Selection of Skin Test Antigens to Evaluate PPD Anergy

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Background. A purified protein derivative (PPD) tuberculin skin test may be nonreactive because of cutaneous anergy, technical problems with the test, or absence of tuberculosis infection. This study investigated the sensitivity and specificity of five test agents in measuring cutaneous anergy when the PPD test is nonreactive. Agents evaluated include antigens for *Candida*, mumps, histoplasmin, tetanus, and *Trichophyton*.

Methods. Delayed-type hypersensitivity skin test records were analyzed in 1113 patients admitted to the University of Texas Health Center at Tyler from December 1988 through June 1993. These patients were admitted with initial diagnoses of diseases other than active tuberculosis or human immunodeficiency virus infection.

Results. Patients with a negative PPD test reacted most often to the control skin test *Candida* (63.5%), followed by mumps (52.2%), histoplasmosis (37.2%), tetanus (35.7%), and *Trichophyton* (6.1%). Analysis of these data indicates that the use of more than three of the four most commonly reactive control tests (*Candida*, mumps, and histoplasmin or tetanus) yielded minimal additional precision in the determination of skin test anergy compared with using all five control skin tests. This finding remained constant whether the PPD was considered negative at <5 mm, <10 mm, or <15 mm of induration.

Conclusions. In controlling for false-negative PPD tests, the use of three skin test antigens, *Candida*, mumps, and tetanus, should provide reliable control for delayed-type hypersensitivity anergy.

Key words. Tuberculin test; delayed hypersensitivity; skin tests; anergy (cutaneous). (*J Fam Pract 1995; 41:59-64*)

The incidence of tuberculosis in the United States increased by 18% between 1985 and 1991, representing a marked change after a steady decline from 1953 to 1984.[1,2] This increase is multifactorial, including the advent of the human immunodeficiency virus (HIV) epidemic, the increased number of tuberculosis cases resulting from immigration and travel, and the difficulty in diagnosing and managing this illness within our health care system.

The Mantoux purified protein derivative (PPD) tuberculin skin test continues to play a vital role in the diagnosis of new tuberculosis cases in the United States. The sensitivity of the PPD test varies, however, from 96% in the general population,[3] to 59% in the population infected with HIV,[4] to only 50% in critically ill patients with disseminated tuberculosis.[5] False-negative PPD test reactions may result from problems with the tuberculin test itself, such as administration of the tuberculin material used; errors in retrieving the results; or host factors, such as altered cellular immunity. Conditions associated with cutaneous anergy include malnutrition, cachexia, advanced age, metabolic diseases, cancer, infection (especially HIV), drugs, stress, and live-virus vaccinations.[6]

There is ongoing controversy concerning the most reliable method for separating a negative PPD test due to nontuberculous infection from negative tuberculin reactivity secondary to cutaneous anergy. Skin test anergy panels are currently accepted as reasonable tests for determining the patient's ability to respond to delayed-type hypersensitivity (DTH).[5,7-9] A patient who fails to respond to any of these antigens is presumed to be anergic. These anergy panels are composed of antigens, such as fungi, bacteria, chemicals, and viruses, to which healthy people frequently demonstrate cutaneous response. However, the choice of a specific skin test antigen or group of antigens to best control for anergy has not been

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clearly defined. Although there are many antigens to choose from, only mumps, coccidioidin, histoplasmin, and tuberculin have been standardized for use in the Mantoux-type format of injecting 0.1 mL of antigen intradermally. Other antigens used to measure DTH skin test anergy include tetanus, *Proteus, Trichophyton, Candida*, diphtheria, *Streptococcus*, old tuberculin, dinitrochlorobenzene, and streptokinase-streptodornase. This study surveys cutaneous reactivity to five antigens: *Candida*, mumps, tetanus, histoplasmin, and *Trichophyton*.

Methods

The results of 3575 intradermal skin tests (Mantoux test), including PPD and five other antigen controls administered in a variety of combinations, were collected from a skin test logbook of 1113 patients treated at the University of Texas Health Center at Tyler from December 1988 to June 1993. Specially trained nurses administered intradermally 0.1 mL of one or more of the following skin test antigens: PPD (5 TU, Aplisol, Parke-Davis, Morris Plains, NJ); tetanus toxoid USP diluted to 2 Lf units/ mL (Connaught Laboratories, Inc, Swiftwater, Pa); mumps skin test antigen (Connaught Laboratories, Inc); histoplasmin (Parke-Davis); *Candida* antigen diluted from 1:10 to 1:100 (Hollister-STIER, Miles [HSM], Spokane, Wash); and *Trichophyton* (Dermatophytin diluted from 1:1 to 1:100, HSM).

The choice and number of skin tests selected for controls were determined by the ordering physician. Skin tests were read by the same nurse at 48 and 72 hours, and millimeters of induration were recorded in a designated skin test logbook. Control skin tests were interpreted as positive (nonanergic) if >5 mm induration in any dimension was noted in any non-PPD test. Data were collected from this logbook and described. Data were excluded from analysis if the data recorded in the logbook were incomplete, missing, duplicated, or unacceptable for interpretation.

Before 1985, the medical institution where the study was conducted served only as a tertiary referral center for pulmonary and cardiac patients; however, at present, it provides primary care for a broad patient base, including the family practice center of the institution's family practice residency. During the years our data were collected, most patients treated were self-referred and are considered more representative of a general hospital population than of a pulmonary referral population. For example, during the representative year of 1993, 74% of the skin tests were performed in the outpatient setting. There were 80,194 visits, only 10% of which involved the specialty services of pulmonary and infectious disease. Although not actually tabulated, it is probable that the patients receiving DTH testing represented a general population for a large multidisciplinary group practice. The source of the data used by this study did not include patients admitted to the floor reserved for patients suspected of having tuberculosis or acquired immune deficiency syndrome, or both.

Table 1. Percentage of Positive Delayed-Type Hypersensitivity, by Antigen Group

Negative PPD Test Result	Antigen Group, %				
	Candida (+)	Mumps (+)	Histoplasmin (+)	Tetanus (+)	*Trichophyton* (+)
Any 1 antigen applied (n=301–436)	63.5	52.2	37.2	35.7	16.1
All 5 antigens applied (n=253)	64.0	64.8	40.7	37.9	21.3

NOTE: Positive PPD = induration ≥5 mm.
PPD denotes purified protein derivative.

Statistical Analysis

Although single descriptive statistics (Table 1) give the percentage of patients who respond to any one test, they do not consider the interaction between multiple tests. A method using conditional probability was used to estimate the best order for the least number of tests to effectively eliminate anergy. Since not all tests were performed on all patients, the denominators for each test varied. If the anergy level of a negative PPD reaction was labeled P_1, the anergy level of the test with the highest proportion of unique positive (nonanergic) responses was calculated, included, and labeled P_2. The joint probability of anergy for the combination of tests was then calculated as the product of P_1 and P_2. This process was continued for the next test with the highest proportion of positive responses not found in the tests selected so far. The entire process was repeated until all tests were entered, yielding an optimal ordering.

Results

The logbook contained 1113 names of patients to whom 3575 skin tests were administrated and recorded. Of these, 928 PPD tests were administered and recorded. The remaining 185 either did not have a PPD test performed or did not meet inclusion criteria. Six hundred eighty-two of the 928 (73.5%) patients had a negative PPD reaction (<5 mm). As shown in Table 1, these patients reacted most often to the control skin test for *Can-*

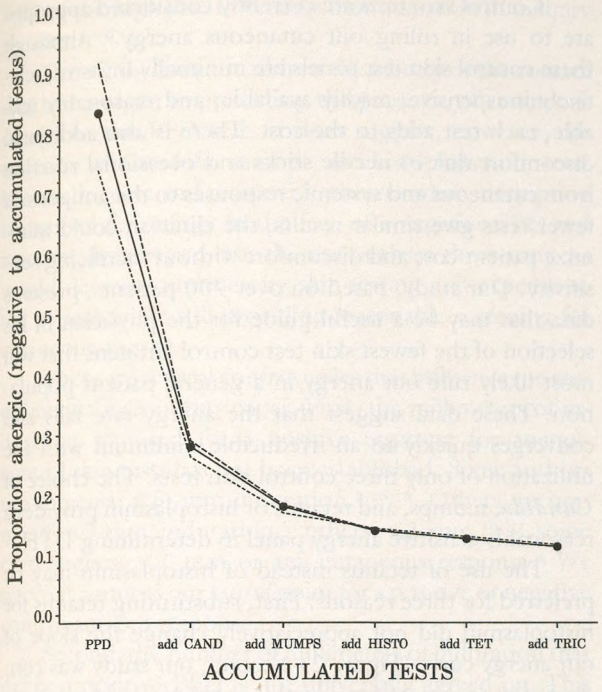

Figure 1. The estimated anergy curve shows decreasing percentage of anergy as more skin tests are added. The lower dashed line represents positive PPD test based on a minimum of 5-mm induration. The solid line represents positive PPD test based on a minimum of 10-mm induration. The upper dashed line represents positive PPD test based on a minimum of 15-mm induration. PPD denotes purified protein derivative tuberculin skin test, Mantoux method; CAND, *Candida;* HISTO, histoplasmin; TET, tetanus; TRIC, *Trichophyton.*

dida (277/436, 63.5%), followed by mumps (233/446, 52.2%), histoplasmin (112/301, 37.2%), tetanus (129/361, 35.7%), and *Trichophyton* (55/342, 16.1%). Similar percentages were noted in a subset of 253 patients with negative PPD reactions who had all five antigens applied: *Candida* (162/253, 64.0%), mumps (164/253, 64.8%), histoplasmosis (103/253, 40.7%), tetanus (96/253, 37.9%), and *Trichophyton* (54/253, 21.3%).

Further statistical analysis using conditional probability confirms the order of reactivity of antigens, as suggested by the above data. Given a negative PPD reaction, *Candida* antigen had the most unusual number of positive tests, followed in decreasing order of reactivity by mumps, histoplasmin, tetanus, and *Trichophyton.* Only three of the four most reactive antigens (*Candida,* mumps, and histoplasmin or tetanus) were shown to be necessary in determining cutaneous anergy. As shown in Figure 1, the estimated anergy for the population decreased very rapidly with the addition of the second and third test. Although anergy initially ranged between 75% and 92%, depending on measured induration, the percentage quickly converged around the 28% level with one additional test. The slope quickly leveled off after adding *Candida*, mumps, and histoplasmosis or tetanus, in that order. The addition of further tests yielded very little decrease in the determination of anergy. A subset of data involving 253 patients in which all five antigens were applied was analyzed with the same statistical methodology and produced the same ordering of antigens.

In Table 2 the estimated anergy in our population based on a negative PPD reaction (<5 mm) is recorded in relation to the control antigens. Figure 2 shows that these results are independent of the millimeters of induration (<5, <10, or <15 mm) used to determine a positive PPD reaction.

Figure 2 demonstrates that the inclusion of tetanus before histoplasmin antigen does not appreciably alter the slope of the curve and hence the amount of anergy determined. Even though anergy is slightly less common to tetanus than to histoplasmin, the insertion of tetanus before histoplasmin does not seem to noticeably change the slope of the anergy curve.

Seventeen percent of patients (48/288) with a neg-

Table 2. Estimated Anergy in Study Population Based on 5-mm Induration for PPD Test

Antigen Skin Tests with Negative Outcome	Conditioned on Jointly Negative Outcomes for	Estimated Conditional Probability	Estimated Joint Probability
PPD	(No other tests)	682/928=0.74	0.74
Cand	PPD	151/425=0.36	0.26
Mumps	Cand, PPD	86/133=0.65	0.17
Histo	Mumps, Cand, PPD	56/66=0.85	0.14
Tetanus	Histo, Mumps, Cand, PPD	49/54=0.91	0.13
Tric	Tetanus, Histo, Mumps, Cand, PPD	44/49=0.90	0.12

PPD denotes purified protein derivative; Cand, Candida; Histo, histoplasmin, Tric, Trichophyton.

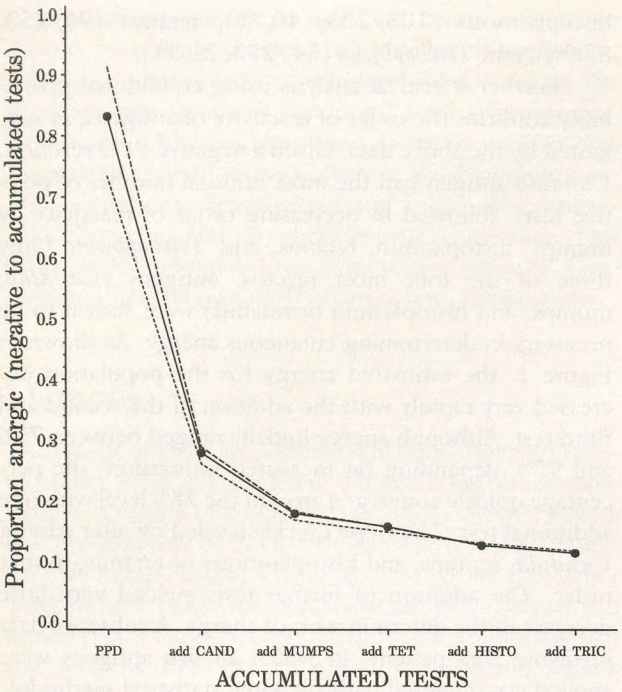

Figure 2. The modified order of the estimated anergy curve (tetanus before histoplasmin) shows decreasing percentage of anergy as more skin tests are added. The lower dashed line represents positive PPD test based on a minimum of 5-mm induration. The solid line represents positive PPD test based on a minimum of 10-mm induration. The upper dashed line represents positive PPD test based on a minimum of 15-mm induration. PPD denotes purified protein derivative tuberculin skin test, Mantoux method; CAND, *Candida;* TET, tetanus; HISTO, histoplasmin; TRIC, *Trichophyton.*

ative PPD reaction (<5 mm) were also unresponsive to all five control skin test antigens. Twenty-seven percent (246/928) of patients reacted positively (>5 mm) to PPD, but three of these patients were nonreactive (<5 mm) to all five skin test control antigens.

Discussion

If the PPD test is negative, the question frequently facing the clinician is: is the patient free of tuberculosis infection, or is the PPD test result a false negative secondary to cutaneous anergy? Estimates of nonreactivity to tuberculin in patients with microbiologically proven tuberculosis typically range between 10% and 25%.[10–13] Our study population showed an anergy rate of 12% after the administration of five control skin test antigens (Figures 1 and 2). This anergy should be considered *relative* anergy since three patients who had a positive (>5 mm) PPD reaction did not react to any of the five control antigens, as evidenced by a <5-mm reaction.

Control skin tests are currently considered appropriate to use in ruling out cutaneous anergy.[6] Although these control skin test panels are minimally invasive, relatively inexpensive, readily available, and reasonably reliable, each test adds to the cost. There is also additional discomfort due to needle sticks and occasional reaction from cutaneous and systemic responses to the antigens. If fewer tests give similar results, the clinician could minimize patient cost and discomfort without sacrificing sensitivity. Our study, based on over 900 patients, presents data that may be a useful guide for the physician in his selection of the fewest skin test control antigens that will most likely rule out anergy in a general patient population. These data suggest that the anergy rate falls and converges quickly to an irreducible minimum with the utilization of only three control skin tests. The choice of *Candida*, mumps, and tetanus or histoplasmin provides a reasonably sensitive anergy panel in determining DTH.

The use of tetanus instead of histoplasmin may be preferred for three reasons. First, substituting tetanus for histoplasmin did not appreciatively change the slope of our anergy curve (Figure 2). Second, our study was conducted in an area of considerable subclinical histoplasmosis: histoplasmin sensitivity is known to be lower in geographic areas where the fungus is not as prevalent. Finally, many physicians are hesitant to use histoplasmin as a skin test antigen because it may interfere with the serology and diagnosis of histoplasmosis.[14] Exposure to *Candida*, mumps, and tetanus is felt to be generally distributed equally throughout the United States; therefore, data concerning these two antigens should not vary with geography.

The mathematical model used in this paper, which maximized the likelihood of positive test results by making assumptions about the missing data, could be subject to selection bias. It is possible to get a different order of tests if all patients received all tests. However, similar results were obtained from an analysis of the subset of 253 patients who received the PPD and all five skin tests, thus supporting the model assumptions and making a difference unlikely.

The results of our study are supported by several recent studies,[8,15–17] which also recommend using a combination of two or three of the following three antigens: *Candida*, mumps, and tetanus to screen for PPD anergy in the high-risk HIV population. While our study population was not at high risk for HIV infection, our conclusions for antigen choice and number were quite similar. In patients studied from a population of military recruits infected with HIV, tetanus was more sensitive than *Candida* and mumps in establishing the presence of DTH.[15] This higher sensitivity to tetanus would seem logical in

light of the high rate of tetanus immunization in military recruits.

Our study does not address the efficacy of the control skin test antigen in predicting the presence of cutaneous anergy to the PPD test. It does suggest, however, the selection of *Candida*, mumps, and histoplasmin or tetanus as adequate and reasonable choices when attempting to control for anergy in the face of a negative PPD test reaction. It is hoped that the use of these three antigens would decrease unnecessary skin testing and expense to the patient while still providing reasonable screening for cutaneous anergy.

There are several controversies that influence the recommendations of this paper. First, the millimeters of induration required for a positive response for anergy-control skin tests has not been established. Some authors recommend <5 mm-induration.[8,15,18] Others recommend <3-mm induration,[5] others <2 mm,[16,19] some even suggest <1 mm or any cutaneous response.[4] We selected 5 mm as our cutoff point for a positive or negative control antigen response.

Second, the number of millimeters of induration that define a positive PPD is not universally agreed on. This controversy is particularly important in the patient with HIV infection in whom the suggested size of a positive PPD reaction has ranged from 2 mm to 10 mm induration.[20–25] However, the American Thoracic Society has recently suggested >5-mm induration should be considered positive for a patient with HIV infection. Furthermore, they suggest that the mumber of millimeters of induration required for a positive PPD test result in other patients should be determined by the patient's risk of acquiring tuberculosis. Patients are placed in three risk categories with corresponding millimeters (>5, >10, and >15 mm) of induration required for a positive PPD reaction; these recommendations are summarized in Table 3.[7]

If DTH controls remain clinically relevant, use of multiple antigen delivery devices could potentially save cost and time. A multitest antigenic device called Multitest CMI (Connaught Laboratories, Inc) has been used for several years to deliver intradermally seven control antigens (tetanus, diphtheria, *Streptococcus,* old tuberculin, *Candida, Trichophyton,* and *Proteus*) with a glycogen control at one application. Use of this device may be limited, however, by the differences in the amount of antigen delivered to each patient's skin, which may vary according skin texture, thickness, and other factors.[25]

Finally, clear-cut indications for the application of skin test control antigens has not been established. Most workers would agree that antigen panels are not appropriate for routine skin testing or surveillance. They are most helpful when evaluating populations at risk for tuberculosis who have debilitating comorbid conditions known to be associated with anergy. Common examples are patients with HIV infection, malnutrition, immunosuppression due to drug therapy, cancer, collagen vascular diseases, and debilitation due to a host of other causes.

Table 3. Quantification of Positive PPD Reaction According to Likelihood of Tuberculous Infection

Group I (≥5 mm of induration)
1. Persons who are positive for human immunodeficiency virus (HIV) or at risk for HIV infection
2. Persons who are close contacts to tuberculosis patients
3. Persons who have chest radiographs compatible with old healed tuberculosis
Group II (≥10 mm of induration)
1. Persons from Asia, Africa, or Latin America where tuberculosis is highly prevalent
2. Intravenous drug users
3. Medically underserved and low-income minorities
4. Residents of institutions, such as correctional and nursing facilities
5. Persons with high-risk medical illnesses, such as silicosis, diabetes mellitus, gastrectomy, and renal failure
6. Persons who work or attend health care facilities, child care facilities, etc
Group III (≥15 mm of induration)
All others not in Group I or II.

Table is based on information from Bass JB Jr, Farer LS, Hopewell PC, Jacobs RF, Snider DE Jr. Diagnostic standards and classification of tuberculosis. Am Rev Respir Dis 1990; 142:725–35.

In a recent state-of-the-art article, Pesanti[26] reviewed the negative tuberculin test and suggested that skin test control antigens may not substantially improve the interpretation of a properly applied tuberculin test, especially in the patient infected with HIV. In our study population, however, 88% of our patients with negative PPD skin tests responded to at least one control antigen. In a small study of nursing home residents,[27] only 7% (2 of 29) showed anergy when tested with the Multitest device using >2-mm induration as a positive reaction. In a larger study of 262 nursing home residents,[28] 55 (21%) were nonreactive to PPD (including booster testing), *Candida*, and *Trichophyton*. However, when retested 6 months later, 6 of 55 patients were positive (>10 mm) to PPD. Future studies are needed to address the efficacy of skin test control antigens in determining the presence of DTH in relation to the PPD test.

Conclusions

Our recommendation is to use three skin test antigens (*Candida*, mumps, and tetanus or histoplasmin) for anergy control when administering a PPD to patients who may have active tuberculosis infection and who are at risk for being anergic to PPD.

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