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Comparative studies of diagnostic tests of tuberculouslymphadenitis: culture for TB (BACTEC), polymerasechain reaction and histopathology

P. Hirunwiwatkul

S. Tumwasorn

N. Udomsantisuk

U. Sirichai

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Comparative studies of diagnostic tests of tuberculous lymphadenitis: culture for TB (BACTEC), polymerase chain reaction and histopathology

Prakobkiat Hirunwiwatkul* Somying Tumwasorn**

Nibondh Udomsantisuk** Ukkaporn Sirichai*

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Objective : *To compare diagnostic tests of tuberculous lymphadenitis by culture (BACTEC) with histopathology and polymerase chain reaction in sensitivity, specificity and predictive value.*

Setting : *King Chulalongkorn Memorial Hospital.*

Design : *Prospective analytic study.*

Methods : *Clinically suspected cervical tuberculous lymphadenitis patients underwent excisional biopsy. Specimens were sent for culture (BACTEC), PCR and histopathology. "POSITIVE" culture of Mycobacterium tuberculosis by BACTEC technique was assumed as positive control in the study.*

Results : *Sensitivity, specificity, positive and negative predictive values of histopathology results of caseous granulomatous lymphadenitis with positive AFB stain was 26.32 %, 88.89 %, 83.33 % and 36.36 %, respectively. If the criteria to diagnose TB lymph node was only caseous granulomatous lymphadenitis, then sensitivity, specificity, positive and negative predictive values of histopathology results were 100 %, 66.67 %, 86.36 % and 100 %, respectively. The sensitivity, specificity, positive and negative predictive value of PCR results was 84.21%, 100 %, 100 % and 75.00 %, respectively.*

* Department of Otolaryngology, Faculty of Medicine, Chulalongkorn University.

**Derpartment of Microbiology, Faculty of Medicine, Chulalongkorn University.

Conclusions : *For diagnosis of tuberculous lymphadenitis, polymerase chain reaction has the best reliability as a confirmatory test. Histopathology by criteria of caseous granulomatous lymphadenitis is the best screening test. Therefore, both tests are recommended to be prescribed in every suspected patients. Culture of Mycobacterium tuberculosis by BACTEC technique is also reliable to give definite diagnosis; it also provides susceptibility test to antimicrobial agents.*

Key words : *Tuberculous lymphadenitis, Polymerase Chain Reaction, BACTEC, Culture, Histopathology.*

Reprint request : Hirunwiwatkul P. Department of Otolaryngology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

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ประกอบเกียรติ หิรัญวิวัฒน์กุล, สมหญิง ธัมวาสร,นิพนธ์ อุดมสันติสุข, อรรคพร ศิริชัย. ประสิทธิภาพของการตรวจวินิจฉัยวัณโรคต่อมน้ำเหลืองโดยการตรวจหาเชื้อวัณโรค *Mycobacterium tuberculosis* ด้วยวิธี Polymerase Chain Reaction และวิธีตรวจทางพยาธิวิทยา เปรียบเทียบกับวิธีเพาะเลี้ยงเชื้อวัณโรค โดยวิธี BACTEC. จุฬาลงกรณ์เวชสาร 2545 ส.ค; 46(8): 619 – 29

วัตถุประสงค์ : *เพื่อศึกษาเปรียบเทียบความไว ความจำเพาะ ความสามารถของการทำนายของการตรวจวินิจฉัยวัณโรคต่อมน้ำเหลืองโดยวิธีเพาะเลี้ยงเชื้อวัณโรค โดยวิธี BACTEC เปรียบเทียบกับวิธี Polymerase Chain Reaction และวิธีตรวจทางพยาธิวิทยา*

สถานที่ศึกษา : *โรงพยาบาลจุฬาลงกรณ์*

รูปแบบการวิจัย : *Prospective analytic study*

วิธีการศึกษา : *ผู้ป่วยที่สงสัยว่าเป็นวัณโรคต่อมน้ำเหลืองจากการตรวจทางคลินิกได้รับการผ่าตัดเอาต่อมน้ำเหลืองออก ชิ้นเนื้อต่อมน้ำเหลืองที่ตัดออกมาได้รับการตรวจเพาะเลี้ยงเชื้อวัณโรค โดยวิธี BACTEC, วิธี Polymerase Chain Reaction และวิธีตรวจทางพยาธิวิทยา โดยถือว่ากลุ่มที่ผลการเพาะเลี้ยงเชื้อพบเชื้อวัณโรคเป็นกลุ่มควบคุมชนิดบวก*

ผลการศึกษา : *ถ้าถือว่าการตรวจทางพยาธิวิทยาพบมีลักษณะต่อมน้ำเหลืองอักเสบชนิด caseous granulomatous และพบเชื้อวัณโรคจากการย้อม AFB การตรวจนี้จะมีความไวต่ำ (26.32 %) และความจำเพาะสูง (88.89 %) ความสามารถของการทำนาย ถ้าผลการตรวจให้ผลบวก ผู้ป่วยมีโอกาสที่จะเป็นโรคสูง (83.33 %) และความสามารถของการทำนาย ถ้าผลการตรวจให้ผลลบ ผู้ป่วยมีโอกาสที่จะไม่เป็นโรคได้ต่ำ (36.36 %)*

แต่ถ้าถือว่าการตรวจทางพยาธิวิทยาพบมีลักษณะต่อมน้ำเหลืองอักเสบชนิด caseous granulomatous ไม่ว่าจะพบเชื้อวัณโรคจากการย้อม AFB หรือไม่ก็ตาม การตรวจนี้จะมีความไวสูงมาก (100.00 %) และความจำเพาะระดับปานกลาง (66.67 %) ความสามารถของการทำนาย ถ้าผลการตรวจให้ผลบวก ผู้ป่วยมีโอกาสที่จะเป็นโรคระดับสูง (86.36 %) และความสามารถของการทำนาย ถ้าผลการตรวจให้ผลลบ ผู้ป่วยมีโอกาสที่จะไม่เป็นโรคได้สูงมาก (100.00 %)

ส่วนการตรวจโดยวิธี Polymerase Chain Reaction มีความไวระดับสูง (84.21%) แต่มีความจำเพาะสูงมาก (100.00 %) ความสามารถของการทำนาย ถ้าผลการตรวจให้ผลบวก ผู้ป่วยมีโอกาสที่จะเป็นโรคระดับสูงมาก (100.00 %) ถ้าผลการตรวจให้ผลลบ ผู้ป่วยมีโอกาสที่จะไม่เป็นโรคได้ระดับปานกลาง (75.00 %) ตามลำดับ

สรุป : *ในการวินิจฉัยวัณโรคต่อมน้ำเหลือง การตรวจโดยวิธี Polymerase Chain Reaction มีความจำเพาะสูงมาก เหมาะสำหรับใช้เป็นการทดสอบยืนยัน ส่วนการตรวจทางพยาธิวิทยา โดยถือว่าการตรวจพบลักษณะต่อมน้ำเหลืองอักเสบชนิด caseous granulomatous ไม่ว่าจะพบเชื้อวัณโรคจากการย้อม AFB หรือไม่ก็ตาม เป็นเกณฑ์ในการวินิจฉัย มีความไวสูงมาก เหมาะที่สุดสำหรับใช้เพื่อคัดกรอง ดังนั้นการวินิจฉัยวัณโรคต่อมน้ำเหลืองจึงควรส่งตรวจทั้งสองอย่างนี้ ส่วนการตรวจเพาะเลี้ยงเชื้อวัณโรค โดยวิธี BACTEC ใช้เวลาในการตรวจนาน แต่มีความสำคัญในการให้การวินิจฉัยที่แน่นอนและยังให้ข้อมูลเกี่ยวกับความไวของเชื้อวัณโรคต่อยาต้านจุลชีพ*

คำสำคัญ : *วัณโรคต่อมน้ำเหลือง, Polymerase Chain Reaction, BACTEC, การเพาะเลี้ยงเชื้อ, การตรวจทางพยาธิวิทยา*

Tuberculosis is one of the most common communicable diseases in Thailand; its incidence has risen significantly due to the widespread HIV infection. Although medical knowledge in this area grows up rapidly, the incidence of tuberculous lymphadenitis, especially in the cervical region, is still present and high prevalence that the past.

Cervical tuberculous lymphadenitis shares its course of pathogenesis with other diseases such as malignant lymphoma, Kikushi (histiocytic necrotizing lymphadenitis), metastatic lymphadenopathy, other infectious or neoplastic lymph node diseases. Granulomatous lymphadenitis, in particular, has been known to be caused by *Mycobacterium tuberculosis* or atypical *Mycobacterium* - - the infection which requires different treatment. *M. tuberculosis* infection is a medically curable disease, but atypical *Mycobacterium* infection needs surgical treatment.[1]

Diagnosis of tuberculous lymphadenitis has long been problematic because the gold standard of diagnosis needs tissue culture (BACTEC technique), which is highly specific and sensitive. Normally, the culture technique also notifies the result of drug susceptibilities, which is important information for an accurate anti-microbial treatment. However, the culture and susceptibility test by BACTEC technique is time-consuming (about 2 months), expensive and hardly available in most hospitals (except in major university hospitals).

Most Thai physicians, who are primary caregivers for patients with tuberculous lymphadenitis, often ignore the culture test before starting antituberculous drugs. Usually, they merely rely upon clinical findings (history taking and physical examination) to diagnose and treat their patients. There is a great chance for the diagnoses to be wrong. Some physicians diagnose TB lymph node by using Fine Needle Aspiration Cytologic examination which is known to have a certain degree of false positive and false negative results. Some physicians treat TB lymph node by using histopathological results that are compatible with TB such as Granulomatous Inflammation (caseating or non-caseating). Without the identification of AFB positive organisms, the diagnosis of tuberculous lymphadenitis is not definite. In most cases of tuberculous lymphadenitis, the chance to find AFB positive organism is rare, because normally there are few organisms in the infected lymph node. Although positive AFB stained organisms are found in the specimen of a lymph node, it can be other type of AFB stained organisms (such as atypical mycobacterium, Nocardia, etc.). An accurate diagnosis of tuberculous lymphadenitis, therefore, depends upon reliable tests apart from compatible histopathological findings.[2]

Since the identification of *M. tuberculosis* by polymerase chain reaction (PCR) technique was developed to find out some trace of *M. tuberculosis* DNA in the specimen. The specificity of the test is high. It detects even a minor trace of DNA in a specimen that contains only few *M. tuberculosis* cells (viable or nonviable cells). Moreover, the technique does not need a long time to provide its result, so it is beneficial for clinicians to give an accurate diagnosis and a proper treatment.

Another advantage of the technique is that it can be used to confirm the diagnosis of *M. tuberculosis* infection given by other methods such as histopathology test. There is no need for clinicians to wait for BACTEC culture result, they can immediately

start the antituberculous drugs to the patients. PCR technique can also be used in the paraffin-embedded specimens;[3-5] so it is beneficial in the cases of suspected *M. tuberculous* infected patients who were not set up for PCR test at the time of excisional biopsy or in those who have been partially treated with anti-microbial drugs. BACTEC culture technique cannot be employed in both situations.

Because of the fact that the technique only requires few organisms in a tiny specimen, fine-needle aspiration biopsy may also be used for cytologic examination, as a combination of the PCR test for an accurate diagnose of TB.[6-8]

Our study aims to find out the efficiency of PCR technique for *M. tuberculosis* detection, histopathological diagnosis and clinical diagnosis compared with gold standard technique (*M. tuberculosis* culture by BACTEC technique). The sensitivity, specificity and predictive value of PCR test and histopathological diagnosis were studied.

Goal of study

To determine the efficiency of polymerase chain reaction (PCR) and histopathological diagnosis as tools for diagnosis of *M. tuberculosis* cervical lymphadenitis by comparing the results obtained from positive culture of *M. tuberculosis* by BACTEC technique.

Material and method

Prospective analytical study was designed and started from Jan 2000. All cases were selected from patients in ENT clinic of King Chulalongkorn Memorial Hospital with the following criteria.

Inclusion criteria

- Studied cases were only selected from patients who presented with neck mass and who were clinically suspected of cervical tuberculous lymphadenitis.
- Criteria of neck mass which was suspected of tuberculous lymphadenitis were the following:
  1. Neck mass of more than 1 month duration.
  2. Characteristics of the mass were likely of a lymph node.
  3. Location of the mass was occipital or supraclavicular area.
  4. No evidence of primary cancer in the head and neck was detected from complete ENT examination.
  5. FNA cytology showed lymphoid tissue with no malignancy or other findings compatible with TB.
- Patients gave their informed consent.

Exclusion criteria

- Previous treatment with anti-tuberculous drugs within 6 months
- Positive anti-HIV test

Every patient who complained with neck mass either on occipital or a supraclavicular area more than 1 month was completely examined by ENT doctors, to exclude primary cancer in upper aerodigestive tract, and then underwent fine needle aspiration biopsy. Cytological examination was done by attending staff of the Department of Pathology, Faculty of Medicine, Chulalongkorn University. If a malignancy was detected, the patient would be sent to Tumor Clinic for further management. If no malignancy was found or cytology compatible with TB, such as granulomatous inflammation with or without caseous necrosis, the patient would be recruited and assumed

to be clinically diagnosed of cervical tuberculous lymphadenitis in this study.

The patients were scheduled for excisional biopsy of lymph nodes (1 or 2 nodes). One part of the lymph node specimen was wrapped in saline-soaked sterile gauze and kept in a sterile container and sent for the detection of *Mycobacterium tuberculosis* and TB culture and susceptibility test (BACTEC technique). PCR test and TB culture of every specimen was done at the Department of Microbiology, Faculty of Medicine, Chulalongkorn University, under the supervision of an authorized microbiologist. The PCR technician did not know any clinical information about the patients and the histopathology result of each specimen. The PCR results were reported as "POSITIVE" or "NEGATIVE". The other part of specimen was fixed in formalin and sent for histopathological study. Every specimen was pathologically reviewed by attending pathologists.

PCR and histopathology results were reported within 1 week, whereas the result of TB culture and susceptibility test (BACTEC) were reported about 2 months. If the histopathology result was compatible with TB (granuloma with or without caseation and with or without positive AFB stain) or the result of PCR test was "POSITIVE", the patient would be treated as tuberculous lymphadenitis with short-course of anti-TB drug regimen (2HRZE + 2HR). The short-course regimen consisted of 4 drugs (isoniazid 5 mg/kg po, rifampicin 10 mg/kg po, ethambutol 15-25 mg/kg po and pyrazinamide 15 -30 mg/kg po) in the first 2 months; then 2 drugs (isoniazid 5 mg/kg po, rifampicin 10 mg/kg po) were given in the following 4 months. If the histopathology result was otherwise (such as "lymphoma") and PCR test was "NEGATIVE", the patient would be properly managed further according to the diagnosis. All patients were followed up at ENT Clinic every month. When TB culture (BACTEC) results were reported, anti-TB drugs were modified according to the susceptibility results and continued for at least 6 months (total course duration).

Polymerase chain reaction (PCR) technique

DNA extraction

After tissue specimen was crushed, digestion was performed overnight at 55°C. DNA was purified by phenol-chloroform-isoamyl alcohol (25:24:1) extraction, precipitated by ethanol, and dissolved in 50 µl of TE buffer (10 mM Tris HCl, 1mM EDTA, pH8). Five microliters of the sample were used for the PCR assay. Each sample was spiked with *Mycobacterium tuberculosis* DNA (100 fg) as a control to detect the presence of amplification inhibitors. A positive control contained 100 fg of *M. tuberculosis* DNA; a negative control, that contained no DNA were included in each run.

PCR analysis

PCR was performed by the amplification of *IS 6110* insertion sequence as described by Kolk *et al.*[9] with modification. Primers INS1 (5′ CGTGAG GGCATCGAGGTGGC) and INS2 (5′ GCGTAGGCGT CGGTGACAAA) were used to amplify a 245-bp fragment, then nested primers Pt3 (5′ GAACGGCTG ATGACCAAACT) and Pt6 (5′ ACGTAGGCGAACCC TGCCCA) were used to amplify a 188-bp fragment situated within the 245-bp fragment.

The first PCR solution (final volume 50 µl) contained 50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 0.01% (W/V) gelatin, 0.2 mM each of dATP, dCTP and

dUTP (dUTP instead of dTTP), 0.2 U of uracil-N-glycosylase (BRL), 0.4 μM of primers INS1 and INS2 and 1 U of Taq polymerase (Perkin Elmer Cetus). The second PCR solution contained primers Pt3 and Pt6 and other reagents of the first PCR solution except uracil-N-glycosylase.

Treated sample was added in a volume of 5 μl and the reaction mixture was left at room temperature for 10 minutes. The reaction was carried out in a DNA thermal cycler (Perkin Elmer Cetus). The temperature of the sample was first raised to 94°C for 10 minutes to inactivate uracil-N-glycosylase and to denature the DNA. Amplification of 40 cycles consisted of denaturing at 94°C for 90 seconds, annealing at 65°C for 90 seconds, and extension at 72°C for 90 seconds. For the second PCR, 10 μl of the reaction solution containing the amplified first PCR product was added into 40 μl of the second PCR solution and the reaction mixture was amplified for 40 cycles with the same PCR condition as of the first PCR. The PCR products were electrophoresed in 2 % agarose gel containing 0.5 μg/ml of ethidium bromide and visualized by UV transillumination.

Results

"POSITIVE" culture of *Mycobacterium tuberculosis* by BACTEC technique was used as gold standard and positive control for the study. The histopathology, culture and PCR results of individual cases were shown in Table1. The culture and PCR results were statistically analyzed by *chi*-square test (Table 2). The culture and histopathology results of caseous granulomatous lymphadenitis with positive AFB stain were statistically analyzed by chi-square test. (Table 3). The culture and histopathology results of caseous granulomatous lymphadenitis with or without positive AFB stain were statistically analyzed, using chi-square test. (Table 4).

Table 1. Histopathology, culture and PCR results of individual cases.

Patient No.	Histopathology	Cuture for TB (BACTEC)	PCR result
1	Caseous granulomatous lymphadenitis with AFB positive	*M. tuberculosis*	POSITIVE
2	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	POSITIVE
3	Caseous granulomatous lymphadenitis with AFB positive	Negative	Negative
4	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	POSITIVE
5	lymphoma	Negative	Negative
6	lymphoma	Negative	Negative
7	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	POSITIVE

Table 1. Continuous.

Patient No.	Histopathology	Cuture for TB (BACTEC)	PCR result
8	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	POSITIVE
9	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	POSITIVE
10	Caseous granulomatous lymphadenitis with AFB negative	Negative	Negative
11	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	POSITIVE
12	Caseous granulomatous lymphadenitis with AFB positive	*M. tuberculosis*	POSITIVE
13	Caseous granulomatous lymphadenitis with AFB positive	*M. tuberculosis*	POSITIVE
14	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	POSITIVE
15	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	Negative
16	Necrotizing lymphadenitis with AFB negative	Negative	Negative
17	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	POSITIVE
18	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	Negative
19	Hodgkin's lymphoma	Negative	Negative
20	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	Negative
21	Caseous granulomatous lymphadenitis with AFB positive	*M. tuberculosis*	POSITIVE
22	Reactive hyperplasia of lymph node	Negative	Negative
23	Caseous granulomatous lymphadenitis with AFB positive	*M. tuberculosis*	POSITIVE
24	Caseous granulomatous lymphadenitis with AFB negative	Negative	Negative
25	Necrotizing lymphadenitis with AFB negative	Negative	Negative
26	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	POSITIVE
27	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	POSITIVE
28	Caseous granulomatous lymphadenitis with AFB negative	*M. tuberculosis*	POSITIVE

Table 2. Culture results vs. PCR results.

	Culture positive	Culture negative
PCR +	16	0
PCR -	3	9
Sensitivity (ความไว)	= 84.21 %	
Specificity (ความจำเพาะ)	= 100.00 %	
Positive predictive value	= 100.00 %	
Negative predictive value	= 75.00 %	

Table 3. Culture results vs. histopathology results of caseous granulomatous lymphadenitis with AFB+ve.

	Culture positive	**Culture negative**
Caseous granuloma with AFB +	5	1
Others	14	8
Sensitivity (ความไว)	= 26.32 %	
Specificity (ความจำเพาะ)	= 88.89 %	
Positive predictive value	= 83.33 %	
Negative predictive value	= 36.36 %	

Table 4. Culture results vs. histopathology results of caseous granulomatous lymphadenitis.

	Culture positive	**Culture negative**
Caseous granuloma	19	3
Others	0	6
Sensitivity (ความไว)	= 100.00 %	
Specificity (ความจำเพาะ)	= 66.67 %	
Positive predictive value	= 86.36 %	
Negative predictive value	= 100.00 %	

Discussion

In the study we accepted "POSITIVE" culture of *Mycobacterium tuberculosis* by BACTEC technique as the gold standard. The sensitivity, specificity, positive and negative predictive value of histopathology results of caseous granulomatous lymphadenitis with positive AFB stain was: 26.32 %, 88.89 %, 83.33 % and 36.36 %, respectively. The low sensitivity means that it could not be a good screening diagnostic tool for TB lymph node. If histopathological diagnostic criteria of TB lymph node was less strict to be only caseous granulomatous lymphadenitis, then the sensitivity, specificity, positive and negative predictive value of histopathology results were: 100 %, 66.67 %, 86.36 % and 100 %, respectively. The high sensitivity enable it to serve as the best screening test.

Sensitivity, specificity, positive and negative predictive values of the PCR results were: 84.21 %, 100 %, 100 % and 75.00 %, respectively. The false positive of the test might result from contamination of DNA fragments (IS 6110) from the previous test. It can be eliminated by using uracil-N-glycosylase & dUTP. False negative can also occur because of the presence of inhibitor (Taq polymerase) in the specimen which can be prevented by using proteinase K, phenol-chloroform extract. All of these confounding factors were eliminated in the study. The high specificity and positive predictive values made PCR one of the two best confirmatory tests. It is also a less time-consuming test (within 1 week) in comparison to culture technique (about 2 months). Since PCR can not give information about susceptibility to antimicrobial agents, the culture technique especially BACTEC method still remain essential in every case.

Sugita [7] *et al.* reported that PCR helped diagnosis of tuberculous lymphadenitis in patients who were receiving steroid. Narita [8] *et al.* presented that PCR technique was able to detect a minor trace of DNA in lymph node specimens that contained only nonviable *M. tuberculosis* cells which the culture technique was unable to be positive.

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