Usefulness of time-to-positivity in aerobic and anaerobic vials to predict the presence of *Candida glabrata* in patients with candidaemia

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Objectives: To determine whether time-to-positivity (TTP) in aerobic and anaerobic blood culture vials is useful to predict the presence of *Candida glabrata* in patients with candidaemia.

Methods: TTP was recorded for both aerobic and anaerobic vials for each blood culture set of monomicrobial candidaemia. We considered TTP as the shortest time registered for any positive vial. Two diagnostic criteria were evaluated: the cut-off TTP value as obtained from a receiver operating characteristic curve and the detection of growth only or with a shorter TTP in anaerobic vials.

Results: A total of 157 episodes were analysed of which 19 (12.1%) were due to *C. glabrata*. The TTP for *C. glabrata* was longer than that for other species. *C. glabrata* grew more frequently than other species in anaerobic vials [9/19 (47%) versus 19/138 (14%); *P=* 0.001] and also more often exclusively or earlier in anaerobic vials [7/19 (37%) versus 5/138 (4%); *P*, 0.0001]. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of a TTP $\leq$ 56.5 h for predicting the presence of *C. glabrata* were 47%, 88%, 36% and 92%, respectively. Growth detection only or earlier in anaerobic flasks had a sensitivity of 37%, a specificity of 96%, a PPV of 58% and an NPV of 92%.

Conclusions: Using the BACTEC 9240 system, a TTP $\leq$ 56.5 h is useful to rule out *C. glabrata*. In addition, in settings with an ~12% prevalence of *C. glabrata* candidaemia, yeast detection exclusively or earlier in anaerobic vials increases the probability of the presence of *C. glabrata* to 58%, which may be useful for early treatment optimization.

Keywords: time to blood culture positivity, culture media, bloodstream infections

Introduction

Time-to-positivity (TTP), as provided by automatic blood culture processing machines, can be useful to disclose the kinds of microorganism involved and the presence of some resistant microorganisms, to differentiate contaminants from true pathogens, and to predict the source and prognosis of bacteraemia. There are, however, few data on these issues in relation to candidaemia. It has been previously reported that TTP is significantly longer for *Candida glabrata* than for other species. In addition, in vitro studies have recognized that this species is particularly prone to growing more frequently or more quickly in anaerobic vials. A presumptive diagnosis of *C. glabrata* at the time when growth is detected by the blood culture processor may help to shorten the time for optimizing antifungal therapy, due to the usual low susceptibility or resistance of this species to fluconazole. The aim of the present study was to assess the diagnostic accuracy of TTP and differential growth in aerobic and anaerobic blood culture vials for the diagnosis of *C. glabrata* in patients with monomicrobial candidaemia.

Methods

From 1 January 2008 to 31 March 2012, the microbiology laboratory registered the TTP for all blood culture vials positive for yeast obtained at a 700 bed university hospital in Barcelona, Spain. Blood cultures were processed using the BACTEC 9240 system (Becton-Dickinson, MD, USA). The vials used were the resin-containing BACTEC Plus Aerobic/F and BACTEC Plus Anaerobic/F, and the non-resin-containing BACTEC Standard/10 Aerobic/F and BACTEC Lytic/10 Anaerobic/F. The recommendation in our hospital is to inoculate 8–10 mL of blood into each vial, but the exact volume of blood was not recorded. Only patients with monomicrobial candidaemia were included in the study.
candidaemia were included. We considered TTP as the shortest time (measured in h) registered for any positive vial. If a patient had persistent candidaemia, only the first positive sets of blood cultures were taken into account.

For TTP, means, standard deviations, medians and IQRs were calculated. Pairwise comparisons of TTP between C. glabrata and other species were carried out using the Student’s t-test. Proportions were compared by the χ² or Fisher’s exact test. A receiver operating characteristic (ROC) curve was used to assess the diagnostic value of TTP for C. glabrata and to identify the cut-off value. Statistical analysis was undertaken using the SPSS statistical package for Windows (version 18.0; Chicago, IL, USA).

Results

A total of 157 episodes of monomicrobial candidaemia were analysed. The prevalence of the different species is shown in Table 1. The mean and standard deviation of the TTP for all isolates were 39.12 (21.4). The means and medians of TTP for the different species of Candida are shown in Table 1. The TTP for C. glabrata was significantly longer than that for the other species.

The performance with aerobic, anaerobic or both types of vials for each Candida species is shown in Table 2. C. glabrata grew more frequently than other species in anaerobic vials [9/19 (4.7%) versus 19/138 (14%), \( P = 0.001 \)] and also more often exclusively or earlier in anaerobic vials [7/19 (37%) versus 5/138 (4%), \( P = 0.0001 \)].

The cut-off TTP value obtained from the ROC curve to identify C. glabrata was 56.5 h, which was the same as the median of the distribution of TTP for this species. The performance characteristics of a TTP of >56.5 h for predicting the presence of C. glabrata were as follows: sensitivity, 47%; specificity, 88%; positive predictive value (PPV), 36% and negative predictive value (NPV), 92% [with an area under the ROC curve (AUC) of 0.66 (95% CI 0.52–0.8)]. Exclusive or earlier detection in anaerobic vials showed 37% sensitivity, 96% specificity, 58% PPV and 92% NPV. Combining both criteria (a TTP >56.5 h or exclusive or earlier detection in anaerobic vials) yielded a sensitivity, specificity, PPV and NPV of 79%, 85%, 42% and 97%, respectively.

Discussion

The main finding of the present study is that the exclusive or earlier growth of yeast in anaerobic vials is predictive of the presence of C. glabrata. This species grew more frequently than others in anaerobic media and, when growing in both types of vial, the TTP was shorter for the anaerobic vial in 60% of cases. The present study also confirms that a shorter TTP is predictive to rule out C. glabrata. The ability of the TTP and exclusive or earlier growth in anaerobic vials to predict the presence of C. glabrata seem to be unrelated, the latter being a little less sensitive, but more specific. In our setting, exclusive or earlier yeast detection in anaerobic vials increased the chances of C. glabrata being present from a pre-test probability of 12% to 58%. Combining the TTP (>56.5 h) with an exclusive or earlier detection in anaerobic vials had as a main effect an increase in sensitivity (from 37% to 79%) and in NPV (from 92% to 97%).

In the present study, the cut-off value derived from the ROC curve (56.5 h) for predicting the presence of C. glabrata was higher than that proposed in two previous studies (27.7 and 45.1 h). In addition, the diagnostic value of TTP observed in the present study, defined by the area under the ROC curve, was only moderate (0.66) in comparison with the better values obtained by Lai et al. (0.83) and Huang et al. (0.8). This was due to the fact that, in our study, the difference in the distribution of TTP between C. glabrata and Candida albicans was less striking than that previously observed.

Although all three studies used the BACTEC system, many of these discrepancies probably lie in the different criteria and types of vial used. It has previously been reported that C. glabrata has a shorter TTP in anaerobic than aerobic vials and also that the Myco/F lytic vial is an aerobic medium with the fastest TTP for

Table 1. TTP of different Candida spp. included in the study

<table>
<thead>
<tr>
<th>Species</th>
<th>n (%)</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>81 (51.6)</td>
<td>41.5 (19.4)</td>
<td>39.1 (28.4–49.9)</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>31 (19.7)</td>
<td>36.2 (17)</td>
<td>34.8 (25.1–46.5)</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>20 (12.7)</td>
<td>28.3 (19.9)</td>
<td>22 (16.6–31.8)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>19 (12.1)</td>
<td>53.4 (26.8)</td>
<td>56.5 (29.7–77.3)</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>4 (2.5)</td>
<td>23.3 (17.1)</td>
<td>21.3 (8.3–40.25)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (1.3)</td>
<td>19.9 (4.6)</td>
<td>19.8 (16.5–19.9)</td>
</tr>
<tr>
<td>Total</td>
<td>157 (100)</td>
<td>39.12 (21.4)</td>
<td>34.8 (25.1–48.7)</td>
</tr>
</tbody>
</table>

Table 2. Performance of aerobic, anaerobic or both types of vial for the different Candida spp. included in the study

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Growth in aerobic and anaerobic vials</th>
<th>Growth in aerobic vials only</th>
<th>Growth in anaerobic vials only</th>
<th>Growth exclusively or earlier detection in anaerobic vials</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>81</td>
<td>70 (86%)</td>
<td>1 (1%)</td>
<td>3 (4%)</td>
<td></td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>31</td>
<td>28 (90%)</td>
<td>0</td>
<td>2 (7%)</td>
<td></td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>20</td>
<td>16 (80%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>C. glabrata</td>
<td>19</td>
<td>10 (53%)</td>
<td>4 (21%)</td>
<td>7 (37%)</td>
<td></td>
</tr>
<tr>
<td>C. krusei</td>
<td>4</td>
<td>3 (75%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>2 (100%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>157</td>
<td>129 (82%)</td>
<td>5 (3%)</td>
<td>12 (8%)</td>
<td></td>
</tr>
</tbody>
</table>
simulated candidaemia in the BACTEC 9240 system.\textsuperscript{12} This could explain why Lai et al.,\textsuperscript{7} who exclusively used Myco/F lytic vials, reported a mean TTP for \textit{C. albicans} that was lower than that in our study, and a mean TTP for \textit{C. glabrata} that was similar. For the same reason, Huang et al.,\textsuperscript{8} who evaluated only regular aerobic vials, obtained a TTP for \textit{C. glabrata} that was much longer than the one found in our setting. On the other hand, according to our data, using only aerobic vials would be inappropriate since this could dismiss the 21\% isolates of \textit{C. glabrata} that grew only in anaerobic vials.

Part of the observed variability can also be explained by the different distribution among the studies of other variables that may influence TTP, such as the volume of blood inoculated, ongoing antimicrobial therapy, delay until the onset of incubation, the incubation system,\textsuperscript{12} the source of fungaemia,\textsuperscript{13} and some clinical conditions such as shock, neutropenia or cirrhosis.\textsuperscript{1} Due to these confounders, it may be difficult to apply what is a useful cut-off TTP value in a particular study to other settings. We suggest that exclusive or earlier growth in anaerobic vials may essentially depend on an intrinsic property of \textit{C. glabrata} less prone to being affected by the above-mentioned variables, and therefore of more general application.

The clinical relevance of predicting \textit{C. glabrata} remains unclear. It is currently recommended that an echinocandin be used as empirical therapy in patients with suspected invasive candidiasis and severe sepsis or septic shock, and in those with risk factors for having potentially fluconazole-resistant species.\textsuperscript{11} However, in those patients in which either empirical antifungal therapy was not given or fluconazole was started empirically, measuring and reporting the TTP for each aerobic and anaerobic positive vial may lead to an early optimization of antifungal therapy without complications of the extra cost or availability of rapid techniques such as PCR or matrix-assisted laser desorption/ionization-time of flight mass spectrometry. The clinical relevance of predicting \textit{C. glabrata} might also be compromised in the future if resistance to fluconazole in other species such as \textit{Candida parapsilosis} or \textit{Candida tropicalis} became a frequent event.

Some limitations of this study are the low number of \textit{C. glabrata} isolates, the absence of antimicrobial susceptibility testing, the lack of control over variables that may influence TTP and the use of a particular blood culture processing system, which makes it necessary to undertake further studies with a higher number of isolates and other types of blood culture systems to confirm our findings.

In conclusion, using the BACTEC 9240 system, either a TTP ≤56.5 h or growth only or earlier in aerobic flasks is useful to rule out the presence of \textit{C. glabrata}. In addition, in settings with an ~12\% prevalence of \textit{C. glabrata} candidaemia, yeast detection exclusively or earlier in anaerobic vials increases the probability of \textit{C. glabrata} being present to 58\%. Knowing the type of medium in which the growth is detected would improve the value of TTP in predicting the presence of \textit{C. glabrata} and could be useful for early treatment optimization.

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\textbf{Transparency declarations}

None to declare.

\textbf{References}