

Cytological diagnostic of lymphadenitis tuberculosis by eosinophilic material

Delyuzar^{1*}, Z Amir² and L Kusumawati³

¹Anatomical Pathology Department, Universitas Sumatera Utara, Medan, Indonesia

²Pulmonology Department, Universitas Sumatera Utara, Medan, Indonesia

³Microbiology Department, Universitas Sumatera Utara, Medan, Indonesia

*Corresponding author: dr_delyuzar@yahoo.com

Abstract. AFB sputum and chest X-ray are used to identify patients with pulmonary TB. For extrapulmonary TB, fine needle aspiration cytology is needed, even though occasionally found not atypical feature in the form of eosinophilic material with dark brown particles, suspected as TB. This research was to show that eosinophilic material with dark brown particles is accurate as new criteria for the cytological diagnosis of TB. By performing fine needle aspiration biopsy stained with Giemsa, if an eosinophilic material with dark brown particles was encountered, we continued with Ziehl-Neelsen AFB stain and confirmed with PCR. To assess accuracy, we used a diagnostic test to evaluate sensitivity and specificity of eosinophilic material with dark brown particles by using AFB and PCR as the gold standard. The sensitivity and specificity of cytological diagnosis in tuberculosis of eosinophilic material with dark brown particles were 93.65% and 70.99%, respectively if confirmed with AFB. On the other hand, if confirmed with PCR using *Mycobacterium tuberculosis* DNA, the sensitivity and specificity were 98.95% and 96.79%, respectively. In conclusion, eosinophilic masses with dark brown particles is accurate as new criteria of TB diagnostic cytology with high sensitivity and specificity confirmed with AFB and PCR test.

1. Introduction

Tuberculosis or TB is often called a major problem in Indonesia. As the fourth country to have the most cases in the world (Total case notification 321,308 cases of 242 million population, in 2011). TB also ranks fourth in the cause of death in Indonesia. Therefore it needs to be investigated more deeply for both diagnostic and therapeutic.[1]

The ability to accurately detect *M. tuberculosis* infection becomes very important to control the epidemic. Increased human resources are needed including the ability of health workers in case detection and mitigation in order to reduce transmission.[2]

A quick way to detect *M. tuberculosis* infection will help speed up early diagnosis in clinically suspected patients with tuberculosis and prompt follow-up of management.[3]

Tuberculous lymphadenitis (LTB) may be cytologic examination through Fine Needle Aspiration Biopsy (FNAB) aspiration. The diagnostic criteria that have been used for diagnosing tuberculosis cytologically are the clustering of epithelial cell type histiocytes and multinucleated cells of the Langhans type.[4] Sarwar A. et al. [5] describes in addition to Langhans cells (giant cell multinuclear) also contains necrosis caseous.

Gupta SK. et al. [6] describes the morphological picture of cytology of lymph node TB, which is divided into 1. Dominant necrosis, 2. Granulomas that contain epithelioid cell carcinoma and datia



cells (giant cells), 3. Granulomas with non-cultured epitheloid cells without datum cells, 4. Very few epitheloid cells. Culture results are often found to be positive on type 1 of 2, 3 and 4. Staining Acid-fast bacilli (AFB) is also highest in type 1, followed by type 2, 3, whereas in type 4 staining and culture are found to be negative. Also found 7.8% smear AFB positive but culture negative. Negative cultures do not rule out the presence of tuberculosis for it needs other techniques to become gold standard even though histopathology has been a grip but cannot exclude abnormalities due to non-tuberculosis infection.[6] Das DK. et al. [7] also divided the results of TB cytology examination of epitheloid granulomas without necrosis, epitheloid granulomas with necrosis and necrosis without epitheloid granulomas.

In addition to tissue examination (classical histopathology), molecular biology tests for TB such as PCR (Polymerase Chain Reaction) and immunohistochemistry (IHC) continue to grow. Krishna, Singh et al. [8] examined the classical histopathological examination compared with PCR as a standard gold obtained 92% sensitivity, 37% specificity, 60% positive predictive value and negative predictive value 81. The immunohistochemical examination had more accurate accuracy than classical histopathology.[9]By using PCR as the gold standard, obtained immunohistochemical anti-MPT64 technique on TB in abdomen and lymph nodes respectively sensitivity, specificity, positive predictive value and negative predictive value were 92%, 97%, 98%, and 85%.[10] Immunohistochemistry with anti-MPT64 antiserum can be done relatively quickly, sensitively, and specifically to establish a diagnosis of TB.[11] To establish a diagnosis of tuberculosis lymphadenitis can also be done culture, smear with Ziehl-Neelsen coloration in addition to histopathology both classical and immunohistochemical images.[12]

There is still some doubt about the accuracy of diagnostic aspiration of fine needle biopsy of TB lymphadenitis, especially some doctors on the part of the child so as to use a scoring system in the diagnosis of tuberculosis; it is necessary to prove the accuracy of biopsy aspiration diagnostic by using PCR gold standard. It is also necessary to find what percentage of diagnoses with cytology of TB lymphadenitis originated from *Mycobacterium tuberculosis*. High percentages can conclude that cytologic features according to criteria such as epithelioid, lymphocytes and necrotic backgrounds deserve to be diagnosed as tuberculosis lymphadenitis.

This study used a Ziehl-Neelsen microscopic examination for AFB as a benchmark and biomolecular examination of PCR as a gold standard, so as to prove the accuracy of the cytology diagnosis of fine needle biopsy is superior to the Ziehl-Neelsen examination for the diagnosis of tuberculosis lymphadenitis.

Whether the diagnosis of tuberculosis cytology via eosinophilic mass imagery with dark brown particles can be used in the diagnosis of TB lymphadenitis caused by *Mycobacterium tuberculosis*. In this study PCR examination as standard gold standard and examination Ziehl-Neelsen as a comparison.

The results of this study are expected to increase the confidence in the diagnosis of tuberculosis cytology through a picture of eosinophilic mass with dark brown particles in the diagnosis of cytology of tuberculosis. Thus it can improve the success of tuberculosis lymphadenitis treatment in relation to the presence of *M. tuberculosis* through the cytology of a fine needle aspiration biopsy (FNAB).

2. Methods

The study was conducted from February 2016 to July 2016 by microscopic examination of Ziehl-Neelsen for AFB and PCR biomolecular examination to confirm the diagnosis of 97 inflammatory cases with a picture of eosinophilic amorphous mass suspected TB lymphadenitis and 97 inflammatory cases without eosinophilic amorphous mass suspected of non-TB lymphadenitis after doing a cytology examination of a Fine Needle Aspiration Biopsy (FNAB).

Type of Research Diagnostic Test with Cross-sectional Study. Samples of cytology research were obtained from the Adam H. Adam Malik hospital. Pirngadi, a private hospital or clinic in the Municipality of Medan and its surroundings or who came to examine himself in the Anatomy Pathology Section of FK USU, while the microscopic examination of AFB with Ziehl-Neelsen

method as recommended by WHO was conducted in TB Laboratory of Microbiology Department of FK USU Medan, PCR performed at Integrated Laboratory FK USU. This study used 194 samples.

Through a fine needle aspiration biopsy technique, it is stained with Giemsa, when the eosinophilic mass is present with dark brown particles, followed by staining of Ziehl-Neelsen AFB and confirmed by PCR examination. As a comparison is other inflammation that is not TB is also confirmed with AFB and PCR. To assess the accuracy of the diagnostic tests assess the sensitivity and specificity of eosinophilic mass with dark brown particles with a gold standard of AFB and PCR examination.

3. Results and Discussion

Table 1 showed that 59 cases of 97 aspirate preparations showing eosinophilic amorphous mass with dark brown particles were tested positive for AFB examination, while 93 cases of 97 aspirate preparations without eosinophilic amorphous mass with dark brown particles showed negative results on smear examination.

Table 1. Results of BTA examination in groups of inflammatory cases with eosinophilic amorphous mass suspected TB lymphadenitis and inflammatory cases without eosinophilic amorphous mass suspected not to be TB lymphadenitis.

Aspiration Type	AFB (+)	AFB (-)
Eosinophilic amorphous mass with dark brown particles	59	38
Without eosinophilic amorphous mass with dark chocolate particles	4	93

This suggests that tuberculosis cytostatic diagnostics through eosinophilic mass imagery with dark brown particles provides 93.65% sensitivity and 70.99% specificity when confirmed with AFB examination as standard gold TB diagnostic tool.

Table 2. PCR examination results in the inflammatory case group with eosinophilic amorphous mass suspected TB lymphadenitis and inflammatory cases without eosinophilic amorphous mass suspected not to be TB lymphadenitis.

Aspiration Type	PCR (+)	PCR (-)
Eosinophilic amorphous mass with dark brown particles	94	3
Without eosinophilic amorphous mass with dark chocolate particles	1	96

The above table shows that 94 cases of 97 aspirate preparations showing eosinophilic amorphous mass with dark brown particles were tested positive for PCR examination, while 96 cases of 97 aspirate preparations without eosinophilic amorphous mass with dark brown particles showed negative results on PCR

This suggests that tuberculosis cytostatic diagnostics through eosinophilic mass imagery with dark brown particles gives 98.95% sensitivity and 96.97% specificity when confirmed by PCR examination using *Mycobacterium tuberculosis* DNA.

The results showed significant sensitivity and specificity for using cytological examination via eosinophilic features with dark brown particles as one of the diagnostic tools for tuberculosis.

4. Conclusion

Eosinophilic amorphous mass can be used to prove the presence of *Mycobacterium tuberculosis* bacteria in suspected as a confirmed tuberculosis lesion with Ziehl-Neelsen AFB examination obtained 93.65% sensitivity and 70.99% specificity, while confirmation with PCR obtained 98.95% sensitivity and specificity of 96.97%. This figure is quite accurate for use, so it is suggested that eosinophilic amorphous mass be one of the diagnostic criteria for tuberculosis lymphadenitis by using fine needle aspiration biopsy.

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