Lack of Utility of the Lysis-Centrifugation Blood Culture Method for Detection of Fungemia in Immunocompromised Cancer Patients

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We retrospectively compared the utility of a fungal isolation device (Isolator) versus conventional techniques for recovering fungal organisms from blood cultures obtained from neutropenic cancer patients. Positive cultures were deemed true pathogens, possible pathogens, or contaminants according to laboratory and clinical criteria. Fifty-three patients had 66 positive blood cultures for fungi, nine on multiple occasions. In 20 episodes true pathogens were recovered, 6 from broth medium alone, 4 from the Isolator system alone, and 10 from both systems. False-negative cultures were noted in 4 of 20 (20%) cases in which broth medium was used and in 6 of 20 (30%) cases in which the Isolator system was used. Possible pathogens were detected in 4 of 66 blood culture-positive cases. Forty-two positive cultures were considered contaminants, 1 collected from standard medium and 41 of 42 (98%) which grew only in Isolators. Eleven of 18 patients with true fungal infections expired as a result of infection, while 4 of 33 patients with a contaminant expired, none from a fungal cause. We do not advocate the routine use of Isolator tubes in the evaluation of the febrile, neutropenic patient due to the high rates of false positives and of contamination.

Fungal infections are an increasing problem in immunocompromised patients, especially in leukemia or bone marrow transplant patients who experience prolonged periods of neutropenia and protracted courses of antibiotics (5, 9, 16, 17, 31). Fungal pathogens commonly encountered in these situations are often disseminated by the time they are recognized clinically (16, 19, 34, 37). The antemortem diagnosis of fungal infections in immunocompromised patients remains a difficult problem, and it has become routine practice to administer amphotericin B or fluconazole empirically to patients who remain febrile despite the use of broad-spectrum antibacterial agents (10, 23, 25, 27, 29, 41). Amphotericin B therapy has numerous toxic side effects; fluconazole is ineffective in Aspergillus sp. infections, and its use may lead to the emergence of resistant species (20, 21, 24, 34, 40, 41).

Although a definitive diagnosis of invasive fungal infection can be made histopathologically from biopsies of lung, skin, and liver, diagnostic tests which use cultures are less accurate and sensitive, and it is often difficult to distinguish between colonization and invasion (34, 37). In an effort to increase the detection of fungal infections, various approaches have been developed to improve the efficiency of blood cultures and to detect fungal elements in blood or tissues. These techniques include enzyme-linked immunosorbent assay, radioimmunoassay, latex agglutination, monoclonal antibody methods, and PCR to detect Aspergillus-specific DNA sequences (1, 11, 14, 22, 26, 30, 33, 38).

A lysis-centrifugation method, using a blood concentrate specimen plated onto solid medium, has been used to increase the yield of blood cultures when fungal infections are suspected (2–4, 7, 13). A commercially available test (Isolator; Wampole Laboratories, Cranbury, N.J.) has been reported to provide greater sensitivity than conventional broth blood culture systems in recovering fungal organisms from the blood (2–4, 7, 13). We report our 56 months’ experience with immunosuppressed cancer patients who had fungal blood cultures performed in order to evaluate the Isolator method and compare it with conventional broth techniques.

The computer-based files of the Microbiology Laboratory of the Pathology Department of the University Hospitals of Cleveland were reviewed in order to identify all patients on the combined inpatient adult bone marrow transplant and leukemia unit who had blood cultures positive for fungi during the 56-month period of January 1991 through August 1995. During this period, it was policy to obtain simultaneous blood cultures using both the Isolator system and conventional broth culture bottles (Bectec; Becton Dickinson Diagnostic Systems, Towsen, Md.) when febrile, neutropenic patients were evaluated for infection. These samples formed the basis for this comparison.

At the time of analysis, the significance of the positive fungal blood culture was based on the following criteria. A positive blood culture result was deemed a true pathogen (i) if the same organism was isolated from tissue by biopsy (e.g., of skin, liver, or lung), (ii) if there was an associated mucosal fungal infection, (iii) if a change in antifungal therapy resulted in clinical improvement, (iv) if large numbers of colonies of fungi were recovered from the blood, (v) if simultaneous blood cultures obtained from both the conventional broth method and the lysis-centrifugation method recovered the same organism, or (vi) if multiple sequential blood cultures yielded the same organism.

A positive fungal blood culture result was deemed a possible pathogen if there was clinical evidence of fungal infection, such as appearance of new pulmonary infiltrates, but corroborating microbiological or histopathological evidence was not present.
A positive fungal blood culture result was defined as a contaminant (i) if none of the above criteria were met, (ii) if only one or two colonies were detected with the Isolator system, or (iii) if only one of a series of conventional broth cultures was positive.

Blood for fungal culture was processed in a biosafety class II hood by using the Isolator lysis-centrifugation system according to the manufacturer’s instructions. Blood for conventional culture was collected in Bactec NR6A (aerobic) and NR7A (anaerobic) broth and examined daily on a Bactec NR660 instrument (Becton Dickinson) for 6 days. Positive blood cultures which demonstrated fungal elements on microscopic examination were plated on Sabouraud dextrose agar and incubated aerobically at 30°C.

Identification of yeast-like fungi was based on microscopic morphology on corn meal agar and carbon assimilation using the API 20C yeast identification system (bioMeRieux Vitek, Hazelwood, Mo.). Filamentous fungi were identified by colonial and microscopic morphology.

During the 56-month study period, 53 patients had fungi isolated from blood cultures using conventional medium, the Isolator system, or both, and their data formed the basis for this report. These patients, 24 males and 29 females ranged in age from 32 to 77 years (median, 46 years). Leukemia (n = 30), lymphoma (n = 9), and breast cancer (n = 7) were the most frequent diagnoses. Other diagnoses included multiple myeloma (n = 5), germ cell tumors (n = 2), sarcoma (n = 1), and malignant melanoma (n = 1). Twenty-six patients were undergoing bone marrow or peripheral-blood progenitor cell transplantation, while 27 patients were receiving nonmyeloablative chemotherapy.

All patients were treated by indwelling central venous catheters during their hospitalization, and all received corticosteroids at some time during their inpatient confinement, either as an antiemetic or as part of their cancer treatment. Patients given amphotericin B therapy also received hydrocortisone (50 mg) intravenously for the prevention of adverse reactions. At the time that blood cultures were noted to be positive for fungi, all patients were receiving broad-spectrum antibacterial agents that were begun as therapy for neutropenic fever. All but three patients were receiving amphotericin B at the time the first positive fungal blood culture was reported. The median cumulative amphotericin B dose during hospitalization was 900 mg (range, 200 to 8,000 mg). At the time of the documented positive fungal blood culture, the median duration of neutropenia was 29 days (range, 16 to 165 days) for patients who had true pathogens, 23 days (range, 10 to 35 days) for patients with possible pathogens, and 14 days (range, 0 to 54 days) for patients who had contaminants identified in blood cultures.

In 9 of the 53 patients, two or more blood cultures collected with the Isolator were positive for fungi. Five patients had two positive cultures, and four patients had three positive cultures. Each positive culture was analyzed separately, resulting in a total of 66 positive cultures, i.e., 1 each from 44 patients, 2 each from 5 patients, and 3 each from 4 patients. Forty-nine of 66 positive cultures were detected with the Isolator system, 7 were detected with the conventional culture medium, and 10 were detected with both systems. Since 12,600 cultures were performed during the 56-month period of study, the Isolator positivity rate was 0.52% (66 of 12,600).

Twenty positive culture isolates obtained from 16 patients represented true pathogens; these organisms were recovered in 6 situations from the broth medium only, in 4 instances from the Isolator system only, and in 10 cases from both systems (Table 1). These results did not differ significantly, since blood cultures using standard medium were successful in recovering 16 of 20 (80%) organisms compared to 14 of 20 (70%) with the Isolator system (P = 0.715 by the chi-square test). Fungi isolated that were deemed to be true pathogens are listed in Table 1. False negatives were obtained in 4 of 20 (20%) cases from conventional broth cultures and in 6 of 20 (30%) instances from Isolators.

Three patients were considered to have possible pathogens; they accounted for 4 of the 66 culture-positive cases. All four fungi (Candida parapsilosis [n = 1] and Aspergillus fumigatus [n = 3]) were recovered by using Isolators only; one patient had two Aspergillus sp. isolates.

Forty-two positive cultures obtained from 33 patients were considered contaminants (Table 2). Four patients had more than one organism recovered from a single culture. One of the 42 fungal cultures was recovered from conventional medium only, while 41 (98%) grew only in Isolators. Therefore, for our patient population, the standard procedure yielded only a 1% false-positive rate, compared to 62% with the Isolator system.

Patients deemed to have a true pathogen in the blood had a higher mortality rate than those for whom the positive culture was classified as a possible infection or a contaminant. Eleven of the 18 patients thought to have true fungal infections died of the opportunistic infection or its complications. None of the patients who were considered to have a possible fungal infection died. Four of 33 patients with isolates considered contamin-

### Table 1. Positive blood culture isolates considered true pathogens

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total</th>
<th>Isolator only</th>
<th>Standard technique only</th>
<th>Isolator and standard techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida tropicalis</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Candida lusitaniae</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>4</td>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>

### Table 2. Organisms recovered from blood cultures with the Isolator system that were considered contaminants

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>11</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>8</td>
</tr>
<tr>
<td>Nonsporulating molds</td>
<td>3</td>
</tr>
<tr>
<td>Aspergillus spp.</td>
<td>4</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>2</td>
</tr>
<tr>
<td>Cryptococcus albidus</td>
<td>2</td>
</tr>
<tr>
<td>Paecilomyces spp.</td>
<td>2</td>
</tr>
<tr>
<td>Aureobasidium pullulans</td>
<td>1</td>
</tr>
<tr>
<td>Cladosporium sp.</td>
<td>1</td>
</tr>
<tr>
<td>Curvularia sp.</td>
<td>1</td>
</tr>
<tr>
<td>Engerodontium album</td>
<td>1</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>1</td>
</tr>
<tr>
<td>Hormonema sp.</td>
<td>1</td>
</tr>
<tr>
<td>Philaphora sp.</td>
<td>1</td>
</tr>
<tr>
<td>Rhodotorula glutinis</td>
<td>1</td>
</tr>
<tr>
<td>Sporobolomyces salmonicolor</td>
<td>1</td>
</tr>
</tbody>
</table>

*Only one isolate (C. albicans) considered a contaminant was recovered from blood cultures with the standard technique.*
Although Candida and Aspergillus spp. are the most common fungal organisms recovered from intensively treated cancer patients, saprophytic filamentous fungi, once thought to be nonpathogenic in humans, are emerging as significant pathogens (17, 21). Local fungal infections in sites such as mucosa or skin are more easily diagnosed, but recognition of systemic fungal infections is difficult. Since disseminated fungal infections often are recognized postmortem, antifungal therapy frequently is administered empirically (10, 23, 25, 27, 29, 35, 39, 41). Newer methods of detection which have shown promise, such as PCR-DNA detection, are not in widespread use and are not routinely available to clinicians (6, 36).

We compared the Isolator system to conventional blood culture methods and demonstrated the former approach to be no more effective in detecting true fungal infections than routine broth blood cultures. Furthermore, the Isolator technique was associated with significantly more false-positive results. Only 20 of the 66 positive cultures (30%) were interpreted to be true pathogens; 14 of 20 fungemias (70%) were detected with Isolator tubes, while standard blood culture techniques demonstrated 16 of 20 fungemias (80%).

Clinicians frequently are required to decide whether a positive fungal culture represents contamination, colonization, or a true fungal infection. This decision-making process has significant clinical implications: antifungal therapy is a toxic and expensive treatment. Adverse events, including renal dysfunction, anemia, thrombocytopenia, sepsis, electrolyte disturbances, and phlebitis, usually are not well tolerated by immunosuppressed patients who already have been debilitated by their underlying disease and cytotoxic therapy (20, 34). Newer agents such as fluconazole and different amphotericin B formulations have been used increasingly, but the emergence of resistant pathogens and the economic impact of administering extremely expensive agents which have not been shown to be cost-effective also must be taken into consideration in the new medical economic environment (12, 15, 28, 32).

Investigators may often dismiss positive fungal blood cultures as contaminants (20). Standard blood cultures are inherently poor at detecting molds, prompting the use of newer laboratory techniques such as the Isolator blood culture system. The high rate of false-positive blood cultures, which was demonstrated in our study, is a serious problem in evaluation of the immunocompromised, febrile patient. Forty-one of 42 false-positive blood cultures we detected were obtained with Isolator tubes, while only one false-positive result was obtained with conventional techniques. Three other groups similarly reported high blood culture contamination rates with lysine-citrfugation (8, 18, 32). Telenti and Roberts (32) noted a 17% false-positive rate for Candida species. Morrell and co-workers (18) evaluated 5,196 separate fungal blood cultures, 84 of which were positive for fungi. Twenty-four cultures were positive with the Isolator system; 19 of these were considered to be contaminants, and no clinical interventions were undertaken. These investigators evaluated the Isolator system according to four criteria: the fraction of tests producing new information, the turnaround time of the test, the differential value of the test, and the potential harm of false-positive tests. This group concluded that while there may be specific cases that would require specific fungal blood culture systems, their general use should be discouraged. Furthermore, in a retrospective review of candidemia at their institution, Fraser et al. (8) reported that the Isolator system was the sole method of fungal detection in only 9 of 59 episodes of candidemia; on the other hand, 92% of candidemia episodes were documented solely with the use of routine blood culture techniques. They could find no difference in risk factors or outcome for patients who had documentation of candidemia by use of Isolators compared to standard blood cultures. These studies strongly suggest that fungal blood cultures add little to patient management, that they are not cost-effective, and that their use should be restricted. Although a positive blood culture result may be helpful under certain circumstances, a negative blood culture result does not eliminate the possibility of a fungal infection. Newer techniques such as PCR hopefully will soon become available to the clinician, and these will facilitate an increase in the diagnosis of fungal infections (6).

In our analysis, we found that the possibility of using the Isolator system conferred no advantage over standard blood culture methods in patient evaluation. Because use of the Isolator system resulted in a large number of false-positive fungal cultures, we do not advocate the routine use of Isolator tubes in the evaluation or management of the febrile neutropenic patient.

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REFERENCES

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