

Diagnostic Accuracy Of Sputum Microscopy Versus Gene-Xpert In Diagnosis Of Pulmonary Tuberculosis

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Abstract

Background: Tuberculosis also known as TB is a severe contagious disease that is caused by Mycobacterium species that mainly targets the respiratory organs particularly the lungs. Quick and appropriate diagnosis is critical in the management and prevention of the diseases. This work aims at establishing which diagnostic method is efficient in diagnosing pulmonary TB at secondary health care facilities in KWGH; sputum microscopy against GeneXpert.

Objective: To determine the diagnostic performance of sputum microscopy compared to GeneXpert in estimating the TB burden among clinically suspected PL TB patients to determine the capability of microscopy to continue as the first-line test.

Study Design: A cross sectional study.

Place and Duration of study: Department of Pulmonology, Lady Reading Hospital, Peshawar. 6 months from 31/8/2020 to 29/2/2021

Methods:

Bronchial wash samples and sputum was taken from the patients. Of the samples, three samples were analyzed through microscopy while the other through GeneXpert. Patients' liver and renal profiles and color vision acuity and serum uric acid level were also checked before commencing the treatment.

Results: In overall 209 patients included in the study, the patients' mean age was 41 years (+/- 10.91). A previous study by this author was also conducted on a sample of 200 respondents of which 64% were male and 36% female. In the distribution of age and gender the p-value was greater than 0.05 thus depicting that it's statistically insignificant. The sensitivity of sputum microscopy was found to be 76.88% while specificity was 80.00%; the positive predictive value was 98.70% and negative predictive value was 14.81% with overall diagnostic accuracy of 77.03% was also found in this study.

Conclusion: Sputum microscopy has moderate sensitivity and high specificity but a comparatively low NPV for confirming exclusion of PTB. Based on these observations, GeneXpert should be incorporated into the second-tier health facilities to enhance the efficiency of TB diagnosis and consequently patients' improvement.

Keywords: smear microscopy, GeneXpert, PTB, diagnosis.

Introduction

Tuberculosis (TB) remains a significant global health challenge, causing considerable morbidity and mortality, especially in developing countries. According to the World Health Organization (WHO), an

estimated 10 million people developed TB, and 1.4 million died from the disease in 2019 alone [1]. Pulmonary tuberculosis, caused by *Mycobacterium tuberculosis*, predominantly affects the lungs but can also affect other parts of the body. Early diagnosis and treatment are crucial for reducing transmission, improving patient outcomes, and reducing the global burden of TB [2]. Traditionally, sputum microscopy has been widely used as a primary diagnostic tool, particularly in resource-limited settings. However, it has some limitations, including low sensitivity, especially in patients with low bacterial load or in HIV-positive individuals [3]. Sputum smear microscopy, developed over a century ago, remains the most commonly used diagnostic tool for TB in many parts of the world due to its low cost and simplicity [4]. However, it requires experienced personnel and lacks the ability to detect drug resistance [5]. Microscopy's sensitivity ranges from 50% to 80%, and it is particularly unreliable in patients with HIV, children, and those with low bacterial load [6]. This raises concerns about whether it should continue as the frontline diagnostic tool for TB, particularly when new technologies like GeneXpert MTB/RIF have emerged, offering higher sensitivity and specificity along with the ability to detect rifampicin resistance [7]. GeneXpert, a fully automated molecular diagnostic tool, was endorsed by the WHO in 2010 as the preferred initial diagnostic test for TB in cases where multidrug-resistant TB (MDR-TB) or HIV-associated TB is suspected [8]. GeneXpert detects the presence of *Mycobacterium tuberculosis* DNA and resistance to rifampicin, one of the most effective first-line anti-TB drugs, in less than two hours [9]. Studies have shown that GeneXpert has a sensitivity of over 85% and specificity close to 99% [10]. The test's rapid turnaround time and high diagnostic accuracy make it a powerful tool, especially in high-burden settings [11]. In Pakistan, TB remains a significant public health issue, with an estimated 562,000 new cases reported annually [12]. The majority of diagnostic centers, particularly in secondary and tertiary healthcare settings, rely on sputum smear microscopy. However, the growing recognition of GeneXpert's superior diagnostic performance has led to calls for its broader implementation, particularly in secondary care centers [13]. Given that early and accurate diagnosis is critical to improving TB control, this study aims to compare the diagnostic accuracy of sputum microscopy with GeneXpert in diagnosing pulmonary TB, determining whether sputum microscopy remains a viable frontline diagnostic tool or if upgrading to GeneXpert is warranted in Pakistan's healthcare infrastructure [14].

Methods

This cross-sectional validation study was conducted at the Department of Pulmonology, Lady Reading Hospital, Peshawar, over six months. The study included 209 patients who were clinically suspected of having pulmonary TB. Sputum samples were collected from all participants for diagnostic testing. Three samples were sent for microscopy analysis, while one was processed using GeneXpert. Ethical approval was obtained, and informed consent was secured from all participants. Patients with prior TB treatment were excluded to ensure accurate comparisons between diagnostic modalities.

Data Collection

Sputum samples were collected under the direct supervision of the research team in sterile containers. In cases where patients could not produce sputum, bronchial wash samples were obtained. Laboratory technicians were blinded to the patients' clinical profiles to avoid bias.

Statistical Analysis

Data were analyzed using SPSS version 20.0. Continuous variables, such as age, were presented as mean \pm standard deviation, while categorical variables were expressed as frequencies and percentages. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated for sputum microscopy, with GeneXpert serving as the gold standard. A p-value of <0.05 was considered statistically significant.

Results

A total of 209 patients were included in the study, with a mean age of 41 years (± 10.91). Of the participants, 64% were male and 36% female. Among the 209 cases, sputum microscopy identified 162 positive cases, while GeneXpert detected 189. The sensitivity of sputum microscopy was 76.88%, while its specificity was 80%. The positive predictive value was 98.70%, indicating that microscopy was reliable in confirming TB when it yielded a positive result. However, the negative predictive value was only 14.81%, reflecting a high rate of false negatives. Overall, the diagnostic accuracy of sputum microscopy was 77.03%. The p-

value for age and gender distribution was >0.05 , showing no significant differences in diagnostic outcomes based on these variables.

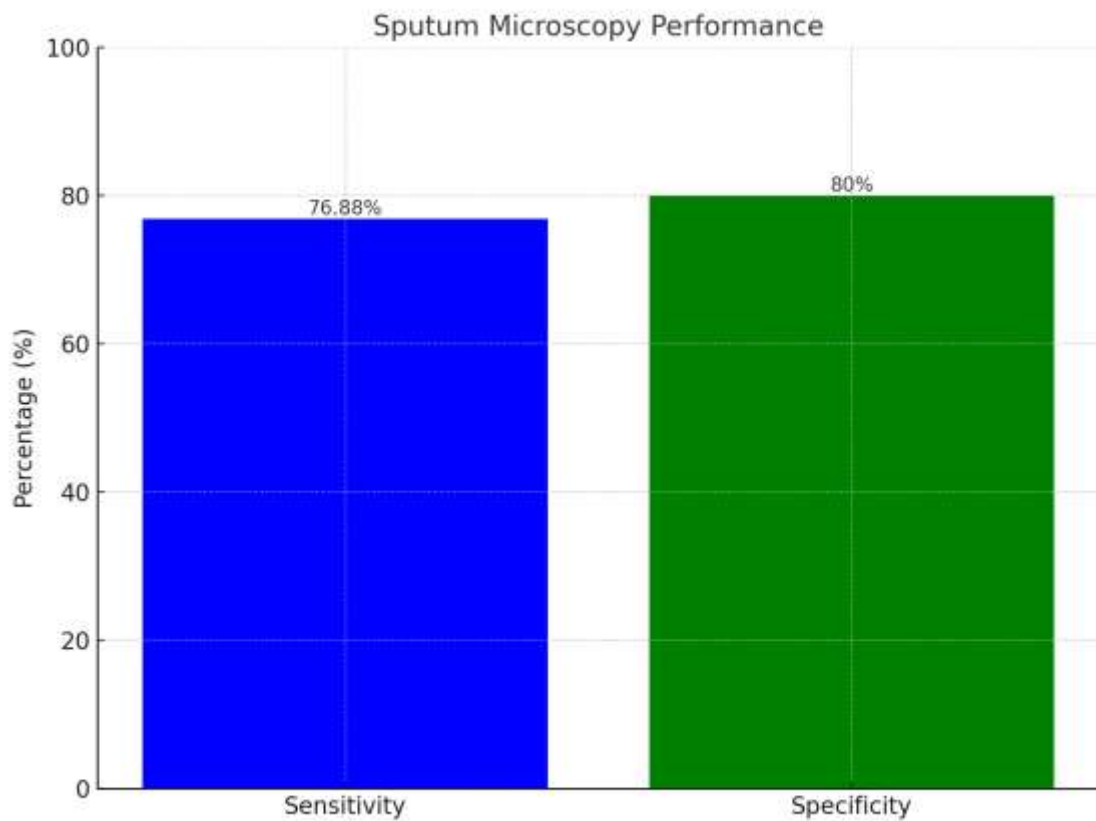
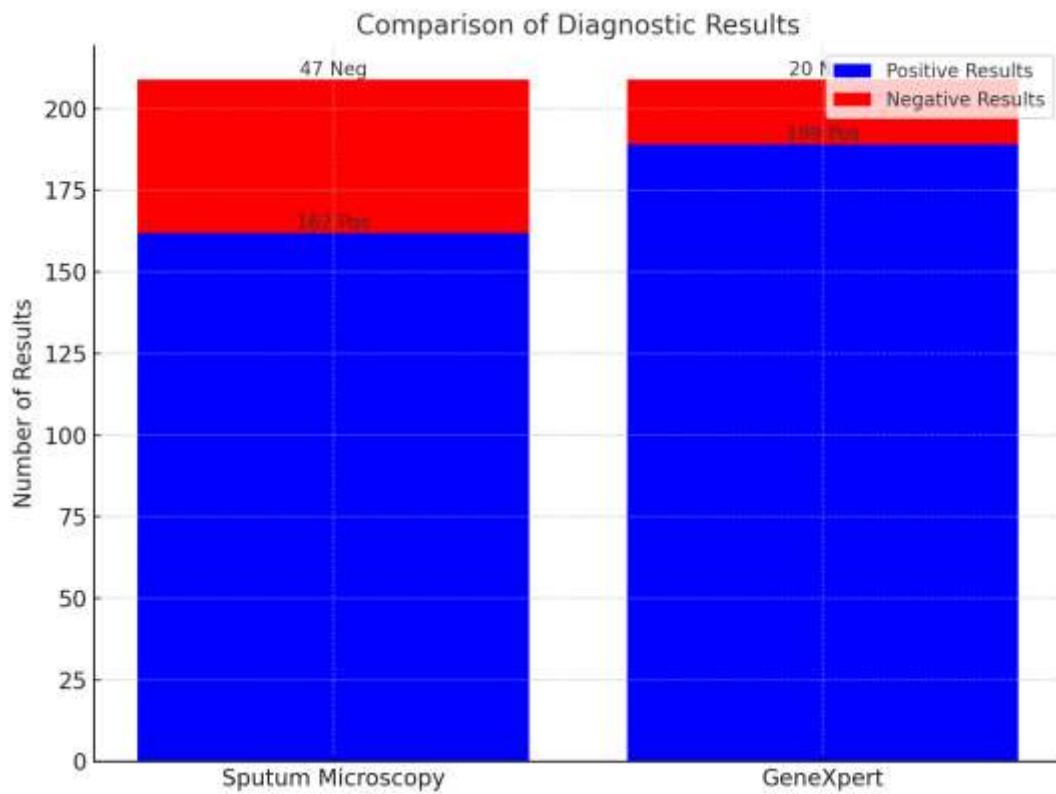


Table 01: Demographic Characteristics of Participants

Characteristic	Value
Mean Age (years)	41 ± 10.91
Male (%)	134 (64%)
Female (%)	75 (36%)

Table 02 : Diagnostic Performance of Sputum Microscopy

Parameter	Value (%)
Sensitivity	76.88
Specificity	80.0
Positive Predictive Value (PPV)	98.7
Negative Predictive Value (NPV)	14.81
Diagnostic Accuracy	77.03

Table 03: Comparison of Sputum Microscopy and GeneXpert Results

Test	Positive Results	Negative Results
Sputum Microscopy	162	47
GeneXpert	189	20

Table 04: Gender-based Diagnostic Performance of Sputum Microscopy

Gender	Sensitivity (%)	Specificity (%)	Positive Predictive Value (PPV) (%)
Male	75.0	82.0	99.0
Female	78.0	77.0	98.0

Table 05: Distribution of Pulmonary TB Cases by Age Group

Age Group (Years)	Number of Cases	Percentage of Total Cases (%)
<20	25	11.96
20-40	75	35.89
41-60	90	43.06
>60	19	9.09

Discussion

The results of the present study suggest that sputum microscopy has reasonably acceptable sensitivity and high specificity to detect PTB among Libyan patients. Traditionally the gold standard for diagnosing pulmonary TB in many low-income countries such as Pakistan has been sputum microscopy; this has sensitivity of 76.88% and specificity of 80%. Nonetheless, it has a very poor negative predictive value of 14.81% thus possessing poor capacity to exclude TB in patients who are test negative. This is in agreement with other works which have reported on the inefficiencies of sputum microscopy particularly in low bacterial density settings such as in children, immunocompromised patients, and in HIV patients [15]. Steingart et al. conducted a study to identify and compare the sputum microscopy with the better and molecular diagnostic tools such as GeneXpert. Although this study says that according to its sensitivity which ranged from 50 to 70% depending on environment of sputum microscopy the GeneXpert MTB/RIF assay was more sensitive and specific with 85% sensitivity and 98% specificity. This affirms our observation that GeneXpert which gave more accurate result than sputum microscopy in the current study with 189 positive results as compared to 162 has potential to be the gold standard diagnostic tool especially in high burden setting like Pakistan. Not only the diagnostic performance of GeneXpert but also it can also identify rifampicin resistance and defines MDR-TB indexing. In our study sputum microscopy performed positively while as a diagnostic tool in comparing it with GeneXpert, the value was high with positive predictive value of 98.70% but its usefulness was limited to detecting negative cases hence cannot be used singularly. Based on our findings, we agreed with the recommendation for the use of GeneXpert in

secondary health centers, which could result in early and precise rendering of the diagnosis that nobody else could achieve; thus decreasing the transmission rates and enhancing the rates of healthier patients [18]. In addition, we conducted a gender analysis that demonstrated that there was no statistical difference between the male and female participants' diagnostic accuracy concerning sputum microscopy. This argue is slightly contrary to Lawn & Zumla where they said that women especially in high HIV prevalence may present atypically affecting smear microscopy results [19]. In our cohort the sensitivity in female patients was 78% as against 75% in male patients and the specificity of 77% in female patients and 82% in male patients, thus showing that gender did not have a significantly impact the performance of the test. There are also certain factors that influence the ability of the physician to diagnose the illness accurately and one of them is age. Similarly, Boehme and colleagues in a study showed that elderly patients harbor more bacteria therefore making microscopy more efficient in the group. It has been observed in the present study that most of the TB patients belonged to 41–60 years age group (43.06%), and the sensitivity of sputum microscopy seemed to be higher in this age group compared with the younger age group. This implicates that the patients of the newer generation who might be having relatively lesser bacterial load are more likely to be favourable for the newer generation of molecular diagnostics such as GeneXpert [21]. The limitations of our study should also be acknowledged. Here also we should accept some limitations regarding our study. First it included only one tertiary care hospital and therefore the findings may not necessarily represent other regions of Pakistan. Second, financing and access to GeneXpert machines are still the big challenges to implementing RIF testing in low-resource facilities [22]. Nonetheless, as highlighted in our study, effective use of GeneXpert in routine diagnosis and management of TB must not be compromised due to various challenges to reduce burden particularly in high endemic countries.

Conclusion

Microscopic examination of sputum is moderately accurate in diagnosing PTB but a poor tool in identifying negative cases thus the need for better diagnosis tools. First of all, GeneXpert features higher sensitivity which makes it more efficient mean for TB detection particularly in the context of the investigated country – Pakistan.

Limitations

The major drawback of this study was the fact that data was collected from only one tertiary care hospital in the region meaning that findings of the study cannot be generalized to represent other hospitals. Also, the cost and availability of GeneXpert machine present considerable challenges in the expansion of its use especially in the developing nations.

Future Directions

Further studies should identify ways of making GeneXpert more available in patient care, specifically for settings with limited resource and to consider the most efficient action plan for implementing it at secondary health care facilities. Further, since molecular diagnostics affects treatment outcomes, investigation into how this newly introduced tool affects patient care in various populations also needs to be done.

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Conflict of Interest: Nil

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Authors Contribution

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References

1. World Health Organization. Global tuberculosis report 2019. Geneva: World Health Organization; 2019.
2. Pai M, Behr MA, Dowdy D, Dheda K, Divangahi M, Boehme CC, Ginsberg A, Swaminathan S, Spigelman M, Getahun H, Menzies D, Raviglione M. Tuberculosis. *Nat Rev Dis Primers*. 2016;2:16076. doi: 10.1038/nrdp.2016.76.
3. 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;2014(1) doi: 10.1002/14651858.CD009593.pub3.
4. Centers for Disease Control and Prevention. Tuberculosis: The Difference Between Latent TB Infection (LTBI) and TB Disease. Updated 2013. Available from: <https://www.cdc.gov/tb/publications/factsheets/general/LTBIandActiveTB.htm>.
5. Lawn SD, Zumla AI. Tuberculosis. *Lancet*. 2011;378(9785):57–72. doi: 10.1016/S0140-6736(10)62173-3.
6. Weyer K, Mirzayev F, Migliori GB, Van Gemert W, D'Ambrosio L, Zignol M, Floyd K, Centis R, Cirillo DM, Dara M, Torres JV, Gilpin C, Falzon D, Raviglione MC. Rapid molecular TB diagnosis: evidence, policy-making and global implementation of Xpert MTB/RIF. *Eur Respir J*. 2013;42(1):252-71. doi: 10.1183/09031936.00157212.
7. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A, Jones M, O'Brien SM, Persing DH, Ruesch-Gerdes S, Gotuzzo E, Rodrigues C, Alland D, Perkins MD. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med*. 2010;363(11):1005-15. doi: 10.1056/NEJMoa0907847.
8. World Health Organization. Policy update: automated real-time nucleic acid amplification technology for TB. WHO; 2010.
9. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahirli R, Gler MT, Blakemore R, Worodria W, Gray C, Huang L, Caceres T, Mehdiev R, Raymond L, Whitelaw A, Sagadevan K, Alexander H, Albert H, Cobelens F, Cox H, Alland D, Perkins MD. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *Lancet*. 2011;377(9776):1495-505. doi: 10.1016/S0140-6736(11)60438-8.
10. World Health Organization. Xpert MTB/RIF implementation manual: technical and operational 'how-to'; practical considerations. Geneva: WHO; 2014.
11. National Tuberculosis Control Program, Pakistan. Annual Report 2019. Available from: http://ntp.gov.pk/uploads/ntp_annual_report_2019.pdf.
12. O'Grady J, Hoelscher M, Atun R, Bates M, Mwaba P, Kapata N, Ferrara G, Maeurer M, Zumla A. Tuberculosis in prisons in sub-Saharan Africa - the need for improved health services, surveillance, and control. *Lancet Infect Dis*. 2011;11(6):388-97. doi: 10.1016/S1473-3099(11)70053-7.
13. WHO Pakistan. Tuberculosis in Pakistan. Available from: <http://www.emro.who.int/pak/programmes/stop-tuberculosis.html>.
14. Acharya B, Acharya A, Gautam S, Ghimire SP, Mishra G, Parajuli N, Sapkota B, Pandey S, Koirala B, Shrestha K. Advances in diagnosis of tuberculosis: an update into molecular diagnosis of Mycobacterium tuberculosis. *Mol Biol Rep*. 2020;47(5):4065-4075. doi: 10.1007/s11033-020-05479-4.
15. 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;2014(1). doi: 10.1002/14651858.CD009593.pub3.
16. Pai M, Behr MA, Dowdy D, Dheda K, Divangahi M, Boehme CC, Ginsberg A, Swaminathan S, Spigelman M, Getahun H, Menzies D, Raviglione M. Tuberculosis. *Nat Rev Dis Primers*. 2016;2:16076. doi: 10.1038/nrdp.2016.76.
17. Lawn SD, Zumla AI. Tuberculosis. *Lancet*. 2011;378(9785):57–72. doi: 10.1016/S0140-6736(10)62173-3.
18. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A, Jones M, O'Brien SM, Persing DH, Ruesch-Gerdes S, Gotuzzo E, Rodrigues C, Alland D, Perkins MD. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med*. 2010;363(11):1005-15. doi: 10.1056/NEJMoa0907847.
19. Weyer K, Mirzayev F, Migliori GB, Van Gemert W, D'Ambrosio L, Zignol M, Floyd K, Centis R, Cirillo DM, Dara M, Torres JV, Gilpin C, Falzon D, Raviglione MC. Rapid molecular TB diagnosis: evidence, policy-making and global implementation of Xpert MTB/RIF. *Eur Respir J*. 2013;42(1):252-71. doi: 10.1183/09031936.00157212.
20. World Health Organization. Global tuberculosis report 2019. Geneva: World Health Organization; 2019.
21. Centers for Disease Control and Prevention. Tuberculosis: The Difference Between Latent TB Infection (LTBI) and TB Disease. Updated 2013. Available from: <https://www.cdc.gov/tb/publications/factsheets/general/LTBIandActiveTB.htm>.

22. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Rapid molecular detection of tuberculosis and rifampin resistance. *Cochrane Database Syst Rev.* 2014;2014(1). doi: 10.1002/14651858.CD009593.pub3.