Table 1—Ratio of Isoniazid-Preventable Tuberculosis to Isoniazid-Associated Hepatitis*

<table>
<thead>
<tr>
<th>Race, Sex</th>
<th>Age in Years</th>
<th>25</th>
<th>35</th>
<th>45</th>
<th>55</th>
<th>65</th>
</tr>
</thead>
<tbody>
<tr>
<td>W, M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.7</td>
<td></td>
<td>4.2</td>
<td>1.9</td>
<td>0.9</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>W, F</td>
<td></td>
<td>19.5</td>
<td>3.7</td>
<td>1.8</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>N, M</td>
<td></td>
<td>25.8</td>
<td>3.9</td>
<td>1.7</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>N, F</td>
<td></td>
<td>24.7</td>
<td>4.3</td>
<td>1.8</td>
<td>0.9</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Data from Comstock and Edwards.4

Against those risks, we have to consider the risk of preventive therapy. Let me show you the results of a large-scale surveillance undertaken cooperatively with 21 health departments. It is clear that with age there is an increase in the rate of isoniazid-associated hepatitis (Fig 8).2 The rate quickly goes up in older people within the first three months of therapy. Note that in the younger age group there is little increase during the first months, and the curve is entirely different from the others with such a long lag period. This could suggest that some of the hepatitis is not related to isoniazid administration. There are other causes for elevated levels of serum glutamic oxaloacetic transaminase and other symptoms. Unfortunately, there is no way at present to distinguish between those cases caused by isoniazid and those caused by viral infections.

Table 1 represents data that Dr. George Comstock3 has evaluated to weigh the risk of tuberculosis against the risk of isoniazid-associated hepatitis. Up to the age of 45 years, the tuberculosis risk outweighs the hepatitis risk in all four race-sex groups. But in the 55-year age group, the balance is shifted to the other side for men but stays close to equal for women.

REFERENCES


Tuberculin Testing Antigens and Techniques

Robert A. MacLean, M.D.*

The first “tuberculin test” was administered by Robert Koch. At the Tenth International Congress of Medicine held in Berlin in 1890, he announced the discovery of a curative agent for tuberculosis.1 This “agent” was, in fact, a broth culture filtrate of tubercle bacilli to which Koch gave the name, tuberculin. The announcement was greeted with great interest and some skepticism by the medical profession. It was soon determined that tuberculin was not curative. However, the significant discovery was the difference in reactions observed when tuberculin was injected subcutaneously in tuberculous and nontuberculous patients. In the former a febrile response from 102°F (38.9°C) to 104°F (40.0°C) occurred in 10 to 12 hours, along with vomiting, rigors, and other constitutional symptoms. In nontuberculous patients the only reaction to the injection was slight pains in the limbs, a sense of transient fatigue, and a small rise in temperature, if any. This observation by Koch laid the foundation for one of the most valuable and widely used diagnostic tests in both veterinary and human medicine and in the field of public health.1

It may surprise physicians to learn that veterinarians were the first to use tuberculin testing, in the control of tuberculosis in cattle. In a series of dramatic demonstrations, a high degree of correlation was shown between tuberculin sensitivity and lesions of tuberculosis at autopsy. Until recently, the usefulness of tuberculin testing in man was limited because of an infection rate of about 80 percent. Today, with only an estimated 16 million tuberculous infected persons in the United States and new infections in children occurring at a rate of only one in 10,000 per year, the test has become a most useful tool for diagnosis and public health control.2 Identifying infected high-risk individuals who may be candidates for preventive treatment is a major part of modern tuberculosis control programs.

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CHEST, 68: 3, SEPTEMBER, 1975 SUPPLEMENT

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TYPES OF TESTS

The discovery that the administration of tuberculin onto or into the skin (rather than subcutaneously) eliminated the generalized reaction in favor of a localized one at the injection site led to the development of a number of different testing techniques. Today, the cutaneous, or Moro, patch test has been discarded as unreliable. Survivors are the various types of multiple-puncture tests (Tine, Heaf, and MONO-VACC) and intracutaneous tests by intradermal needle or jet injector.

Multiple-Puncture Tests

There are three currently available multiple-puncture tests, the Heaf test, the Tine Test, and MONO-VACC. All of these use concentrated tuberculin with no way to standardize the amount of tuberculin introduced. They are intended to be used as screening tests only. All positive reactions should be confirmed with an intradermal Mantoux test.

Heaf. The Sterneedle modification of the Heaf test uses a spring-loaded gun and reusable cartridges. Heaf solution is concentrated purified protein derivative of tuberculin (PPD). A sterile cartridge is attached to the gun, dipped into an inverted cap containing Heaf solution, pressed perpendicular to the volar surface of the arm, and rotated to evenly distribute the material; the trigger is then pulled, driving six points through the film of liquid tuberculin 1 mm into the skin. Advantages for screening are the speed of administration, patient acceptance, and the minimal training required to give the test. Reading is best done at 48 to 72 hours. Induration at the puncture sites can be measured, or the results can be more simply quantitated into the following five categories: grade 0, no induration; grade 1, discrete papules that do not coalesce; grade 2, induration coalesces to form a ring; grade 3, a solid plaque; and grade 4, a solid plaque with blisters.

Virtually all grade 1 Heaf reactions represent cross-reactivity with atypical mycobacteria, since retesting with a 5 tuberculin-unit (TU) intradermal Mantoux test will almost always produce a negative (0 to 4 mm) or doubtful (5 to 9 mm) reaction. Grades 2 through 4 Heaf reactions are less likely to represent cross-reactions but, except for extremely strong reactions, should always be confirmed.

Tine. The Tine-Test cartridge has four metal prongs, 2 mm long, coated with dried US standard old tuberculin. It is also a convenient screening test that requires minimal skill to apply. The test is best read at 48 to 72 hours, with the amount of induration present measured in millimeters. Two millimeters or more of palpable induration with the Tine Test has been shown to correlate well with 6 mm or more of induration from a 5-TU test with reference-standard PPD (PPD-S). Doubtful or positive results need to be confirmed with a 5-TU intradermal test. In my experience the Tine Test is more likely to produce both false-positive and false-negative results.

MONO-VACC. The MONO-VACC test uses liquid old tuberculin applied with nine plastic points in an area of approximately one-sixteenth of an inch mounted on a plastic ring that fits onto the thumb. Old tuberculin is applied to the points, which are then pressed into the skin. Again, since the dose administered cannot be measured, doubtful or positive reactions should be confirmed with an intradermal 5-TU Mantoux test.

Intracutaneous Tests

Jet Injection. This method uses a jet gun with a special intradermal nozzle to deliver 5 TU in 0.1 ml of tuberculin intracutaneously under high pressure. However, unless the gun is precisely calibrated and properly applied, it often fails to deliver the recommended dose. This test has little practical use in the United States today.

Intracutaneous Injection (Mantoux). This is the most reliable tuberculin test available and should always be used in high-risk situations and to confirm positive multiple-puncture tests. It is performed by the intracutaneous injection of 0.1 ml of PPD, usually containing 5 TU (0.001 mg), into the skin of the volar or dorsal surface of the forearm. The injection is made with a short (one-half inch), bluntly beveled, platinum (26-gauge) or steel (27-gauge) needle with a glass or plastic tuberculin syringe. The injection should be made just beneath the surface of the skin with the needle bevel upward. A discrete wheal 6 mm to 10 mm in diameter should be produced. If the injection is made too deep and no wheal appears, the test should be immediately reapplied at a site at least 2 inches away. The test is read at 48 to 72 hours by measuring the maximum transverse diameter of induration in millimeters. Reading should be done with good lighting and with careful palpation of the test site.

ANTIGENS

The currently available material for intracutaneous (Mantoux) testing today is PPD, stabilized in solution with the addition of an antiabsorbant, polysorbate 80 (Tween 80), and calibrated to contain 5 TU in each 0.1 ml. Current lots of PPD are standardized for biologic activity against PPD-S. Portions of the original lot (No. 46908) produced by Dr. Florence Seibert in 1939 were sent to the Division of
Biologic Standards, National Institutes of Health, Bethesda, Md, to serve as the US reference standard. This lot was adopted in 1952 as the International Standard for Purified Protein Derivative of Mammalian Tuberculin by the World Health Organization Expert Committee on Biological Standardization.9

Nonstabilized dilute solutions of PPD have been shown by a number of investigators to rapidly lose their potency by adsorption to glass or plastic surfaces of vials or syringes. Landi et al10 demonstrated that nonstabilized tuberculin in solution in a syringe could lose up to 25 percent of tuberculin activity after only 20 minutes and more than 80 percent after 24 hours. More recently Landi and Held11 have demonstrated the effect of various wavelengths of light in reducing potency of stabilized tuberculins stored in clear glass vials. Polysorbate 80 effectively blocks adsorption to glass or plastic surfaces without interfering with the test itself.12,13 Grzybowski et al14 demonstrated that fresh solutions of nonstabilized tuberculin varied considerably from one manufacturer to another, and from one lot to another of the same manufacturer. Fortunately, these products are no longer available, having been replaced by stabilized tuberculins with a high degree of comparability between different manufacturers and lots.15

Atypical Antigens

During the course of epidemiologic studies to determine the causes of low-grade tuberculin sensitivity in the United States, the Tuberculosis Branch of the Center for Disease Control produced multiple, atypical, skin test PPD antigens. When standardized against 5 TU of PPD-S, these antigens were used to help differentiate those individuals sensitized to one or more atypical mycobacteria from those infected with M tuberculosis. By giving dual or multiple simultaneous tests and comparing the different reaction sizes, it was shown that most doubtful reactors to PPD-S (6 to 9 mm induration) could be demonstrated to exhibit significantly stronger reactions to one or more atypical antigens. Differential skin testing with multiple antigens in diseases caused by atypical mycobacteria is practically never of value in establishing which particular Mycobacterium is responsible for the disease. This is because of the high cross-reactivity (low specificity) exhibited by these antigens. These special PPD antigens are not available for general use. However, the information gained from nationwide studies of various population groups gave us the basis for current recommendations on skin test interpretation.16-18

Second-Strength PPD

Both first-strength (1-TU) and second-strength (250-TU) PPDs have limited use in current practice. In our experience, even in cases of active tuberculosis, reaction size and severity to intermediate-strength (5-TU) PPD is rarely so strong that a weaker testing solution would have been preferred. Since current guidelines on interpretation of skin test reactions have been based on the size of reaction to a 5-TU test, this strength should be used routinely. Because of its potency, second-strength (250-TU) PPD will elicit a nonspecific sensitivity to any mycobacterial antigen. A positive test, therefore, is of limited value. A negative test in a patient with unimpaired delayed hypersensitivity may be of some value in excluding tuberculosis as a diagnostic possibility. However negative second-strength tuberculin tests have been shown to occur occasionally in proved cases of tuberculosis.19

INTERPRETATION OF SKIN TEST REACTIONS

The following interpretations are recommended by the American Thoracic Society Committee on Diagnostic Skin Testing:8

Intracutaneous Mantoux and Jet Injection Tests

With the standard test dose, the following interpretations are recommended:

Induration 10 mm or More: Positive Reaction. This is interpreted as positive for past or present infection with M tuberculosis, because reactions this large most likely represent specific sensitivity. The test does not need to be repeated for confirmation in ordinary circumstances, unless there is reason to question the validity of the test.

Induration 5 mm to 9 mm: Doubtful Reaction. Reactions in this size range reflect sensitivity that can result from infection with either atypical mycobacteria or M tuberculosis; hence, they are classed as doubtful. However, a person with a doubtful reaction who is known to have been in close contact with a person sputum positive for M tuberculosis or a person having radiographic or clinical evidence of disease compatible with tuberculosis should be regarded as probably infected with M tuberculosis. For all other persons, if an appropriate antigen for atypical mycobacteria is available, an intracutaneous test with such an antigen may be applied at the same time as a repeat tuberculin test.

Induration 0 mm to 4 mm: Negative Reaction. This reflects either a lack of tuberculin sensitivity or a low-grade sensitivity that most likely is not caused by M tuberculosis infection. No repeat test is necessary unless there is also suggestive clinical evidence.

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of tuberculosis. If the person is the contact of a person with tuberculosis, he should be followed according to the established routine for contacts.

**Multiple-Puncture Test**

In determining the size of induration, measure the diameter of the largest single reaction. If the reaction consists of discrete papules, the diameters of separate areas of induration should not be added. For screening tests, the following interpretation is suggested:

**Vesiculation: Positive Reaction.** If vesiculation is present, the test may be interpreted as positive, in which case the management of the patient is the same as that for one classified as positive to the Mantoux test.

**Induration 2 mm or More: Doubtful Reaction.** Even though such reactions may be due to *M tuberculosis*, a significant proportion of them may not be confirmed by a positive standard Mantoux test. This is particularly true of small reactions. Therefore, a standard Mantoux test should be done on all persons in this group, and management should be based on the reaction to the Mantoux test or on the results of dual testing, using PPD and PPD derived from Battey bacilli (PPD-B). Commercial PPD-B is not available at this time.

**Induration Less than 2 mm: Negative Reaction.** There is no need for retesting unless the person is a contact of a patient with tuberculosis or there is clinical evidence suggestive of the disease.

**Problems in Interpretation**

Not all patients with active tuberculosis will have a positive standard (5-TU) tuberculin test. A few will also have a negative second-strength (250-TU) test. Sensitivity generally develops six to eight weeks after initial infection and, once acquired, tends to persist. It may wane with advancing age. Sensitivity may decrease or disappear temporarily during any severe or febrile illness, measles or other exanthems, administration of live viral vaccines, sarcoidosis, overwhelming tuberculosis, and the administration of immunosuppressive drugs. Sensitivity to tuberculin may decrease or disappear if treatment of the infection is given in its earliest stages. If tuberculosis sensitivity has not been challenged by testing in many years, the initial test may be negative or doubtful. A repeat tuberculin test may then "boost" or increase the size of the reaction, sometimes being interpreted as a "conversion." This booster phenomenon is primarily a problem in persons over 55 years of age. Skin test "converters" should have a difference in reaction size of at least 6 mm or more before being considered true converters (see Dr. Comstock's report on page 465).

With the improvement in reliability of tuberculin testing antigen, the largest problem that remains is unreliable results due to improper test administration or interpretation. Testing should be done only by individuals who have had proper training and experience. Unfortunately, it is too often the least qualified member of the medical team to whom this responsibility is delegated. The problems with tuberculin testing were well summarized by Edwards:

Like any diagnostic test, the test result must be considered in context. Test material, method of administration and the condition of the person tested are all of consequence to the reaction. Of what value would an electrocardiogram be if the clinical history were unknown, the leads improperly placed, the stylus faulty, or the reader inexperienced or improperly trained.

**References**

15. Kent DC: Memorandum to all American Tuberculosis Association members. February 1972
16. Hsu KH, Jenkins DE: The significance of low-grade
Parent Reading and Reporting of Children’s Tuberculin Skin Test Results

Russell S. Asnes, M.D.;** and Sajid Maqbool, M.D.†

Tuberculin skin testing, an established method of detecting tuberculosis infection, is widely employed in pediatric practice. Although there has been recent debate concerning the value of routine tuberculin skin testing, the American Academy of Pediatrics recently renewed its recommendation that such testing be performed on all children during the first year of life and annually or biennially thereafter.1,2

The tuberculin skin tests used most commonly are the Tine, Heaf, MONO-VACC, and the Mantoux tests. The successful use of these tests is dependent on the employment of proper technique in the intradermal placement of a tuberculin antigen and the accurate reading and interpretation of test results. Tuberculin skin tests are tests of delayed hypersensitivity, and the test results are not read for at least 48 hours following administration of the antigen. The reading of test results by trained personnel presents few problems when one is dealing with a captive population, such as school children or hospitalized patients. However, when evaluating children in the setting of a pediatric clinic or emergency room, the usual procedure has been to depend upon parents to keep a return appointment to have the child’s skin test read. Thus, parental compliance with return appointments is essential for successful testing.

Broken appointments are a common and significant problem in pediatric clinics, particularly those which provide predominantly acute episodic care and which lack continuity in physician-patient relationships. Such is the case with the Pediatric Clinic of the Columbia-Presbyterian Medical Center in New York City. This clinic serves a culturally diverse and economically disadvantaged population in upper Manhattan and the southeast Bronx. The overall broken appointment rate for the clinic exceeds 40 percent. In 1973, approximately 70,000 clinic visits were recorded, and more than 3,000 tuberculin Tine Tests were administered for specific indications. Children who received skin tests were given an appointment to return to the clinic 72 hours following the administration of the Tine Test for the reading of results.

The clinic staff became increasingly concerned about a seemingly high rate of noncompliance with appointment keeping for tuberculin skin test reading. This concern prompted an investigation of the actual rate of noncompliance and of factors which might contribute to it.3 Four hundred eighty-eight consecutive children to whom Tine Tests were administered were studied. The indications for testing included a history of persistent fevers, failure to thrive, pneumonia, and adenopathy. In 28 percent of the children tested, there was noncompliance, i.e., the child did not keep a return appointment for test reading. We were somewhat surprised that the rate of noncompliance was this low. A number of variables which could possibly affect compliance were studied. These included the distance of the patient’s residence from the clinic, race, age of the mother, source of payment for medical services, previous broken appointment rate, parental understanding of the purpose of the Tine Test, and family size. Only the latter factor, family size, could be shown to have

18 Edwards PQ: Significance of the tuberculin test today. Clin Notes Respir Dis 8, Fall (No. 2) 1969