

Case Report

A Retrospective Study on Xpert MTB and Comparison with Smear Microscopy

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Abstract

Tuberculosis still constitutes a major health problem worldwide with an 8.8% incidence and 1.3% mortality rate reported in 2010. Traditionally the diagnosis of active TB is mainly based on chest X Ray, microscopy and body fluid culture whilst the diagnosis of latent TB depends on the tuberculin skin test or haematology tests. Acid fast bacilli smear microscopy remains the most used and widely used TB diagnostic method available in low income and middle-income countries. However, as many as 40-50% of active TB cases were smear negative TB culture requires 2-6 weeks for interpretation and has less than perfect sensitivity. GenXpert showed both high sensitivity and high specificity and suggested its high value in TB diagnosis. This study confirms the superiority of GenXpert on TB test compared to ZN staining in the detection of tuberculosis and in the prediction of multi resistance. Its systematic use coupled with ZN staining would better control tuberculosis in Sub-Saharan Africa.

Keywords: GenXpert; TB; AFB.

Aim

A retrospective study on Xpert MTB and microscopic smear examination for detection of tuberculosis in Mwangezi Hospital at leprosis and tuberculosis Provincial Coordination Centre Lualaba.

Introduction

In Sub Saharan Africa, diagnostic methods for tuberculosis are inadequate and are essentially

based on microscopy. They constitute a real obstacle for the diagnosis and treatment of tuberculosis, control of tuberculosis. Genxpert MTB assay is an automated molecular test that is designed to simultaneously detect Mycobacterium tuberculosis complex and Rifampicin resistance. However, there are relatively few studies on this method. GenXPert has been routinely used at Hospital De General Mwangezi since 2014. Thus, the aim of this study was to evaluate the performance of GenXpert MTB test as compared to classical ZN staining

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at Mwangeji Hospital, DR Congo and provide a reference and guidance for the detection and diagnosis of TB in non-specialized TB hospitals.

Methods

The medical records of results of ZN staining and GenXpert performed at 3816 patients at Mwangeji Hospital from 10/12/2014 to 31/03/2021 were collected. This study compares the validity of different diagnostic test in the detection of tuberculosis. Cepheid GenXpert equipment was used in the study.

Result

In the entire group, the frequency of pulmonary tuberculosis was 28.8%. The positivity rate was significantly higher in GenXpert than in ZN staining in the entire group (28.8% vs 17.4%) and in HIV zero positive patients (13.3%). The sensitivity of GenXpert MTB test compared to ZN wasn't maximum (100%). Finally, GenXpert test detected Rifampicin resistance in 11.8% cases.

Conclusion

GenXpert showed both high sensitivity and high specificity and suggested its high value in TB diagnosis. This study confirms the superiority of GenXpert on TB test compared to ZN staining in the detection of tuberculosis and in the prediction of multiresistance. Its systematic use coupled with ZN staining would better control tuberculosis in Sub-Saharan Africa.

Background:

Tuberculosis still constitutes a major health problem worldwide with an 8.8% incidence and 1.3% mortality rate reported in 2010. Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* complex usually affects the lungs but also affects other parts of the body. The typical symptoms of active pulmonary TB are chronic cough and haemoptysis, fever, night sweats and weight loss. Currently, about a quarter of the world population are carriers of TB. Notably, extrapulmonary TB is also a serious clinical problem and comprises increased population of total TB cases in the past few decades.

Traditionally the diagnosis of active TB is mainly based on chest X Ray, microscopy and body fluid culture whilst the diagnosis of latent TB depends on the tuberculin skin test or haematology tests. Acid fast bacilli smear microscopy remains the

most used and widely used TB diagnostic method available in low income and middle income countries. However, as many as 40-50% of active TB cases were smear negative TB culture requires 2-6 weeks for interpretation and has less than perfect sensitivity.

Several Polymerase chain reactions (PCR) based molecular methods have recently been developed for early TB diagnosis and rapid detection of drug resistance from clinical specimen. The GenXpert MTB assay is one of these methods and consist of heminested real time PCR test that simultaneously identifies *Mycobacterium tuberculosis* and detects Rifampicin resistance as a surrogate of multidrug resistance directly from clinical specimens. This assay requires less than 2 hours and its key advantage over other PCR methods is that it is a fully automated process designed to run on the GenXpert Dx system (Cepheid). This system incorporates DNA extraction often considered the critical step along with real time PCR Amplification and detection in a single handsfree process, then acting as a real labonchip device.

Since December 2010, WHO has recommended the Xpert MTB assay as a bonafide follow on test due to its high quality performance compared to microscopy whenever MDR-TB or HIV are of lesser concern and specially in cases of smear negative specimens. This conditional recommendation does in fact exclusively concern sputum samples. Finally, there have been few studies on performance of expert MTB compared to microscopy in high TB burden countries.

Methods

Study design: This is a retrospective survey and analysis of data collected from inpatients simultaneously tested with Xpert MTB, AFB smear microscopy from November 2014 to March 2021 enrolled at Coordination Leprosy and Tuberculosis Provincial Centre, Lualaba at Mwangeji Hospital.

Inclusion criteria: All patients with valid data who underwent GenXpert test and AFB smear test at Mwangeji Hospital, Kolwezi, Lualaba.

Exclusion criteria:

- Invalid
- No results
- Error

Acid Fast bacilli (AFB) Smear Microscopy

Smear microscopy was performed according to the clinical and laboratory standards Institute guidelines. Specimens were stained for acid fast

microscopic examination using the ZN stain. Smears positive specimens were graded 1+ to 4+ according to the American Thoracic Society Scale.

Negative 0 AFB / 300 fields

Positive 1+ 1-9 AFB/100 fields

2+1-9

AFB/10 fields

3+1-9 AFB/field

4+>9 AFB/field

Xpert MTB

Xpert MTB was performed on the GenXpert Instrument System according to the manufacturers recommendation. The Xpert software was used to interpret the results and cases were classified in various categories. A sputum sample was collected from the patient with suspected TB which was mixed with reagent provided with the assay kit and a cartridge containing the test mixture placed in Xpert machine. All processing from this point on is fully automated.

Classification of the patients

Patient type was classified as:

NP = Nouveau patient

R = Rechute

E = Retraitment apres echec

PV = Retraitment apres predi de vere

Au = Autre retraitment

Nc = Inconnu

Liste Des Presumes TB MR

1. Retraitment
 - (a) Rechutes
 - (b) Eches
 - (c) Chroniques
 - (d) Predus de vue
2. NP
 - (a) Contacts des MDR Connus known
 - (b) Patients CoInfected TB along with HIV
 - (c) Patients AVEC F2/F3 + along with

Records were available for only 3113 patients.

Broadly it could be categorized as:

Patients under Retraitment =124 (3.98%)

Rechute=170=5.46%

Echec=147=4.72%

Case contact=356=11.43%

PVV=594=19.08%

NP=1722=55.3%

Results

Total no. of patient samples enrolled between November 2014 to March 2021 were 4263.

Cases excluded due to Invalid, noresults, error etc. Were 447.

Total no. of cases included were 3816.

Predominant age group involved was 20-40 years.

Males were slightly more affected than females.

MTB not detected were 2635.

GenXpert Results:

MTB+ Rifampicin (Sensitive)	MTB + Rifampicin (Resistant)
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Very Low = 153	Very Low = 17
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Medium = 316	Medium = 54
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High =281	High = 33
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Low = 220	Low = 26
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Total Distribution

Very Low = 153+17=170

Medium = 316+54=370

High = 281+33=314

Low = 220+26=246

Total = 1100

$1100 \times 100 / 3816 = 28.8$

%AFB+ case=192= 17.4%

Only 17.4% of all positive case were detected by microscopy.

Obviously GenXpert is a method to look forward to as a diagnostic modality.

Distribution of AFB + cases

+ =161=83.8%

++=21=10.9%

+++ =6=3.12%

++++=4=2.08%

HIV+ Cases=147 (13.3%)

About 13.3% of positive cases were simultaneously HIV infected.

Comparison of xpert MTB and Smear microscopy:

The positive ratios of AFB and xpert MTB were 5.03% and 28.8% respectively. Xpert showed the better diagnostic performance.

Prediction of Xpert positive

PPV	Confirmed TB	Total sample	Type of sample
100%	314	314	Positive High
100%	370	370	Positive Medium
98.3%	242	246	Positive Low
98.8%	168	170	Positive Very Low
95.5%	124	2635	Negative
75.4%	1218	3735	Total

AFB Smear Results:

Total Sample	4+	3+	2+	1+	Number of Cases	Type
30	0	6	11	13	294	Positive High
55	1	9	7	38	315	Positive Medium
26	0	0	3	23	220	Positive Low
19	0	0	3	16	151	Positive Very Low

Discussion

The study of 3816 patients from November 2014 to March 2021 shows that GenXpert shows the best performance. The high specificity of Xpert across all specimens highlights its utility as a rule in test for TB diagnosis and can be reliably used to inform the clinicians about the start of TB treatment when positive.

This study shows that Xpert showed high sensitivity.

There were 71 Xpert false positive cases in this study. According to previous studies, the false positive results of Xpert may occur in patients with prior TB and Xpert may detect cell free DNA rather than DNA in cells.

This study has some limitations.

First, this is a single centre retrospective study and thus the overall relevant scope of our findings was limited. Second, Mwangeji Hospital is a non-TB specialized Govt Hospital and TB culture was not carried out for comparison, culture based MTB antimicrobial susceptibility test was not conducted. However, it can act as a guide for future studies considering the different methods used.

Conclusions

The microscopic examination of AFB by ZN stain showed low sensitivity and high sensitivity thus cannot be used as a single tool in the diagnosis of tuberculosis. GenXpert showed both high sensitivity and high specificity compared to traditional smear microscopy. The simultaneous use of both needs to be done.

Availability of data and material

The data sets used and analyzed during the current study are available from the corresponding author upon request.

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