Three Cases of Fungemia in HIV-Infected Patients Diagnosed Through the Use of Mycobacterial Blood Culture Bottles

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Abstract

We treated three cases of fungemia in HIV-infected patients. These cases were caused by *Candida albicans*, *Cryptococcus neoformans*, and *Penicillium marneffei*, respectively, and all were diagnosed through the use of mycobacterial blood culture bottles. Although the detection of the etiologic agents of fungal infection is difficult, it has been shown that blood culture media for mycobacteria are more effective for the detection of fungemia than media for aerobes and anaerobes. Some reports have shown that Bactec Myco/F lytic bottles were useful for the diagnosis of fungemia in clinical samples. Here, we report the successful use of BacT MB bottles.

Key words: mycobacterial blood culture bottle, BacT MB bottle, fungemia, HIV

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Introduction

The incidence of fungemia, and especially of that caused by *Candida* spp., has recently been increasing. Because diagnosis and treatment with antifungal agents tend to be delayed in such cases, the mortality rate is high (1, 2). Part of the reason for this is that the estimated sensitivity of candidemia detection methods using standard aerobic and anaerobic blood culture bottles is only about 50% (3-6). Blood culture media for mycobacteria, however, are more suitable for detecting fungi *in vitro* than these traditional media are (7). In addition, a few reports have shown that, in clinical situations, Bactec Myco/F Lytic bottles manufactured by Becton Dickinson (Franklin Lakes, NJ, USA) yield better accuracy in detecting fungemia (8, 9). In the cases reported here, we used BacT MB bottles manufactured by bioMérieux (Marcy l'Etoile, France), and showed that they, too, were useful in the diagnosis of three different fungemia

cases in human immunodeficiency virus (HIV)-positive patients.

Case Report

Case 1

A 53-year-old Japanese HIV-infected man with a 1-month history of dizziness was referred to us. Four months previously, he had started trimethoprim-sulfamethoxazole for *Pneumocystis* pneumonia and anti-retroviral therapy (lopinavir/ritonavir+ tenofovir/emtricitabine). On admission, his CD4 lymphocyte count was 12/μL and his viral load was under 50 copies/mL. Brain MRI showed a 3-cm ring-enhanced tumor in his cerebellum. After open biopsy, he was diagnosed with malignant lymphoma (diffuse large B cell type). Whole-brain radiation therapy was started. One month later, he was treated with meropenem against extended-spectrum beta-lactamase (ESBL)-producing *Kleb-*

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Figure 1. The timing of blood cultures and detection for the three cases. BT: body temperature ($^{\circ}\text{C}$), β -DG: serum $1 \rightarrow 3$ - β -D-glucan (pg/mL), FA, FN, and MB, BacT FA (aerobic bottle), BacT FN (anaerobic bottle), and BacT MB (mycobacterial bottle), respectively. - represents negative in culture. () indicates the number of incubation days.

siella pneumoniae sepsis, and with vancomycin against methicillin-resistant *Staphylococcus epidermidis* (MRSE) sepsis. Initially his fever abated, but after antibiotic treatment he experienced another spike fever (defined hereafter as day 1). We took blood cultures in six bottles: two bottles for aerobes (BacT FA), two for anaerobes (BacT FN), and two for mycobacteria (BacT MB). Two days later (day 3), yeast was growing in one mycobacterial bottle (Fig. 1). We drew another set of blood cultures on day 3 to determine whether the yeast growth indicated a true fungemia or a contamination. Because the patient was in severe distress, we started voriconazole empirically, though it is known to interact with lopinavir/ritonavir. Yeast was found on day 5 in the mycobacterial bottle cultured on day 3, and on day 11 in the aerobic bottle cultured on day 3. All three yeasts were identified as *Candida albicans*. Since the same organism was detected in each of three bottles which had been taken

on different days, we considered this case to be a true fungemia. They were sensitive to fluconazole, so we switched the patient from voriconazole to fluconazole. Serum $1 \rightarrow 3$ - β -D glucan was not elevated, measuring 14.9 pg/mL at most. An ophthalmologist confirmed no endophthalmitis. The patient had no central venous catheter, and the entry point of candidemia was unknown. After treatment for candidemia, he was found to have a brain abscess, cellulitis, and a skin abscess at the site of bone marrow examination. He recovered from these serious infections and was discharged home.

Case 2

A 44-year-old Japanese HIV-infected man with a 3-week history of fever and headache was referred to us. He had chronic hepatitis B virus (HBV) infection. His CD4 lymphocyte count was 40/ μL , and his viral load was 40,000 copies/

Figure 2. Gram-stained yeasts (*C. neoformans*) from a mycobacterial bottle in Case 2 (×1000).

Figure 3. Gram-stained hyphae (*P. marneffei*) from a mycobacterial bottle in Case 3 (×1000).

mL. On admission (defined hereafter as day 1), we took one set of blood cultures including one bottle each for aerobes (BacT FA), anaerobes (BacT FN), and mycobacteria (BacT MB) (Fig. 1). Encapsulated yeasts were detected from the cerebrospinal fluid. We suspected *Cryptococcus* meningitis and accordingly started both liposomal amphotericin-B and flucytosine. We also used ampicillin/sulbactam for aspiration pneumonia. The patient's 1→3-β-D glucan was slightly elevated at 40.4 pg/mL. On day 4, yeasts were found growing in the only mycobacterial bottle (Fig. 2). We took two additional sets of blood cultures, which were all negative, probably because antifungal therapy had already been started. These yeasts were identified as *Cryptococcus neoformans*. After treatment for *Cryptococcus* meningitis, we started anti-retroviral therapy (atazanavir+ritonavir+ tenofovir/emtricitabine), and the patient was discharged home.

Case 3

A 30-year-old Japanese HIV-infected man with a 10-day history of fever, cervical and subclavian lymphadenopathy was referred to us. He had traveled to Thailand several months previously. His CD4 lymphocyte count was 10/μL, and his viral load was 140,000 copies/mL. On the day after admission (defined hereafter as day 1), we took a single blood culture in a mycobacterial bottle (BacT MB) to test for *Mycobacterium avium* complex (Fig. 1). On day 2, we also drew blood cultures into aerobic (BacT FA) and anaerobic (BacT FN) bottles. These three bottles were all negative. Another mycobacterial bottle was taken on day 5, and aerobic, anaerobic, and mycobacterial bottles were taken on day 8. At this point we started antimycobacterial therapy empirically. Hyphae were observed on day 11 growing from the mycobacterial bottle taken on day 5 (Fig. 3), on day 12 from the anaerobic bottle taken on day 8, and on day 14 from the mycobacterial bottle taken on day 8. All of those hyphae were identified as *Penicillium marneffei*. The culture from a subclavian lymph node biopsy tested positive for the same organism. The patient's serum 1→3-β-D glucan was elevated at 57.6 pg/mL. We started liposomal amphotericin-B and he became afebrile. After anti-retroviral therapy

(fosamprenavir+tenofovir/emtricitabine), he was discharged home.

Discussion

The incidence of fungemia, especially that caused by *Candida* spp., has recently been increasing (1, 2). The diagnosis of candidemia is frequently difficult, however, because the efficacy of fungemia detection using traditional aerobic and anaerobic bottles is estimated at only 50% (3-6). In general, serum 1→3-β-D-glucan is not sufficiently sensitive or specific to serve as a diagnostic marker for fungemia (4). Delayed diagnosis leads to poor prognosis: the mortality rate is over 40 percent (1, 2, 5). *Cryptococcus* meningitis is somewhat easier to diagnose, because in most cases it can be detected in cerebrospinal fluid. One report, however, has described a case of *Cryptococcus* meningitis that was not detected in cerebrospinal fluid but only through blood culture (8). *Penicillium marneffei* infection is rare in Japan but common in Southeast Asia. In cases of delayed diagnosis, the mortality rate is about 75% (9).

We have described the detection of three different fungal species, *Candida albicans*, *Cryptococcus neoformans*, and *Penicillium marneffei*, through the use of BacT MB bottles. The positivity rates of *C. albicans* detection were 33% (1/3 bottles) using aerobic bottles (BacT FA), 0% (0/3 bottles) using anaerobic bottles (BacT FN), and 67% (2/3 bottles) using mycobacterial bottles (BacT MB) (Table 1). The aerobic bottles (BacT FA) required 8 days of incubation before yielding results, while the mycobacterial bottles (BacT MB) required only 2 days. Mycobacterial bottles therefore exhibited the highest sensitivity and the shortest incubation period. The positivity rates of *C. neoformans* detection were 0% (0/3 bottles) using BacT FA bottles, 0% (0/3 bottles) using BacT FN bottles, and 33% (1/3 bottles) using BacT MB bottles (Table 1); in other words, *C. neoformans* was detected only when a mycobacterial bottle was used. The positivity rates of *P. marneffei* detection were 0% (0/2 bottles) using BacT FA bottles, 50% (1/2 bottles) using BacT FN bottles, and 67% (2/3 bottles) using BacT MB bottles (Ta-

Table 1. Positivity Rates and Detection Times for Aerobic, Anaerobic, and Mycobacterial Bottles

	Case 1 <i>Candida albicans</i>	Case 2 <i>Cryptococcus neoformans</i>	Case 3 <i>Penicillium marneffei</i>
Positivity rate of aerobic bottle(BacT FA)	0.33 (1/3bottles)	0 (0/3bottles)	0 (0/2bottles)
anaerobic bottle(BacT FN)	0 (0/3bottles)	0 (0/3bottles)	0.5 (1/2bottles)
mycobacterial bottle(BacT MB)	0.67 (2/3bottles)	0.33 (1/3bottles)	0.67 (2/3bottles)
Detection time of aerobic bottle(BacT FA)	8 days	—	—
anaerobic bottle(BacT FN)	—	—	4 days
mycobacterial bottle(BacT MB)	2 days	3 days	6 days

ble 1). The mean number of days required for incubation was 4 days for BacT FN and 6 days for BacT MB. For *P. marneffei*, therefore, the mycobacterial bottle again exhibited the highest sensitivity, while the anaerobic bottle required the shortest incubation period. All three of these cases were completely cured through treatment with appropriate antifungal therapies. No other organisms were found in any other blood culture bottles.

About 200 HIV-positive patients are admitted to our hospital each year. When these HIV patients are febrile, we routinely take six bottles of blood culture, two each for the detection of aerobes, anaerobes, and mycobacteria. The required amounts of blood are 10 mL for each aerobic or anaerobic bottle and 5 mL for each mycobacterial bottle. Our laboratory uses the BacT/ALERT 3D automated blood culture system.

Between 2000 and 2005, we took 552 sets of aerobic and anaerobic blood cultures and 390 sets of mycobacterial blood cultures from 684 HIV-positive patients. The positivity rate among aerobic and anaerobic cultures was 3.81% (21/552 sets); three of the 21 positive results were considered to have been contaminations. The organisms involved in the true-positive cases were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Cryptococcus neoformans*, and *Candida guilliermondii*. The positivity rate among mycobacterial cultures was 1.74% (7/390 sets); all of these involved only *Mycobacterium avium*.

The main reason for culturing blood in mycobacterial bottles is to detect miliary tuberculosis or *Mycobacterium avium* complex. In the three cases described here, however, the routine use of mycobacterial bottles for these other purposes led to early diagnosis of fungemia, not mycobacteremia. In Case 1, the first *Candida* culture grew in only one mycobacterial bottle, but because we had suspected opportunistic infection, we did not assume that it represented a contamination. Because continuous fungemia was demonstrated in another set of blood cultures, *Candida* was determined to be the etiologic agent. Having noticed that the *Candida* grew well in the mycobacterial bottle, we were not surprised when *Cryptococcus* and *Penicillium* were also detected through the use of mycobacterial bottles in Cases 2 and 3. Before witnessing these three cases, when fungi or bacteria other than mycobacteria grew in a mycobacterial

bottle, we had suspected that the bottle had been contaminated.

Two kinds of blood culture bottles for mycobacteria are available: the Bactec Myco/F Lytic bottle manufactured by Becton Dickinson, and the BacT MB bottle manufactured by bioMérieux. Bactec Myco/F Lytic bottles are designed to detect both mycobacteria and fungi, but BacT MB bottles are designed to detect mycobacteria only, and not fungi. Nevertheless, both mycobacterial bottles were superior to aerobic and anaerobic bottles for fungal detection *in vitro* (7). One report has stated that, in a clinical situation, Bactec Myco/F Lytic bottles exhibited higher sensitivity and shorter incubation periods in the detection of *Candida albicans* and *Candida glabrata* than aerobic bottles (Bactec Plus Aerobic/F) did (5). Another report has shown that the routine use of Bactec Plus Aerobic/F, Plus Anaerobic/F and Myco/F Lytic bottles for immunocompromised hosts, such as patients in the ICU, permitted highly efficient *Candida albicans* detection (6). That study examined 1,253 blood culture sets (3,759 bottles) in two years. From these sets, 62 yeasts were isolated. The positivity rates were 7.33% among Plus Aerobic/F bottles (44/600 bottles), 1.13% among Plus Anaerobic/F bottles (5/441 bottles), and 25.4% among Mycotic IC/F bottles (48/189 bottles).

Because the present report includes only three fungemia cases, it may not be appropriate to compare these results with those of their reports, but our data correspond well with those from the larger studies in showing that mycobacterial blood cultures can detect fungi with a higher sensitivity than aerobic or anaerobic cultures offer.

Nevertheless, it is very difficult to estimate the true positivity rate of fungemia detection through the use of these blood cultures. Especially among HIV-positive patients, even if serum 1→3-β-D-glucan is elevated, this is frequently caused by *Pneumocystis pneumonia*, not by fungemia. Thus, the direct detection of fungi from blood cultures is particularly important in this area.

Some antibiotics are included inside Bactec Myco/F Lytic bottles to inhibit the growth of bacteria other than mycobacteria or fungi. No antibiotics are included inside BacT MB bottles. The reason why fungi grow so well in mycobacterial bottles is unclear. Both fungi and mycobacteria grow well in aerobic environments, and fungi grow faster than mycobacteria. Accordingly it is possible that, in cases of co-infection with fungi and mycobacteria, the mycobacteremia will be

overlooked.

Each mycobacterial bottle requires an extra 5 mL of blood from the patient, as well as laboratory space for its storage. The mycobacterial bottles also cost three times as much as typical aerobic or anaerobic bottles (7, 10). The efficacy of aerobic and anaerobic detection is so low that the regular use of mycobacterial bottles is not recommended in the case of community-acquired infection. Mycobacterial bottles are suitable for patients with high risk of fungemia, including immunocompromised hosts and patients with central venous catheters in place. The prognosis of fungemia is still not very good, but early diagnosis leads to early anti-fungal treatment which is more likely to result in a complete cure.

We have encountered three cases of fungemia in HIV-infected patients, caused by *Candida albicans*, *Cryptococcus neoformans*, and *Penicillium marneffei*, respectively, all of which were diagnosed through blood culture in BacT MB bottles. Blood culture in aerobic and anaerobic bottles alone would not have been sufficient in these cases. We have found that BacT MB bottles are also useful for the isolation of fungi in clinical situations. More data are required to confirm the usefulness of these mycobacterial bottles for the detection of fungemia in immunocompromised hosts.

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