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## Czułość rozmazu AFB, wskaźnika opadania erytrocytów i testu Mantoux w diagnostyce gruźliczej limfadenopatii szyjnej

Sensitivity of acid-fast bacilli smear, erythrocyte sedimentation rate, and Mantoux test in diagnosing tuberculous cervical lymphadenopathy

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### Streszczenie

**Cel:** Ocena czułości rozmazu AFB, wskaźnika opadania erytrocytów i testu Mantoux w diagnostyce gruźliczej limfadenopatii szyjnej w porównaniu z uznanym za złoty standard badaniem histopatologicznym z użyciem cytologii aspiratu cienkoigłowego. **Materiał i metody:** W latach 2001–2016 przeprowadzono retrospektywne badanie w szpitalu o III stopniu referencyjności, z udziałem pacjentów z potwierdzoną gruźliczą limfadenopatią rozpoznaną na podstawie wyników badania cytologicznego aspiratu cienkoigłowego. Zgromadzono wyniki badań pacjentów z użyciem testu Mantoux, wskaźnika opadania erytrocytów i rozmazu AFB, a dane analizowano z zastosowaniem programu SPSS (IBM) w wersji 22.0. **Wyniki:** Test Mantoux uzyskał najwyższą czułość (77,37%) w rozpoznawaniu gruźliczej limfadenopatii, natomiast najniższą czułość stwierdzono w przypadku wskaźnika opadania erytrocytów (32,31%). Rozmaz AFB również okazał się słabym testem (51,32%) w diagnostyce gruźlicy, gdyż umożliwił zróznicowanie jedynie około połowy przypadków z wynikiem dodatnim. **Wniosek:** Najwyższą czułością charakteryzuje się test Mantoux, a w dalszej kolejności rozmaz AFB i wskaźnik opadania erytrocytów.

**Słowa kluczowe:** gruźlica pozapłucna, zapalenie węzłów chłonnych szyjnych, aspiracja cienkoigłowa, wskaźnik opadania erytrocytów

### Abstract

**Objective:** To evaluate the sensitivity of acid-fast bacilli smear, erythrocyte sedimentation rate and Mantoux test in diagnosing tuberculous lymphadenitis in comparison to the gold standard confirmatory histology examination using fine needle aspiration cytology. **Materials and methods:** Retrospective study conducted in a tertiary hospital between 2001 and 2016, involving patients confirmed as tuberculous lymphadenitis diagnosed via fine needle aspiration cytology. Results of Mantoux test, erythrocyte sedimentation rate and acid-fast bacilli in each of the patients were collected and data were analysed using IBM SPSS version 22.0. **Results:** Mantoux test had the highest sensitivity (77.37%) in diagnosing tuberculous lymphadenitis. On the other hand, erythrocyte sedimentation rate had the lowest sensitivity (32.31%). Acid-fast bacilli smear also showed to be a poor prognostic test (51.32%) for tuberculous as it was only able to differentiate around half of the positive cases. **Conclusion:** Mantoux test has the highest sensitivity followed by acid-fast bacilli smear and erythrocyte sedimentation rate.

**Keywords:** extrapulmonary tuberculosis, cervical lymphadenitis, fine needle aspiration, erythrocyte sedimentation rate

## INTRODUCTION

Aspiration of lymph nodes for diagnostic purposes was first done in patients with sleeping sickness at the beginning of the twentieth century<sup>(1)</sup>. Fine needle aspiration (FNA) developed gradually until 1921, when Guthrie tried to correlate FNA results with various disease processes<sup>(2)</sup>. The use of fine needle aspiration cytology (FNAC) in the investigation of lymphadenopathy has become an acceptable and widely practised minimally invasive technique, which is safe, simple, rapid and relatively painless. FNAC is highly cost-effective and accurate as a first line investigative technique, with differential diagnoses including reactive hyperplasia/inflammatory conditions, granulomatous disorders and malignancy, stratifying cases requiring further investigations, surgical intervention or clinical follow-up<sup>(3)</sup>.

The Mantoux test is used to show delayed-type hypersensitivity reactions against mycobacterial antigen, in which the reagent is mostly protein purified derivative (PPD). The test becomes positive 2–10 weeks after mycobacterial infection. Positive reactions (>10 mm induration) can occur in *Mycobacterium tuberculosis* infections. Suspicious reactions (5–9 mm induration) can occur after BCG vaccination, *M. tuberculosis* infection or non-tuberculous mycobacterial infections. Negative reactions (<4 mm induration) represent a lack of tuberculin sensitisation. False-negative reactions can occur in at least 20% of all people with active tuberculosis. The test may also be false positive in different conditions, like other infections, metabolic disease, malnutrition, live virus vaccination, malignancy, immunosuppressive drugs, newborns, elderly people, stress, sarcoidosis and inadequate test application<sup>(4)</sup>.

This study aims to evaluate the sensitivity of erythrocyte sedimentation rate (ESR), Mantoux test and acid-fast bacilli (AFB) smear test in the diagnosis of tuberculous lymphadenopathy.

## MATERIALS AND METHODS

We analysed cases of patients with cervical lymphadenopathy referred to the Department of Otorhinolaryngology, Hospital Selayang between 2001 and 2016. All patients were subjected to routine Mantoux test, sputum AFB and ESR. FNAC was performed aseptically, using a 24 G needle and a 10 mL syringe. The smears were stained with Giemsa, haematoxylin & eosin (H&E) and by Ziehl–Neelsen (ZN) technique for AFB. The contents of the needle were also inoculated on Löwenstein–Jensen medium. The morphology of growth appearing after 2–3 weeks of incubation was checked. The diagnostic criteria for tuberculosis included the presence of granulomas comprised of epithelioid cells and/or ZN smear positivity for AFB and/or positive culture for mycobacteria confirmed by a pathologist.

All cases of patients above 18 years of age diagnosed positive for tuberculosis, as confirmed with FNAC of cervical lymphadenopathy, were included in the study.

	Frequency [n]	Sensitivity [%]
<b>ESR:</b>		
• positive	63	32.31
• negative	132	
<b>Mantoux test:</b>		
• positive	106	77.37
• negative	31	
<b>Sputum AFB:</b>		
• positive	78	51.32
• negative	74	

Tab. 1. The sensitivity of each diagnostic test in diagnosing outcome tuberculous lymphadenitis

## Statistical analysis

The data were cleaned and analysed using IBM SPSS version 22.0. Since this was an aggregate data, data were weighted before the analysis. The gold standard in this study was FNAC and pathologist's review. Sensitivity analysis was computed for the respective diagnostic, tests using the following formula:

$$\text{Sensitivity} = \frac{\text{Number of positive cases identified by the diagnostic test}}{\text{Total cases with positive tuberculous lymphadenitis}} \times 100\%$$

## RESULTS

The results of the sensitivity test are shown in Tab. 1. The higher the sensitivity, the better the ability of a diagnostic test to give a positive result when the person has the disease. It was shown that the Mantoux test had the highest sensitivity in diagnosing tuberculous lymphadenitis. On the other hand, the ESR had the lowest sensitivity (32.31%). This also indicated that ESR is not suitable to serve as a test to diagnose tuberculous lymphadenitis as it failed to diagnose more than half of the positive cases. Sputum AFB also showed to be a poor diagnostic test for tuberculosis as it was only able to differentiate around half of the positive cases (51.32%) (Fig. 1).

## DISCUSSION

Tuberculosis, a deadly bacterial infection, can spread to other body tissues and organs through the bloodstream and the lymphatic system<sup>(5)</sup>. With the global increase in the incidence of human immunodeficiency virus (HIV), there has been a steady increase in extrapulmonary tuberculosis as exemplified in the United States<sup>(6)</sup> and Malaysia, where 21% of extrapulmonary tuberculosis cases were associated with HIV infection<sup>(7)</sup>. Tuberculous lymphadenitis, also known as scrofula (King's Evil)<sup>(8)</sup>, was first described 3,000 years ago and is one of the common forms of extrapulmonary tuberculosis. The microbiological cause of scrofula was first appreciated by Bollinger, May and Demme in the mid

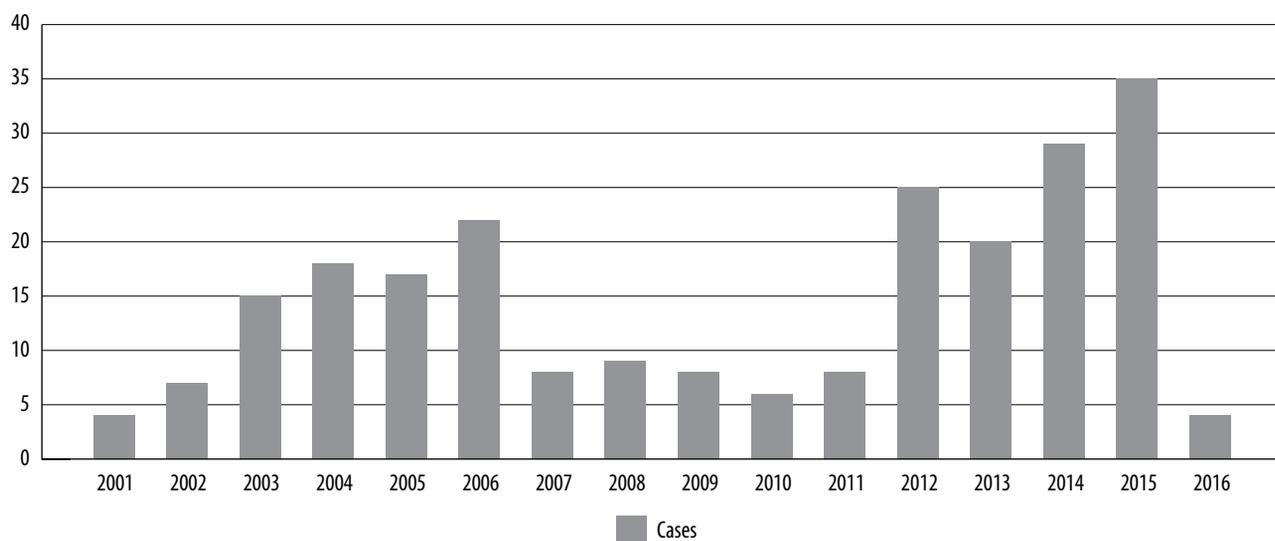


Fig. 1. Number of cases of tuberculous lymphadenitis confirmed by FNAC results from year 2001 to 2016

to late 19<sup>th</sup> century, when they noted that *Mycobacterium bovis* from cows was the cause of this ailment<sup>(9)</sup>.

Cervical lymph node was the most common site of tuberculous lymphadenitis involvement in most of the studies, followed by axillary lymph nodes<sup>(10)</sup>. FNAC has been proven to be an excellent diagnostic tool for tuberculosis in patients with lymphadenopathy. It is simple, safe, quick, reliable, accurate, minimally invasive, cost-effective and suitable in countries with increasing incidence rates of tuberculosis. FNAC is minimally invasive and can avoid the risk of fistula formation and complications associated with biopsy<sup>(11-13)</sup>. FNAC was hence chosen as the standard in diagnosing tuberculous lymphadenopathy in our study. The sensitivity and specificity of FNAC in the diagnosis of tuberculous lymphadenitis were 88% and 96%<sup>(14)</sup>.

In our study, 77.37% of patients were Mantoux test positive. Other studies reported reactivity of Mantoux from none to very strong. A study by Furcolow et al.<sup>(15)</sup> and Johnston et al.<sup>(16)</sup> showed up to 99% of positive tests. Tuberculin contains more than 200 proteins, widely shared among the mycobacteria. In another study on tuberculous lymphadenitis conducted in South Korea, the sensitivity and specificity of Mantoux test were 86% and 67%, respectively<sup>(17)</sup>. Due to this cross-reactivity, it has a poor specificity in population extensively vaccinated with BCG<sup>(18)</sup>. Hence, despite having poor specificity, but high sensitivity, as shown in our study and other previous studies, it should be used for the diagnosis of tuberculosis as it is the cheapest, safest and most productive diagnostic method.

Positive AFB smear was seen in 51.32% of our patients. Previous study by Bezabih et al.<sup>(19)</sup> showed similar results (59.4%), while Dua et al.<sup>(20)</sup> (27.11%) reported lower AFB smear positivity. This variation may be due to the fact that AFB positivity depends upon exposure and patient's immunological status. A positive AFB stain result denotes of mycobacterial aetiology which has excellent specificity for

*M. tuberculosis* in adults. In another study from California, from a total of 180 patients diagnosed via FNAC, 18% of FNAC smear specimens yielded positive results. However, when positive results were combined with detection of AFB, the sensitivity of FNAC specimens was 46%, while specificity was 100%<sup>(21)</sup>. The presence of histologic features such as nonspecific lymphoid infiltrates, noncaseating granulomas, or Langerhans giant cells support the diagnosis of probable tuberculosis in AFB-negative, culture-negative cases.

In our study, ESR had only 32.31% sensitivity for tuberculous lymphadenopathy. ESR has been used generally to indicate the presence of active inflammation and is commonly used as a nonspecific screening tool to indicate inflammation or a chronic disease state. Having said that, it has very low specificity and sensitivity on its own to diagnose cervical tuberculous lymphadenopathy. In a previous study involving 68 children diagnosed with tuberculosis, ESR was found to be normal in one-third of patients<sup>(22)</sup>. The latest CPG for Management of Tuberculosis, Malaysia, 3rd Edition, has not mentioned ESR as a screening or laboratory investigation tool in diagnosing tuberculous lymphadenopathy<sup>(18)</sup>.

## CONCLUSION

The Mantoux test has the highest sensitivity followed by AFB smear and finally ESR. The Mantoux test and AFB smear should be performed as a standard screening procedure and for the diagnosis for tuberculous lymphadenitis. ESR, on the other hand, is not a standard procedure in the screening and diagnosis of tuberculous lymphadenopathy, as shown in our study.

### Conflict of interest

The authors do not report any financial or personal connections with other persons or organisations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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