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Radiometric Detection of Yeasts in Blood Cultures of Cancer Patients

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During a 12-month period, 19,457 blood cultures were collected. Yeasts were isolated from 193 cultures derived from 76 cancer patients. *Candida albicans* or *Candida tropicalis* accounted for 79% of isolates. Of the three methods compared, the radiometric method required 2.9 days to become positive, "blind" subculture required 2.6 days, and Gram stains required 1 day. However, the radiometric method was clearly superior in detecting positive cultures, since 73% of all cultures were first detected radiometrically, 22% were detected by subculture, and only 5% were detected by Gram stain. Although 93% of the isolates were detected by aerobic culture, five (7%) isolates were obtained only from anaerobic cultures. Seven days of incubation appear to be sufficient for the radiometric detection of yeasts.

Yeasts can be detected in blood cultures of 25% of cancer patients with disseminated candidiasis (2). The use of biphasic media (9) or liquid media with transient venting (1, 7) appears to improve yeast detection. The BACTEC radiometric system has been shown to detect bacteremia more rapidly than conventional blood culture systems (3-6, 8). It was of interest, therefore, to determine the efficiency of the radiometric detection system for yeasts.

In this study, we compared the average time required for yeast detection by the radiometric method with the "blind" subculture method and microscopic detection of Gram-stained blood smears. The cultures were performed using both the BACTEC aerobic (6B) and the anaerobic (7B) bottles (Johnston Laboratories, Cockeysville, Md.). We also investigated length of incubation and transient aeration of BACTEC bottles to improve the detection of yeasts.

MATERIALS AND METHODS

Routine BACTEC blood culture method. At the patient's bedside, 3 ml of blood was aseptically transferred to BACTEC bottles (6B and 7B) containing 30 ml of tryptic soy broth with 0.025% sodium polyanethol sulfonate, according to standard institution procedure. In the laboratory we processed blood cultures in the following manner. Aerobic culture bottles were placed on a BACTEC 225 after an incubation with shaking of 6 to 8 h at 37°C. The 6B bottles were then tested at 3-h intervals for the next 6 to 12 h and radiometrically monitored once daily on the BACTEC thereafter. The 7B bottles were monitored once each day. The blood cultures were considered to be positive when a growth index of 25 or more for the 6B bottles and of 13 or more for the 7B bottles was obtained. All negative 6B cultures were subcultured and Gram stained after 12 to 24 h and after 7 days of incubation. In addition to the daily BACTEC monitoring, negative 7B cultures were subcultured to aerobic media and Gram stained on day 2. Subcultures from 7B bottles to anaerobic media were performed on day 3.

Fungal BACTEC blood culture medium. When specific requests for fungal culture of blood specimens were received, bottles were processed as previously described for the first 7 days except that subcultures were incubated for the first 2 days at 37°C and for an additional 5 days at room temperature. One milliliter, rather than 0.1 ml, of BACTEC medium was subcultured to Sabouraud dextrose agar plates after 7 days of radiometric monitoring. The final reading and subcultures were performed after an additional 7 days of incubation at 37°C. Airways were in place in the 7B culture bottles from days 7 to 13. The airways were removed on day 13, and the cultures were tested on the BACTEC the next day.

RESULTS

Our laboratory received 19,457 blood cultures during the 12-month period of 1 July 1978 to 30 June 1979. Yeasts were isolated from 193 cultures derived from 76 patients. As shown in Table 1, 20 of the positive cultures were obtained from postmortem heart blood specimens. *Candida albicans* or *Candida tropicalis* was isolated from 70% of the individuals who had positive blood cultures, and these strains accounted for 79% of the positive cultures. *Torulopsis glabrata* was isolated from 10 patients, and 6 of these isolates were from postmortem specimens. Two or more positive blood cultures were obtained from 52% of the 56 patients who were alive at the time the blood was collected.

Many patients in the study population had

TABLE 1. *Yeast isolates from the blood of 76 patients*

Organism	Total no. (%) of cultures	Total no. (%) of patients	No. of patients with multiple positive blood cultures	No. of autopsy isolates
Candida albicans	82 (42.5)	34 (45)	16	10
Candida tropicalis	70 (36.3)	19 (25)	12	2
Torulopsis glabrata	14 (7.2)	10 (13)	3	6
Candida parapsilosis	10 (5.2)	7 (9)	3	2
Cryptococcus neoformans	9 (4.7)	1 (1)	1	0
Trichosporon cutaneum	6 (3.1)	3 (4)	3	0
Candida krusei	1 (0.5)	1 (1)	0	0
Rhodotorula rubra	1 (0.5)	1 (1)	0	0

indwelling catheters; therefore, one cannot exclude the possibility that positive cultures resulted from a colonized catheter tip or site in a few asymptomatic cases.

Yeasts were detected in 35 anaerobic cultures derived from 27 cancer patients, 19 of whom were alive at the time blood was drawn. As shown in Table 2, five isolates were obtained only from anaerobic bottles, 49 were obtained only from the aerobic bottles, and 22 were obtained from both. Of the 22 cases, 6 were first detected anaerobically, 9 were detected aerobically, and 7 were detected in both kinds of bottles on the same day. Of the 19 anaerobic isolates from living patients, 16 were first detected radiometrically, 2 were detected by Gram stain, and 1 was detected by subculture. Ninety-three percent of the yeast isolates were detected by aerobic culture. Only 36% of the yeasts were detected anaerobically; however, regardless of the culture conditions, there were no significant differences between the 6B and 7B bottles in the time required for the detection of yeasts.

Table 3 presents a comparison of the average time required for the detection of the first positive blood culture in 56 patients who were alive at the time of sampling. To detect a positive culture, the radiometric method required an average of 2.9 days, subcultures required 2.6 days,

TABLE 2. *Analysis of isolates based on culture conditions*

Organism	Conditions of isolation		
	Aerobic only	Anaerobic only	Aerobic and anaerobic
Candida albicans	21	3	10
Candida tropicalis	11	1	7
Candida parapsilosis	6	0	1
Torulopsis glabrata	6	1	3
Trichosporon cutaneum	2	0	1
Cryptococcus neoformans	1	0	0
Candida krusei	1	0	0
Rhodotorula rubra	1	0	0

TABLE 3. *Method and time required for detection of positive cultures from 56 living patients*

Organism	No. of isolates first detected		
	BACTEC	Subcultures	Gram stain
Candida albicans	14 (2.7)[a]	7 (2.3)	3 (1.0)
Candida tropicalis	11 (2.1)	4 (2.0)	2 (1.0)
Candida parapsilosis	3 (4.0)	2 (2.5)	1 (1.0)
Torulopsis glabrata	4 (4.7)	0	0
Trichosporon cutaneum	3 (2.0)	0	0
Cryptococcus neoformans	1 (6.0)	0	0
Rhodotorula rubra	0	1 (7.0)	0
Total	36 (2.9)	14 (2.6)	6 (1.0)

[a] Numbers in parentheses indicate average number of days to detect positive cultures.

and Gram-stained blood smears required 1 day. However, the radiometric method was first in detecting 64% of the positive cultures, whereas subculturing was first in 25%, and the Gram stain was first in 11%. It should also be noted that all 20 cases that were first detected by the subculture method or Gram stain became positive by the radiometric method within the next 24 h. Moreover, only 1 of these 20 isolates was detected on day 7 of testing. The average time required to detect positivity for all 193 cultures studied did not differ significantly from that calculated from the cultures derived from the 56 patients who were alive at the time of sampling.

Seventy-three percent of all positive cultures were first detected by the radiometric method, whereas only 22% and 5% were first detected by subculturing or by microscopic examination, respectively. The higher rate of detection of yeast growth by the radiometric method could be due to the more frequent radiometric monitoring used in this study.

We also processed 283 blood cultures from 131 patients specifically for fungi. Cultures from five

of these patients became positive for yeasts. However, none of the 283 fungal blood cultures became positive during week 2 of incubation, with the exception of two cultures (derived from one patient) that grew *Histoplasma capsulatum*.

DISCUSSION

Venting liquid media improves the recovery rate for fungi from blood cultures, and as might be expected, the majority of the isolates in this study were detected radiometrically in aerobic cultures. Interestingly, 36% of the isolates were detected and cultured anaerobically with no difference in the average time for appearing positive. Moreover, 14% of the yeasts either were cultured only anaerobically or were first detected anaerobically. This finding may be due to our use of a lower growth index value for anaerobic cultures as a criterion of positivity. In addition, the anaerobic medium contains added nutrients and may provide a better growth environment for certain strains. Incubation and subculture of BACTEC cultures beyond 7 days appears to be unnecessary, since all positive cultures were detected during the first 7 days of incubation (with the exception of *H. capsulatum*). Since with the BACTEC culture method, the gas content of the aerobic bottles is flushed out and replaced daily with 95% air and 5% CO_2, one should expect that the radiometric method is as efficient as vented liquid cultures. Although all cultures eventually become positive by radiometric monitoring, it is most important that fungal sepsis be detected at the earliest possible time in the critically ill immunosuppressed patient. Therefore, in our patient population, optimization of yeast detection in blood cultures includes the performance of blind subculture and Gram stain of all negative BACTEC culture bottles (6B and 7B) particularly after 12 to 24 h of incubation.

The poor correlation between systemic candidiasis and the ability to detect yeasts in blood, regardless of the method of culture, is very disturbing. Extensive disseminated candidiasis must involve the circulatory system. The rate of budding of the yeast could be modulated by the biochemical environment peculiar for each patient; therefore, the detection may vary regardless of severity of infection. Also, the infectivity of the yeasts might be greatly enhanced in immunosuppressed cancer patients. Very few viable yeast cells may be present in the blood at any one time, making their detection difficult; however, because of the immunosuppressed state of the host, each yeast cell may likely colonize and cause tissue invasion in vivo. These factors, as well as others unidentified, may explain the paradox of finding some patients with extensive fatal candidiasis who never have positive blood cultures and, conversely, repeatedly obtaining positive blood cultures from other patients.

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