SHORT COMMUNICATION

Purified protein derivative tuberculin and delayed-type hypersensitivity skin testing in migrant farm workers at risk for tuberculosis and HIV coinfection

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Objective: To assess the joint use of purified protein derivative (PPD) and delayed-type hypersensitivity (DTH) antigens in screening individuals of unknown HIV serostatus for tuberculosis (TB) preventive therapy eligibility.

Design: Population-based survey.

Methods: A group of migrant farm workers were screened for HIV and skin-tested with PPD, tetanus toxoid (TET), *Candida albicans* (CAN) and mumps (MUM) antigens by the Mantoux method. Anergy was defined as a ≤2 mm reaction to all four antigens. Eligibility for preventive therapy was defined as a reaction of ≥5 mm to PPD among HIV-seropositive individuals, ≥10 mm among HIV-seronegatives, or anergy.

Results: A total of 253 out of 271 individuals had sufficient data for analysis. Of these, 15 (5%) were HIV-seropositive; 183 (75%), 175 (72%) and 157 (65%) reacted to TET, CAN, and MUM, respectively, and 113 (47%) were eligible for preventive therapy [108 (44%) PPD-positive, five (2%) anergic]. Use of PPD alone was 95% sensitive for detecting preventive therapy eligibility; PPD plus one DTH antigen was more sensitive (99%) but less specific (range, 69–85%); PPD plus two DTH antigens was most specific (CAN + MUM, 84%; TET + MUM, 93%; and TET + CAN, 100%).

Conclusions: In this population with 5% HIV seroprevalence, testing for anergy did not significantly increase the detection of preventive therapy eligibility. The use of two DTH antigens is very sensitive and specific. These results support the recommendation of joint PPD and anergy testing for the screening of HIV-seropositive individuals. Our data also suggest, however, that for individuals whose HIV serostatus is unknown, anergy testing should be considered as a screening tool only if the prevalence of anergy is expected to exceed the prevalence of PPD positivity.

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Keywords: Tuberculosis (TB) screening, purified protein derivative testing, anergy testing, TB and HIV coinfection

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Introduction

The prevention of tuberculosis (TB) in the United States is most reliably accomplished by screening population groups at high risk for TB [1]. Screening is performed in these groups to detect individuals with latent *Mycobacterium tuberculosis* infection who would benefit from therapy to prevent active TB. Several factors increase the risk of latent *M. tuberculosis* infection progressing to active disease. The most significant factor is immunodeficiency, such as that caused by coinfection with HIV [2]. On this basis, the Centers for Disease Control and Prevention (CDC) and the Advisory Council for the Elimination of Tuberculosis have recommended that individuals infected with HIV be screened for TB infection and that any HIV-infected individual, regardless of age, who has a tuberculin skin test reaction ≥ 5 mm induration should be offered a 12-month regimen of isoniazid preventive therapy [3].

An important issue in screening HIV-infected patients with the tuberculin skin test is cutaneous anergy to delayed-type hypersensitivity (DTH) testing [4]. Cutaneous anergy renders a negative tuberculin skin test uninterpretable and complicates decisions about preventive therapy. For this reason, in 1991 the CDC recommended that HIV-infected individuals be evaluated for anergy in conjunction with tuberculin skin testing and that anergic individuals whose risk of TB infection was estimated to be ≥ 10% be considered for isoniazid preventive therapy after active TB has been excluded [5]. The CDC guidelines also recommend that anergy testing be considered for individuals of unknown HIV serostatus who are at risk for TB and HIV coinfection. However, there are no clear data defining the risk of anergy and the usefulness of anergy testing for determining whether persons at risk for HIV infection, but of unknown HIV serostatus, are eligible for preventive therapy.

In order to address this question we conducted a study to evaluate the joint use of purified protein derivative (PPD) tuberculin and DTH antigens in screening individuals at risk for HIV infection to determine their eligibility for preventive therapy.

Methods

The population, data collection and laboratory methods for HIV testing have been described previously [6]. Subjects were farm workers who at the time of the study were based at migrant camps in Immokalee, an agricultural community in southwest Florida. The survey, which took place between 19 February and 14 March 1992, involved blood sampling to detect HIV antibodies, standardized interviewing to assess HIV risk factors and TB history, and skin testing to detect TB or anergy. Voluntary participants gave written informed consent.

The Mantoux technique was used for the intracutaneous injection of 0.1 ml each of four antigens: five tuberculin units of PPD (Tubersol; Connaught Laboratories, Swiftwater, Pennsylvania, USA), tetanus toxoid adsorbed USP (TET; Connaught Laboratories) in 1 : 5 dilution with human serum albumin, *Candida albicans* (CAN; Miles Allergy Products, Spokane, Washington, USA; 1 : 100 Oidiomycin), and mumps skin test antigen (MUM; MSTA; Connaught Laboratories). The skin test response was measured after 48–72 h. A positive PPD skin test was defined as a response of ≥ 5 mm induration to PPD in HIV-seropositive individuals or ≥ 10 mm induration in HIV-seronegative individuals. Anergy was defined as a skin test response of ≤ 2 mm induration to all four antigens. Individuals whose test results were positive for TB or HIV infection were referred to appropriate health-care agencies for further evaluation.

The outcome measures for our study were the estimates of the sensitivity and the specificity of skin testing with PPD alone, or skin testing with PPD plus DTH antigens, to determine their eligibility for preventive therapy. Eligibility for preventive therapy (true positives) was defined as anergy or a positive reaction to a PPD skin test. False positives would occur if all the skin testing results were not used for classification and a subject was misclassified as anergic when he or she reacted to one of the DTH antigens not used for classification. Positive predictive values were calculated using a hypothetical model in which the prevalence (prior probability) of anergy in a population ranged from 0 to 100%.

Results

Of an estimated 518 individuals aged > 15 years residing in the migrant camps, 310 (60%) agreed to participate in the study. Participants were predominantly men [247 (80%)] and foreign-born [93 Haitians (30%), 83 Mexicans (27%), and 44 Guatemalans (14%)]. Fifteen (5%) were HIV-antibody-positive. We evaluated the history of injecting drug use among the subjects because of its association with anergy: none of the HIV-seropositives and few of the HIV-seronegatives admitted to injecting drug use.

Of the 310 subjects, 27 had a history of TB and were not skin tested. Of those tested, 266 (86%) returned within 48–72 h to have the PPD skin-test reaction interpreted. The 266 subjects included all 15 HIV-seropositive individuals in the study. Overall, 118 (44%) of the 266 had positive PPD skin test reactions. The rate of positive PPD skin test results was lower among individuals with HIV infection

Table 1. Skin-test reaction sizes by HIV serostatus.

	Antigen [n (%)]							
	PPD		TET		CAN		MUM	
Induration (mm)	HIV+ (n = 15)	HIV– (n = 251)	HIV+ (n = 15)	HIV– (n = 228)	HIV+ (n = 15)	HIV– (n = 228)	HIV+ (n = 15)	HIV– (n = 228)
0	8 (53)	109 (43)	6 (40)	43 (19)	6 (40)	57 (25)	8 (53)	71 (31)
1–2	1 (7)	1 (0.4)	2 (13)	9 (4)	0 (0)	5 (2)	1 (7)	6 (3)
3–4	2 (13)	4 (2)	0 (0)	32 (14)	0 (0)	13 (6)	3 (20)	20 (9)
5–9	1 (7)	23 (9)	3 (20)	87 (38)	1 (7)	48 (21)	0 (0)	48 (21)
10–14	0 (0)	43 (17)	3 (20)	37 (16)	4 (27)	51 (22)	1 (7)	46 (20)
15–19	1 (7)	45 (18)	0 (0)	15 (7)	2 (13)	31 (14)	1 (7)	15 (7)
≥ 20	2 (13)	26 (10)	1 (7)	5 (2)	2 (13)	23 (10)	1 (7)	22 (10)

PPD, purified protein derivative; TET, tetanus toxoid; CAN, *Candida albicans*; MUM, mumps.

than among those without HIV infection [four out of 15 (27%) versus 104 out of 228 (46%); relative ratio (RR), 0.6; 95% confidence interval (CI), 0.2–1.4]. However, the small sample size provided little statistical power (28%) to detect a significant difference ($\alpha/2 = 0.025$). The rates of PPD positivity did not differ significantly between individuals whether immunized with bacillus of Calmette and Guerin (BCG) or not [47 out of 102 (46%) versus 71 out of 164 (43%); RR, 1.1; 95% CI, 0.8–1.4].

Of the 266 individuals tested with PPD, 243 were also anergy tested with DTH antigens. The distribution of the skin-test reaction sizes by HIV serostatus is shown in Table 1. Of these 243 subjects, 183 (75%) reacted (induration ≥ 3 mm) to TET, 175 (72%) to CAN and 157 (65%) to MUM antigens. Age, HIV serostatus, place of birth, and sex were not statistically associated with reactivity to any one DTH antigen. Individuals with HIV infection were significantly more likely to be anergic than those without [three out of 15 (20%) versus two out of 228 (1%); RR, 11.9; 95% CI, 4.8–29.4]. In addition to four (27%) PPD-positive subjects, three of the 15 (20%) HIV-positive individuals were found eligible for preventive therapy on the basis of anergy testing. Of 228 HIV-seronegative subjects, 113 (49%) were found eligible for preventive therapy [108 (47%) PPD-positive; five (2%) anergic].

Assuming that subjects' HIV serostatus was unknown and that skin-test screening was performed with PPD alone, individuals enrolled in this study would be considered appropriate candidates for preventive therapy if their skin-test response to PPD was ≥ 10 mm induration [7]. Using this criterion, the sensitivity of skin testing with PPD alone was 95%; by definition, the specificity was 100%. Anergy testing with PPD plus any one DTH antigen was 99% sensitive and 69, 77, and 85% specific for PPD + MUM, PPD + CAN and PPD + TET combinations, respectively. A combination of PPD and any two DTH antigens was also 99% sensitive and 84, 93 and 100% specific for PPD + CAN + MUM, PPD + TET + MUM and PPD + TET + CAN combinations, respectively.

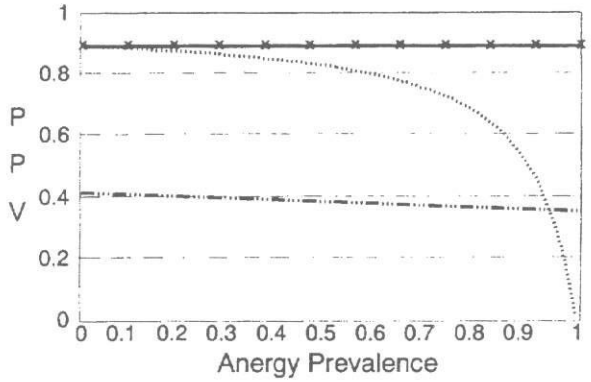

Fig. 1. Positive predictive value (PPV) of skin tests by different levels of cutaneous anergy, in a hypothetical population in which the prevalence of tuberculosis infection is 47%. PPD, purified protein derivative; DTH, delayed-typed hypersensitivity. (–•–•–), PPD + 2 DTH antigens; (– – – –), PPD + 1 DTH antigen; (·····) PPD alone.

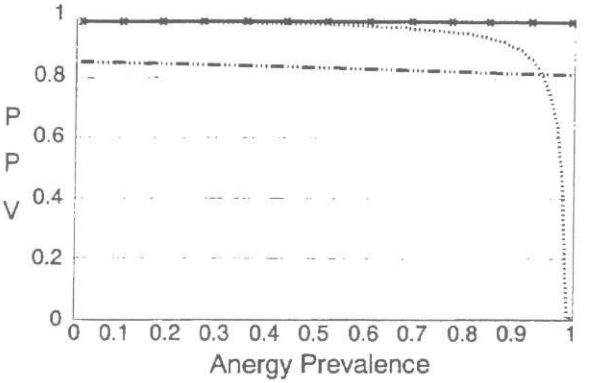

Fig. 2. Positive predictive value (PPV) of skin tests by different levels of cutaneous anergy, in a hypothetical population in which the prevalence of tuberculosis infection is 10%. PPD, purified protein derivative; DTH, delayed-typed hypersensitivity. (–•–•–), PPD + 2 DTH antigens; (– – – –), PPD + 1 DTH antigen; (·····) PPD alone.

The effect of the prevalence of TB infection and anergy in the population tested on the positive predictive value of skin testing for recognizing preventive

therapy eligibility was examined in a hypothetical model. For this model, we first used the best estimates of sensitivity and specificity from our study and a 47% prevalence rate of TB infection. Skin testing with PPD plus one DTH antigen had a lower positive predictive value than PPD alone, regardless of the anergy rate. However, skin testing with PPD plus two DTH antigens had a higher positive predictive value than PPD alone at high (for example, ≥60%) rates of anergy (Fig. 1). Again using the best estimates of sensitivity and specificity but in a model that assumes a 10% prevalence of TB infection, the positive predictive value of skin testing was lower for PPD used alone or in combination with one or two DTH antigens than it was for the model that assumed a 47% rate of TB infection (Fig. 2). In the model that assumed a 10% prevalence, skin testing with PPD plus two DTH antigens had a better positive predictive value for recognizing preventive therapy eligibility than skin testing with PPD alone, beginning at lower (for example, ≥30%) rates of anergy.

Discussion

This study was conducted to assess the joint use of PPD and DTH antigens in screening individuals of unknown HIV serostatus for preventive therapy eligibility.The high (47%) prevalence of PPD skin-test positivity found in this population-based study of farm workers in Florida is consistent with previous reports [8,9]. Although our study was limited by a small sample of HIV-seropositive subjects, the results suggest a relationship between HIV seropositivity and the impaired ability to mount a DTH response to PPD. Of the HIV-seropositive subjects, 20% had a reaction to PPD of ≥10 mm, and 60% were non-reactive (induration ≤2 mm) to PPD. Of the HIV-seronegative subjects, however, 45% had a reaction to PPD of ≥10 mm and 43% were non-reactive. Although not statistically significant, the differences in these rates may suggest that the DTH to PPD wanes in HIV-seropositive individuals. These findings support the current recommendation for using a lower (≥5 mm induration) reaction size cut-off to define PPD skin-test positivity and eligibility for isoniazid preventive therapy [6] in a population subgroup (HIV-infected individuals) that is at high risk for active TB if infected with *M. tuberculosis*. In our study population with a 5% HIV seroprevalence, screening for anergy did not find significantly more individuals eligible for preventive therapy than screening with PPD alone; however, anergy testing found 20% of the (seven out of 15) HIV-positive subjects eligible for preventive therapy. In this population known for its high prevalence rate of TB infection, these anergic, HIV-seropositive subjects are at very high risk for active TB [4]. Thus, isoniazid preventive therapy should be strongly considered for these individuals. Of the HIV-seronegative subjects in our study, less than 0.5% were anergic. This finding differs from previous reports that approximately 10% of apparently healthy individuals without HIV infection would not have a detectable DTH response to a panel of antigens [5].

More people reacted to the TET (75%) and CAN (72%) than to the MUM (65%) antigens. Skin testing with PPD plus a combination of any two companion DTH antigens achieved excellent sensitivity (99%). The combination of PPD + TET + CAN achieved the best specificity (100%) of all the combinations for determining the study subjects' eligibility for preventive therapy. The use of PPD plus any one companion DTH antigen achieved low specificity and thus is not recommended for anergy testing. We also studied the positive predictive value of skin testing for determining preventive therapy eligibility. In the model that assumed a 47% prevalence rate of TB infection, the positive predictive value of skin testing with PPD alone was good, and it was not affected until the prevalence rate of cutaneous anergy was greater than 50%. The overall positive predictive value of skin testing was lower in the model assuming a 10% prevalence rate of TB infection because the false-positive rate of a test is likely to increase as the prevalence of the condition decreases in the population tested. Thus, anergy testing increases the sensitivity of skin testing for determining preventive therapy eligibility. However, anergy testing should not affect the positive predictive value when the prevalence rate of TB infection is high and the prevalence of anergy does not exceed the prevalence of PPD positivity.

Our study supports the CDC recommendation that two DTH antigens should be used in conjunction with PPD for the routine screening of individuals known to be HIV-seropositive to determine eligibility for preventive therapy. Our data also suggest, however, that for individuals whose HIV serostatus is unknown but who are at risk for TB and HIV coinfection, anergy testing should be considered as a screening tool only if the prevalence of anergy is expected to exceed the prevalence of PPD positivity. For example, anergy testing may be appropriate for some groups of injecting drug users, among whom the prevalence of anergy is expected to be high.

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