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 CD_4 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 TCD_4^+ count at the time of *M. avium* bacteremia ranged from 7cells/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 CD_4 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.

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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 Imunotech-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 37ºC for 42 days and continuously monitored for microbial growth (BacT/ALERT Microbial Detection Systems). Samples positive for microbial growth were screened by Ziehl-Gabbet staining, 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 37ºC 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 $TCD_4^+ \leq$ 100.0 cells/μL and six patients (7.5%) had $TCD_4 >$ 100.0 cells/μL. *Mycobaterium avium* bacteremia was detected with TCD_4^+ ranging from 7.0 cells/mL, with an average of 48.5 cells/mL, while bacteriemia by *M. tuberculosis* was detected with TCD_4^+ 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 $CD_4 <$100 cells/μL. It is well know that susceptibility to opportunistic infections increases as HIV-induced immunodeficiency becomes more severe.

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

Sample identification	Age (years)	Sex	TCD_4^+cells/μL	Mycobacterial species
515	34	M	7	*M. avium*
583	46	F	25	*M. avium*
539	41	M	34	*M. avium*
505	39	F	38	*M. avium*
513	12	F	82	*M. avium*
582	31	M	112	*M. avium*
521	40	M	311	*M. avium*
519	23	F	50	*M. tuberculosis*
522	33	M	55	*M. tuberculosis*
509	32	M	94	*M. tuberculosis*
541	25	F	95	*M. tuberculosis*
569	27	M	108	*M. tuberculosis*

M. = *Mycobacterium*.

Among patients with HIV infection, T^+CD_4 lymphocyte counts continue to be the best-validated predictor of opportunistic infection [2]. Before HAART was available, patients with CD_4 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 CD_4 cell count [2]. Nightingale et al. observed that 39% of patients with *M. avium* infection presented $CD_4 \leq 10.0$ cells/μL, while the infection occurred in only 1 to 3% of patients with CD_4 100 to 199 cells/μL. On the other hand, infection by *M. tuberculosis* seems to occur when the CD_4 cell count is around 200 to 300 cells/μL [11], and the extra-pulmonary form can appear when CD_4 is even lower, around 15 to 114 cells/μL [12]. In our investigation, one of the inclusion criteria was TCD_4^+ count ≤ 100 cells/μL. Mycobacteremia by *M. avium* was detected in patients with TCD_4^+ 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 CD_4 count of 311 cells/μL. On the other hand, *M. tuberculosis* infection occurred in patients with TCD_4^+ 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 CD_4<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].

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 enviroment 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

1. Montessori V., Philips P., MontanerJ., et. al. Species distribution in human imunodeficiency virus-related mycobacterial Infections: implications for selection of initial treatment. Clin Infec Dis **1996**;22:989-92.
2. Kovacs J.A., Masur H. Prophilaxis against opportunistic infections in patients with human immunodeficiency virus infection. N Engl J Med **2000**;346:1416-29.
3. Wallace J.M., Hannah J.B. *M. avium* complex infection in patients with the acquired immunodeficiency syndrome. A clinicopathologic study. Chest **1988**;93:926-8.
4. Nihtingale S., Byrd L.T., Southern P.M., et. al. Incidence of *M. avium-intracellulare* complex bacteremia in human immunodeficiency virus-positive patients. J Infect Dis **1992**:1082-1085.
5. Reimer L.G., Wilson M.L., Weinstein M.P. Update on detection of bacteremia and fungemia. Clin Microbiol Rev **1997**;10:444-65.
6. Ole K., Gatell M.J., Mocrofoft A., et. al. **I**nfection with m*ycobacterium tuberculosis* and *Mycobacterium avium* among HIV-infected Patients after the Introduction of Highly Active Antiretroviral Therapy. Am J Respir Crit Care Med **2000**;162:865-72.
7. Lopes-Santoro G., Pinho A.M.F., Harrison L.H. Schecter M. Reduced risk of tuberculosis among Brazilian patients with advanced human immunodeficiency virus infection treated with highly active antiretroviral therapy. Clinical Infectious Disease **2002**;34:543-6.
8. Costa H.C., Souza L.C., Martini J.P., et. al**.** Comparative study of various staining methods for mycobacteria. Hansenol Int. **1988**;3:37-41.

9. Metchock B.G., Nolte F.S., Wallace R.J. *Mycobacterium*, p.399-439, In: P. R. Murray, E. J. Baron, M. A. Pfaller,F.C Tenover, and R.H. Yolken(ed.), Manual of clinical microbiology, 7th ed. Washington, D.C: American Society for Microbiology, **1999**.
10. Nakatani S.M., Burger M., Messias-Reason I.J.T., et. al. Efficiente method for mycobacterial DNA extraction in blood cultures aids rapid identification of M. tuberculosis and M. avium. Euro J Clin Microbiol Infect Dis **2004**;23:851-4.
11. Cock K.M. Research issues involving HIV-associated tuberculosis in resource poor countries. JAMA **1996**;276:1502-7.
12. Reyn C.F. The significance of bacteremic tuberculosis among persons with HIV infection in developing countries. AIDS **1999**;13:2193-5.
13. Aily D.C., Camargo S.S., Paro H.S., et al. Systematic mycobacteriosis in AIDS patients as determined by blood cultures on biphasic medium. Rev Argent Microbiol **1999**;31:53-7.
14. Ramos M.C., Vilares M.C.B., Moraes M.J., et al. A retrospective bacteriological study of mycobacterial infections in patients with acquired immune deficiency syndrome (AIDS). Brazilian J Infec Dis **2000**;2:86-90.
15. Barreto J.A., Palaci M., Ferrazoli L., et al. Isolation of Mycobacterium avium complex from bone marrow aspirates of AIDS patients in Brazil. J Infect Dis **1993**;168:777-9.
16. Palella F.J. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med **1998**;338:853-60.
17. Horsburgh C.R.*M. avium* complex infection in the acquired immunodeficiency syndrome. N Engl J Med **1991**;324:1332-8.
18. Jacomo V., Musso D., Gevaudan M.J., Drancourt M. Isolation of blood-borne *M. avium* by using the nonradioactive Bactec 9000 MB System and comparison with a solid- culture system. J Clin Microbiol **1998**;36:3703-6.
19. Hadad DJ., Palaci M., Pignatari A.C., et. al. Mycobacteraemia among HIV-1-infected patients in São Paulo, Brazil:1995 to 1998. Epidemiolo Infect **2004**;132:151-5.
20. Gotteberg A., Sacks L., Mahala S., et. al. Utility of blood cultures and incidence of micobacteremy in patients with suspected tuberculosis in South African infectious disease referral hospital. Int J Tuberc Lung Dis **2000**;5:80-6.