Detection of Mycobacteria in the Bloodstream of Patients With Acquired Immunodeficiency Syndrome in a University Hospital in Brazil

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This study was done to determine the occurrence of mycobacteria in the bloodstreams of patients with fever and advanced AIDS in a Brazilian hospital. We also verified the capability of an automated method for recovering these bacteria. During a period of 19 months, 254 patients with AIDS were evaluated. Blood cultures were generally submitted in pairs and drawn separately. Blood cultures were processed by the BACTEC 460TB System (Becton Dickinson Microbiology Systems, Sparks, MD), using the Bactec 13A media (Becton Dickinson Microbiology Systems, Sparks, MD). Of the 530 vials submitted, 77 (14.5%) from 41 (16%) patients were positive. *Mycobacterium avium* complex was recovered from 45 (58.4%) of the 77 positive vials, corresponding to 22 (53.6%) patients with positive blood cultures. The average time to detect *Mycobacterium avium* complex was 15 days. *Mycobacterium tuberculosis* was recovered from 26 (33.8%) of the 77 positive vials, corresponding to 15 (36.6%) patients with positive blood cultures, with an average detection time of 24 days. Other species of mycobacteria were recovered from 6 (7.8%) of the 77 vials, corresponding to 4 (9.8%) patients. *M.avium* complex was fairly prevalent (8.7%) in severely ill patients with AIDS in our hospital. *M. tuberculosis* was also an important (6.0%) agent of systemic bacterial infections in these patients. The rapid diagnosis of mycobacteremia was possible with the implementation of this automated technology.

Key Words: Mycobacterial infection, HIV, automated diagnosis, AIDS.

Mycobacteremia is an important issue in clinical microbiology laboratories. With the AIDS epidemic, these infections have become more important, with *Mycobacterium avium* complex being the most important agent of these infections [1-3]. *M. tuberculosis* has emerged in many countries and the disseminated form of the disease has proven to be important [4]. In Brazil, little is known about the dissemination of *M. avium* and *M. tuberculosis* [5,6]. Disseminated *M. avium* complex (DMAC) disease is a common debilitating and potentially fatal infection of advanced human immunodeficiency virus (HIV) disease. Since 1987, the prevalence of this infection among such patients has steadily increased. However, with the introduction of antiretroviral and prophylactic therapies that have delayed the onset of AIDS defining events and prolonged survival, the incidence of this infection has decreased [7]. Currently, the prevalence of *M.avium* bacteremia is approximately 5% at the time of AIDS diagnosis, and it increases approximately 20% in 1 year, and up to 40% within 2 years following the diagnosis of AIDS. The incidence of DMAC infection has been described as unrelated to age, sex, race, or risk groups for HIV infection [3,7,8].

Only a few studies related to DMAC infection in developing countries have been published in the medical literature [5,6,9-12]. Studies from Mexico, Uganda and Brazil show the existence of this mycobacteria in the environment with different prevalence. This is probably

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a reflection of the difficulties in the diagnosis of the disease, the high prevalence of tuberculosis, and the shorter survival time of these patients in some developing countries, when compared with most of the developed countries.

M. tuberculosis bacteremia was rarely described until the advent of HIV infection. Recently, *M. tuberculosis* bacteremia has appeared to be relatively frequent in HIV-infected patients with variable prevalence depending on the country. Esteban, et al., observed an incidence of *M.tuberculosis* bacteremia of 2.2%, in contrast with other studies where the incidence of *M.tuberculosis* was much higher (64%) [4,13]. Even though there have been several advances in the detection of *M. tuberculosis*, little is known about *M.tuberculosis* bacteremia because the appropriate diagnostic technology is not applied in most laboratories in our country.

We conducted this study to determine the occurrence of different mycobacterial species in blood cultures of patients with advanced forms of AIDS after the introduction of automated technology for culture and identification of mycobacteria. All patients were hospitalized or were being followed up at the Outpatient AIDS Clinic – Casa da AIDS – of the Hospital das Clínicas, School of Medicine, University of São Paulo. This University Hospital is an institution with 1,200 beds and outpatient clinics, located in the city of São Paulo, Brazil.

Materials and Methods

From June, 1995, to January, 1997, a total of 530 blood specimens were obtained from 254 patients with HIV/AIDS, as part of a clinical trial to compare two drugs for the treatment of DMAC. All patients were investigated for other opportunistic diseases as well. The criteria for blood collection to investigate mycobacteremia was a previous positive serology for HIV and at least 1 of the following signs or symptoms: fever, hepatic and/or spleen enlargement, diarrhea, abdominal pain, loss of weight, lymphadenopathy, and hematological abnormalities.

Blood specimens for culture were generally submitted in pairs obtained by two separate venipunctures. Some patients had several blood samples collected on different days during the study. Up to 5mL of blood were inoculated into each of 2 Bactec 13A vials (Becton Dickinson, Sparks, MD). After inoculating the blood sample, 0.5mL of Enrichment Supplement (Becton Dickinson, Sparks, MD) containing 15% of bovine serum albumin, was added to each bottle as indicated by the manufacturer. The vials were incubated at 37°C and read on the Bactec 460TB System, twice weekly in the first two weeks and weekly thereafter for the next 8 weeks. When the growth index (GI) was ≥ 50, or there was a significant difference between 2 consecutive readings ($\Delta GI > 10$), an acid-fast stain from the sample was performed and examined for the presence of acid-fast bacilli. The time to detection was defined as the time period from collection until a positive growth index was indicated by the Bactec 460TB System. Lowenstein-Jensen (LJ) agar and Bactec 12B medium (Becton Dickinson Microbiology Systems, Sparks, MD) were inoculated with 0.1 ml of the positive Bactec 13A vial. All LJ medium was incubated during the first week with the screwcaps loose at 37°C in a 5% CO_2 incubator. After this period, the caps were tightened and LJ medium was incubated at 37°C in an air incubator and examined weekly for growth. The Bactec 12B bottles were incubated at 37°C in an air incubator and read daily after the initial 24 hours of incubation. When the GI was between 50 and 100, the NAP differentiation test (Becton Dickinson Microbiology Systems, Sparks, MD) was setup according to the manufacturer's instructions, and tested daily for 5 days. A decreased or an unchanged GI in the NAP vials indicates the presence of *M. tuberculosis* complex. Differentiation among the species of the *M. tuberculosis* complex was done by traditional biochemical tests (Nitrate, Niacin, Urease) [14]. An increased GI in the NAP vial indicates the presence of other mycobacteria species, not *M. tuberculosis* complex. These mycobacteria were identified using DNA probes for *M. avium* complex, *M. gordonae*, *M. kansasii* (Gen-Probe, San Diego, CA, U.S.) and traditional biochemical tests [14]. The

Table 1: Results of the positive blood cultures for *M. avi*um of HIV positive patients detected by the Bactec 460TB System

Patient ID	CD4 count (cells/mL)	Collection date (dd/mm/yy)	Days for detection in each vial*
4	NA	13/07/95	7, 7
8	2	01/08/95	8, 8
		15/03/96	18, -
26	1	15/09/95	10, 10
38	41	11/10/95	46, -
		29/04/96	- , -
		19/06/96	50
42	31	31/10/95	8, 8
		25/04/96	13, 13
		13/05/96	21, 21
44	NA	08/11/95	8, 8
103	NA	02/10/95	7, 37
119	14	23/02/96	-
		23/05/96	-
		10/06/96	9, 9
122	74	27/03/96	12
		23/05/96	-
		19/08/96	- , -
139	5	24/03/96	37, -
181	97	21/05/96	12, 7
182	32	22/05/96	13
184	43	30/05/96	10, 10
186	4	03/06/96	28, 49
189	NA	07/06/96	5, 5
197	12	17/06/96	6, 6
213	12	15/08/96	11
219	61	03/09/96	20, 20
222	9	10/09/96	13, 13
234	58	18/11/96	18, 21
236	13	12/11/96	9, 9
249	5	01/02/97	35, 37, -, -

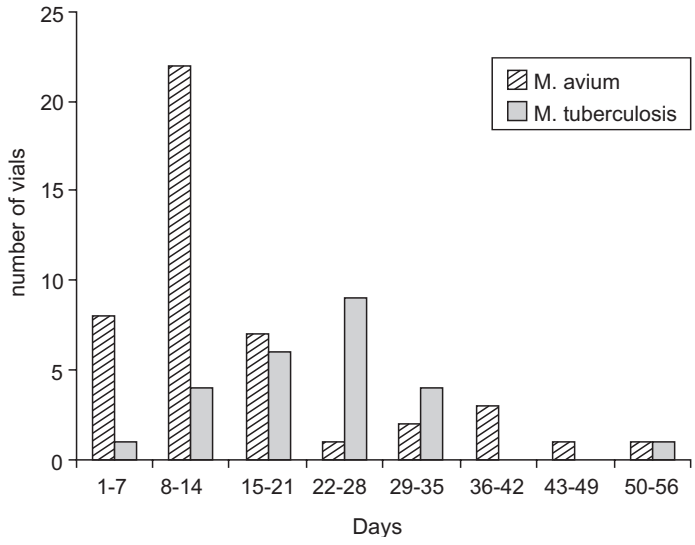
NA: not available; -: negative; *: time from collection to detection of acid-fast bacilli in each vial.

Table 2. Results of the positive blood cultures for *Mycobacterium tuberculosis* of HIV positive patients by the Bactec 460TB System

Patient ID	CD4 count (cells/mL)	Collection date (dd/mm/yy)	Days for detection in each vial*
18	14	15/08/95	21
25	NA	31/08/95	13, -, -
27	2.4	15/09/95	23, 23
29	30	19/09/95	15, 29
47	NA	26/10/95	30
		22/11/95	-
57	NA	21/12/95	20, 8
		22/12/95	12
85	NA	18/08/95	53
140	14	22/03/96	26, 26, 33, -
153	42	08/04/96	-
		05/08/96	69
155	34	09/04/96	22, 22
179	27	20/05/96	33, -
196	97	18/06/96	27, 27
206	20	25/07/96	19, 24
224	18	19/09/96	20, 20
229	NA	21/10/96	14, 14

NA: not available; -: negative; *: time from collection to detection of acid-fast bacilli in each vial.

Figure 1. Days for detection of the *M. avium* and *M. tuberculosis* in blood cultures



Bactec 12B vial used as a control for the NAP test was allowed to grow until the GI was > 700. After that, 1.0 mL of the sample was transferred to an Eppendorff tube and centrifuged at 10,000rpm for 5min. The pellet was then used with the probes mentioned above following the manufacturer's recommendation.

Results

Of the 254 patients (530 vials) studied, mycobacterial growth was recovered in 77 (14.5%) vials from 41 (16%) patients. *M. avium* was recovered in 45 (58.4%) of the positive vials corresponding to 22 (53.6%) patients and *M. tuberculosis* in 26 (33.8%) of the positive vials corresponding to 15 (36.6%) patients. *M. gordonae* was recovered from 1 (2.4%) patient in 2 vials, and *M. bovis* was recovered in only 1 vial from 1 (2.4%) patient.

The average time in days for detection of MAC was 15 days ranging from 5 to 50 days (Table 1). For *M. tuberculosis*, the average time for detection was 24 days with a range between 8 to 69 days (Table 2). Nearly half (48.4%) of the MAC positive vials became positive in the second week of incubation (8 to 14 days). Of the *M. tuberculosis* positive vials, 23% were detected in the second week of incubation and 34.6% in the third week. Only 1 vial was positive for *M. tuberculosis* after 69 days of incubation (Table 2).

M. gordonae was detected after 22 days of incubation in both vials collected from this patient. This patient presented a CD_4 count of 2 cells/ml. *M. bovis* was detected in 1 vial after 13 days of incubation. This patient had 2 additional vials submitted on different days following the first positive culture, with negative results.

Two patients showed positivity by the Bactec 460TB System after 54 (1 vial of 2 collected) and 46 (2 vials of 2 collected) days of incubation, but no mycobacteria was recovered in solid medium. A Bactec 12B vial was then inoculated with a sample of the Bactec 13A vial of each patient and supplemented with hemin. The detection of growth in this media suggested *M. haemophilum* but no further identification was done.

Twenty two (8.7%) patients, of the 254 studied, showed growth for other species of bacteria. Fungi was recovered in 3 vials of 2 patients; 1 was identified as *Histoplasma capsulatum* (after 9 days of incubation), and the other two as *Cryptococcus neoformans* (after 9 to 12 days of incubation).

Of the 530 vials collected, 448 vials from 189 patients were drawn at the same time from different venipunctures. Of the 41 patients with positive blood cultures, 35 (85%) had paired vials, and 21 (51.2%) had both vials detected at the same time. Of the 22 patients with *M. avium*, 19 (86%) had vials submitted in pairs. Of these 19 patients, 10 (52.6%) had both vials positive at the same time, five (26.3%) had positive vials on different days, and 4 (21%) had only 1 vial positive. Of the 5 patients with cultures detected on different days, the two major differences were observed in patient number 103 and patient number 186 (Table 1). Patient 103 had 1 vial detected after 7 days of incubation, and the other after 37 days of incubation. For this patient, there was no other specimen submitted before or after collection of the blood culture. In patient 186, the difference between detection time in both vials was 21 days and, as with patient 103, no other cultures were submitted.

Of the 15 patients with positive blood cultures for *M. tuberculosis*, 11 (73.3%) had vials drawn in pairs. Of these, 5 (45.4%) were detected at the same time in both vials, 3 (27.3%) patients had *M. tuberculosis* detected on different days, and 3 patients (27.3%) had it detected in only 1 of the vials collected. Of the 3 vials detected on different days, the major difference was observed in patient 29 (Table 2) where the difference between detection time was 14 days.

Of all patients with bacteremia due to *M. tuberculosis*, 8 (53.3%) had no other positive specimens in an acid-fast stain or culture, prior to the detection in the blood culture. One patient (number 27) had a positive acid-fast stain from a sputum sample that was obtained 2 days before the blood culture was collected. Four patients (26.7%) had other specimens collected after the positive blood culture, that turned out to be positive.

Eighteen patients (81.8%) with DMAC had the CD_4 cell counts available and the media was 28.5cell/mL. Ten (66.6%) patients with disseminated tuberculosis already had the CD_4 cell counts and the media was 29.8cells/mL.

Discussion

The detection of mycobacteremia in blood has been available for a long time, but the importance of this procedure was recognized only in recent years with the advance of the AIDS epidemic. The prevalence of bacteremia due to mycobacteria varies in different countries. Esteban, and co-workers, found 10.3% of positive cultures in 541 samples analyzed [4]. Stone, and co-workers, found 13% of positivity for *M. avium* in 1,047 cultures evaluated [15]. In our study, the mycobacteremia occurrence of 16% in patients with advanced AIDS, demonstrates the importance of this bacteria in the morbidity of AIDS in a university affiliated public hospital, and the need for updating diagnostic methods.

Of the 41 patients with positive blood cultures, *M.avium* was isolated in 53.6%, and *M. tuberculosis* was isolated in 36.6% of the patients. These numbers probably reflect the high prevalence of *M. tuberculosis* in developing countries.

Morrisey, and co-workers, observed a complete absence of MAC bacteremia in AIDS patients from Uganda [16]. It is not known if the BCG vaccination or the *Mycobacterium tuberculosis* infection offers protection against MAC infection in developing countries, because we do not have epidemiological data on the prevalence of MAC and other mycobacteria infections in Brazil and other developing countries [17].

There are several non-automated methods for detecting mycobacteria growth in blood specimens. However, some require long time periods for detection, which is a very significant disadvantage, particularly for AIDS patients. The automated systems present several advantages, with the most important being a decrease in the length of time for detection.

In our study, the average time for detection of *M. avium* complex was 15 days, with nearly half of the vials being detected during the second week of incubation (8 to 14 days). If we summarize this in terms of patients, *M. avium* was detected after 8 days in three (13.6%) patients, and in another three (13.6%) patients after 9 days of incubation. The average time for detection of *M. tuberculosis* was 24 days, with 9 vials detected during the third week of incubation (15 to 21 days), and 6 vials detected during the second week. In terms of patients, there was not a particular day during the second or third week of incubation with a higher incidence of positive cultures. The diagnosis of tuberculosis with a positive blood culture represents an exception in clinical practice, but this practice can increase the diagnosis of this kind of infection since the positivity of acid-fast stains in sputum samples is low in AIDS patients. The rapid diagnosis of this type of infection was made possible by the introduction of an automated method. This is not possible with the traditional methods available in most laboratories in Brazil.

The recovery of *H. capsulatum* and *Cryptococcus neoformans* in Bactec 13A vials demonstrates that these organisms must also be considered in AIDS patients.

Even though the volume of blood processed in our study was almost the same (10mL) as the volume processed in Stone and colleagues study (8.3mL), we had a lower percentage of concordant results when drawing 2 vials. Our study presented 77.2% concordant results, and Stone, et al., showed 96% concordant results. This data may lead us to think more carefully about submitting 2 vials per patient instead of 1 vial, since our percentage of concordant results was low. In our study, there were 15 patients with discordant results between the 2 vials, representing 6.0 % of all patients. These results demonstrate that, if only 1 sample was drawn from each patient, the result might be negative or there might have been a delay in detecting mycobacterial growth in 6.0% of the patients studied. More studies are necessary to determine if 1 or 2 samples should be drawn from each patient. This issue also requires careful consideration because the cost of the vials.

In 80% (12 patients) of the patients with tuberculosis included in this study, blood cultures were the only laboratory test that confirmed the diagnosis, even though other specimens were obtained before and after the blood specimen .

A multivariate analysis of risk factors for DMAC in patients with AIDS, including CD_4 count, age, sex, race, and HIV transmission category, have shown that the CD_4 cell count was the only significant factor for development of DMAC [3.] In our study, we obtained the CD_4 count in 18 of the 22 patients with DMAC, and the media was 28.5cells/mL, correlating with the literature. In patients with disseminated tuberculosis, the mean of CD_4 cell count was 29.8cells/mL, showing the possibility of *M. tuberculosis* bacteremia in very advanced forms of AIDS.

We must stress the great difficulties for the diagnosis of DMAC in most of the public laboratories in Brazil, related to the complexity of biochemical tests, and the high cost (probe tests) for the identification of MAC strains. These difficulties probably falsely result in a lower level of DMAC diagnoses in Brazil. Even though only 3 other different species of mycobacteria were isolated, *M gordonae*, *M. bovis* and *M. haemophilum*, they should be considered important pathogens in disseminated infections.

This study demonstrates that *M. avium* is a common agent of systemic bacterial infection in severely ill AIDS patients in our hospital, with a prevalence of 8.7% of the cases. *M. tuberculosis* complex is almost as common (5.9%) and must be considered, particularly in patients with advanced form of AIDS.

Only after introducing automated techniques for culture and species identification, the study of the prevalence of mycobacteremia in patients with advanced AIDS was possible.

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