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The Role of Rapid Diagnostic Tests for Tuberculosis in Singapore

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ABSTRACT

There have been major advances in molecular techniques which rapidly identify mycobacterial DNA in clinical specimens. This has culminated in the approval by the United States Food and Drug Administration (FDA) of two rapid diagnostic tests (RDT) for tuberculosis. The FDA only licensed these tests for use in AFB smear positive patients. The role of these tests in smear negative disease is undefined. This article reviews the data on efficacy of RDT in the diagnosis of AFB smear negative PTB and proposes an alogrithm which incorporates RDT in the routine diagnosis of PTB.

Keywords: tuberculosis, rapid diagnosis, polymerase chain reaction, AMPLICOR

INTRODUCTION

Early diagnosis is important for both the effective treatment of individual patients and global control of pulmonary tuberculosis (PTB). The traditional rapid diagnostic test for PTB is the acid fast bacillus (AFB) smear. It is a well established, inexpensive, simple and reliable test. The AFB test fulfills the public health need to treat and isolate the most highly infectious patients. Patients who expectorate AFB negative sputum have pauci-bacillary disease (below 5,000 organisms per mL) and do not pose a major public health hazard. They need not therefore, be isolated. For the individual patients with PTB however, early diagnosis may reduce morbidity (less delay, fewer expensive and potentially dangerous tests) and mortality. In this regard, the AFB smear is less than satisfactory because of its low sensitivity.

We found in a recent study of hospitalised patients with PTB, that the majority (64%) were AFB smear negative[1]. In only half of the smear negative patients was a clinical suspicion of PTB considered high enough to warrant empirical anti-tuberculous treatment before culture results were known. Thus, in nearly 30% of patients, PTB was not recognised early and there was a delay of about 6 weeks before appropriate treatment was commenced following positive culture results[1].

In the past decade, the application of gene amplification techniques have resulted in major advances in the rapid identification of mycobacterial DNA in clinical specimens. The medical literature is replete with reports which describe good to excellent correlation between a variety of new amplification tests and gold standard culture positive diagnosis of PTB[2-6]. The most widely tested methods have incorporated the polymerase chain reaction either as an in-house modification or a commercial kit. This has culminated in the approval by the United States Food and Drug Administration (FDA) of two nucleic acid amplification rapid diagnostic tests (RDT) for PTB[7]. They are the Mycobacterial Direct Test (Gen-Probe; San Diego) and AMPLICOR (Roche Diagnostic Systems, Inc., NJ).

The perception that PTB is a rising problem, the pressing need for an early diagnosis, the belief that high tech tests are best and economic forces have fuelled the demand for RDT in Singapore in recent years. As a result, there is a growing number of laboratories which offer these tests on demand and for a price. These new RDTs are important breakthroughs that will have an impact on clinical practice. They are not perfect however and will not replace traditional gold standards. The appropriate use of these new diagnostic test should therefore, follow formal rules of clinical decision making[8]. Furthermore, these rules should be applied with a clear appreciation of the local conditions and the individual patients in question. Failure to differentiate between the real advantages and abiding problems and uncertainties inherent in these new tests will result in costly errors.

This review will therefore summarise current recommendations by official publications on the role of RDT for PTB, discuss the clinical efficacy of these tests in diagnosing AFB smear negative TB, highlight the relevant uncertainties and present an algorithm for the diagnosis of suspected TB in Singapore which incorporates RDT. The comments and recommendations refer primarily to FDA approved tests.

Official positions

Presently, all official national and international bodies which have examined the published data on the reliability of RDT have declined to recommend the use of these tests in the routine diagnosis of PTB. These include the United States Center for Disease Control (CDC)[7], FDA[7], American Thoracic Society (ATS)[9], British Thoracic (BTS)[10] and European Respiratory Societies (ERS)[11]. The FDA only licensed the Gen-Probe and AMPLICOR tests for use in AFB smear positive patients in order to distinguish infections by mycobacterial tuberculosis (MTB) from

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mycobacteria other than tuberculosis (MOTT) primarily for the purpose of quarantine. The ATS[9], BTS[10], ERS[11] and CDC[7] still recommend that treatment of PTB should be based on the interpretation of clinical information, chest radiographs, AFB smear and culture results.

The efficacy of locally developed in-house RDT are dependent upon the performance of individual laboratories and technicians. Noordhoek et al, in a collaborative study of 30 reputable laboratories, found that quality control was poor, results highly variable and in general, not good enough for routine application[12]. We therefore urge extreme caution in the interpretation of results from non-standardised in-house RDT for PTB unless they have been verified by blind sampling against international reference laboratories and tested in large numbers of consecutive patients in Singapore.

By contrast, the two FDA approved RDTs, Gen-Probe and AMPLICOR have well defined processing protocols, reliable internal controls, have undergone extensive clinical testing and are likely to become widely available. In patients with AFB smear positive disease, these two RDTs consistently show sensitivities and specificities of above 95%[13-19]. Gen-Probe probably has a higher sensitivity but lower specificity than AMPLICOR[14,19].

In Singapore, where prevalence of tuberculosis is higher than in developed countries, the majority of AFB smear positive cases are from patients with MTB and not MOTT. The use of RDT under FDA guidelines may therefore have little clinical relevance here. The thoughtful off label application of these tests may however be appropriate in selected patients suspected of having AFB smear negative PTB.

Smear negative disease (Fig 1)

The majority of patients with tuberculosis (both pulmonary and extra-pulmonary) have AFB smear negative (pauci-bacillary) disease. Studies which evaluate RDT (AMPLICOR RDT being most widely studied) in smear negative PTB, report sensitivities of 40% to 70% (overall ~ 60%) and specificity of above 95%[9,11,13-16,18,19]. It is not widely appreciated that a test which purports to amplify logarithmically even a single fragment of bacteria has such a low sensitivity of detection. Thus, it is a mistake to use a negative RDT to absolutely rule out PTB.

The appropriate interpretation of RDT in smear negative PTB requires an accurate estimate of pre-test probability of disease and the use of Bayes' Theorem to calculate post-test probability from the test result[8]. Fig 1 is a graphic solution of Bayes' Theorem which assumes a sensitivity of 60% and specificity of 95% (consistent with averaged results of the AMPLICOR RDT in AFB smear negative PTB). The critical first step in Bayesian analysis and everyday diagnostic decision making is a correct estimation of the likelihood of PTB (dotted line in Fig 1) based upon all relevant information. A positive RDT (upper curve) has a greater impact on disease probability (greater change from dotted line after test) than a negative RDT (lower curve). These tests should

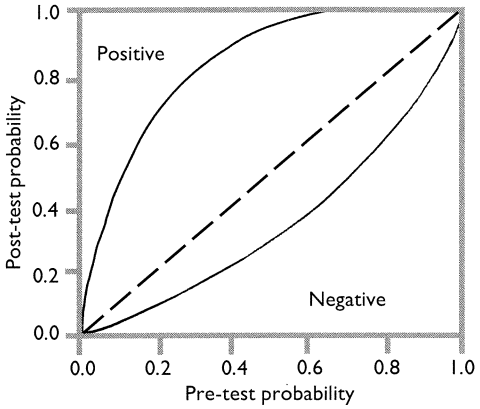

Fig 1 – This figure describes the effect of positive (upper curve) and negative (lower curve) RDT results on the probability of PTB in patients who are AFB smear negative. The pre-test probability is represented by the dotted line. The post-test curves have been calculated from Bayes' Theorem[8] by assuming test sensitivity of 60% and specificity of 95%. These are representatives values for the AMPLICOR test in AFB smear negative cases[9,11].

not however, be ordered at the extreme ends of the pre-test probability range since management steps should then be taken independent of further testing (ie. pre-test probability close to 1.0 = start treatment and pre-test probability close to 0 = no further work up for PTB). Moreover, in these extreme cases, a test result which is contrary to pre-test estimate (say negative test with pre-test > 0.8 = likely false negative) is likely to be misleading. Furthermore, even in cases where test results concur with pre-test estimates (say positive test with high pre-test probability or negative test with low pre-test probability), there may be errors of 5% – 10%.

The accuracy of RDT in specimens other than sputum is less well-defined. Its specificity is high in exudative fluid from normally sterile compartments such as pleural, pericardial spaces and in cerebrospinal fluid. A positive result, in the appropriate clinical case, is an indication for prompt anti-tuberculous treatment[20-22]. As in AFB smear negative PTB, negative RDT result should not however be used to rule out tuberculosis in these potentially life threatening diseases. Our centres as well as other centres have reported a relatively low sensitivity ($< 80\%$) of RDT in these pauci-bacillary infections which are often both smear and culture negative[23,24]. The best RDT for tuberculous infection in these three compartments is probably the adenosine deaminase level[24,25]. This is an old, cheap biochemical test which deserves much more attention in Singapore.

RDTs have also been applied to a variety of biopsy specimens including paraffin embedded ones with variable results. Shim et al[26] have reported a sensitivity of 87.5% (7/8) in aspirated specimens from patients with solitary pulmonary nodules using a nested polymerase chain reaction. These should be seen as preliminary studies in small number of patients and not extrapolated to routine practice.

Fig 1 is based upon data from FDA approved RDT applied to patients with AFB smear negative PTB from developed countries. The demography, ethnic

background and risk factors for PTB among these patients (Caucasian with large Black and Hispanic minorities, HIV infection, alcohol and drug abuse) are different form those in Singapore (older age and diabetes mellitus). This may affect its validity and practical application here. It should therefore, be used as a model for discussion and not a graph for formal decision making.

We have examined the accuracy of the AMPLICOR test in over 650 respiratory specimens from over 480 consecutive patients suspected of smear negative PTB in Singapore[27]. Interim results suggest excellent concordance of AMPLICOR with culture results (~ 95%) and sensitivity of ~ 60%. These results are encouraging and compare favorably with previous reports.

An algorithm (Fig 2)

Despite failure of the FDA to endorse the use of RDT in AFB smear negative PTB, loose guidelines and an alogrithm for immunocompromised patients have been suggested by North American[9,28,29] and European[11] experts. These guidelines have been evolved for developed countries where the overall prevalence of PTB is lower than in Singapore and have indicated RDT even for patients with very high or low pre-test probabilities. Some even omit discussing the intermediate risk group where the uncertainty is greatest and where RDT may have the most impact on estimate of disease probability (Fig 1: greatest movement away from the dotted line when the pre-test value is around 0.5)[9-11]. We therefore propose an approach to the diagnosis of PTB in patients with intermediate risk which incorporates RDT. This is summarised as an algorithm in Fig 2.

a) Very high or low risk

Patients in the extreme ends of the risk spectra should probably not be tested with RDT. Clinically "active" patients with apical cavitary disease should be promptly treated irrespective even of the AFB smear results. On the other extreme, patients who are well and have normal or near normal chest radiographs should also not be tested.

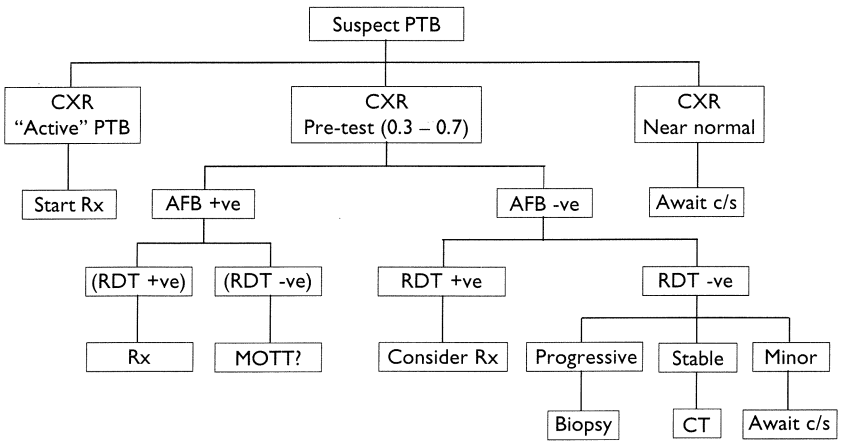

Fig 2 – An algorithm for patients suspected of having PTB.

b) Intermediate risk, positive AFB

Patients with positive AFB in the sputum should in general, be treated for PTB. Fig 2 includes suggestions (in brackets) for the outcomes of RDT in AFB positive patients. Infection with MOTT should be considered in patients who return negative RDT results. It may be more cost effective however, to only order RDT when there is reason to suspect MOTT such as in elderly female patients with bronchiectasis and multiple pulmonary nodules[30].

c) Intermediate risk, negative AFB, positive RDT

Anti-tuberculous treatment would also be indicated for most patients in this category. Caution however, should be exercised in the interpretation of positive RDT in two sub-groups of patients: (1) those with a prior history of PTB and chest radiographs suggestive of healed fibrotic disease and (2) those who have just (within 6 to 12 months) received curative anti-tuberculous treatment. Querol et al[31] reported a false positive rate of 23% (10/44) in patients with old PTB while Hellyer et al[32] reported a similarly high positive rate in recently treated patients. The clinical significance of a positive RDT in these 2 groups of patients is uncertain.

d) Intermediate risk, negative AFB, negative RDT

A concordance of negative AFB and negative RDT makes active PTB unlikely. But because of the lack of RDT sensitivity in this category of patients, PTB cannot be ruled out absolutely (Fig 1). Again clinical judgment must come into play. We suggest an approach based upon the severity and evolution of the clinical course (best assessed by serial radiographs). Patients with minor abnormalities and a resolving course should be watched. A definitive diagnosis and specific treatment should be the aim in acutely ill patients with progressive disease. Where appropriate, invasive biopsy tests may be necessary. Patients with more indolent disease may be worked up at a more leisurely pace with non-invasive imaging tests first.

This management plan was generated in order to facilitate the education of clinicians on practical issues, stimulate discussion and especially to point out caveats and uncertainties for further research. It is not a road map on which to use the RDT as a convenient overdrive.

Cost-efficacy and case-mix

Another important issue which deserves consideration is the cost-efficacy of adding RDT to the conventional approach. There is no systematic data but this is a question which needs to be resolved for each hospital and laboratory. We estimate that, if RDT (assumed sensitivity – 60%) were ordered in all smear negative patients suspected of PTB (incidence of active disease $< 10\%$)[27], over 50 patients will have to be tested in order to make an early diagnosis on a single patient who would otherwise have been missed[33]. One out of three patients with culture positive PTB will still be missed and only treated 6 weeks later. This analysis includes neither the costs of errors introduced by false positive tests nor the benefits of early diagnosis in culture negative PTB.

We also estimate that, based upon the 1,600 new cases of PTB notified per year in Singapore (Epidemiological News Bulletin 1998, 24, No. 2), a sputum AFB smear positive rate of 40%[1], an incidence of < 10% among smear negative specimens[27] and per test cost of $100 (National University Hospital), it would cost nearly $1 million per year just to test all smear negative patients suspected of PTB in Singapore. This does not include extra-pulmonary disease which is even more likely to be smear negative. Application of the algorithm in Fig 2 may reduce the cost by – 50% since majority of patients in this category have low pre-test prevalence for PTB and should not have been tested[27]. In this cost conscious era and especially with the plan to introduce elements of diagnosis related grouping in the case-mix program in Singapore, the need to resolve these issues are more urgent than ever.

CONCLUSIONS

The new, licensed RDT are important new tools in the fight against PTB. They represent a landmark achievement in applying molecular techniques directly to patent care. We are only beginning to understand how to use these new tests to supplement the traditional approach to diagnosing PTB. More research is needed to resolve the many outstanding uncertainties in the application of these molecular techniques.

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