Diagnosis of invasive mould infection in patients receiving antifungal prophylaxis/treatment:

Galactomannan

Martin Hoenigl, PD, MD, FECMM
Section of Infectious Diseases and Tropical Medicine, Department of Medicine,
Medical University Graz, Austria
AND
Division of Infectious Diseases, University of California San Diego, USA
▪ Structure of my Talk

- Background
- GM as Biomarker for Aspergillosis and influence of antifungals:
  - Serum GM
  - BALF GM
  - GM in Urine and CSF
  - Neutropenia and GM
  - Suggested GM Testing Algorithm

- Conclusion
• Invasive Aspergillosis (IA): Crude mortality of 80-90% in absence of adequate treatment

Key factors in survival
– Timely diagnosis!
– Early start of antifungal therapy

• In vivo diagnosis also essential to estimate Prevalence of Invasive Aspergillosis.
Autopsy Rates in Europe

- Russia
- Austria
- EU
- EU members before 2004
- Italy
- Germany? No data


Big Difference between countries. Overall Trend: DECREASE!

Martin Höningl
84% of the IFIs diagnosed postmortem in the first 5 years of the study; the rate decreased to 49% in the last 4 years of the study.

Lewis, R.. Kontoyiannis, D. Mycoses 2013; 638-45
### In Vivo Diagnosis

- **Early In vivo Diagnosis** relies on early markers of IA:
  - GM from Serum / BALF
  - PCR
  - BDG, LFD

<table>
<thead>
<tr>
<th>Test (combination) and condition</th>
<th>Sensitivity (n = 16)</th>
<th>Specificity (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR (BALF)</td>
<td>7/16 (43.8%)</td>
<td>37/37 (100%)</td>
</tr>
<tr>
<td>PCR (whole blood)</td>
<td>0/16 (0%)</td>
<td>37/37 (100%)</td>
</tr>
<tr>
<td>GM 0.5 ODI (BALF)</td>
<td>6/16 (37.5%)</td>
<td>34/37 (91.9%)</td>
</tr>
<tr>
<td>GM 0.5 ODI (serum)</td>
<td>5/16 (31.3%)</td>
<td>37/37 (100%)</td>
</tr>
<tr>
<td>GM (BALF) 1.0 ODI</td>
<td>5/16 (31.3%)</td>
<td>35/37 (94.6%)</td>
</tr>
<tr>
<td>Culture (BALF)</td>
<td>3/16 (18.8%)</td>
<td>37/37 (100%)</td>
</tr>
</tbody>
</table>

Increasing number of studies indicating that diagnostic performance of these early biomarkers/tests for IA are impacted by Antimould prophylaxis/Treatment
Aspergillus Galactomannan

- Galactomannan (GM): polysaccharide component of the cell wall of *Aspergillus* spp.
  - released into host’s circulation by growing hyphae

- GM EIA test positive in IA
  - Also positive in *Rasamsonia argillacea*, *Penicillium* spp., *Blastomyces* spp., *Fusarium* spp. (?)
  - Negative in e.g. *Mucorales*

Galactomannan: Serum

- **SERUM:**

  Overall sensitivity 78% (61 – 89%), overall specificity 81%

  - **Cut-off 0.5 ODI**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sensitivity (%, IC 95%)</th>
<th>Specificity (%, IC 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematological disease</td>
<td>70 (62-77)</td>
<td>92 (90-93)</td>
</tr>
<tr>
<td>HSCT</td>
<td>82 (70-90)</td>
<td>86 (83-88)</td>
</tr>
<tr>
<td>Allogeneic transplants</td>
<td>84 (69-91)</td>
<td>87 (82-89)</td>
</tr>
</tbody>
</table>

Serum Galactomannan and AF

- Four year study:
  - 262 hematological malignancy patients at high risk for IPA receiving Posaconazole prophylaxis
  - GM screening twice a week:
    - 2972 Serum GMs, 96.7% negative
- 5 breakthrough IPA infections, all positive serum GMs results (1.9% of all episodes)
  - Prophylaxis highly effective (1.9% of all episodes versus 7-20% without prophylaxis)!
- 30 episodes with false positive GM results

PPV GM „diagnostic driven“=suspicion of breakthrough IPA 89.6%
PPV GM screening during ongoing mouldactive AF prophylaxis 11.8%

NPV in both scenarios near to 100%
Serum Galactomannan and AF Study 2

- 146 evaluable episodes in pts with hematological malignancies receiving micafungin prophylaxis & GM serum Screening AND diagnostic driven GM
  - 4 episodes true-positive in the context of probable breakthrough IA (incidence of breakthrough IA, 2.7%); screening performed only in one of these
  - 111/146 high-risk episodes (76%) true-negative
  - 31/146 (21.2%) false-positive; No false-negative episodes
  - Positive predictive and negative predictive values of screening GM: 3.2% (1/31) and 100% (110/110)
  - Of diagnostic GM: 75% (3/4) and 100% (1/1)

What is earlier?

All depends on Prevalence

- Prevalence of IA/breakthrough IA decisive for PPV

Naturally prevalence of IA under mould active prophylaxis (1-4%) is lower, than in high risk settings without mould active prophylaxis (7%-20%)

Assuming GM Sensitivity 80%, Specificity 80%

IPA Prevalence 20%, positive GM: PPV 16/32 = 50%
IPA Prevalence 10%, positive GM: PPV 8/26 = 31%
IPA Prevalence 5%, positive GM: PPV 4/23 = 17%
### Serum GM and AF: Sensitivity?

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Week -2</th>
<th>Week -1</th>
<th>Week 0 (day of diagnosis until day +7)</th>
<th>Week 1 (week 2 after diagnosis)</th>
<th>Week 2 (week 3 after diagnosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n positive / n tested (percentage)</td>
<td>n positive / n tested (percentage)</td>
<td>n positive / n tested (percentage)</td>
<td>n positive / n tested (percentage)</td>
<td>n positive / n tested (percentage)</td>
</tr>
<tr>
<td><strong>All patients</strong></td>
<td></td>
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</tr>
<tr>
<td>Serum &gt;0.5</td>
<td>3/14 (21%)</td>
<td>10/18 (56%)</td>
<td>15/19 (79%)</td>
<td>9/13 (69%)</td>
<td>5/8 (63%)</td>
</tr>
<tr>
<td>BAL &gt;1.0</td>
<td>2/2</td>
<td>1/1</td>
<td>0/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Not receiving antifungal therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum &gt;0.5</td>
<td>0/4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAL &gt;1.0</td>
<td>1/1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Receiving antifungal therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum &gt;0.5</td>
<td>3/10 (33%)</td>
<td>10/13 (77%)</td>
<td>15/19 (79%)</td>
<td>9/13 (69%)</td>
<td>5/8 (63%)</td>
</tr>
<tr>
<td>BAL &gt;1.0</td>
<td>1/1</td>
<td>1/1</td>
<td>0/1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Targeted/Pre-emptive Antifungal Therapy** reduces Sensitivity of serum GM; Marr, CID 2005

**Antifungal Prophylaxis** may not reduce Sensitivity of serum GM for Breakthrough Infections; Hoenigl, Mycoses 2013; Duarte CID 2014; Vena CMI 2017
Proof of concept

- 100 episodes with diagnostic driven serum GM strategy during Posaconazole prophylaxis
- Only 39 out of 100 episodes required serum GM testing, and only 78 serum GM tests were performed overall with this approach (i.e. 6.9% of the total number of assays performed per 100 episodes with serum GM screening despite posa prophylaxis (P<0.001)
- 11 serum GM tests positive in 6 episodes; all false positive.
- 2 breakthrough infections: Candida glabrata and Fusarium solanii
- Costs for serum GM testing 3,147 € in the diagnostic driven strategy versus 45,757 € in the screening strategy
Proof of concept

- Duarte et al: 100 episodes with diagnostic driven serum GM strategy during Posaconazole prophylaxis.
  - Only 39 out of 100 episodes required sGM testing, and only 78 serum GM tests were performed overall with this approach (i.e. 6.9% of the total number of assays performed per 100 episodes with the serum GM screening despite posaconazole prophylaxis (P<0.001)).
  - 11 serum GM tests positive in 6 episodes; all false positive.
  - 2 breakthrough infections: Candida glabrata and Fusarium solani.
  - Costs for serum GM testing 3,147 € in the diagnostic driven strategy versus 45,757 € in the screening strategy.

Serum GM Screening can be safely removed in patients receiving posaconazole prophylaxis and replaced by a diagnostic driven GM testing strategy!
**Hintergrund**

- **Aspergillus GM**
- **LFD**
- **Neutropenia**

**Conclusion**

Afolter et al AJRCCM 2014 vs. Prattes et al AJCCM 2014

**Hematology Patients:**
- 62% Mould active Prophylaxis/therapy at the time of bronchoscopy
- BAL GM not considered for IPA grading
- Serum GM only in 1/3 the patients
- Goldstandard: BAL Culture (?)

**Pulmonology Patients:**
- Nobody on AF
- BAL GM considered for IPA grading
**BAL Galactomannan and AF**

**GM cut-off 0.5:**
- Sensitivity without AF 95%
- Sensitivity with AF 71%

**GM cut-off 1.0:**
- Sensitivity without AF 81%
- Sensitivity with AF 52%

**BAL GM:**
- Sensitivity might be reduced in case of mould active AF prophylaxis / treatment
- In patients receiving mould active AF 0.5 ODI cutoff preferable.

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**Table:**

<table>
<thead>
<tr>
<th></th>
<th>BALF GM sensitivity (cut-off 0.5 ODI)</th>
<th>BALF GM sensitivity (cut-off 1.0 ODI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>87% (55/63)</td>
<td>71% (45/63)</td>
</tr>
<tr>
<td>Under mould active systemic antifungals</td>
<td>71% (15/21)</td>
<td>52% (11/21)</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>90% (9/10)</td>
<td>70% (7/10)</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>0% (0/4)</td>
<td>0% (0/4)</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>80% (4/5)</td>
<td>60% (3/5)</td>
</tr>
<tr>
<td>Liposomal Amphotericin B</td>
<td>67% (2/3)</td>
<td>33% (1/3)</td>
</tr>
<tr>
<td>Without Antifungals</td>
<td>95% (40/42)</td>
<td>81% (34/42)</td>
</tr>
</tbody>
</table>
**BAL Galactomannan**

**Background**

- Aspergillus GM
- LFD
- Neutropenia

**Conclusion**

- Prattes J et al J Infect 2015
- Gils S et al JCM 2016

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**Table 1**  
Galactomannan levels in native and pretreated bronchoalveolar lavage fluid samples.

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Initial GM (median and range)</th>
<th>GM native from frozen sample (median and range)</th>
<th>GM pretreated from frozen sample (median and range)</th>
<th>p-Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall study population</td>
<td>15</td>
<td>0.73 (0.15–16.79)</td>
<td>0.51 (0.08–16.79)</td>
<td>0.01 (0–0.01)</td>
<td>0.001</td>
</tr>
<tr>
<td>Proven IPA</td>
<td>1</td>
<td>&gt;16.79</td>
<td>&gt;16.79</td>
<td>0.01 (all 0.01)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Probable IPA</td>
<td>4</td>
<td>16.6 (15.53–16.79)</td>
<td>&gt;16.79 (all &gt;16.79)</td>
<td>0.01 (all 0.01)</td>
<td>0.046</td>
</tr>
<tr>
<td>Possible IPA</td>
<td>1</td>
<td>0.73</td>
<td>0.51</td>
<td>0.01 (all 0.01)</td>
<td>n.a.</td>
</tr>
<tr>
<td>No IPA</td>
<td>9</td>
<td>0.19 (0.15–0.75)</td>
<td>0.22 (0.08–0.67)</td>
<td>0.01 (0–0.01)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Abbreviations: GM = galactomannan, IPA = invasive pulmonary aspergillosis, n.a. = not applicable.

a p-Values were calculated for GM native versus GM pretreated.

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**CAVE:** BALF pretreatment with dithiothreitol (Sputasol AND Copan SLsolution; used for liquifying BALF) and N-Acetyl-L-Cysteine agents reduce GM levels dramatically

**SOLUTION:** Liquiillizer (Dithiothreitol-free and odorless) no impact on GM levels
Imperfect Diagnostics - SOLUTION

All diagnostic lab tests for IA have IMPERFECT SENSITIVITIES WHICH MAY BE FURTHER REDUCED UNDER MOULD ACTIVE AF!!

- Blood samples more affected than BAL samples!

SOLUTION: BIOMARKER/TEST COMBINATIONS!!

- GM +/- PCR +/- LFD from BALF AND Blood
  - +/- BAL culture +/- Blood BDG
- Markedly increased sensitivity, specificity mostly unchanged

CSF and Urine Galactomannan

CSF: Future studies needed

- CSF GM recommended in current guidelines for patients with CNS IA
- Based on case reports indicating promising performance
Other materials: Urine

Advantages of biomarker detection from urine samples
- Non-invasive collection
- May reduce blood drawings from (anemic) patients
- Large sample volume
- Point of care home testing???

Current limitations
- Low evidence for clinical use
- Only few biomarkers have been studied in urine samples (galactomannan, LFD, TAFC)
- Urine dilution as potential confounding factor

References:
- Duettmann et al Med Mycol 2014
- Reischies et al JCM 2015
- Dufresne et al Plos ONE 2012
Urine Galactomannan

Urine: Future studies needed

- GM in native urine: Significant positive correlation found between urine & serum GM results

- Urine GM/Urine creatinine index
  - takes into account urine dilution

<table>
<thead>
<tr>
<th>Urine GM Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>DOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 ODI</td>
<td>0.19</td>
<td>0.937</td>
<td>0.2</td>
<td>92</td>
<td>3.48 (1.03-11.74)</td>
</tr>
<tr>
<td>0.15 ODI</td>
<td>0.333</td>
<td>0.905</td>
<td>0.2</td>
<td>92</td>
<td>4.76 (1.73-13.11)</td>
</tr>
<tr>
<td>0.1 ODI</td>
<td>0.476</td>
<td>0.86</td>
<td>0.2</td>
<td>51</td>
<td>5.57 (2.18-14.22)</td>
</tr>
</tbody>
</table>

Duettmann et al, Med Mycol 2014
Reischies JCM 2016
Creatinine as a marker for dilution

Background
Serum GM
BALF GM
Other Materials
Neutropenia
Conclusion

Serial dilution of urine samples: GM ODI levels reduced proportional to urine creatinine levels

Reischies et al JCM 2016
Reischies ICAAC 2015, oral communication
GM/Creatinine Index - IPA vs no IPA

- GM ODI 0.746
- GM-CreaIndx 0.801

AUC

Reischies et al JCM 2015
Reischies ICAAC 2015, oral communication
Results

- Sensitivities, specificities, positive (PLR) and negative likelihood ratio (NLR) for TAFC/crea index for probable versus no IA

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PLR (95% CI)</th>
<th>NLR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Per Patient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAFC/crea</td>
<td>0.86 (0.49 – 0.97)</td>
<td>0.88 (0.64 – 0.97)</td>
<td>6.86 (1.81 – 25.96)</td>
<td>0.16 (0.03 – 1.01)</td>
</tr>
<tr>
<td><strong>Per Sample</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAFC/crea</td>
<td>0.81 (0.60 – 0.92)</td>
<td>0.90 (0.71 – 0.97)</td>
<td>8.5 (2.2 – 32.3)</td>
<td>0.21 (0.09 – 0.51)</td>
</tr>
</tbody>
</table>
# Neutrophil count

## Background
- Neutrophil count
- Serum GM
- BALF GM
- Other Materials

## Neutropenia

<table>
<thead>
<tr>
<th>Neutropenic Patients</th>
<th>Non-neutropenic Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouldactive Prophylaxis frequent</td>
<td>Mouldactive Prophylaxis rare (e.g., GVHD, secondary prophylaxis, lung transplant recipients...)</td>
</tr>
<tr>
<td>Angioinvasive growth of Aspergillus</td>
<td>Airway invasive growth of Aspergillus</td>
</tr>
</tbody>
</table>
Radiology versus Galactomannan (2)

### IA Animal Model:

**Serum**
GM significantly higher in Ara-C induced Neutropenia vs immunosuppression with Cyclosporin/Prednislon (i.e. non-neutropenic)
Similar trend for beta-D-Glucan!

**BAL**
Increased in both: neutropenic and non-neutropenic; no difference
Serum GM: Influence of Neutropenia

Cordonnier et al CMI 2008

In non-neutropenic patients with invasive Aspergillosis Serum GM is less reliable!!

Bronchoscopy and BAL GM recommended!

<table>
<thead>
<tr>
<th></th>
<th>Neutropenic</th>
<th>non-Neutropenic</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL, 1.0</td>
<td>100%</td>
<td>94.7%</td>
<td>0.99</td>
</tr>
<tr>
<td>Serum, 0.5</td>
<td>90%</td>
<td>36.8%</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Courtesy of K. Lagrou

Galactomannan: When and where from?

In patients at “lower” risk for IA (e.g. COPD, solid tumours, liver cirrhosis, long time high dose corticosteroids)

GM testing in case of Clinical Suspicion

i.e. diagnostic driven GM testing

- Preferably BAL (mostly one time testing)
- Additionally Serum GM (NPV??) (at least twice)
Galactomannan: When and how often?

In patient groups at “high” risk for IA (e.g. alloSCT recipients, induction chemotherapy for AML)

Depends on whether patient is receiving mould active AF prophylaxis/treatment
Galactomannan: When and how often?

In patient groups at “high” risk for IA (e.g. alloSCT recipients, induction chemotherapy for AML, Lung TX)

- If ongoing mould active prophylaxis / empirical therapy
  - Diagnostic Driven GM testing for breakthrough infection

- If no mould active prophylaxis / empirical therapy
  - GM screening 2-3 times a week
Serum GM for Treatment Stratification and Outcome prediction in those with preemptive/targeted AF Treatment:

Once serum GM is positive may test 2-3 times a week until GM is negative

Seeber K et al. Usefulness of the Serum Galactomannan Assay for Early Response Assessment and Treatment Stratifications of Invasive Aspergillosis Article in Current Fungal Infection Reports 6(3) · September 2012
**Take home messages**

- GM important tool for diagnosis of breakthrough IA in neutropenic patients with AF prophylaxis
  - AF prophylaxis: diagnostic driven serum GM testing superior
  - BAL (GM, LFD and PCR) or CT guided puncture whenever possible (discriminate from Mucorales etc.)

**Biomarker combinations in case of AF treatment!!**

- Non-neutropenic patients at risk for IA
  - Blood Biomarkers – in particular serum GM – lower sensitivity
  - BAL GM preferred

Urine GM and other biomarkers: bigger role in the (near) future

**Thank You For Your Attention!**