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ORIGINAL ARTICLE

Clinical significance of time to positivity for yeast in candidemia

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Purpose: Candidemia is an important issue of nosocomial bloodstream infections, and is associated with a high mortality rate. However, little information is available before final species identification, which takes days after the episode of candidemia. This study tried to determine whether time to positivity (TTP) for yeast helps in predicting the species of candidemia.
Methods: A retrospective cohort study was conducted in Taiwan, which included 434 episodes of nonduplicated candidemia during the period between 2006 and 2009. The demographic features, clinical characteristics, TTP for yeast, and acute illness scores were included for analysis.
Results: The mean age of patients with candidemia was 70.4 ± 15.2 years, and the 30-day crude mortality rate was 48.2%. Forty-five percent of patients suffered from shock status with a mean Acute Physiological and Chronic Health Evaluation II score of 27.0 ± 8.7 and a mean Sequential Organ Failure Assessment score of 9.7 ± 4.5, whereas 50% were admitted to the intensive care units. *Candida albicans* was still the most commonly identified pathogen (58.1%), followed by *C. tropicalis* (14.7%), *C. parapsilosis* (13.1%), and *C. glabrata* (8.3%). Results of multivariate logistic regression showed that TTP for yeast within 48 hours would more

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favor *C. tropicalis* ($p = 0.044$), and less favor *C. glabrata* ($p = 0.025$) and *C. parapsilosis* ($p < 0.001$). Patients with parenteral nutrition usage were more frequently associated with a TTP for yeast within 48 hours, whereas those with previous exposure to an antifungal agent had a longer TTP for yeast.

Conclusion: The TTP for yeast might provide a hint of the responsible *Candida* species before final identification among critical patients with candidemia. The association between antifungal agents and TTP would need more evidence for elucidation.

Introduction

Invasive fungal infections in hospitalized patients are associated with significant morbidity and mortality.[1] The mortality rate associated with invasive candidiasis was as high as 40—50%.[2—4] *Candida* species are the most common cause of invasive fungal infections, accounting for approximately 15% of total hospital-acquired infections and more than 72% of nosocomial fungal infections.[1,5] Moreover, candidemia has become the fourth most common cause of nosocomial bloodstream infections in the United States and in much of the developed world.[1,2,6]

The time to positivity (TTP) of blood culture in patients with candidemia was less discussed in the clinical field, although it is generally agreed that the mean time for detection of *Candida* species growth varies among species. Most species were identified positive for yeasts within 48 hours in an *in vitro* blood-culture system, but there was a delay in final species identification.[7,8] Thus, the TTP for yeast might be a simple indicator for predicting the invaded *Candida* species, and would help clinicians to make a decision on the antifungal treatment required. A recent study by Fernandez et al reported that *Candida glabrata* would take long TTP for yeast by the BacT/ALERT system, which delayed appropriate antifungal therapy.[8] Another study by Ben-Ami et al reported that the TTP for yeast could be a marker for catheter-related candidemia.[9]

However, the mortality and associated clinical presentation among patients with candidemia were no fully explored in the previous two studies. To clarify the importance of TTP for yeast, a retrospective cohort study was conducted to evaluate its impact on mortality, final species identifications, and associated risk factors in a tertiary medical center in Taiwan.

Materials and methods

Patient identification and data collection

This retrospective cohort study examined all patients with a positive blood culture for yeasts (confirmed by microbiological laboratory testing) at a 2900-bed tertiary medical center during the period between January 1, 2006 and December 31, 2009. The blood cultures were collected in paired aerobic and anaerobic bottles by standard sterile procedures via the peripheral vessels. Every episode was identified as true candidemia by reviewing the medical records of those patients with concomitant symptoms or signs of systemic inflammatory response syndrome.[10] Demographic characteristics and clinical presentation among patients as well as acute illness severity scores, such as the Acute Physiological and Chronic Health Evaluation II (APACHE II) and Sequential Organ Failure Assessment (SOFA) scores, were collected for analysis.[11,12] The TTP for yeast, final species identification, and the susceptibility tests results were also evaluated. This study was approved by the Institutional Review Board of the hospital.

Patients were excluded from participating in the study if they were younger than 16 years, were identified as having two or more *Candida* species at the same time, or had another episode diagnosed within 30 days. Patients with two episodes of candidemia diagnosed 30 days apart were considered as having acquired another infectious episode unless a failed primary focus eradication was identified.[13]

Microbiological identification

Pathogens were initially isolated from aerobic blood cultures using the BacT/ALERT 3D system (bioMérieux, Inc., Marcy-l'Étoile, France) during the study period. The laboratory receipt time and the TTP for yeast were recorded manually by the technicians. The data were reported online in the hospital computerizing system and medical staff had open access to these data. Species were identified by standard biochemical testing using an ATB ID 32C kit (bioMérieux, Hazelwood, MO, USA) and the VITEK 2 system with ID-YST Card (bioMérieux VITEK, Hazelwood, MO, USA). The susceptibility results were interpreted based on the criteria specified by the Clinical and Laboratory Standards Institute.[14]

Definition

The TTP for yeast was defined by the manually recorded time to growth detection for yeast minus the laboratory receipt time accessible on website by physicians. Because most blood cultures reported positive for yeasts within 48 hours in clinical experience, this duration was chosen as the cutoff value of TTP. The underlying comorbidities were identified by the medical records with clear documentation. Catheter-related infection was defined by semi-quantitative tip culture of indwelling catheter with growth $\geq$15 colonies that are identical to the species identified from the peripheral blood culture. Chronic renal insufficiency was identified based on serum creatinine levels

>1.5 mg/dL or an estimated serum creatinine clearance < 30 mL/minute/1.73 m^2 for >6 months, and end-stage renal disease was defined as serum creatinine levels >3.0 mg/dL or estimated serum creatinine clearance < 10 mL/minute/1.73 m^2 for over 6 months. Immunosuppressive therapy was defined by the use of immunosuppressants or corticosteroids in equivalent dosage to prednisolone (20 mg/day or more) for at least 3 days. Candidemia patients were defined neutropenic if their absolute neutrophil count was <1000 cells/mm^3 and were defined thrombocytopenic if their platelet count was <100,000 cells/mm^3. Peripheral parenteral nutrition only included those with the administration of lipid-containing formulation. Colonization was defined as positive growth of yeast from at least one surveillance site.[13,15]

Statistical analysis

The Chi-square test or Fisher exact test was used for categorical comparisons of data and differences in continuous variables were analyzed by the independent Student *t* test. A p value < 0.05 was considered statistically significant. Significant predictors in the univariate analysis with a p value < 0.2 were included for further evaluation. A logistic regression model was used to identify the most important risk factors. All analyses were performed using the Statistical Package for the Social Sciences for Windows (version 17.0; SPSS, Inc. Chicago, IL, USA).

Results

A total of 485 episodes of candidemia were collected during the study period. Fifty-one episodes were excluded due to double species disclosed in the same event or incomplete clinical data collection. Finally, 434 episodes in 410 patients were included for analysis. The mean age of patients with candidemia was 70.4 ± 15.2 years, and 291 (67.1%) were male. The overall 30-day crude mortality was 48.2%. Forty-five percent of the patients suffered from shock status, and 50% of them were admitted in the intensive care unit. The mean APACHE II score was 27.0 ± 8.7, and the mean SOFA score was 9.7 ± 4.5 among patients with candidemia. *C. albicans* is the most common pathogen identified during this study (252 episodes, 58.1%, TTP 58.4 ± 18.2 hours), followed by *C. tropicalis* (64, 14.7%, TTP 50.6 ± 13.9 hours), *C. parapsilosis*(57, 13.1%, TTP 71.3 ± 26.9 hours), and *C. glabrata*(36, 8.3%, TTP 90.7 ± 36.5 hours). Overall demographic and clinical characteristics, and results of univariate analysis are presented in Table 1. All patients had received broad-spectrum antibacterial agents at least 3 days before the antifungal therapy and thus the usage of antibacterial agents was not included for further analysis.

In multivariate logistical regression analysis, the antifungal agent exposure in previous 14 days, the *Candida* species, comorbidities of diabetic mellitus, use of mechanical ventilation, and total usage of parenteral nutrition showed significant difference after adjustment by age, sex, and APACHE II score (Table 2). An interaction term between APACHE II score and use of mechanical ventilation was created for further regression analysis, and the effect of ventilation factor weaned after this adjustment.

Discussion

The TTP for yeast showed major difference between species on final identification. As reported here, *C. tropicalis* was the most frequently identified yeast within 48 hours, whereas *C. glabrata* and *C. parapsilosis* were more commonly detected over 48 hours, which was similar to previous studies (Table 2).[8,9,13,16] Inevitably, the TTP for yeast would be closely related to the natural growth speed of each *Candida* species by previous results, and it might provide a clue on species prediction in candidemia.[17,18] There is a marked difference between species distribution within or over 48 hours, and this might aid a clinician to decide whether to keep fluconazole as the first-line therapeutic agent or shift to other antifungal agents in general practice. For patients with candidemia of TTP over 48 hours, with limited clinical improvement after fluconazole administration, and high prevalence of resistant pathogens, echinocandin would be a reasonable option to cope with candidemia.[19–22]

Usage of parenteral nutrition was more frequently associated with a TTP for yeast within 48 hours. Parenteral nutrition was long recognized as an important risk factor for invasive candidiasis,[23] and lipid emulsion in parenteral nutrition was recently recognized as a risk factor for biofilm formation and catheter-related *Candida* infection.[24] As presented in our study, total use of parenteral nutrition containing lipid formulation might be an enrichment factor for *Candida* growth and shortened the TTP for yeast as presented by Swindell et al.[24] Moreover, Ben-Ami et al also reported that shortened TTP for yeast might be an early predictor for catheter-related candidemia.[9] We had evaluated whether there would be any interaction between nontunneled central venous catheter usage and the *Candida* species, but no such interaction was found by statistical analysis. We believed that the use of parenteral nutrition would be an independent factor for shortening TTP for yeast among patients with candidemia.

Previous exposure to antifungal agents 14 days before candidemia was associated with a longer TTP for yeast. One possible explanation is that antifungal agent administration, either on purpose of prophylaxis or pre-emptive treatment, would suppress the most susceptible species, such as *C. albicans*, and would increase the identification of other resistant *Candida* strains or species.[25] Another possible explanation is that decreased colony counts on blood culture sampling due to antifungal agent administration might also lengthen the TTP for yeast. However, the relationship between antifungal agents and TTP still needs more evidence for further elucidation.

Several limitations were encountered in our study. First, the definition of TTP for yeast used here was different from those of previous reports by Ben-Ami et al, Fernandez et al, and Horvath et al that defined TTP using an automatic machine.[8,9,17] A major reason for this study design is that the TTP for yeast defined here had open access for most clinicians in daily practice, rather than that defined by automatic machine, which is only assessed by specialized

Table 1 Demographics and clinical characteristics of patients with candidemia and results of univariate analysis

Variable	Overall (n = 434)	TTP for yeast within 48 hours (n = 229)	TTP for yeast over 48 hours (n = 205)	p
Hours to yeasts	60.6 ± 22.2	44.8 ± 5.6	81.1 ± 21.6	<0.001
Age (years)	70.4 ± 15.2	66.6 ± 16.2	70.5 ± 15.0	0.009
Male sex	291 (67.1)	151 (65.9)	140 (68.3)	0.602
Onset in ICUs	150 (34.6)	66 (28.8)	84 (41.0)	0.008
Precandidemia LOS (days)	35.6 ± 35.3	45.2 ± 102.2	33.8 ± 29.8	0.091
LOS (days)	65.3 ± 60.3	83.1 ± 143.9	71.3 ± 59.0	0.273
30-day mortality	209 (48.2)	108 (47.2)	101 (49.3)	0.661
APACHE II score	27.0 ± 8.7	23.7 ± 8.3	25.6 ± 9.2	0.020
SOFA score	9.7 ± 4.5	6.3 ± 4.6	7.3 ± 4.9	0.043
Underlying comorbidity				
CAD	73 (16.8)	33 (14.4)	40 (19.5)	0.156
CHF	58 (13.4)	24 (10.5)	34 (16.6)	0.062
CVA	88 (20.3)	34 (14.8)	54 (26.3)	0.003
COPD	46 (10.6)	20 (8.7)	26 (12.7)	0.182
CRI	166 (38.2)	70 (30.6)	96 (46.8)	0.001
DM	132 (30.4)	51 (22.3)	81 (39.5)	<0.001
Liver cirrhosis	23 (5.3)	16 (7.0)	7 (3.4)	0.097
Pancreatitis	33 (7.6)	23 (10.0)	10 (4.9)	0.043
Collagen vascular disease	15 (3.5)	4 (1.7)	11 (5.4)	0.039
Conditions within previous 30 days				
ICU admission	223 (51.4)	108 (47.2)	115 (56.1)	0.063
Surgery	140 (32.3)	66 (28.8)	74 (36.1)	0.105
Immunosuppressive therapy	66 (15.2)	26 (11.4)	40 (19.5)	0.018
Thrombocytopenia	222 (51.3)	127 (55.7)	95 (46.3)	0.052
Procedures				
Hemodialysis	74 (17.1)	30 (13.1)	44 (21.5)	0.021
NTCVC	285 (65.8)	150 (65.5)	135 (66.2)	0.883
CVC removal	206 (72.5)	113 (76.9)	93 (67.9)	0.090
Mechanical ventilation	180 (41.5)	78 (34.1)	102 (49.8)	0.001
Arterial line	124 (28.6)	57 (24.6)	67 (32.7)	0.073
Parenteral nutrition	214 (49.3)	137 (59.8)	77 (37.6)	<0.001
Urinary catheters	231 (53.2)	108 (47.2)	123 (60.0)	0.007
Candida species				<0.001
C. albicans	252 (58.1)	144 (62.9)	108 (52.7)	
C. tropicalis	64 (14.7)	50 (21.8)	14 (6.8)	
C. parapsilosis	57 (13.1)	16 (7.0)	41 (20.0)	
C. glabrata	36 (8.3)	8 (3.5)	28 (13.7)	
Fluconazole susceptibility[a]	355 (87.2)	201 (92.2)	154 (81.5)	0.002
Catheter-related candidemia	181 (41.8)	110 (48.2)	71 (34.6)	0.004
Antifungal agent exposure in previous 14 days	44 (10.2)	8 (3.5)	36 (17.6)	<0.001
Concurrent bacteremia	69 (15.9)	30 (13.1)	39 (19.0)	0.092

[a] Fluconazole susceptibility was available among 407 strains.

Data were n (%) or mean ± SD unless otherwise indicated.

APACHE II = Acute Physiological and Chronic Health Evaluation II; CAD = coronary arterial disease; CHF = congestive heart failure; CRI = chronic renal insufficiency; COPD = chronic obstructive pulmonary disease; CVA = cerebrovascular accident; CVC = central venous catheter; DM = diabetes mellitus; ICU = intensive care unit; LOS = length of hospital stay; NTCVC = nontunneled central venous catheter; SD = standard deviation; SOFA = Sequential Organ Failure Assessment; TTP = time to positivity for yeasts.

technicians or microbiologists. Therefore, the TTP reported in our study was much longer than that reported in previous studies. However, we believe that this definition of TTP for yeast might be beneficial for most clinicians in clinical practice. Second, there might be a time lag between the real time of yeast detection by machine and the recorded time for positive finding by a technician due to the manual processing procedure on sequential cultures. Third, in this retrospective study, it was difficult to adjust the blood volume sampled in each episode, which might influent the inoculation amount in the culture bottle. Finally, the distribution of *C. parapsilosis* and *C. glabrata* was relatively lower than the other *Candida* species, and this might cause some bias on statistical analysis. Further prospective study to elucidate the role of TTP might be needed in the clinical field.

Table 2 Associated factors for time to positivity for yeast within 48 hours by multivariate analysis adjusted by age, sex, and APACHE II score

Variable	Odds ratio (95% confidence interval)	*p*
Antifungal agent exposure in previous 14 days	0.119 (0.032—0.434)	0.001
Candida species[a]		
C. tropicalis	2.497 (1.025—4.415)	0.044
C. glabrata	0.269 (0.086—0.845)	0.025
C. parapsilosis	0.122 (0.046—0.324)	<0.001
Mechanical ventilation	0.464 (0.227—0.950)	0.036
Parenteral nutrition	3.514 (1.892—6.527)	<0.001

[a] *C. albicans* was taken as the reference category for comparison between species.
Time to positivity for yeast over 48 hours was taken as reference group.

The TTP for yeast might be a hint towards species prediction before final species identification among patients with candidemia. Use of parenteral nutrition was associated with TTP for yeast within 48 hours, whereas previous exposure to antifungal agents seemed to prolong the TTP for yeast detection among patients with candidemia. However, the association between antifungal agents and TTP would need more evidence for further elucidation.

Conflicts of interest

The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the manuscript.

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