

Immunocytochemical Study of Rabbit-Polyclonal to Mycobacterium Tuberculosis, AB905: Improving Diagnostic of Tuberculous Lymphadenitis

Kajian Imunositokimia Rabbit-Polyclonal to Mycobacterium Tuberculosis, AB905: Meningkatkan Diagnostis Limfadenitis Tuberkulosis

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Abstract: Tuberculous lymphadenitis (TBLN) is the most common form of extrapulmonary tuberculosis. However, the optimal diagnosis using Fine-Needle Aspiration Cytology (FNAC) or excisional biopsy is uncertain. This research aims to improve the diagnostic of TBLN with FNAC and immunocytochemistry (ICC) compared to the response to antituberculosis therapy. The cross-sectional study involved 43 patients with the criteria for TBLN diagnosis based on the appropriate clinical history of tuberculosis and indicative cytological results. Immunocytochemical examination employed rabbit-polyclonal to Mycobacterium tuberculosis (MTB) antibody (AB905). The MTB expression was found in 35 out of 43 cases (81%) that appropriate cytological features of the tuberculosis process. Meanwhile, eight out of 43 cases (19%) did not express MTB. Diagnostic tests for lesions with a positive cytologic appearance of TBLN and ICC were compared to the response to anti-tuberculosis therapy, revealing the sensitivity, specificity, positive predictive value, and negative predictive value of 95.2%, 75%, 95.2%, and 17%, respectively. Besides, Fisher's exact tests utilized to identify the relationship between two variables; $p < 0.05$ was considered significant. This research found immunocytochemical study was a sensitive and specific tool for improving the diagnostic of TBLN.

Keywords: cytology; fine-needle aspiration biopsy; immunocytochemistry; diagnostic; tuberculous lymphadenitis

Abstrak: Limfadenitis tuberkulosis (LNTB) merupakan bentuk tuberkulosis ekstrapulmonal yang paling umum. Diagnosis menggunakan sitologi aspirasi jarum halus (FNAC) atau biopsi eksisi. Penelitian ini bertujuan untuk membuktikan diagnosis LNTB berdasarkan pemeriksaan FNAC dan imunositokimia (ISK) dengan membandingkan respons terapi antituberkulosis. Metode cross sectional yaitu 43 pasien dengan kriteria diagnosis LNTB didasarkan pada riwayat klinis tuberkulosis dan hasil sitologi indikatif. Pemeriksaan ISK dilakukan dengan rabbit-polyclonal Mycobacterium tuberculosis (MTB) antibody (AB905). Ekspresi MTB terlihat pada 35 dari 43 kasus (81%) menunjukkan gambaran sitologi dari proses tuberkulosis. Sedangkan 8 dari 43 kasus (19%) tidak mengekspresikan MTB. Uji diagnostik lesi dengan gambaran sitologi LNTB dan ISK positif. Respons pengobatan anti tuberkulosis diperoleh sensitivitas, spesifisitas, nilai duga positif, dan negatif sebesar 95.2%, 75%, 95.2%, dan 17%. Tes Fisher menentukan hubungan antara dua variabel $p < 0,05$. Penelitian menemukan imunositokimia adalah alat yang sensitif dan spesifik untuk diagnosis limfadenitis tuberkulosis.

Kata Kunci: limfadenitis tuberculosis; sitologi aspirasi jarum halus; sitologi; imunositokimia; diagnosis

INTRODUCTION

Tuberculous lymphadenitis (TBLN) is the most common form of extrapulmonary tuberculosis, with approximately one million cases worldwide each year. The disease is caused by *Mycobacterium tuberculosis* (MTB) and is often associated with the reactivation of latent infections.^{1,2,3}

The diagnosis of tuberculous lymphadenitis is a challenge for pathologists because of various cytological features, such as cases of non-MTB infection, other systemic disease diagnoses, and neoplastic. Another difficulty is because MTB shows paucibacillary properties and an irregular distribution that tends to be clustered, resulting in very low sensitivity to Acid Fast Bacilli (AFB) smear and conventional culture.⁴

The diagnosis is usually confirmed by Fine Needle Aspiration, core biopsy, or open biopsy for microscopy, culture, cytology, or histology.⁵ Fine Needle Aspiration is a simple examination technique. It is relatively painless, but the tissue obtained for examination is much less so that the diagnosis of lymphadenitis is sometimes difficult to establish. In contrast, core biopsy or open biopsy requires complex resources, such as operating rooms, surgical and anesthesia teams, and often leaves unpleasant scars on the patient despite being very informative about the histological structure.⁶ Hence, the optimal diagnostic approach is still unclear, and there is no diagnostic algorithm for testing tuberculous lymphadenitis, so the choice of examination rests with the clinician. In addition, not infrequently, patients with complaints of swollen lymph nodes with a clinical history showing the tuberculosis process are immediately given antituberculosis drug therapy and usually always respond well.

On the other hand, immunocytochemistry (ICC) is a sensitive and simple procedure. It is known that immunoreactive substances in the cytoplasm and cell wall of mycobacterium consist of proteins, lipids, and polysaccharides. Therefore, the present study aims to assess the presence of mycobacterial antigens by utilizing this examination. Recent studies have examined the performance of an immunocytochemical staining method (ICC) to detect MTB complex specific antigen using MPT64 in the extrapulmonary tuberculosis diagnostic. In that research, the UTI showed a sensitivity of 74.5% and specificity of 89.5%.⁷ Meanwhile, a study conducted by Prapanna and Srivastava detected MTB antigen using a polyclonal antibody to mycobacteria with a sensitivity and specificity of 96.92% and 95%.⁸

Thus, this research aimed to improve the diagnosis of tuberculous lymphadenitis with FNAC and immunocytochemistry compared to the response to antituberculosis therapy in patients who clinically show symptoms of extrapulmonary tuberculosis disease.

MATERIALS AND METHOD

Study Design and Participants

Between April 1, 2019, and December 31, 2020, a cross-sectional study was conducted by the Department of Anatomical Pathology, Universitas Muhammadiyah Sumatera Utara, Indonesia. Forty-three patients presented with lymphadenopathy of the neck and were clinically suspected to be of tubercular disease. All patients who had recently been diagnosed with tuberculosis and were on antitubercular treatment were withdrawn from the study. The participants were informed about the study procedure and the Health Research Ethical Committee of the Faculty of Medicine Universitas Sumatera Utara/H. Adam Malik General Hospital Medan granted ethics clearance.

Fine-Needle Aspiration Cytology

Under aseptic conditions, FNAC of the LN was performed under clinical guidelines with a needle of 23-gauge.^{9,10} The aspirate was separated into three parts. For May-Grunwald Giemsa staining, one portion was smeared onto a slide and fixed immediately with 95% alcohol. For immunostaining of rabbit polyclonal to *Mycobacterium tuberculosis* antibody, two smeared slides were air-dried prepared.

Cytological Evaluation

An experienced pathologist examined a cytological analysis of lymph node aspirates by May Grunewald Giemsa stain. The appearance of dark specks (Group I) and darkened elliptical objects in lymph node aspirates was used to diagnose TBLN (Group II).¹¹

Immunocytochemistry Evaluation

After removal, the slides were treated with phosphate-buffered saline (PBS) for a brief amount of time; they were incubated with hydrogen peroxide for half-hour to initiate the block of endogenous peroxidase activity. Once ingesting using the microwave antigen, the things were blocked with 5% normal serum horse (NHS) for fifteen minutes. Then, the primary antibody was applied (rabbit-polyclonal of MTB antibody, from Abcam, London, UK, diluted by 1/100) Liquid treatment) overnight and abruptly treated with Dako Real EnVision. The antigen was observed with diaminobenzidine (DAB), and hematoxylin was used for anti-staining.

Immunostaining Evaluation

Stained stuff was classified as positive and negative assessment. A positive assessment is if there is an expression appearance of brown color on the *M. tuberculosis*. Meanwhile, the assessment is negative if it fails to express a brown color, where at the same time, the positive control (from pleural fluids) displays a brown color.

Antituberculous Drug Response

Assessment of therapy response was based on neck lump reduction after administration of antituberculosis drugs for six months of use, and the patient did not complain of any abnormalities. First-line antituberculous agents for the treatment of tuberculosis consisted of isoniazid, rifamycin (usually rifampin), ethambutol, and pyrazinamide.

Statistical Analysis

The results of cytological diagnosis and immunocytochemical staining were analyzed. For the anti-tuberculosis drug response, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the accuracy of cytology and immunocytochemistry were calculated. Fisher's exact test was used to statistically analyze the correlation between FNAC results, immunocytochemical staining, and anti-tuberculosis drug response. If the p-value is <0.05 , the difference is considered significant.

RESULTS

From April 2019 to December 2020, 43 lymph node aspirate samples were collected from patients who attended the anatomical pathology laboratory from April 2019 to December 2020. The age of the patients ranged from four to 70 years (median 28 years). In this study, females dominated, namely 27 females and 16 males (male to female ratio = 0.6). Extrapulmonary sites involved were lymph nodes (32), supraclavicular (4), inguinal (2), breast (1), submandibular (1), malleolus (1), submental (1), and wrist joints (1).

All patients showed signs and symptoms based on the disease location. The main local symptoms were neck lumps, night sweats, loss of appetite, chest discomfort, and headaches.

The cytological findings of this study displayed the Dark Specks Features with Finely Granular Eosinophilic Amorphous Mass in the background and the darkened elliptical objects in the macrophage assembly.¹¹ Table 1 summarizes the correlation between signs and symptoms and cytological findings.

Table 1. The Correlation between Signs, Symptoms, and Cytological Findings

Signs and symptoms	Dark specks	Darkened elliptical objects
Neck lumps	21 (49%)	11 (26%)
Night sweats	2 (5%)	1 (2%)
Loss of appetite	3 (7%)	1 (2%)
Chest discomfort	1 (2%)	0 (0%)
Headaches	2 (5%)	1 (2%)

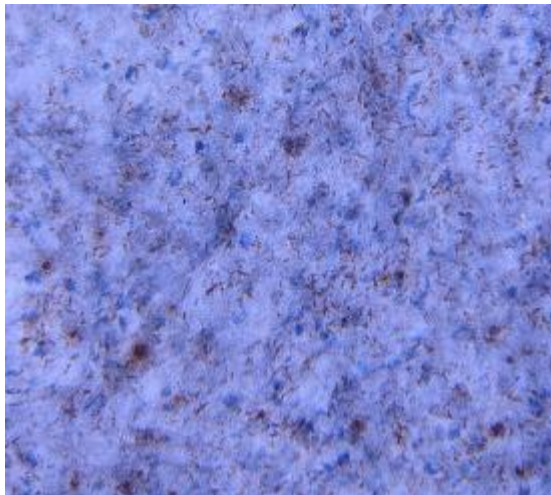


Figure 1. Positive Expression of Rabbit Polyclonal of Mycobacterium Tuberculosis Antibody in Dark Specks in the Entire Field of View (ICC, 400x)

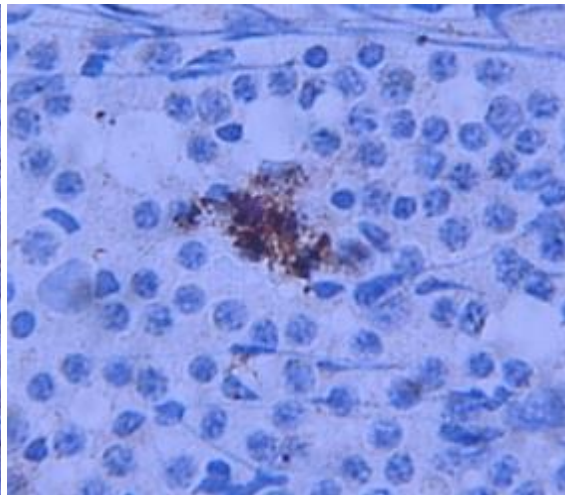


Figure 2. Positive Expression of Rabbit Polyclonal of Mycobacterium Tuberculosis Antibody in Darkened Elliptical Objects (ICC, 400x)

Table 2. The Correlation between Cytology Findings, Immunocytochemical Staining, and Antituberculous Drug Responses

Category	Cytology	Immunocytochemical positivity	Antituberculous drug responses positivity
Dark specks	25 (58%)	21 (60%)	20 (62%)
Darkened elliptical objects	18 (42%)	14 (40%)	12 (38%)
Total	43	35	32

Twenty-five of forty-three cases had dark specks features; twenty-one were immunocytochemically positive by rabbit-polyclonal to MTB antibody (AB905) (Figure 1); twenty were positive antituberculous drug responses. Eighteen of forty-three cases had dark oval body features; fourteen were immunocytochemically positive by rabbit-polyclonal to MTB antibody (AB905) (Fig. 2); twelve were positive antituberculous drug responses.

Therapeutic response was assessed from the same patient who was given antituberculosis drugs without any additional drugs for six months. The assessment was carried out by looking at the lump reduction every two weeks of re-control. From the results obtained, it was seen that 20 cases with dark specks cytology and 12 cases with cytological images of darkened elliptical objects showed a satisfactory therapeutic response. Overall displayed that the lump disappeared, and the patient did not complain of any abnormalities.

Table 2 presents the correlation between cytological findings, immunocytochemical staining, and antituberculous drug response.

Table 3. Immunocytochemical Staining with Dark Specks Features, Taking Antituberculous Drug Response as the Gold Standard

Immunocytochemical staining ab905	Category	Antituberculous drug response		Total
		Positive	Negative	
	Positive	20 TP	1 FP	21
	Negative	1 FN	3 TN	4
		21	4	25

TP, true-positive; FP, false-positive; FN, false-negative; TN, true-negative

Table 4. Immunocytochemical Staining with Darkened Elliptical Objects Features, Taking Antituberculous Drug Response as the Gold Standard

Immunocytochemical staining ab905	Antituberculous drug response		Total	
	Positive	Negative		
	12 TP	2 FP	14	
	2 FN	2 TN	4	
		14	4	18

*TP, true-positive; FP, false-positive; FN, false-negative; TN, true-negative

Compared to the antituberculous drug response, there were 20 cases of dark specks features (positive immunocytochemical staining and positive antituberculous drug response), one case (positive immunocytochemical staining and negative antituberculous drug response), one case (negative immunocytochemical staining and positive antituberculous drug response), and three cases (negative immunocytochemical staining and negative antituberculous drug response).

While in aspirates cytology of darkened elliptical objects features, there were 12 cases with positive immunocytochemical staining and positive antituberculous drug response, two cases (positive immunocytochemical staining and negative antituberculous drug response), two cases (negative immunocytochemical staining and positive antituberculous drug response), and two cases (negative immunocytochemical staining and negative antituberculous drug response) (Table 3,4).

The efficacy of FNAC of dark specks and immunocytochemical staining compared to antituberculous drug response as the gold standard was found to be good, with a sensitivity of 95.2%, specificity of 75%, PPV of 95.2%, and NPV of 17%.

On the other hand, the efficacy of FNAC of darkened elliptical objects and immunocytochemical staining compared to antituberculous drug response as the gold standard obtained a sensitivity of 85.7%, a specificity of 50%, a PPV of 85.7%, and an NPV of 14.3%. Fisher's exact tests were used to determine the relationship between two variables. $P < 0.05$ was considered significant.

DISCUSSION

Tuberculosis is the most common cause of adenopathy, aside from primary malignant tumors. Patients with TBLN are predominantly young and primarily present in cervical lymph nodes. Most participants are women, and it often highlights gender differences in the literature.¹² The frequency of TBLN, which is more often found in women, cannot be explained until now.

Compared with the literature, most patients have unilateral cervical lymphadenopathy, especially on the left side; 75% complained of a mass in the neck, and none complained of cough. Recent studies have suggested that nearly 90% of TBLN cases appeared without TB symptoms elsewhere.¹³ In this study, it was only based on the data obtained on patient complaints without further examination of TB involvement elsewhere. As a result, the possibility of this infectious disease was more common than previously thought. Three-quarters of the cases in this study affected the cervical gland, which is similar to many other studies.¹⁴

TBLN is also the most common form of extrapulmonary tuberculosis. Behr and Waters proposed a theory that tuberculosis is regarded as a lymphatic disease with access to the lungs, explaining the specific involvement of the lymphatic system. This theory denies that TB is a primary lung disease.¹⁵ Unfortunately, this research does not prove this theory, and further research should be done.

On the other hand, Fine-Needle Aspiration Cytology (FNAC) is a simple, reliable, and inexpensive method to confirm the TBLN diagnosis.^{8,16,17} The superficial lymph nodes are easily collected by FNAC examination; the collected specimens can be used for cytology assessment, viewing ZN stained acid-fast bacilli, culture. Tuberculosis is diagnosed based on the presence or absence of necrotic granulomas and epithelioid cell granulomas, and dark specks and darkened elliptical objects are sometimes seen.^{11,18}

Moreover, a definite diagnosis of TB is made based on the finding of MTB by an examination of ZN staining or culture. However, in patients with negative AFB, diagnosis is difficult. Prapanna and Srivastava's study used polyclonal antibodies to establish the tuberculosis diagnosis with negative ZN staining and culture to prove the existence of MTB in necrotic areas, the cytoplasm of macrophages, epithelioid cells, and giant cells.⁸ In this study, aspirate with dark specks image showed 60% of cases, and 40% of Darkened elliptical objects showed positive immunocytochemistry with rabbit-polyclonal to MTB antibody (AB905) stained in necrosis area and the distribution of macrophages. Previous studies have used more tissue material for immunohistochemical techniques because MTB antigen and tissue morphology can be appropriately evaluated. Unexpected background can be removed to give a specificity figure of 100%.¹⁹ Nevertheless, this technique takes a long time compared to ICC that can be processed immediately after sampling.

In this study, the gold standard used was the response to anti-tuberculosis therapy based on an ex juvantibus diagnosis. We used this diagnosis based on several reasons. (1) Even though ZN staining is still considered the gold standard for tuberculosis, we did not use it. It is because none of the preliminary studies of the 20 cases reviewed with ZN were positive. This negative result is probably due to the length of time the aspirate preparation was fixed in alcohol. In the general procedure for ZN staining, it is best if the preparations are processed as soon as possible in a fresh smear condition because TB germs will die quickly or have difficulty growing at a temperature of 30^o-40^oC.²⁰ Meanwhile, the aspirates we had collected so far were fixed with 96% alcohol and done a few months later, and it is likely that TB germs had died and could

not be detected by ZN staining. (2) From the literature we obtained, ex juvantibus therapy can be given if several drugs that have been given do not show the results as expected. For example, in this study's case, we administered anti-tuberculosis drugs, and so far, all patients recovered (swelling disappeared) within six-nine months. This therapy is supported by the research by Lubis et al., which found the presence of TB bacilli in the two structures above.²¹

Further, our study verified the accuracy of immunocytochemical in the tuberculous lymphadenitis diagnosis. This study also found patients with positive cytological dark specks and immunocytochemical compared with therapeutic response, a sensitivity of 95.2%, and a specificity of 75%, respectively. Whereas with a cytological picture of darkened elliptical objects, a sensitivity of 85.7% and a specificity of 50% were obtained. Besides, Fisher's exact tests were used to determine the relationship between two variables; $p < 0.05$ was considered significant. In the literature, the FNAC sensitivity and specificity in the tuberculous lymphadenitis diagnosis vary greatly, ranging from 43 to 100%.^{22,23} This difference can be seen in the cytological diagnosis of tuberculosis lymphadenitis. Based on experience, we can confirm that FNAC is a reliable, fast, and accurate method for diagnosing tuberculous lymphadenitis and can replace invasive biopsy.

CONCLUSION

In cases of lymphadenitis tuberculosis, FNAC and immunocytochemical may help to improve diagnostic accuracy.

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CONFLICT OF INTEREST

There is no conflict of interest of this publication

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