Assessment of the agglutination test for tuberculosis

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Geddes, D., Emerson, P., and Lacey, B. (1977). Thorax, 32, 349–351. Assessment of the agglutination test for tuberculosis. A new whole cell agglutination test for active tuberculosis, for which encouraging results have recently been reported, has been carried out on the serum of 112 subjects. The results have been disappointing; the test had no predictive value in the diagnosis of active tuberculosis.

Encouraging results have recently been claimed for a new whole cell agglutination test to diagnose active tuberculosis (Nicholls, 1975; Nicholls and Horsfield, 1976). Because the test is now being increasingly used in clinical practice, we feel it important to report our own less favourable experience with the same agglutination test in 27 control subjects and in 85 patients suspected of suffering from tuberculosis.

Patients

The test was done on the serum of 85 of our patients in whom diagnosis of active tuberculosis was initially considered possible. In 32 of these active pulmonary tuberculosis was diagnosed—by positive sputum culture in 23, by histology in two, and on clinical and radiological grounds in seven. The remaining 53 patients had no evidence of active tuberculosis and a definite diagnosis of another condition was made in 39; the other 14 are well with no evidence of tuberculosis after 10 months’ follow-up.

A further 27 control sera were tested, 20 from healthy women attending the antenatal clinic and seven from children aged from 3 months to 2 years. There was no suspicion of tuberculosis in any of these patients.

Methods

ANTIGENS

Most of the tests were done with a suspension kindly supplied by Dr. Nicholls and prepared from H37Ra according to Nicholls’ (1975) method. Other tests were made with a new antigen, also supplied by Dr. Nicholls and prepared from H37Rv. In both cases the stock suspension was kept at 5°C and diluted in 0·3% saline to give an opacity equal to Browns tube No. 1 immediately before use (C.1/3 with the H37Ra antigen and C.1/45 with H37Rv antigen). One attempt to prepare a suspension in our laboratory from H37Ra according to Nicholls’ (1975) method resulted in an unusable translucent viscous solution.

SERA

Sera were separated from red cells as soon as they were received and either stored at 5°C for testing within five days or at −18°C until thawed and tested or retested later.

TITRATIONS

Doubling dilutions of serum were made in 0·3% NaCl in glass-distilled water in 2½×⅜ inch glass tubes. To each tube an equal volume of diluted antigen suspension was added. The mixture was shaken, incubated at 50°C for two hours, and read after standing for 48 hours at 5°C. Attempts to improve the end points by using microtitre plates, by varying the diameter of the tubes, concentration of antigen, temperature, and time of incubation were all unsuccessful. With most of the sera the antigen prepared from H37Ra gave the clearer end-point.

In order to validate our laboratory’s methods, sera from 85 of our patients and controls were sent to Dr. Nicholls’ laboratory at the Midhurst Medical Research Institute for parallel titration. The results obtained there were in agreement with ours (either the same titre or only one tube differ-
ence) in 74 (88%). The titrations were repeated in our laboratory on 85 sera using a new antigen prepared from H37Rv. The results of the tests with the two antigens were the same in 77 (92%) of the 85 sera. Use of the new antigen therefore had no significant effect on the results.

Results

Table 1 shows the agglutination titres of the sera of patients according to whether the final diagnosis was active tuberculosis or not.

<table>
<thead>
<tr>
<th>Agglutination titre</th>
<th>Active</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/640</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>1/320</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>1/160</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>1/80</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>1/40</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>53</td>
</tr>
</tbody>
</table>

Table 2 shows a series of decision matrices indicating the results that are obtained with the test when different titres are taken as the cut-off point.

<table>
<thead>
<tr>
<th>Cut-off titre</th>
<th>1/640</th>
<th>1/320</th>
<th>1/160</th>
<th>&lt;1/80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test + ve</td>
<td>Test - ve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>7</td>
<td>16</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>None</td>
<td>8</td>
<td>18</td>
<td>35</td>
<td>38</td>
</tr>
</tbody>
</table>

Table 3 is derived from the decision matrices and shows in the first two columns the true positive and false positive ratios for each of the titres. The third column shows the probability of active tuberculosis given a positive test result, and in the fourth column the probability of no active tuberculosis, given a negative test result, for each of the titres.

It will be seen that none of the titres provided a diagnostically useful test. The cut-off titre used by Nicholls was 1/125. Our nearest titre to this was 1/160. Using this titre, false negative results would have been reported in 10 of our patients with undoubtedly active tuberculosis. Of these, eight had positive cultures, one had positive histology, and in one the diagnosis was on clinical and radiological grounds alone. Protein electrophoresis was done on eight of these 10 false negative sera and agammaglobulinaemia was excluded as a cause of the misleading results.

Table 4 shows the results of the control group. These were also unsatisfactory; at a cut-off titre of 1/160 the test would be read as positive in 18 of 20 healthy women and in four of seven healthy children.

<table>
<thead>
<tr>
<th>Agglutination titre</th>
<th>Antenatal</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/640</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>1/320</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>1/160</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1/80</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>7</td>
</tr>
</tbody>
</table>

Discussion

We were disappointed not to be able to confirm the encouraging results originally reported using this agglutination test. It seems most unlikely that our results are due to difficulties with the technique. The test is not difficult to do and the results have previously been shown to be reproducible between different laboratories (Nicholls and Horsfield, 1976). Furthermore, the test on our sera gave substantially the same results at the Midhurst Medical Research Institute and in our laboratory.

Of our 85 patients, 32 (38%) had active tuberculosis and 53 (62%) had no active tuberculosis. Thus if a serum was selected at random the probability of the patient having active tuberculosis, P (active tuberculosis), would be 0.38 and the probability of his not having tuberculosis, P (no active tuberculosis), would be 0.62. These probabilities are very near to those for any of the titres in Table 3 and strongly suggest that the test has no predictive value in the diagnosis of active tuberculosis.

The high incidence of false positive results in the control sera is unexplained. Although previous
Assessment of the agglutination test for tuberculosis

BCG vaccination might conceivably account for the results in the women attending the antenatal clinic, this cannot be the explanation in the children. There was a higher incidence of false positive results among young women in the patient group and the test may therefore be detecting hormonal differences.

Our results are remarkably similar to those of agglutination tests reported in the 1920s. Topley and Wilson (1929) concluded that agglutination tests were unreliable for the diagnosis of tuberculosis. On the present evidence there appears to be no reason to modify this conclusion.

References


Requests for reprints to: Dr. P. A. Emerson, Westminster Hospital, London SW1P 2AP.
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Thorax 1977 32: 349-351
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