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SHORT REPORT
Mycobacteraemia among HIV-1-infected patients in São Paulo, Brazil: 1995 to 1998

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SUMMARY

From July 1995 to August 1998, mycobacterial blood cultures were obtained from 1032 HIV-infected patients seen at the Centro de Referência e Treinamento de AIDS (CRTA), Hospital São Paulo (HSP), and Centro de Referência de AIDS de Santos (CRAS). Overall, 179 episodes of mycobacteraemia were detected: 111 (62·0%) at CRTA, 50 (27·9%) at HSP, and 18 (10·1%) at CRAS. The frequency of positive cultures declined sharply from 22·6% in 1995 to 6·9% in 1998, consistent with the decrease in opportunistic infections following the publicly funded distribution of highly active antiretroviral therapy. In 1995, mycobacteraemia was more frequently due to *Mycobacterium avium* complex (59·2%) than *Mycobacterium tuberculosis* (28·6%), whereas in 1998 the relative frequencies were reversed (28·6 *vs.* 64·3% respectively), probably justified by the increased virulence of *M. tuberculosis* and the greater risk of invasive infection in less-immunocompromised patients, including patients unaware they are infected with HIV.

In Brazil, the total number of cases of tuberculosis infection diagnosed in 1998 was 82 931, which corresponded to an incidence rate of 51·3/100 000 people. In São Paulo State, 21 356 cases were notified in 1999 [1]. The incidence of non-tuberculous mycobacterial disease among the general population is not known. Disseminated *Mycobacterium avium* complex (MAC) disease among HIV-infected patients who attended an AIDS reference centre in São Paulo, Brazil was reported in 23 (18·4%) out of 125 patients with persistent fever, anaemia and leucopaenia [2].

Bacteraemia due to *Mycobacterium tuberculosis* (MTB) or MAC was rarely documented prior to the HIV pandemic. However, the importance of disseminated mycobacterial infection in HIV-infected persons was rapidly appreciated [3, 4], particularly in areas with higher endemic rates of tuberculosis, such as in Latin America. Early reports from Brazil were based on non-automated culture methods, which were infrequently utilized due to limited laboratory resources. From January 1989 to February 1991, 26 cases of mycobacteraemia (18 due to MAC and 8 due to MTB) were diagnosed among 122 selected HIV-infected patients at Instituto de Infectologia Emílio Ribas and Centro de Referência e Treinamento de AIDS (CRTA) in São Paulo City (SP) using a biphasic system [Middlebrook 7H9/Löwenstein–Jensen (7H9/LJ)] [5]. From November 1992 to July 1995, 30 cases (11 MAC and 19 MTB) were documented among 50 selected AIDS patients in Rio de

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Table. *Number and frequency of cultures yielding for mycobacteria among blood cultures of HIV-1-infected patients submitted by three treatment centres in São Paulo state from 1995 to 1998*

	Number of positive cultures/total number submitted (%)			
Centre	1995	1996	1997	1998
CRTA	29/147 (19·7%)	58/299 (19·4%)	16/113 (14·2%)	8/143 (5·6%)
HSP	17/66 (25·8%)	17/93 (18·3%)	10/69 (14·5%)	6/61 (9·8%)
CRAS	3/4 (75·0%)	10/31 (32·3%)	5/14 (35·7%)	0/0
Total	49/217 (22·6%)	85/423 (20·1%)	31/196 (15·8%)	14/204 (6·9%)

CRTA, Centro de Referência e Treinamento AIDS (São Paulo); HSP, Hospital São Paulo; CRAS, Centro de Referência de AIDS de Santos.

Janeiro using Middlebrook 7H9 broth media and LJ slants [6].

In July 1995, the Instituto Adolfo Lutz (IAL) in SP implemented an automated system for culturing mycobacteria from blood which allowed the assessment of substantially larger numbers of HIV-infected patients in the surrounding area. This report describes the results of >1000 mycobacterial blood cultures performed from July 1995 to August 1998, with particular emphasis on the frequencies of mycobacterial species isolated over that period.

Since July 1995, a BACTEC 460 TB (Becton Dickinson Instrument Systems, Sparks, MD, USA) has been available in the Mycobacteria Laboratory at IAL. Blood cultures from HIV-1-infected patients have been submitted primarily from the CRTA and the Hospital São Paulo (HSP), both located in SP, and the Centro de Referência de AIDS de Santos, located in Santos city (CRAS). Blood samples were collected from any HIV-infected patient, with opportunistic infections, a total lymphocyte count $<2000/mm^3$ or a CD4 lymphocyte count $<100/mm^3$, indicative of advanced AIDS disease [7, 8], who reported more than 2 weeks of fever. Specimens were submitted from both ambulatory and hospitalized patients seen at CRTA and HSP, but only from ambulatory patients at CRAS.

At each site, 5 ml of blood were inoculated into 30 ml vials of BACTEC 13A medium. The vials were transported to IAL, typically within 24 h, supplemented with 0·5 ml of enrichment fluid according to the manufacturer's instructions, and incubated at 36 °C for up to 12 weeks. Isolates were identified as MTB based upon presence of cording and reactivity in the NAP (*p*-nitro-α-acetylamino-β-hydroxypropiophenone) test, and as MAC or other species based on biochemical tests and growth on specific media [9–11]. An episode of mycobacteraemia was defined as the isolation of mycobacterium species, or complex, from blood collected from an individual patient.

Overall, among 1032 patients cultured, there were 179 (17·3%) episodes of mycobacteraemia. There were two patients who each had two episodes of mycobacteraemia. CRTA consistently cultured 2–3 times as many patients as HSP; however, the overall frequency of positive cultures was comparable at the two sites (Table). The number of cultures submitted from CRAS was relatively modest and the frequency of positives somewhat higher, suggesting the technique was applied more selectively.

Overall, there was a dramatic decrease in the frequency of mycobacteraemia over the course of the study period, with rates of over 20% during 1995 and 1996, dropping to below 7% in 1998 (Table). In addition, among the positive cultures, there was a dramatic shift in the relative frequency of the particular species isolated (Fig.). At the beginning of the study period, almost 60% of the isolates were MAC and <30% were MTB. By the end of the study period, the ratio was reversed, with MTB representing 64% of the isolates and MAC only 28·6%.

Cell-mediated immunity, which is essential for host resistance against mycobacteria and other intracellular pathogens, is directly impaired as a consequence of viral replication during HIV infection. In areas with a low incidence of tuberculosis, e.g. the United States and the countries of Western Europe, disseminated infection and mycobacteraemia due to MAC emerged as the most common invasive bacterial infection in patients with advanced HIV infection [12, 13]. In sub-Saharan Africa and Latin America, HIV-infected patients more typically developed

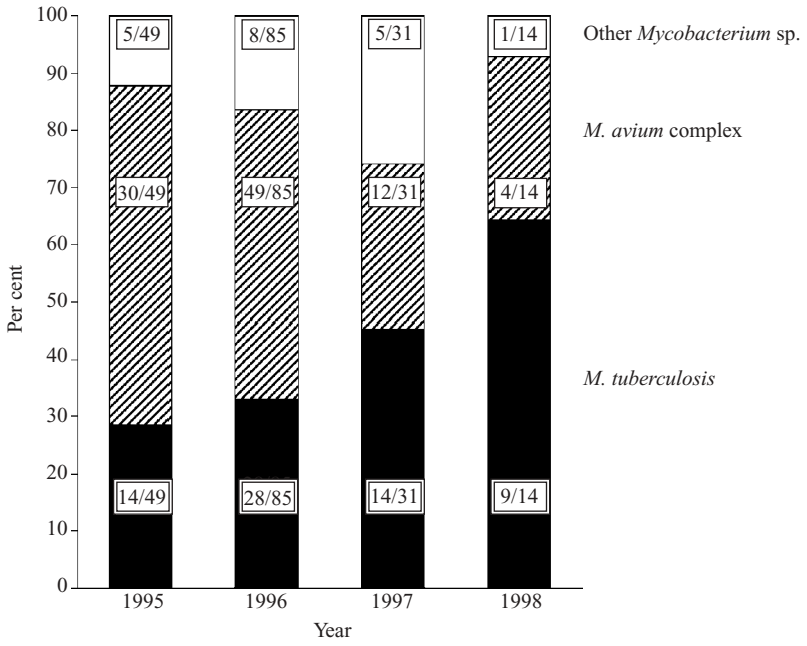

Fig. Distribution of different species of mycobacteria isolated from blood of HIV-1-infected patients in São Paulo, Brazil, from 1995 to 1998. The number of positive cultures was 49 (MTBC 14; MAC 30; *Mycobacterium* sp. 5) in 1995; 85 (MTBC 28; MAC 49; *Mycobacterium* sp. 8) in 1996; 31 (MTBC 14; MAC 12; *Mycobacterium* sp. 5) in 1997; and 14 (MTBC 9; MAC 4; *Mycobacterium* sp. 1) in 1998.

rapidly progressive tuberculosis, including both pulmonary disease and disseminated infection with mycobacteraemia [6, 13].

Several epidemiological studies of HIV-infected patients in multiple parts of the world have now demonstrated that the relative risk of disease with MTB *vs.* MAC is directly related to the risk of exposure to MTB and degree of residual immunocompetence as reflected in CD4 counts [13, 14]. At CD4 counts of $>100/\mu$l, there is little risk of disseminated MAC, whereas, because MTB is appreciably more virulent [15, 16], patients exposed to MTB or carrying latent infection are at a greatly increased risk of progressive primary infection or reactivation respectively [17]. If patients are not at risk from exogenous or endogenous MTB and progress to levels of severe immunosuppression (typically CD4 $<25/\mu$l), their risk of MAC increases dramatically, regardless of their location, consistent with evidence that MAC are ubiquitous in the environment [13, 14].

The BACTEC 460 TB combined with vials containing 30 ml of 13A medium and supplemented with enrichment fluid is a highly sensitive and selective automated culture system for detecting mycobacteraemia [4, 12]. When applied to large numbers of patients with AIDS in São Paulo, Brazil in 1995 and 1996, this system permitted detection of significant numbers of patients with disseminated MAC. Consistent with the high endemic levels of tuberculosis, appreciable numbers of patients with mycobacteraemia due to MTB were also detected.

Over the next 3 years, the absolute frequency of positive blood cultures for mycobacteria declined significantly. This was despite continued use of the same culture system, with submission of cultures from the same clinical settings and patient populations. While detailed clinical and epidemiological data regarding all the patients cultured are not available, a substantial portion of the cultures were submitted by the authors of this paper and their colleagues, and there was no change in their clinical practice.

One possible explanation for the observed decrease in mycobacteraemia is the change in treatment available to the typical HIV-infected patient seen at these clinics. In December 1996, the National Programme of Sexually Transmissible Diseases and AIDS (Brazilian Ministry of Health) began to provide highly active antiretroviral therapy (HAART), including the protease inhibitors, at no cost to the patient [18]. This treatment regimen commonly results in marked and sustained improvements in CD4 levels, even in patients who have progressed to counts as

low as $<50/\mu$l. Several reports have documented that such changes are associated with a dramatic reduction in morbidity and mortality among HIV-infected patients [19–21].

The increased availability of effective antiretroviral therapy may also explain the decrease in the fraction of mycobacteraemia due to MAC and the corresponding increase in MTB. Advanced AIDS patients receiving HAART are less likely to be infected with MAC due to the rapid increase of CD4 count levels. As noted above, MTB is appreciably more virulent than MAC and the risks of both reactivation and progressive primary infection are significantly increased at relatively modest levels of immunosuppression [17]. Further, HIV-infected patients may reach such levels of immunosuppression without any significant previous illness. Consequently, among HIV-1-infected persons in São Paulo, disseminated MTB may represent their first major opportunistic infection. Many such patients have not sought antiretroviral therapy or are not even aware they have HIV infection [3, 19].

In conclusion, the analysis of the results of blood cultures for mycobacteria among HIV-1-infected patients in São Paulo, Brazil demonstrates several important features. Only a few years ago, episodes of mycobacteraemia due to MAC were relatively common and appreciably more frequent than those due to MTB. By 1998, however, the frequency of positive blood cultures for mycobacteria had decreased, probably reflecting the nearly universal availability of new potent antiretroviral drugs. However, among the residual cases, more than 60% are now due to MTB. These, as well as other observations, highlight that in Brazil and elsewhere co-infection with HIV and MTB continue to have broad public health implications [22]. In areas with high rates of endemic tuberculosis, programmes to make antiretroviral therapy more widely available need to be paralleled by programmes directed at the control of tuberculosis.

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