Recent advances of fungal diagnostics and application in Asian laboratories

Professor Arunaloke Chakrabarti
Head, Department of Medical Microbiology
Postgraduate Institute of Medical Education and Research
Chandigarh, India
Co-chair, ISHAM Asia Fungal Working Group
Recent advances of fungal diagnostics and application in Asian laboratories

Arunaloke Chakrabarti
Professor & Head, Department of Medical Microbiology
In-charge, Center for Advanced Research in Medical Mycology & WHO Collaborating Center
Postgraduate Institute of Medical Education & Research, Chandigarh, India
• **Mortality** due to invasive fungal infection
  - 97-100% if not treated
  - ~50% even after proper treatment
  - Why so poor outcome despite antifungal?

• **Can early therapy improve the outcome?**
• **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
1. Inaccurate diagnosis of **fungal sepsis** - resulting in inappropriate use of broad-spectrum antibacterial drugs

2. Most serious fungal infections are ‘**hidden**’, occurring as a consequence of other health problems such as asthma, AIDS, cancer, organ transplant & corticosteroid therapies

3. **Misdiagnosis** resulting in unnecessary antibacterial drugs
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?**
You may not get answer always

- 1,017 patients with haematological malignancies autopsied
  - 31% were found to have invasive fungal infections
  - 75% were not diagnosed before death
Advances in diagnostics

• In conventional techniques
• In culture & identification
• Biomarkers
• Nucleic acid detection
• Unmet needs & problems with present development
• In the pipeline
• Scenario in Asia
Laboratory diagnosis – some success

- **Sample collection** –
  - Improvement in invasive procedure (FNAC/lung biopsy), bronchoscopy

- **Direct microscopy, culture & Histopathology** –
  - Very important (especially PJP), can see mycelial fungi, takes few minutes

- **Identification** – important, as you can choose the drug
  - MALDI & sequencing – revolutionized

- **Ag detection** – excellent in Cryptococcus, Histoplasma (urine – 80-90% positive)
Identification of fungus in tissue

- Immunohistochemistry
- In situ hybridization
- Extraction of DNA from tissue & sequencing

Success – fresh tissue (95%), formalin fixed tissue – 60%
T2 magnetic resonance nanoparticle mediated detection for Candida

- Improved turnaround
- BACTEC – 2.6d, T2 – 3-4h

- Identify only 5 Candida species
- Contamination
- Can’t perform susceptibility
- Can’t replace conventional
- Not available in developing countries

MALDI-TOF-MS

- Identification within few minutes (yeast)
- Susceptibility testing
- Molecular typing

- Database needs improvement
- Not available in majority labs of developing countries
Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for the rapid identification of yeasts 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
Rapid detection of fluconazole resistance in *Candida tropicalis* by MALDI-TOF MS

Saikat Paul, Pankaj Singh, Shananth A S, Shivaprasad M. Rudramurthy, Arunaloke Chakrabarti and Anup K Ghosh*

*Medical Mycology, 2017, 0, 1–8*

doi: 10.1093/mmy/myx042
“Okay—who put my lunch through the mass spectrometer...?”
But what would be real success?

Current diagnostic methods

Candida Ag/Ab
Candida PCR
Glucan
Aspergillus PCR
Aspergillus GM
Aspergillus LFD

Targeted prophylaxis/
Pre-emptive therapy

Empirical/targeted therapy

Biological infection
Clinical infection
Pathological changes

INFECTION
Culture independent methods – proteomic vs. genomic approach

Proteomic approach

• Detection in clinical sample – promising, but success limited

• Limitation
  ➢ presence of biomarker in pg
  ➢ No scope of prior amplification before detection

Genomic approach

• Pre-amplification possible

• Higher sensitivity & specificity, low turn-around time

• GM released in active growth, PCR better in prophylaxis
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
Procalcitonin, C-reactive protein and serum lactate dehydrogenase in the diagnosis of bacterial sepsis, SIRS and systemic candidiasis

Fabio Miglietta¹, Maria Letizia Faneschi¹, Giambattista Lobreglio², Claudio Palumbo¹, Adriana Rizzo¹, Marco Cucurachi³, Gerolamo Portaccio³, Francesco Guerra³, Maria Pizzolante²

Le Infezioni in Medicina, n. 3, 230-237, 2015

<table>
<thead>
<tr>
<th>Variables</th>
<th>All patients (n=145)</th>
<th>SIRS (n=42)</th>
<th>P</th>
<th>Bacterial sepsis (n=70)</th>
<th>P</th>
<th>Systemic candidiasis (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 0</td>
<td>0.9 (0.4-9.4)</td>
<td>0.38 (0.26-0.64)</td>
<td>&lt;0.001</td>
<td>10.2 (1.2825.3)</td>
<td>&lt;0.001</td>
<td>0.55 (0.360.9)</td>
</tr>
<tr>
<td>day 2</td>
<td>0.6 (0.3-4.9)</td>
<td>0.28 (0.12-0.5)</td>
<td>&lt;0.001</td>
<td>4.9 (0.711.9)</td>
<td>0.001</td>
<td>0.5 (0.20.6)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 0</td>
<td>91.7 (55.7164)</td>
<td>68.6 (48.5139)</td>
<td>&lt;0.001</td>
<td>128.6 (77-254.7)</td>
<td>&lt;0.001</td>
<td>60.5 (54.4-96.5)</td>
</tr>
<tr>
<td>day 2</td>
<td>69.8 (52.3117)</td>
<td>58.5 (47.183)</td>
<td>0.001</td>
<td>99.9 (58-180.2)</td>
<td>0.046</td>
<td>67.4 (50-78.8)</td>
</tr>
</tbody>
</table>

Intern Emerg Med
DOI 10.1007/s11739-017-1627-7
Published online: 04 February 2017

Usefulness of procalcitonin in differentiating Candida and bacterial blood stream infections in critically ill septic patients outside the intensive care unit

Filippo Pieralli¹ · Lorenzo Corbo¹ · Arianna Torrigiani² · Dario Mannini³ · Elisa Antonielli¹ · Antonio Mancini¹ · Francesco Corradi² · Fabio Arena³ · Alberto Moggi Pignone⁴ · Alessandro Moretti³ · Carlo Nozzoli¹ · Gian Maria Rossolini³,⁵,⁶,⁷
LDH can help in diagnosis of pneumocystis pneumonia

<table>
<thead>
<tr>
<th>Variables</th>
<th>Clinical diagnosis</th>
<th></th>
<th></th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PJP (n = 19)</td>
<td>CAP (n = 18)</td>
<td>Other (n = 23)</td>
<td></td>
</tr>
<tr>
<td>(1-3)-β-D-Glucan in serum (pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>183</td>
<td>29.8</td>
<td>52.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>240.8 (± 185.7)</td>
<td>36.3 (± 34.2)</td>
<td>67.3 (± 60.7)</td>
<td></td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Median</td>
<td>761</td>
<td>419</td>
<td>441</td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>762.47 (± 433.18)</td>
<td>379.5 (± 98.9)</td>
<td>442.6 (± 217.6)</td>
<td></td>
</tr>
<tr>
<td>Viral load (copies/mL)</td>
<td></td>
<td></td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>Median</td>
<td>62,609</td>
<td>3800</td>
<td>74,892</td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>801,171 (± 2,194,157)</td>
<td>449,525 (± 270,015)</td>
<td>482,859 (± 135,562)</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte T CD4+ (cell/mm³)</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Median</td>
<td>40</td>
<td>230</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>73 (± 107)</td>
<td>303 (± 267)</td>
<td>217 (± 312)</td>
<td></td>
</tr>
<tr>
<td>Time since diagnosis of HIV infection (years)</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Median</td>
<td>0</td>
<td>15</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td>1.94 (± 6.83)</td>
<td>14 (± 7.67)</td>
<td>10.7 (± 8.32)</td>
<td></td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
<td>0.62</td>
</tr>
<tr>
<td>Discharge</td>
<td>15</td>
<td>15</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Mean time of hospitalization (in days)</td>
<td>22.9 (± 22.8)</td>
<td>13.4 (± 8.9)</td>
<td>16.1 (± 12.3)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

PJP, Pneumocystis jirovecii pneumonia. PJP group included: P. jirovecii + community acquired pneumonia: 4 patients; P. jirovecii + Mycobacterium non-tuberculosis: 2 patients. CAP: community acquired pneumonia. Other: lower respiratory infection: 9 patients; tuberculosis: 5 patients; histoplasmosis: 2 patients; cryptococcosis: 1 patient; disseminated strongyloidiasis: 1 patient; nocardiosis: 1 patient; pulmonary embolism: 1 patient and undiagnosed: 3 patients.
Galactomannan

- GM for serum, BAL – FDA approved, can also be detected in CSF, urine
- But, many false positive and negative issues
- Better performance in patients undergoing intensive chemotherapy compared to solid-organ transplant patients

<table>
<thead>
<tr>
<th>Patients</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

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>94</td>
<td>Guo, <em>Chest</em> 2010</td>
</tr>
<tr>
<td>90</td>
<td>96.4</td>
<td>Avni, <em>JCM</em> 2011; 4:665-70</td>
</tr>
<tr>
<td>87</td>
<td>94</td>
<td>Zou, <em>PlosOne</em> 2012; 7:e52833</td>
</tr>
</tbody>
</table>

Cut-off of 0.5
Pros & Cons of GM test

• 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
• Possibly we may use it also in CSF & urine
• Not yet standardized in ICU patients

• Limitation
  ➢ Cross-reaction with some fungi (Geotrichum, 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 Cryptococciosis
- Positive before clinical symptoms; Helps to monitor therapy
- Good performance in suspected *Pneumocystis* & *Candida* infection
- False positivity, difficulty to test, cost

---

## False positivity of bio-marker tests

<table>
<thead>
<tr>
<th></th>
<th>Beta-D-glucan</th>
<th>Galactomannan</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Medication</strong></td>
<td>i.v. amoxycillin-clavulanate or ampicillin-sulbactam</td>
<td>Piperacillin-Tazobactum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other beta-lactam antibiotics</td>
</tr>
<tr>
<td><strong>Infusion</strong></td>
<td>i.v. immunoglobulin</td>
<td>Plasmalyte (electrolyte infusion)</td>
</tr>
<tr>
<td></td>
<td>Cellulose filter for i.v. infusion</td>
<td>i.v. solution with sodium gluconate</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td></td>
</tr>
<tr>
<td><strong>Medical intervention</strong></td>
<td>Hemodialysis with cellulose filter</td>
<td>Enteral feeding with soybean proteins</td>
</tr>
<tr>
<td></td>
<td>Gauze packing in serosal surface</td>
<td></td>
</tr>
<tr>
<td><strong>Other infections</strong></td>
<td><em>Pneumocystis</em> infection</td>
<td><em>Penicillium</em> spp., <em>Histoplasma capsulatum</em>, <em>Geotrichum</em>, <em>Neosartoria</em>, <em>Bifidobacterium</em></td>
</tr>
</tbody>
</table>
• 42y-F – HLA matched HSCT from unrelated donor
• Serum GM accessed twice weekly from Day 0 of Tx
• GM index increased to 2.22 & 3.01 on D32 & D34
• At that time she had GVHD
• But, she was afebrile with no pulmonary/sinus symptoms
• CT scan of brain, sinus, abdomen – normal
• Voriconazole started on D35
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

White PL *et al.* Clin Infect Dis 2015; 61: 1293

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GM-EIA</th>
<th>B-D-glucan</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methodological</td>
<td>Single commercial assay with SOP:</td>
<td>5 commercial assays:</td>
<td>Pathonostics Aspergenius,</td>
</tr>
<tr>
<td>recommendation</td>
<td>Platelia Aspergillus antigen (BioRad)</td>
<td><strong>Fungitell</strong> (Associates of Cape Cod)</td>
<td>Roche Septifast,</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Fungitec G-Test MK</strong> (Seikagaku Corporation)</td>
<td>Myconostica MycAssay,</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>B-G Star</strong> (Maruha Corporation)</td>
<td>Ademtech Mycogenie,</td>
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<td></td>
<td><strong>B-Glucan Test Wako</strong> (Wako Pure Chemicals)</td>
<td>Renishaw Fungiplex,</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Dynamiker Fungus</strong> (1–3)-β-D-Glucan Assay</td>
<td>Procedural recommendations for DNA extraction <strong>(EAPCRI)</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quality control</td>
<td>Internal – BioRad Proficiency panel</td>
<td>No</td>
<td>Independent – QCMD &amp; EAPCRI Panels</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BAL: 83.6–85.7</td>
<td></td>
<td>BAL: 76.8–79.6</td>
</tr>
<tr>
<td>Specificity %</td>
<td>Blood: 80.5–86.3</td>
<td>Blood: 81.3–97.0</td>
<td>Blood: 75–76</td>
</tr>
<tr>
<td></td>
<td>BAL: 89.0–89.4</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>Yes</td>
<td>Limited</td>
<td>yes</td>
</tr>
</tbody>
</table>
# 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 Ag</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>Candidasis</td>
<td>Routine</td>
<td>Investigational (anti-mannan)</td>
<td>PCR/mannan/B DG</td>
<td>No</td>
</tr>
</tbody>
</table>
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
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.
- Temptation – 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.
Are we ready with *Candida* biomarkers?

Leon et al, Crit Care 2016; 20: 149

Single or combined biomarker screening in prospective ICU cohort (candidiasis incidence, 13%)

Patients with (medical or surgical) severe abdominal condition, & expected ICU stay ≥7 days

<table>
<thead>
<tr>
<th>Colonization</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not colonized</td>
</tr>
<tr>
<td></td>
<td>N = 48</td>
</tr>
<tr>
<td>BDG ≥ 80 pg/mL, no. (%)</td>
<td>16/46 (34.8)a</td>
</tr>
<tr>
<td>BDG ≥ 100 pg/mL, no. (%)</td>
<td>16/46 (34.8)a</td>
</tr>
<tr>
<td>BDG ≥ 200 pg/mL, no. (%)</td>
<td>10/47 (21.3)a</td>
</tr>
<tr>
<td>CAGTA positive, no. (%)</td>
<td>10/47 (21.3)a</td>
</tr>
<tr>
<td>Mannan-Ag positive, no. (%)</td>
<td>10/48 (20.8)a</td>
</tr>
<tr>
<td>Mannan-Ab positive, no. (%)</td>
<td>6/48 (12.5)</td>
</tr>
<tr>
<td>C-PCR positive, no. (%)</td>
<td>14/23 (60.9)</td>
</tr>
</tbody>
</table>

- Single assays are highly nonspecific (≈80% of positive results are false)
- Positive *Candida albicans* germ tube antibody & β-D-glucan in a single blood sample or β-D-glucan positivity in two consecutive blood samples allowed discriminating invasive candidiasis
- Sensitivity still low in very high risk (≈30% of cases missed)
- A negative test does not rule out candidiasis in a high-risk patient
Galactomannan and Polymerase Chain Reaction–Based Screening for Invasive Aspergillosis Among High-Risk Hematology Patients: A Diagnostic Meta-analysis

Marios Arvanitis,1,2,3 Theodora Anagnostou,1,2 and Eleftherios Mylonakis1,2

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>84 (71–92)</td>
<td>76 (64–85)</td>
</tr>
<tr>
<td>2 PCRs</td>
<td>57 (40–72)</td>
<td>93 (87–97)</td>
</tr>
<tr>
<td>GM</td>
<td>92 (83–96)</td>
<td>90 (81–95)</td>
</tr>
<tr>
<td>2 GMs</td>
<td>62 (48–74)</td>
<td>95 (91–97)</td>
</tr>
<tr>
<td>GM or PCR</td>
<td>99 (96–100)</td>
<td>64 (49–77)</td>
</tr>
<tr>
<td>GM and PCR</td>
<td>68 (54–80)</td>
<td>98 (94–100)</td>
</tr>
</tbody>
</table>

Weekly screening with GM and PCR

- No positive tests
- One positive GM or PCR
  - IA highly unlikely
  - IA cannot be confirmed or excluded
- At least 2 positive tests in the same patient
  - Diagnosis of IA highly likely
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.

Biomarkers may monitor therapy

- 18 centres (US & Belgium) – 47 patients with IA (9 proven + 38 probable)
- GM & BDG – twice weekly for six weeks

---

<table>
<thead>
<tr>
<th>Response</th>
<th>GM+BDG (mean z-score)</th>
<th>BDG (pg/mL)</th>
<th>GM (ng/mL)</th>
<th>GMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline to W-2</td>
<td>Baseline to W-6</td>
<td>Baseline to W-2</td>
<td>Baseline to W-6</td>
</tr>
<tr>
<td>Week 6</td>
<td>R, Mean (N)</td>
<td>-0.10 (25)</td>
<td>-0.12 (25)</td>
<td>929 (24)</td>
</tr>
<tr>
<td></td>
<td>NR, Mean (N)</td>
<td>0.24 (22)</td>
<td>0.31 (22)</td>
<td>2174 (20)</td>
</tr>
<tr>
<td></td>
<td>Mean Difference</td>
<td>0.34</td>
<td>0.43</td>
<td>1245</td>
</tr>
<tr>
<td></td>
<td>90% CI</td>
<td>-0.17, 0.84</td>
<td>-0.11, 0.97</td>
<td>-1825, 4615</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.13</td>
<td>0.09</td>
<td>0.25</td>
</tr>
<tr>
<td>Week 12</td>
<td>R, Mean (N)</td>
<td>-0.12 (27)</td>
<td>-0.16 (27)</td>
<td>873 (26)</td>
</tr>
<tr>
<td></td>
<td>NR, Mean (N)</td>
<td>0.42 (14)</td>
<td>0.55 (14)</td>
<td>3555 (12)</td>
</tr>
<tr>
<td></td>
<td>Mean Difference</td>
<td>0.54</td>
<td>0.71</td>
<td>2682</td>
</tr>
<tr>
<td></td>
<td>90% CI</td>
<td>-0.01, 1.10</td>
<td>0.12, 1.3</td>
<td>-1083, 6447</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td><strong>0.05</strong></td>
<td><strong>0.02</strong></td>
<td>0.12</td>
</tr>
</tbody>
</table>


Commercial platforms for diagnosis of Candidiasis

• Detection from clinical samples
  - Biomarkers – fungal antigen detection – beta-D-glucan
    - Fungitell assay (Cape Cod, USA)
  - PCR based methods from whole blood (Serum)
    - SeptiFast (Roche)
    - Fungiplex Candida – RT-PCR (Bruker)
    - T2 magnetic resonance (T2 Biosystem)

• Identification from culture
  - Candida PNA-FISH (AvanDx)
  - FilmArray multiplex PCR (BioFire Dx, BioMerieux)
  - MBT Sepsityper (MALDI-TOF) (Bruker)
Commercial platforms for diagnosis of Aspergillosis

• Detection from clinical samples
  ➢ Biomarkers – fungal antigen detection – Galactomannan
    ○ Platelia Aspergillus (Bio-RAD)
  ➢ PCR based methods from whole blood (Serum)
    • Pathonostics Aspergenius
    • Roche Septifast
    • Myconostica MycAssay
    • Ademtech Mycogenie
    • Renishaw Fungiplex
New techniques

POCT tests
Lateral flow assay

- Cryptococcosis – well standardized
- Used in many laboratories, cost effective

- *Aspergillus* specific extracellular glycoprotein
- Secreted during active growth of fungi
- Mab (JF5) developed
- Lot of variability in sensitivity & specificity

- Use of test with BAL fluid >> serum
- Most promising in non-neutropenic patients
- Use in combination with PCR +/- GM
Serum specificity tests

1. Cryptococcus neoformans
2. Candida albicans
3. Fusarium solani
4. Rhizopus oryzae
5. Aspergillus fumigatus
Proximity ligation assay for the early detection of invasive aspergillosis

G. Johnson\textsuperscript{1}, M. Shannon\textsuperscript{1}, C. Thornton\textsuperscript{1}, S. Agrawal\textsuperscript{2}, C. Lass-Flörl\textsuperscript{3}, W. Mutschlechner\textsuperscript{3}, S. Bustin\textsuperscript{1}

- 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; 25\textsuperscript{th} ECCMID congress, Copenhagen, 2015
Mucorales-Specific T Cells in Patients with Hematologic Malignancies


Without mucormycosis

With mucormycosis

Presented at MMTN Conference, 1-3 Dec 2017
Copyright of speaker
Electronic Nose Technology for Detection of Invasive Pulmonary Aspergillosis in Prolonged Chemotherapy-Induced Neutropenia: a Proof-of-Principle Study

Highly effective in invasive aspergillosis in neutropenic patients

Sensitivity 100%
Specificity 83%

A Breath Fungal Secondary Metabolite Signature to Diagnose Invasive Aspergillosis

Sophia Koo,1,2,3,8 Horatio R. Thomas,1,3,8 S. David Daniels,1 Robert C. Lynch,1 Sean M. Fortier,1 Margaret M. Shea,1 Preshious Rearden,4 James C. Comolli,4 Lindsey R. Baden,1,2,3 and Francisco M. Marty1,2,3

Volatile Organic Compounds
A. β-trans-bergamotene
B. Trans-gerranylacetone
C. β-valerenene
D. α-trans-bergamotene
E. Limonene
F. Camphene
G. α-pinene
H. β-pinene

Clinical Infectious Diseases® 2014;59(12):1733–40

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Invasive Aspergillosis</th>
<th>Other Pneumonia</th>
<th>Total Patients</th>
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<tbody>
<tr>
<td>Aspergillus metabolite signature b +</td>
<td>32</td>
<td>2</td>
<td>34</td>
</tr>
<tr>
<td>Aspergillus metabolite signature –</td>
<td>2c</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>Total patients</td>
<td>34</td>
<td>30</td>
<td>64</td>
</tr>
</tbody>
</table>

Test parameters
- Sensitivity (95% CI) 0.94 (.81–.98)
- Specificity (95% CI) 0.93 (.79–.98)
- Positive likelihood ratio (95% CI) 14.1 (3.69–54.0)
- Negative likelihood ratio (95% CI) 0.063 (.02–.24)
Present scenario in Asian countries; 241 laboratories surveyed

Chindamporn et al. Med Mycol 2017 (accepted)
### Present scenario in Asian countries

<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=4 (%)</th>
<th>Taiwan n=18 (%)</th>
<th>Thailand n=7 (%)</th>
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</thead>
<tbody>
<tr>
<td>Crypto 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>
<td>50.0</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>
<td>0</td>
</tr>
<tr>
<td>Candida Ag</td>
<td>14.8</td>
<td>43.8</td>
<td>7.1</td>
<td>22.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GM</td>
<td><strong>22.8</strong></td>
<td>25.4</td>
<td>26.9</td>
<td>9.1</td>
<td>11.5</td>
<td>25.0</td>
<td>27.8</td>
<td>14.3</td>
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<tr>
<td>BDG</td>
<td><strong>10.0</strong></td>
<td>25.4</td>
<td>3.8</td>
<td>0</td>
<td>3.8</td>
<td>0</td>
<td>0</td>
<td>14.3</td>
</tr>
<tr>
<td>PCR</td>
<td><strong>37.8</strong></td>
<td>43.8</td>
<td>46.2</td>
<td>0</td>
<td>1 lab</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>TDM</td>
<td><strong>38.1</strong></td>
<td>58.3</td>
<td>16.7</td>
<td>0</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

Chindamporn et al. Med Mycol 2017 (accepted)
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