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Underestimation of *Mycobacterium tuberculosis* infection in HIV-infected subjects using reactivity to tuberculin and anergy panel

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Background This study aimed to evaluate purified protein derivative (PPD) reactivity and its interrelationship with anergy panel and CD4+ lymphocytes in HIV-infected subjects as compared to PPD reactivity in HIV-uninfected individuals in a tuberculosis endemic and high Bacillus Calmette-Guérin (BCG) coverage environment.

Methods Clients of four Mexico City HIV detection centres were screened for HIV-1 antibodies (ELISA or haemagglutination, Western Blot); reactivity to PPD (Mantoux PPD, 5TU RT-23), Candida (1:1000, 0.1 ml), and tetanus toxoid (10Lf, 0.1 ml); and CD4+ T cells. Active tuberculosis was excluded. Informed consent was obtained.

Results From 5130 clients 1168 subjects were enrolled; of these 801 (68.6%) were HIV positive. Reactivity to PPD among HIV-positive subjects was found in 174 (22%), 261 (32.6%), and 296 (37%), at PPD cutoff levels of ⩾10 mm, ⩾5 mm, and ⩾2 mm as compared to 224 (61%) of 367 HIV-negative individuals' reactors to PPD (⩾10 mm) ($P < 0.001$). After exclusion of anergic individuals using two cutoff levels for cutaneous allergens (⩽2 mm and ⩽5 mm), PPD reactivity between HIV-infected and uninfected individuals continued to be significantly different. Only HIV-infected individuals with CD4+ T cells ⩾500 cells/mm^3 had similar reactivity to PPD as HIV-uninfected individuals. Variables associated with PPD reactivity were CD4+ T cell counts, BCG scar, HIV infection and age.

Conclusions PPD reactivity was useful to diagnose tuberculosis infection only among HIV-infected individuals with CD4+ counts ⩾500 cells/mm^3. Among individuals with lower counts, lowering cutoff levels or using anergy panel did not permit comparable reactivity as that observed among HIV-uninfected individuals.

Keywords Tuberculin test, HIV, anergy, BCG vaccine, CD4 lymphocytes, delayed-type hypersensitivity

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HIV/AIDS epidemic has had a major impact on the global epidemiology of tuberculosis, especially in developing countries.[1] In Mexico, tuberculosis continues to be endemic,[2] with increasing incidence rates over the last 10 years,[3] and an estimated rate of 50 cases per 100 000.[4] Rates of tuberculosis have been found to be higher among populations characterized by crowded living conditions, low family income, illiteracy and low access to health care.[2] HIV infection, although not of the same magnitude as in other regions, is spreading into the heterosexual population and rural areas.[5] The interaction between tuberculosis and HIV/AIDS is reflected in the high frequency of tuberculosis in patients with AIDS, in whom it is the third most frequent infection after candidiasis and *Pneumocystis carinii* pneumonia.[6] The frequency of tuberculosis among hospitalized AIDS patients, ranging from 7.7%,[7] 25.6%[8] to 50%,[9] is greater among patients from the lowest socioeconomic strata and often goes undiagnosed during the patient's lifetime.[10,11] HIV-1 infection prevalence among newly diagnosed tuberculosis

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patients is 3.1%,[12] several times greater than that found among blood donors (0.05–0.09%).[5]

To prevent the development of active tuberculosis due to reactivation of latent infection, isoniazid chemoprophylaxis has been recommended based on the results of tuberculin testing.[13,14] Health authorities in Mexico have adopted international recommendations;[15] however, it has been recognized that as no field evaluation has been conducted in Mexico, this recommendation is provisional and pending the results of specific research.[16] The objective of this study was to assess the usefulness of tuberculin reactivity in HIV testing centres located in a region with a high prevalence of tuberculosis infection and high coverage of Bacillus Calmette-Guérin vaccine (BCG) as a criterion for diagnosis of *Mycobacterium tuberculosis* infection in HIV-infected subjects. The proportion of HIV-infected subjects, who had positive reactivity to PPD was compared to PPD reactors among HIV-uninfected individuals. Interrelationship of PPD reactivity with anergy panel and CD4+ lymphocytes was examined.

Methods

Recruitment and selection of participants

This study was conducted as part of the recruitment for a tuberculosis chemoprophylaxis study. Individuals seeking HIV testing at one of four Mexico City HIV testing centres were invited for study enrolment. Age above 18 years and Karnofsky score greater than 60% were required for study entry. The Karnofsky scale is used to measure performance status. A Karnofsky performance status of 60% implies a patient who is unable to work but able to live at home and perform most personal tasks only requiring occasional assistance. As the objective of the study was to evaluate the tuberculin test for its use as a criterion for diagnosis of *M. tuberculosis* infection, subjects with a history of active tuberculosis or a current diagnosis of active tuberculosis, as determined by clinical history, chest x-ray, sputum acid fast bacilli smear and culture, were excluded from the study. All study participants signed a letter providing informed consent.

Procedures

For subjects who previously tested positive for HIV-1 prior to study entry, a second test was performed. A physician conducted a structured questionnaire providing information about the patient's general health and risk factors. All subjects were given a physical examination. Cutaneous tests were administered (purified protein derivative [PPD], Candida, and tetanus toxoid) and blood cell count, blood chemistry, chest x-ray, acid fast bacilli smear and culture of sputum or other appropriate samples, and CD4+ lymphocyte count tests were performed. Subjects with abnormal results on any of these tests were referred for appropriate medical care.

Tuberculin test

The cutaneous test for tuberculin was conducted using the Mantoux method, using tuberculin PPD stabilized with Tween-80 PPD-RT23. The product was diluted locally from a concentration of 50 000 TU to 5 TU.[17] PPD batch RT23 was produced and standardized by the Statens Seruminstitut (17 July 1981) of Copenhagen, Denmark. Vials were kept at 4–8ºC. Opened vials were not used for more than 48 hours and unused vials were discarded 6 months after preparation.

Specifically trained nurses performed PPD reactivity readings, 48–72 hours after application, by measuring the transversal axis of induration (not erythema) with a flexible ruler. The exact number of millimetres was recorded. Clinical staff responsible for the administration and reactivity reading of PPD tests were required to take a course, standardized by the PanAmerican Health Organization, for instruction on how to administer and evaluate the test.

Candida and tetanus toxoid

Candida (1:1000, 0.1 ml) and tetanus toxoid (10Lf, 0.1 ml) were each administered by intradermic injection into the foreside of the forearm. The number of millimetres of induration was recorded. Candida was locally prepared from a smooth strain of *Candida albicans* using the method described by INDRE's Department of Immunology.[18] The preparation was compared with Candida (batch 10J 21K 4685 07–22–93 Hollister-Stier, Spokane, Washington). Aliquots of the final product were produced in 1 ml amber bottles, refrigerated and discarded if 6 months of shelf life had passed. The tetanus toxoid was produced by the Instituto Nacional de Higiene using tetanus toxin, in accordance with the specifications of the Pharmacopoeia Mexicana. Tetanus toxoid was stored at 4–8ºC and discarded accordingly 6 months after preparation.

Collection of mycobacteria samples for culture

Three sputum samples were collected in clean flasks that were refrigerated until transportation to the laboratory. In the event that extrapulmonary tuberculosis was suspected, subjects were referred to a specialized clinic for collection of appropriate samples.

Typification and susceptibility tests of *M. tuberculosis*

The sputum smears were processed at the mycobacteriology laboratory of INDRE. Smears were pretreated with a mucolytic agent and a 4% solution of sodium hydroxide following Petroff procedure. Ziehl-Neelsen and rodamine stains were performed. The samples were cultured in Lowenstein-Jensen medium and were examined on a weekly basis until growth was detected. Cultures were reported as negative if there was no growth after 8 weeks. Cultures with visible growth were processed for biochemical identification at the species level according to standardized methods.[19]

Blood collection and processing

Blood was collected by venepuncture. Samples were processed for cell blood count and blood chemistry in an autoanalyser (Beckman) using standardized procedures.

Detection of HIV-1 antibodies

Sera samples were processed for the detection of HIV-1 antibodies using an immuno-absorbent enzyme assay (ELISA, Abbott, second-generation or haemagglutination equipment, Miles). All double-positive samples were confirmed by Western Blot. Tests were processed at the Instituto Nacional de Diagnostico y Referencia Epidemiologicos and at the Human Retrovirus Unit of Universidad Nacional Autonoma de Mexico/Secretaria de Salud.

Quantification of CD4+ lymphocytes

The Instituto Nacional de Nutricion Salvador Zubiran Immunology Laboratory quantified CD4+ lymphocytes. The analysis was

processed using a flow cytometer (Becton-Dickinson), following quality control procedures recommended by the American Association of Histocompatibility and Immunogenetics. Samples collected were forwarded to the immunology laboratory every day. A total of 3 hours elapsed between reception of the samples and preparation.

Analysis

The categorical variables were compared using an χ^2 test, continuous variables with a normal distribution with a t-test, and skewed data with the non-parametric Kruskall-Wallis test. The ⩾2 mm, ⩾5 mm, and ⩾10 mm tuberculin cutoff levels were analysed. For Candida and tetanus toxoid, cutoff levels of ⩾2 mm and ⩾5 mm were evaluated. Individuals were stratified according to their CD4+ lymphocyte counts <200, ⩾200 to <500, and ⩾500 cells. Both odds ratios and confidence intervals (95% CI) associated with PPD reactivity were calculated. In the crude and adjusted analysis, a positive tuberculin result was recorded if an HIV-positive subject was found to have a ⩾5 mm PPD reaction or if an HIV-negative subject was found to have a ⩾10 mm reaction.

Unconditional logistic regression analysis was used to determine the factors independently associated with a positive PPD reaction. Variables were entered into the models according to their statistical significance in univariate analysis and their biological relevance. Both the SAS and EPINFO programs (US Centers for Disease Control) were used.

Results

Between January 1992 and December 1993, 5130 people requested an HIV test at one of the four HIV testing centres in Mexico City. Of these, 1276 (24.8%) patients agreed to participate in the study. The rest received the services routinely offered by the HIV detection centres. There were 108 patients (8.5%) excluded either because of a history of tuberculosis or ingestion of antituberculosis drugs for more than 2 months (n = 25); or because of a positive *M. tuberculosis* culture at the time of the medical screening (n = 29); or because of the presence of one or more abnormalities compatible with tuberculosis at the time of the medical screening, as determined by clinical history evaluation, chest x-ray, acid fast bacilli smear, and/or sputum culture (n = 54).

Study population (n = 1168) were mostly young and middle aged men, with middle level education. Main HIV transmission category was men having sex with men (Table 1). Compared to the characteristics of all individuals seeking services from the four Mexico City HIV testing centres during the time of this study (n = 5130), there were statistically significant differences between the HIV testing centre clients and study participants. Rate of HIV infection was higher among study participants (801, 68.6% versus 2229, 43.5%, $P < 0.01$); average age was higher among study participants (31.0, SD 8.0, years versus 28.4, SD 8.6, years, $P < 0.001$); proportion of men who reported having sex with other men was significantly higher among study participants (851, 72.9% versus 2442/3617, 67.5%, $P = 0.001$).

Among the 1168 participants, there were also differences between HIV-positive subjects (n = 801) and HIV-negative (n = 367) participants. HIV-positive subjects were mostly men (92.1% versus 79.6%, $P < 0.001$); slightly older (average age 31.8, SD 8.7, versus 29.3, SD 8.8, years, $P < 0.001$); with fewer years of formal education (53.1% had finished college versus 65.1%, $P < 0.001$) and higher probability of coming from lower socioeconomic level (17.2% versus 7.4%, $P < 0.001$). A higher proportion of infected men referred were having sex with other men (79.4% versus 58.6%). The frequency of BCG scar was similar between both groups, (81.0% versus 82.6%, $P = 0.5$).

Table 1 Characteristics of study participants

	Participants (N = 1168)	
Characteristic	No./Total	(%)
Men	1030	88.2
Age (years, SD)	31.0	8
College education	664	56.8
Low socioeconomic level	165	14.1
Alcohol usage	636	54.7
Drug usage	192	16.5
Intravenous drug usage	13	1.1
Men having sex with men	851	72.9
HIV-1 antibodies	801	68.6
BCG scar	952	81.5
Reactivity to PPD[a]	485	41.5
Reactivity to Candida[b]	425	36.4
Reactivity to tetanic toxoid[b]	1081	92.6
Cutaneous anergy	51	4.4
Clinical symptomatology (unrelated to TB)	800	68.5
Antiretroviral agents	143	12.4
CD4+ lymphocyte count (cells/mm^3, SD)	503	418

[a] ⩾5 mm in HIV infected, ⩾10 mm in HIV uninfected.

[b] ⩾2 mm.

Three cutoff levels were considered for interpreting PPD reactivity. Reactivity to PPD among HIV-infected subjects was found in 174 (22%), 261 (33%), and 296 (37%), at PPD cutoff levels of ⩾10 mm, ⩾5 mm, and ⩾2mm. In HIV-negative subjects, PPD reactivity (⩾10mm) was found in 224 (61%) of 367 individuals, ($P < 0.001$). HIV-infected subjects had significantly lower reactivity than HIV-uninfected individuals at all three cutoff levels (Table 2).

Excluding individuals whose responses were below the cutoff level, mean of induration to PPD was significantly higher among HIV-uninfected subjects than among HIV-infected subjects (Table 3).

Two cutoff levels were considered for tetanus toxoid and Candida (⩾2 mm and (⩾5 mm) among HIV-infected individuals. The number of anergic individuals among HIV-positive subjects was: 49/801 (6.1%) for cutoff level of 2 mm for Candida and tetanic toxoid and 5 mm for PPD and 77/801 (9.6%) for cutoff

Table 2 Reactivity to PPD among HIV-negative subjects compared to reactivity among HIV-positive individuals

PPD cutoff	HIV negative No. (%)	HIV positive No. (%)	*P*[a]
⩾2 mm	297 (81)	296 (37)	<0.01
⩾5 mm	279 (76)	261 (33)	<0.01
⩾10 mm	224 (61)	174 (22)	<0.01

[a] χ^2 test.

Table 3 Induration to PPD among individuals with reactivity above the cutoff level

	HIV negative		HIV positive		
PPD cutoff	No.	Mean (SD)	No.	Mean (SD)	***P*[a]**
⩾2 mm	297	16.4 (±9.3)	296	12.8(±8.5)	<0.01
⩾5 mm	279	17.2 (±9.0)	261	14.1 (±8.2)	<.001
⩾10 mm	224	19.7 (±8.4)	174	17.8 (±7.6)	<.05

[a] t test.

Table 4 Reactivity to PPD among individuals responding to Candida and tetanic toxoid at ⩾2 mm and at ⩾5mm

PPD cutoff	**HIV negative No. (%)**	**HIV positive No. (%)**	***P*[a]**
Candidin and tetanic toxoid ⩾2 mm			
⩾2 mm	297 (81)	296/757 (39)	<0.001
⩾5 mm	279 (76)	261/752 (35)	<0.001
⩾10 mm	224 (61)	174/748 (23)	<0.001
Candidin and tetanic toxoid ⩾5 mm			
⩾2 mm	297 (81)	296/729 (41)	<0.001
⩾5 mm	279 (76)	261/724 (36)	<0.001
⩾10 mm	224 (61)	174/717 (24)	<0.001

[a] χ^2 test.

level of 5 mm for Candida, tetanus toxoid and PPD. After excluding subjects who did not respond to any of the three antigens, reactivity to PPD was still significantly different between HIV-infected and HIV-uninfected individuals at each cutoff level for PPD (Table 4).

The mean CD4+ lymphocyte count in subjects infected with HIV was 324.4 cells/mm^3 (SD 273.41 cells/mm^3), compared to a mean CD4+ lymphocyte count of 895.5 cells/mm^3 (SD 411.7 cells/mm^3) among HIV-uninfected study subjects ($P < 0.001$). In Figure 1, the distribution of PPD reactivity in four groups is reported: HIV-uninfected subjects (n = 367), HIV-infected subjects with CD4+ counts ⩾500 cells/mm^3 (n = 167, 20.8%), HIV-infected subjects with CD4+ counts ⩾200 to <500 cells/mm^3 (n = 325, 40.5%), and HIV-infected subjects with CD4+ counts <200 cells/mm^3 (n = 309, 38.5%). It was found that the distribution of reactivity to PPD was significantly different in all groups ($P < 0.01$, Kruskall-Wallis test). PPD reactivity as measured in millimetres had a bimodal distribution in all the groups, among HIV-infected individuals the proportion of subjects having decreased or negative responses increased as the count of CD4+ lymphocytes was lower.

When reactivity to PPD was analysed according to levels of CD4+ lymphocytes (Table 5) it was found that only among HIV-positive subjects with CD4+ T cell counts ⩾500 cells was the

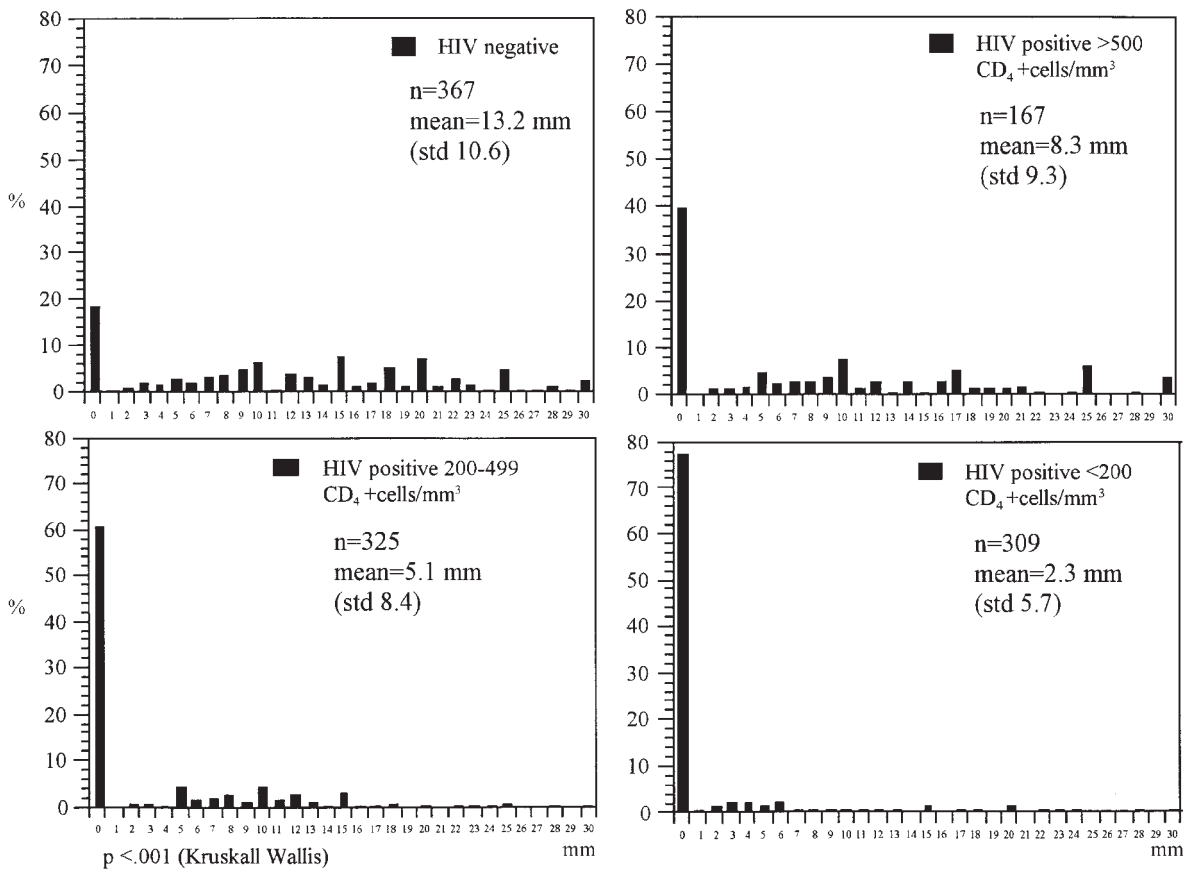

Figure 1 Distribution of reactivity to PPD (mm) according to HIV serology and CD4 T lymphocyte count (cells/mm^3) was bimodal and different in every group, the proportion of subjects having decreased or negative responses increased as CD4 lymphocyte counts went down

Table 5 Interaction between reactivity to PPD and CD4+ lymphocytes in HIV infected as compared to reactivity to PPD in HIV-uninfected

CD4+ lymphocytes in HIV infected	Reactivity to PPD[a]	*P*[b]
CD4+ <200	47/309 (15.2%)	<0.001
CD4 ⩾200 <500	120/325 (37%)	<0.001
CD4 ⩾500	94/167 (56%)	0.3
HIV-uninfected	224/367 (61%)	

[a] HIV-positive PPD ⩾5 mm; HIV-negative PPD ⩾10 mm.
[b] χ^2 test.

reactivity not significantly different from that observed among HIV-negative subjects. Among individuals with lower counts, 167/634 (26.3%) were PPD reactive.

Crude and adjusted analysis of variables associated with reactivity to PPD (⩾10 mm among HIV-uninfected individuals and ⩾5mm among HIV-infected subjects) was performed. Crude analysis showed statistical significant association with sex, BCG scar, HIV infection and CD4+ T cell counts. We did not find association with socioeconomic level, number of years of formal education, alcoholism, drug usage or sexual practices. Multivariate analysis showed that having CD4+ T cell counts ⩾500 cell/mm^3 (OR 2.6, 95% CI : 1.9–3.6, $P < 0.001$), having a BCG scar (OR 2.3, 95% CI : 1.6–3.2, $P < 0.001$), not being HIV infected (OR 1.9, 95% CI : 1.3–2.6, $P < 0.001$), and older age (⩾31 years) (OR 1.5, 95% CI: 1.1–1.9, $P < 0.001$), were associated with positive reactivity to PPD.

Discussion

The proportion of tuberculin reactivity in this study's HIV-uninfected subjects is high (41.5%) and comparable to other studies conducted in Mexico,[20,21] and other developing countries, such as Haiti,[22] Brazil[23] and Uganda.[24] This high prevalence of tuberculin reactivity appears to reflect both tuberculous infection, based on the prevalence of tuberculosis in this region, and the high coverage of BCG vaccination in this population. The tuberculosis prevention and control programme in Mexico is based on BCG vaccination at birth, chemoprophylaxis upon contact, and early diagnosis and treatment in accordance with the guidelines indicated by the World Health Organization.[16] This country has achieved very high rates of BCG vaccination with BCG, which was first used in 1965 and has resulted in coverage rates of greater than 95% as of 1993.[25] Interpretation of PPD in BCG vaccinated subjects is a controversial issue. In this context, it was considered important to assess the usefulness of tuberculin and anergy panel to evaluate tuberculosis infection among HIV-1 infected subjects.

The tuberculin test is the only currently licensed tool available for the diagnosis of *M. tuberculosis* infection. However, when interpreting the results, the characteristics of sensitivity and specificity of the test, and the population being studied, must be taken into consideration.[26] The absence of tuberculin reactivity in HIV-infected subjects could be attributed to anergy, and not necessarily to a lack of *M. tuberculosis* infection.[27] Considering the lower degree of reactivity to cutaneously applied antigens in HIV-infected subjects, it has been recommended that the cutoff levels be reduced to 5 mm,[13] or even 2 mm[28] and that other cutaneous allergens be used to identify anergic individuals.[29]

The results of our study show that the use of PPD in ambulatory HIV-infected subjects who attend HIV testing centres underestimates the PPD reactivity found in HIV-uninfected subjects. Reducing the cutoff level from 5 mm to 2 mm did not improve the sensitivity of the test. The difference was not only due to a greater number of subjects with a non-response, but also to a decreased reactivity in subjects with positive results. These data contrast with results found in Haiti[22] where mean PPD reactivity was slightly greater for HIV-infected individuals than for HIV-uninfected subjects.

It has been recommended that other cutaneous allergens be used to differentiate between the presence of non-reactivity to tuberculin due to an absence of infection or due to anergy[26] as delayed-type hypersensitivity (DTH) skin test responsiveness reflects the body's ability to mount an effective defence against antigens of invading organisms. Unresponsiveness to the administration of three cutaneous allergens has been considered an indicator of inability to mount a skin test reaction and response to one of these allergens in the presence of a negative reactivity to tuberculin is interpreted as absence of infection with *M. tuberculosis*. Our data support the existence of specific anergy to tuberculin without generalized anergy as has been reported by others.[30] We found that Candida and tetanus toxoid were not useful for differentiating between the absence of reactivity to tuberculin caused by HIV-associated immunodeficiency and that due to absence of latent *M. tuberculosis* infection. After excluding anergic subjects considering two cutoff levels for Candida and tetanus toxoid, HIV-infected subjects continued having lower reactivity to PPD than subjects uninfected with HIV. Usefulness of anergy testing has been questioned recently and its use to guide administration of chemoprophylaxis has been discontinued in the United States.[31]

On the other hand, the CD4+ lymphocyte count, using a cutoff level of 500 cells/mm^3, allowed us to discriminate between a lack of reactivity to tuberculin due to non-infection with *M. tuberculosis* or to immunodeficiency. This occurred although the curves of distribution of PPD reactivity were different among HIV-uninfected and HIV-infected subjects with CD4+ lymphocyte counts above 500 cells/mm^3. Therefore this tool is useful in our context for interpreting tuberculin reactivity. However, the availability of CD4+ lymphocyte counts in Mexico and in most developing countries is limited.

The greatest limitation in this study was the self-selection of subjects, thus possibly preventing extrapolation of our data to the much larger HIV-infected population. The population we studied was ambulatory, and was selected from subjects who were requesting an HIV antibody test at institutions designed for that purpose. We found differences between our study population and all the clients of the HIV detection centres that indicated that participants were more likely to be HIV infected or that considered themselves at higher risk of being infected. Participants were older, with greater probability, among those referred, of having sex with other men, and with higher rates of HIV infection. The need to comply with the required visits to the HIV care centres could have meant that those subjects who completed the assessment may have already known that they were infected with HIV or had symptoms and therefore had the highest degree of immunodeficiency. We found that most

HIV-infected subjects enrolled presented with low CD4+ lymphocyte counts, although the number of anergic individuals was low. We used the reactivity found among HIV-uninfected subjects as a reference point for that observed among HIV-infected individuals as both groups were recruited from the same population of clients of HIV detection centres. However, HIV-infected individuals differed from HIV-uninfected subjects as the former were older, from a lower socioeconomic level, with fewer years of formal education and were more likely to be having sex with other men. Age and sexual practices have been described as being associated with HIV infection in Mexico;[5] lower socioeconomic level and older age are more likely to determine exposure to tuberculosis.[32] On the other hand, HIV-infected and HIV-uninfected individuals were similar regarding the frequency of BCG scar. Therefore, participants and in particular HIV-infected subjects in our study population might have had a greater probability of exposure to tuberculosis (because they were older, and in the case of HIV-infected, had fewer years of formal education and came from lower socioeconomic status) and therefore our study might overestimate the frequency of tuberculosis infection in this population. This limitation underlines the lack of usefulness of tuberculin reactivity. Although HIV-infected individuals might have been more heavily exposed to tuberculosis, tuberculin reactivity among them was lower than among HIV-uninfected individuals.

The variables found to be independently associated with PPD reactivity were a lower degree of immunodeficiency as determined by CD4+ lymphocyte counts, presence of a BCG vaccination scar, absence of HIV infection, and older age. The association with BCG vaccination is noteworthy. Other studies have found higher PPD reactivity rates among adults vaccinated at birth;[33,34] however, conflicting results have been found by other authors.[35] Although vaccination coverage rates are very high in Mexico (>95%), from the operational viewpoint we do not consider that BCG constitutes an obstacle to interpretation of the reactivity to tuberculin. Association with age has been found in other studies.[21,28] We infer that this association reflects a cohort effect resulting from a greater probability of exposure to *M. tuberculosis* with increasing age. Inverse association with HIV infection and low counts of CD4 cells were described in a study performed in several US cities.[36]

Results of this study support use of tuberculin administration to diagnose tuberculosis infection only among HIV-infected individuals with CD4+ counts $\geqslant$500 cells/mm^3. Among individuals with lower counts of CD4+ cells, lowering the tuberculin reactivity cutoff levels or using anergy panel did not permit comparable PPD reactivity as that observed among HIV-uninfected individuals. Therefore administration of tuberculin to these patients is not useful for the diagnosis of tuberculosis infection as a negative PPD test will not mean that the person is not infected with *M. tuberculosis*. From the operational point of view, we consider that if CD4 lymphocytes are not available, PPD reactivity should not be used to guide control measures such as administration of isoniazid for the prevention of tuberculosis among HIV-infected individuals in regions with high prevalence of tuberculosis infection. Therefore, recommendations such as the one formulated by the PanAmerican Health Organization[37] that advises on tuberculous chemoprophylaxis to all HIV-infected individuals, regardless of PPD reactivity, should be considered.

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Informed consent was obtained from patients or their parents or guardians. Human experimentation guidelines of the US Department of Health and Human Services and of Mexican institutions were followed. Approval by IRB committees both from the US and Mexican participating institutions was obtained.

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