Is Xpert MTB/RIF Assay in Gastric Lavage Aspirate Useful for Diagnosis of Smear-negative Childhood Pulmonary Tuberculosis?

SOURCE CITATION: *Pang Y, Wang Y, Zhao S, Liu J, Zhao Y, Li H. Evaluation of the Xpert MTB/RIF assay in gastric lavage aspirates for diagnosis of smear-negative childhood pulmonary tuberculosis. Pediatr Infect Dis J. 2014;33:1047-51.*
SECTION EDITOR: ABHIJEET SAHA

SUMMARY

This study evaluated the Xpert MTB/RIF assay for diagnosis of smear-negative childhood pulmonary tuberculosis (TB) using gastric lavage aspirates (GLA) in 211 Chinese children. The sensitivity in detecting children with a clinical diagnosis of TB for MGIT and Xpert was 12.1% (95% CI 9.3%, 14.9%) and 48.6% (95% CI 44.4%, 52.8%), respectively. The authors concluded that Xpert MTB/RIF assay is an excellent tool for the diagnosis of smear-negative childhood with GLA samples. The high proportion of very low mycobacterial load in the GLA samples from smear-negative TB cases may increase the frequency for obtaining indeterminate RIF resistance results by Xpert.

COMMENTARIES

Evidence-based-medicine Viewpoint

Relevance: Childhood tuberculosis poses unique diagnostic challenges different from adults. These are related to paucibacillary infection, absence of hallmark symptoms/signs such as hemoptysis or a pulmonary cavity on radiography, overlap of clinical symptoms with various differential diagnoses, logistic/technical challenges in obtaining sufficient quantity of appropriate biological specimens and poor specificity of Mantoux test. These challenges create debates on whether to 'prove and treat' or 'treat and prove' the condition. Although many physicians give a therapeutic trial when in doubt, the practice has also led to indiscriminate prescription of anti-tuberculosis drugs (ATD) resulting in inadequate/insufficient anti-tuberculosis therapy (ATT), thereby contributing to the problem of drug resistance. Therefore, it would be welcome to have diagnostic tests that are sensitive enough to identify tuberculosis disease (as opposed to infection) and specific enough to lead to correct therapeutic decisions (starting or stopping therapy). The GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA), has been suggested as a test fulfilling these criteria [1]. The test received a shot in the arm when the World Health Organization (WHO) recently recommended [2,3] that it *may* (note emphasis) be used as the initial test in children with suspected tuberculosis instead of attempting to identify/detect Mycobacteria through smear and culture. WHO also recommended it as the first test for children with suspected multi-drug resistant tuberculosis or disease associated with HIV infection, however data on these conditions in Indian children are lacking. A recent Cochrane review [4] also suggested that the test is very promising both as an initial diagnostic test as well as an add-on test in those who are smear negative. However, the review did not examine studies in children. The Revised National Tuberculosis Control Programme (RNTCP) has chosen a more cautious position and merely lists it as one of the tests endorsed by it [5]. In 2012, the Indian Academy of Pediatrics expert committee rightly emphasized that all efforts should be made to detect Mycobacteria through smear, culture, or the Xpert assay [6]. Against this backdrop, the recent study by Pang, *et al*. [7] evaluating the diagnostic utility of the test in gastric lavage samples of children with suspected TB but negative smear examination, is highly relevant.

Critical appraisal: ***Table* I** summarizes the methodological aspects of the study [8]. Additional points to be considered are as follows. Ideally, Xpert MTB/RIF assay should be shown to be comparable with the reference standard (either culture or a clinical diagnosis leading to treatment). However, this study shows that it is inferior to culture and perhaps clinical diagnosis (***Table* I**), although the latter is relatively non-specific. Inferiority to culture has been demonstrated in several other studies as well [9-11]. This is surprising because the principle of nucleic acid amplification tests (NAAT) is to detect even miniscule amounts of nucleic acids (through amplification), hence theoretically NAAT

TABLE I CRITICAL APPRAISAL OF THE STUDY

Validity	
Are the results of the study valid?	The investigators applied the index test (Xpert MTB/RIF assay) in 211 children with suspected tuberculosis and compared the test results against two reference standards. Children who were smear positive (n=15) were not included. Tuberculosis was suspected in the presence of any one of the following: cough longer than 2 weeks, fever lasting beyond 2 weeks, weight loss (magnitude undefined), history of contact with TB (undefined) and suggestive radiography. It is unclear whether eligible participants were enrolled consecutively or an element of selection bias existed.
Was the reference standard applied regardless of the index test result?	Two reference standards were used: (1) Culture confirmed tuberculosis and (2) Clinical diagnosed tuberculosis. Cases were labelled culture confirmed if they had cough+fever >2weeks and Mycobacterial culture positive using Bactec MGIT 960 system. Clinical diagnosed tuberculosis was defined as cough+fever >2weeks and two of the following three viz (i) contact with active TB, (ii) positive tuberculin skin test (undefined) and (iii) Effective for anti-TB regimen (undefined). Presumably, the reference standard was applied regardless of the index test result. However, it should be noted that 'Culture confirmed' and 'Clinical diagnosis' appear to be mutually exclusive in the sense that an individual child could have only one of the two. The authors have not considered a reference standard combining the two criteria
Was there an independent, blind comparison between the index test and an appropriate reference ('gold') standard of diagnosis?	Culture confirmed TB is an appropriate gold standard. Many physicians also rely on Clinical Diagnosis as an appropriate reference, although both have limitations. The authors do not specify whether the index test and reference tests were undertaken independently by examiners blinded to the results
Test characteristics and measures	Xpert assay *vs* culture: Sn 64.7%, Sp 70.1%, PPV 15.9%, NPV 95.7%, LR+ 2.16, LR- 0.50. Xpert assay *vs* clinical diagnosis: Sn 46.3%, Sp 98.6%, PPV 98.3%, NPV 51.4%, LR+ 33.1, LR- 0.54 Xpert assay *vs* Diagnosis by culture or clinical criteria: Sn 48.5%, Sp 98.6%, PPV 98.6%, NPV 49.3%, LR+ 33.1, LR- 0.54.
Do the methods described permit replication?	The methods described for smear, culture and Xpert assay are standard, well-accepted laboratory techniques. The method for collecting and processing gastric lavage specimens are also appropriate. Therefore, the methodological aspects in the study can be applied in the Indian setting. However, the results call for cautious optimism and further refinements in the test before it can replace the current reference standards for diagnosis.

LR- = Likelihood ratio of a positive test, LR- = Likelihood ratio of a negative test, NPV=Negative predictive value. Sn=Sensitivity, SP=Specificity, PPV=Positive predictive value

are expected to be more sensitive than culture. This is the basis for the high sensitivity of polymerase chain reaction (PCR) based diagnosis of infection (and superiority over culture). In this study [7], the Xpert MTB/RIF assay failed to identify 6/17 (35%) smear negative, culture positive cases. Some or all of these 17 children may not have fulfilled the criteria for 'clinically diagnosed TB case' as per the authors' definition. Therefore, they would not have received treatment until the culture results became available. This suggests that reliance on Xpert MTB/RIF instead of smear and culture as the initial diagnostic test could result in non-treatment of these children. Rather than exploring the relatively poor sensitivity of Xpert MTB/RIF assay, Pang, *et al*. have chosen to downplay it citing comparable results in other studies. This poses a real danger that in future also, this serious limitation of the assay will simply be ignored.

On the other hand, the assay appears to diagnose TB in only about half of those clinically labeled as tuberculosis. This appears to be a significant advantage in the sense that it could reduce the burden of unnecessary treatment. However, a noteworthy point is that the criteria used for 'clinical diagnosis' in this study [7] make it very difficult to ignore TB, and most physicians would still opt for treatment (irrespective of the assay results). In that sense, the assay has not really demonstrated superiority over the clinical diagnosis, although a negative result appears to rule out infection. Further, among the 123 clinically diagnosed cases, Pang,

et al. have not provided a detailed break-up of the criteria these cases fulfilled. This could be important because one of the criteria is 'effective for anti-TB regimen.' Presumably this implies adequate therapeutic response, and can be determined only several weeks after initial presentation. It would be interesting to analyze the performance of the Xpert MTB/RIF assay separately in those diagnosed on this basis, because if the assay performs well in them, a correct treatment decision (initiation or withholding) could be made at presentation.

Pang's analysis of MGIT sensitivity and specificity against the reference standard of 'both microbiologically and clinically diagnosed TB' is inappropriate because the index test (here MGIT) is also part of the reference test. Therefore, MGIT could only be compared against 'clinically diagnosed TB'.

Another important (but oft-ignored issue) is that the Xpert MTB/RIF assay does not distinguish between infection and disease, and also active versus inactive disease (whereas clinical diagnosis almost always points towards active disease). Likewise culture-based diagnosis indicates live bacilli (and presumably active disease). Therefore, it would have been very valuable if Pang's study had reported the outcome of children whose therapeutic decision (not to treat) was based on the assay result despite a clinical diagnosis compatible with diagnosis.

Perhaps the strength of the Xpert MTB/RIF assay lies in the speed of obtaining results especially with regard to Rifampicin resistance. For the diagnosis of tuberculosis, speed is of the essence and a positive test can result in faster initiation of therapy. This could be particularly useful in smear negative cases, wherein the theoretical time to diagnosis is shorter than the assay. The Xpert assay has been developed as a sturdy kit requiring 'minimal hands-on technical time' [2]. This sounds encouraging but can lead to problems associated with quality control when performed without standardization (as happens with many molecular diagnostic tests in India today). The superiority of Xpert assay in terms of rapidity of diagnosing Rifampicin resistance has to be balanced with the cost and availability at the point-of-care.

It should also be noted that drug resistance detected by the Xpert MTB/RIF assay is restricted to detection on one (albeit the most important) gene responsible for it. Of course, Rifampicin resistance is not synonymous with INH (and thereby multi-drug) resistance. In such a scenario, the therapeutic regimen for a Rifampicin resistant case is not fully elucidated [3].

Extendibility: It would appear that this study bolsters the WHO recommendation [2, 3] to consider the Xpert MTB/RIF assay as an alternative to the conventional methods for diagnosis. On the face of it, the milieu is ripe for introducing a new test. The clinical setting of Pang's study (developing country with relatively low burden of pediatric HIV-associated as well as multi-drug resistant tuberculosis), the diagnostic challenges, and the current algorithms for diagnosis are similar to India. Based on this, it is relatively easy to replicate a similar study in our setting. However, the data and the study limitations elucidated above suggest that though we can be optimistic about molecular diagnostic methods, there is a considerable ground to be covered before the Xpert assay can replace the current diagnostic methods (despite their relative limitations).

Conclusions: Molecular diagnostic methods such as the Xpert MTB/RIF assay appear promising, but data do not suggest that it can be used as a replacement for current diagnostic methods (smear/culture or clinical diagnosis) in children suspected to have tuberculosis (not associated with HIV or suspected to be drug-resistant).

JOSEPH L MATHEW
Department of Pediatrics,
PGIMER, Chandigarh, India.
dr.joseph.l.mathew@gmail.com

Pediatric Pulmonologist's Viewpoint

After World Health Organization endorsing the GeneXpert MTB/RIF assay, several studies were conducted on smear-negative/culture-positive childhood pulmonary and all of them agreed the advantage of the above tool in the rapid diagnosis of tuberculosis (TB) and rifampicin-resistant TB [2]. The present study by Pang, *et al.* [7] has concluded that GeneXpert MTB/RIF showed significantly better performance (sensitivity and specificity of 48.6% and 98.6%) than MGIT (the sensitivity and specificity 12.1% and 100.0%) with gastric lavage aspirates (GLA) samples and recommends Xpert may serve as a useful tool for the diagnosis of childhood TB, especially for smear-negative cases.

Though MGIT 960 system reports the growth of tubercle bacilli fast, conventional culture is mandatory to know the drug resistant status and to formulate an effective regimen. Including conventional culture in randomized clinical trials can contribute additional information on this subject.

Though many diagnostic tests for TB have been identified, most of them are used for research purpose only and a cost effective rapid diagnostic test is the need of the hour. Rapid tests in children with suspected

tuberculosis would not only improve the management protocol of the affected child but also allow greater integration of pediatric tuberculosis into national tuberculosis control programs. The result of the TB diagnostic test depends both upon the test and also on the clinical specimen used.

Since children below 6 years are not able to expectorate the sputum, specimens like gastric lavage aspirate (GLA), induced sputum (IS), bronchoalveolar lavage (BAL) are used and each one will have its own advantages and disadvantages. In the present study GLA samples were used but a trial comparing all the available specimens against Gene Xpert MTB/RIF add more information. It has been highlighted that neutralization of GLA sample with sodium bicarbonate would have inactivated a part of tubercle bacilli. Since PCR based technology can detect nucleic acids from both dead and live bacilli, neutralization aspects need a revision. More than this, a delay in the transportation and storage of GLA samples would have contributed their own share for the low detection rate of MGIT culture in the present study. Again the yield of GLA varies widely in various studies (with or without vancomycin to reduce contamination, with or without nasogastric tube insitu overnight, frozen against fresh samples, hospitalized versus ambulatory patient, pulmonary versus adenopathy, extensive versus mild disease,) emphasizing the fact that GLA technique needs further standardization [12].

Unlike the adult type, pediatric tuberculosis have many challenges like paucibacillary nature of infection, poor clinical expression, equal affection of both pulmonary and extra pulmonary system and difficulty in obtaining good specimens. All these factors make microbiologic diagnosis of the pediatric TB a difficult task. Lot of money is wasted on many unnecessary investigations in TB diagnosis and unfortunately poor and downtrodden are the victims.

WHO play a great role by providing timely information on useful diagnostic tests and treatment regimen for effective TB management which is evidenced by its constant recommendation. Abandoning TB serodiagnosis, promoting Xpert MTB/RIF for smear-negative TB, simplifying all TB treatment regimens into two categories and implementing DOTS are the measures which contributed effectively to reduce the burden of tuberculosis.

Since Xpert assay can determine only Rifampicin resistance and not resistance to other first and second-line anti-TB drugs, this fact needs consideration in the evaluation of suspected resistance. The study concludes by saying that Xpert MTB/RIF assay is an excellent tool for the diagnosis of smear-negative childhood TB with GLA samples but our fervent appeal is all precautions should be duly followed during GLA collection and if the treating physician involves himself directly, the bacteriologic yield will increase. Since inadequate specimen is the major drawback in pediatric TB diagnosis, combining both specimens like GLA and IS taken on the same day, may further increase the yield.

D VIJAYASEKHARAN
Former Professor,
Madras Medical College,
Chennai, India
vijsekar@hotmail.com

Microbiologist's Viewpoint

World Health Organization (WHO) estimated the global burden of tuberculosis at 8.6 million new cases and 1.3 million deaths in 2012, with up to 15% burden in pediatric cases [13]. Tuberculosis (TB) in children has remained relatively neglected mainly due to lack of sensitive diagnostic methods [14]. However, recent invention of the Xpert MTB/RIF assay has significantly transformed the diagnostics algorithm [15]. After its endorsement by WHO, several workers have started using Xpert MTB/RIF assay for the diagnosis of pediatric tuberculosis [16]. Nevertheless, very few studies are published on its utility in smear negative pediatric samples such as gastric aspirates (GA).

In this study, Pang, *et al*. [7] report that in smear negative culture positive GA samples, the sensitivity of Xpert MTB/RIF was 64.7% which is on expected lines. However, the unexpected finding in this study is high discordance between MGIT culture and Xpert MTB/Rif results. The study also shows unexpectedly high (29.9%, 58/194) false positivity of Xpert MTB/Rif in smear and culture negative samples. We have observed that 92.2% smear and culture negative GA samples will also be Xpert MTB/RIF negative (concordance) and only 7.8% bacteriologically negatives samples will be Xpert MTB / RIF positive (unpublished data). This discrepancy was most likely due to very low culture yield (8.1%) in their samples, which were probably not truly negative. Low culture yield was result of harsh treatment given to the samples, i.e. first neutralized with $NaHCO_3$ and then frozen at $-20^{\circ}C$ and transported to the reference laboratory after 3-4 days. The paper does not mention that after receiving when these samples were processed for culture and Xpert MTB/Rif testing by the reference laboratory. Second, who performed smear microscopy – referral laboratory or source laboratory? Third, paper is silent on why smear microscopy was not done from decontaminated samples as a standard protocol?

Fourth, how many cultures were contaminated and how many of these were Xpert MTB/RIF assay positive? It is presumed that $NaHCO_3$ neutralized samples would have had more chances of contamination in cultures [17].

The authors highlight limitations of their study and emphasize that Xpert MTB/Rif assay is not ideal for GA samples due to its low negative predictive value which means overall low (70.1%) specificity. They also emphasize that $NaHCO_3$ neutralization of GA samples is not advisable if samples have to be stored and then cultured in MGIT960 system, though these samples may be used for DNA-based tests, as the DNA of dead bacilli can be amplified by later methods. The study also shows that 11.6% Xpert MTB/Rif samples yielded indeterminant Rifampicin-resistance results, which is an important cost implication.

SARMAN SINGH
Department of Laboratory Medicine, AIIMS, New Delhi, India
sarman.singh@gmail.com

REFERENCES

1. No authors listed. Cepheid Receives FDA Market Authorization for Xpert MTB/RIF. Available from: *http://ir.cepheid.com/releasedetail.cfm?releaseid=780520*. Accessed November 8, 2014.
2. World Health Organization. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extra-pulmonary TB in adults and children. Policy update. Available from: *http://apps.who.int/iris/bitstream/10665/112472/1/9789241506335_eng.pdf*. Accessed November 5, 2014
3. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children Second edition Available from: *http://apps.who.int/medicinedocs/documents/s21535en/s21535en.pdf*. Accessed November 10, 2014
4. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev 2014;1:CD009593.
5. Government of India. Central TB Division Directorate General of Health Services, Ministry of Health and Family Welfare. TB India 2014 Revised National TB Control Programme. Annual Status Report. Available from: *http://www.tbcindia.nic.in/pdfs/TB%20INDIA%202014.pdf*. Accessed November 10, 2014.
6. Kumar A, Gupta D, Nagaraja SB, Singh V, Sethi GR, Prasad J; Indian Academy of Pediatrics. Updated national guidelines for pediatric tuberculosis in India, 2012. Indian Pediatr. 2013;50:301-6.
7. Pang Y, Wang Y, Zhao S, Liu J, Zhao Y, Li H. Evaluation of the Xpert MTB/RIF assay in gastric lavage aspirates for diagnosis of smear-negative childhood pulmonary tuberculosis. Pediatr Infect Dis J. 2014;33:1047-51.
8. No authors listed. Critical Appraisal Skills Programme (CASP) Diagnostic Test checklist_14.10.10. Available from: *http://www.caspinternational.org/mod_product/uploads/CASP_Diagnostic_Checklist_14.10.10.pdf*. Accessed October 10, 2014.
9. No authors listed. Critical Appraisal Skills Programme (CASP) Diagnostic Test checklist_14.10.10. Available from: *http://www.caspinternational.org/mod_product/uploads/CASP_Diagnostic_Checklist_14.10.10.pdf*. Accessed October 10, 2014.
10. Nhu NT, Ha DT, Anh ND, Thu DD, Duong TN, Quang ND, *et al*. Evaluation of Xpert MTB/RIF and MODS assay for the diagnosis of pediatric tuberculosis. BMC Infect Dis. 2013;23;13:31.
11. Tortoli E, Russo C, Piersimoni C, Mazzola E, Dal Monte P, Pascarella M, *et al*. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. Eur Respir J. 2012;40:442-7.
12. Kabra SK, Lodha R, Seth V. Some current concepts on childhood tuberculosis. Indian J Med Res 2004;120:387-97.
13. World Health Organization, 2013. WHO Global Tuberculosis Control Report: Geneva, Switzerland. *http://www.who.int/tb/publications/factsheet_global.pdf*. Accessed October 16, 2014.
14. Swaminathan S, Rekha B. Pediatric tuberculosis: Global overview and challenges. *Clin Infect Dis.* 2010;50:S184-94.
15. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, *et al*. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med. 2010;363:1005-15.
16. Bates M, O'Grady J, Maeurer M, Tembo J, Chilukutu L, Chabala C, *et al*.. Assessment of the Xpert MTB/RIF assay for diagnosis of tuberculosis with gastric lavage aspirates in children in sub-Saharan Africa: A prospective descriptive study. Lancet Infect Dis. 2013;13:36-42.
17. Parashar D, Kabra SK, Lodha R, Singh V, Mukherjee A, Arya T, *et al*. Does neutralization of gastric aspirates from children with suspected intrathoracic tuberculosis affect mycobacterial yields on MGIT culture? J Clin Microbiol. 2013; 51:1753-6.