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Technical report

Diagnostic tests for tuberculous lymphadenitis: fine needle aspirations using tissue culture in mycobacteria growth indicator tube and tissue PCR

Pakpoom Supiyaphun[a], Somying Tumwasorn[b], Nibondh Udomsantisuk[b], Somboon Keelawat[c], Wilailuck Songsrisanga[a], Punjapon Prasurthsin[b], Ajcharaporn Sawatpanich[b]

[a]Department of Otolaryngology, [b]Department of Microbiology, [c]Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Background: The diagnosis of tuberculous lymphadenitis (TBLN) ranges from therapeutic diagnosis to open biopsy with tissue culture. The open biopsies are accepted as the gold standard to diagnose TBLN, but it requires skin incision that leaves unwanted scars.

Objective: Test the sensitivity and specificity of fine needle aspiration (FNA) using tissue culture in mycobacteria growth indicator tube (MGIT) and tissue polymerase chain reaction (PCR) for comparison with open biopsy using tissue culture.

Subject and methods: Forty patients with clinically suspected cervical tuberculous lymphadenitis were recruited at King Chulalongkorn Memorial Hospital. The patients underwent FNA followed by open biopsies either excisional or incisional. Specimens from FNA were collected for tissue culture in MGIT and for tissue PCR. The specimens from open biopsies were divided into two portions for tissue culture in MGIT (the gold standard) and for hispathology.

***Results*:** FNA for tissue culture in MGIT had a moderate sensitivity (65%) but high specificity (83%) (73% positive and 76% negative predictive value). FNA for tissue PCR had a moderate sensitivity (53%) but very high specificity (96%) (90% positive and 73% negative predictive values). Combination of either FNA for tissue culture or FNA tissue PCR revealed an increase in sensitivity and specificity to 83.6% and 80.0%, respectively. However, a combination of both FNA for tissue culture and FNA tissue PCR revealed a decrease in sensitivity *(*34.5%*)* but a highly increase in specificity (99.0%).

Conclusion: Either the FNA using tissue culture in MGIT or tissue PCR had a moderate sensitivity but high specificity. FNA using tissue culture or FNA tissue PCR may be used as an alternative test for diagnosis TBLN. The techniques may replace the open biopsies because of its effectiveness and low complication rate.

Keywords: FNA, MGIT, PCR, tuberculosis, lymphadenitis

The incidence of tuberculosis (TB) rises significantly, partly from the widespread human immunodeficiency virus (HIV) infection [1], and partly from global migration of infected individuals. Tuberculous lymphadenitis (TBLN) is the most common form of extrapulmonary TB [2], and one of the most frequent causes of lymphadenopathy (30-52%) [3].

Correspondence to: Pakpoom Supiyaphun MD, Department of Otolaryngology, Faculty of Medicine, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand. E-mail: cu_ent@hotmail.com

In general, TBLN is differentiated from other granulomatous lymphadenopathy such as sarcoidosis, lymphoma or sarcoma, viral or bacterial adenitis, fungal infection, toxoplasmosis, and cat-scratch fever that mimics the diagnosis of TB by cytology and/or histopathology [4]. It is hard to distinguish TBLN from atypical mycobacterium infection which needs surgical removal [5]. Therefore, correct diagnosis should be used for proper management.

The definite diagnosis of TBLN is made through bacterial cultures. The sensitivity of mycobacterial isolation is very low, and the isolation process takes a

long time. For this reason, the treatment of cervical TBLN usually begins with the histological diagnosis. However, surgical biopsy through skin incision leaves unwanted scar tissues in the neck, causing aesthetic problems. Moreover, there is a possibility of long-term drainage problems through the incision line [6].

Recently, diagnosis of TBLN using fine-needle aspiration cytology (FNAC) in cervical lymph nodes has been widely used in endemic areas. This method can avoid unwanted effects of surgical biopsies, but the absence of specific cytologic findings of granulomatous lymphadenitis or negative acid-fast bacilli (AFB) smears require additional open biopsy or repeated FNAC. The diagnostic efficacy of FNAC has the sensitivity of only 52.9% and specificity of 100% [7]. It is very variable between institutions. Thus, this method has limitation in clinical situations.

With recent advances in molecular diagnosis using polymerase chain reaction (PCR) and in culture methods using mycobacteria growth indicator tube (MGIT), new techniques have been introduced to identify mycobacterium TB more easily and quickly. These techniques may give a better diagnostic outcome. In this study, we evaluated the efficacy of fine needle aspiration (FNA) using tissue PCR and tissue culture in the MGIT as the diagnostic tools for cervical TBNL and compared the results of tissue culture with open-biopsies.

Patients and method

This prospective and diagnostic study was carried out at King Chulalongkorn Memorial Hospital between August 2003 and November 2006. The study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University.

Forty patients (4 men, 36 women) with cervical lymphadenopathy were recruited for this study. Their ages ranged between 18-69 years (mean ± standard deviation: 34±8 years). The patients signed informed consents. Inclusion criteria included patients presented with mobile, painless cervical lymphadenopathy with diameter greater than 1 cm. There was no evidence of head and neck cancer on physical examination and no recent upper airway infection. The chest X-ray was normal. There was no spontaneous regression of cervical lymphadenopathy after two months of observation. Out-patients FNA cytology reports revealed the epithelioid cell granuloma and caseation necrosis. Exclusion criteria excluded any patients who either were treated with the anti TB drugs, or have cancer or any systemic diseases.

Diagnostic procedure

All diagnostic procedures were carried out in the operating room, under sterile technique. The FNAs were performed before open biopsies. Specimens from FNAs are divided into two portions as follows. One was immediately put in sterile bottle filled with 0.5 mL sterile normal saline solution and sent to the laboratory for tissue PCR (High Pure PCR Template Preparation Kit; Roche, Mannheim, Germany), according to the standard technique described elsewhere [8]. Another was immediately inoculated into Mycobacteria Growth Indicator tube or MGIT (Becton, Dickinson U.K. Limited, Oxford, U.K.) for tissue culture [9]. Following FNAs, the open-biopsy by either total excision or incisional was carried out. The specimens were divided for histopathological examination and for tissue culture in the MGIT.

Results

The demographic data, histopathology, tissue cultures in MGIT and tissue PCR obtained from both FNA and open-biopsies and histopathological diagnoses are shown in **Table 1.**

Histopathological diagnoses of all 40 open-biopsy specimens revealed the caseating granulomatous lymphadenitis in 14 cases (35.0%), necrotizing granulomatous lymphadenitis in 17 cases (42.5%), histiocytic necrotizing lymphadenitis in three cases (7.5%), and necrotic granulomatous inflammation in four cases (10.0%). All but one cases with a diagnosis of granulomatous lymphadenitis/inflammation demonstrated negative AFB staining (34 out of 35 cases). Moreover, even in 14 cases diagnosed as caseating granulomatous lymphadenitis, only one case showed a positive AFB.

Seventeen cases showed positive results while 23 cases were negative. Ten out of 17 cases (58.8%) positive result cases showed positive FNA-tissue cultures. However, four out of 23 cases (17.4%) with negative biopsy-tissue cultures reciprocally showed the positive FNA-tissue cultures (**Table 2**). Then, the sensitivity, specificity, and positive and negative predictive values of the FNA-tissue culture in the MGIT were 65%, 83%, 73%, and 76%, respectively.

Table 1. Histopathology, FNA-tissue for MGIT and PCR and open-biopsy for MGIT in 40 patients (P = positive, N = negative).

Subject	Age (year)	Surgical pathology with AFB stain	FNA tissue MGIT	FNA tissue PCR	Excisional tissue culture MGIT
1	28	Caseating granulomatous Lymphadenitis; AFB+ve	N	P	P
2	42	Caseating granulomatous Lymphadenitis; AFB-ve	N	N	N
3	34	Caseating granulomatous Lymphadenitis; AFB-ve	P	N	P
4	26	Caseating granulomatous Lymphadenitis; AFB-ve	P	P	P
5	21	Necrotizing granulomatous Lymphadenitis; AFB-ve	P	P	P
6	32	Reactive lymphoid hyperplasia	N	N	N
7	51	Caseating granulomatous Lymphadenitis; AFB-ve	P	P	P
8	31	Caseating granulomatous Lymphadenitis; AFB-ve	N	N	P
9	26	Reactive lymphoid hyperplasia	N	N	N
10	20	Caseating granulomatous Lymphadenitis; AFB-ve	P	N	P
11	39	Caseating granulomatous Lymphadenitis; AFB-ve	P	N	P
12	33	Caseating granulomatous Lymphadenitis; AFB-ve	P	P	P
13	30	Caseating granulomatous Lymphadenitis; AFB-ve	N	P	P
14	40	Caseating granulomatous Lymphadenitis; AFB-ve	N	P	P
15	33	Caseating granulomatous Lymphadenitis; AFB-ve	P	N	N
16	43	Caseating granulomatous Lymphadenitis; AFB-ve	P	N	N
17	20	Reactive lymphoid hyperplasia	N	N	N
18	33	Histiocytic necrotizing lynphadenitis	N	N	N
19	24	Caseating granulomatous Lymphadenitis; AFB-ve	P	N	P
20	34	Histiocytic necrotizing lymphadenitis	N	N	N
21	32	Necrotic granulomatous Inflammation; AFB-ve	P	N	P
22	29	Necrotic granulomatous Inflammation; AFB-ve	N	P	N
23	42	Necrotic granulomatous Inflammation; AFB-ve	N	N	N
24	26	Necrotic granulomatous Inflammation; AFB-ve	N	N	N
25	27	Necrotizing granulomatous Lymphadenitis; AFB-ve	N	N	N
26	49	Necrotizing granulomatous Lymphadenitis; AFB-ve	N	N	N
27	29	Necrotizing granulomatous Lymphadenitis; AFB-ve	P	N	N

Table 1. Histopathology, FNA-tissue for MGIT and PCR and open-biopsy for MGIT in 40 patients (P = positive, N = negative) (continued).

Subject	Age (year)	Surgical pathology with AFB stain	FNA tissue MGIT	FNA tissue PCR	Excisional tissue culture MGIT
28	34	Necrotizing granulomatous Lymphadenitis; AFB-ve	N	N	N
29	27	Necrotizing granulomatous Lymphadenitis; AFB-ve	N	N	N
30	44	Necrotizing granulomatous Lymphadenitis; AFB-ve	P	N	N
31	47	Necrotizing granulomatous Lymphadenitis; AFB-ve	N	N	P
32	44	Necrotizing granulomatous Lymphadenitis; AFB-ve	P	P	P
33	57	Necrotizing granulomatous Lymphadenitis; AFB-ve	P	N	P
34	35	Necrotizing granulomatous Lymphadenitis; AFB-ve	N	P	P
35	45	Necrotizing granulomatous Lymphadenitis; AFB-ve	N	N	N
36	69	Necrotizing granulomatous Lymphadenitis; AFB-ve	N	N	N
37	24	Necrotizing granulomatous Lymphadenitis; AFB-ve	N	N	N
38	49	Necrotizing granulomatous Lymphadenitis; AFB-ve	N	N	N
39	25	Necrotizing granulomatous Lymphadenitis; AFB-ve	N	N	N
40	18	Necrotizing granulomatous Lymphadenitis; AFB-ve	N	N	N

Table 2. Culture results *vs.* FNA for culture.

	Culture positive	Culture negative
FNA culture +ve	11	4
FNA culture -ve	6	19

Sensitivity= 65%, Specificity = 83%, Positive predictive value = 73%, Negative predictive value = 76%.

The FNA-tissue PCRs were compared with the gold standard. For 17 cases with a positive open-biopsy tissue culture, only nine cases yields the positive PCR (53%). However, for 23 cases with negative tests for the gold standard, 22 cases showed a negative PCR (96%). The sensitivity, specificity, and positive and negative predictive values of the FNA-tissue PCR were 53%, 96%, 90%, and 73%, respectively (**Table 3**).

When these two diagnostic tests (FNA-tissue culture and FNA-tissue PCR) were combined, we obtained the results as follows (**Table 4**).

1) For combination of only either tests, the sensitivity and specificity were 83.6% and 80.0%, respectively.

2) For combination of both tests together, the combined sensitivity and specificity were 34.5% and 99.0%, respectively.

Table 3**.** Culture results *vs.* FNA for PCR.

	Culture positive	Culture negative
FNA for PCR +ve	9	1
FNA for PCR -ve	8	22

Sensitivity= 53%, Specificity = 96%, Positive predictive value = 90%, Negative predictive value = 73%.

Table 4. Sensitivity and specificity; FNA for culture or FNA for PCR.

	Sensitivity	Specificity
FNA for culture	65.0%	83.0%
FNA for PCR	53.0%	96.0%
FNA culture or FNA PCR*	83.6%	80.0%
FNA culture and FNA PCR**	34.5%	99.0%

*combined Either FNA culture or FNA PCR, **combined both FNA culture and FNA PCR

Discussion

It is very difficult to diagnosis TBLN definitely. It ranges from so-called therapeutic diagnosis to open-biopsy with histopathology and tissue culture [9]. The open biopsies with tissue culture are accepted as the gold standard to diagnose TBLN. However, it requires skin incision that leaves the unwanted scars. Moreover, histopathological examination, even using AFB staining, frequently leads to misdiagnoses of TBLN. In fact, in our experiment, the histopathology reports were often difficult to distinguish caseating granulomatous lymphadenitis and necrotizing granulomatous lymphadenitis. Moreover, the AFB staining was not necessarily helpful. It yielded only one AFB-Positive from total 40 cases or otherwise one from 17 cases with positive open-biopsy tissue culture in MGIT. Since the histopathology with AFB staining is not a good indicator for TBLN, it is important to develop tests with high sensitivity and specificity but that dose less invasion and provided a definite diagnosis.

In this study, we used open-biopsy tissue culture of Mycobacterium tuberculosis by MGIT technique as the gold standard. Since the FNA technique did not require skin incision, we could not obtain histopathology but we did obtain FNA tissue for culture and PCR. Compared to the gold standard, the sensitivity, specificity, and positive and negative predictive values of FNA-tissue culture were 65%, 83%, 73%, and 76%, respectively. The sensitivity, specificity, positive and negative predictive values of FNA-tissue PCR were 53%, 96%, 90%, and 73%, respectively. The low sensitivity of either methods means that any of them cannot be a good screening diagsnotic test for TB lymph node. However, if we combine the test sensitivity of either FNA tissue culture or FNA tissue PCR, the results are higher (83.6%) as shown in **Table 4**. Therefore, we should do both tests and look for the positivity of any tests that fits for the screening. However, if we deen both tests positive, it is not good for screening. This combination should be a good confirmed test (combined specificity: 99%).

Although tissue PCR is a less time-consuming test (one week) compared to the culture technique (MGIT about three weeks), PCR cannot give information about susceptibility to antimicrobial agents. Therefore, the culture techniques, especially the MGIT method, still remains essential in every case.

In our study, four patients had false positive for FNA tissue culture. It is difficult to explain these false positive tests. However, the tissue obtained from FNA is a very small amount compared to open-biopsy specimen. It is likely that the presence of the contamination with resistant micro-organism during FNA may induce changes in MGIT indicator and not reflect the true TBLN . In our FNA-tissue PCR group, one patient had a false positive test. According to Narita et al. [10], PCR technique was able to detect a minor trace of DNA in lymph node specimens. These specimens may contain only nonviable TBNL cells that cannot be detected by the culture technique.

Conclusion

TBLN is hard to diagnose by histopathology with or without ABF staining. For diagnoses of TBLN, the authors suggest FNA tissue culture in MGIT combined with FNA tissue PCR. The combination of either test gives a good screening result without any need of skin incision that leaves unwanted scars.

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