Antifungal therapy: to combine or not combine, that is the question!

*In vitro* antifungal susceptibility and genomics to predict outcome of fungal infections

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Transparency Declaration

Over the last 24 months I have received honoraria for talks on behalf of

- Astellas Pharma
- Gilead Sciences
- Roche
- Sanofi
- Qiagen
- Merck, Sharp & Dohme
Determinants of outcome in invasive fungal infections...

- Antifungal therapy
- Pathogen susceptibility
- Host genomics
- Pathogen genomics
- Candida
- Aspergillus

- Antifungal pharmacogenomics
- In vitro testing
- Innate and adaptive immunity
- Biofilm formation, persistent infection
- Virulence factors
Agenda

• *In vitro* antifungal susceptibility and candidemia outcome

• Biofilm formation in *Candida*

• Genomics and virulence factors

• Conclusions
Agenda

- *In vitro* antifungal susceptibility and candidemia outcome
- Biofilm formation in *Candida*
- Genomics and virulence factors
- Conclusions
96 episodes of candidemia

Treated with fluconazole monotherapy (≥72 hours)

In vitro susceptibility centrally assessed by CLSI (M27-A2) method

<table>
<thead>
<tr>
<th>Multivariate analysis</th>
<th>Odds ratio (IC 95%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluconazole MIC &gt; 64 mg/L</td>
<td>5.3 (0.8 – 33.4)</td>
<td>0.08</td>
</tr>
<tr>
<td>Age &gt; 63 years</td>
<td>8.9 (2.0 – 38.9)</td>
<td>0.004</td>
</tr>
<tr>
<td>APACHE (per 1-point increment)</td>
<td>1.2 (1.03 – 1.3)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Impact of fluconazole exposure (PK/PD)

![Graph showing patient survival (%) against fluconazole AUC\textsubscript{24h}/CLSI MIC]

**Fluconazole AUC\textsubscript{24h}/CLSI MIC**

- **<11.5**: N = 7 (100% survival)
- **11.5-100**: N = 12 (80% survival)
- **100-800**: N = 25 (60% survival)
- **800-1600**: N = 22 (40% survival)
- **>1600**: N = 18 (0% survival)

In vitro antifungal susceptibility and genomics to predict outcome of fungal infections

• **217 episodes** of candidemia (Australia Candidemia Study)

• Treated with **fluconazole monotherapy** (median duration of 14 days)

• *In vitro* susceptibility centrally assessed by **Sensitrite® YeastOne®**

• **Study outcomes:**
  
  • Primary: 30-day all-cause mortality (as assessed by the study investigator)
  
  • Secondary: all-cause 30-day mortality
In vitro antifungal susceptibility and genomics to predict outcome of fungal infections

OR for infection-related mortality [MIC ≥ 2 mg/L]: 8.2 (95% CI: 2.3-29.7); P-value = 0.001

MIC target identified by CART analysis

• 75 episodes of candidemia (single center in Israel)

• Treated with fluconazole monotherapy (≥48 hours)

• In vitro susceptibility centrally assessed by E-test stripes (interpretation by CLSI CBPs)

• Study outcomes:
  • Primary: 30-day all-cause mortality
  • Secondary: 7-day all-cause mortality
In vitro antifungal susceptibility and genomics to predict outcome of fungal infections

C. albicans

Non-albicans species (C. tropicalis, C. parapsilosis, C. guilliermondii)

**In vitro antifungal susceptibility and genomics to predict outcome of fungal infections**

Patients with early response

- Patients with early response

<table>
<thead>
<tr>
<th>C. albicans</th>
<th>Non-albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
<td>#</td>
</tr>
</tbody>
</table>

**P-value = 0.003**

Fluconazole dose/MIC >400

- Fluconazol dose/MIC ≤400

AUC$_{24}$/MIC >400

- AUC$_{24}$/MIC ≤400

**Clin Microbiol Infect 2015;21:1011-7.**
Impact of fluconazole susceptibility on the outcome of patients with candidaemia: data from a population-based surveillance

M. Fernández-Ruiz 1,*, J. Guinea 2, D. Lora-Pablos 3,4, Ó. Zaragoza 5, M. Puig-Asensio 6, B. Almirante 6, M. Cuenca-Estrella 5, J.M. Aguado 1 on behalf of the CANDIPOP Project 7, GEIH-GEOMICOMED (SEIMC) and REIPI

• 257 episodes of candidemia (29 Spanish centers)

• Treated with fluconazole monotherapy (≥72 hours)

• In vitro susceptibility centrally assessed by EUCAST and CLSI methods

• Study outcomes:
  • Clinical failure: 30-day all-cause mortality and/or persistent candidemia for ≥72 hours
  • Secondary: early (day 7) and late (days 7-30) all-cause mortality

Clin Microbiol Infect 2017;23:672.e1-672.e11.
Impact of MIC values (EUCAST)

In vitro antifungal susceptibility and genomics to predict outcome of fungal infections

Clin Microbiol Infect 2017;23:672.e1-672.e11.

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Impact of MIC values (CLSI)

In vitro antifungal susceptibility and genomics to predict outcome of fungal infections
Adjusted\(^a\) HR for clinical failure

- MIC ≥4 mg/L (CLSI)
- MIC ≥0.5 mg/L (CLSI)
- MIC ≥0.15 mg/L (EUCAST)
- MIC ≥4 mg/L (EUCAST)
- MIC ≥2 mg/L (EUCAST)

\(^a\) Adjusted for age, receipt of RRT, parenteral nutrition, Pitt score and early CVC removal (≥48 hours).

*Clin Microbiol Infect* 2017;23:672.e1-672.e11.
In vitro antifungal susceptibility and genomics to predict outcome of fungal infections

Impact of fluconazole exposure (PK/PD)

Fluconazole AUC$_{24h}$/EUCAST MIC (h)

- Early mortality
- Late mortality
- Clinical failure

Clin Microbiol Infect 2017;23:672.e1-672.e11.
Impact of fluconazole exposure (PK/PD)

In vitro antifungal susceptibility and genomics to predict outcome of fungal infections

Patients with outcome (%)

Fluconazole AUC$_{24h}$/CLSI MIC (h)

- Early mortality
- Late mortality
- Clinical failure

Clin Microbiol Infect 2017;23:e672.e1-e672.e11.
Adjusted\textsuperscript{a} HR for clinical failure

\begin{align*}
\text{AUC}_{24}/\text{MIC} & \geq 1,550 \text{ h (EUCAST)} \\
\text{AUC}_{24}/\text{MIC} & > 400 \text{ h (EUCAST)} \\
\text{AUC}_{24}/\text{MIC} & \geq 100 \text{ h (EUCAST)} \\
\text{AUC}_{24}/\text{MIC} & \geq 1,226 \text{ h (CLSI)} \\
\text{AUC}_{24}/\text{MIC} & > 400 \text{ h (CLSI)} \\
\text{AUC}_{24}/\text{MIC} & \geq 50 \text{ h (CLSI)}
\end{align*}

\textsuperscript{a}Adjusted for age, receipt of RRT, parenteral nutrition, Pitt score and early CVC removal (≥48 hours).
<table>
<thead>
<tr>
<th>Author</th>
<th>Sample</th>
<th>Correlation of FLU dose/MIC or AUC/MIC ratio with outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rex (1995)</td>
<td>N = 68</td>
<td>Inverse correlation between FLU MIC and persistence of candidemia ($P = 0.05$)</td>
</tr>
<tr>
<td>Pai (2007)</td>
<td>N = 77</td>
<td>FLU AUC/MIC ratio breakpoint &gt;55.2 associated with mortality ($P = 0.008$)</td>
</tr>
<tr>
<td>Rodríguez-Tudela (2007)</td>
<td>N = 126</td>
<td>FLU dose/MIC ratio of ≥100 associated with therapeutic success (92% vs. 50%)</td>
</tr>
<tr>
<td>Baddley (2008)</td>
<td>N = 84</td>
<td>FLU dose/MIC ratio of 12.5 associated with better survival (74% vs. 43%)</td>
</tr>
<tr>
<td>Eschenauer (2013)</td>
<td>N = 127</td>
<td>No FLU AUC/MIC thresholds associated with mortality in multivariable analysis</td>
</tr>
<tr>
<td>Brosh-Nissimov (2015)</td>
<td>N = 75</td>
<td>No FLU dose/MIC or AUC/MIC breakpoint identified for the entire cohort</td>
</tr>
<tr>
<td>C. albicans: FLUC dose/MIC and AUC/MIC ratios &gt;400 associated with 30-day mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fernández-Ruiz (2017)</td>
<td>N = 249</td>
<td>No FLU dose/MIC of AUC/MIC thresholds associated with therapeutic failure</td>
</tr>
</tbody>
</table>
Potential difficulties to interpret these studies...

- Relatively low number of isolates with high MIC values (most patients achieved PK/PD targets in excess)
- Heterogeneity in dosage regimens and PK/PD parameters
- Most of studies focused on fluconazole (no data for echinocandins)
- Need of adjustment for confounding factors (baseline clinical status, septic shock, type of candidemia, timing of source control...)
- Heterogeneity in analyzed outcomes (mortality vs. clinical failure, all-cause vs. infection-related mortality...)
In vitro antifungal susceptibility and genomics to predict outcome of fungal infections

Agenda

• *In vitro* antifungal susceptibility and candidemia outcome
• Biofilm formation in *Candida*
• Genomics and virulence factors
• Conclusions
• **217 episodes** of candidemia (Scotland, 2012-2013)

• Biofilm formation assessed by **biomass production** (crystal violet) and **metabolic activity** (XTT reduction assay and SYTO9)

• **Study outcome:** 30-day all-cause mortality
Higher biofilm production in *C. albicans* isolates

30-day survival in episodes due to *C. albicans* (n = 107)

![Graph showing cumulative survival over time for low- and high-biofilm producers](image)

- **Low-biofilm producers**
- **High-biofilm producers**

HR for mortality of high biofilm production:

5.99 (95% CI: 1.3 - 28.3); *P*-value = 0.024

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In vitro antifungal susceptibility and genomics to predict outcome of fungal infections

• **349 episodes** of candidemia or invasive candidiasis C

• Multicenter cohort restricted to **ICU patients**

• Biofilm formation assessed by the **BioFilm Ring Test®** (evaluates first step [adhesion])

• **Study outcome:** 28-day all-cause mortality
Lower biofilm production in *C. albicans* isolates

- **High-biofilm producers**
- **Low-biofilm producers**
- **No biofilm producers**


In vitro antifungal susceptibility and genomics to predict outcome of fungal infections
No impact on outcome of biofilm production ability

<table>
<thead>
<tr>
<th>Biofilm data</th>
<th>Survivors at D28 (N = 244)</th>
<th>Non-survivors at D28 (N = 105)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio dose/CMI</td>
<td>41.6 [18.9; 72.3]</td>
<td>48.3 [21.3; 70.3]</td>
<td>0.5696</td>
</tr>
<tr>
<td>Biofilm: High</td>
<td>100 (41.0)</td>
<td>34 (32.4)</td>
<td>0.3518</td>
</tr>
<tr>
<td>Biofilm: Low</td>
<td>49 (20.1)</td>
<td>21 (20)</td>
<td></td>
</tr>
<tr>
<td>Biofilm: No</td>
<td>95 (38.9)</td>
<td>50 (47.6)</td>
<td></td>
</tr>
<tr>
<td>Biofilm Score (Continuous)</td>
<td>7.3 [0; 19.9]</td>
<td>13.1 [0; 20]</td>
<td>0.1195</td>
</tr>
</tbody>
</table>

Is biofilm production a prognostic marker in adults with candidemia?

Biofilm formation assessed by crystal violet and XTT assays

Study outcomes:
- 7- and 30-day all-cause mortality, unfavourable prognosis

In vitro antifungal susceptibility and genomics to predict outcome of fungal infections

- 55 Candida isolates
- Biofilm formation assessed by crystal violet and confocal microscopy
- 7 patients (37 isolates) with persistent candidemia (>72 hours of therapy)
- 18 patients with single, non-persistent episodes

### Clinical impact of Candida spp. biofilm production in a cohort of patients with candidemia

<table>
<thead>
<tr>
<th></th>
<th>High</th>
<th>Moderate/low biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-day mortality:</td>
<td>16.7%</td>
<td>12.6%</td>
</tr>
<tr>
<td>30-day mortality:</td>
<td>37.8%</td>
<td>32.1%</td>
</tr>
<tr>
<td>Unfavourable prognosis:</td>
<td>50.0%</td>
<td>38.4%</td>
</tr>
</tbody>
</table>

(P = 0.0002 for biofilm formation)

7-day mortality: 16.7% vs. 12.6% (P = 0.362)
30-day mortality: 37.8% vs. 32.1% (P = 0.418)
Unfavourable prognosis: 50.0% vs. 38.4% (P = 0.071)

Clin Microbiol Infect. 2018;24:1010-5.


In vitro antifungal susceptibility and genomics to predict outcome of fungal infections
Potential difficulties to interpret these studies...

- Biofilm formation is a dynamic process

- Non-standardized methodology: biomass production (e.g. crystal violet) vs. metabolic activity (e.g. XTT assay), different thresholds...

- Choice of the most appropriate outcome

¿Persistent candidemia better than all-cause mortality?
Agenda

- *In vitro* antifungal susceptibility and candidemia outcome
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- Conclusions
Adhesion and endocytosis of *Candida albicans* hyphae by epithelial cells

ALS (Agglutinin-Like Sequence) adhesins

Structurally similar to bacterial adhesins

HWP (Hyphal Wall Protein) adhesins

Fungal attachment to host epithelial cells

Biofilm formation in *C. albicans* and the transcription regulatory network involved

I. Initial attachment and adhesion

II. Initiation of early stage biofilm

III. Late stage biofilm

IV. Dispersion of planktonic cells

**Adhesion genes**

**Als1**  
Downregulated in low virulent strains

**Als3**  
Downregulated in all strains

**Protease genes**

**Sap1**  
Upregulated only in moderately virulent strains

**Sap4**  
Upregulated in low/moderate virulent strains

**Sap6**  
Upregulated in low/moderate virulent strains

**Hyphal growth regulation and adhesion gene**

**Hwp1**  
Upregulated in moderate/high virulent strains

The mitochondrial protein Mcu1 plays important roles in carbon source utilization, filamentation, and virulence in *Candida albicans*.

Guobo Guan\(^a,1\), Haitao Wang\(^b,1\), Weihong Liang\(^a,c,1\), Chengjun Cao\(^a,c\), Li Tao\(^a\), Shamoon Naseem\(^d\), James B. Konopka\(^d\), Yue Wang\(^b,e\), Guanghua Huang\(^a,9\)

**Mouse model**

Mitochondrial protein involving in using N-Acetyl-Glucosamin as carbon source

**MCU1** gene

**mcu1/mcu1 mutant**

**Kidney fungal burden**

In vitro antifungal susceptibility and genomics to predict outcome of fungal infections

DST659 genotype of *Candida albicans* showing positive association between biofilm formation and dominance in Taiwan

Jang-Jih Lu¹,²,³,¹, Hsiu-Jung Lo⁴,⁵,¹, Yen-Mu Wu⁶, Jia-Yuan Chang⁷, Yin-Zhi Chen⁴ and Shao-Hung Wang⁷,*

- 42 *Candida* isolates from candidemic patients
- MLST typing method
  - DST659 genotype (20 isolates)
  - DST693 genotype (22 isolates)
- Zebrafish egg model
- Clinical outcome

**Survival (%)**

<table>
<thead>
<tr>
<th></th>
<th>DST659</th>
<th>DST693</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-value</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

**Patient survival (%)**

<table>
<thead>
<tr>
<th></th>
<th>DST659</th>
<th>DST693</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-value</td>
<td>= 0.501</td>
<td></td>
</tr>
</tbody>
</table>

In vitro antifungal susceptibility and genomics to predict outcome of fungal infections

Agenda

• *In vitro* antifungal susceptibility and candidemia outcome

• Biofilm formation in *Candida*

• Genomics and virulence factors

• Conclusions
Uncertainties?

- Non-consistent (and limited) data on the direct relationship between in vitro fluconazole MIC values or PK/PD parameters and therapeutic failure in candidemic patients treated with fluconazole monotherapy.
- Some (but not all) studies suggest that the capacity for biofilm formation may increase the risk of clinical failure in candidemic patients.
- What are the best outcomes to assess the clinical effect of in vitro susceptibility and biofilm formation (therapeutic failure, persistent infection, mortality)?
- Difficulties to translate genomic data on Candida virulence factors into clinical outcomes.
- No clear data to potentially target combination therapy in invasive candidiasis.
In vitro antifungal susceptibility and genomics to predict outcome of fungal infections

Thank you for the attention

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