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Usefulness of Two-Step Algorithm with Earlier Growth Detection in Anaerobic Bottle and Time to Positivity to Predict *Candida glabrata* Fungemia

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Background: Fast identification of *Candida glabrata* is important, because empirical antifungal therapy for fungemia with *C. glabrata* and non-*C. glabrata* varies. We proposed an algorithm for rapid presumptive diagnosis to identify fungemia with *C. glabrata* using earlier or only growth from anaerobic bottles and longer time to positivity (TTP) in blood cultures.
Methods: Positivity and TTP using the BacT/Alert 3D system (bioMerieux Inc, USA) with resin bottles (FA Plus and FN Plus) were analyzed in 215 candidemia patients from June 2014 to June 2016 in a university-affiliated hospital in Korea.
Results: A higher proportion of earlier or only growth from anaerobic bottles was observed in *C. glabrata* (38.8%, 7/18) than in *C. albicans* (7.6%, 8/105), *C. parapsilosis* (10.5%, 4/138), and *C. tropicalis* (9.2%, 5/54) (P=0.006). The mean (±standard deviation) TTP for *C. glabrata* was 41.7 h (±16.3 h) compared with 26.7 h (±15.9 h) for *C. albicans*, 33.4 h (±8.4 h) for *C. parapsilosis*, and 23.1 h (±17.3 h) for *C. tropicalis* (P<0.0001). We could predict fungemia with *C. glabrata* with a sensitivity of 94.4%, specificity of 63.9%, positive predictive value of 19.3%, and negative predictive value of 99.2% using a two-step algorithm: earlier or only growth from anaerobic bottles and TTP >31.4 h.
Conclusion: This two-step algorithm in the BacT/Alert 3D system could be the basis for an initial empirical antifungal therapy for fungemia with *C. glabrata* prior to final identification. **(Ann Clin Microbiol 2018;21:23-27)**

Key Words: Blood culture, *Candida glabrata*, Candidemia, Fungemia, Time to positivity

INTRODUCTION

Candida species are the fourth most common cause of nosocomial blood stream infections (BSI) worldwide [1]. *Candida albicans* is the leading pathogen of fungal BSI, followed by *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis*. The risk factors of candidemia are well known includes use of broad spectrum antibiotics, corticosteroids, or chemotherapeutic agents, hematologic or solid-organ malignancy, neutropenia, extensive intra-abdominal surgery or burns, mechanical ventilation or admission to an intensive care unit, indwelling central venous catheter or parenteral nutrition, hemodialysis, and prior fungal colonization [2]. When candidemia is suspected, empirical antifungal therapy should start prior to culture-based etiological identification. Administration of an appropriate antimicrobial therapy is associated with lower mortality [2]. Echinocandin such as caspofungin, micafungin, and anidulafungin is recommended as initial therapy [3,4]. However, fluconazole is an acceptable alternative to an echinocandin as initial therapy in selected patients, including those who are not critically ill and who are considered unlikely to have a fluconazole-resistant *Candida* species. Fluconazole is still preferred in medical environment with limited access to echinochandin. Importantly *C. glabrata* has shown a decreased susceptibility to fluconazole [5]. Therefore, an empirical antifungal therapy should be different between *C. glabrata* and non-*C. glabrata* infections.

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Although *Candida* species are known to be absolute aerobic organisms, sometimes it grows only in the anaerobic bottles [6]. Foster et al. [7] and Hovarth et al. [8] discovered a faster growth of *C. glabrata* characteristically in the anaerobic bottles. *C. glabrata* demonstrated a longer time to positivity (TTP) compared to non-*C. glabrata* both in the Bactec system (Becton-Dickinson, Sparks, MD, USA) and BacT/Alert 3D system (bioMereiux Inc., Durham, NC, USA) [7,9-12]. Time to positivity (TTP) is the time lapse from the insertion of the blood culture bottles into the continuous monitoring blood culture systems to the positive signals of microorganisms. TTP is information obtained without additional efforts or cost. Also, it comes much earlier than the final identification. Number of different studies has tried to facilitate the parameter, TTP, in order to predict either bacterial concentration in the blood or resistance to antibiotics [12,13]. We evaluated positivity and TTP from aerobic and anaerobic bottles using BacT/Alert 3D system to propose a rapid presumptive diagnosis algorithm to initiate antifungal therapy for fungemia with *C. glabrata*. There are very few data of fungemia regarding TTP using recently introduced resin bottles of BacT/Alert 3D system yet.

MATERIALS AND METHODS

1. Review of candidemia

The positive blood cultures with candidemia from June 2014 to June 2016 in a 960-bed university-affiliated hospital in Korea were reviewed. We had used BacT/Alert 3D system with resin bottles (FA Plus and FN Plus). Immediate inoculation into automated blood culture machine was conducted during the business hours. Beside the business hours, the bottles were stored in the room temperature were inoculated in the morning.

Data of pediatric bottles were excluded. *Candida* species was identified with Vitek-2 systems (bioMerieux Inc.). Frequency of identified *Candida* species was investigated. When growth of *Candida* species was detected, earlier growth was analyzed. In addition, TTP was recorded from the stored information in the BacT/Alert 3D system. Medical records such as age, sex, malignancy, intensive care unit admission, and death were reviewed after the approval of institutional review board (IRB) in Gyeongsang National University Hospital.

2. Statistics

Mean and standard deviation were calculated out from numerical data such as TTP. Number and proportion were calculated out from categorical data such as proportion of growth. Proportion of earlier growth or only from anaerobic bottles was analyzed by Fisher's exact probability test. Before analyzing TTP, normal distribution (by Shapiro-Wilk test) and equal variance assumption (by Levene's F test) of each species were tested. As TTP of each species were not normally distributed, they were analyzed by Wilcoxon signed rank test. Kruskall Wallis with *post hoc*. Bonferroni's method was used to compare the differences of mean TTP of each *Candida* species. TTP and mortality were analyzed by Mann-Whitney test. TTP was analyzed with other clinical factors of age, gender, malignancy, intensive care unit admission using multiple regression analysis. A receiver operating characteristic (ROC) curve was facilitated to decide the optimal cut-off value. Diagnostic efficacy using one parameter (earlier growth in the anaerobic bottles) or two parameters (earlier growth in the anaerobic bottles and certain TTP) was evaluated. For all analyses, a P value of <0.05 was considered significant for two-tailed tests. All statistical analyses were performed using SPSS, version 21 (IBM Corp., Armonk, NY, USA).

RESULTS

1. Positivity and TTP

During 2 years of study period, 231 sets of monomicrobial candidemia were identified. The most frequently isolated *Candida* species were as follows; 105 *C. albicans*, 54 *C. tropicalis*, 38 *C. parapsilosis*, and 18 *C. glabrata*, which accounts for 215 (93.1%). Other rare Candida species (5 *C. lusitaniae*, 5 *C. krusei*, 2 *C. guilliermondii*, 2 *C. famata*, 1 *C. intermedia*, and 1 *C. colliculosa*) were excluded from the analysis.

The proportion of growth in the anaerobic bottles only or earlier than in the aerobic bottles was 38.8% (7/18) of *C. glabrata* compared to 7.6% (8/105) of *C. albicans*, 10.5% (4/38) of *C. parapsilosis*, and 9.2% (5/54) of *C. tropicalis* (P=0.006). Growth

Table 1. Comparison of the mean time to positivity (TTP) and standard deviation of BacT/Alert 3D system

Species (n.)	FA Plus (n.)	FN Plus (n.)
Candida albicans (105)	26.9±15.8 (97)	24.0±17.8 (8)
Candida glabrata (18)	49.5±15.7 (11)	29.3±6.8 (7)
Candida tropicalis (54)	23.3±18.2 (48)	21.1±6.8 (5)
Candida parapsilosis (38)	33.2±8.8 (34)	35.0±3.1 (4)

Abbreviations: FA Plus, growth earlier or only from aerobe bottles; FN Plus, growth earlier or only from anaerobe bottles.

earlier or only from anaerobic bottles identified *C. glabrata* with sensitivity of 38.8%, specificity of 91.3%, positive predictive value of 29.1%, and negative predictive value of 94.2% (Table 1).

The mean (±SD) TTP was 41.7 (±16.3) h for *C. glabrata*, compared to 26.7 (±15.9) h for *C. albicans*, 33.4 (±8.4) h for *C. parapsilosis*, 23.1 (±17.3) h for *C. tropicalis*, respectively ($P<0.0001$).

We used ROC curve to decide the optimal cut-off value to discriminate *C. glabrata* from others. Area under ROC curve (AUC) was 0.75 (95% confidence interval, 0.65-0.86) to discriminate *C. glabrata* using the screening algorithm consisting of two-steps (earlier growth or only from anaerobic bottles and TTP >31.4 h). With this two-step algorithm (Fig. 1), we could predict *C. glabrata* with sensitivity of 94.4%, specificity of 63.9%, positive predictive value of 19.3%, and negative predictive value of 99.2% (Table 2).

2. Mortality analysis

Mortality rate revealed 69.5% of *C. albicans*, 44.6% of *C. tropicalis*, 39.0% of *C. parapsilosis*, and 72.0% of *C. glabrata* ($P<0.001$). This data did not include the hopeless discharge. Longer TTP of all *Candida* species and mortality were closely related ($P=0.011$). However, when we separated into each *Candida* species, no relationship of TTP and mortality was observed. Other clinical factors including age, sex, underlying malignancy, admission to intensive care units were not correlated with mortality.

DISCUSSION

We observed a significantly prolonged TTP in the fungemia with *C. glabrata* as described in the previous studies [9-11]. This succeeded to advocate its presence more frequently and even faster in the anaerobic bottles than in the aerobic ones. A previous study using BACTEC 9240 system demonstrated this feature which distinguished fungemia of *C. glabrata* with more than 90% of specificity and negative predictive value, but 33% of sensitivity using only the criteria of earlier growth in the anaerobic bottles [9]. In our study, it was not highly sensitive (38.8%) to presume *C. glabrata* using the same criteria, either. Increase of sensitivity was required in order not to miss out *C. glabrata* which needed echinocandin or high dose of fluconazole/voriconazole for an empirical antifungal treatment. ROC curve was exploited to determine the cut-off TTP which was 31.4 h. Other studies using Bactec 9240 system derived a wide range of cut-off TTP (27.7 h, 45.2 h, and 56.5 h). TTP is easily influenced by various factors including volume of blood, prior use of antifungal agents, host immunity, concentration of organ-

Table 2. Performance of algorithm using growth from anaerobic bottle and TTP (time to positivity)

Algorithm	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Growth earlier or only from anaerobic bottle	38.8%	91.3%	29.1%	94.2%
Growth earlier or only from anaerobic bottle and TTP >31.4 h	94.4%	63.9%	19.3%	99.2%

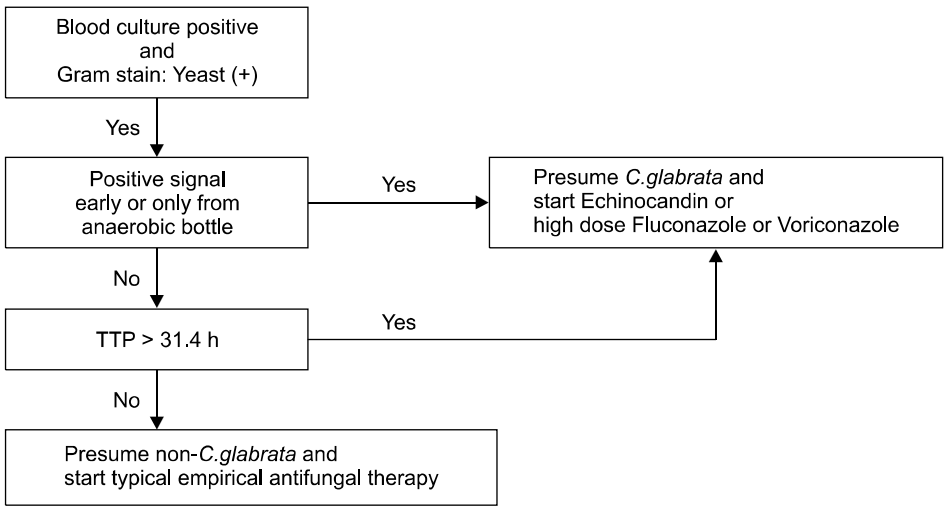

Fig. 1. Two-step algorithm for a presumptive identification of *C. glabrata* to initiate empirical antifungal therapy.

ism [12] and patterns of inoculum. It is the limitation of this study conducted based on TTP and the reason of various cut-off TTP were derived. The cut-off TTP should be calculated in each institute, because the blood culture system, bottles, blood volume, inoculation patterns used may be different from ours [12,14]. The two-step algorithm (Table 2) exhibited lower specificity of 63.9%, but improved sensitivity of 94.4%.

Longer TTP consequently takes more time to report final identification. Delayed administration of adequate antifungal agents is related with high mortality. Therefore, high mortality of patients with *C. glabrata* is partially explained. In contrast, this finding is not in concordant with a previous study: a short TTP (≤24 h) was associated with high mortality, suggesting of high concentration of fungemia. Also, delayed initiation of appropriate antifungal therapy (>72 hr) were independently associated with the 6 week mortality rate [15].

In conclusion, *C. glabrata* has a tendency to proliferate faster in anaerobic bottles in the BacT/Alert 3D system. By using this information, growth earlier or only in anaerobic bottles and longer TTP which can be obtained without additional cost and labor, we can postulate fungemia with *C. glabrata*. Each hospital should check its own automated blood culture system first and apply this algorithm with pertinent cut-off TTP so that an appropriate antifungal therapy could be initiated promptly.

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=국문초록=

혐기병 우선 검출 및 양성시간의 2단계 알고리즘을 통한 *Candida glabrata* 진균혈증의 예측

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배경: *Candida glabrata*와 나머지 *Candida*종에 의한 진균혈증에 대한 경험적 치료가 다를 수 있으므로, 신속한 *C. glabrata* 동정이 필요하다. 저자들은 혈액배양에서 혐기병에서 먼저 배양되고, 균검출시간이 긴 경우 잠정적으로 *C. glabrata* 진균혈증으로 진단하는 알고리즘을 제시하였다.

방법: 2014년 6월-2016년 6월까지 한 대학병원에서 분리된 진균혈증 215예에 대해서 진균의 종류와 균검출시간을 분석하였다. 혈액배양 장비는 BacT/Alert 3D system (bioMerieux Inc, USA), 배양병은 레진병(FA Plus, FN Plus)을 사용하였다.

결과: 혐기병에서 먼저 자라는 비율은 *C. glabrata* 38.8% (7/18), *Candida albicans* 7.6% (8/105), *Candida parapsilosis* 10.5% (4/38), *C. tropicalis* 9.2% (5/54)보다 높았지만, 통계적으로 유의하지는 않았다($P>0.05$). 평균(±표준편차) 균검출시간은 *C. glabrata*가 41.7시간(±16.3)으로서, *C. albicans* 26.7시간(±15.9), *C. parapsilosis* 33.4시간(±8.4), *C. tropicalis* 23.1시간(±17.3)보다 통계적으로 유의하게 길었다($P<0.0001$). 혐기병에서 먼저 자라고, 배양시간 31.4시간 이상의 두 가지 지표를 사용할 경우 *C. glabrata* 진균혈증을 예측할 수 있는 민감도는 94.4%, 특이도는 63.9%, 양성예측률은 19.3%, 음성예측률은 99.2%였다.

결론: BacT/Alert 3D 혈액배양 장비에서 레진병을 사용할 경우, 혐기병에서 먼저 자라는지, 배양시간이 31.4시간 이상되는지 확인한다면 *C. glabrata* 진균혈증을 빠르게 잠정적으로 진단할 수 있어 경험적 항균제 치료에 도움이 될 것이다.

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