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Comparison of bone marrow studies with blood culture for etiological diagnosis of disseminated mycobacterial and fungal infection in patients with acquired immunodeficiency syndrome

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The role of bone marrow examination in the diagnosis of prolonged fever in patients with human immunodeficiency virus infection remains controversial. This study compared the results of bone marrow examination and blood culture in the evaluation of opportunistic infections in 100 patients with acquired immunodeficiency syndrome who presented with prolonged fever and/or pancytopenia from November 1995 through August 1998. Bone marrow and blood specimens were routinely submitted for bacterial, mycobacterial, and fungal cultures, and histopathological examination of the bone marrow specimens was also performed. A total of 33 cases of disseminated mycobacterial infections and 17 cases of disseminated fungal infections were identified. Fifteen cases of mycobacterial infection and 8 cases of fungal infection were identified by using bone marrow culture. The causative organisms included *Mycobacterium avium* complex (9 isolates), *Mycobacterium tuberculosis* (4), *Mycobacterium chelonae* (1), *Mycobacterium kansasii* (1), *Cryptococcus neoformans* (3), and *Penicillium marneffei* (5). Granulomas were seen in only 8 of 33 disseminated mycobacterial infections, and acid-fast bacilli were detected in only 4 patients. Although bone marrow culture did not provide a significantly higher diagnostic yield than blood culture, bone marrow culture plus histopathological examination had a higher diagnostic yield than blood culture alone for patients with disseminated mycobacteriosis. This study demonstrated that bone marrow examination provides an additional aid in the diagnosis of disseminated mycobacterial or fungal infections in patients with human immunodeficiency virus who had prolonged fever.

Key words: Acquired immunodeficiency syndrome, bone marrow aspiration, mycobacteriosis, mycosis

Blood culture and bone marrow examination and culture are important diagnostic methods for the etiologic evaluation of opportunistic infections in patients with human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) [1,2]. However, the comparative usefulness between bone marrow examination plus microbiologic culture and blood culture in HIV-infected patients remains controversial [3,4].

Previous studies conducted in the National Taiwan University Hospital found that mycobacteriosis and mycoses were the 2 most common causes of prolonged fever in HIV-infected patients [5,6]. In this hospital, bone marrow examination and blood cultures are often concurrently performed for etiological diagnosis in HIV-infected patients with prolonged fever. The purpose of this study was to compare the diagnostic yields of blood culture and bone marrow examination plus microbiologic culture, including bacterial, fungal, and mycobacterial culture, in the diagnosis of disseminated mycobacterial and fungal infection in HIV-infected patients.

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Materials and Methods

Participants

Since June 1994, all HIV-infected patients hospitalized at the National Taiwan University Hospital have undergone a series of examinations to determine the etiology of fever. The evaluation includes complete blood count, blood chemistry, cultures of blood, urine, and sputum for bacteria, fungi, and mycobacteria, and

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chest radiography. Invasive diagnostic procedures, such as biopsy of lymph nodes, bone marrow, or liver are performed if the etiology of fever could not be identified with these routine studies [7,8]. From November 1995 through August 1998, 111 HIV-infected patients were hospitalized at NTUH for the management of HIV-associated complications. One hundred of them who received both blood culture and bone marrow aspiration and biopsy for etiological diagnosis of prolonged fever and/or pancytopenia were included in this study. Two sets of routine blood cultures were performed first in patients with fever, and bone marrow examination was subsequently performed after obtaining informed consent from the patient or the patient's guardian if blood culture for common microbes was negative after incubation for 1 week.

Microbiologic cultures of blood and bone marrow specimens

BACTEC NR 6A and NR 7A bottles (Becton Dickinson, Diagnostic Instrument System, Spark, MD, US) were used for blood culture of aerobic and anaerobic bacteria. BACTEC fungal bottles (Becton Dickinson) were used for fungal blood cultures. Isolators (Dupont Isolator system, Dupont, Wilmington, DE, US) were used for blood and bone marrow culture of mycobacteria. After lysis and centrifugation of the blood or bone marrow specimens, the sediment was inoculated onto Lowenstein-Jensen medium (BBL, Microbiology Systems, Cockeysville, MD, US) and 7H11 Middlebrook medium (BBL, Microbiology Systems).

Bone marrow histological examination

For each bone marrow biopsy specimen, the cellularity, hematopoietic maturation, dysplastic changes, stromal changes, and granulomas were assessed by pathologists. The histopathologic stains used included hematoxylin and eosin stains, Gomori methenamine-silver stains, periodic-acid-Schiff stains, and Kinyoun acid-fast stains.

Definitions

An AIDS-defining illness was defined as a positive anti-HIV antibody test result as confirmed by Western blot and the 1993 Centers for Disease Control expanded surveillance case definition of AIDS among adolescents and adults [9]. The definitions of disseminated mycobacteriosis and mycoses were positive cultures from 2 separate body sites.

Statistical analysis

SPSS software (SPSS, Chicago, IL, US) was used to analyze the data. Categorical variables were compared by univariate analysis with chi-square test or Fisher's exact test. A p value of <0.05 was considered statistically significant.

Results

During the 34-month study period, both blood culture and bone marrow examination plus culture were performed 123 times in 100 patients. Ninety-five (95%) of the patients were men with a mean age of 36 years (range, 21-67 years). Among them, 33 patients had disseminated mycobacterial infection and 17 had disseminated fungal infection. Of the mycobacteria causing disseminated infection, 16 (49%) were nontuberculous mycobacteria (NTM) and 17 (51%) were *Mycobacterium tuberculosis* (MTB). Among the 16 NTM cases, *Mycobacterium avium* complex (MAC) was the most common etiologic organism, which accounted for 13 (81%) cases (Table 1). In all 13 disseminated MAC infection cases, the causative microorganism could be isolated from bone marrow and/or blood except for one case that was isolated from sputum, liver biopsy specimens, and stool, and another one that was isolated from biopsy specimens of the liver

Table 1. Distribution of etiologies in 33 patients with disseminated mycobacterial and fungal infection

Pathogen	No. of cases		
	BM/C positive	BC/ positive	Total
Nontuberculous mycobacteria	11	9	16
Mycobacterium avium complex	9	8	13
Mycobacterium chelonae	1	0	1
Mycobacterium kansasii	1	1	2
Mycobacterium tuberculosis	4	3	17
Fungi	8	12	17
Cryptococcus neoformans	3	8	8
Penicillium marneffei	5	3	7
Histoplasma capsulatum	0	1	2

Abbreviations: BM/C = bone marrow culture; B/C = blood culture

and lung. In all disseminated *Mycobacterium chelonae* and *Mycobacterium kansasii* infections, the causative microorganism could be isolated from bone marrow and/or blood except for one case of *M. kansasii* that was isolated from lymph node. Among the 17 cases of disseminated MTB infection, the causative microorganism could be isolated from bone marrow and/or blood in 5 cases and from the lymph node in 13. In one case of disseminated MTB infection, the causative microorganism was isolated from blood, sputum, and lymph node. Of the 17 cases of disseminated mycosis, 8 (48%) were caused by *Cryptococcus neoformans*, 7 (41%) by *Penicillium marneffei*, and 2 (11%) by *Histoplasma capsulatum* (Table 1). In all disseminated fungal infections, the causative microorganism could be isolated from bone marrow and/or blood except for one case of *P. marneffei* and one of *H. capsulatum* that were isolated from lymph node.

CD4 count distribution

All patients with disseminated mycobacteriosis and mycoses had a low CD4 cell count (range, 1-764 cells/μL; median, 14.5 cells/μL). More than 70% (36 patients) had a $CD4^+$ lymphocyte count of less than 20 cells/μL. All patients with NTM infection had a very low CD4 cell count (range, 1-33 cells/μL; median, 9 cells/μL). In patients with disseminated MTB infection, there was a higher CD4 cell count (range, 1-159 cells/μL; median, 13 cells/μL). However, the number of circulating CD4 cells was not significantly different between patients with disseminated NTM infection and patients with disseminated tuberculosis. In all patients with disseminated fungal infections, the CD4 cell count was less than 50 cells/μL except for one patient with *P. marneffei* infection who had a CD4 cell count of 53 cells/μL (range, 2-53 cells/μL; median, 9 cells/μL).

Correlation of histopathology and bone marrow culture in disseminated mycobacteriosis

Among all 123 bone marrow examinations, granulomas were observed in 13 cases and acid-fast bacilli (AFB) were found in the histopathological sections of 4 (3%) of them. Caseous necrosis was noted in only one of the 123 bone marrow examinations. Among the 33 patients with the final diagnosis of disseminated mycobacterial infection, only 8 showed granulomas, and 2 of them showed both AFB and granuloma in their bone marrow sections. Bone marrow specimens from 23 of these 33 patients showed neither AFB nor granulomas (Table 2). Fifteen patients with disseminated mycobacterial infection had a positive mycobacterial bone marrow culture. Among them, 6 had positive histological findings of granulomas and/or AFB on histopathological section. Bone marrow culture was more sensitive than histopathological examination for the detection of mycobacterial infection (Table 2).

Comparison of blood culture and bone marrow culture in disseminated mycobacteriosis

Among the 33 patients with disseminated mycobacterial infection, 15 had positive bone marrow culture and 12 had positive blood culture. Among them, 3 patients had positive blood culture but negative bone marrow culture, and 6 had positive bone marrow culture but negative blood culture. Bone marrow culture had a higher positive yield rate than blood culture, but this difference was not significant (45% vs 36%, $p>0.05$). *Mycobacterium tuberculosis* and MAC could both grow either from blood culture or from bone marrow culture, and there was no significant difference in the positive yield rate between patients with disseminated tuberculosis and patients with disseminated NTM infection.

Fungal infection

Among the 17 patients with disseminated fungal infection, bone marrow culture was positive in 8 patients, including 3 with *C. neoformans* infection and 5 with *P. marneffei* infection. The bone marrow specimen showed positive fungal stain in only 4 of these 8 patients. Among the 9 patients who had a negative bone marrow culture result, only one had positive fungal stain

Table 2. Correlation of bone marrow examination and culture results in 33 patients with disseminated mycobacteriosis

AFB/Granuloma	No. of cases		
	BM/C positive	BM/C negative	Total
+/+	1	1[a]	2
+/-	2	0	2
-/+	3	3	6
-/-	9	14	23
Total	15	18	33

Abbreviations: AFB = acid-fast bacilli; BM/C = bone marrow culture

[a]Sputum and blood cultures yielded *Mycobacterium avium* complex.

on histological sections. A total of 8 patients had positive bone marrow culture, and 10 patients had positive blood culture. Among them, 4 patients had both positive bone marrow culture and positive blood culture. Blood culture had a slightly higher positive yield rate than bone marrow culture, but this difference was not significant.

Discussion

This study demonstrated that both blood culture and bone marrow examination plus culture are important for detecting mycobacterial and fungal infections in HIV-infected patients. For patients with disseminated mycosis, bone marrow examination plus culture did not have a higher diagnostic yield rate than blood culture. But for patients with disseminated mycobacteriosis, bone marrow examination plus culture had a higher diagnostic yield rate than blood culture.

This study confirmed previous findings that granulomas may not be seen by bone marrow examination in AIDS patients with disseminated mycobacterial infection [4,10,11]. In 11 patients, mycobacteria grew from bone marrow cultures but no granuloma formation was found on histopathological examination. This study also supports the conclusions of previous investigators that absence of AFB or granulomas does not exclude eventual growth of mycobacteria in bone marrow culture [12]. Culture of the bone marrow was a more sensitive method than AFB staining and/or granuloma formation in bone marrow histopathological examination. Previous studies have reported that AIDS patients with cryptococcosis or histoplasmosis might have negative results in their bone marrow fungal stain or bone marrow culture [3,13]. In addition, this study confirmed that bone marrow fungal stain may be negative in patients with positive bone marrow culture for fungi. Therefore, in HIV-infected patients with prolonged fever, bone marrow culture for mycobacteria and fungi should be routinely done even when the histopathological examination results are negative.

Several previous studies have compared the results of bone marrow culture and blood culture for fungi and/or mycobacteria in the HIV-positive patient population. For mycobacteria, the positive culture rate ranged from 17% to 50% in blood culture [1,3,13,14] and from 23% to 43% in bone marrow culture [1,3,13,14]. For fungi, the positive culture rate was only around 6% to 8% regardless of whether it was performed in blood culture or in bone marrow culture [3,13]. Previous studies found no significant difference in positive yield rates between blood culture and bone marrow culture either for diagnosis of mycobacterial or fungal infection [1,3,13, 14]. This study also did not find any significant difference in the positive yield rate of blood and bone marrow culture. However, if patients with negative bone marrow culture but positive histopathological findings were included in the analysis, bone marrow examination plus culture did achieve a higher diagnostic yield rate (19/33, 58%) than blood culture alone (12/33, 36%) for patients with disseminated mycobacterial infection. In addition, for patients with negative blood culture results, bone marrow culture and/or histopathological examination could still be positive and the diagnostic yield rate can increase to 54% and 83%, respectively, for disseminated mycobacteriosis and disseminated mycosis if both blood culture and bone marrow study were done. These findings suggest that for HIV-infected patients with prolonged fever, both blood culture and bone marrow examination plus culture should be done for etiological diagnosis if disseminated mycobacteriosis or disseminated mycosis is included in the differential diagnosis.

Individuals with an absolute CD4 cell count below 75 cells/μL are at high risk of developing bacteremia due to MAC [15,16]. Patients with HIV infection should receive chemoprophylaxis against disseminated MAC disease if they have a CD4 cell count of less than 50 cells/μL [15]. In this study, all patients with disseminated NTM infection had a CD4 cell count below 50 cells/μL. The CD4 cell count indicative of the need for prophylaxis of *C. neoformans* and *H. capsulatum* in HIV-infected patients in endemic areas was suggested to be less than 50 cells/μL and less than 100 cells/μL, respectively [10]. In this study, all HIV-infected patients with *C. neoformans* or *H. capsulatum* infection had a CD4 cell count of less than 50 cells/μL. Therefore, prophylaxis for the first episode of MAC and *C. neoformans* infection in HIV-infected patients is necessary if the CD4 cell count is less than 50 cells/μL.

In summary, the results suggest that for HIV-infected patients with a low CD4 cell count, blood culture and bone marrow examination plus culture should be done when patients have prolonged fever and no other etiology can be found by other routine examinations. Bone marrow study provided a better diagnostic yield rate than blood culture for HIV-infected patients with disseminated mycobacteriosis, although it did not provide a better diagnostic yield rate than blood culture for disseminated mycosis. Bone marrow examination provided additional diagnostic yield when the blood culture result was negative. Therefore, bone marrow examination should be considered when patients have a negative blood culture result. Blood

culture plus bone marrow study achieved a much higher diagnostic yield rate in patients with disseminated mycobacteriosis or mycosis.

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