THE IMMUNOSEROLOGICAL DIAGNOSIS OF TUBERCULOSIS: A COMPARISON OF TWO TESTS

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Abstract. A comparative study of the diagnostic value of the ICT-TB test and the TB-Dot test, based on laboratory examination, was carried out in 39 patients suffering from sputum positive pulmonary tuberculosis (25 males and 14 females, aged 16-50 years) and in 48 patients (27 males and 21 females, aged 17-55 years) suffering from non-tuberculosis pulmonary diseases, that had attended the Tembagapura Hospital and the TB Control Health Center Timika-Mimika, Papua. The diagnostic sensitivity of the ICT-TB test was 87.18%, the diagnostic specificity was 81.25%, the diagnostic positive predictive value was 79.07%, the negative predictive value was 88.64%, and the diagnostic efficiency was 83.91%. The diagnostic sensitivity of the TB-Dot test was 93.31%, the diagnostic specificity was 95.83%, the diagnostic positive predictive value was 94.74%, the negative predictive value was 93.85%, and the diagnostic efficiency was 94.25%. The results of the statistical analysis of the data obtained in this study revealed that the diagnostic specificity, the diagnostic positive predictive value and the diagnostic efficiency of the TB-Dot test were significantly higher ($p < 0.05$) than those of the ICT-TB test. However, the diagnostic sensitivity and the negative predictive value of both tests did not differ significantly ($p > 0.05$). Viewed from the point of their practicability, it can be justified that the ICT-TB test is a very practicable test, which needs only 15 minutes and does not require special instruments to perform the test, but is more expensive than the TB-Dot test. On the other hand, though the TB-Dot test is not very practicable and relatively time consuming, it has a significantly higher degree of diagnostic value and is much cheaper when compared to the ICT-TB test.

INTRODUCTION

Tuberculosis (TB) especially pulmonary TB is still a major public health problem in Indonesia as well as in other places of the world. The number of TB cases in Indonesia ranks it as number three in the world behind India and China (Sujudi, 2002). There are 583,000 new TB cases per year in Indonesia and 262,000 of them have positive acid-fast bacilli in their sputum (Aditama, 2002). The case fatality rate for TB in Indonesia is high (140,000 per year) (Dye *et al*, 1999).

It is important to note that primary resistance to the main anti-TB drugs is on the increase (Dye *et al*, 1999). The above mentioned data led to the decision that tuberculosis has to be brought under control. Case identification followed by adequate treatment are the key points in the TB control program. For the purpose of case identification, a reliable and practicable diagnostic tool has to be used (Handojo, 2001). In the last few years, two practicable serologic tests for TB, the locally made TB-Dot and the imported ICT-TB test, have frequently been used to support the diagnosis of tuberculosis.

The aim of this study was to compare the diagnostic value of the two serologic tests for TB in pulmonary tuberculosis.

MATERIALS AND METHODS

There were 2 groups of patients involved in this study. Thirty-nine cases were bacteriologically confirmed (microscopically as well as cultures) pulmonary tuberculosis consisting of 25 male patients and 14 female patients, aged 16-50 years, who had never received anti-TB treatment, and had attended the Tembagapura Hospital and TB Control Health Center Timika-Mimika in Papua. There were also 48 non-TB patients consisting of 27 male patients and 21 female patients, aged 17-55 years, that had attended the same treatment centers as mentioned above. Among them were 24 patients with pneumonia, 13 patients with chronic bronchitis, 4 patients with bronchiectasis, 4 patients with

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chronic obstructive pulmonary diseases (COPD), and 3 patients with chronic allergic pharyngitis. All of them had negative sputum culture as well as negative smears for *M. tuberculosis*. None of the patients in the study had received corticosteroids or other immunosuppressive drugs previously. None suffered from diseases that might interfere with the humoral immune response.

Each of the specimens of serum obtained from the patients in the study were tested with the local made TB-Dot (Mekar Jaya Diagnostika, Indonesia), and the ICT-TB (ICT Diagnostics, NSW, Australia) test that was imported. The procedures for the tests were followed in accordance with the manufacturer's instructions.

The ICT-TB test procedure

The principles of this immunochromatografic test have been described previously (Cole *et al*, 1996). Five highly purified antigens (including one of the 38 kDa) secreted by *M. tuberculosis* during active infection were immobilized in four capture lines on the test strip. The test detects the presence of immunoglobulin G (IgG) to these antigens. A total of 30 µl serum was added to the blue pad and diffused along the test strip. The test card was closed and the conjugate (anti-human IgG labeled with colloidal gold particles) bound any human IgG on the capture lines, producing one or more pink lines of color. The presence of one or more pink lines on the test strips was considered a positive result.

The TB-Dot test procedure

The basic principle of this test is an indirect enzyme immunobinding assay using as the solid phase a piece (12x7 mm) of nitrocellulose paper (Padmidewi, 1996).

The antigen used in this test is a polymerized peptide of the cytoplasm, cytoplasmic membrane and cell surface proteins of *M. tuberculosis* var *bovis* (ultrasonically disintegrated and ultracentrifuged). The conjugate used in this test was a goat antihuman IgG $F(ab)^2$ fragment. The substrate used in this test was a 0.02% solution of H_2O_2 in substrate buffer (pH 5.6) and 3-amino-9-ethylcarbozole (EAC) was used as the chromogen of the substrate. The nitrocellulose test strips, each containing one drop (1 µl) of the polymerized antigen, were distributed into the wells of the plastic tray provided by the manufacturer of the kit. One ml of diluted (1:3,200) sera (patient or control) was added into each well, and the tray was incubated at room temperature for 2 hours on a shaker (60 rotations per minute). Unbound sera components were removed with a micropipette and washed afterwards 3 times for 5 minutes with a washing buffer solution containing 0.15 M NETG (NaCl, EDTA, tris, and gelatin), and 0.05% Tween 20. Subsequently, 250 µl of diluted (1:2,500) conjugate solution was added into the wells and the tray was incubated at room temperature for one hour on a shaker. After another wash cycle, 250 µl of the chromogenic substrate solution was added to the wells. The colorimetric reaction proceeded in the dark for 8 minutes at room temperature. Finally, the reaction was stopped by rinsing the nitrocellulose test strips, twice, with aquadestilata and then the results of the tests were read with the naked eye. The result of the test was positive when a red dot appeared on the nitrocellulose paper and negative when no red dot was observed on the nitrocellulose test strips. In each series, positive and negative control sera were run. The results of the test were judged as valid if the positive control serum gave a positive result and the negative control serum gave a negative result.

For both tests (ICT-TB and TB-Dot), 3 laboratory technicians were assigned to read each test. The reported end-results were those approved by at least 2 of the 3 readers. The diagnostic value of the ICT-TB and TB-Dot tests were assessed based on the determination of the diagnostic sensitivity, the diagnostic specificity, the diagnostic efficiency, diagnostic positive predictive value and negative predictive value (Krieg *et al*, 1975; Galen, 1982). If there were differences in the diagnostic values between the two tests, the McNemar's test was used to recognize the significance ($p<0.05$) (Siegel, 1988).

RESULTS

The results of the ICT-TB and the TB-Dot tests in the group of patients with pulmonary tuberculosis (TB) and non-TB respiratory diseases are summarized in Table 1. As shown in Table 1, out of 39 patients with pulmonary TB, 34 patients (87.18%) had a positive ICT-TB test and 36 patients (92.31%) had a positive TB-Dot test. Based on these results, the diagnostic sensitivity of the ICT-TB test and the TB-Dot tests were 87.17% and 92.31%, respectively. The difference was not statistically significant ($p>0.05$).

Four samples in the pulmonary TB patients had a negative result with the ICT-TB test and a

Table 1
The results of the ICT-TB and the TB-Dot tests on 38 patients with pulmonary tuberculosis and 48 patients with non-tuberculosis respiratory diseases.

Type of the disease	NA	ICT-TB test				TB-Dot test			
		Positive		Negative		Positive		Negative	
		NB	%	NC	%	ND	%	NE	%
Pulmonary-TB	39	34	87.18	5	12.82	36	92.31	3	7.69
Non-TB respiratory diseases	48	9	18.75	39	81.25	2	4.17	46	95.83

NA = Total number of patients; NB = Number of patients with positive ICT-TB test; NC = Number of patients with negative ICT-TB test; ND = Number of patients with positive TB-Dot test; NE = Number of patients with negative TB-Dot test.

positive result with the TB-Dot test, while 2 samples of this group of patients had a positive result with the ICT-TB test and a negative result with the TB-Dot test. One sample of this group of patients had a negative result with both tests.

Out of the 48 samples of the group of patients with non-TB respiratory diseases, 39 samples (81.25%) had a negative ICT-TB test and 46 samples (95.83%) had a negative TB-Dot test. Based on these results, the diagnostic specificity of the TB-Dot test was 95.83%, which was significantly higher ($p<0.05$) than the diagnostic specificity of the ICT-TB test (82.25%). Nine samples (18.75%) had a false positive result with the ICT-TB test and a negative result with the TB-Dot test, while 2 samples (4.17%) had a false positive result with the TB-Dot test and a negative result with the ICT-TB test. The diagnostic positive predictive value of the TB-Dot test (94.74%) in this study was significantly higher ($p < 0.05$) than that of the ICT-TB test (79.07%). The negative predictive value of the TB-Dot test (93.88%), though higher than the ICT-TB test (88.64%) was statistically not significant ($p>0.05$).

The diagnostic efficiency of the TB-Dot test (94.25%), was significantly ($p<0.05$) higher than that of the ICT-TB test (83.91%).

DISCUSSION

Although the diagnostic sensitivity of the TB-Dot test was higher than the ICT-TB test, the difference was not significant ($p>0.05$). This result could be classified as high (80-90%) according to the criterion of Handojo (1988). The polymerized protein antigen used in the TB-Dot test is considered to be the main reason for the higher degree of diagnostic sensitivity of this test. In the process of polymerization, many small antigen molecules containing antigenic determinants are covalently linked to one another, forming large and stable molecules which contain more antigenic determinants when compared with the non-polymerized antigen. The polymerized antigen has a greater chance of catching specific antibodies in the patient's serum resulting in a higher degree of sensitivity than the non-polymerized antigen. During the process of polymerization, which takes place at an acid pH (5.2), the parts of the polypeptide antigen carrying the antigenic determinants are prone to be located on the outer surface of the polymerized antigen (Handojo, 1988). At this location, the antigenic determinants have a greater chance to bind antibody molecules in patient's serum. Thus, its sensitivity is consequently higher.

Tthe high degree of diagnostic sensitivity of the ICT-TB test is attributable to the use of 5 different antigens, consisting of one 38 kDa antigen and 4 other antigens derived from the cytoplasmic membrane of *M. tuberculosis*, immobilized on 4 lines on the test strip. The combined use of these 5 antigens gives rise to the broad spectrum of antigens in the ICT-TB test and consequently the high degree of diagnostic sensitivity of the test.

The 38 kDa antigen used in the ICT-TB test is a very specific antigen for *M. tuberculosis*. However, the molecular weight of this antigen is rather low resulting in a lower degree of immunogenicity and consequently a lower degree of antibody production to this antigen. This can influence the diagnostic sensitivity of the test (Pratanu, 1995).

The results of this study, as shown in Table 1, reveal that the diagnostic specificity of the TB-Dot

test (95.83%) is significantly higher ($p<0.05$) than the ICT-TB test (81.25%).

False positive results for the TB-Dot test were only found in 2 (4.17%) sera; one serum was obtained from a patient with non-tuberculosis chronic bronchitis and another one was obtained from a patient with non-tuberculosis bronchiectasis.

The higher degree of diagnostic specificity of the TB-Dot test compared to the ICT-TB test was attributed to the use of a special conjugate in the TB-Dot test, the $F(ab)^2$ fragment of rabbit antihuman IgG labeled with horse-radish peroxidase. This conjugate does not have an Fc fragment and can not be bound by the rheumatoid factor, which is often found in chronic infectious diseases, especially in viral infection. The high degree of serum dilution (1:3,200) in the TB-Dot test has the advantage that cross-reacting antibodies, which may be present in the patient's serum, are diluted to such a degree which makes them undetectable by the test. If a small amount of cross-reaction antibody is still absorbed by the antigen, it can be removed easily during the washing procedure of the TB-Dot test.

The use of a local strain of *M. tuberculosis* as the antigen and the determination of the cutoff value of the TB-Dot test in the Indonesian population are other contributing reasons for the higher degree of specificity of this test compared with the ICT-TB test in this study. The degree of specificity of the ICT-TB test (81.25%), though lower compared to the TB-Dot test, can still be classified as high according to the classification of Handojo (1988).

The significantly higher ($p<0.05$) degree of the diagnostic positive predictive value and the diagnostic efficiency of the TB-Dot test (94.74% and 94.25%, respectively) compared to the ICT-TB test in this study, are the other superiorities of the TB-Dot test over the ICT-TB test.

Seen from a practical point of view, the ICT-TB test has a higher degree of practicability than the TB-Dot test, especially with regard to the time needed to accomplish the test. The cost of the TB-Dot test is, however, much lower than the ICT-TB test and is thus more compatible with developing countries, like Indonesia.

It can be concluded as based on the analysis of data obtained in this study, that the TB-Dot test has a significantly higher degree of diagnostic value (diagnostic specificity, diagnostic positive predictive value and diagnostic efficiency) and is less expensive, more time consuming and less practicable compared to the ICT-TB test.

ACKNOWLEDGEMENTS

The authors would like to thank Mekar Jaya Diagnostica Research Laboratory for providing the materials for the performance of this study.

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