Time to positivity of blood cultures of different *Candida* species causing fungaemia

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This study investigated the time to positivity (TTP) for different species of *Candida* causing bloodstream infection and whether TTP can help differentiate fluconazole-resistant *Candida glabrata* and *Candida krusei* from other *Candida* species. We conducted this study at the National Taiwan University Hospital, a 2500-bed tertiary care medical centre in northern Taiwan. Patients with candidaemia were identified by central laboratory personnel from July 2010 to March 2011. TTP in each patient was determined using an automated blood culture instrument. Each patient was included only once at the time of detection of the first bloodstream infection. During the study period, a total of 329 sets of blood cultures positive for *Candida* were isolated from 176 patients. The mean TTP for all isolates causing candidaemia was 25.9 ± 24.9 h. The TTP for *C. glabrata* was significantly longer than the TTP of the other species. In contrast, the TTP of *C. tropicalis* was significantly shorter than that of the other three species. The diagnostic sensitivity of TTP for *C. glabrata* isolates in patients with candidaemia was 93.9 % (95 % CI 0.798–0.993), the specificity was 66.4 % (95 % CI 0.581–0.741), the positive predictive value was 39.2 % (95 % CI 0.286–0.509), and the negative predictive value was 97.9 % (95 % CI 0.92–0.996) with a TTP cut-off value of >27.7 h. In conclusion, the different TTP values of different *Candida* species causing bloodstream infection may be helpful in differentiating *C. glabrata* from other *Candida* species.

INTRODUCTION

Invasive candidiasis has become as an important nosocomial infection, especially in critically ill patients with intravascular catheters, on broad-spectrum antibiotics, on mechanical ventilation, on immunosuppressive agents or on parenteral nutrition (Asmundsdottir et al., 2002; Almirante et al., 2005; Ruan et al., 2008). Although *Candida albicans* remains the predominant cause of invasive candidiasis and accounts for >50 % of all cases, incidence rates of fungaemia caused by other species of *Candida*, including *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis*, are increasing (Abi-Said et al., 1997; Wisplinghoff et al., 2004; Ruan & Hsueh, 2009). Most importantly, *Candida krusei* is intrinsically resistant to fluconazole because of a decreased susceptibility to 14a-demethylase (Orozco et al., 1998) and *C. glabrata* is relatively resistant to fluconazole due to an energy-dependent efflux mechanism (Parkinson et al., 1995). The increasing incidence of fungaemia due to *C. glabrata* and *C. krusei* has important implications for therapy. For example, several studies have reported that the mortality rate associated with *C. glabrata* fungaemia ranges from 25 to 50 % (Gumbo et al., 1999; Malani et al., 2005; Wilson et al., 2005) and that delayed administration of appropriate antifungal therapy has significant adverse effects on the outcome of patients with candidaemia (Morrell et al., 2005; Garey et al., 2006). Conventional diagnostic methods take days for yeast identification, and incorrect or incomplete identification has been reported with these methods (Pfaller & Diekema, 2002). Therefore, rapid and accurate methods for identification of *Candida* species are needed so that antifungal agents can be administered in a timely manner. Although several techniques have been shown to achieve earlier identification of pathogenic fungi, their utility and effectiveness in clinical practice is limited (Li

Abbreviations: ROC, receiver operating characteristic; TTP, time to positivity.
et al., 2003; Patterson, 2005; Leaw et al., 2006; Barnes, 2008; Louie et al., 2008; Gherna & Merz, 2009; Hsiue et al., 2010).

Time to positivity (TTP), defined as the time from the start of incubation to the start of the alert signal (as documented by the monitoring system), was assessed by automated blood culture devices and is reported to be dependent on inoculum size (Blot et al., 1998), and may even constitute a surrogate marker of the degree of bacteraemia (Khatib et al., 2005). Furthermore, TTP provides useful diagnostic and prognostic information (Blot et al., 1998; Khatib et al., 2005). For example, we recently showed that TTP was helpful in differentiating between Staphylococcus aureus and coagulase-negative staphyloccocal bacteria (Lai et al., 2011). However, few studies have evaluated the association between TTP and candidaemia (Horvath et al., 2004; Ben-Ami et al., 2008; Park et al., 2010; Taur et al., 2010). Therefore, in this study we investigated the TTP of different species of Candida causing bloodstream infection and whether TTP can help differentiate fluconazole-resistant C. glabrata and C. krusei from other species of Candida.

### METHODS

**Setting and study design.** This study was conducted at the National Taiwan University Hospital, a 2500-bed tertiary care medical centre in northern Taiwan. Patients with candidaemia were identified by central laboratory personnel. Fungal blood cultures obtained from July 2010 to March 2011 at the hospital were analysed. BACTEC Myco/F Lytic bottles (Becton Dickinson) containing 5–10 ml blood from patients were incubated in a BACTEC 9240 culture system at 35 °C. TTP for each patient was determined using the hospital’s automated blood culture instrument. Each patient was included only once at the time of detection of the first bloodstream infection. Patients younger than 18 years of age and patients with polymicrobial bacteraemia or fungaemia were excluded. For patients with multiple sets of blood cultures identified as positive at approximately the same time, the shortest TTP was used.

**Statistical analysis.** Means and SD were calculated for continuous variables, and receiver operating characteristic (ROC) curves were plotted for TTP to identify C. glabrata and C. krusei fungaemia. A P-value <0.05 was considered to represent statistical significance. Data were analysed with the statistical package SPSS version 10.0 for Windows.

### RESULTS

During the study period, a total of 329 sets of blood cultures that were positive for Candida were isolated from 176 patients. The most common Candida species was C. albicans (n=146, 44.3%) followed by C. tropicalis (n=78, 23.7%), C. glabrata (n=57, 17.3%), C. parapsilosis (n=36, 10.9%), Candida guilliermondii (n=8, 2.4%), C. krusei (n=2, 0.6%) and Candida lusitaniae (n=2, 0.6%). Based on the number of patients (176) with Candida isolates, the most prevalent species were C. albicans (n=83, 47.2%), C. tropicalis (n=41, 23.3%), C. glabrata (n=33, 18.8%), C. parapsilosis (n=14, 8.0%), C. guilliermondii (n=2, 1.1%), C. krusei (n=2, 1.1%) and C. lusitaniae (n=1, 0.6%).

The mean TTP for all Candida isolates was 25.9±24.9 h. The TTP of C. albicans, C. tropicalis, C. glabrata and C. parapsilosis isolates are shown in Table 1. The TTP of two species was significantly shorter than that of the other three species. In contrast, the TTP of C. tropicalis was significantly shorter than that of the other three species. In addition, the TTP of C. lusitaniae isolates was 34.5 and 26.2 h. The TTP of C. krusei isolates were 27.6 and 18.7 h, and the TTP of one C. lusitaniae isolate was 14.0 h.

The ROC curves were plotted to evaluate the ability of TTP to identify C. glabrata isolates in patients with Candida bloodstream infections (Fig. 1). The area under the curve (AUC) was 0.852 (95% CI 0.791–0.901). Overall, the diagnostic sensitivity of TTP for detecting C. glabrata isolates in patients with candidaemia was 93.9% (95% CI 0.798–0.993), the specificity was 66.4% (95% CI 0.581–0.741), the positive predictive value was 39.2% (95% CI 0.286–0.509), and the negative predictive value was 97.9% (95% CI 0.92–0.996), with a TTP cut-off value of >27.7 h.

The ROC curves were plotted to evaluate the ability of TTP to differentiate between C. glabrata and C. krusei isolates in patients with Candida bloodstream infections (Fig. 2). The AUC was 0.831 (95% CI 0.768–0.883). Overall, the diagnostic sensitivity of TTP for differentiating between the two species was 97.1% (95% CI 0.851–0.999), the specificity was 58.9% (95% CI 0.503–0.671), the positive predictive value was 36.6% (95% CI 0.270–0.472), and the negative predictive value was 98.8% (95% CI 0.925–0.999), with a TTP cut-off value of >24.7 h.

### Table 1. TTP and number of non-duplicate positive blood culture bottles of four commonly encountered Candida species

<table>
<thead>
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<th>Species</th>
<th>No. of bottles with positive cultures</th>
<th>Mean TTP ± SD (h)</th>
</tr>
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<tr>
<td>C. albicans</td>
<td>83</td>
<td>34.2 ± 25.1*</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>41</td>
<td>16.9 ± 7.7†</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>33</td>
<td>56.5 ± 25.5†</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>14</td>
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In order to evaluate the diagnostic usefulness of TTP in fungaemia, many studies have reported values of different species of Candida, suggesting that TTP differs among different species of Candida. In our study as well as in previous studies, Horvath et al. (2004) reported that the mean TTP values for C. glabrata, C. albicans, C. parapsilosis and C. tropicalis were 50.8 ± 19.6 h, 37.5 ± 11.8 h, 35.3 ± 11.3 h and 22.4 ± 10.3 h, respectively. In addition, Ben-Ami et al. (2008) reported that the TTP was significantly longer for C. glabrata than for other Candida species causing fungaemia (61.3 ± 7 h vs 25.6 ± 2 h; P<0.001). The findings reported in our study as well as those in previous studies suggest that TTP differs among different species of Candida and that longer TTP values for Candida species causing fungaemia may be predictive of C. glabrata.

In order to evaluate the diagnostic usefulness of TTP in patients with candidaemia, we analysed whether TTP could differentiate between C. glabrata and other species of Candida. We found that the negative predictive value of TTP for identifying C. glabrata fungaemia in patients with candidaemia was 97.9% with a TTP cut-off of >27.7 h, suggesting that C. glabrata fungaemia can be excluded if the TTP of Candida isolates is <27.7 h. Thus, the high sensitivity value and negative predictive value of TTP imply that it is a useful adjunct test for excluding C. glabrata fungaemia in patients with candidaemia.

Drug-resistance patterns differed among each species of Candida, including the relatively drug-resistant species C. glabrata and C. krusei. Therefore, it is important for clinicians to know the species of Candida that is causing the bloodstream infection so that appropriate antifungal agents can be administered. However, it normally takes days to identify members of the Candida genus to the species level in clinical practice. In order to investigate the diagnostic usefulness of TTP to identify the two relatively drug-resistant Candida species, C. glabrata and C. krusei, among other species of Candida causing bloodstream infection, we divided all of the Candida species into two groups and compared the difference between the TTP values of these two groups in this study. We found that the negative predictive value of TTP for diagnosing C. glabrata and C. krusei was as high as 98.8% if a TTP cut-off of 24.7 h is used. This indicates that if the TTP of the Candida species is <24.7 h, the probability of Candida isolates being C. glabrata and C. krusei is minimal, indicating that fluconazole may be the appropriate antifungal agent for treatment.

This study had several limitations. Firstly, TTP can be influenced by multiple factors, such as the volume of blood drawn, incubation conditions, time from specimen collection to receipt in the laboratory, presence of growth inhibitors such as antimicrobials, prior use of antifungal agents and concentration of the organism within the blood. However, the influence of these factors in this study may have been minimal because the volume of blood drawn was similar in all adult patients and the incubation conditions were stable due to the use of an automated system. In addition, we did not record the duration of illness before blood samples had been collected for culture, which might have an important influence on fungaemia severity and TTP. Susceptibility testing of C. glabrata isolates to fluconazole might provide a better understanding of whether TTP can differentiate between fluconazole-resistant C. glabrata from other Candida species because not all C. glabrata isolates were resistant to fluconazole. Finally, this study was performed at a medical centre and the sample size was limited, especially with respect to C. krusei cultures. Further large-scale investigations are needed to assess whether these methods can be applied to all hospitals.

In conclusion, our findings suggest that TTP is a useful diagnostic adjunct for candidaemia. The varying TTP values of different Candida species causing bloodstream infections.
infection may be helpful in differentiating C. glabrata infections from those caused by other species of Candida.

REFERENCES


