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Evaluation of bone marrow and blood cultures for the recovery of mycobacteria in the diagnosis of disseminated mycobacterial infections

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ABSTRACT

This study evaluated the validity of bone marrow (BM) and blood specimens for the diagnosis of disseminated mycobacterial infections (DMIs). From 1990 to February 1997, all specimens were processed with the lysis–centrifugation procedure; thereafter (until December 2001), they were processed with the BACTEC Myco/F Lytic system. Twenty-three paired BM–blood specimens with mycobacteria in at least one specimen were studied from 23 patients. The strains isolated were 14 *Mycobacterium avium* complex (MAC) and nine *M. tuberculosis* complex (MTBC). Blood specimens had a statistically significant greater sensitivity for the isolation of MAC than BM (100% vs. 57.1%, respectively), whereas sensitivity for the isolation of MTBC was equal for the two specimen types (66.7%). Although not statistically significant, the times required to detect mycobacteria from blood specimens were lower than those from BM in the MycoF/Lytic system. Overall, blood cultures represented a more sensitive and less invasive alternative to BM cultures for the diagnosis of disseminated mycobacteriosis caused by MAC, especially when the MycoF/Lytic system was used, but provided no advantage for the diagnosis of DMI caused by MTBC.

Keywords Blood cultures, bone marrow, disseminated mycobacterial infections, mycobacteria

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INTRODUCTION

Diagnosis of disseminated mycobacterial infection (DMI) can be achieved by the use of several histopathological and/or microbiological criteria, including the recovery of mycobacteria from bone marrow (BM) and blood specimens [1–4]. Although blood extraction is less invasive than BM aspiration, little is known about the usefulness of blood culture compared with BM culture for the evaluation of patients with suspected DMI [3,5–7]. Therefore, the objective of the present study was to compare the microbiological yields of concurrently obtained BM and blood cultures for the diagnosis of DMI in patients admitted to our institution over a 12-year period.

MATERIALS AND METHODS

Patients

All BM and blood specimens sent to the mycobacteriology laboratory of the Hospital General Universitario Gregorio Marañón (Madrid, Spain) from patients suspected of having DMI during the period 1990–2001 were analysed retrospectively. Specimens with contaminated cultures were excluded from the study. All pairs of BM–blood specimens from the same patient were included in the study, provided that there was a maximum of 10 days between the two specimens being taken. The clinical charts of all patients included in the study were reviewed for empirical or prophylactic anti-mycobacterial therapies in the period from 1 month before the first specimen or between specimens.

Staining procedures

BM smears were taken on glass slides by the haematologist at the bedside and were sent to the laboratory with culture specimens. They were then stained by the auramine technique. Blood samples were not stained.

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Cultures and identification

From January 1990 to February 1997, BM and blood specimens were processed by lysis–centrifugation [8], using Lowenstein–Jensen medium (with and without pyruvate) and Middlebrook 7H9 broth as culture media. Briefly, 8–10 mL of blood was inoculated into an average of three BACTEC PLUS Aerobic/F* bottles (Becton Dickinson, Sparks, MD, USA). After the bottles had been incubated at 35°C for 5 days in the BACTEC 9000 instrument (Becton Dickinson), 10 mL of Middlebrook 7H9 broth was added to bottles which showed no apparent growth. The bottles were then incubated for a further 7 days. After this second incubation period, 10 mL from each bottle for the same patient was transferred to a tube containing 0.5 mL of sodium deoxycholate (0.1 g/mL). Following centrifugation at 3800 *g* for 15 min, the leukocyte layer was inoculated finally on to culture media.

BM specimens (*c.* 2–5 mL) were transferred into glass tubes containing glass beads and vortexed for 10 min. They were then centrifuged at 3800 *g* for 15 min, followed by inoculation of the sediments on to culture media. For both blood specimens and BM, the media were incubated in an aerobic atmosphere at 37°C for 8 weeks, or until positive growth was observed. Cultures were examined on a weekly basis.

During the second period (February 1997 onwards), both specimen types were cultured in MycoF/Lytic bottles (Becton Dickinson) according to standard procedures [8].

Presumptive mycobacterial identification

Isolates were identified using DNA probes (AccuProbe; GenProbe, San Diego, CA, USA) according to the manufacturer's instructions.

Data analysis

Sensitivity rates were calculated with reference to the growth of mycobacteria in any type of specimen. The recovery rates of mycobacteria from both types of specimen were compared by the exact binomial test for paired samples [9]. The median times required for detection by the two culture systems, both globally and by species, were compared with the Mann–Whitney test. In order to allow for the weekly reading of the cultures, the time required for growth of mycobacteria was calculated from the day when samples were first processed in the laboratory until 3 days before growth was first observed. The median times required for detection of both types of specimen were compared by the Wilcoxon signed-rank test for paired specimens [10]. A p value ≤ 0.05 was considered to be statistically significant.

RESULTS

During the study period (1990–2001), 7237 blood specimens were taken from 4759 patients (average number/patient = 1.5), and 1238 BM specimens from 1076 patients were sent to the mycobacteriology laboratory. Of these, 388 paired BM–blood specimens from 356 patients fulfilled the criterion for selection (i.e., a maximum of 10 days between the two specimens). Forty-four pairs of specimens (11.3%) from 42 different patients grew mycobacteria from at least one specimen. However, 21 pairs from 19 patients were excluded from the study because the patients had received antimycobacterial drugs in the previous month or between the two specimens being taken. Therefore, 23 pairs of specimens from different patients remained for analysis. Blood specimens were taken before BM specimens in 18 patients (78.3%; median time, 6 days), two were taken after (8.7%; median time, 3 days), and the remaining three pairs were taken on the same day. The mycobacterial strains isolated from the 23 patients comprised 14 *Mycobacterium avium* complex (MAC) and nine *M. tuberculosis* complex (MTBC). Smears were taken for all 23 BM samples, but these showed a sensitivity of only 8.7%, with only two positive results. No single episode was produced by more than one strain. All but four patients (all MTBC isolates) were HIV-positive.

When the pairs of specimens from the 23 patients were analysed, 11 were positive for both MAC and MTBC (eight MAC and three MTBC isolates), BM grew only three (all MTBC), and blood grew only nine (six MAC and three MTBC). Global sensitivities for the recovery of all species were 60.9% from BM and 87.0% from blood (not significant) (Table 1). These differences were greater when compared with those pairs from patients suffering from MAC infection (57.1 and 100%, respectively; p 0.03). However, in pairs from patients with disseminated tuberculosis,

Table 1. Rates of recovery of mycobacteria from BM and blood cultures for the 23 patients analysed

	Number of positive specimens		
Isolates	**Lysis–centrifugation 1990–1997**	**MycoF/Lytic 1997–2001[a]**	**Total**
All			
Total	15	8	23
BM	10 (66.7%)	4 (50%)	14 (60.9%)
Blood	14 (93.3%)	6 (75%)	20 (87.0%)
MAC			
Total	11	3	14
BM	7 (70.0%)	1 (33.3%)	8 (57.1%)
Blood	11 (100%)	3 (100%)	14 (100%)
MTBC			
Total	4	5	9
BM	3 (75.0%)	3 (60%)	6 (66.7%)
Blood	3 (75.0%)	3 (60%)	6 (66.7%)

[a]There were no statistically significant differences between the rates of recovery of mycobacteria from BM and blood specimens globally, during each period and by species, except for MAC during the entire period.
BM, bone marrow; MTB, *Mycobacterium avium* complex; MTBC, *M. tuberculosis* complex.

Table 2. Time required for detection of mycobacteria from bone marrow and blood cultures for the 23 patients analysed

	Number (range) of days required for detection of mycobacteria		
Isolates	Lysis–centrifugation 1990–1997	MycoF/Lytic 1997–2001[a]	Total
All			
BM	27 (18–60)	23.5 (12–35)	26 (12–60)
Blood	37.5 (20–70)	17.5 (11–28)	31.5 (11–70)
MAC			
BM	27 (18–60)	25	26 (18–60)
Blood	40 (26–70)	22 (12–28)	30.5 (12–70)
MTBC			
BM	31 (25–36)	22 (12–35)	28.5 (12–36)
Blood	34 (20–52)	13 (11–26)	23 (11–52)

[a]There were no statistically significant differences between the median times to detection of mycobacteria by both systems, either globally or by species, except for blood specimens during the entire period (p 0.001) and for the isolation of MAC (p 0.019).

both specimens had the same sensitivity (66.7%). These recovery rates were similar in each period studied.

When the median times required for detection of mycobacteria by both systems were compared (Table 2), the MycoF/Lytic system recovered mycobacterial isolates earlier from both types of specimen, but the differences were only statistically significant for blood cultures. When the median time required for detection of mycobacteria from BM vs. blood cultures was analysed using the data for the 11 paired specimens that both grew mycobacteria, there were no statistically significant differences between the values. However, blood cultures processed by the MycoF/Lytic system revealed mycobacteria in approximately half the time needed for BM.

DISCUSSION

DMIs are serious illnesses with a fatal outcome in the absence of treatment. For this reason, prompt diagnosis is essential in the management of DMI. Moreover, isolation of mycobacteria from affected organs or tissue is required for a definitive diagnosis. Traditionally, BM has been an efficient tissue specimen for the diagnosis of DMI, although invasive procedures are necessary to obtain specimens [2,4,5,11,12]. Therefore, blood culture could represent an alternative for the diagnosis of this illness. The present study compared the usefulness of BM and blood cultures for the diagnosis of DMI over a 12-year period.

To our knowledge, only four studies have performed a parallel evaluation of the diagnostic yields of blood and BM cultures in DMI, using either solid media (Lowenstein–Jensen and/or Middlebrook 7H11 agar) [3,5–7], 7H12 Bactec vials [5,7], or other liquid media [6,7]. In the present study, two different culture systems were used for the recovery of mycobacteria during the study period. A lysis–centrifugation procedure, with Lowenstein–Jensen pyruvate and Middlebrook 7H9 media as the final culture media, was performed during the first part of the study, after which the MycoF/Lytic system was used. The global sensitivity of blood culture in the present study (87.0%) was similar to that reported earlier (62–83%), but the global sensitivity of BM culture (60.9%) was lower than reported elsewhere (78–93%) [3,5–7]. As some patients took antimycobacterial drugs before specimen extraction in at least one previous study [3] (no information was available from the other studies), a higher sensitivity might have been expected in the present study. However, other variables, such as the time between the two specimens being taken (6 weeks [3], 2 weeks [6], 3 days [5], unspecified [7]), the number of pairs, the mycobacterial species isolated and the processing method used, could explain these differences.

The low number of cases included in some data comparisons in the study made statistical analysis difficult. However, differences in sensitivity were observed between BM and blood cultures for paired specimens that grew MAC isolates, especially during the MycoF/Lytic period. In contrast, there were no differences when comparing paired specimens that yielded MTBC. It is not known whether two sets of blood cultures have a greater diagnostic yield for the isolation of MTBC than only one set. Unfortunately, the three patients (two of whom were HIV-positive) diagnosed with disseminated tuberculosis infections only on the basis of positive BM cultures did not have other simultaneous blood cultures taken, although one patient had a positive blood culture taken 18 days before the extraction of the BM specimen.

The results showed that the MycoF/Lytic system was generally faster than lysis–centrifugation for both types of specimen, whether for MAC or MTBC isolates, with a decrease in median time required for detection of 33.5–22 days (decreasing average, 34%; range, 7–62%). A previous study performed with blood specimens showed a reduction in the time required for diagnosis of 61% [8]. Other studies have shown the utility of the MycoF/Lytic system for the diagnosis of DMI

from blood specimens, whether for MAC infections (median time range, 10–16 days) [8,13,14] or MTBC infections (median time range, 19–26 days) [8,15]. When positive paired specimens were analysed, the MycoF/lytic system detected mycobacteria from blood specimens in about half the time required for BM. Although smears from BM could provide a rapid presumptive diagnosis of DMI, the study showed a low sensitivity for this technique.

In summary, blood culture represents a more sensitive and less invasive procedure than BM culture for the diagnosis of DMI caused by MAC isolates, especially when using the MycoF/Lytic system. In this respect, a single blood culture using the MycoF/Lytic system could be the first step in the diagnosis of DMI caused by MAC. However, sensitivity was similar for the recovery of MTBC strains, so one-third of the episodes would have been misdiagnosed without the BM culture. The use of BM and blood cultures in combination would provide the maximum sensitivity for diagnosis of disseminated tuberculous infections.

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