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Quantitation of *Candida* CFU in Initial Positive Blood Cultures▽

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One potential limitation of DNA-based molecular diagnostic tests for *Candida* bloodstream infection (BSI) is organism burden, which is not sufficiently characterized. We hypothesized that the number of CFU per milliliter (CFU/ml) present in an episode of *Candida* BSI is too low for reliable DNA-based diagnostics. In this study, we determined *Candida* burden in the first positive blood culture and explored factors that affect organism numbers and patient outcomes. We reviewed records of consecutive patients with a positive blood culture for *Candida* in the lysis-centrifugation blood culture system (Isolator, Wampole Laboratories, Cranbury, NJ) from 1987 to 1991. Descriptive statistics and logistic regression analyses were performed. One hundred fifty-two episodes of *Candida* BSI were analyzed. Patient characteristics included adult age (72%), indwelling central venous catheters (83%), recent surgery (29%), neutropenia (24%), transplant (14%), and other immune suppression (21%). Rates of treatment success and 30-day mortality for candidemia were each 51%. The median CFU/ml was 1 (mode 0.1, range 0.1 to >1,000). In the multivariate analysis, pediatric patients were more likely than adults to have high organism burdens (odds ratio [OR], 10.7; 95% confidence interval [95% CI], 4.3 to 26.5). Initial organism density did not affect patient outcome. *Candida* CFU/ml in the first positive blood culture of a BSI episode varies greatly; >50% of cultures had ≤1 CFU/ml, a concentration below the experimental yeast cell threshold for reliable DNA-based diagnostics. DNA-based diagnostics for *Candida* BSI will be challenged by low organism density and the need for sufficient specimen volume; future research on alternate targets is warranted.

Candida is the fourth most common cause of bloodstream infection (BSI) in much of the developed world and is responsible for significant morbidity and mortality in hospitalized patients (19). The standard of care for detecting *Candida* BSI is the blood culture, a method that is hampered by a sensitivity of only 50 to 60% and a lag time of up to 5 days for identification (2). This poor performance has led to the development of new molecular and protein-based diagnostics for *Candida* BSI. Unfortunately, no test has emerged as a clear improvement over blood culture, which remains the standard despite several studies demonstrating need for early therapy to improve outcome (6, 17).

A potentially limiting factor in developing molecular testing for *Candida* BSIs is organism burden. If the number of *Candida* CFU per milliliter in blood is low early in disease, then the expected sensitivity of a DNA-based test would be poor. The complexity of detecting a few organisms is compounded by the difficulty of breaking open the fungal cell wall to access DNA. The estimated burden of yeasts required for reliable DNA-based PCR detection is 5 to 10 CFU/ml (4, 13, 14). To date, the organism burden early in clinical candidemia has not been well established. Because automated blood culture systems (BACTEC 9240, Becton Dickinson, Sparks, MD and BacT/Alert 3D, bioMérieux, Durham, NC) have largely replaced the lysis-centrifugation system (Isolator, Wampole Laboratories, Cranbury, NJ) for detecting *Candida* BSI, quantitative blood cultures are no longer routinely performed. Before this transition, however, quantitative data were collected with the Isolator system, and limited earlier evaluations reported marked variations in the burden of yeasts in *Candida* BSIs (3, 7, 9, 10, 14, 20).

We hypothesized that the CFU/ml in the initial positive blood culture with *Candida* BSIs is too low for reliable fungal DNA-based diagnostics. Consequently, we assessed *Candida* CFU/ml in the first positive blood culture in candidemia and explored factors that affect organism burden and clinical outcome.

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MATERIALS AND METHODS

Subjects were identified by review of Duke University Clinical Microbiology Laboratory records from September 1987 through December 1991 for patients who had a positive blood culture for *Candida* with the fungal Isolator system. During the study period, Isolator tubes were centrifuged and the sediment was distributed equally among three agar plates (brain heart infusion, inhibitory mold, and chocolate) that were incubated at 35°C and examined daily for 21 days for growth. CFU/ml were calculated by dividing the total number of colonies visualized on all three plates by the precentrifugation blood volume.

The positive fungal blood culture sequence (first, second, etc.) and each quantitation in CFU/ml were recorded. A clinical chart review was performed to extract pertinent data, including age, sex, hospital ward, comorbid disease, days of suspected fungemia, culture source, *Candida* species isolated, presence of indwelling central venous catheters (CVCs), source of candidemia, treatment outcome, and 30-day mortality. This study and its retrospective collection of clinical information were approved by the Duke University Institutional Review Board.

Definitions. The first positive fungal blood culture was limited to the primary episode of candidiasis in each patient; subsequent episodes of relapse and rein-

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fection were excluded from this study. The CFU/ml were designated according to the source and number of fungal blood cultures as follows: if only one culture was positive, the CFU/ml of that culture was selected; if a peripheral blood culture was paired with a CVC culture, the peripheral CFU/ml was preferentially chosen; and if more than one positive culture was obtained from peripheral (or unknown) sites, then the average CFU/ml of the positive cultures was utilized.

The source of candidiasis was based on investigator assessment. Specifically, a catheter-related source of infection was defined according to the Infectious Diseases Society of America guidelines (16). At our institution, catheter tip cultures were not routinely performed during the study period. When a quantitative catheter tip culture was available, catheter-associated BSI (CA-BSI) was defined as either >15 CFU/ml from a 5-cm segment of the catheter tip by semiquantitative (roll-plate) culture or a 3-fold greater quantitative blood culture CFU count between peripheral vein and catheter hub blood cultures. In the absence of quantitative methods, CA-BSI was defined as having an intravascular device and a positive blood culture plus clinical manifestations of infection (e.g., fever, chills, and/or hypotension) and no apparent alternate source of infection.

Days of suspected fungemia prior to the index positive blood culture were summed based on the preceding presence of: (i) temperature of >100.5°F (>38 °C) or <96.8 °F (36 °C); (ii) hypotension (systolic blood pressure of <90 mm Hg or a decrease of >30 mm Hg from baseline); and/or (iii) blood culture(s) obtained for suspected infection. Breakthrough infection was defined as a new episode of candidemia in a patient who had received a minimum of 2 days of prior systemic antifungal therapy. Treatment success was defined as a negative culture from the original site of infection (if available) and clinical resolution of symptoms and signs of infection at 2 weeks.

Statistical analysis. Sample size was determined by the data available during the study period. Descriptive statistics were performed: chi-square and Fisher's exact tests were employed as indicated to compare baseline variables between groups. Potential predictors were correlated using Spearman's rank correlation prior to association with the outcome to select indicator variables for the multivariate models. Potential predictors included age (adult versus pediatric [<18 years old]), days of suspected fungemia, hospital ward (intensive care unit [ICU] or not), neutropenia, other immune suppression, breakthrough candidemia, blood culture site, source of candidemia, the presence of indwelling CVCs, and the *Candida* species isolated. Because only 5 degrees of freedom were available for the adjusted model (18), correlations of potential predictors prior to their association with outcome were reviewed and the following were selected: age, neutropenia, *Candida* spp. (*C. albicans* versus non-*albicans Candida* spp.), and blood culture source (peripheral, central, or unknown).

Univariate and multivariate regressions were performed. Multivariate logistic regression was employed to evaluate predictors of CFU/ml on the first positive blood culture using high (≥5) versus low (<5) CFU/ml as the binary outcome. Model discrimination and calibration were evaluated by the c-statistic and Hosmer-Lemeshow goodness-of-fit tests, respectively. Sensitivity analyses were performed using different CFU/ml thresholds (1 and 10 CFU/ml) and a different pediatric age cutoff (1 year). Model validation was achieved by bootstrapping 99 iterations; a validated predictor required a statistical significance of ≥95 of these replications.

Secondary analyses using multivariate logistic regression were performed to evaluate the impact of CFU/ml on the binary outcomes "treatment success" and "30-day mortality." These hypothesis-driven models evaluated CFU/ml only; CFU/ml was added to already adjusted models with the predefined potential predictors of age, source of infection, antifungal therapy, and ICU status.

All analyses were performed using SAS version 9.2 (Cary, NC). The Wald method was chosen for logistic regression, and two-tailed *P* values of <0.05 were considered statistically significant.

RESULTS

One hundred seventy-four subjects had 369 Isolator blood cultures positive for *Candida* during the study period. Twenty-two subjects were excluded because of insufficient data to determine CFU/ml ($n = 16$), reinfection ($n = 3$), and interhospital transfer with candidemia ($n = 3$).

Thus, the first positive cultures of 152 subjects were analyzed. Patient characteristics and outcomes are summarized in Table 1. Those associated with a CFU/ml of <5 (via individual chi-square tests) included adult age, neutropenia, recent major surgery, end-stage liver disease, renal replacement therapy, an interrupted gastrointestinal tract, and an indwelling CVC; those associated with a CFU/ml of ≥5 included receipt of total parenteral nutrition (TPN), successful treatment, and lower 30-day mortality.

TABLE 1. Patient characteristics ($n = 152$)[a]

Characteristic	No. of patients with a CFU/ml of:		*P* value[b]
	<5 [$n = 104$ (%)]	≥5 [$n = 48$ (%)]	
Age (yr, median)	51	1.5	N/A
Age category			
Adult	**93 (89)**	17 (35)	**<0.001**
Pediatric (<18 years)	11 (11)	**31 (65)**	**<0.001**
Male sex	53 (51)	30 (63)	0.184
Comorbid disease			
Acute leukemia	15 (14)	2 (4)	0.094*
Other active malignancy	23 (22)	6 (13)	0.161
Recent major surgery	**37 (36)**	7 (15)	**0.008**
ESLD (nontransplant)	**12 (12)**	0	**0.010***
Receiving RRT	**25 (24)**	4 (8)	**0.026***
Neutropenia	**31 (30)**	5 (10)	**0.008***
Hematopoietic stem cell transplant	8 (8)	2 (4)	0.506*
Solid organ transplant	9 (9)	1 (2)	0.172*
Receipt of exogenous steroids	12 (11)	1 (2)	0.063*
Receipt of other immune suppressive medication	10 (10)	8 (17)	0.211*
Interrupted GI tract	**50 (48)**	13 (27)	**0.015**
Indwelling CVC	**93 (89)**	33 (69)	**0.002**
Receiving TPN	44 (42)	**29 (60)**	**0.038**
Breakthrough infection	10 (9)	2 (4)	0.341
Patient in ICU	63 (61)	33 (69)	0.332
≤2 Days suspected fungemia	63 (61)	35 (73)	0.140
Candida species			**<0.001***
C. albicans	63 (61)	23 (48)	
C. glabrata	**16 (15)**	2 (4)	
C. tropicalis	16 (15)	6 (13)	
C. parapsilosis	4 (4)	**13 (27)**	
Other/mixed[c]	5 (5)	4 (8)	
Blood culture site			**0.001**
Peripheral	24 (23)	23 (48)	
CVC	12 (12)	9 (19)	
Unknown	**68 (65)**	16 (33)	
Source of infection			**0.011**
CVC	15 (14)	**17 (33)**	
Extravascular	**52 (50)**	14 (29)	
Unknown	37 (36)	18 (38)	
Treatment			0.061*
None	17 (16)	4 (8)	
Amphotericin B	81 (78)	35 (73)	
Amphotericin B plus flucytosine	5 (5)	7 (15)	
Other[d]	1 (1)	2 (4)	
Treatment success ($n = 130$ received antifungal therapy)	36 (41)	**30 (70)**	**0.002**
Alive at 30 days	46 (44)	**31 (65)**	**0.020**

[a] Abbreviations: GI, gastrointestinal; CVC, central venous catheter; TPN, total parenteral nutrition; ICU, intensive care unit; ESLD, end-stage liver disease; N/A, not applicable; RRT, renal replacement therapy. Boldface data represent statistically significant differences.

[b] *P* values are 2-tailed via uncorrected chi-square or, when noted, Fisher's exact test (*).

[c] Mixed/other species included mixed cultures (6), *C. krusei* (1), and *C. lusitaniae* (2).

[d] Other treatments included fluconazole ($n = 2$) and cilofungin ($n = 1$).

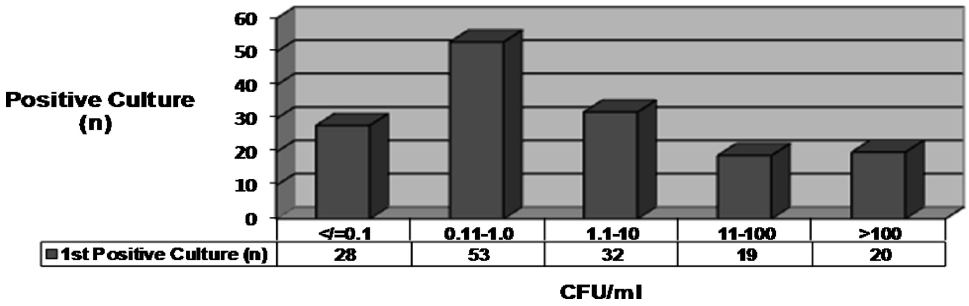

FIG. 1. *Candida* CFU/ml distribution of the first positive blood culture.

The median CFU/ml of the first positive culture was 1 (mode 0.1, interquartile range 0.2 to 12.1, range 0.1 to >1,000). Figure 1 demonstrates these data sorted into quintiles; 53% and 68% of cultures had CFU/ml values of ≤1 and ≤5, respectively.

The distribution of *Candida* species in the study is shown in Fig. 2. Notably, subjects with *C. glabrata* were more likely to have <5 CFU/ml, while those infected with *C. parapsilosis* were more likely to have ≥5 CFU/ml.

As shown in Table 2, while blood culture source, presence of neutropenia, and age were each predictive in univariate analyses, in the multivariate analysis only pediatric age status was a significant predictor of high organism burden (odds ratio [OR], 10.7; 95% confidence interval [95% CI], 4.3 to 26.5; $P < 0.001$). This adjusted model demonstrated reasonable discrimination (c-statistic = 0.82) and calibration (Hosmer-Lemeshow, $P = 0.38$). The model was robust as demonstrated by the sensitivity analyses; pediatric age predicted high CFU/ml using thresholds of both 1 CFU/ml (OR, 21.2; 95% CI, 5.8 to 77.3; $P < 0.001$) and 10 CFU/ml (OR, 8.1; 95% CI, 3.2 to 20.4; $P < 0.001$), while other predictors had no significant impact on either threshold. Similarly, adjusting the pediatric age cutoff to <1 year did not significantly alter this variable's impact on initial organism burden (OR, 10.8; 95% CI, 3.8 to 31.1; $P < 0.001$). Bootstrapping confirmed that the impact of pediatric age was highly reproducible; in all 99 iterations pediatric age predicted high CFU/ml, while in only 19, 23, and 26 cases did the infecting *Candida* spp., blood culture source, and presence of neutropenia, respectively, do so.

Overall, 51% of subjects were successfully treated and 51% were alive at 30 days. Although treatment outcome and mortality appeared to differ between groups (Table 1), after adjusting for age, source of infection, antifungal therapy, and ICU status in the multivariate nested model, CFU/ml was associated with neither treatment success (OR, 1.2; 95% CI, 0.36 to 3.8; $P = 0.80$) nor 30-day mortality (OR, 0.79; 95% CI, 0.27 to 2.3; $P = 0.66$).

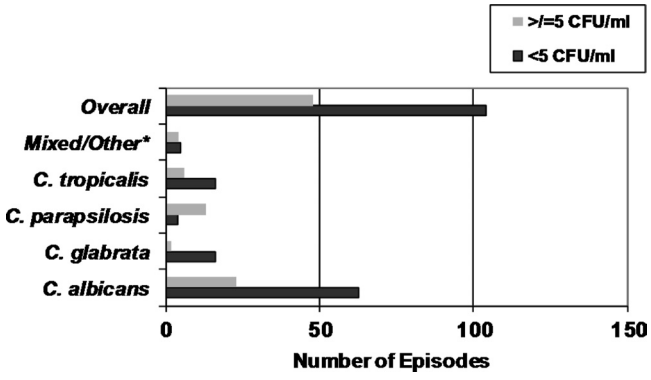

FIG. 2. *Candida* species distribution by CFU/ml. *, Mixed/Other includes mixed cultures (6), *C. krusei* (1), and *C. lusitaniae* (2).

DISCUSSION

In this report, we found that over half of initial *Candida* spp. blood cultures have a CFU/ml of ≤1 and that the range of CFU/ml varied widely among patients. Our findings confirm several prior publications that also utilized quantitative methods (Table 3): combined data from these prior studies demonstrated a "low" organism burden in 50% (207/415) of all fungal blood cultures (3, 7, 9, 10, 20). Furthermore, of the two studies in which only initial cultures were included, 58% (134/233) of patients had <1 CFU/ml (7, 20). The findings are striking when juxtaposed with the current limit of detection (LOD) for DNA-based diagnostic tests for candidemia, the majority of which have LOD values of 5 to 10 CFU/ml. Thus, the low organism burden in most *Candida* BSI episodes appears to be too low for reliable detection with DNA-based tests.

The reported sensitivity of PCR for candidemia has varied widely; two representative examples in the literature include Lau et al. (11) and McMullan et al. (15), whose groups described sensitivities of 75% and 91%, respectively. A recently published meta-analysis of *Candida* PCR included 54 studies and evaluated 101 unique molecular tests (1). In this study, across all comparisons the sensitivity of PCR ranged from 58 to 100%. Greater sensitivity was associated with an *in vitro* detection limit of ≤10 CFU/ml, multicopy gene targets, and the use of whole blood. The general higher-than-expected PCR sensitivity can be explained in several ways. First, dead yeasts cannot grow in culture but their DNA can be detected by PCR. Also, DNA from dead organisms may be easier to access than that from living yeasts due to a leaky cell wall. Additionally, multicopy gene (rRNA) targets have higher sensitivity because of the larger number of target molecules per yeast cell (5). Finally, the use of whole blood, in which cellular components are retained, appears to maximize fungal DNA burden per sample. Although these factors probably help to overcome the low organism burden demonstrated in our study, it is because of this organism burden that DNA-based PCR has yet to prove

TABLE 2. Logistic regression predictors of ≥5 CFU/ml in the first positive *Candida* blood culture[a]

Potential predictor	Univariable OR (95% CI)	*P* value	Multivariable OR (95% CI)[c]	*P* value
Blood source[b]				
Peripheral vs. CVC	1.28 (0.45–3.6)	0.64	0.82 (0.23–2.9)	0.76
Unknown vs. CVC	**0.31 (0.11–0.87)**	**0.03**	0.46 (0.14–1.6)	0.21
Neutropenia	**0.27 (0.10–0.76)**	**0.01**	0.44 (0.13–1.5)	0.52
Non-*albicans Candida* spp.	1.6 (0.84–3.3)	0.14	1.7 (0.70–4.1)	0.24
Pediatric (age <18)	**15.4 (6.5–36.5)**	**<0.01**	**10.7 (4.3–26.5)**	**<0.01**

[a] Abbreviations: CI, confidence interval; CVC, central venous catheter; OR, odds ratio. Boldface data represent statistically significant differences.
[b] Global null hypothesis *P* value was < 0.01 for "Blood Source" as a predictor in the univariate model.
[c] Overall global null hypothesis *P* value was <0.01 for the multivariate model.

clinically useful as an alternative to current blood culture systems.

In earlier studies, catheter-associated bloodstream infection (CA-BSI) appeared to be associated with high organism load (3, 20). In our cohort, CVCs were common in both groups, but CVC presence was not associated with higher CFU/ml. In fact, in univariate analysis, the presence of a CVC was associated with a lower fungal burden. When a CVC was judged to be the source of infection, the organism burden was more likely to be high (≥5 CFU/ml), whereas low organism burden (<5 CFU/ml) was associated with an extravascular source such as an abdominal site. Collection of the blood culture through an infected catheter would be expected to affect the density of organisms recovered, but our analyses were limited because the site of collection was delineated in only 45% of patients.

The infecting *Candida* spp. also appeared to influence organism burden. *C. glabrata* and *C. parapsilosis* were associated with lower and higher organism densities, respectively. The *C. parapsilosis* association with high burden may have been confounded by its coassociation with pediatric age.

Compared with adults, pediatric subjects had higher organism burdens and were more likely to have the triad of a catheter-associated fungemia, receipt of TPN, and infection with *C. parapsilosis.* One important issue in the pediatric population was the difference in the absolute volume of blood collected per culture (adults [8 to 10 ml] versus infants [1.5 ml]). The theoretical minimum CFU/ml in an adult is 0.1 CFU/ml (1 colony/10 ml); for an infant that value is 0.7 (1 colony/1.5 ml). Therefore, it was not surprising that the sensitivity analysis using a high/low cutoff of 1 CFU/ml (instead of 5 CFU/ml) demonstrated the highest association of pediatric age with organism burden; thus, it is reassuring that a second sensitivity analysis using a CFU/ml of 10 confirmed the significance of this variable. Similar to prior findings from Bille et al., in our study initial organism density did not affect clinical outcome (3). Although an apparent association with improved patient out-

TABLE 3. Summary of prior studies evaluating organism density in *Candida* BSI[a]

Study/yr	Total first positive cultures	First positive only?	CFU/ml category range	No. (%) of cultures in category range	Comments
Telenti et al. 1991 (20)	172	Yes	<1	112 (65)	CA-BSI was suspected in 90% and 18% of subjects with CFU/ml values of >2.5 and <1, respectively
			1–2.5	12 (7)	
			>2.5	48 (28)	
Henry et al. 1983 (7)	61	Yes	<1	22 (36)	
			1–10	16 (26)	
			>10	23 (38)	
Kiehn et al. 1983 (10)	91	No	<1	43 (47)	
			1–10	27 (30)	
			11–100	14 (15)	
			>100	7 (8)	
Kiehn 1989 (9)	68	No	<1	18 (26)	Yeasts, mostly *Candida*
			1–10	19 (28)	
			11–100	16 (24)	
			101–1000	6 (9)	
			>1000	9 (13)	
Bille et al. 1984 (3)	23	Yes	<5	12 (52)	Non-*albicans Candida* spp. and CA-BSI associated with "high-grade" infection; fungal burden not associated with clinical outcome
			>5	11 (48)	

[a] Abbreviations: BSI, bloodstream infection; CA-BSI, catheter-associated BSI.

come and survival was observed, this finding was confounded by the high fungal burden that was also associated with pediatric age and possibly CA-BSI.

Limitations of this study stem from its retrospective nature. Ideally, the sample size would have been increased to allow the exploration of more predictors in the adjusted model. Certain variables of interest had inconsistent documentation and other important variables (Acute Physiology and Chronic Health Evaluation [APACHE] scores) were unavailable. Also, patient characteristics and care have changed in the past 2 decades, e.g., antifungal prophylaxis was not employed during the study period but is more commonly practiced today. Furthermore, the epidemiology of *Candida* infection has evolved; the species distribution in this series had a relatively high percentage of *C. albicans* and low proportion of both *C. parapsilosis* and *C. glabrata*, which is quite different from the current species distribution (8, 12).

In summary, we have demonstrated that the burden of organisms in the first positive *Candida* blood culture ranges widely and that over half of the cultures demonstrate ≤1 CFU/ml, an organism density below that currently required for reliable DNA-based diagnostics. Close attention to specimen volume, preparation, and the possible capture of free DNA may be necessary to enable robust DNA-based diagnostics. Future research that targets the more abundant fungal RNA or proteins or gene or protein expression of the host is warranted.

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