Prevalence of *Mycobacterium avium* and *Mycobacterium tuberculosis* in Blood Cultures of Brazilian AIDS Patients After Introduction of Highly Active Retroviral Therapy

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The use of highly active antiretroviral therapy (HAART) for the treatment of HIV infection has been associated with a marked reduction in the incidence of most opportunistic infections. From April 2001 to February 2002, 80 blood samples from patients who were suspected to have disseminated mycobacterial infection, presenting fever and (preferably) a CD₄ T cell count ≤100.0 cell/µL were investigated. Twelve (15%) of the 80 blood cultures were positive for mycobacteria, with *Mycobacterium avium* being identified in 7 (8.8%) samples and *M. tuberculosis* in 5 (6.2%). The TCD₄⁺ count at the time of *M. avium* bacteremia ranged from 7 cells/µL (average of 48.5 cell/µL), while in *M. tuberculosis* bacteremia it ranged from 50.0 cells/µL (average of 80.0 cell/µL).

The prevalence of *M. avium* bacteremia in our study follows the expected decline in opportunistic infections observed after the introduction of HAART; however, mycobacteremia by *M. tuberculosis* still indicates a high prevalence of tuberculosis infection in AIDS patients.

**Key Words:** Bacteremia, *M. avium*, *M. tuberculosis*, AIDS.

Infections with mycobacteria are frequent in individuals with AIDS [1]. *Mycobacterium avium* and *M. tuberculosis* are both common in opportunistic infections. The introduction of highly active antiretroviral therapy (HAART), defined as a combination of antiretroviral regimens that include either a potent viral protease inhibitor or a non-nucleoside reverse transcriptase inhibitor, has dramatically changed the clinical prognosis for human immunodeficiency virus (HIV)-infected patients in terms of decreased mortality, morbidity, and need for hospitalization [2]. In the pre-HAART era, infection with *M. avium* was detected in 50% of autopsied patients [3], but only 12% of the patients with a suspicion of *M. avium* infection had positive blood cultures [4]. The detection of *M. tuberculosis* in blood samples is less frequent; its determination is only indicated for patients with severe immunosuppression or with suggestive clinical symptoms [5]. A decline in the frequency of *M. avium* and *M. tuberculosis* infections has been associated with the introduction of HAART and changes in CD₄ cell count in various studies, although these studies also analyzed various types of samples other than blood [6]. However, it is still uncertain whether after antiretroviral regimens, the prevalence of *M. tuberculosis* infection in AIDS patients would be comparable in regions with a low prevalence of latent TB infection and a low risk of transmission of *M. tuberculosis*, compared with developing countries, where co-infection of HIV with *M. tuberculosis* is very common [7]. We determined the prevalence of *M. avium* and *M. tuberculosis* mycobacteremia in AIDS patients in the Brazilian state of Paraná, where *M. tuberculosis* is still highly prevalent.
Material and Methods

Patients

From April 2001 to February 2002, 80 patients (49 men and 31 women), with a median age of 32 years (range 7 to 64 years), who were suspected to have disseminated mycobacterial infection, presenting fever, and preferably a CD4 T cell count ≤100 cells/µL, were investigated. All patients were under HAART therapy and originated from infectious disease clinics at different hospitals in Curitiba, Paraná, Brazil. The diagnosis of HIV infection was established on the basis of two positive enzyme-linked immunosorbent assays (ELISA) and confirmed by Western blot (immunoblot) analysis. The study was approved by the ethics committee of the Hospital de Clínicas, and informed consent was obtained from all patients before their inclusion in this study.

CD4 Lymphocyte counts

They were made using fluorescein-labelled murine monoclonal antibodies (Becton-Dickinson Immunotech-Caxter San Jose Calif. USA) in a flow cytometer (FACS Count™ Reagents, Becton-Dickinson-San Jose Calif. USA).

Processing of clinical samples

Five milliliters of peripheral blood was inoculated into MB/BacT® bottles (BacT/alert Microbial Detection Systems, Bio Merieux, Durham, NC, USA), 1 mL of enrichment fluid was added, and the bottles were incubated at 37°C for 42 days and continuously monitored for microbial growth (BacT/ALERT Microbial Detection Systems). Samples positive for microbial growth were screened by Ziehl-Gabbet bacilloscopy, a modified Ziehl-Neelsen method [8] and submitted to DNA extraction immediately after the system signaled positive growth. Approximately 250 µL of the blood culture fluid was inoculated onto two solid Löwenstein-Jensen slants. The slants were incubated at 37°C in ambient air for up to eight weeks. Mycobacteria were identified by classical biochemical tests [9].

PCR

All the samples were processed by PCR, which was performed as previously described [10].

Results

Twelve (15%) of the 80 blood cultures were positive for mycobacteria, with M. avium being identified in seven samples and M. tuberculosis in five. Positive blood cultures were detected by the BacT/ALERT system after a mean incubation time of 9.5 days for M. avium and of 16.7 days for M. tuberculosis. All 12 positive blood cultures were confirmed by Ziehl-Gabbet bacilloscopy and after culture on solid Löwenstein-Jensen medium using the appropriate biochemical tests. In addition, two cultures (2.5%) were positive for Cryptococcus sp. and one culture (1.25%) was positive for Candida sp.

Seventy-four patients (92.5%) presented TCD4+ ≤100.0 cells/µL and six patients (7.5%) had TCD4+ >100.0 cells/µL. Mycobacterium avium bacteremia was detected with TCD4+ ranging from 7.0 cells/mL, with an average of 48.5 cells/mL, while bacteriemia by M. tuberculosis was detected with TCD4+ counts from 50 cells/mL, and average of 80 cells/mL (Table 1).

Detection of M. avium and M. tuberculosis from positive blood cultures by PCR showed 100% agreement with the culture results, as has been previously described [10].

Discussion

We found mycobacteremia in 15% (12/80) of the AIDS patients who had clinical symptoms, such as fever and preferably CD4<100 cells/µL. It is well know that susceptibility to opportunistic infections increases as HIV-induced immunodeficiency becomes more severe.
Among patients with HIV infection, TCD4+ lymphocyte counts continue to be the best-validated predictor of opportunistic infection [2]. Before HAART was available, patients with CD4 cell counts above 200 cells/µL were found to be at low risk for the majority of AIDS-defined opportunistic infections, with an increased individual risk with decreases in the CD4 cell count [2]. Nightingale et al. observed that 39% of patients with M. avium infection presented CD4 ≤10.0 cells/µL, while the infection occurred in only 1 to 3% of patients with CD4 100 to 199 cells/µL. On the other hand, infection by M. tuberculosis seems to occur when the CD4 cell count is around 200 to 300 cells/µL [11], and the extra-pulmonary form can appear when CD4 is even lower, around 15 to 114 cells/µL [12]. In our investigation, one of the inclusion criteria was TCD4+ count ≤100 cells/µL. Mycobacteremia by M. avium was detected in patients with TCD4+ counts ranging from 7.0 cells/µL, with an average of 48.5 cells/µL (Table 1). This average does not include a patient who presented an M. avium infection with a CD4 count of 311 cells/µL. On the other hand, M. tuberculosis infection occurred in patients with TCD4+ higher than 50 cells/µL, with an average of 80 cell/µL. Our data indicates that as immunosuppression becomes more intense, the patients were more susceptible to M. avium and extra-pulmonary forms of M. tuberculosis infections.

There are only a few studies on the prevalence of mycobacteremia in AIDS patients in Brazil. Some of these reports are retrospective studies analyzing different types of clinical samples [14]. Our calculated prevalence of mycobacteria infection (15%) was higher than that observed by Aily et al. [13], who analyzed blood samples of 1,521 patients with CD4<100cells/µL from Instituto Adolfo Lutz (São Paulo) from 1994 to 1997, and found 9.9% mycobacteremia. In addition, Ramos et al. [14] in 1996 and Barreto et al. [15] from 1990 to 1992 found 22.0% and 25.6% mycobacterial infection, respectively, in patients from São Paulo. The difference in the prevalence of mycobacteremia between earlier and recent studies is probably related to the introduction of HAART in 1997. After the introduction of this therapeutic scheme, various studies have demonstrated a significant decrease (>50%) in opportunistic infections between 1992 and 1997, especially those caused by M. avium and Cytomegalovirus [2]. In addition, HAART has had a strong impact on the decline of mortality, which fell from 21.9/100 in 1994 to 3.7/100 in 1997 [16].

Table 1. Demographic profile, TCD4+ count, and mycobateria species isolated from blood cultures of AIDS patients

<table>
<thead>
<tr>
<th>Sample identification</th>
<th>Age (years)</th>
<th>Sex</th>
<th>TCD4+cells/µL</th>
<th>Mycobacterial species</th>
</tr>
</thead>
<tbody>
<tr>
<td>515</td>
<td>34</td>
<td>M</td>
<td>7</td>
<td>M. avium</td>
</tr>
<tr>
<td>583</td>
<td>46</td>
<td>F</td>
<td>25</td>
<td>M. avium</td>
</tr>
<tr>
<td>539</td>
<td>41</td>
<td>M</td>
<td>34</td>
<td>M. avium</td>
</tr>
<tr>
<td>505</td>
<td>39</td>
<td>F</td>
<td>38</td>
<td>M. avium</td>
</tr>
<tr>
<td>513</td>
<td>12</td>
<td>F</td>
<td>82</td>
<td>M. avium</td>
</tr>
<tr>
<td>582</td>
<td>31</td>
<td>M</td>
<td>112</td>
<td>M. avium</td>
</tr>
<tr>
<td>519</td>
<td>23</td>
<td>F</td>
<td>50</td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>522</td>
<td>33</td>
<td>M</td>
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<td>M. tuberculosis</td>
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<tr>
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<td>M</td>
<td>94</td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>541</td>
<td>25</td>
<td>F</td>
<td>95</td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>569</td>
<td>27</td>
<td>M</td>
<td>108</td>
<td>M. tuberculosis</td>
</tr>
</tbody>
</table>

M. = *Mycobacterium.*
Regarding the species of mycobacteria observed in our study, the prevalence of M. avium (8.8%) was similar to that observed for M. tuberculosis (6.2%). Although the prevalence of M. avium mycobacteremia was lower than those reported in previous investigations, in which it ranged from 15% to 24% [17], the rate was similar to that recently reported by Jacomo et al. [18], with only 5% of blood samples tested being positive for M. avium.

On the other hand, when we analyzed the frequencies of M. avium (7/12, 58%) and M. tuberculosis (5/12, 42%) in positive samples, they were similar to those reported by Aily et al., with 53.8% of blood samples positive for M. avium. Using various clinical samples, such as spit, blood, biopsies and cerebral-spinal fluid, Ramos et al., found 45% positivity for M. avium and 55% for M. tuberculosis. This difference in incidence is likely to be due to the type of sample used and indicates that the source of material can strongly influence the results in the detection of mycobacterial species. In another study in 1995 (pre HAART), positive mycobacteremias were more frequently due to M. avium (59.2%) than M. tuberculosis (28.6%); whereas in 1998 (HAART era), the relative frequencies were reversed (28.6 vs. 64.3% respectively [19]. While M. avium mycobacteremia has been reported by various authors, data on M. tuberculosis mycobacteremia are scarce. In industrialized countries, mycobacteremia is mainly caused by M. avium, and in developing countries, it is generally caused by M. tuberculosis [12]. In Africa, mycobacteremia by M. tuberculosis is very prevalent (28% to 46%), while bacteremia by M. avium occurs in 10% of the cases [20]. The high prevalence, and consequently the high degree of exposure of M. tuberculosis in developing countries, may be the main factor for this rate. On the other hand, M. avium is ubiquitous in nature and only causes disease in individuals with immune deficiencies, such as AIDS [17].

In the present study, the findings of M. avium and M. tuberculosis infection detected are in agreement with the decline in opportunistic infections observed after the introduction of HAART, specially due to M. avium. Bacteremia caused by M. tuberculosis was uncommon until the advent of AIDS, and most studies have been based on a small number of patients. Our data of M. tuberculosis mycobacteremia suggest that although therapy, the infection is frequent particularly in AIDS patients who are not compliant to HAART or have resistance to current antiretroviral therapy. These findings more likely are related to the high prevalence of the disease in our environment with consequently more reactivation cases. However, for continuous monitoring of the incidence of AIDS-defining events in the post-HAART era, larger studies are needed, in order to established the real reduction of mycobacterial infection in these patients.

Reference


