Four-Stage Tuberculin Testing in Elderly Subjects Induces Age-Dependent Progressive Boosting*

Raul Van den Brande, M.D.; and Maurits Demedts, M.D., F.C.C.P.

We administered four sequential tuberculin skin tests (5 TU, PPD) with intervals of one week to 223 subjects older than 65 years of age to evaluate whether elderly subjects demonstrated progressive boosting. Indurations of at least 10 mm with increases of at least 6 mm (over the previous test) were considered significant reactions, and these were found in 29 percent of the subjects after test 1, in 43 percent after test 2, in 53 percent after test 3, and in 57 percent after test 4 (p<0.05), ie, only about 50 percent of all the positives were detected after the first test. The percentage of positive reactors was inversely related to age (p<0.001), yet this age-dependent difference decreased with increasing number of tests. For the 65- to 74-year-old age group, 44 percent reacted positively after the first test and after three tests almost a plateau of 65 to 70 percent positive reactors was reached, suggesting that a minority only of about 30 to 35 percent of these geriatric patients might have outlived their bacilli or were never infected. For the 75- to 84-year-old age group, 24 percent reacted after the first test and 55 percent reacted after the fourth one. For the older than 85-year-old age group, 19 percent positive reactors were found after the first test and 46 percent were found after the fourth test, without clear-cut leveling off toward a plateau value, suggesting that additional tests would induce further boosting. Mean diameters of positive reactions were 15 to 24 mm, and were mostly at least 12 mm larger than in the previous tests. These data support the hypothesis that the negative tuberculin reaction, which is often found in elderly subjects, is mainly due to the failing immune response to tuberculin antigen that can be restored progressively by repeated administrations. These findings, furthermore, emphasize that especially in elderly, care should be taken not to interpret a boosting reaction as a conversion and especially that neither a two-step testing as recommended by the ATS and CDC (Am Rev Respir Dis 1990; 142:723-35) nor even a four-step testing may suffice to detect all positives in this type of population.

Although there is a striking decline in the incidence of tuberculosis in Western countries, the prevalence of disease is increasing among elderly patients and in this latter group, the relative contributions of recrudescence of an old infection vs a new infection have been questioned.

Stead et al2 reported nosocomial spread of the disease among persons in nursing homes and stated that elderly patients could develop a "second" primary infection, having outlived their initial infection. This interpretation was based on the high prevalence of "unusual" roentgenographic presentations and on the conversion of the tuberculin skin test.2,3

In elderly subjects, the percentage of significant reactors on tuberculin declines with age.4,5 This is attributed to a specific waning of cell-mediated immunity for tuberculin antigen, rather than to a generalized anergy or a diminished cutaneous reactivity of the elderly patient.5,6 Furthermore, it has been shown that a booster phenomenon that can be seen in a second test done as soon as a week after the first test is frequently encountered among persons older than 55 years of age.7 This boosting effect is probably the reason for an inordinately high conversion rate, which has been reported in some testing programs. Failure to make a distinction between boosting and conversion may lead to a needless treatment with antituberculous drugs and may subsequently expose a number of patients to the possible risks of drug toxic reactions.8,9 The recent Official Statement of the American Thoracic Society (ATS) and the Centers for Disease Control (CDC)9 stipulates in this respect that a positive reaction to a third test (with an increase of more than 10 mm) is likely to represent the occurrence of infection.

We performed four sequential tuberculin skin tests with intervals of one week in elderly subjects to evaluate the age-dependent progressive boosting and to investigate whether a plateau value of positive reactors could be reached and what percentage of elderly appeared to have positive reactions after sufficient boosting.

Patients and Methods

We examined 223 subjects, older than 65 years, staying in a geriatric hospital: 71 were 65 to 74 years old; 104 were 75 to 84 years old; and 48 were at least 85 years old. None of these subjects had any evidence of active pulmonary tuberculosis at the moment of the tests nor were any suspected of being silent carriers. They have been followed up for one year and, to our knowledge, active pulmonary tuberculosis has not developed in any of them. Most of them were convalescing from surgical procedures, cerebrovascular accidents, or medical illnesses (mostly ischemic heart disease), yet all were in good and stable clinical condition at the time of testing.

To their knowledge, no prior tuberculin skin test had been

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performed for at least one year. All skin tests were applied by us with 5 tuberculin units (TU) of commercially prepared polysorbate 80 (Tween 80) stabilized purified protein derivative (PPD) Tuberculin Berna, Bern, Switzerland. The tests were applied by trained and experienced staff nurses, using the intracutaneous method (Mantoux) on the volar side of the forearm. If a test did not produce a wheal, it was repeated 10 cm away from the first application place. The results, which were read by one of us (F.V.D.B.) at 48 to 72 hours, were recorded as millimeters of induration measured transversely to the long axis of the forearm.\textsuperscript{9} Indurations of 10 mm or more were considered positive and those less than 10 mm were considered negative. The patients with an initial tuberculin test of less than 10 mm of induration were retested after seven days. The skin test was then applied to the other forearm. This procedure was repeated up to a total of four tests or until a boosting reaction occurred. We defined a boosting reaction as an increase of at least 6 mm in reaction size (from an initial reaction of less than 10 mm of induration) to at least 10 mm in a subsequent test.\textsuperscript{10}

Statistical analysis consisted of the calculation of the effect of age on the number of tests needed to obtain a positive result, using an ordinal logistic regression model\textsuperscript{11} on all individual values. The procedure "logistic" of the SAS package was applied for this.\textsuperscript{12}

**RESULTS**

In Table 1, the number of positive reactions to four sequential tests for three age groups are shown, \textit{i.e.}, the ratio of positive reactors over the number of tests for each sequence. Each test sequence brings about an additional number of reactors, although a declining rate of additional positive reactions with an increasing number of tests is apparent, however, without reaching zero even at test 4. This decline is age dependent: it is lowest in the oldest age group and highest in the youngest age group. This age-dependent difference in percentage of positive reactions decreases from the first to the third test and it is even reversed for the fourth test: the difference between the youngest and oldest age group is 25 percent \textit{(i.e.,} 44 percent to 19 percent \textit{)} for test 1, 12 percent for test 2, 2 percent for test 2, and −6 percent for test 4.

The cumulative percentage of positive reactors is shown in Table 2 and Figure 1: after the first test, 29 percent of the 223 patients present a significant reaction; after the second test, the cumulative percentage of positive reactions is 43 percent \textit{(i.e.,} an increase of 14 percent), after the third test 53 percent \textit{(i.e.,} increase of 10 percent), and after the fourth test 57 percent \textit{(i.e.,} increase of 4 percent). The increases are significant from test 1 through test 4 \textit{(p<0.05)}, with an overall doubling of the percentage of positive reactors between the first and fourth test. The highest overall percentage of reactors is found in the youngest age group, \textit{i.e.}, 66 percent and a leveling off between test 3 and 4 is encountered. For the oldest age group, positive reactions are found in 46 percent only, but even with test 4, no leveling off occurs, suggesting that no maximum has been approached.

The age effect on the tuberculin reaction was calculated by an ordinal logistic regression model\textsuperscript{11} applied on all individual values. This indicated a significant positive correlation between age and the number of tests needed to show a positive reaction \textit{(p<0.001)}.

The mean induration for each positive test and for each age group is shown in Table 3. No significant differences in indurations for the positive tests are noted depending on sequence or age. In most subjects,

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**Table 1 — Ratios of Positive Reactors for Each of Four Tests**

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Test 1 (%)</th>
<th>Test 2 (%)</th>
<th>Test 3 (%)</th>
<th>Test 4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65-74</td>
<td>31/71 (44)</td>
<td>14/40 (25)</td>
<td>5/30 (17)</td>
<td>1/25 (4)</td>
</tr>
<tr>
<td>75-84</td>
<td>25/104 (24)</td>
<td>16/79 (20)</td>
<td>13/63 (20)</td>
<td>3/50 (6)</td>
</tr>
<tr>
<td>≥85</td>
<td>9/48 (19)</td>
<td>53/9 (13)</td>
<td>5/34 (15)</td>
<td>3/29 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>65/223 (29)</td>
<td>31/158 (20)</td>
<td>23/127 (18)</td>
<td>7/104 (7)</td>
</tr>
</tbody>
</table>

**Table 2 — Cumulative Numbers of Positive Reactors**

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Test 1 (%)</th>
<th>Test 2 (%)</th>
<th>Test 3 (%)</th>
<th>Test 4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65-74</td>
<td>31/71 (44)</td>
<td>14/40 (25)</td>
<td>5/30 (17)</td>
<td>1/25 (4)</td>
</tr>
<tr>
<td>75-84</td>
<td>25/104 (24)</td>
<td>16/79 (20)</td>
<td>13/63 (20)</td>
<td>3/50 (6)</td>
</tr>
<tr>
<td>≥85</td>
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<td>Total</td>
<td>65/223 (29)</td>
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<td>23/127 (18)</td>
<td>7/104 (7)</td>
</tr>
</tbody>
</table>
Table 3—Mean Induration of the Positive Reactions, mm*

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>65-74</td>
<td>24 (10-45)</td>
<td>18 (11-24)</td>
<td>17 (10-24)</td>
<td>16 (13-21)</td>
</tr>
<tr>
<td>75-84</td>
<td>19 (12-30)</td>
<td>16 (15-26)</td>
<td>15 (12-21)</td>
<td>16 (13-20)</td>
</tr>
<tr>
<td>≥85</td>
<td>19 (14-24)</td>
<td>24 (12-42)</td>
<td>15 (12-20)</td>
<td>16 (14-18)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses represent range of induration for positive reactions. Negative reactions are not shown.

the positive test was at least 12 cm larger than the preceding negative test.

**Discussion**

This study was undertaken to provide more insight in the value, the interpretation, and the pitfalls of tuberculin skin testing in elderly subjects. Our main finding was that in subjects older than 65 years without active tuberculosis, the number of positive reactors on 5 TU PPD increases progressively with each of four sequential weekly tests. Since our subjects have been followed up for one year and since not one has developed active pulmonary tuberculosis, we are confident that our findings are attributable to a continued boosting of the tuberculin reaction and not to a true conversion of the tuberculin skin test.

We conclude that our subjects are representative of the general population as a whole because the percentages of positive reactors after the first test were not lower and even higher than in other published series. Indeed, for all age groups combined, we obtained a positivity of 29 percent after the first test, which is clearly higher than the 12.3 percent reported by Burnst in et al. in institutionalized patients or the 12 percent of Stead et al. in newly admitted nursing home residents, and is comparable to the 30 percent found by Alvarez et al. in a domiciliary population.

In our 65- to 74-year-old age group, 44 percent positive reactors were found after the first test, while Stead reported about 20 percent positives in this age group at entry in a nursing home and about a 40 percent point prevalence in all residents. Our 75- to 84-year-old age group presented 24 percent positive reactors after the first test vs about 15 percent and 30 percent, respectively. In our group of at least 85-year-old subjects, we registered 19 percent positives vs about 12 percent and 25 percent, respectively. Therefore, we may conclude that our finding of a progressive boosting was not due to a temporary anergy or loss of overall or cutaneous immunologic reactivity in our subjects at the time of the first test, which was progressively restored during the one or five weeks of investigation. The general and nutritional status of our subjects was rather good even at the time of the first PPD test, which also guarantees a representative age-related immunologic response. The finding of a significant age dependency (p<0.001) of the tuberculin reactivity conforms with the data of the literature and has been attributed to a falling immune response as a major cause for the negative tuberculin reaction in the geriatric subject, which can be restored when providing repeatedly tuberculin antigen.

This progressive boosting effect makes the interpretation of the tuberculin skin test in the elderly patients especially difficult and puts its clinical diagnostic relevance into question. Indeed, this boosting should not be confounded with a real conversion due to the occurrence of a primary or even "second" primary infection in the elderly, in which case it is recommended to start chemotherapy because of the risk of clinical tuberculosis developing. In the recent ATS and CDC statement, it is emphasized that a two-step testing procedure in the elderly reduces the likelihood of interpreting a boosted reaction as representing recent infection, and that a positive reaction to a third test (with an increase of more than 10 mm) is likely to represent the occurrence of infection. However, Welty et al. suggested that in elderly subjects, more than two skin tests are needed to fully elicit the booster phenomenon. This was confirmed by Gordin et al. who showed a progressive boosting on three sequential tests. We now demonstrate that only after three, four, or even more tests, a leveling off of the continuing boosting effect may occur and a plateau value apparently corresponding with the real positives is formed. In the subjects older than 85 years of age, even after four tests, no leveling off of additional responses occurs, indicating that subsequent tests would still induce further boosting. In favor of this hypothesis is the fact that after four tests, the total percentage of responders detected in these subjects of at least 85 years is less than in those 65 to 74 years old.

In the 65- to 74-year-old age group, the cumulative percentage of responders is 66 percent and almost a plateau level is reached between the third and fourth test, suggesting that this 65 to 70 percent represents the real positives; and thus that there are 30 to 35 percent real negatives, ie, who had never been infected with tubercle bacilli or who "outlived" these. This percentage of real negatives is lower than what could be assumed from other publications. These differences, however, may be due not only to the number of boosters applied but also to demographic differences in tuberculin prevalence between the US and European countries 40 years ago possibly due to World War II. Indeed, in the United States, the incidence of tuberculosis was 53.10^-5 in 1953 and 14.10^-5 in 1974, while in Belgium it was still 28.10^-5 in 1972 and 23.10^-5 in 1980. It may be estimated that at least 80 percent of the elderly subjects of the present study have been positive at one time in their life. Indeed, although only scarce historical data are available, it is apparent that in 1950, 50 percent of the 20-year-old...
Belgian subjects and almost 100 percent of the 50-year-olds were tuberculin positive, and those latter now belong to the elderly group of 60- to 90-year-olds. Even in the Netherlands, the percentage of positive tuberculin reactors among subjects 60 years old in 1975 was estimated around 80 percent (and those subjects should be almost 75 years old at the time of this study). Also, Rich20 forwards the assumption that 80 to 90 percent of elderly persons had been infected earlier in life.

The boosting reactions after the fourth as well as after the third or second test show a mean induration diameter of 15 to 24 mm, and in most of them an increase of clearly more than 12 mm in comparison with the previous negative test is found. This means that the statement of the ATS and CDC13 that an increase of more than 10 mm at a third test is likely to represent the occurrence of infection is not applicable to our subjects. Our findings are at variance with those of Stead and To.15 They found in populations in nursing homes with closed environment that minor increases in reaction size appeared to be due to immunologic recall and large increases of at least 12 mm indicated spread of infection. Our findings are also at variance with the statement of the ATS and CDC that an increase of more than 10 mm at a third test is likely to represent the occurrence of infection.

Although in our study, the mean size of induration in the ultimate test is larger than the figures given by Gordin et al.,16 and by Welty et al.,8 the difference may be seemingly only. Indeed, our mean number of 16 mm at test 4 (Table 3) represents the positive reactors of at least 10 mm only (range, 12 to 21 mm) while in the other two studies,8,16 the mean size for the nonzero reactors was given and thus also negative reactions (ie, of less than 10 mm) were included.

Finally, we must emphasize that an additional question that can be raised after our study is whether the booster reaction is mainly determined by the number of administrations of tuberculin antigen or rather by the total amount of tuberculin antigen administered. In this respect, it could be interesting to compare the end result of the administration of a given total dose of tuberculin over multiple repeated tests vs over one single booster test.

In summary, our findings of progressive boosting put the practical value of single or even dual tuberculin testing in elderly subjects into question. Although the tuberculin test, perhaps adjusted for dosage and interval, will retain some value in epidemiologic studies, it progressively loses its clinical diagnostic value in the elderly. We agree with Cauthen and Snider21 that "No really satisfactory solution to these problems seems possible without the development of a better test for identifying persons at risk of developing disease."

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