CHAPTER 3

RESEARCH METHODOLOGY

1. Research questions

1.1. Primary research question:

1.1.1. What is the diagnostic performance of the combined FNAC and TT for tuberculous lymphadenitis in the group of inconclusive diagnosis by FNAC?

1.1.2. What is the incremental ratio of cost-effectiveness of FNAC+TT comparing with FNAC alone for the correct diagnosis of tuberculous lymphadenitis? Is it efficient on patient's perspective?

1.2. Secondary research questions:

1.2.1. What are the pitfalls of diagnosis by FNAC?

1.2.2. What is the efficient model for diagnosis of TB lymph node by combined FNAC and TT?

2. Research objectives

2.1. To determine sensitivity, specificity, positive and negative predictive values and likelihood ratio of the combined test (FNAC+TT) in helping diagnosis of cervical tuberculous lymphadenitis at King Chulalongkorn Memorial Hospital.

2.2. To calculate the incremental ratio of cost-effectiveness of FNAC+TT and FNAC in the diagnosis of cervical tuberculous lymphadenitis, on the patient perspective, at King Chulalongkorn Memorial Hospital.

2.3. To determine the pitfalls of false positive and negative diagnoses by FNAC.

2.4. To determine the efficient model for diagnosis of cervical tuberculous lymphadenitis by combined FNA and TT, on the patient perspective, at King Chulalongkorn Memorial Hospital.

3. Research design & Sample specification

Research design: Diagnostic test

Methodology: Prospective, cross-sectional study, economic evaluation

Target population: Thai patients presenting with palpable neck masses (nodes) for more than two weeks' duration and FNA gives inconclusive result.

Studied population: OPD patients presenting with palpable neck masses (nodes) for more than two weeks' duration at King Chulalongkorn Memorial Hospital and FNA gives inconclusive result.

Sample size: 69

4 Sample size calculation

Formula: $n= Z^2_{\alpha/2} P(1-P)/d^2$

n= number of patients with TB node

$Z_{\alpha/2}$ =Z-value at the level of α error (0.05) = 1.96

P = expected sensitivity of the test = 90% (from reference9)

d = precision = 10% (see comment)

n= 3.84x0.9x0.1/0.01 = 34.56

The prevalence of TB node in this study is 50%

N= number of suspected TB node patients need to find the n cases of TB node

N= 34.56x100/50 = 69

Comment:

d is acceptable error =10%. It means that the sensitivity of the test (FNA+TT) can be accepted at 80%, since the sensitivity of FNA alone is estimated at 70%. It is reasonable that the combined test is better in term of sensitivity.

5. Eligibility criteria

5.1. Inclusion criteria:

1. OPD patient, older than 14 years of age.
2. Palpable neck node(s), persistent for more than 2 weeks.
3. At least one node ≥ 1 cm by palpation.
4. FNA gives inconclusive result.
5. The same node that has been investigated with FNA is excised.

5.2. Exclusion criteria:

1. Not consent
2. Partial treatment with anti-TB or related drugs
3. Skin lesions that are not suitable to have TT

6. Methods

6.1. Patient care and procedure

1. OPD patients with chief complaints of superficial palpable masses (clinically presumed nodes) ≥ 1 cm for more than two weeks received FNA at first encounter.
2. When FNA result was conclusive i.e. malignant nodes, TB nodes with positive acid-fast bacilli, cyst, etc. these patients would refer to specialty doctors of the fields.
3. The patients with inconclusive result by FNA were the candidate group that the author explained for his research protocol. When patients who were willing to enter this project study, they were our studied population.
4. Patients in the group of studied population received ENT exam to exclude ENT malignancy. After that they received both TT and excisional biopsy of the investigated lymph node. Patients outside the study were advised to have ENT exam, node excision and / or follow-up at ENT department as usual line of management.
5. FNA was performed by the author and TT was performed by the well-trained staff of Chest Unit, Department of Internal Medicine.

6. FNA was performed by using gauge 23 needle attached to a 10 ml syringe. Clean and sterile the skin with 70% ethanol and puncture the needle. When the needle was inside the node, negative pressure was applied, forwarded the needle just a little bit like jerking, released negative pressure within the syringe before removed the needle out of the node. Direct smears were made. Most slides were immediately put into the jar containing 95%ethanol for a 5-min fixation. These smeared slides were stained according to Papanicolaou method. One air-dried slide or one Papaniculaou's stained slide of each case was sent for acid-fast stain according to Kinyoun's method. Two or three aspirations were undertaken. The puncture sites were pressed until bleeding stopped.

6.2. **Cytology, Tuberculin test and Biopsy specimens**

1. Cytomorphologic change was evaluated by the author. Six feature categories were described in the column of cytologic diagnosis. There were i. epithelioid cell aggregate with or without granuloma feature (Figure 2) , ii. caseating necrotizing feature (Figure 3) , iii. non-specific necrotizing feature (Figure 4) , iv. non-specific lymphoid cell feature (Figure 5) , v. reactive lymphoid feature (Figure 6) , and vi. reactive lymphoid feature with appreciable tingible body macrophages (Figure 7).
2. TT was performed with Mantoux method by intradermal injection of 0.1 ml PPD at the ventral aspect of forearm 2 inches below the elbow line. Observed results in the following two days (48 hours). The size of tuberculin reaction induration was measured in mm, made by a well-trained staff of Chest Unit.
3. The excisional nodes were fixed in 10% neutral buffered formalin and processed according to usual histological technique. Caseating granulomatous lymphadenitis was diagnostic criteria for TB node [20]. The diagnosis was agreed by two pathologists.

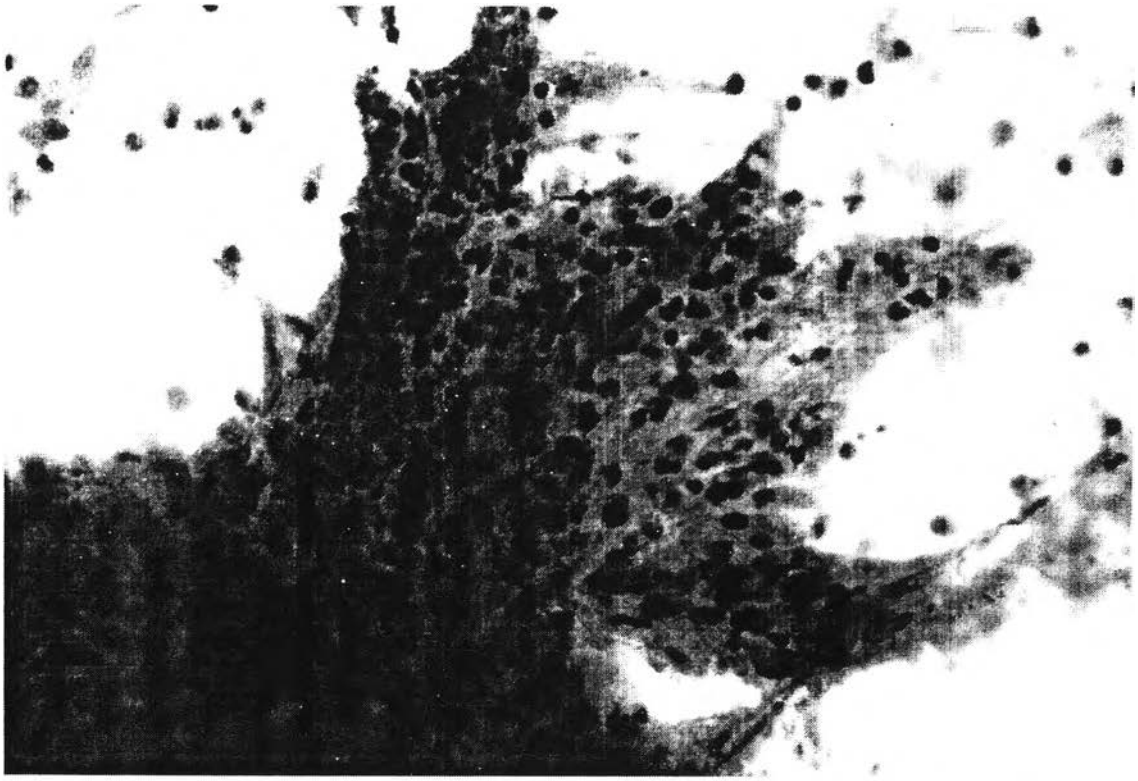

Figure 2: Epithelioid cell aggregates feature (original magnification x400)

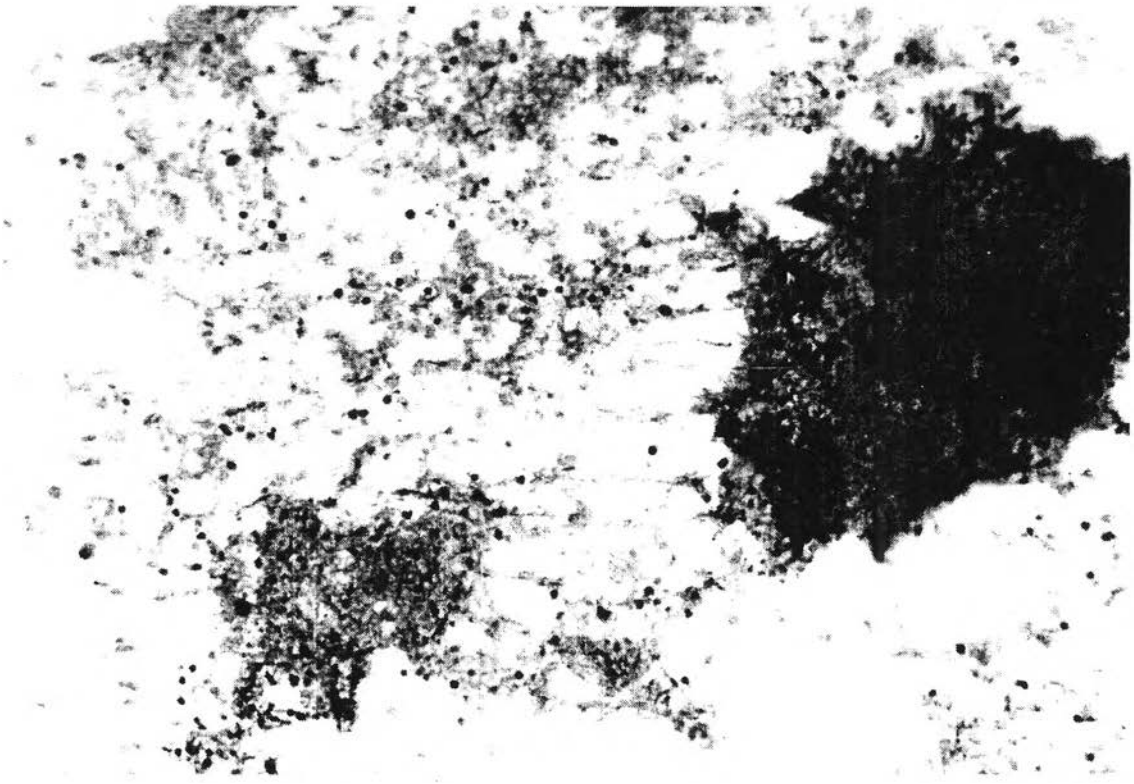

Figure 3: Caseating necrotizing feature (original magnification x200)

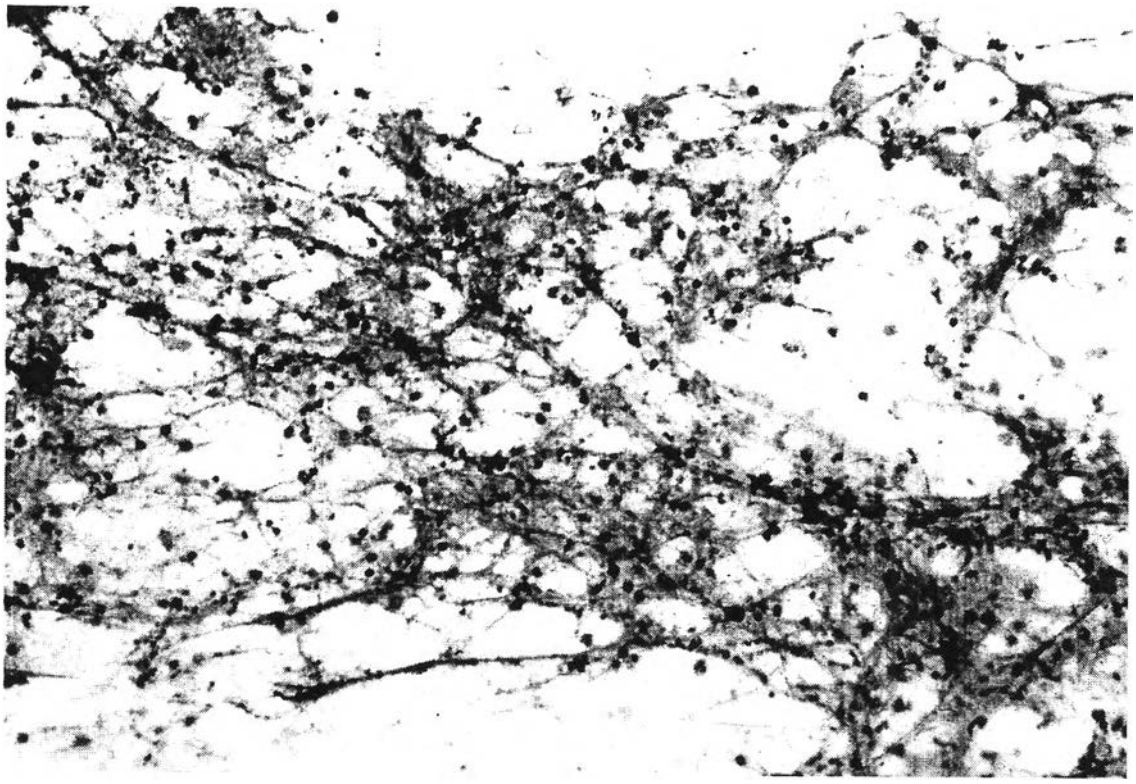

Figure 4: Nonspecific necrotizing feature (original magnification x200)

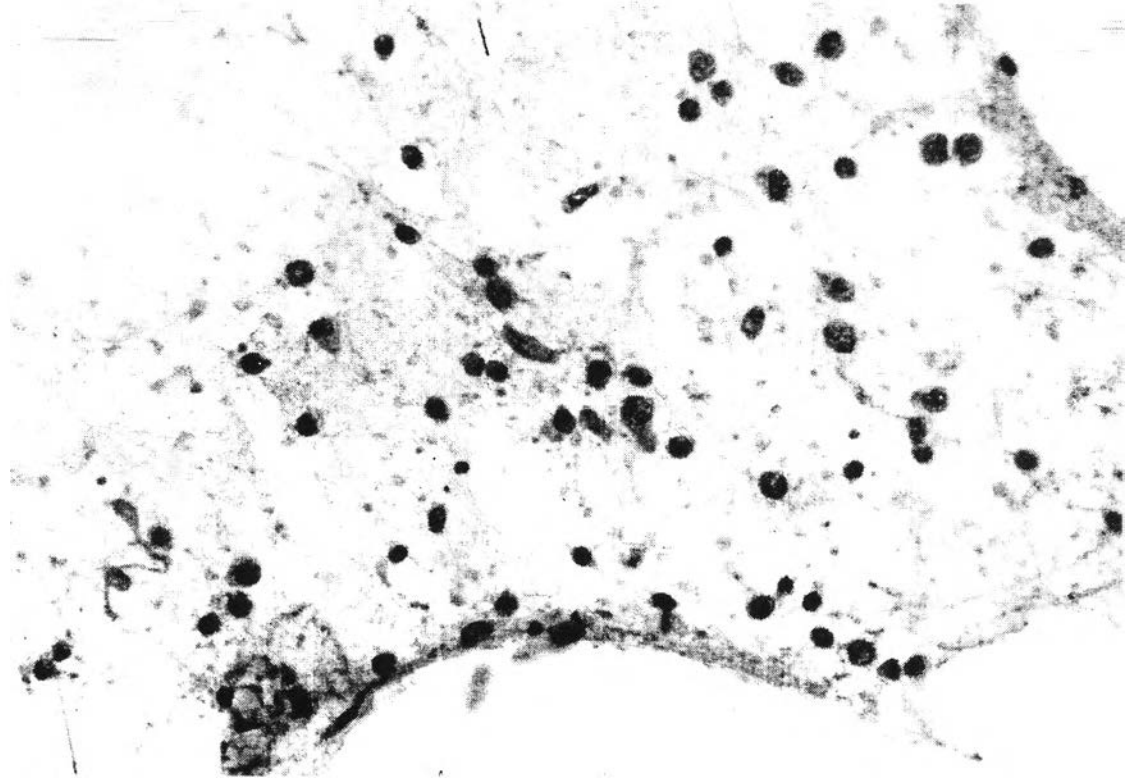

Figure 5: Lymphoid cells, not specified (original magnification x400)

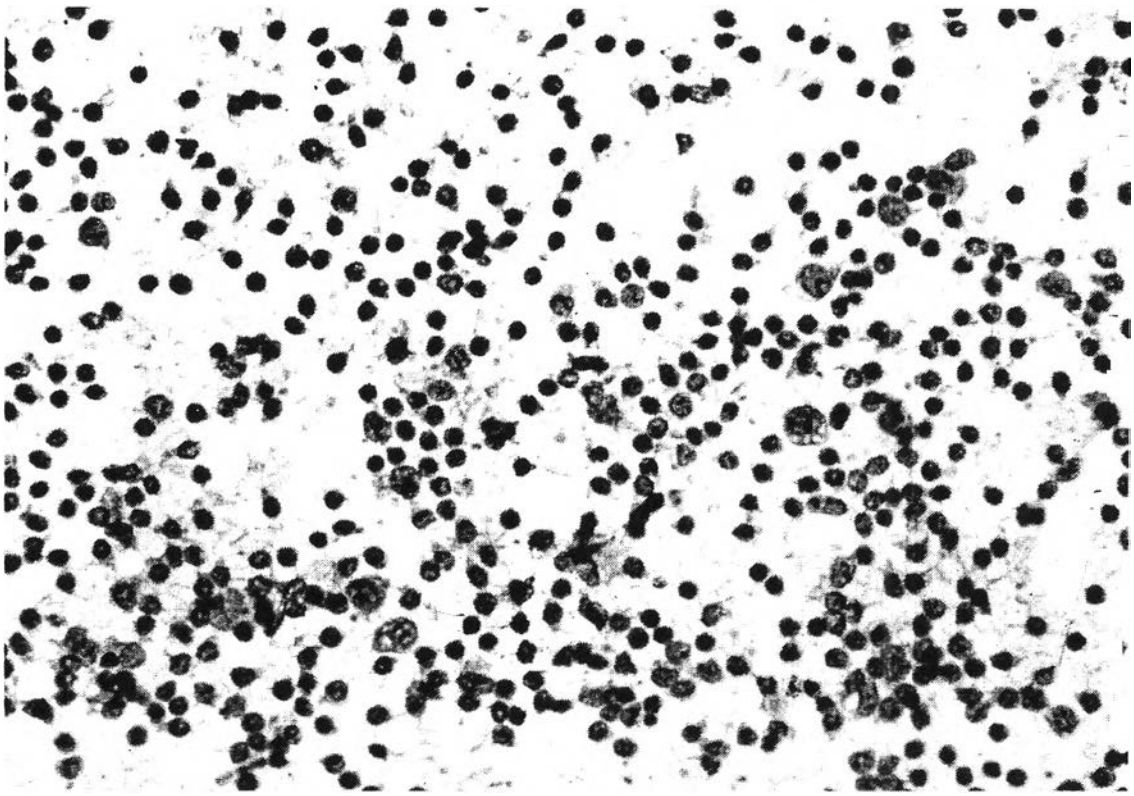

Figure 6: Reactive lymphoid feature (original magnification x400)

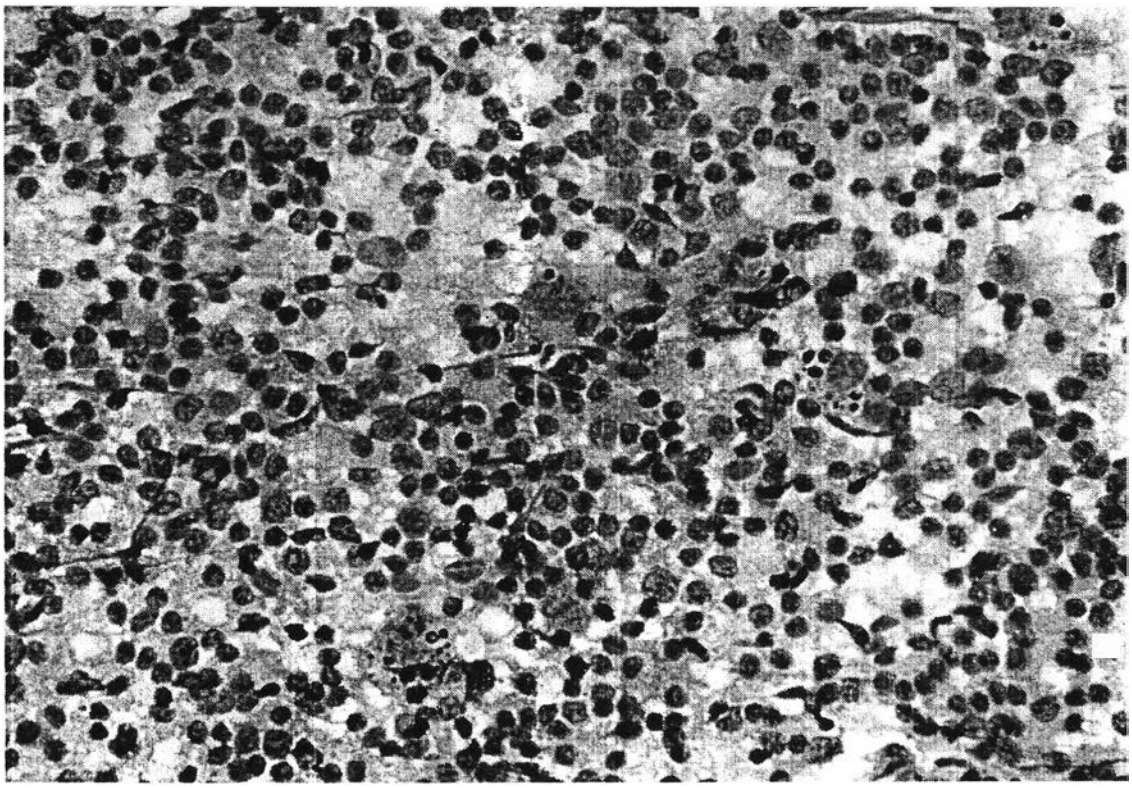

Figure 7: Reactive lymphoid with tingible-body macrophage feature

(original magnification x400)

6.3. Economic evaluation

Patient's viewpoint was considered. The cost of performing tuberculin test, travelling expense and indirect cost of work loss were calculated. The visiting scheme was proposed and evaluated for the number of extra days needed to have the combined test. Complications and care for reaction were observed. Analyses concentrated on the incremental cost-effectiveness ratio.

7. Operational Criteria

7.1. Conclusive diagnoses by FNAC are as following:

1. Detectable AFB or other specific microorganisms.
2. Cyst which has disappeared after aspiration and no recurrence.
3. Malignant neoplasm.

7.2. Inconclusive diagnoses by FNAC are as following:

1. Presence of granuloma image and/or caseous necrosis material but without detectable AFB.
2. Nonspecific necrotizing inflammation without detectable AFB.
3. Reactive or non-specific lymphoid cell feature.

7.3. Cytologic features that are components of granulomatous inflammation:

1. Epithelioid cell aggregate with or without giant cells
2. Caseating necrotizing feature

7.4. Cytologic features that are suggestive or suspicious for tuberculosis:

1. Epithelioid cell aggregate with or without granuloma feature
2. Caseating necrotizing feature
3. Necrotizing liquefaction (cheesy pus-like material)

7.5. Positive test for combined test is as following:

1. Cytology consistent with granulomatous inflammation AND tuberculin test positive (induration ≥15 mm)
2. Cytology NOT consistent with granulomatous inflammation AND tuberculin test is strongly positive (induration ≥25 mm)

8. Data collection

FNA-No.	Sex	Age	HIV	No-node	Location	Size	FNA-finding	AFB	TT	Final-Dx

9. Data analysis

9.1. Items for analysis

1. Demographic characteristics
2. Cytologic features and pitfalls
3. Diagnostic performance of cytology
4. Diagnostic performance of TT (cut-off value ≥25 mm)
5. Diagnostic performance of combined test
6. Economic evaluation

9.2. Methods for diagnostic performance

A. Based on true and false positive and negative cases

$$\text{Sensitivity (\%)} = 100 \times \frac{TP}{TP+FN}$$

$$\text{Specificity (\%)} = 100 \times \frac{TN}{TN+FP}$$

(TP= true positive, FN= false negative, TN= true negative, FP= false positive)

B. Based on case and non-case, test positive and test negative table

Biopsy / FNA+TT	TB -Node	Non TB - Node	Sum
Test-Positive			
Test-Negative			
Sum			

10. Economic Evaluation

10.1. Items of patient expense

Number of day visits ..... Days

Travelling expense ..... Baht

Medical charges

FNA ..... Baht

TT ..... Baht

Biopsy with Histology ..... Baht

Indirect cost for work absence ..... Baht

Complication and treatment ..... Baht

10.2. Methods of analysis

Cost-effectiveness analysis: Analyse for incremental cost-effectiveness ratio

$$= \frac{C1-C2}{E1-E2}$$

whereas

C1 = Cost of FNAC+TT in diagnosis of TB node

C2 = Cost of FNAC in the diagnosis of TB node

C1-C2 = Cost of TT in the diagnosis of TB node

E1 = Number of cases correctly diagnosed by FNAC+TT

E2 = Number of cases correctly diagnosed by FNAC

E1-E2 = Number of cases that can be detected more by FNAC+TT than FNAC

11. Ethical consideration

This study is aimed to improve the diagnostic mean that has been used in practice. Both FNA and TT are safe. Mild complications such as hemorrhage and limited skin necrosis from TT hypersensitivity reaction might occur. They can be detected and manageable. False negative and false positive tests will do no harm to the patients. They will be revealed by surgical biopsy. The treatment will start only when gold standard criterion is fulfilled. The aim and content of the research will be explained to the patients and consent form will be signed.

The proposal of this study has passed the Faculty Ethical Board.

12. Limitations

1. The efficiency of the combined test may have influence from the prevalence of HIV. But this will reflect the real situation in Thai population if no selection bias occurs.

2. Atypical tuberculosis cannot separate from typical tuberculosis without culture and microbiologic identification test. All AFB positive cases or cases with evidence of granulomas and/or caseous necrosis will regard as tuberculosis unless culture is proved to be atypical tuberculosis group.

13. Time Schedule

1 year

	Mar	Apr	May.....Dec	Jan	Feb	Mar	Apr
Preparation	------						
Staff meeting	------						
Data collection		----------					
Data analysis					----------		
Thesis writing					----------		
Presentation							*