Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert MTB/RIF assay: A meta-analysis

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Accepted 21 February 2012
Available online 27 February 2012

Summary

Objectives: Xpert MTB/RIF (Cepheid) assay has been introduced for the diagnosis of tuberculosis (TB) and RIF-resistance. The meta-analysis was used to establish the overall accuracy of Xpert MTB/RIF assay for diagnosing TB and RIF-resistance.

Methods: Based on comprehensive searches of the Pubmed and Embase, we identified outcome data from all articles estimating diagnostic accuracy with Xpert MTB/RIF assay. A summary estimation for sensitivity, specificity, diagnostic odds ratios (DOR) and the area under the summary ROC curve (AUC) was calculated by using the bivariate random-effects approach.

Results: The meta-analysis included 18 studies (10,224 suspected specimens). The summary estimate was 90.4% (95%CI 89.2%–91.4%) for sensitivity, 98.4% (95%CI 98.0%–98.7%) for specificity and 328.3/0.9822 for DOR/AUC in pulmonary tuberculosis (PTB). The sensitivity, specificity and DOR/AUC of detecting RIF-resistance were 94.1%, 97.0% and 177.8/0.9832, respectively. For extrapulmonary tuberculosis, the overall pooled sensitivity was 80.4% and specificity was 86.1%. The findings in subgroup analysis were as follows; the accuracy of Xpert MTB/RIF assay is higher in smear-positive specimens and the sensitivity of diagnosing PTB in adults was higher than that in children (90.8% versus 74.3%).

Conclusions: TB and RIF-resistance can be rapidly and effectively diagnosed with Xpert MTB/RIF assay.

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Introduction

Tuberculosis (TB) constitutes a serious threat to public health in the world, with nearly 10 million new cases and 1.7 million deaths annually. The incidence of multidrug resistant (MDR) TB is increasing with almost 0.5 million reported new cases in 2008. The global control of TB and MDR-TB has created an urgent need for timely and effectively diagnostic method.

Rapid and effective diagnosis of patients suspected of having TB remains a challenge. The conventional TB diagnosis techniques, including methods based on direct microscopic examination by Ziehl–Neelsen staining, culture, chest radiography and tuberculin skin testing have limitations and are thus not always helpful in diagnosing TB. Smear microscopy alone, although cheap and easy to perform, has a highly false-negative result and cannot identify drug-resistance. Currently, only 28% of expected incident cases of tuberculosis are detected and reported as smear positive. Although the culture is more sensitive than the smear microscopy, culture generally provides results in at least 2–8 weeks requires biosafety measures, and needs specialized laboratory personnel. This leads to a diagnostic delay that impedes disease control, and increases healthcare costs. Since the discovery of the polymerase chain reaction (PCR), a large number of molecular techniques have been developed. However, their sensitivity is greatly dependent on the efficiency of the sample preparation, DNA extraction and the presence of PCR inhibitors. Therefore, a simple, rapid and effective method for TB diagnosis remains to be developed.

One of latest assay, Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) assay, was evaluated in large studies recently. The assay detects Mycobacterium tuberculosis (Mt) and RIF-resistance by PCR amplifying five overlapping probes complementary to the rifampin resistance-determining region (RRDR) of the Mtb rpoB gene, and subsequently probes this region for mutations that are associated with RIF-resistance. The PCR amplification process is heminested to minimize cross-amplification of nontuberculosis mycobacterium (NTM) species, and to maximize mutation detection. RIF-resistance can serve as a marker for MDR-TB. Therefore, the assay may fulfill the requirement of diagnosing TB and MDR-TB. Compared to conventional diagnosis methods, the Xpert MTB/RIF assay could detect TB and RIF-resistance in one sputum sample within 2 h. In addition, the assay can diagnose TB with a nearly fully automated manner, including bacterial lysis, nucleic acid extraction and amplification, and amplicon detection. Furthermore, the assay requires minimal biosafety measures.

The diagnostic performance of Xpert MTB/RIF assay has been investigated in several studies, which have variable results. The aim of this meta-analysis is to establish the overall diagnostic accuracy of Xpert MTB/RIF assay and identify potential confounders or effective modifiers of its value in TB, thus providing the important up-to-date information on Xpert MTB/RIF assay for TB diagnosis.

Methods

Data sources and search strategy

The Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines for the conduct of meta-analyses of observational cohort studies was followed. Two investigators (K.C. and W.L.) independently performed a systematic electronic search of the Pubmed and Embase databases for original articles published until 1 October 2011 to identify potentially relevant articles and abstracts. The following search terms were used: “Xpert MTB/RIF assay” OR “Xpert MTB” OR “Xpert MTB RIF” OR “Xpert” AND “tuberculosis” OR “TB” OR “TB infection” OR “TB disease” OR “Mycobacterium tuberculosis”. There were no language restrictions. We reviewed the bibliographies of all selection articles to identify additional relevant studies.

Selection of publications

Two reviewers (K.C. and W.L.) independently screened titles and abstracts of all studies for relevancy. Disagreements were resolved by a third opinion (M.C.). Full-text publications were retrieved for relevant articles. The strength of the individual studies was weighed for relevance, based on the following items: (1) the clinical domain should include patients with suspected TB, meanwhile the study was prospective rather than a case–control design; (2) the reference standards were clearly described and all specimens were diagnosed by using the reference tests; (3) completeness of data (availability of absolute numbers of true-positive, false-positive, true-negative and false-negative Xpert MTB/RIF assay results to allow reconstruction of the diagnostic 2 by 2 table) were reported; (4) the studies were written in English.

Methods appraisal and data extraction

The final set of English articles was assessed independently by two reviewers (K.C. and J.W.). The retrieved data included author, publication year, the number of included specimens and the proportion of smear-positive specimens, specimen types, TB prevalence, sensitivity and specificity. The quality of the studies was assessed using the guidelines published by the QUADAS (quality assessment for studies of diagnostic accuracy, maximum score 14).

Statistical analysis

The studies were analyzed by using Q test to determine whether there was heterogeneity and I^2 to estimate the degree of heterogeneity. According to the result of heterogeneity analysis, the appropriate statistical analysis model for meta-analysis was chosen. From the 18 studies included, we extracted the numbers of patients with a true-positive, false-positive, true-negative, and false-negative test result either directly or through recalculation based on reported measures of accuracy in combination with the incidence and specimen size of the study. Sensitivity, specificity and diagnostic odds ratios (DOR) together with 95%CI were calculated for each study based on the reconstructive 2 by 2 table. The pooled sensitivity and specificity were calculated with bivariate random-effects approach. Based on this model, the elliptical joint confidence region for sensitivity and specificity were constructed, which combined data on sensitivity and specificity to give an indication of a test’s ability to rule
in or rule out a condition. We plotted all results from 18 included studies on a receiver operating characteristic (ROC) plot of sensitivity against specificity, with the specificity axis reversed. In addition, area under the summary ROC curve (AUC)-ROC values were determined.

The publication bias of included studies was assessed by using effective sample-size funnel plot. The Beggar's test and Egger's test were used to assess publication bias statistically. The Spearman correlation coefficient of sensitivity and 1-specificity was calculated to estimate the threshold effect. Subgroup analysis and meta-regression were used to analyze the sources of heterogeneity.

Data were analyzed by using the software for statistical analysis Stata 8.0 (StataCorp, College Station, TX, USA) and MetaDisc software, version 1.4.21

Results

Search result

A total of 90 studies were identified in the Pubmed and Embase databases. After we evaluated these citations and the bibliographies of the potential studies, 18 unique studies were eventually included in our meta-analysis. The main reasons of excluding 72 other studies were as follows: the study was a duplicate between the PubMed and Embase database, the study was not diagnostic, or the study cannot reconstruct the diagnostic 2 by 2 table.

Study characteristics and quality assessment

Overall, the selected 18 studies included 10,224 suspected specimens of having TB, which included 2983 TB specimens (Patients with microbiologically confirmed TB), 6183 non-TB specimens (Patients with bacterial chest infection who were smear- and culture-negative and responded to antibiotics with full recovery. No anti-TB treatment was given to these patients), 335 clinical TB specimens (Patients with lacking microbiological confirmation of MTB who were diagnosed as TB based on clinical and radiologic findings after failure to respond to antibiotics. All patients showed effective anti-TB treatment in the follow-up), 680 suspected specimens of MDR-TB and 43 indeterminate specimens. We enrolled 9166 specimens (8108 respiratory specimens and 1058 non-respiratory specimens) in the meta-analysis. The study sizes ranged from 25 to 4742 specimens (median 223.5, IQR 142.8–459.0). The proportion of smear-positive specimens ranged from 4.7% to 89.3% (median 18.7%, IQR 12.4%–41.5%). The TB incidence ranged from 2.6% to 81.7% (median 29.7%, IQR 17.1%–63.1%). These results are reflected in Table 1.

The quality of the 18 studies was generally high, satisfying the majority of the criteria. The scores of QUADAS ranged from 9 to 14 (median 12, IQR 10–14). The quality assessment of included studies was shown in Table 51. The culture, current gold standard, is a highly sensitive method identifying MTB and drug-resistance. The culture methods of solid and liquid-medium were widely used in TB diagnosis. In this meta-analysis, seventeen studies clearly describe the solid and liquid culture as the reference standard. In the remaining one study, the liquid culture was used.

Diagnostic accuracy in pulmonary tuberculosis

Fifteen of the 18 included studies estimated the diagnostic accuracy of Xpert MTB/RIF assay in pulmonary tuberculosis (PTB).14,22–35 The forest plot of sensitivity and specificity for Xpert MTB/RIF assay in diagnosing PTB was shown in Fig. 1A. The heterogeneity analysis showed $I^2$ of 89.0% for sensitivity and 76.0% for specificity. The existence of significant heterogeneity occurred in the 15 studies, thus the random effects model approach was selected in this study. The overall pooled sensitivity of 15 studies was 90.4% (95%CI 89.2%–91.4%), pooled specificity was 98.4% (95%CI 98.0%–98.7%). The DOR was 328.3 (95%CI 154.3–698.3). The AUC was 0.9822. The sensitivity, specificity and 95% confidence region (precision of estimation of pooled sensitivity and specificity) of 15 studies were showed in a summary ROC curve (pooled sensitivity against 1-(pooled specificity)) (Fig. 3A).

Diagnostic accuracy in RIF-resistant PTB

Four of the 18 included studies estimated the diagnostic accuracy of Xpert MTB/RIF assays in diagnosing RIF-resistant PTB were showed in Fig. 1B.14,22,24,27,28,33,34 The sensitivity of diagnosing RIF-resistance ranged from 16.7% to 100.0% (median 80.0%, IQR 50.0%–97.6%), and the specificity ranged from 72.0% to 100.0% (median 98.1%, IQR 94.1%–99.3%). The overall pooled sensitivity was 94.1% (95%CI 91.6%–96.0%), pooled specificity was 97.0% (95%CI 96.0%–97.7%) and pooled DOR was 177.8 (95%CI 41.7–757.7). The AUC was 0.9832. The summary ROC curve (pooled sensitivity against 1-(pooled specificity)) was showed in Fig. 3B.

Diagnostic accuracy in HIV-PTB co-infection

Four of the 18 included studies estimated the diagnostic accuracy of Xpert MTB/RIF assay in PTB with HIV co-infection,22,24,27,28 The sensitivity and specificity were showed in Fig. 1C. The overall pooled sensitivity was 81.7% (95%CI 77.0%–85.8%), pooled specificity was 98.0% (95%CI 96.6%–99.8%). The DOR to diagnose PTB with HIV co-infection was 217.0 (95%CI 30.3–1554.3).

Diagnostic accuracy in extrapulmonary tuberculosis

Fig. 2 showed the diagnostic accuracy of Xpert MTB/RIF assay in extrapulmonary tuberculosis (EPTB).23,29,31,32,36–38 The overall pooled sensitivity was 80.4% (95%CI 75.0%–85.1%), pooled specificity was 86.1% (95%CI 83.5%–88.4%). The DOR to diagnose EPTB was 59.2 (95%CI 11.5–304.9).

Subgroup analysis

For the PTB specimens, the fifteen studies were divided into subgroups by different smear status and age. The results of subgroup analysis presented some variability in sensitivity, specificity and DOR. Compared with that in smear-negative specimens (75.0% for sensitivity, 98.2% for specificity, 91.7 for DOR, 0.9395 for AUC), the accuracy of Xpert MTB/RIF assay to diagnose PTB in smear-positive specimens (98.7% for sensitivity, 98.2% for specificity, 1917.2 for DOR, 0.9964 for AUC) was substantially higher.
The I² of heterogeneity test is 48.3% for sensitivity in smear-positive specimens, 80.8% for sensitivity in smear-negative specimens. When stratified by age, the higher diagnostic accuracy of Xpert MTB/RIF assay was found in adults specimens (90.8% for sensitivity, 98.4% for specificity) than in children specimens (74.3% for sensitivity, 98.4% for specificity) (Table 2).

For the EPTB specimens, the meta-analysis was performed to assess diagnostic accuracy of Xpert MTB/RIF assay in smear-positive and smear-negative specimens. The accuracy of Xpert MTB/RIF assay to diagnose EPTB in smear-positive specimens is higher. The results were showed in Table 2.

The heterogeneity analysis revealed that between-study variation was substantial and not attributable to chance. To explain heterogeneity in results, we investigate the threshold effect. The Spearman correlation coefficient of sensitivity and 1-specificity was 0.200, and P value was 0.475. By meta-regression analysis, the heterogeneity sources were attributed to the number of specimens, the incidence of TB in the specimens, QUADAS scores, and the proportion of smear-positive specimens. However, the four above-mentioned factors had no significant impact on sensitivity and specificity.

Publication bias

Funnel plots, Egger’s test and Begg’s tests were performed to access the publication bias of studies. The shape of
funnel plots showed asymmetry to some degree due to the limited number of studies (Fig. 4). Then, Egger’s tests and Begg’s tests were used to provide statistical evidence of funnel plot asymmetry. The result still did not suggest any evidence of publication bias. The $P$ value of Egger’s tests was 0.472 and Begg’s tests was 0.676.

**Discussion**

In this study, we clarified the diagnostic accuracy of Xpert MTB/RIF assay for PTB and EPTB. We evaluated the diagnostic accuracy of Xpert MTB/RIF assay for RIF-resistant PTB and HIV-PTB co-infection. In addition, the diagnostic accuracy in different smear status and age were also explored. To our knowledge, this is the first systematic review and meta-analysis to estimate the diagnostic accuracy of Xpert MTB/RIF assay in TB.

The results of these 15 studies showed that Xpert MTB/RIF assay was accurate to diagnose PTB. Using the bivariate random-effects approach, we found a summary AUC of 0.9822, a summary estimate of 90.4% for sensitivity and 98.4% for specificity, which indicated that the assay may result in a 9.6% false-negative test results in TB patients and a 1.6% false-positive test result. The false-negative result may attribute to the analytical limit of detection of Xpert MTB/RIF assay, which was reported to be 131 CFU/ml. However, the culture can detect very low concentrations of organisms (as low as 10 to 100 CFU/ml). A sub clinical relapse or excretion of residual persistent DNA from dead organisms was possible reasons for the false-positive result to the patient with history of TB.

A key advantage of Xpert MTB/RIF assay over smear microscopy is the simultaneous assessment to RIF-resistance. The Xpert MTB/RIF assay was highly sensitive (94.1%) and specific (97.0%) for RIF-resistance. However, the assay can detect RIF-resistance by only probing the \( rpoB \) gene, and the mutation points in approximately 5% RIF-resistant \( Mtb \) isolates occur outside core \( rpoB \) gene region. A sub clinical relapse or excretion of residual persistent DNA from dead organisms was possible reasons for the false-positive test result to the patient with history of TB.

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Furthermore, the sensitivity of smear microscopy decreased significantly in HIV-infected compared to uninfected patients. The same pattern was seen in Xpert MTB/RIF assay. However, the sensitivity of Xpert MTB/RIF assay is higher than that of smear microscopy.

EPTB accounts for more than 20% of tuberculosis (TB) cases. The frequently atypical clinical presentation simulating other inflammatory and neoplastic conditions is the major challenge in the diagnosis of EPTB. Compared with that in PTB, the accuracy of Xpert MTB/RIF assay to diagnose EPTB (80.4% for sensitivity, 86.1% for specificity, 59.2 for DOR, 0.8923 for AUC) was substantially lower. The results indicate that the assay may be more suitable to pulmonary specimens than to extrapulmonary specimens. The sample preparation and DNA extraction may be designed in accordance with the requirements of pulmonary specimens. Therefore, a preincubation of proteinase K, enhancing the capacity of the provided lysis buffer, may be helpful to improve the sensitivity in tissue specimens.

In this research, the diagnostic accuracy of Xpert MTB/RIF assay was assessed in different smear status. As for diagnosing smear-positive PTB, the Xpert MTB/RIF assay possessed the highest accuracy. The reason may be that smear-positive specimens with higher prevalence of PTB have recruited all specimens under much more selective conditions than smear-negative specimens. Compared with the smear-negative specimens, smear-positive specimens, with higher TB prevalence, may result in an overestimated sensitivity and specificity. Although our analysis showed a substantially lower sensitivity in smear-negative specimens than that in smear-positive specimens, the assay was better than conventional assay in diagnosing smear-negative PTB.

The interpretation of the pooled sensitivity and specificity existed significantly heterogeneity among the studies. The result variation range from a test result with a high sensitivity and specificity to the test result that is neither sensitive nor specific. The Spearman correlation coefficient of sensitivity and 1-specificity indicated that the heterogeneity was not related to threshold effect. Subgroup analysis revealed that different smear status was important source of the heterogeneity. Compared with the smear-negative specimens, the smear-positive specimens leads to an overestimated sensitivity and specificity. We added the four factors (the number of specimens, the incidence of TB in

Figure 2  Forest plots of sensitivity and specificity for Xpert MTB/RIF assay in diagnosing EPTB. The pooled sensitivity was 80.4% (95%CI 75.0%–85.1%; I² 86.5%, n = 7); pooled specificity of two studies was 86.1% (95%CI 83.5%–88.4%; I² 96.1%, n = 7). The point estimates of sensitivity and specificity from each study are shown as solid circles. Error bars indicate 95%CI. Numbers indicate the studies included in the meta-analysis, as cited in the reference list.

Figure 3  Summary receiver operating characteristic (SROC) curves for Xpert MTB/RIF assay in PTB (A) and rifampicin-resistant PTB (B). ROC, receiver operating characteristics curve.
the study specimens, QUADAS scores, and the proportion of smear-positive specimens) to the meta-regression, but these did not explain the heterogeneity. Owing to the limited number of studies in this meta-analysis, we restricted meta-regression analysis to these four factors, which are the most likely to cause the heterogeneity between studies.

A major strength of this meta-analysis is that we excluded the case–control studies, only included the studies addressing patients suspected of having TB. The case–control studies tend to overestimate values of diagnostic accuracy because of existing diagnostic suspicion bias. Some limitations should be considered when interpreting the results. Firstly, subgroup analysis was restricted by limited original data. Secondly, the retrieved study is potentially not comprehensive enough. Our search scope was limited to published studies that had probably missed some of the gray literature, such as conference papers cannot get. Thirdly, the sample sizes of several included studies are rather small and they do not have adequate ability to assess the diagnostic accuracy.

Conclusions

The Xpert MTB/RIF assay fulfills the requirements of rapidly and effectively diagnosing TB and RIF-resistance. The single Xpert MTB/RIF assay diagnosed 90.4% of culture-confirmed PTB patients (98.7% in smear-positive specimens, 75.0% in smear-negative specimens) and 80.4% of EPTB patients (95.2% in smear-positive specimens, 70.7% in smear-negative specimens). RIF-resistant PTB was diagnosed with 94.1% for sensitivity and 97.0% for specificity. In a word, the introduction of Xpert MTB/RIF assay would be helpful for the reduction of the infectious pool and the improvement in TB control.

Conflict of interest

We have no conflict of interest for this article.

Acknowledgments

The authors would like to express thanks to Prof. Wei Sun to review the manuscript. This work was supported by the Major Project Chinese National Programs for High Technology Research and Development (863 Program, 2007AA02Z416), the Chinese National Natural Science Foundation (No. 81071428, 30400107) and the National Infectious Disease Prevention and Cure Special Project (No. 2008ZX10003-012).

Appendix A. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.jinf.2012.02.012.

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