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# CLINICAL ARTICLE

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## Clinical utility of tissue polymerase chain reaction in the diagnosis of spinal tuberculosis

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### Abstract

#### Background

An estimated 14 million people worldwide have active tuberculosis (TB). About 3% of these patients will have osteoarticular involvement and approximately 25% to 60% will have an infectious focus in the spine. Early diagnosis is essential as prompt treatment is associated with improved outcome and reduced mortality. This is particularly true within a high HIV-1 seroprevalence setting.

#### Materials and methods

All patients admitted to Kalafong District Hospital from January 2008 to December 2010 with a clinico-radiological diagnosis of spinal TB were included in this study. In all cases Ziehl-Nielsen (ZN) microscopy, TB culture, TB polymerase chain reaction (PCR), and histology with ZN stains were collected, and the turnaround times for these assays recorded. HIV testing was performed on patients who gave consent for the procedure.

#### Results

In total, 29 patients were included in this study. Seventeen patients consented to HIV testing of which 11 were confirmed to be positive. It was determined that sensitivity for culture and PCR were comparable at 77% and 72% respectively. Furthermore, when looking at the subgroup of HIV-1 positive patients specifically, both assays performed better, with sensitivities of 88% and 82% respectively. The TAT for assays was highly variable, with PCR and histology having comparable times.

#### Conclusions

PCR testing for spinal TB shows promising results especially within the HIV-1-positive population. Although this type of testing theoretically offers a shorter turnaround time, results were available in similar time frames as for histology. Therefore, on-site testing should be offered in hospitals with high case loads of TB, and combination testing should be used rather than opting for a single testing modality.

**Key words:** *Mycobacterium tuberculosis*, molecular diagnosis, skeletal TB

## Introduction

Infection with *Mycobacterium tuberculosis* remains a major health problem worldwide.<sup>1,2</sup> The global incidence, as estimated by the World Health Organization (WHO), is said to have increased by 0.4% per annum.<sup>3</sup> South Africa has also seen a significant rise in incidence, with rates increasing from 190 cases per 100 000 in 1980 to 339 per 100 000 in 2001.<sup>3</sup> This is largely driven by the HIV-1 pandemic<sup>4</sup> but other causes of immunosuppression, including malnutrition, IV drug abuse, alcoholism, cirrhosis, diabetes mellitus, pharmacological suppression,<sup>5,6</sup> ageing<sup>7-13</sup> and transplants<sup>14</sup> may also precipitate activation of latent TB.

The WHO estimates that of the 14 million patients worldwide with active TB, approximately 3% will have skeletal infection.<sup>15</sup> Of these, approximately 25% to 60% will have the infectious focus in the spine.<sup>16</sup> Spinal TB produces an indolent and slow-growing infection<sup>17</sup> and is characteristically paucibacillary.<sup>18</sup> For this reason, diagnosis by demonstration of the micro-organisms is often problematic.<sup>19</sup>

Despite the diagnostic pitfalls, early accurate identification of the organism and determination of antibiotic sensitivity is essential, as early appropriate treatment is associated with improved outcome and reduced mortality.<sup>20</sup> This is particularly true within a high HIV-1 seroprevalence setting.<sup>20</sup> Solid-media culture-based testing can require up to 8 weeks for identification, which is reduced to approximately 3 weeks with the use of liquid culture assays.<sup>19</sup> The promise of rapid diagnosis exists with the wide implementation of molecular platforms like polymerase chain reaction assays (PCR). Turnaround times are being reported to be as short as 24 hours.<sup>21</sup>

In our clinical setting, patients are investigated utilising various modalities, including culture, histology, TB PCR and microscopy. Each of these modalities has varying sensitivities and specificities. The aim of this study was to evaluate the clinical utility of each of these modalities, both in terms of diagnostic accuracy as well as turnaround times.

## Materials and methods

### Patient population

All patients admitted to the Kalafong District Hospital from January 2008 to December 2010 with a diagnosis of spinal TB, were included in this study. For the purpose of this study, a diagnosis of spinal TB was based on a combination of suggestive clinical features, in conjunction with typical radiological findings associated with spinal TB. HIV-1 serology results were included where available. All patients were evaluated regarding the site of infection. This was described as both the number of vertebrae affected in each patient, as well as the level of infection.

### Laboratory parameters

HIV-1 status was determined using the HIV Combi Assay (Roche Diagnostics, Mannheim, Germany). All positive results were confirmed using the HIV Ag/Ab Combo Assay (Abbott, Santa Clara, USA). Direct microscopy for *M. tuberculosis* was performed with a Ziehl-Nielsen stain (ZN).<sup>22</sup> Quantification of acid-fast bacilli was performed using parameters specified by the Centers for Disease Control and Prevention (CDC).<sup>22</sup> Mycobacterial cultures were performed the MGIT system (BD Diagnostic Systems, Sparks, MD) and subsequent sensitivities were determined using the agar proportion method by BACTEC MGIT 960 (BD Diagnostic Systems, Sparks MD). Histological examination of biopsy samples obtained from the affected spinal structures was performed using both haematoxylin and eosin (H&E) stains as well as ZN stain. The H&E stained samples were examined for features of mycobacterial infection including granulomata and Langerhans cells. The ZN stain was examined for acid-fast bacilli. Biopsy samples were submitted for molecular testing using the GeneXpert Diagnostic System (Cepheid, Sunnydale, CA). This platform utilises real-time PCR technology and has been validated for direct use on diagnostic samples.<sup>23,24</sup>

Of note, the local laboratory, situated at the study site, performed microscopy and cultures. The biopsy samples were sent to the nearest academic centre for evaluation and the PCR was performed by a local private laboratory, as a referred test.

Results were obtained retrospectively for all assays (microscopy, culture, histology and PCR testing) from the laboratory database. The turnaround times were documented in terms of days from submission of the sample to availability of verified results.

### Statistics

In order to determine the sensitivity and specificity of each of the assays, laboratory-based positivity was defined as either a positive finding on culture or histology compatible with tuberculous infection with acid-fast bacilli noted on ZN stain of the biopsy. ZN positivity in isolation was not considered as a positive result as various environmental mycobacteria may lead to false positivity. PCR positivity was not included as this was the assay used for comparison of diagnostic utility.

## Results

### Patient population

In total, 29 patients were included in this study. The median age was 46 years (28 to 70 years). In total, 11 of the patients were confirmed to be HIV-1-positive, six were HIV-1-negative and, in 12 cases, the HIV-1 status was not determined. Site of infection affected predominantly the lower thoracic spine, lumbar and sacral spine (*Figure 1*).

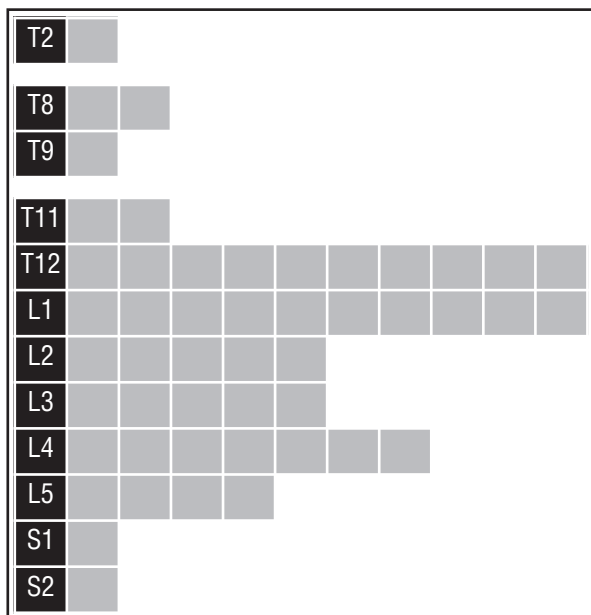


Figure 1. Distribution of vertebrae affected by infection with *M tuberculosis*

Only 11 of the patients had infection in only one vertebra, with the remainder having two vertebrae affected.

Of the patients included, 18 had confirmed spinal TB by either histology, ZN stain or culture positivity. Patients considered to be truly negative for spinal TB were diagnosed with a range of other conditions. Malignant disease was the most common, accounting for more than half of these patients. Two patients were diagnosed with multiple myeloma, one patient with thyroid carcinoma, one with B-cell lymphoma and one with adenocarcinoma. In three patients, no histological diagnosis could be made and in one case, chronic osteomyelitis was diagnosed.

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**Diagnostic accuracy (Table I)**

As expected, ZN staining performed very poorly as a stand-alone diagnostic test. Although a positive result was highly specific in this setting, the negative predictive value was only 45%. The sensitivity for both culture and PCR were in excess of 70%, but this was found to be even higher in the HIV-1 positive population group (82% and 88% respectively). Culture had a higher method efficiency and negative predictive value. As per study definition, no false positive results were noted for platforms other than PCR testing, rendering the specificity at 100%. Of note, the single case of a positive PCR with negative culture, histology and ZN was not investigated further, and is only considered false positive based on the case definition defined in this study. This finding is further supported by the fact that *Mycobacterium tuberculosis* was not isolated in any of the cases where an alternative diagnosis was obtained.

**Clinical utility**

Culture results were obtained between 10 days and 6 weeks from submission to the laboratory (mean of 27 days). All samples with positive cultures were further investigated for possible resistance, and all strains identified were fully susceptible to rifampicin and INH. Although ZN stains generally yield a rapid result, sensitivity and specificity are so poor that they cannot be utilised in this clinical context.

These results reflect the markedly large range in turnaround times for all the assays. This is understandable in culture, as a negative result can only be reported after a six-week incubation period. However, for the other assays, the reason for this variability is unknown. Contrary to what was expected, the mean and average turnaround times for histology and PCR were comparable at 5.5 to 6 and 7.5 to 9 days respectively (Table II).

**Discussion**

The confirmation of infection with *M. tuberculosis* remains a diagnostic dilemma despite advances in radiological and laboratory testing. In fact, radiological findings are often so very similar for TB and various malignancies,<sup>25-28</sup> that some authors advocate the use of microbiological or histological confirmation in all cases.<sup>29</sup>

Table I: Comparison of performance characteristics of various testing platforms in the patient population

	Sensitivity		Specificity	Efficiency	PPV*	NPV**
	Cohort	HIV-1 positive				
<b>Culture</b>	77	88	100	86	100	77
<b>Histology</b>	100	100	100	100	100	100
<b>ZN</b>	33	45	100	57	100	45
<b>PCR</b>	72	82	100	82	100	67

\* Positive predictive value  
 \*\* Negative predictive value

**Table II: Turnaround times of diagnostic modalities**

	Range (in days)	Average (in days)	Mean (in days)
Histology	3–28	9.2	5.5
Culture	10–42	29	27
ZN	1–21	3.3	2
PCR	2–35	7.5	6

Confirmation with culture can take as long as eight weeks with solid media culture, and alternative testing platforms are therefore sorely needed.<sup>7,10,30-36</sup> Recently, various forms of serological testing including the interferon-gamma assay<sup>37</sup> have been suggested for diagnosis. This has very limited utility in a high prevalence setting, as most individuals will show some degree of reactivity, irrespective of disease activity.<sup>17</sup> Molecular testing seems to be more promising and is performed by amplifying and detecting nucleic acids specific to the micro-organism in question. These assays are often capable of delivering results within 24 hours.<sup>38</sup> They also promise superior sensitivities and specificities, depending on number and actual sites targeted for amplification,<sup>16,39,40</sup> as well as clinical sites sampled<sup>41-43</sup> and the HIV-1 status of the patient.<sup>44</sup> Use of molecular methods has the added advantage of improved laboratory safety, as live, infectious organisms are not amplified by culture.

Histology is still considered to be the gold standard for diagnosis by some authors, as the diagnosis is often made within the context of a local tissue reaction in conjunction with acid-fast bacilli.<sup>18</sup> Histology requires good sampling techniques, and poor quality biopsies often have poor diagnostic utility. In this setting, molecular testing on tissue samples may render superior results, as very little genetic material is needed to be amplified and detected.<sup>45</sup>

Suffice it to say that the diagnostic test of choice should not only provide accurate results, but should also do so in a timely manner, to ensure the early initiation of appropriate therapy.<sup>46</sup> Both histology and tissue PCR requires invasive sampling. Therefore, PCR testing will only be preferred if it offers a quicker turnaround time. In this study, histology and PCR turnaround times were very similar. Davies and co-workers suggested that provided the case load is sufficient, testing should be offered on site, as this had a big impact on acquiring timely results.<sup>19</sup> Furthermore, utilising various diagnostic assays in a complementary fashion, rather than considering any one assay as a gold standard, may further improve diagnostic yield.<sup>45</sup>

*Histology is still considered to be the gold standard for diagnosis as the diagnosis is often made within the context of a local tissue reaction in conjunction with acid-fast bacilli*

In conclusion, PCR testing for spinal TB shows promising results especially within the HIV-1 positive population. Although this type of testing theoretically offers a shorter turnaround time, results were available in similar time frames as for histology. Therefore, on-site testing should be offered in hospitals with high case loads of TB, and combination testing should be used rather than opting for a single testing modality.

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