy
  - Good performance in suspected Pneumocystis & Candida infection
  - False positivity, difficulty to test, cost

---

**Nucleic acid detection - Real challenge in clinical sample**

- PCR based detection assay - Real time PCR or qPCR
- Large number of PCR protocols published over 20 years, but absence of consensus standardized technique
- PCR is not included in EORTC/MSG guideline

**Comparison with virology**

- Different protocol published for viruses, but this does not hamper acceptance of PCR in diagnostic virology
- For viruses – we deal with $>10^3$
**Challenges in fungal PCR**

- Too few fungal DNA in sample
- PCR inhibitors – heparin, haemoglobin, lactoferrin
- Contamination is a big issue - environment
  - 10-20% tube may have Aspergillus DNA contamination
  - 18% commercial tubes with anticoagulant have fungal DNA

**Recommendation EAPCRI**

- Serum may be used, plasma best – blood volume >3ml
  - Elution in small volume
  - Mechanical lysis better than enzymatic lysis of cell wall
  - Internal control, ITS target

**Diagnosis of aspergillosis – comparison GM/BDG/PCR**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GM-EIA</th>
<th>B-D glucan</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Methodological recommendation</strong></td>
<td>Single commercial assay with 10F: Planeta Aspergillus antigen (BioRad)</td>
<td>Commercial assay: Fungitell (AdvanDx), Fungitell G-Test M, Diagnostic Enzyme Test, B-G Test (SRL), Dynamizer Forus (Microgen Biotechnology Co. LTD)</td>
<td>Presence of Aspergillus, Pahe Septifog, Pahecomics Mycocheck, Advantech Mycocheck, Renkebe Fungible, Procedural recommendations for DNA extraction (EAPCRI)</td>
</tr>
<tr>
<td><strong>Quality control</strong></td>
<td>Internal – BioRad, Proficiency panel</td>
<td>Independent – QCMD &amp; EAPCRI Panels</td>
<td></td>
</tr>
<tr>
<td>Sensitivity %</td>
<td>Blood: 79.3</td>
<td>Blood: IA: 56.8-77.1</td>
<td>Blood: 64-68</td>
</tr>
<tr>
<td></td>
<td>BAL: 81.6-85.7</td>
<td></td>
<td>BAL: 76.8-79.6</td>
</tr>
<tr>
<td>Specificity %</td>
<td>Blood: 94-98</td>
<td>Blood: IA: 81.3-97.0</td>
<td>Blood: 75-76</td>
</tr>
<tr>
<td></td>
<td>BAL: 93.3-94.5</td>
<td></td>
<td>BAL: 93.7-94.5</td>
</tr>
<tr>
<td>False-positive</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>False-negative</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Clinical utility</td>
<td>Limited</td>
<td>yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Interpretation of non-culture diagnostic tests**

- If blood culture is negative due to low level of candidemia, beta-glucan & PCR assays unlikely to make diagnosis reliably
- If a patient in low-risk group (ICU admission), positive result does not help, but negative result excludes the disease
- If a patient in high-risk group (repeated ileal leak or pancreatitis), a positive result increases the likelihood of invasive candidiasis
- Tempation – shorter turn around time & early therapy
- We tend to believe - non-culture diagnostic tests can identify blood culture negative primary or secondary deep-seated candidiasis
- Two high positive results are compelling
- Similarly multiple negative results are compelling

**Galactomannan and Polymerase Chain Reaction-Based Screening for Invasive Aspergillosis Among High-Risk Hematology Patients: A Diagnostic Meta-analysis**

- **Presented at MMTN Conference, 5-6 Aug 2017**
- Copyright of Speaker

**Clin Infect Dis 2015; 61: 1263**

**Pre-emptive approach can:**
(i) direct antifungal therapy (reduce empiric therapy);
(ii) allow earlier detection of IA
GM + PCR better than GM alone

- 941 patients, 5146 serum samples
- 51 patients – proven/probable IA
- PCR – 66.7% sens., 98.7% spec.
- GM – 78.4% sens., 87.5% spec.
- PCR+GM – 88.2% sens.

Aspergillus PCR is highly predictive of 90d mortality

New techniques
POCT tests

Current diagnostics: consensus

<table>
<thead>
<tr>
<th>Infection</th>
<th>Culture/ Histo</th>
<th>Biomarker (Ab)</th>
<th>Biomarker (Ag)</th>
<th>Response to Rx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillosis</td>
<td>Yes invasive</td>
<td>No</td>
<td>GM/BDG/PCR</td>
<td>Increasing evidence</td>
</tr>
<tr>
<td>Cryptococcosis</td>
<td>Routine</td>
<td>No</td>
<td>AG/PCR</td>
<td>Yes (CSF Ag)</td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td>Culture - delay</td>
<td>Limited</td>
<td>Ag</td>
<td>Yes (Ag)</td>
</tr>
<tr>
<td>Mucormycosis</td>
<td>Yes - invasive</td>
<td>No</td>
<td>Investigational</td>
<td>No</td>
</tr>
<tr>
<td>Other moulds</td>
<td>Yes – invasive</td>
<td>No</td>
<td>Investigational</td>
<td>No</td>
</tr>
<tr>
<td>Candida</td>
<td>Routine</td>
<td>Investigational</td>
<td>PCR/mannan/B</td>
<td>No</td>
</tr>
</tbody>
</table>

Cryptococcal meningitis

- CrAg Lateral flow assay (Immun,Mannan)
- No equipped lab or skill staff required
- Can identify disease before symptoms
- Temperature stable, rare cross-reaction
- Takes 10 minutes; Costs $2, cost-effective
- Cannot monitor therapy – clearance slow

Study in Uganda, Suspected cryptococcal meningitis


<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Number</th>
<th>Number positive/number tested (%)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF culture</td>
<td>401</td>
<td>186/401 (46.3)</td>
<td>26/26 (100)</td>
<td>36/36 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 µL culture</td>
<td>516</td>
<td>105/516 (20.1)</td>
<td>391/391 (100)</td>
<td>391/391 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 µL culture</td>
<td>252</td>
<td>103/252 (40.8)</td>
<td>159/159 (100)</td>
<td>159/159 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total culture</td>
<td>809</td>
<td>391/809 (48.5)</td>
<td>391/391 (100)</td>
<td>391/391 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF LFA</td>
<td>903</td>
<td>186/903 (20.6)</td>
<td>391/391 (100)</td>
<td>391/391 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF LFA (Biodot)</td>
<td>279</td>
<td>176/279 (63.3)</td>
<td>176/176 (100)</td>
<td>176/176 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF LFA (MFY)</td>
<td>761</td>
<td>452/761 (59.3)</td>
<td>452/452 (100)</td>
<td>452/452 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Aspergillus specific extracellular glycoprotein Ag
- Secreted during active growth of fungi
- Mab (JF5) developed
- Lateral-flow device (point of care)
- Useful in BAL
- Lot of variability in sensitivity & specificity among the laboratories

Aspergillosis diagnosis – BALF Aspergillus LFD

<table>
<thead>
<tr>
<th>Study</th>
<th>Risk group</th>
<th>Serum specificity</th>
<th>Lateral flow specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berg 2015</td>
<td>HM</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Berg 2015</td>
<td>HL</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>Berg 2015</td>
<td>HM and non-HM</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>Berg 2015</td>
<td>SOT</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Berg 2014</td>
<td>SOT</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>Fein 2015</td>
<td>ICU</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Prates 2014</td>
<td>Respiratory</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Rod 2015</td>
<td>HCT</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Wite 2015</td>
<td>HM</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Aspergillus LFA: current status

- Use of test with BAL fluid >> serum
- Most promising in non-neutropenic patients (no data on serum LFA in this group)
- Use in combination with PCR +/- GM
- Non-specific binding evident even with the “CE marked” strips observed in some countries
- Till more data, for now, small but potential role in IA diagnostics
• PLA 10-100 fold higher sensitivity to GM
• 1000 fold higher sensitivity to lateral flow assay (LFD)
• No cross reaction with other fungal species

Biomark Med 2014; 8: 429-51; 25th ECCMID congress, Copenhagen, 2015

• Fusicaridine C (FsC) & triacetylfusarine C (TAFC) – major siderophore of A. fumigatus released after spore germination
• Not validated yet in clinical samples


Electronic Nose - Cyranose®

Highly effective in invasive aspergillosis in neutropenic patients

Sensitivity 100%
Specificity 83%


A Breath Fungal Secondary Metabolite Signature to Diagnose Invasive Aspergillosis

Clinical Infectious Diseases 2014;59(12):1733-40
Present scenario in Asian countries; 241 laboratories surveyed

<table>
<thead>
<tr>
<th>Tests</th>
<th>Overall n=241 (%)</th>
<th>China n=71 (%)</th>
<th>India n=10 (%)</th>
<th>Indonesia n=11 (%)</th>
<th>Philippines n=26 (%)</th>
<th>Singapore n=1 (%)</th>
<th>Thailand n=7 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crypt Ag</td>
<td>65.2</td>
<td>66.7</td>
<td>58.3</td>
<td>50.0</td>
<td>75.0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Histo Ag</td>
<td>2.6</td>
<td>5.0</td>
<td>2.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Candida Ag</td>
<td>14.8</td>
<td>43.8</td>
<td>7.1</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GM</td>
<td>22.8</td>
<td>25.4</td>
<td>26.9</td>
<td>11.5</td>
<td>25.0</td>
<td>27.8</td>
<td>14.3</td>
</tr>
<tr>
<td>BDG</td>
<td>10.0</td>
<td>25.4</td>
<td>3.8</td>
<td>3.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PCR</td>
<td>37.8</td>
<td>43.8</td>
<td>46.2</td>
<td>1 lab</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TDM</td>
<td>38.1</td>
<td>58.3</td>
<td>15.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Almost no access biomarker tests in Indonesia, Philippines, Thailand

Summary

- Areas of interest – detection of fungi in blood & tissue, rapid identification of fungi & antifungal drug resistance in clinical samples
- Proteomic approach – MALDI, biomarkers – promising
- Genomic approach – more promising, but majority are in house & not standardized
- EAPCRI is a bold initiative, but commercial closed system required
- New initiatives – genetic susceptibility, POCT (lateral flow, proximity ligation assay, microarray, nano technology, T2)
- Asian laboratories – investment required, LFA – cheaper option, need to develop reference lab with availability of all biomarker tests

Thank you!

Presented at MMTN conference, 5-6 Aug 2017

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