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Original Article

Xpert MTB/RIF and Microscopic Cytology of FNAB in Tuberculosis

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ABSTRACT

Background: The symptom of lymphadenopathy can describe several disorders, including tuberculosis (TB) lymphadenitis. The suspicious gland will undergo supportive investigations to confirm the diagnosis in patients with suspected TB lymphadenitis. Fine Needle Aspiration Biopsy (FNAB) is a minimally invasive treatment frequently used to collect samples from individuals with lymphadenopathy. With these specimens, Xpert MTB/RIF detects DNA of *Mycobacterium tuberculosis* (MTB) in addition to the cytological evaluation, which examines tissue reactions on the host. This study will evaluate the discrepancies between microscopic descriptions of FNAB and Xpert MTB/RIF investigation result.
Method: The patients with lymphadenopathy who underwent *Xpert MTB/RIF* examination and FNAB cytology at Dr. Soetomo Hospital Surabaya, Indonesia, between September 2021 and September 2022, are the subjects of this retrospective analytical observational study. The scoring system is based on FNAB microscopic descriptions and the *Xpert MTB/RIF* nominal data. The Wilcoxon, Mann-Whitney, and McNemar tests were used to analyze both.
Result: The results of the Xpert MTB/RIF and the FNAB were not significantly different according to the McNemar test ($p=0.118$; $p<0.05$).
Conclusion: The positive Xpert MTB/RIF results group had a higher microscopic description score than the negative Xpert MTB/RIF results group. There was no difference in the Xpert MTB/RIF examination results with the FNAB end conclusions

GRAPHICAL ABSTRACT

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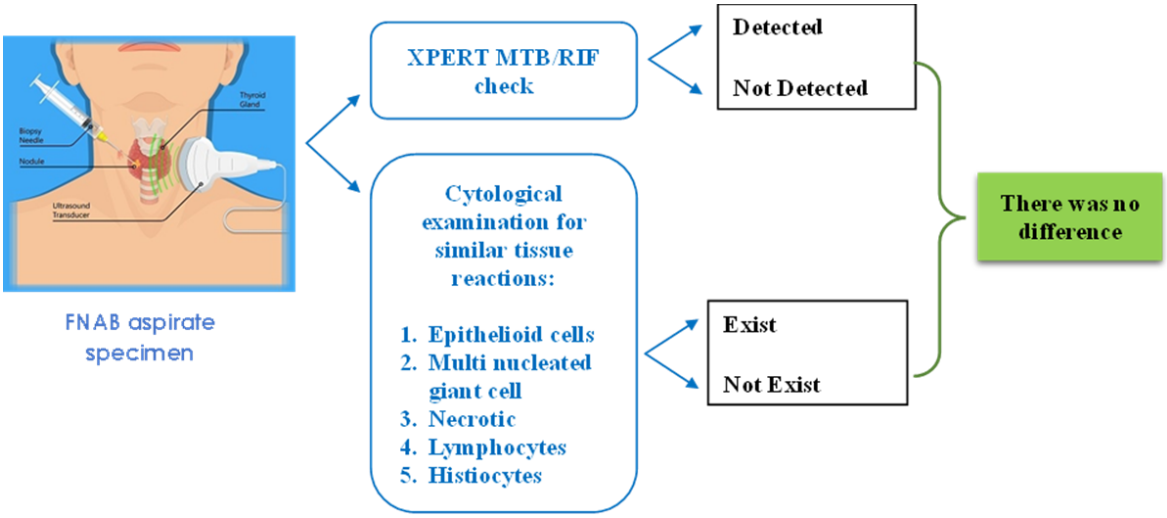

Introduction

A picture of the condition of lymph nodes that are abnormal in terms of size and consistency is called lymphadenopathy. One of the clinical manifestations of many diseases is lymphadenopathy [1-3]. A common cause of lymphadenopathy in tuberculosis (TB) endemic areas is tuberculous lymphadenitis [4, 5]. Lymphadenopathy is 43% caused by TB, and patients typically range in age from 11 to 30. In Indonesia, especially in Bandung, tuberculous lymphadenopathy was reported to be 68.7% of all existing extrapulmonary TB. In Padang, 11.6% of extrapulmonary TB cases are tuberculous lymphadenopathy, the second disease with the highest cases after meningitis TB [6-8].

The inadequate volume of specimens, the specimens division for various tests, the non-uniform distribution of microorganisms, as well as the paucibacillary nature of the specimens, are some reasons why the diagnosis is thought to be interesting [9]. The primary or secondary cause of tuberculous lymphadenopathy is latent TB infection. Typically, a fine needle aspiration biopsy (FNAB) specimen from the affected lymph node is used to make the diagnosis [10-13].

In 2013, the World Health Organization (WHO), for the first time, recommended using Xpert to diagnose TB in extrapulmonary specimens, including lymph nodes. In 2021, the WHO updated its guidelines and recommended the use of Xpert and Xpert Ultra as the initial diagnostic tests instead of acid-fast rod microscopy and/or culture for pulmonary and extrapulmonary specimens. The International Standard for Tuberculosis Care (ISTC) suggests collecting samples for histological analysis and microbiological testing for suspected extrapulmonary TB [12]. Xpert MTB/RIF, in diagnosing LNTB, has sensitivity and specificity; 78%, 74% [14]; 82.6%, 85% [15]; and 79%, 90% [16]. For the diagnosis of tuberculous lymphadenopathy, Xpert MTB/RIF Ultra has 91%, 72% sensitivity and specificity, respectively [11].

For diagnosing tuberculous lymphadenopathy, FNAB is the best test because it provides a less invasive, less traumatic, and more practical alternative [17]. FNAB has a sensitivity of 85.78%, a specificity of 70.73%, and an accuracy of 80.95% when diagnosing lymphadenopathy [18]. Finding epitheloid cells, multi-nucleated giant cells, necrotic material, and the presence of lymphocytes and histiocytes are FNAB characteristics suspected to be TB symptoms [19, 20]. The diagnosis of nontuberculous Mycobacterium lymphadenitis with features of granulomatous inflammation can also be made using the results of a FNAB cytological examination [21].

By confirming the association of these two diagnostic methods, we are aiming to ensure the actual infection itself. We hypothesize that true infection of TB, detected by Xpert, should be followed by its tissue reaction, seen by the FNAB method. There is still a curiousity in the lack of

similar research that connects these variables, especially in TB endemic countries like Indonesia.

Materials and Methods

This research is a retrospective study. The design used was a cross-sectional study comparing the Xpert MTB/RIF examination results with microscopic description of FNAB. FNAB conclusions are categorized into granulomatous and non-granulomatous inflammation. FNAB description is also detailed into the existence of epitheloid cell, multi-nucleated giant cell, necrotic substance, lymphocyte, and histiocyte. It is then analyzed by McNemar and Wilcoxon test. Mann-Whitney analysis is used to know the association of microscopic features in each group Xpert MTB/RIF examination result.

Results and Discussion

There are two categories of FNAB examination results in this study, granulomatous and non-granulomatous inflammation (Table 1). The McNemar test showed no significant difference between Xpert MTB/RIF and the FNAB conclusion ($p=0.118$; $p<0.05$). It means the pathology report of FNAB is statistically consistent with Xpert MTB/RIF result.

Based on the scoring system above (Table 2), statistical analysis was carried out to compare the Xpert MTB/RIF and microscopic description of FNAB using the Wilcoxon Test. The test results showed that there was a significant difference between Xpert MTB/RIF and the microscopic description of FNAB ($p=0.000$; $p<0.05$). It means, after being detailed, Xpert MTB/RIF and description of pathology report are not consistent when it is processed into the scoring system. On the other hand, the appearance of epitheloid cell ($p=0.143$; $p<0.05$), granuloma formation ($p=0.064$; $p<0.05$), and histiocyte ($p=0.064$; $p<0.05$) are consistent to Xpert MTB/RIF result (Table 3). Neither necrotic material nor lymphocyte appearance gives Xpert MTB/RIF result consistency.

The results of the Mann-Whitney test showed a significant difference in the average score of microscopic descriptions of FNAB between the two groups on Xpert MTB/RIF results ($p=0.036$; $p<0.05$). The positive Xpert MTB/RIF results group had higher microscopic description scores than the negative Xpert MTB/RIF results group.

15 (29.4%) of the 51 samples in this research had tuberculous lymphadenopathies detected bacteriologically using Xpert MTB/RIF. Patients who have received empiric antibiotic therapy are not disqualified from this research. Empirical non-anti-TB antibiotic therapy lowers the likelihood that microbiological testing will prove tuberculous lymphadenopathy [22].

Table 1: Granulomatous and non-granulomatous inflammation

Granulomatous Inflammation	Xpert MTB/RIF Detected	Xpert MTB/RIF Not-Detected
Yes	11	8
No	4	28

Table 2: The results of each presentation

Score	Score 5	Score 4	Score 3	Score 2	Score 1	Score 0
Xpert MTB/RIF Detected	1 (1.96%)	4 (7.84%)	5 (9.8%)	3 (5.89%)	2 (3.92%)	0 (0%)
Xpert MTB/RIF Not-Detected	2 (3.92%)	5 (9.8%)	7 (13.73%)	4 (7.84%)	17 (33.33%)	1 (1.96%)
Total	3 (5.89%)	9 (17.65%)	12 (23.53%)	7 (13.73%)	19 (37.25%)	1 (1.96%)

Table 3: Re-observations of the FNAB description and presence of epitheloid cells, multi-nucleated giant cells, necrotic material, lymphocytes, and histiocytes

Description	Epitheloid cell	Multi-nucleated giant cell	Necrotic material	Lymphocyte	Histiocytes
Xpert MTB/RIF Detected	10 (66.67%)	1 (6.67%)	12 (80%)	11 (73.33%)	10 (66.67%)
Xpert MTB/RIF Not-Detected	12 (33.33%)	5 (13.89%)	16 (44.44%)	27 (75%)	14 (38.89%)
Total	22 (43.14%)	6 (11.76%)	28 (54.9%)	37 (72.55%)	24 (47.01%)

Furthermore, it can be more difficult to identify extrapulmonary tuberculosis than pulmonary TB. This is because bacteriological evidence has shown it, and the amount of microbes outside the lungs is typically very small [18]. The sensitivity and specificity of FNAB cytology in the diagnosis of tuberculous lymphadenopathy were 88% and 96% [4] as well as 92.50% and 96.49%, respectively [23] while Xpert MTB/RIF has sensitivity and specificity; 78%, 74% [14]; 82.6%, 85% [15]; and 79%, 90% [16].

This study results showed that the pathology report of FNAB is statistically consistent with Xpert MTB/RIF result, while the description does not. Only some of the detailed microscopic descriptions are consistent (appearance of epitheloid cells, granuloma formation, and histiocyte). On the other hand, the positive Xpert MTB/RIF group had a mean microscopic description score higher than the negative Xpert MTB/RIF group. This might be closely related to the pathogenesis of *Mycobacterium tuberculosis* (MTB).

MTB is a bacterium that has a preference for tissues rich in oxygen supply. MTB enters the body and will encounter resistance from the natural and adaptive immune system [24]. Exploring the bacterial dynamics of MTB in human lymph nodes is very difficult because the time of MTB infection is usually unknown. MTB will travel ipsilaterally (same side) from the lungs to lymph node. The node would respond to the MTB in several ways. Macroscopically, the lymph node becomes swollen and gray-red in color. Histologically, lymphoid follicles are noticeable and have a big germinal center with lots of mitoses inside. They have enlarged lymph nodes due to lymphocyte proliferation or macrophage hyperplasia resulting from MTB infiltration [24-26].

The process of macrophage phagocytosis is the primary source of resistance to MTB. Macrophages that deliver antigens to T lymphocytes are mycobactericidal in addition to produce cytokines. T cells and macrophages both generate TNF-α, a cytokine that promotes inflammation. Phagocytes, lymphocytes, and other cells move toward the infection's source as a result of this cytokine's stimulation of inflammation cells. The second proinflammatory cytokine, IL-1, is generated by monocytes, macrophages, and dendritic cells, which also contribute to the development of granulomas [24, 25]. Small groups of epitheloid, histiocytes, and lymphocytes congregate to create granulomas [27, 28]. MTB burden increases with increasing granuloma size [25]. The active phase of TB will occur if changes in the immune system cause granuloma damage [24, 25]. The presence of necrosis is associated with the proliferation of tubercle bacilli, while lymphocytes, epithelioid cells, and multi-nucleated giant cells have a role in inhibiting MTB proliferation.

Patients with TB in earlier research in 1992-1993 had a variety of tissue characteristics, including type I (presence of epithelioid granuloma without necrosis), type II (presence of epithelioid granuloma with necrosis), and type III (presence of necrosis without epithelioid granuloma). Type II is the most prevalent of the three kinds [18]. Histological and cytological comparisons were performed in 23 cases, caseous granulomas consisted of necrotic material, polymorphonuclear cells, histiocytes, epithelioid

cells, and multi nucleated giant cells. Meanwhile, non-caseating granulomatic contains aggregates of epithelioid cells, necrotic material, and a number of other inflammatory cells [29]. Part of the histological granuloma consisting of necrosis surrounded by epithelioid cells, histiocytes or multi-nucleated giant cells can be due to Mycobacterium tuberculosis or Mycobacterium non-tuberculosis [18].

Various appearances of microscopic descriptions are observed which are categorized as having different values for each individual. The presence of agent factors (virulence factors), host (immune response, nutrition, and genetic conditions), and environment (medical history and lifestyle) will influence the MTB discovery and the description of existing tissue reactions [24]. Then, there are several factors in making the diagnosis that is considered, such as inadequate volume of specimens, distribution of specimens for different tests, non-uniform distribution of microorganisms, and the paucibacillary nature of the specimens [30].

Our study results showed that there was no significant difference between Xpert MTB/RIF and the FNAB conclusions. Further analysis shows that these two diagnostic modalities are compatible, especially when the epitheloid cell, granuloma formation, and histiocyte are found.

As we know, in the National Guidelines for Medical Services for the Management of Tuberculosis, there are two clinical diagnoses for TB infection, a clinically proven TB diagnosis and a bacteriologically proven TB diagnosis. FNAB and Xpert MTB/RIF examinations describe the infection process at the tissue and cellular levels, and then find the agent. The compatibility of these two modalities can strengthen the TB diagnosis, which is proven bacteriologically and causing the tissue effect.

Conclusion

The positive Xpert MTB/RIF results group had a higher microscopic description score than the negative Xpert MTB/RIF results group. There was no difference in the Xpert MTB/RIF examination results with the FNAB end conclusions. The compatibility of these two modalities can strengthen the TB diagnosis, which is proven bacteriologically and causing the tissue effect.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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