Diagnosis of Aspergillosis: Progress in Technologies and Approach

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Disclosures

• Consultant / Advisory Board
  – Amplyx, Cidara, Merck

• Royalties / Equity
  – MycoMed Technologies
Background

• Focus on invasive aspergillosis
• This talk will address use of *non-culture based biomarkers* to:
  – Predict disease risk
  – Indicate presence of infection
Nucleic acids

PCR

• Many years of research on PCR in tissue, BAL, blood
• Many in-house studies with non-standardized assays varied performance on BAL and blood, meta-analyses
• Several recent large studies on BAL:
  – 1555 samples / 3 yrs, tested using multiple in house and commercialized assays. Sensitivity 61 – 74% (GM 87%), up to 100% with GM combined\(^1\)
  – 1248 samples / 12 yrs: lower sensitivity (40%), increased with combined (74%) \(^2\)
• Results suggest utility with BAL, especially combined with GM, multiplexing enabling species ID & resistance genotype
• Recent efforts to optimize and standardize sample processing in EU cooperative

\(^1\)Guegan et al. JI 2018; 76
\(^2\)Hardak e al. IJID 2019; 3557
Cruciani et al. Cochrane DSR. 2015
Boch et al. CMI 22 (2016) 862
Chong et al. JAC 71 (2016) 3528
Nucleic acids
PCR

- Blood / plasma / serum complicated
  - Critical issues described for DNA extraction
    - Volume, buffers
    - Variation in commercial kits
    - Elution volumes
    - and more...

Protocol

- Sample Volume Critical
  - Volume < 0.5 ml
    - Positivity when detecting 10 genomes/sample = 78.9%*
    - irrespective of elution volume
  - Volume > 0.5 ml
    - Positivity when detecting 10 genomes/sample = 100%**
    - Elution volume < 100 µl

Notes

- Nucleic Acid Extraction using commercial kit
  - Kits successfully used:
    - Manual: Qiagen Qiamp DNA, Qiagen Ultrasens Virus, Qiagen Circulating NA Kit, Roche High Pure LV Roche High Pure Template PCR.
    - Automated: Roche MagNA Pure NA, Roche MagNA Pure Compact, Roche MagNA Pure LV, Qiagen EZ1 Virus 2.0, Qiagen QIASymphony, BioMerieux EasyMag

- False Positivity Rates in EAPCRI serum study:
  - Biomerieux EasyMag 1/4
  - Qiagen QIASymphony 0/1
  - Qiagen EZ1 Kits 2/4
  - Qiagen Manual Kits 2/7
  - Roche MagNA Pure Kits 1/8
  - Roche Manual Kits 0/2
  - Promega Maxwell 0/1
  - Promega Wizard Genomic DNA 1/1

- Elution Volume Critical
  - Eluting in < 100 µl
    - Positivity when detecting 10 genomes/sample = 100%*
    - Sample volume 0.5 – 1.0 ml
  - Eluting in ≥ 100 µl
    - Positivity when detecting 10 genomes/sample = 62.5%**

- Perform PCR in Duplicate
- Perform an IC PCR
- A Cq threshold of 43 cycles is optimal

Barnes et al. Med Mycol 2018; 56
Immunodiagnostics

- Platelia galactomannan EIA widely studied in serum & BAL
  - Reported performance continues to be variable
    - Serum sensitivity ranging from 19% - 80%
    - BAL sensitivity ranging from 43% - 90%
      - Positive predictive value highest in BAL (highest prevalence)
  - Many variables impact performance
    - Hosts (neutropenic vs. not)
    - Other drugs (antifungals, contamination)
    - Biology (colonization vs. invasion)
    - Technical (cut-off variation)
  - Recent attention to cutoffs, “false positives”, and antigen

Boch et al. Clin Microbiol Infect 2016; 22A
BAL Cut-offs

- Multiple cut-offs used: 0.5, 0.7 vs. 1.0?
  - Perhaps depends on host, and goal
  - Subject to a lot of bias: disease definitions, antifungal exposure (time) and math
- Lavage dilutions, variation in kit threshold control

BMT – 0.5  
Musher JCM2004

Heme – 1.0  
Maertens CID2004

Mix – 0.8  
D’Haese JCM 2012

Non-neut – 0.7  
Zhou JCM 2017
Consecutive patients (n=134) with BAL GM EIA to assess real-world predictive value
- 42% of BAL positive at >0.5 were falsely positive, PPV = 58%
- Is 0.5 too low?

How do we really distinguish between FP test vs. FN gold standards, or definitions that are too conservative?

Biology is vexing: airway antigen not necessarily disease
- Understanding nature of antigen critical
Immunodiagnostics

• “galactomannan” is a complex polymer (mannan & gal\textit{f} side chains)
  – Secreted in differing amounts depending on growth conditions

• Antigenic moiety = galactofuranose
  – In mammals, galactose is common but only found in the hexopyranosyl form (Gal\textit{p})
  – Galactofuranose (Galf) is found in bacteria, fungi, protozoa, starfish, sponges and green algae (and lichens)- abundant
  – Equilibrium favors Gal\textit{p}

![Diagram of galactofuranose and galactose structures]

**β-Galactofuranose (galf)**

- *A. fumigatus* cell wall contains many galf-glycoconjugates, including
  - Fungal-type galactomannan (GM)
  - O-glycans
  - N-glycans

**galf - epitope recognition**

- EB-A2 reactivity tested against galf-glycoarray
- Dimeric β5 (galf-galf) recognition a surprise
  - Terminal β5 (galf-galf)
- Potential specificity and sensitivity implications
  - Broader than previously thought but some limits on glycan recognition
- galf epitope specificity the most important driver – mAb comparisons needed
Immunodiagnostics

- Multiple LFDs simplifying detection of Ag’s in BAL
  - JF5 monoclonal reactive to “glycoprotein” released during active growth (OLM)
    - Different versions evaluated; new CE marked BAL LFD 71% sensitive; 100% specific\(^1\)
    - Large BAL study with 247 heme / BMT patients\(^2\)
      - BAL – sensitivity 82%; specificity 86% - 96% (visual vs. digital)
      - Correlation with serum GM EIA
      - Late reads – increased sensitivity, decreased specificity
    - “Galactomannan” LFD (IMMY)
  - Comparative studies
    - Non-neutropenic cohort
      - sensitivity 58 – 69%; specificity 68 – 75%\(^3\)

\(^1\) Hoenigl et al. Mycoses 2018; 61
\(^2\) Mercier et al. J Clin Microbiol; 2019: 57
\(^3\) Jenks et al., Mycoses 2019; 62
Immunodiagnostics

Comparative study with digital readouts: both with good performance

GM – higher sensitivity, more processing

JF5 – easier, lower sensitivity

Mercier et al. *ECCMID 2019*
Immunodiagnostic

- mAb476 –recognizes *Aspergillus* glycans in infected animal serum, BAL, lung homogenate *but most antigen rapidly excreted in urine*
  - Proof of concept shown with human samples after urine processing step to optimize antigen recovery¹

- LFD tested in 120 IA patients (MycoMed Tech.)²
  - Sensitive 89.5% (69-98); 92% specific (74-99)
  - Early in sequential samples from ‘possible’ cases

- Semi-quantitative ELISA developed (ASM, 2019)

- ECCMID 2019 (Tues): novel epitope specificity to include monomeric and terminal non β(1→5)-Galβf enables recognition of urinary Ag

¹Dufresne et al., *PLOS ONE*, 7: e42736, 2012
²Marr et al., Clin Infect Dis 2018
**β-D-Glucan**

- Activates *Limulus* amebocyte lysate
- Factor G initiates cascade. Output measured by multiple substrates in multiple kits: Wako BDG (Fujifilm Wako); Fungitec G test (Seikagaku); Fungitell (Assoc. Cape Cod); Dynamiker Fungus (Dynamiker BT); GKT-25M (Tianjin Era BT); Goldstream Fungus (Gold Mountain River Test Development)
β-D-Glucan

- Highly sensitive, yet non-specific assays
- Likely different cut-offs needed for different IFIs – none yet optimized for IA
  - Fungitell and Goldstream assay – IA with lower values
  - Dynamiker – not as much variation
- More comparisons, ROCs needed
• Exhaled VOCs
  – Unique metabolic profiles predict IA (esp. sesquiterpenes)
    • Detected by MS-GC & Enose
  – Complicated studies and outputs
    • Growth conditions change VOCs
• Fungal siderophores including triacetylfusararine C (TIAC) in urine and serum

Koo et al., Clin ID 2014; de Heer J Breath Res 2016
Skriba et al. Frontiers Microbiol 2018
Rees et al. J Breath Res 11(3) 2018
• MS – disaccharide (glycan) secreted during growth
  – Per-sample performance (blood) – less sensitive compared to BDG but more specific
  – Signals ? (trehalose)
And more....

• Next Generation Sequencing of cell-free DNA in plasma (Karius)
• Interpreting fungal markers in context of host inflammatory markers (Hoenigl)
• New technologies...
Historical Prevention Paradigm

Empirical

Pre-emptive

Clinical symptoms/signs
Antigenemia / PCR

Requires belief in negative tests

Granulocytes

Day

ESCMID eLibrary © by author
The Math of (Negative) Tests

• Negative predictive value
  – NPV is the probability a negative test is correct – either stop a drug (or don’t start it)
• A good test might have sensitivity & specificity = 85%
  – If likelihood is ~ 10% (low risk), NPV is ≥ 98% (wrong 1 in 50)
  – If likelihood is ~ 33% (medium risk), NPV is ≥ 92% (wrong 1 in 11)
• To get to an NPV of ≥ 98% at likelihood of 33%...
  – You need sensitivity and specificity of 96%
  – We don’t have that with any test

We also need to think about risks differently with screening (10%) vs. suspected disease (33%) → NPV vs. PPV
Separate tests according to need
Tailoring diagnostics

Screening assays: High NPV
Easy, convenient: think fecal occult blood
Broad, sensitive, non-invasive predictor of risk

Diagnostic assays: High PPV
More invasive: think colonoscopy
Combined assays specific to pathogen

Granulocytes

Prophylaxis?

Day

10
1
0.1
-14 -7 0 7 14 21 28 35 42 49 56 63
Conclusions

• Much learned about old tests
• New assays use easier technology
  – Need characterized Abs to understand biology
• New sample systems to enable screening (urine, breath)
• Need to think about NPV vs. PPV and combine tests when disease is highly likely
Thank you