# DIAGNOSTIC ACCURACY OF GENEXPERT ON HISTOPATHOLOGICAL EXAMINATION OF LESION TISSUE SAMPLES IN TB SPONDYLITIS CASES AT DR. M. DJAMIL HOSPITAL PADANG

**MUAMMAR AQIB MUFTI[1], RONI EKA SAHPUTRA[2], HENDRA MASKA[2]**

[1] Department of Surgical, Faculty of Medicine, Andalas University/ Dr. M. Djamil Hospital, Padang

[2] Division of Orthopedic Surgery, Department of Surgery, Faculty of Medicine, Andalas University/ Dr. M. Djamil Hospital, Padang

## ABSTRACT

**Introduction:** Tuberculosis spondylitis (TB) or Pott's is a disease that often occurs in developing countries but is relatively difficult to diagnose. Since 2013, the Xpert MTB/RIF test has been used to diagnose extrapulmonary TB, but data regarding its accuracy is still lacking in Indonesia.

**Objective**: To determine the diagnostic accuracy of Genexpert in histopathological examination of lesion tissue samples in cases of TB spondylitis at Dr.M.Djamil General Hospital, Padang.

**Method**: This is a cross-sectional with a retrospective study approach at Dr. M. Djamil Hospital in July – October 2023. This study uses medical record data from patients suspected of suffering from tuberculous spondylitis at Dr. M. Djamil Hospital Padang from January – December 2022. This study assessed the accuracy, sensitivity and specificity of the Gene Xpert in TB spondylitis lesion tissue samples during surgery.

**Results**: There were 19 respondents with suspected TB spondylitis underwent surgery. In the gene Xpert and histopathology examination, 15 positive samples and 4 negative samples were obtained. Of the gene Xpert positive samples, 14/15 samples also received positive results, but 1/14 received negative results on histopathological examination. Meanwhile, in the 4 negative samples from Gene Xpert, 3/4 of the samples were also negative on histopathological examination but 1 sample had a positive result. The accuracy, sensitivity and specificity of the Gene Xperton histopathology of TB spondylitis lesion tissue were 89.47%, 93.33% and 75% respectively.

**Conclusion:**Genexpert has quite high accuracy in diagnosing TB spondylitis so that it can be used as an early detection modality in cases of TB spondylitis.

**KEYWORDS:** Gene Xpert, TB spondylitis, Histopathology, tissue lesions

## INTRODUCTION

Tuberculous (TB) spondylitis or Pott's disease is one of the most common spinal infections, especially in developing countries which is the most common cause of non-traumatic spinal injuries.[1] Worldwide, as much as 80% of TB spondylitis are found in developing and poorcountries.[2,3] TB spondylitis has prevalence of 0.1 to 1.4% and occur more frequently in those with low socioeconomic status.[4]

Tuberculous spondylitis is a chronic and slowly progressive disease which relatively difficult to diagnose.[2] Clinical manifestations include mass effect and neurological deficits caused by cold abscess, and kyphotic deformity caused by damage to the anterior vertebral body. Laboratory and typical imaging findings could lead to diagnosis, but culture, histopathology, and Polymerase Chain Reaction (PCR) is the gold standard.[5] After the patient diagnosed with suspected TB spondylitis, the patient is indicated for surgery. During surgery, tissuesare taken for histopathological examination as the gold standard for diagnosing TB spondylitis with the results are obtained after 6-7 days.

In October 2013, WHO published new Policy Guidance providing revised recommendations on the use of Xpert MTB/RIF to diagnose pulmonary TB, childhood TB, extrapulmonary TB and rifampicin resistance. Xpert MTB/RIF made a significant leap with rapid detection of TB by NAAT as well as Rifampicin resistance in less than 2 hours. In addition to rapid sputum tests, DSTs (drug susceptibility tests), and triage tests, non-sputum biomarkers are identified as a priority.[6]

Department of Spine Orthopaedicswas trying to develop health services by developing brief and faster diagnostic examinationof TB spondylitis so it can accelerate the administering of anti-tuberculosis drug. GeneXpert could generate the result in only 2 hours with lesion samples taken during surgery. This study aims to determine the diagnostic accuracy of GeneXpert towards histopathological examination of lesion tissue samples in TB spondylitis at Dr.M.Djamil General Hospital, Padang.

## METHOD

This is an analytical study using a cross-sectional design. The study was conducted at Dr. M. DjamilGeneral Hospital Padang in July – October 2023 which included all patients diagnosed with TB Spondylitis who had undergone histopathological and GeneXpert examination of lesion tissue samples at Dr.M.Djamil Hospital Padang in 2022. Inclusion criteria were patients whichundergone both of histopathology and GeneXpert examination of tissue samples around the lesion. Patients with only had one of examination and having unclear or incomplete medical records were excluded. The primary results were accuracy, sensitivity and specificity of GeneXpert in TB spondylitis.

## RESULT

In this study, there were 19 subjects suspected of suffering from TB spondylitis. In Table 1, the mean age of respondents is 43.89 ± 18.8 years with the largest age groups being 21-40 and 41-60 years old. Most respondents were male (73.7%), and underweight (42.1%). As many as 78.9% of patients had positive result in histopathology examination andGeneXpert examination also found positive results in 78.9% patients.

**Table 1. Characteristic of suspected Tuberculosis spondylitis patient in Dr. M. Djamil General Hospital Padang**

|  | Mean ±SD (Min-max) | f(%) |
|---|---|---|
| **Age (years)** | 43,89 ± 18,8 (13-76) |  |
| <=20 |  | 2 (10,5) |
| 21-40 |  | 6 (31,6) |
| 41-60 |  | 6 (31,6) |
| >60 |  | 5 (26,3) |
| **Gender** |  |  |
| Male |  | 14 (73,7) |
| Female |  | 5 (26,3) |
| **Body Mass Index** |  |  |
| Underweight |  | 8 (42,1) |
| Normoweight |  | 6 (31,6) |
| Overweight and Obesitas |  | 5 (26,3) |
| **Hasil Histopatologi** |  |  |
| Positif |  | 15 (78,9) |
| Negatif |  | 4 (21,1) |
| **Hasil GeneXpert** |  |  |
| Positif |  | 15 (78,9) |
| Negatif |  | 4 (21,1) |

In Table 2, 15patients with GeneXpertMTB positive are shown, which 14 are positive and 1 is negative by histopathology examination. In 4 patients of GeneXpert MTB negative, 3 patients are also negative on histopathology examination and 1 person is positive on histopathology. From the calculation of the accuracy formula, we found the accuracy 89.47% with 1 false positive case and 1 false negative sample.

In Table 3, the sensitivity of the GeneXpert examination is 93.33% and the specificity is 75%.

**Table 2. The diagnostic accuracy of GeneXpert towards histopathological examination of the tissue around the lesion in suspected cases of TB spondylitis at Dr. M. DjamilGeneralHospital Padang**

| GeneXpert | Histopathology | | Accuracy |
| --- | --- | --- | --- |
| | Positive (n,%) | Negative(n,%) | |
| Positive | 14 (93,3) | 1 (6,7) | 89,47% |
| Negative | 1 (25) | 3 (75) | |

**Table 3. The sensitivity and specificity of GeneXpert towards histopathological examination of the tissue around the lesion in suspected cases of TB spondylitis at Dr. M. Djamil GeneralHospital Padang**

| GenExpert | Histopathology | | Sensitivity | Specificity |
| --- | --- | --- | --- | --- |
| | Positive | Negative | | |
| Positive | 14 | 1 | 93,33% | 75% |
| Negative | 1 | 3 | | |

## DISCUSSION

Tuberculosis (TB) spondylitis is the most common spinal infection with most patients found in developing countries and poor areas. TB spondylitis can occur in teenagers, most in their 30s, and rarely occurs after the age of 45 years. [2,3] In this study,the mean age of respondents was $43.89 \pm 18.8$ years old, with the largest age group are 21-40 years and 41-60 years. The incidence of TB spondylitis is dominated by young to middle-aged adults (productive age) because they are more likely to live in crowded places and have a greater risk of being exposed to environments with poor hygiene. [7] Most of respondents were male (73,

7%) where this may be influenced by work and lifestyle factors.[8] Most of the patients were underweight (42.1%). Malnutrition in patient suffering from TB infection caused by increasing of anabolic processes and additional energy consumption. In addition, TB infection often causes decreased appetite and nutritional malabsorption, which increases the risk of being underweight.[9]

As many as 78.9% patients had positive histopathology results. Li et al stated that even if histopathology is the gold standard for TB spondylitis, the pathological finding are often atypical. The general characteristics of TB spondylitis include caseous necrosis, multinuclear giant cells, granulomatous inflammation and tubercles. In addition, multiple pathological characteristics are frequently seen in TB spondylitis patients as well as atypical preparations. Therefore, pathological examination must be accompanied by appropriate clinical

manifestations and other modalities to increase the diagnostic accuracy of TB spondylitis.[10]

The GeneXpert assay (Xpert MTB/RIF assay) is a new approach using DNA analysis in the detection of MTB and rifampicin resistance. Molecular techniques are major revolution in the diagnosis of tuberculosis which provides fast results with quite high sensitivity in the detection of MTB and drug resistance. In addition, Gene Xpertalso starting to be used in many types of extrapulmonary samples.[11,12]

This study found the accuracy of the GeneXpert was 89.47% with 1 false positive and 1 false negative case. Charisma et al (2021) also reported good accuracy of the GeneXpert TB in extrapulmonary samples.[14] Solanki et al (2019) obtained a higher accuracy which is 96.25% in TB spondylitis samples.[15] Meanwhile, study by Patel et al (2020) in 360 patients diagnosed with spinal TB obtained 14 false positive and 13 false negative cases which confirmed that the GeneXpert examination can only be used as an initialexamination, and must still be followed by gold standard examination.[16]

Rapid and accurate methods for diagnosing MTB are essential for early treatment initiation, preventing complications and improving patient outcomes. The importance of early detection for MTB, especially in developing countries, is the basis for using polymerase chain reaction-based technique,Gene Xpert, which has been developed. GeneXpert examination can be obtained within 2 hours, compared to 8 weeks in culture. Meanwhile, in

the histopathological examination which is the gold standard for TB spondylitis, experts are needed for the examination which needa long-time to assess the preparation. [17] Based on the interviews, histopathological examination of TB spondylitis samples takes 4-5 days at Dr. M. Djamil Padang. Because histopathology examinationdepends on experts, so the diagnostic time can be affected by the number of samples that hadto be examined at the same time. This causes a long time to diagnose TB spondylitis which leads to treatment delay.

In addition tofaster result, GeneXpert MTB/RIF also has another advantage. First, the amplification system is closed so itreduces the potential of cross-reaction. Second, the GeneXpert can give information about rifampicin resistance encoded in the rpoB gene. The rpoB gene is responsible for 96% of cases of rifampin resistance in MTB infection. Resistance to rifampicin is the main predictor of multidrug-resistant TB, because most bacteria that are resistant to rifampicin are also resistant to isoniazid. Third, it can be used as a substitute examination for microscopic testing because it can detect the presence of bacteria in negative and positive smears. Fourth, the GeneXpert test is able to identify the presence of M. tuberculosis taken from different specimens.[18,19]

Technically, no single examination for TB is perfect, where each modality such as microscopic examination, conventional culture and sensitivity testing had limitations related to accuracy, effectiveness, operator-dependent, resource requirements and biosafety.[15] However, seeing several benefits from this examination, Gene Xpert can be used as a rapid initial diagnostic test compared to histopathological examination in cases of TB spondylitis so early therapy initiation can be achieved to

avoid further complications. However, negative results on GeneXpert still need to be reconfirmed through assessment of clinical symptoms, radiology, culture and histopathology examination.

The sensitivity test results for the GeneXpert was 93.33% and the specificity was 75%. Similar to this study, Salim et al (2021) found GeneXpert sensitivity and specificity results of 88.2% and 33.3% respectively, where high sensitivity but low specificity was obtained.[14]Study by Yu et al (2020) in 106 subjects with suspected spinal TB found the sensitivity of GeneXpert examination being lower, which is 86.7%. Study by Yu et al (2020) stated that the GeneXpert is not recommended as a substitute for conventional methods for diagnosing spinal TB due to poor sensitivity.[20] Another study by Held et al (2014) found higher sensitivity and specificity of GeneXpert in Spinal TB which were 95.6% and 96.2%.[21]Study by Arockiaraj et al (2017) found that the sensitivity and specificity of the Gene Xpert examination in detecting MTBwere 80.9% and 80.6% respectively.[22]

The relatively low specificity of the GenXpert found in many studies can occur because the GeneXpert detects both live and dead MTB so that patients with a history of TB infection who have recovered are still detected as positive.For this reason, Gene Xpert cannot be used as a follow-up modality because the results will always be positive even if the MTB had died. GeneXpert coulddetect live and dead MTB because the examination is DNA-based so even if the MTBwere dead, the results still positive because the DNA of dead MTB is still detected.[23]

The high sensitivity obtained in this study and previous studies could prove the effectiveness of the Gene Xpert examination in early detection of TB spondylitis with faster results so that therapy in patients can be started earlier. However, in negative results, other tests must still be followed before excluding the diagnosis of TB spondylitis.[15]

In this study, one false positive and false negative were found on the GeneXpert examination. False positive are often caused by the wrong amplification target while false negative results are often related to problems with the sample, reagent, or amount of PCR inhibitor. Susilawati et al stated that errors in sample processing can be minimized by using Bacillus globigii spores as an internal control. Spores were loaded into cartridges to ensure lysis of M. tuberculosis and to detect PCR inhibitors. Additionally, misinterpretation may also come from the instrument which required stable power, appropriate temperature (<30°C), and routine calibration of the cartridge module.[19]

GeneXpert requires an uninterrupted power supply, annual calibration and an operating temperature of 15°C–30°C. The machine needs to be connected to a computer and cartridges require adequate storage space and have a shelf life of 18 months which stable if stored at 2°C–45°C for <6 weeks at 75% relative humidity. The cartridge must be used within 4 hours after opening the cartridge cap. Inappropriate conditions can be the factor in misinterpretation. In addition, patients with a history of a cured MTB infection or the presence of non-infective MTB bacteria (damaged or non-viable MTB) in the spine, still had positive result even after appropriate therapy because GeneXpert detects DNA from the living and non-livingbacilli.[19,23]

As many as 4 histopathology samples were found to be negative. This is because of the samples taken are not only came from patients with suspected TB spondylitis but also from the patients with suspected intradural tumors (1 patient), lumbar tumors (1 patient), lumbo sacral tumors (1 patient) and lumbar bone metastases (1 patient) based on CT scan or MRI examination. Specimens from this patient were also collected because the lesion findings during surgery were similar to TB spondylitis, so histopathology and GeneXpert examination were also carried out. Lesions in TB spondylitis usually consist of damaged perivertebral tissues, accumulation of pus and secretions which are also found in spinal neoplasms or pyogenic spondylitis so it requires a gold standard examination, such as histopathology, to rule out the diagnosis of TB. [24]

This study was severely limited. As a single-center study, it is unable to characterize the excessive lack of accuracy in the Gene X examination throughout Indonesia's diverse demographics. However, given that the GeneXpert examination can be used as a quick screening tool in instances of TB spondylitis, this is the first study discussing the accuracy of GeneXpert in TB spondylitis using tissue lesions, conducted in West Sumatra, and one of the few studies in Indonesia.

## CONCLUSION

High accuracy was discovered in the GeneXpert examination. Given the various benefits of the GeneXpert examination, including its ability to detect resistance and yield speedier results, it is possible to utilize this test as a screening tool for TB spondylitis. Negative results on GeneXpert, however, must still be validated with assessment of clinical symptoms, radiographic examination, culture, and histology in compliance with diagnostic standards. Furthermore, as this is a single-center study, multi-institutional research is required to raise the caliber of the findings.

## REFERENCE

[1]. Sharma, A., Chhabra, H. S., Chabra, T., Mahajan, R., Batra, S., &Sangondimath, G. Demographics of tuberculosis of spine and factors affecting neurological improvement in patients suffering from tuberculosis of spine: a retrospective analysis of 312 cases. Spinal Cord. 2016; 55(1): 59–63.

[2]. Liu Z, Wang J, Chen GZ et al. Clinical characteristics of 1378 in patients with spinal tuberculosis in general hospitals in South-Central China. BioMed Res Int. 2019: 9765253.

[3]. Javed G, Laghari AA, Ahmed SL et al . Development of criteria highly suggestive of spinal tuberculosis.WorldNeurosurg. 2018; 116:e1002–e1006.

[4]. Kwon SR, Kim TH, Kim TJ, Park W, Shim SC. The Epidemiology and Treatment of Ankylosing Spondylitis in Korea. J Rheum Dis. 2022;29(4):193-199.

[5]. Khanna, K., & Sabharwal, S. (2019). Spinal Tuberculosis: A Comprehensive Review For The Modern Spine Surgeon. The Spine Journal. 2019; 19: 1858- 1870.

[6]. Denkinger CM, Schumacher SG, Gilpin C, Korobitsyn A, Wells WA, Pai M, et al. Guidance for the evaluation of tuberculosis diagnostics that meet the world health organization (WHO) target product profiles: an introduction to who process and study design principles. J Infect Dis. 2019; 220: S91–8.

[7]. Ismiarto AF, Tiksnadi B, Soenggono A. Young to Middle-Aged Adults and Low Education: Risk Factors of Spondylitis Tuberculosis with Neurological Deficit and Deformity at Dr. Hasan Sadikin General Hospital. AMJ. 2018;5(2):69–76

[8]. Novita BD, Muliono AC, Wijaya S, Theodora I, Tjahjono Y, Supit VD, Willianto VM. Managing spondylitis tuberculosis in a patient with underlying diabetes and hypothyroidism: A case report. World J Clin Cases. 2022; 10(21): 7451-8

[9]. Feleke BE, Feleke TE, Biadglegne F. Nutritional status of tuberculosis patients, a comparative cross-sectional study. BMC Pulm Med. 2019; 19:182. https://doi.org/10.1186/s12890-019-0953-0

[10]. Li y, Wang Y, Ding H, et al. Pathologic characteristics of spinal tuberculosis: analysis of 181 cases. Int J Clin Exp Pathol. 2020;13(5):1253-1261.

[11]. World Health Organization. Xpert MTB Implementation Manual. Genevea. 2014

[12]. Sahana, K. S., Prabhu, A. S., & Saldanha, P. R. Usage of Cartridge Based Nucleic Acid Amplification Test (CB-NAAT/GeneXpert) test as diagnostic modality for pediatric tuberculosis; case series from Mangalore, South India.

Journal of Clinical Tuberculosis and Other Mycobacterial Diseases. 2018; 11: 7–9

[13]. Charisma AN, Koesoemoprodjo W. Diagnosis and Outcome of Tuberculosis of Knee Joint (Gonitis Tuberculosis) with Pulmonary Tuberculosis after Completing Anti-Tuberculosis Therapy: A Case Report. JurnalRespirasi. 2021; 7(1): 19-26

[14]. Salim DR, Ramdan A, &Alpharian GT. Comparison of Diagnostic Accuracy of Tuberculosis Spondylitis between GeneXpert and Histopathological Examination. MajalahKedokteran Bandung. 2021; 53 (1).

[15]. Solanki AM, Basu S, Biswas A, Roy S, Banta A. Sensitivity and Specificity of Gene Xpert in the Diagnosis of Spinal Tuberculosis: A Prospective Controlled Clinical Study. Global Spine Journal. 2020;10(5):553-558. doi:10.1177/2192568219858310

[16]. Patel JYK. Can GeneXpert MTB/RIF (Mycobacterium Tuberculosis/Rifampicin) assay replace acid-fast bacilli (AFB) smear and histopathology as a diagnostic test for spinal tuberculosis. The Lancet Infectious Disease. 2020

[17]. Jabber Benellam Y K, Ozkan O, Saadoon Aziz Z . Diagnosis of Mycobacterium tuberculosis using GeneXpert MTB / RIF and TB-LAMP techniques from pulmonary and extra-pulmonary TB patients in Iraq. Revis Bionatura. 2022;7(2): 50.

[18]. Mechal Y, Benaissa E, El Mrimar N, et al. Evaluation of GeneXpert MTB/RIF system performances in the diagnosis of extrapulmonary tuberculosis. BMC Infect Dis. 2019;19(1):1069. Published 2019 Dec 19. doi:10.1186/s12879-019-4687-7

[19]. Susilawati TN, Larasati R. A recent update of the diagnostic methods for tuberculosis and their applicability in Indonesia: a narrative review. Med J Indones 2019;28(3):284–91

[20]. Yu, Y., Kong, Y., Ye, J., & Wang, A. Performance of conventional histopathology and GeneXpert MTB/RIF in the diagnosis of spinal tuberculosis from bone specimens: A prospective clinical study. Clinical Biochemistry. 2020; 85: 33- 37

[21]. Held M, Laubscher M, Zar HJ, Dunn RN, GeneXpert polymerase chain reaction for spinal tuberculosis. Bone Joint J. 2014; 96-B: 1366– 1369.

[22]. Arockiaraj J, Michael JS, Amritanand R, David KS, Krishnan V. The role of Xpert MTB/RIF assay in the diagnosis of tubercular spondylodiscitis. Eur Spine J. 2017;26(12):3162-3169. doi:10.1007/s00586-017-5076-9

[23]. Garg A, Agarwal L, Mathur R. Role of GeneXpert or CBNAAT in diagnosing tuberculosis: Present scenario. Med J DY Patil Vidyapeeth. 2022; 15:14-9.

[24]. Lu M, Chen W, Lei Z, et al. Image-guided percutaneous biopsy and pathological diagnosis in atypical tuberculous spondylitis: a case series and clinical outcomes. Ann Transl Med. 2022;10(20):1140. doi:10.21037/atm-22-4661