Metabolic fingerprinting as a diagnostic tool for invasive aspergillosis: a pilot study

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The authors have **no** actual or potential conflicts of interest to report in relation to this presentation.
Despite significant advances associated with the recent introduction of standardized diagnostic criteria for IA over 1/3 of *Aspergillus* infections still remain undiagnosed ante mortem.

- Conventional histological, microbiological and radiological techniques remain the cornerstone of diagnosis but are not easily applicable, sufficiently sensitive and specific, respectively.
- Serological assays and molecular techniques offer additional information and help provide the diagnostic clues.

<table>
<thead>
<tr>
<th>Test</th>
<th>Indication</th>
<th>Specimen</th>
<th>Challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactomannan antigen</td>
<td>Early diagnosis of IA</td>
<td>Serum, BAL</td>
<td>• Decreased performance in patients receiving antifungal agents (low sensitivity)</td>
</tr>
<tr>
<td>1,3-β-D glucan</td>
<td>Diagnosis of systemic mycoses</td>
<td>Serum</td>
<td>• Non-specific for <em>Aspergillus</em> spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• False positives occur with gram negative bacteria</td>
</tr>
<tr>
<td>PCR</td>
<td>Detection of fungal genetic material</td>
<td>Serum, Whole blood, BAL etc</td>
<td>• Is not included in the EORTC/MSG criteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Non-standardized methodology</td>
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<td></td>
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<td>• High cost</td>
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**Metabolomics**: systematic quantification of the low molecular weight biochemicals (~50-1500 Da) known as **metabolites**, which are sensitive indicators of homeostatic imbalances.

**Metabolomic profiling**: sum of metabolite concentrations that reflect changes in their synthesis in **body fluids** and in the **site of infection** that can be detected **early** so as to allow the **appropriate patient treatment**.
Identification of a disease-specific metabolic profile of serum samples from haematological patients in conjunction with current surrogate biomarkers for the diagnosis of IA

- **Question 1:** Is there a specific metabolic fingerprint of the infection?
- **Question 2:** Can this unique metabolic profile enable the early diagnosis of the infection?
271 serum samples from 104 haematological patients

Detection of GM, BDG and Aspergillus DNA

Detection of circulating galactomannan with a sandwich enzyme-linked immunoassay

Quantification of 1,3-β-D glucan using a chromogenic kinetic assay


GM index

≥0.5 positive

<0.5 negative

≥80 pg/mL

<60 pg/mL

Positive

Negative

Positive

Negative
271 serum samples from 104 haematological patients

39 patients were selected based on GM index values

<table>
<thead>
<tr>
<th>Total number of patients</th>
<th>39 patients</th>
<th>25 men</th>
<th>14 women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control group (GM ≥0.5)</td>
<td>10 patients</td>
<td>7 men</td>
<td>3 women</td>
</tr>
<tr>
<td>Negative control group (GM &lt;0.5)</td>
<td>29 patients</td>
<td>18 men</td>
<td>11 women</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>&lt;40</th>
<th>40-49</th>
<th>50-59</th>
<th>&gt;59</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>5</td>
<td>2</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Women</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>

✓ For the patients in the positive control group, was selected the sample that was detected positive to GM and if available a sample acquired 15 days to a month earlier to investigate whether metabolomic analysis could be discriminatory of changes that have started earlier than the time of detection with the conventional serological assay

✓ Evaluable serum samples: 72
Experimental procedure

Metabolite extraction
Derivatization (MSTFA)

Data acquisition

Gas chromatography- Mass spectrometry (GC-MS)
(Kanani and Klapa Metabo. Eng. 2007; Gkourogianni et al. Plos One 2014)

Identification and quantification

Data validation, normalization and filtering
(Kanani and Klapa Metabo. Eng. 2007; Gkourogianni et al. Plos One 2014)

Extraction of biologically relevant conclusions

Correlation between metabolic profile-IA with Fisher’s exact test

Multivariate statistical analysis
A total of 333 chromatograms were analyzed (72 serum samples*2 replicates*≥3 aliquots)

161 peaks were identified → 80 peaks of metabolite derivatives

After normalization and filtering, 38 metabolites were selected
Positive control group: +GM
Negative control group: -GM

Metabolic profiles of 72 serum samples with respect to the GM assay

26 out of 38 metabolites differentiate the two groups with positive and negative GM for the extraction of biologically relevant conclusions
Positive control group: +GM
Negative control group: -GM

26 out of 38 metabolites differentiate the two groups with positive and negative GM for the extraction of biologically relevant conclusions.
Principal component analysis (PCA) for detecting diagnostically relevant metabolites (n=38)

Positive control group: +GM
Negative control group: -GM

Young patients: robust metabolism

High risk patients with early diagnosis

Patients with BDG or/and PCR + or treated with Vfend

Metabolic profiles of 72 serum samples with respect to the GM assay

26 out of 38 metabolites differentiate the two groups with positive and negative GM for the extraction of biologically relevant conclusions
Diagnostically important metabolites (n=26)

Patients with profile A (n=19)
- Higher concentration
- Lower concentration

Patients with profile B (n=26)

Patients with GM+ (n=8)
- BDG/PCR/Vfend + (n=8)
- Young age (n=2)

Patients with GM- (n=11)

Patients with GM- (n=23)

Patients with GM+ (n=3)

Diagnostically important metabolites (n=26)
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Hierarchical clustering analysis of metabolic profiles-samples
The metabolic profiles of all samples were separated in two clear sets (Fisher’s exact test p=0.038).

The metabolic profiles for all serum samples from 8/10 patients with probable IA clustered together (80% sensitivity), whereas 21/29 patients without IA did not demonstrate the specific metabolomic profile (72% specificity).

Serum metabolomics: 50% PPV and 91% NPV.
Reliable diagnosis of IA early enough to be of value in patient management continues to be a challenge and novel methodologies, such as metabolomics, holds promise for enhanced diagnostic accuracy.

First pilot study to evaluate the metabolomics as a diagnostic tool for IA in sera of patients with hematological malignancies.

- A high discriminatory power of the metabolic profiles of the patients’ serum samples with probable IA (80%) was found with 91% NPV, while serum metabolomics may detect earlier more high-risk patients with an IA-like profile compared to the conventional method.

Further investigation (more samples, detailed study of the patients’ medical history) is required to validate the usefulness of untargeted GC-MS metabolomics as a sensitive and accurate monitoring tool for early diagnosis of the infection.
Dad... Dad...
We learned how
to make bread, beer,
and wine in school
today!

Yawn!
Any old yeast can
do that! Come back
when you cured
thousands of diseases
and saved countless
human lives like our
relative Penicillin!

Thank you for
your attention