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Research article

Frequency of tuberculous and non-tuberculous mycobacteria in HIV infected patients from Bogota, Colombia

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Abstract

Background: The prevalence of infections by *Mycobacterium tuberculosis* and non-tuberculous *Mycobacterium* species in the HIV-infected patient population in Colombia was uncertain despite some pilot studies. We determined the frequency of isolation of *Mycobacterium tuberculosis* and of non-tuberculous *Mycobacterium* species in diverse body fluids of HIV-infected patients in Bogota, Colombia.

Methods: Patients who attended the three major HIV/AIDS healthcare centres in Bogota were prospectively studied over a six month period. A total of 286 patients were enrolled, 20% of them were hospitalized at some point during the study. Sixty four percent (64%) were classified as stage C, 25% as stage B, and 11% as stage A (CDC staging system, 1993). A total of 1,622 clinical samples (mostly paired samples of blood, sputum, stool, and urine) were processed for acid-fast bacilli (AFB) stain and culture.

Results: Overall 43 of 1,622 cultures (2.6%) were positive for mycobacteria. Twenty-two sputum samples were positive. Four patients were diagnosed with *M. tuberculosis* (1.4%). All isolates of *M. tuberculosis* were sensitive to common anti-tuberculous drugs. *M. avium* was isolated in thirteen patients (4.5%), but only in three of them the cultures originated from blood. The other isolates were obtained from stool, urine or sputum samples. In three cases, direct AFB smears of blood were positive. Two patients presented simultaneously with *M. tuberculosis* and *M. avium*.

Conclusions: Non-tuberculous *Mycobacterium* infections are frequent in HIV infected patients in Bogota. The diagnostic sensitivity for infection with tuberculous and non-tuberculous mycobacteria can be increased when diverse body fluids are processed from each patient.

Background

Mycobacterium infections are frequent opportunistic pathogens associated with the acquired immunodeficiency syndrome (AIDS). Its relative virulence and potential for person-to-person transmission distinguishes *Mycobacterium tuberculosis*. Persons infected with the human immunodeficiency virus (HIV) are particularly susceptible to tuberculosis, either by the reactivation of latent infection or by a primary infection with rapid progression to active disease [1–4]. The annual incidence rate of tuberculosis in Colombia during 1998 was 19.6 per 100,000 persons [5], but rates 1,000-fold higher have been reported in some HIV-seropositive populations [6–14]. In addition, disseminated infections with *Mycobacterium avium* complex are increasingly common in advanced human immunodeficiency virus (HIV) disease and cause substantial morbidity [15,16]. Persons with HIV infection and CD4 lymphocyte counts less than 100 cells/mm^3 have a probability of 10% to 20% per year of developing *M. avium* complex disease or bacteremia [17,18]. Bacteremia involving *M. avium* complex produces a wide array of clinical signs and symptoms, including wasting, fever, and night sweats, and is associated with decreased survival [17–20]. The recovery of mycobacteria in blood cultures can help to discover bacteremia that frequently goes unrecognized. We undertook a prospective survey in three AIDS health care units in Bogota, Colombia, in order to determine the prevalence of *Mycobacterium* species in HIV-infected patients and to evaluate the diagnostic value of different types of body fluids to detect the presence of mycobacteria.

Methods

Patients and study design

This study was a multicentric prospective study of the frequency of *Mycobacterium* infections in persons infected with HIV. The study was conducted in three major AIDS health care programs of Bogota ("Hospital Universitario San Juan de Dios", "Hospital Regional Simon Bolívar" and "Centro Atención Básica Chapinero del Seguro Social"). From October 1999 to March 2000, a total of 289 HIV-seropositive patients were recruited. Most of the participants met the definition of AIDS, or were in clinical stage C (CDC, 1993). The institutional review board at each site approved the study, and each participant gave written informed consent.

Specimen collection and culture media

For all patients blood, urine, stool and sputum samples were collected. In brief, 5 to 8 mL of blood was obtained in previously labeled Vacutainer™ tubes containing 1.7 mL of heparin as anticoagulant. All patients attempts were made to collect two paired blood cultures samples (n = 648), 2 urine cultures (n = 497), 2 stool cultures (n = 374) and 2 sputum samples (n = 101). In one patient a sample of ascitic fluid, and in other case cerebrospinal fluid (CSF) were obtained. Bacteriologic methods were used as previously described [21,22]. Briefly, blood samples were centrifuged 15 min at 2,000 g and leukocytes recovered by standard methods with Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) and then mixed with a lysing solution that contained 0.45% sodium deoxycholate. After manual mixing, tubes were centrifuged at 3,500 g for 30 minutes in a refrigerated centrifuge at 4,000 g and the supernatant was discarded. The pellet was resuspended, and 0.2% bovine albumin was added to yield a final volume of 2.5 mL.

One gr of stool samples was placed in 5 ml of albumin Dubos media and mixed in Vortex™ for 2 min, then incubated at room temperature overnight. Afterwards, equal volumes of a solution of 2% NaOH were mixed in Vortex™ for 2 min and incubated at room temperature in continuos mixing for 15 min. Distilled water was added to complete 50 ml and the mixture was centrifuged at 6,000 g for 30 min. One drop of phenol red was added to the pellet and then neutralized with 2 drops of HCl 2 N. The pellet was resuspended in 2 ml of saline solution, mixed and finally 0.2 ml used for culture.

Urine samples were mixed in a ratio of 3:1 with a solution of 20% $Na_3PO_4.12H_2O$. The mixture was incubated at room temperature for 2 h and then centrifuged at 6,000 g for 30 min. The pellet was used for culture after being decontaminated by Ogawa-Kudoh method. Briefly, sterile slabs were placed in a solution of 4% NaOH during 1 or 2 min and then used to culture samples.

Sputum was mixed in equal volume with a solution of 4% $Na_3PO_4.12H_2O$, incubated at room temperature during 2 h and centrifuged at 6,000 g for 30 min. The pellet was decontaminated by the Ogawa-Kudoh method, as described above, and used for culture. The same procedure was used for the ascitic fluid and CSF samples.

All samples were placed in 2 tubes with a biphasic medium: a solid phase Ogawa-Kudoh with iron citrate and a liquid phase of Sauton Twen albumin modified medium and 2 tubes with Stonebrink-Giraldo medium. Readings were made after day 6 and at 2, 4, 6, 8, 12 and 16 week. A positive culture was identified following the criteria of the Centers for Prevention and Disease Control (CDC) [22]. Resistance assay was applied at all *Mycobacterium tuberculosis* isolates using the proportion method for streptomycin (S), isoniazide (H), thioacetazone (Tb), ethambutol (E) and rifampicin (R).

All samples were stained and examined for AFB after centrifugation.

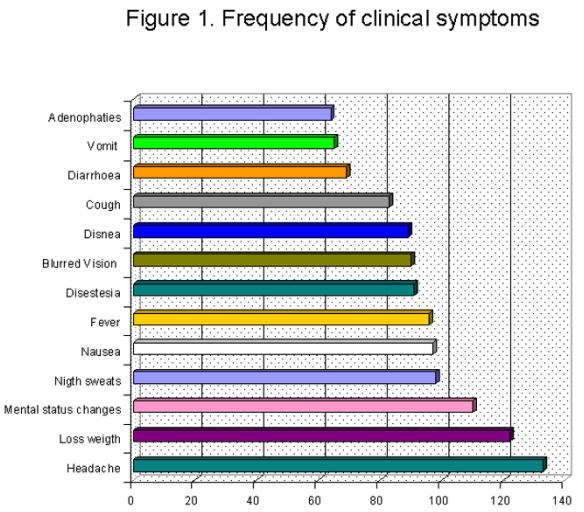

Figure 1
Frequency of clinical symptoms

Statistical analysis

Statistical significance for comparison of rates was determined by an exact test when sample sizes were insufficient and by an asymptotic test when sizes were sufficient. All tests were two-sided, and a P value of 0.05 was considered significant. Exact 95% confidence intervals (CI) were calculated for rates by assuming the numerator to be a Poisson variable [23] and for rate ratios using a modified binomial model [24]. Adjusted rate ratios for comparisons among groups defined by demographic variables were calculated by using a Mantel-Haenszel type estimator for incidence-rate data with approximate 95% confidence limits based on the tests [23].

Results

Demographic characteristics

In total, 286 patients were studied, 251 (87.8 %) were men, and 35 (12.2%) were women. The men:women ratio was 7:1. The age range was 3 to 78 years with a median of 35 years (Table 1). Ten patients (3%) were from "Hospital San Juan de Dios"; one-hundred thirty nine patients (49%) were from "Hospital Simón Bolívar" and one hundred thirty seven patients (48%) were from the "Instituto de Seguro Social". Fifty-eight patients were hospitalized at the time of enrollment (20%), and two hundred twenty eight patients (80%) were enrolled at an outpatient clinic. One hundred ninety five patients (68.5%) were in treatment with antiretroviral medication. Sexual preferences were informed in 269 (94%) patients as follows: in males, ninety (33.4%) were homosexual, eighty-six (31.9%) were bisexual and sixty-one (22.7%) were heterosexual. Of the women, twenty-nine (90.6%) were heterosexual and three (9.4%) were bisexual. CDC staging in these patients is shown in Table 1. Viral load was obtained in 180 patients and results varied in a range between less than 50 and 13 millions copies/mL. The median of viral load was 23,420 copies/mL.

The symptoms most frequently complaint of at the time of initial enrollment were headaches (48%), loss of weight (43%), and mental status changes (38%). The frequency of each symptom is showed in Figure 1. 29% patients complaint of respiratory symptoms. 13% patients had a history of diagnosis of tuberculosis, and 12% gave a history of previous treatment with antituberculous drugs.

Table 1: Demographic characteristics of patients

Distribution by center	n	%
Hospital San Juan de Dios	**10**	
Male	**9**	**90**
Women	**1**	**10**
Hospital Simon Bolívar	**139**	
Male	**128**	**95**
Women	**21**	**15**
Seguro Social	**137**	
Male	**124**	**90**
Women	**13**	**10**
Age groups		
0 – 15	**1**	**0.35**
16 – 25	**38**	**13.3**
26 – 45	**211**	**73.8**
46 – 65	**35**	**12.2**
66 – 78	**1**	**0.35**
1993 CDC stages		
A	**31**	**11**
B	**69**	**24**
C	**186**	**65**

Mycobacterium infection

Overall, sixteen of 286 patients (5.6%) were positive for any Mycobacterium species by acid fast staining or culture of any of their samples. In these sixteen cases, fifteen (94%) were positive by culture, and one patient was detected only by acid fast bacilli staining (6%). Fourteen patients (87.5%) were men and two (12.5%) were women. M. tuberculosis was identified in four cases (1.4%). M. avium was isolated in thirteen patients (4.2%). Two patients presented simultaneous infection by M. tuberculosis and M. avium.

Tuberculosis was diagnosed in its pulmonary form in two cases, and in another two patients as extrapulmonary. In the patients with pulmonary tuberculosis, the AFB smear of the sputum was positive in one case, and in another

Table 2: Staining and culture of *M. tuberculosis*

Patient	Clinical form	Type of Sample	Number of Samples	AFB SMEAR POSITIVE	CULTURE POSITIVE
1	Disseminated	Sputum	2	2	2
		Urine	2	0	2
		Ascitic fluid	1	1	1
2*	Pulmonary	Sputum	2	2	2
		Stool	2	2	2
3	Pulmonary	Sputum	2	0	2
		Stool	2	1	2
4	Gastrointestinal	Stool	2	2	2
		TOTAL	15	10	15

2* This patient also presented positive culture for *M. avium*

Table 3: Staining and culture of *M. avium* in blood samples

Patient	Sample	Number of Samples	AFB smear		Culture	
			Positive	Negative	Positive	Negative
1	blood	2	1	1	2	0
2	blood	2	1	1	2	0
3	blood	2	1	1	2	0
3*	blood	2	0	2	2	0
	urine	2	1	1	2	0
	stool	2	2	0	2	0
	TOTAL	**12**	**6**	**6**	**12**	**0**

3* Sample after one month of follow. This patient had past history of tuberculosis

case the AFB smear was positive in his stool sample. Culture of stool samples was positive in both cases of pulmonary and in one case of extrapulmonary tuberculosis. *M. tuberculosis* was detected in different samples of body fluids as well: in sputum, urine, stool samples, and in ascitic fluid (Table 2). All isolates of *M. tuberculosis* were sensitive to the drugs assayed: streptomycin (S), isoniazide (H), thioacetazone (Th), ethambutol (E) and rifampicin (R).

The only species identified as non-tuberculous mycobacteria was *M. avium* which was detected in 13 patients (4.2%). Two patients had coinfection by *M. tuberculosis* and *M. avium* in their sputum culture. One patient was positive only for acid fast bacilli staining in two stool samples but no growth was observed in culture tubes after 16 weeks of incubation. Interestingly, in three patients the direct AFB staining in blood was positive, and *M. avium* was later identified in these blood cultures (Table 3). *M. avium* was also detected in other clinical samples: urine, stool and sputum (Table 4).

The percent of positive AFB smears and cultures was greater in samples of sputum followed by the samples of stool (Table 5). Samples of urine and stool were valuable to identify most of patients with a *M. avium* infection, only three patients infected by *M. avium* were identified solely by blood cultures (Table 6 and 7).

Association between Mycobacterium avium infection and clinical data

The following factors (Table 6) were significantly associated with a culture positive for *M. avium* infections: death during time of follow up (odds ratio [OR], 9.7 [95% CI, 2.8–32]), loss of weight (OR, 8 [95% CI, 1.6–54]), and diarrhea (OR, 3.9 [95% CI, 1.1–14]). The mean time of survival during the study was significantly reduced in patients infected with *Mycobacterium avium* compared to the ones not infected (154 ± 44 versus 170 ± 37 days, p = 0.01). There were no statistically significant differences in age, sex, clinical stage, CD4 cell count, viral load, use of antiretrovirals drugs or time of diagnosis since HIV infection between patients with or without myco-

Table 4: Staining and culture of *M. avium* in clinical samples other than blood

			AFB Smear		Culture	
PATIENT	**SAMPLE**	**Number of Samples**	**POS**	**NEG**	**POS**	**NEG**
1	Stool	2	0	2	1	1
2	Stool	2	0	2	1	1
3	Stool	2	2	0	2	0
4	Urine	2	0	2	2	0
5	Urine	2	0	2	2	0
6	Urine	2	0	2	1	1
7	Urine	2	0	2	2	0
8*	Sputum	2	2	0	2	0
9*	Sputum	2	2	0	1	0
	TOTAL	**18**	**6**	**12**	**14**	**3**

8* Patient with past history of TB 9* Patient with simultaneous finding of *M. tuberculosis*

Table 5: Positivity for *Mycobacterium* infection and type of sample (n = 1622)

SAMPLE	**CULTURE**				**AFB Smear**			
	POSITIVE		**NEGATIVE**		**POSITIVE**		**NEGATIVE**	
	N	%	n	%	n	%	n	%
Blood	8	1.2	640	98.8	3	0.5	645	99.5
Stool	12	3.2	362	96.8	11	3	363	97
Sputum	8	8	93	92	6	6	93	94
CSF	0	0	1	100	0	0	1	100
Ascitic fluid	1	100	0	0	1	100	0	0
TOTAL	**40**	**2.5**	**1582**	**97.5**	**22**	**1.3**	**1600**	**98.7**

Table 6: Clinical findings associated with *Mycobacterium avium* infection

Clinical findings	**Odds Risk**	**CI 95%**	**p**
Death*	9.7	(2.8 – 32)	**0.0005**
Loss of weight*	8.0	(1.6 – 54)	**0.004**
Diarrhea*	4.5	(1.1 – 14)	**0.017**
Hemoptysis	3.6	(0 – 16)	0.1
Cough	2.1	(0.6 – 6.9)	0.2
Hospitalization	2.5	(0.7 – 8.3)	0.1
History of past TB	2.1	(0.4 – 7.9)	0.2
Dyspnea	1.9	(0.5 – 6.8)	0.2
Fever	1.7	(0.5 – 5.5)	0.3
Lymphadenopaties	1.0	(0.2 – 5.7)	1.0

* Statistically significant associations $p < 0.05$

bacterial infection (Table 7). However, there were no patients with *M. avium* infections in clinical stage A or with a CD4 cell count higher than 500 cells/mm3 (Tables 8 and 9).

Discussion

Our study was a prospective study in HIV infected patients in whom an indiscriminate search *Mycobacterium* infection was done. Clinical suspicion of tuberculosis existed previous to enrollment only in the two patients with pulmonary *Mycobacterium tuberculosis* infection but not in the other two patients with extrapulmonary tuberculosis.

Most of *Mycobacterium* species isolated were *M. avium*

The frequency that we found was lower than that of previously reported studies for *Mycobacterium* in HIV in-

Table 7: Demographic and clinical characteristics in patients with and without *Mycobacterium avium* infection

	Patients with *M. avium* infection		1st and 3rd quartile	Patients without *M. avium* infection		1st and 3rd quartile	p
	N (patients with data)	Median or percent		N (patients with data)	Median or percent		
Age	13	33	[23–39]	273	35	[29–40]	0.2
Sex male	13	92%		273	88%		0.5
CD4 cell counts	13	115	[30–223]	219	164	[60–325]	0.2
Viral load	8	52,000	[5,967–227,941]	172	23,000	[746–147,131]	0.5
Use of antiretrovirals	13	61.5%		272	68.7%		0.8
Time with antiretroviral treatment (months)	10	29	[18–60]	175	22	[12–32]	0.2
Time with diagnosis of VIH infection (months)	10	18.5	[15–28]	175	27	[13–50]	0.2

Table 8: Clinical stage of HIV infection and positivity for *Mycobacterium* infection

CLINICAL STAGE	POSITIVE	TOTAL
A	0 (0%)	0/31 (0%)
B	3 (19%)	3/69 (4.4%)
C	13 (81%)	13/186 (7%)
TOTAL	**16 (100%)**	**16/286 (5.6%)**

Table 9: Relation between CD4 cell count and infection with *Mycobacterium*

CD4+ cells	POSITIVES	TOTAL
(> 500/mm^3)	0 (0%)	0/24 (0%)
(200–499/mm^3)	6 (37%)	6/83 (7.2%)
(< 200/mm^3)	8 (50%)	8/126 (6.3%)
ND	2 (13%)	2/53 (3.7%)
TOTAL	**16 (100%)**	**16/262 (6.1%)**

fected patients in Colombia. A previous study at the "Hospital San Juan de Dios" in 1996 performed in 92 HIV infected patients found a prevalence of 8% for *M. tuberculosis* infection [25]. In another study in 101 patients in Cali, Colombia, the prevalence was only 2% [26]. In the present study it was 1.4%.

The prevalence of non-tuberculous *Mycobacterium* species in Cali was 5% [27], and in the current study it was 4%. The lower prevalence of *M. tuberculosis*, can be related to the introduction of highly active antiretroviral therapy (HAART) that was practically absent in the previous study in Bogota in 1996 but that was being used to treat 68% of the patients in the current study. Our results are also different to the ones reported in a General Hospital, AIDS reference center, in Rio de Janeiro, Brazil [28] where mycobacteria were recovered from 20.6% (313 of 1,517) of all patients, and *M. tuberculosis* was identified in 94.2% (295/313), and non-tuberculous mycobacteria in 5.8% (18/313). Our results suggest that when antiretroviral therapy is introduced the relative frequency of *M. tuberculosis* infections is reduced and the one of *M. avium* infections increases [28].

We found that *Mycobacterium avium* infection was related to symptomatic diarrhea and greater mortality as has been reported previously [29]. Our study shows that frequency of isolation of *M. avium* was greater in urine and stool samples. This emphasizes the need to take multiple body fluid samples in order to enhance the opportunity of isolation increasing the microbiologic diagnostic sensitivity. Interestingly, we found that blood AFB smears can be useful to rapidly confirm a clinical diagnosis of disseminated *Mycobacterium avium* infection.

In the present study there were no resistant strains of *M. tuberculosis* found. In a previous survey in Colombia it was found that *M. tuberculosis* isolates among new cases in patients without HIV infection showed resistance in 9.5% (4 of 42) [30].

Conclusions

Our study found that 5% of HIV infected patients in Bogota were infected with *Mycobacteria* and that *M. Avium* was the predominant species. It becomes evident the need to improve the preventive measures and prompt treatment of this type of opportunistic infection in the HIV infected individuals.

Competing interests

None declared

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