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Short Communication

Evaluation of a Rapid Serological Chromatographic Immunoassay for the Diagnosis of Pulmonary Tuberculosis in Accra, Ghana

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SUMMARY: Advances in serologic assays for tuberculosis (TB) have made serology an attractive surveillance tool. The sensitivity, specificity, and predictive value of these new assays have been well established. We employed an immunochromatographic serodiagnostic test for detecting antibodies (immunoglobulin [Ig] G, IgA, and IgM) specific for mycobacteria-specific antigen 60 in the serum of suspected TB patients (n = 310) attending the Chest Clinic of the Korle-Bu Teaching Hospital, Accra, Ghana. Compared to the gold standard, sputum culture, the performance indexes of the test kit indicated a sensitivity of 80% and a specificity of 98.2%, respectively. The positive predictive value and the likelihood ratios for positive and negative results were 96.6, 44.4, and 0.2%, respectively, when compared to sputum culture. Our results suggest that the serodiagnostic test kit may be a valuable tool for the diagnosis of TB, and can provide simple and satisfactory results, particularly in cases in which TB is clinically difficult to diagnose by the usual clinical and laboratory tests.

The rapid and accurate diagnosis of tuberculosis (TB), a chronic infectious disease caused by *Mycobacterium tuberculosis*, is crucial if effective treatment is to reduce the morbidity and mortality associated with it. For decades, diagnosis has relied on the microscopic examination of sputum smears, and the cost effectiveness of sputum smear microscopy (SSM) in the detection of pulmonary tuberculosis (PTB) has been well documented (1). The technique is simple, requires minimal resources, and can be mastered by relatively untrained personnel. However, it has some notable limitations; it cannot detect bacilli at a low count and gives false negative rates of 20 - 80%, depending on the prevalence of TB in the particular population (1,2). Furthermore, it cannot be used in the diagnosis of extrapulmonary tuberculosis (EPTB) or in the diagnosis of children who do not cough out sputum (2,3). In developed countries where the prevalence of TB is low, SSM is less reliable in the detection of PTB (3). Indeed, in these countries the screening of the population for TB now relies on Mantoux skin testing (4).

The gold standard for PTB diagnosis is sputum culture. This is the most sensitive and accurate method for confirming a case of PTB, and it provides minimal false negative rates. It is, however, relatively expensive, takes a longer time to provide results, requires more qualified personnel, and is not readily available. In Ghana, there are only two centers, that can perform TB cultures. Additionally, TB culture cannot be used for EPTB unless tissue samples are obtained.

Recently, the diagnosis of TB has been facilitated by serological enzyme-linked immunosorbent assay (ELISA) and immunochromatographic analysis (ICA) with thermostable macromolecular antigens (TMAs), which are immunological active complexes present in all mycobacteria (5-8). The best-known TMA is antigen 60 (A60) of *M. bovis* and *M. tuberculosis* (9,10). Current interest in the A60 ICA and ELISA is due to the fact that the A60 complex represents a fraction of the total mycobacterial antigens that is immunodominant and is strongly recognized by infected patients' sera (5,11,12). This A60 has been shown to differentiate active TB disease from both TB infection and BCG vaccination (5). Compared to microscopy, the ICA is effective, quick, cheap, and easy to use. The specificity (86%) and sensitivity (78%) of the test are already well established (13,14). Serological tests may prove useful in Ghana, as they have in other developing countries (15) where TB is endemic and of high prevalence; BCG vaccination is very common; and exposure to environmental mycobacteria is high. A serological test would be particularly useful in the diagnosis of childhood TB, based on the fact that combining immunoglobulin (Ig) M and IgA antibody titers of A60 has been shown to yield very good specificity (92%) and reasonably good sensitivity (75.5%) in childhood TB (16). Additionally, in such populations where TB prevalence is high and BCG vaccination is common, pleural fluid anti-A60 IgM can facilitate the diagnosis of tuberculous pleurisy (17). However, A60 serological tests seem to be of no value in the diagnosis of TB in patients infected with the human immunodeficiency virus (HIV) (18).

To determine the usefulness of a commercially available serological ICA test in the diagnosis of TB, we carried out a prospective study to compare the results obtained with this kit alone with those of this kit combined with SSM and sputum culture in suspected PTB patients attending the laboratory of the Chest Clinic of the Korle-Bu Teaching Hospital in Accra, Ghana, for the diagnosis of PTB. Given that TB culture is the most sensitive, accurate, and reliable method of confirming a case of PTB, culture results were used to assess the results of ICA and/or SSM.

A total of 310 suspected TB patients attending the laboratory of the Chest Clinic of the Korle-Bu Teaching Hospital for SSM were recruited for participation in the study. The study group consisted of 202 (65.2%) males and 108 (34.8%)

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females, with mean ages of 38.5 ± 14.4 and 37.1 ± 14.6 ($P <$ 0.39) years, respectively. Three sputum samples were obtained from each of the 310 patients using routine techniques, and the samples were processed in the Chest Clinic according to established norms (1,19). In brief, the sputum samples were decontaminated by the Petroff's method in a class 1 safety cabinet (20). Smears from the resultant final deposit were screened for acid-fast baccilus (AFB) by the Ziehl-Nelsen method (19). TB culture capacity was limited; therefore, 92 patients (30% of all patients) were randomly selected and their sputa were cultured for *M. tuberculosis* using standard culture techniques (19). In summary, sputum was digested and concentrated with N-acetyl-L-cysteine and sodium hydroxide, then cultured in Lowenstein-Jensen medium at 37°C. Samples showing no growth of AFB after 8 weeks of incubation at 37°C were discarded as negative whereas those showing slopes with growth were confirmed as AFB by the Ziehl-Nelsen method (19). In addition, blood samples (about 3 ml) were collected from all patients into 5 ml heparinized tubes. Plasma was separated and kept at −20°C until analysed. Plasma was screened for the presence of *M. tuberculosis* antibodies with a commercially available ICA test (Humana Diagnostica, Human Gesellschaft mbH, Wiesbaden, Germany) in accordance with the manufacturer's instructions. In brief, the test employs anti-human IgG, IgA, and IgM antibodies (rabbit) dye conjugate, highly purified A60 from *M. bovis*, and strain BCG (cell culture), fixed in the test line; anti-rabbit antibodies (goat) are used in the control line. As the plasma sample flows through the absorbent pad, human Igs are bound by the anti-human Ig-dye conjugate to form an immunocomplex. This binds to the A60 proteins in the test line, and, if anti-TB antibodies are present in the sample, produces a red-violet test line. In the control line, excess conjugate reacts with anti-rabbit antibodies, forming a second red-violet line to demonstrate the correct function of the reagents.

SSM was considered positive when AFB were seen in at least two out of the three specimens. If AFB were seen in only one of three sputum specimens, then three additional specimens were examined; the test was considered positive when AFB were seen in at least one of these last three specimens. SSM was considered negative if no AFB was seen in any of the respective three sputum specimens. Culture was said to be positive when at least one colony of *M. tuberculosis* was grown, and negative if there was no growth after 8 weeks. Only cultures reported as growing *M. tuberculosis* were included in this report. Comparison of results of the tests was made using application of Bayes' theorem (21,22). The Ethical and Protocol Review Committee of the University of Ghana Medical School, Accra, Ghana, approved the study.

The results of the three tests and comparisons among them are summarized in Tables 1 and 2.

The ICA test compared to sputum culture gave a sensitivity (true positive rate [TPR]) of 80.0%, specificity (true negative rate [TNR]) of 98.2%, positive predictive value (PPV) of 96.6%, likelihood ratio for positive results of 44.4%, and likelihood ratio for negative results of 0.2% (Table 1). These results were comparable to those between SSM and sputum culture.

The results of the ICA and SSM were combined, then compared to those of sputum culture; results of this comparison are shown in Table 2. In this combination, if either ICA or SSM test was positive, then the combined result was regarded as positive. The combined test was regarded as negative if both ICA and SSM results were negative. Compared to culture, the combined tests gave a sensitivity (TPR) of 100%, specificity (TNR) of 98.2%, PPV of 97.2%, FPR (false positive rate) of 1.8%, FNR (false negative rate) of 0%, and the likelihood ratio for positive results was 55.6%. The likelihood ratio for negative results could not be calculated because of division into zero.

Table 1. Comparison of immunochromatographic analysis (ICA) with sputum culture

ICA TEST	Sputum culture		
	Positive	Negative	Total
Positive	28	1	29
Negative	7	56	63
Total	35	57	92
Sensitivity (True Positive Rate)	80.0%		
Specificity (True Negative Rate)	98.2%		
Positive Predictive Value	96.6%		
Negative Predictive Value	88.9%		
False Positive Rate	1.8%		
False Negative Rate	220.0%		
Prevalence Rate	38.0%		
Likelihood ratio for positive results	44.4%		
Likelihood ratio for negative results	0.2%		

Table 2. Comparison of immunochromatographic analysis (ICA) combined with sputum smear microscopy (SSM) with sputum culture

ICA Test + SSM	Sputum culture		
	Positive	Negative	Total
Positive	35	1	36
Negative	0	56	56
Total	35	57	92
Sensitivity (True Positive Rate)	100.0%		
Specificity (True Negative Rate)	98.2%		
Positive Predictive Value	97.2%		
Negative Predictive Value	100.0%		
False Positive Rate	1.8%		
False Negative Rate	0.0%		
Prevalence Rate	38.0%		
Likelihood ratio for positive results	55.6%		
Likelihood ratio for negative results	–		

To our knowledge, this is the first report on a serodiagnostic test based on A60 for detection of IgG antibodies specific for *M. tuberculosis* in the serum of suspected TB patients in Ghana. A previous report on a serological test based on anti-A60 IgG that utilized serum from pulmonary smear-positive TB patients yielded a sensitivity and specificity of 78% and 86%, respectively, and suggested very limited usefulness of the A60 test in the diagnosis of TB in a high-incidence area like Ghana (18). The higher sensitivity and specificity of 80% and 98.2%, respectively, obtained herein was in comparison to sputum culture; that is, not to the less reliable SSM with which it was compared in the previous study (18).

This study, although limited by the small number of volunteers, highlights the usefulness of rapid and accurate diagnostic assay for detection of TB, which may be particularly significant in situations where TB is clinically difficult to diagnose. Historically, diagnosis of PTB has relied on the microscopic examination of sputum and culture or isolation of *M. tuberculosis* from sputum, but alternative tests have by

now been developed (5-8,11,12,19). The ICA test, which detects circulating antibodies specific for *M. tuberculosis*, has been shown to be effective, quick, easy to use, and cost-effective (14,23). That test is based on the use of A60 from *M. bovis* strain as a capture antigen. This antigen is species-specific and reacts with antibodies produced during mycobacterial infection; it is generally negative in healed or vaccinated patients, and detects IgG, IgM, and IgA antibodies. The results showed that ICA is a very good test for the diagnosis of PTB, and is comparable to SSM. The sensitivity of 80% obtained with ICA was similar to that of 91.4% obtained with SSM (data not shown). The likelihood ratio of positive results (44.4%) with ICA was very high, while that of negative results (0.2%) was very low, indicating a very discriminating test (data not shown).

In our laboratory, SSM was very sensitive and specific in the detection of mycobacterium, showing a very low FNR of 8.6%. The reason(s) for this high sensitivity and specificity could not be directly discerned from our study. However, the high values may be due to the high prevalence of PTB in the studied population and the frequent exposure of these volunteers to environmental mycobacteria. Combining the results of SSM and ICA enhanced these results; the sensitivity was improved and the FNR was decreased. Significantly, all cases that were falsely negative on sputum smear were detected by ICA as positive. Taken together, the results demonstrate that combining ICA with SSM reduces false negative results and improves the detection of TB.

Our results suggest to us that ICA could be a valuable tool in the diagnosis of TB, and that it can provide simple and satisfactory results, particularly in cases of sputum- negative PTB, which are difficult to diagnose by the usual clinical and laboratory tests. Thus, ICA could be used in the diagnosis of TB or as an adjunct to SSM in the diagnosis of sputum smear-negative PTB. The results reported herein are comparable to those in a previous study based on A60 ELISA, conducted in France (23). The serology, based on A60, detects only active diseases (13); hence, it is not surprising that higher percentages (96.6 and 97.2%) of PPVs are found among our cases. It is assumed that the majority of anti-mycobacterial Igs in the blood, pleural fluid, and cerebrospinal fluid of patients with TB are directed against A60 (11,12,17).

In conclusion, we found that ICA could be suitable or profitable for routine serologic diagnosis of TB in Ghana. Further work needs to be done to consider the results of ICA relative to clinically diagnosed PTB (both sputum-negative and sputum-positive) and those of clinical outcome relative to treatment. A larger community-based study correlating ICA results with different groups of subjects, differentiated by clinical and laboratory means into normal, TB contacts, and TB patients, is needed.

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