

Accuracy of Fine Needle Biopsy (FNAB) in Lymph Nodes Tuberculosis

Aditya Sita Sari^{1,2}, Nila Kurniasari^{1,2,3*}, Anny Setijo Rahaju^{1,2,3},
Etty Hary Kusumastuti^{1,3}, Alphania Rahniayu^{1,2,3}

¹Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

²Department of Anatomical Pathology, Universitas Airlangga Hospital, Surabaya, Indonesia

³Department of Anatomical Pathology, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

Email : nila-k@fk.unair.ac.id

Abstract

Introduction: Tuberculosis (TB) is an infection caused by Mycobacterium Tuberculosis (Mtb) that can attack various organs such as peripheral lymph nodes. Fine needle aspiration biopsy (FNAB) is often used as one of the modalities of diagnosis of lymph node tuberculosis to determine the management of patients. **Objective:** The objective of this study is to evaluate the accuracy, efficiency, and effectiveness of FNAB in diagnosing lymph node tuberculosis. The findings will assess whether FNAB can be adopted as a standard diagnostic and management tool for patients with this condition. **Methods:** This is an observational analytic cross-sectional study in the population of patients with suspicion of lymph node tuberculosis at the Anatomical Pathology Unit of Dr. Soetomo General Academic Hospital Surabaya in one year period. FNAB examination was performed with Modified Grunwald Giemsa (MGG) and Ziehl Nielsen (ZN) staining to detect AFB (Acid Fast Bacilli) organisms, as well as PCR (Polymerase Chain Reaction) of peripheral blood. The results were analysed using Chi square statistic test. **Results:** The sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of FNAB examinations were 100%, 33%, 59% and 100%, respectively, meanwhile the sensitivity and specificity of PCR peripheral blood examination were 8% and 70% compared to the ZN examination of the FNA aspiration material. **Conclusion:** FNAB had a higher sensitivity than PCR of peripheral blood to detect Mycobacterium Tuberculosis infection; on the other hand PCR of peripheral blood had a higher specificity than FNAB to detect Mycobacterium Tuberculosis infection.

Keywords: FNAB, Lymph node, Tuberculosis, Infection

Abstrak

Pendahuluan: Tuberkulosis (TB) adalah infeksi yang disebabkan oleh Mycobacterium Tuberculosis (Mtb) yang dapat menyerang berbagai organ seperti kelenjar getah bening perifer. Biopsi aspirasi jarum halus (FNAB) sering digunakan sebagai salah satu modalitas diagnosis tuberkulosis kelenjar getah bening untuk menentukan penanganan pasien. Tujuan: Tujuan penelitian ini adalah untuk mengevaluasi akurasi, efisiensi, dan efektivitas FNAB dalam mendiagnosis tuberkulosis kelenjar getah bening. Temuan akan menilai apakah FNAB dapat diadopsi sebagai alat diagnostik dan manajemen standar untuk pasien dengan kondisi ini. Metode: Ini adalah studi observasional analitik potong lintang pada populasi pasien dengan dugaan tuberkulosis kelenjar getah bening di Unit Patologi Anatomi Rumah Sakit Umum Akademik Dr. Soetomo Surabaya selama satu tahun. Pemeriksaan FNAB dilakukan dengan pewarnaan Modified Grunwald Giemsa (MGG) dan Ziehl Nielsen (ZN) untuk mendeteksi organisme AFB (Acid Fast Bacilli), serta PCR (Polymerase Chain Reaction) darah perifer. Hasilnya dianalisis menggunakan uji statistik Chi kuadrat. Hasil: Sensitivitas, spesifisitas, Nilai Prediktif Positif (PPV), dan Nilai Prediktif Negatif (NPV) dari pemeriksaan FNAB masing-masing adalah 100%, 33%, 59%, dan 100%, sedangkan sensitivitas dan spesifisitas pemeriksaan PCR darah tepi masing-masing adalah 8% dan 70% dibandingkan dengan pemeriksaan ZN dari bahan aspirasi FNA. Kesimpulan: FNAB memiliki sensitivitas yang lebih tinggi daripada PCR darah tepi untuk mendeteksi infeksi Mycobacterium Tuberculosis; di sisi lain, PCR darah tepi memiliki spesifisitas yang lebih tinggi daripada FNAB untuk mendeteksi infeksi Mycobacterium Tuberculosis.

Keywords: FNAB, Lymph node, Tuberculosis, Infection

I. INTRODUCTION

Tuberculosis (TB) is an infection caused by *Mycobacterium Tuberculosis* bacteria. It can attack various organs in humans, such as lungs, lymph nodes, skin and other organs. Indonesia is an endemic area for tuberculosis cases. In 2023, the number of Acid Fast-stain Bacilli (AFB) positive lung tuberculosis cases in Indonesia were 821,200 people, which has increased when compared to the number of tuberculosis cases found in 2022 of 677,464 cases, majority were found in West Java and East Java.¹ Over a one-year period at the Dr. Soetomo General Academic Hospital's FNAB unit, 247 cases of tuberculosis (TB) were diagnosed across various organs. Of these, lymphadenitis TB (tuberculous lymphadenitis) accounted for the majority (186 cases, 75.3%), underscoring its clinical significance and the need for reliable diagnostic tools like FNAB.

Early diagnosis of lymphadenitis TB is important to provide early intervention. Delay in diagnosis of this disease can result in the formation of localized abscesses that form sinuses in the skin if the abscess ruptures. This can reduce the patient's quality of life and affect the patient's appearance. Early diagnosis of this disease requires invasive specimen acquisition.² FNAB is a fast and affordable method for diagnosing lymphadenitis TB, especially in developing countries like Indonesia. This method plays a role in reducing unnecessary surgery.³

FNAB can be performed to determine the diagnosis of the disease in patients with lymph node nodules, including lymphadenitis TB.⁴ This examination provides a good clinical specimen and is suitable for initial pathological examination.⁵ Sampling with FNAB not only provides specimens for cytological examination but also for Ziehl-Neelsen (ZN) staining for acid fast bacilli (AFB).⁶ The typical feature found

in patients with tuberculosis is a group of epithelial-like histiocytes or so-called epithelioid that form granuloma, surrounded by lymphoid cells with various maturities, caseous necrosis, and Datia Langhans giant cells.^{7,8} Some of these cases are difficult to diagnose due to the fact that the aspiration material is only necrotic material. This limitation causes the FNAB examination results to be less helpful for clinicians in making the diagnosis. In such cases, further investigation is required to find *Mycobacterium Tuberculosis* in the FNAB specimens by performing ZN staining.⁹

In several trials, the suspicious smear was found to be positive for AFB organisms with ZN staining. The aim of this study is to determine that FNAB is an accurate, highly efficient and effective tool, thus it can be used as an examination tool in the management of patients with suspicion of lymphadenitis TB in order to shorten the examination time of the patient resulting in immediate treatment.

II. METHOD

This research was an analytic observational using cross-sectional design on a population of the patients in the FNAB Unit of Dr. Soetomo General Academic Hospital Surabaya with suspicion of lymphadenitis TB in one year period. The study included patients presenting with lymph node enlargement who agreed to undergo FNAB examination. All participants underwent cytopathological evaluation using Modified Grunwald-Giemsa (MGG) and Ziehl-Neelsen (ZN) staining at the Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga/Dr. Soetomo General Academic Hospital. Additionally, peripheral blood PCR testing for tuberculosis was performed at the Institute of Tropical Diseases, Universitas Airlangga, Surabaya. Written informed consent was obtained from

all participants prior to their inclusion in the study.

The diagnosis was established through clinical assessment of lymph node enlargement combined with cytopathological findings from FNAB examination. Diagnostic confirmation required the presence of granulomatous inflammation and/or caseous necrosis on FNAB smears. All aspirates underwent Ziehl-Neelsen (ZN) staining for acid-fast bacilli (AFB) detection using the following protocol: (1) application of carbol fuchsin-phenol solution with heat fixation to enhance mycobacterial wall penetration, (2) acid-alcohol decolorization to remove background staining while preserving AFB coloration, and (3) counterstaining with methylene blue.

Concurrently, peripheral blood samples were collected for Mycobacterium tuberculosis PCR testing using ELISA-based detection. Diagnostic accuracy was evaluated through 2x2 contingency table analysis, with ZN staining of lymph node aspirates serving as the reference standard.

FNAB examination with Modified Giemsa (MGG) staining was evaluated using a light microscope with 100-400x magnification. It was diagnosed as a positive result if there was a granuloma and/or caseous necrosis. AFB smear examination with ZN staining was evaluated using emersion oil under a light microscope with 1000x magnification, and then it was diagnosed as a positive result if we found red rod organisms. The data obtained was then analyzed using a 2x2 table to obtain the accuracy of the FNAB examination compared to smear smear of aspirate and PCR TB blood.

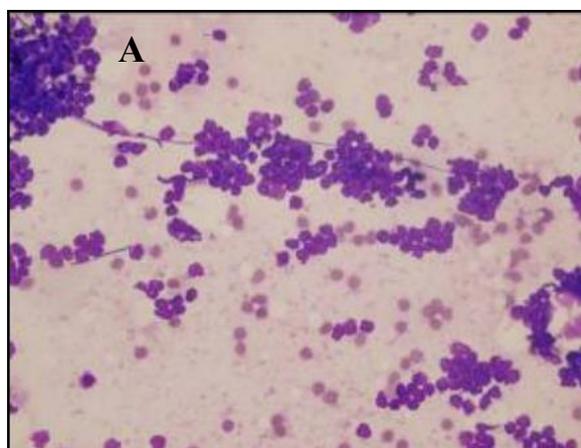
III. RESULT

During the one-year study period, 23 cases of lymphadenopathy met the inclusion criteria. The cohort demonstrated an age range of 3-

62 years (mean \pm SD: 27.52 \pm X years), with a female predominance (15 cases, 65.2%) over males (8 cases, 34.8%). Age distribution analysis revealed the highest incidence of tuberculous lymphadenitis occurred in adolescents and young adults, with peak frequencies in the 11-20 year (30.4%) and 21-30 year (26.1%) age groups. The pediatric population (0-10 years) showed the lowest occurrence (8.7%). Cervical lymphadenopathy was the most frequent presentation (14 cases, 60.9%), followed by submental (2 cases, 8.7%) and pre/intra-auricular (1 case, 4.3%) nodal involvement. Multisite lymphadenopathy was observed in 6 cases (26.1%).

TABLE 1. CHARACTERISTIC OF THE SAMPLE

Age	Male	Female
0-10	2 (8.70%)	0 (0%)
11 - 20.	2 (8.70%)	5 (21.8%)
21-30	2 (8.70%)	4 (17.4%)
31-40	1 (4.30%)	3 (13%)
41-50	0 (0%)	2 (8.7%)
>51	1 (4.3%)	1 (4.3%)
Total	8 (35%)	15 (65%)



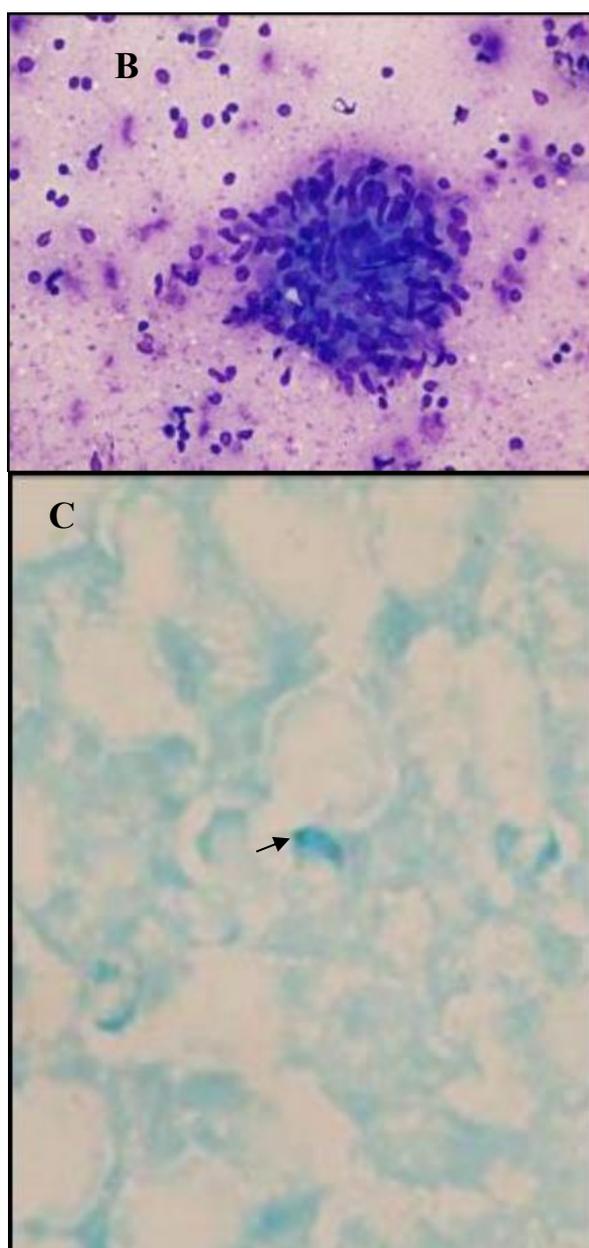


FIGURE 1. TUBERCULOSIS CYTOLOGY A. LYMPHOID HYPERPLASIA WITH MATURE LYMPHOID CELLS, B. GRANULOMA, C. ZN SMEAR OF AFB (ARROW) (400X)

FNAB examination identified 22 of 23 specimens (95.7%) as positive for tuberculous lymphadenitis, demonstrating high diagnostic sensitivity. Comparative analysis showed acid-fast bacilli (AFB) staining detected mycobacteria in 20 specimens (86.9%), revealing two FNAB-positive cases that were AFB-negative. In contrast, peripheral blood PCR exhibited significantly lower detection rates, with only

4 positive specimens (17.4%).

TABLE 2. FNAB EXAMINATION COMPARED TO ZN STAINING IN FINDING AFB

FNAB	AFB		Total
	Positive	Negative	
Positive	20	2	22
Negative	0	1	1
Total	20	3	23

The FNAB method showed no false negative results, while PCR testing failed to detect 16 true positive cases (69.6% of FNAB-confirmed diagnoses).

TABLE 3. BLOOD PCR COMPARED TO FNAB EXAMINATION COMPARED TO ZN STAINING IN FINDING AFB

PCR	AFB		Total
	Positive	Negative	
Positive	4	0	4
Negative	16	3	19
Total	20	3	23

In this study, FNAB had a higher sensitivity but lower specificity than PCR. The sensitivity, specificity, PPV and NPV of FNAB were 100%, 33% 91% and 100% respectively. While the sensitivity, specificity, PPV and NPV of PCR were 20%, 100%, 100% and 16% respectively.

TABLE 4. ACCURACY OF FNAB AND PCR

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
FNAB	100	33	91	100
PCR	20	100	100	16

IV. DISCUSSION

Tuberculosis is a major health problem in several countries, especially developing countries. Some of the risk factors that lead to the high spread of tuberculosis in developing countries include false positive early detection tests, non-compliance with treatment, delays in the development of new therapy plans, and high incidence of HIV.¹⁰ In Indonesia, there are various factors that support the high incidence of TB, such as smoking, malnutrition, diabetes, HIV, alcohol consumption, and air pollution.¹¹

Lymphadenitis tuberculosis is one of the common form of extra pulmonary TB with up to 35% of the incidence of all extra pulmonary TB cases.^{12,13} Early diagnosis is very important for the treatment of this disease, especially in endemic areas. The awareness of health workers against this disease is the key that leads to early diagnosis and early therapy.¹⁴

Females with lymphadenitis TB were higher in number than males; this was in accordance with various literatures which also suggested that the incidence rate in females was higher than males, with the average age being the productive age group.^{15,16} Contrary to pulmonary tuberculosis, which is more common in men, allegedly due to smoking habits.¹⁷ Lymphadenitis TB occurs more frequently in women, presumably because of the tropism of mycobacterial genotypes, hormonal factors, to socioeconomic factors in developing countries leading to lower nutritional status and standard of living in women.¹⁸

In this study, the highest incidence of lymphadenitis TB was found in the age range of 11-30 years with an average age of 27.52 years. This result is in line with other studies which show that the highest incidence of lymphadenitis TB is at the age of 15-30 years (47.62%).¹² However, age is reported to have no effect on the manifestations of extrapulmonary TB or pulmonary TB.¹³

Lymphadenitis sites most involved in this study were the cervical lymph nodes of 61%, followed by sub-mental (8.7%) and pre-infraauricular lymph nodes (4.3%). A study showed similar results that cervical lymphadenitis were the most common presentation of lymphadenitis TB.¹⁹ Another research also stated that cervical lymph nodes were the most common location of lymphadenitis TB.²⁰ More frequent involvement of the cervical lymph nodes due to the presence of lymphatic drainage and

close connection with the lungs.¹⁹ The Mtb portal of entry to the cervical lymph nodes can also be through the tonsils.²¹ Lymphadenitis TB may occur due to spread of Mtb from the primary focus of infection in the Ghon complex or from tonsillar, adenoid, sinonasal or osteomyelitis of the ethmoid bone. Mtb bacilli multiply in the lymph nodes causing swelling, hyperemia, necrosis, and caseous necrosis of the involved lymph nodes. Inflammation that continues to cause adhesions with adjacent skin then rupture into the surrounding tissue or form a sinus into the skin.²²

In this study, the sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of FNAB were 100%, 33%, 91%, and 100% respectively. It showed that cytological examination of the FNAB aspiration material showed higher sensitivity than blood PCR; on the other hand, it had a low specificity (33%). This result was in accordance with a study which stated that cytology had the highest sensitivity and that low specificity (48.1%) correlated with the culture method.²³ In general, the cytomorphology of FNAB has low specificity, but it has high sensitivity, because granulomatous lesion other than tuberculosis can also be diagnosed as LTB (Lymphadenitis TB). In the cytology specimen, LTB diagnostic criteria show the presence of epithelioid cells cluster forming granulomas, as well as Langhans type multinucleated giant cells. LTB can also show caseous or fibrinoid necrosis, as well as suppurative inflammation. However, non-caseous granulomatous inflammation can be found in other diseases such as sarcoidosis, foreign body reactions, brucellosis, and leprosy, moreover, granulomatous inflammation containing caseous necrosis can also be found in fungal infections.²⁴

Lymphadenitis TB is characterized by the presence of painless swelling of the lymph nodes with a size of up to 8-10 cm. This swelling may be accompanied by fever, weight loss, and cold sweats.^{1,2} However, in

cases that do not show pathognomonic radiographic manifestations, isolation of MTB bacilli or identification of caseous granulomas from biopsy samples is an important clue to diagnose TB lymphadenitis. Therefore, accurate and sufficient tissue retrieval from lymph nodes is very important for the process of establishing the patient's diagnosis.¹⁰

The cytological examination also depends on the experience of a pathologist in interpreting the cyto-morphologic features.¹⁰ In this study, two cases were falsely positive; the first case showed granuloma in the absence of caseous necrosis, making it difficult to find AFB on ZN staining. Meanwhile, the second case showed granuloma, necrosis, suppurative inflammation, and fungal colonies. These false positive results are caused by non-tuberculous granuloma or epithelioid cells.²⁵ Lymphadenitis tuberculosis (LTB) has some unique features; the infection is naturally paucibacillary²¹, and the organism tends to accumulate and does not spread evenly. Granulomas composed of epithelioid cells are common pathological reactions to LTB²⁶, whereas the formation of abscesses does not always appear in all cases. Granulomas are a characteristic manifestation characterized by displacement of macrophages and lymphocytes. The initial formation of granulomas begins with the presence of primary lesions in the lungs which are formed by the presence of an immune response of T cells which activate macrophages to phagocytize the causative bacteria. Bacteria from the lungs migrate to the lymph nodes by penetrating the granulomas and alveolar epithelium. Granulomas are dynamic structures formed by interactions between T cells, B cells, activated macrophages, causative agents, and several cytokines. Granulomas can break off and spread to more distant places if there is a decrease in T cells.²⁶

In this study, there were no false negative results on the FNAB examination. Meanwhile, in research by Nur et al. The results of obtaining false positives are thought to be difficult to help granulomas or epithelioid cells in suppurative inflammation due to the inhibitory effect of broad-spectrum antibiotics that have been given before. Nur et al also mentioned that patients with a cytological diagnosis of tuberculosis but negative MTB culture may be due to the absence of bacteria living in the lymph nodes.²⁷

In our study, we found that the sensitivity and specificity of peripheral blood PCR 20% and 100% respectively compared to the ZN examination of FNAB aspirates. These results are in accordance with the results of study which showed that blood specimens were not good examination specimens for diagnosing tuberculosis.²⁷ This was in accordance with the study by Mustafa *et al.*, where PCR of aspirate FNA showed low sensitivity. In that study, it was stated that the possible cause of low PCR detection was small amount of specimen which caused a lack of organisms, especially after dividing the specimens by cytological examination. Specimen storage temperature also affects sensitivity, especially in the tropics where temperatures are higher. Internal variations of DNA concentration and extraction also affect sensitivity.²⁸

The study compared PCR examination of pus specimens, biopsies and cytology stated that PCR sensitivity of aspiration FNA was also low compared to the biopsy specimens. This was caused by the small amount of FNA aspirate specimens and the MTB is not evenly distributed over the specimen. In that study, the highest sensitivity was found in PCR examination of pus specimens with a sensitivity of 93.2% because pus specimens are more fluid and easily homogenized, making DNA extraction easier to perform.²⁸

The diagnosis of extra pulmonary TB can be obtained from various specimens such as pleural fluid, lymph node aspiration, CSF, or depending on the location. Samples of lymphadenitis TB can be obtained from fine needle aspiration or lymph node biopsy, samples to diagnose Pleural TB can be obtained from pleural fluid, and TB meningitis can be diagnosed by PCR examination with CSF samples.¹³ Research conducted by Rahman et al. showed that peripheral blood samples were not the right choice of specimen for detecting MTB by PCR examination. In that study, 0% of 655 samples were positive for MTB.²⁹

The study by Bwanga *et al.* mentioned that the amount of peripheral blood examined may also affect the sensitivity of blood PCR, for which, in the 1 ml sample of the blood, the sensitivity was 33% and the specificity was 97%.³⁰ Meanwhile, if a 9 ml sample of the blood was aspirated, then the specificity rose to 71% and the specificity was 97%. Blood has many PCR inhibitors, one of which is human DNA. In general, the removal of human DNA rather than microbial DNA will affect the sensitivity of PCR amplification and sequencing of target sites in a broad-range or specific manner. Minimum quantities of MTB loads that can be detected in a blood specimen are quite large (median 38 cfu/ml), which can also cause TB PCR from blood sample to be frequently falsely negative.

FNAB cytological examination alone in diagnosing LTB might be at risk of misdiagnosis and errors in the management of patient therapy. Because FNAB has the highest sensitivity, we recommend FNAB as a screening tool. In dubious cases, it should be supplemented by ZN examination of aspirate FNAB for AFB confirmation. This method will result in better disease management services.²⁸

V. CONCLUSION

FNAB had a higher sensitivity than PCR from peripheral blood compared to AFB examination from aspiration material as the gold standard. The PCR from peripheral blood had a higher specificity than FNAB compared to AFB examination from aspiration material as the gold standard.

Examination of FNAB cytology is needed as a screening tool, and additional ZN examination of FNAB aspiration material can be performed in dubious cases in order to result in better disease management services. Further research is required with the PCR method from aspirate or tissue material compared to bacterial culture.

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