FR 1652

Pilot Study on the Role of Fine Needle Aspiration in the Diagnosis of Tuberculous Lymphadenitis

Final Report

RG/2004/M/15

Dr. L K B Mudduwa

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University of Ruhuna

Final Report: RG/2004/M/15

Pilot Study on the role of fine needle aspiration in the diagnosis of tuberculous lymphadenitis

Section 1

Information regarding Project / Project Personnel:

i. Contract Number: RG/2004/M/15

ii. Title of the Project: Pilot Study on the role of fine needle aspiration in the diagnosis of tuberculous lymphadenitis

iii. Principal Investigator: Dr. LKB Mudduwa

iv. Co-Investigator: Dr. A de S Nagahawatte

v. Institute where research was carried out:

Department of Pathology and Department of Microbiology,
Faculty of Medicine,
University of Ruhuna, Galle.

vi. Date of Award: 01/11/2004

vii. Date of Completion of the Project: 01/01/2008

viii. Total Allocation of funds: Rs. 216,100/-

ix. Total Spent: Rs. 1,99080/-

x. Number of research students : None

xi. Postgraduate degrees completed : None

xii. Number of Technical Assistants/Labourers employed: None

xiii. Publications/communications arising during :

Diagnosis of tuberculous lymphadenitis; Combining cytomorphology, microbiology and molecular techniques – A study from Sri Lanka
Accepted for publication in the Indian Journal of Pathology and Microbiology

Section 2 & 3

Executive Summary of the report
Report in detail.

The findings of the project are presented in the article I have submitted to the Indian Journal of Pathology and Microbiology. It has been accepted to be published in the April-June issue. I have annexed the copy I received from the publishers for proof reading. A re-print will be submitted once I receive from the publishers.

Section 4

Impact of research results:

i. Relevance of results achieved to scientific advancement:

The study highlights the value of fine needle aspiration cytology as a simple reliable diagnostic test. It also emphasizes on the best diagnostic criteria to use in the diagnosis. Therefore the results of this project substantiate the value of fine needle aspiration in the diagnosis of tuberculous lymphadenitis. It also highlights the combination of cytological and microbiological features to diagnose tuberculous lymphadenitis with high sensitivity and specificity. This will increase the early diagnosis of tuberculous lymphadenitis.

ii. Relevance of results achieved to national and socio economic development:

Tuberculosis is a major health problem in Sri Lanka. Use of fine needle aspiration with more reliable diagnostic criteria will enhance early detection of tuberculous lymphadenitis. Hence it will contribute to decrease the morbidity and mortality and disease burden on the country at large. The commonly used histopathology assessment for tuberculous lymphadenitis is time consuming and costly. Therefore the use of fine needle aspiration will reduce the cost and the time taken for diagnosis.

iii. Dissemination and application of research out put

Pathologists can make use of the diagnostic criteria highlighted in the publication in routine practice. Clinicians can utilize fine needle aspiration as a first line investigation for the diagnosis of tuberculous lymphadenitis

Section 5

Miscellaneous

Equipment acquired: Micro pipette – functioning well

Publications: Diagnosis of tuberculous lymphadenitis; Combining cytomorphology, microbiology and molecular techniques – A study from Sri Lanka
Accepted for publication in the Indian Journal of Pathology and Microbiology 51(2), April-June 2008

Section 6

Summary Statement of Expenditure - Annexed.

Section 7

I. Grantee's Signature: ..... LKB Mudduwa
Dr. L K B Mudduwa

II. Comments of the Head of the Department: Grantee is the Head

III. Signature/Head of the Institution: .....
Professor T R Weerasooriya,
Dean, Faculty of Medicine,
University of Ruhuna, Galle.

Dean
Faculty of Medicine
University of Ruhuna
Galle.

Financial Statement

The financial position of the grant No. RG/2004/M/15 awarded to Dr. L.K.B. Mudduwa : Foundation is as follows

	Funds Received by University/ Institution	Total Expenditure Rs.	Balance Available Rs.
Personnel	-	-	-
Research Student	-	-	-
Technical Assistant	-	-	-
Other			
Equipment			
Foreign			
Local	15,000.00	15,000.00	Nil
Consumable			
Foreign			
Local	195,100.00	171,845.00	23,255.00
Travelling and Subsistence			
Miscellaneous	6,000.00	12,235.00	-6235.00
Total	216,100.00	199,080.00	17,020.00

Balance as at 30.06.2008 Rs. 17,020.00

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Deputy Bursar
University of Ruhuna
Faculty of Medicine
Galle
30.06.2008

DEPUTY BURSAR
UNIVERSITY OF RUHUNA
FACULTY OF MEDICINE
GALLE.

Original Article

Diagnosis of tuberculous lymphadenitis: Combining cytomorphology, microbiology and molecular techniques - A study from Sri Lanka

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ABSTRACT

Background: Fine needle aspiration is a simple technique to obtain material for early diagnosis of tuberculous lymphadenitis. **Objective:** To assess the value of fine needle aspiration cytology (FNAC) in the diagnosis of tuberculous lymphadenitis. **Materials and Methods:** A total of 43 aspirates from patients who were clinically suspected to have tuberculous lymphadenitis were included in the study. Apart from FNAC, aspirates were smeared for Ziehl Neelsen stain and cultured on Middlebrook 7H9 and 7H10 media. Culture was considered the gold standard. Positive and negative predictive values and agreement between the gold standard and five diagnostic criteria were assessed. Kappa value was used to calculate the agreement. **Results:** The presence of epithelioid cells either with caseation or positive Ziehl Neelsen had the highest agreement (kappa value 0.84), with high positive and negative predictive values (85.71% and 96.55%). Positive Ziehl Neelsen alone and presence of epithelioid cells with positive Ziehl Neelsen had 100% positive predictive values, but the kappa values were lower (0.62 and 0.52) with lower negative predictive values (83.33% and 81.08%). Epithelioid cells alone had a high negative predictive value (93.35%), but the positive predictive value was lower (84.62%). When epithelioid cells were taken together with caseation, the positive predictive value reduced further (83.33%). **Conclusion:** The presence of epithelioid cells either with caseation or positive Ziehl Neelsen stain appears to be the best diagnostic criteria, with a very good agreement with the gold standard and high positive and negative predictive values.

KEY WORDS: Fine needle aspiration cytology, lymph node, tuberculosis

INTRODUCTION

Tuberculosis remains a major threat to global health, with an estimated 8,000,000 new cases and 3,000,000 deaths annually worldwide.[1] It is a notifiable disease in Sri Lanka. Around 6500 to 7000 new cases of tuberculosis are detected annually, and tuberculosis still continues to be a major public health issue in Sri Lanka.[2] In 2003 a total of 9312 patients were diagnosed with tuberculosis, and 18.35% were in extrapulmonary sites. A 34% of them had tuberculous lymphadenitis.[3]

Diagnosis of extrapulmonary tuberculosis is often made on histopathology. Histopathological diagnosis is made when there is caseous necrosis in a granulomatous lymphadenitis. Ziehl Neelsen staining on the histopathology section is an adjunct. However, the chances of identifying acid-fast bacilli in a tissue section are much lower. The effect of formalin and xylene on the stainability of mycobacteria by Ziehl Neelsen method results in extreme low sensitivity of detection of mycobacteria in histopathology sections.[4]

Fine needle aspiration is a simple, less time consuming, less expensive outpatient procedure to obtain material for examination. Most often, superficial lymph nodes are affected in tuberculous lymphadenitis and are accessible for fine needle aspiration without radiological guidance. The aspirates obtained from the affected lymph nodes can be used for cytological assessment, Ziehl Neelsen stain, and culture. However, mycobacteria are slow growing and culture is not routinely done as it takes 6 to 8 weeks to grow on conventional Lowenstein-Jensen (LJ) medium. Commercially available Middlebrook liquid and solid media recover mycobacteria rapidly, yet it takes a few weeks.[5]

The main aim of this study was to assess the diagnostic role of cytology of fine needle aspirations performed on clinically suspected cases of tuberculous lymphadenitis.

We intended to determine the agreement between the cytological and microbiological criteria in the diagnosis of tuberculous lymphadenitis and assess the positive and negative predictive values of these criteria.

MATERIALS AND METHODS

This study was carried out over a period of 1 year. All patients who were clinically suspected of having tuberculous lymphadenitis, referred by clinicians of the local hospital and the Chest Clinic, during this period were included in the study. The

microbiological tests and the staining of cytology smears were carried out at the Departments of Microbiology and Pathology of our institution.

Informed express consent was obtained from all patients before including them in the study. Ethical approval was obtained from the Ethical Review Committee of the institution where the study was carried out.

Fine needle aspirations were performed by the first author under sterile aseptic conditions on the suspected lymph nodes using sterile, disposable 23 gauge needles and 10-cc syringes. The skin over the lymph node was cleaned with 70% ethyl alcohol. Aspiration technique was used first to obtain material for culture. The aspirates were cultured on Middlebrook 7H9 (broth) and Middlebrook 7H10 (solid) media. Needle-jab technique was used to obtain material for cytology and Ziehl Neelsen staining. For each jab, a new sterile disposable needle was used. A minimum of two smears for cytology and one smear for Ziehl Neelsen stain were prepared. Smears for cytology were air dried and stained with Leishman stain. Inoculating the culture media and Ziehl Neelsen staining of direct smears and the staining of cytology smears were done by two experienced technical officers working in the Departments of Microbiology and Pathology respectively. The first author interpreted the cytological features, while the second author interpreted the microbiological tests. Both were blind to each other's findings until a common interpretation was done to issue a report for each patient. Polymerase chain reaction (PCR) for *Mycobacterium tuberculosis* was done on the positive cultures to confirm the etiology.

Kappa coefficient was used to analyze the agreement between the diagnostic criteria. Positive and negative predictive values were calculated taking the prevalence of tuberculous lymphadenitis into consideration. For both analyses, culture was considered the gold standard.

RESULTS

During a period of 1 year, 41 patients with an age range of 3 to 70 years were enrolled for the study. Eighteen (42.8%) of them were less than 30 years of age. Two patients had bilateral cervical lymphadenopathy, and the aspirations were performed on both sides. They were considered four sets of aspirates for the analysis of agreement and the calculation of predictive values.

Out of the 43 aspirates, 13 yielded mycobacterial growth in either Middlebrook 7H9 (broth) or Middlebrook 7H10 (solid) media. The time taken to give a positive culture was 2 to 3 weeks (mean duration 3 weeks). All of them were confirmed as *Mycobacterium tuberculosis* by PCR. The 13 aspirates with confirmed tuberculous etiology were from 11 patients; hence the prevalence of tuberculous lymphadenitis in the study sample of patients suspected of having tuberculous lymphadenitis was 25.58%. There were 2 patients with cytological features of Hodgkin lymphoma, which was confirmed by histopathology. Five patients revealed metastatic deposits of malignancy. A reactive population of lymphoid cells was seen in15 patients. Abscess and infected cysts were the other diagnoses made on the cytological features.

Out of the 43 aspirates acid-fast bacilli were identified in 7 aspirates, all of which were culture positive, giving 100% positive predictive value. Caseation was identified in 7 aspirates only. Epithelioid cells were present in 13 aspirates.

Kappa value was calculated to analyze the agreement between microbiological and cytological diagnostic criteria against culture. Positive Ziehl Neelsen stain was also compared as a stand-alone criterion and also in combination with the cytological criteria [Table 1].

DISCUSSION

Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis,* which mainly affects the respiratory tract. But extrapulmonary TB is also common. Pulmonary TB is diagnosed by demonstration of acid-fast bacilli in the sputum. Detection of acid-fast bacilli in sputum is a simple diagnostic test. However, a trained technician should carefully examine the smears to detect the few bacilli in the smears. Though culture and isolation of the organism is considered the gold standard in the diagnosis of most infections, culturing *Mycobacterium tuberculosis* as a diagnostic test is often not feasible in the clinical setup as the organism takes about 6 to 8 weeks to grow in conventional Lowenstein-Jensen medium. Although Middlebrook medium recovers mycobacteria rapidly, it takes a few weeks. The mean duration taken to give a positive culture in the present study was 3 weeks. Even this is too long as it is necessary to commence treatment as soon as possible. Therefore, quicker methods need to be established to diagnose tuberculous lymphadenitis.

In Sri Lanka, histopathologists confidently diagnose tuberculous lymphadenitis when there are epithelioid cells/granulomata

Table 1: Positive and negative predictive values and strength of agreement between the gold standard and the cytological and microbiological diagnostic criteria

Diagnostic criteria	*Kappa* Value	*Agreement*	*PPV %*	*NPV %*
EC with caseation	0.41	Moderate	83.33	78.38
EC with positive ZN	0.52	Moderate	100	81.08
EC with either caseation or positive ZN	0.84	Very good	85.71	96.55
Positive ZN	0.62	good	100	83.33
EC alone	0.76	good	84.62	93.35

EC - epithelioid cells; ZN - Ziehl Neelsen stain; PPV - positive predictive value; NPV - negative predictive value

with caseous material in the aspirate, as the commonest cause of caseation in Sri Lanka is tuberculosis. Other causes of caseation, e.g., histoplasmosis, are extremely rare. However, caseous material may be absent in the aspirate due to sampling error. If the caseation is minimal in the affected lymph node, it could be absent in the smears. Detection of caseous material in the cytology smears could vary among pathologists, depending on the experience they have in interpreting such smears. In the present study, a moderate agreement was observed between culture and the presence of epithelioid cells together with caseation. Its negative predictive value was the lowest among the five diagnostic criteria compared in the study, probably due to such sampling errors.

Although 13 aspirates yielded positive culture, only 7 aspirates were positive for acid-fast bacilli in the Ziehl Neelsen stain. This is probably due to the low concentration of mycobacteria in the aspirate. The concentration of organisms in the sputum in pulmonary tuberculosis has a direct relationship to the sensitivity of the Ziehl Neelsen stain, and a concentration of $\geq 10^{[4]}$ organisms/mL would guarantee a positive smear.[5] This is applicable to the aspirates from tuberculous lymphadenitis as well. The overall acid-fast bacilli positivity in fine needle aspiration smears can vary from 37.4% to 59.4%.[6-8] In the present study, acid-fast bacilli were identified in 16.27% of the total sample and in 53.84% of all culture-positive aspirates. In HIV patients with tuberculosis, the detection of acid-fast bacilli by Ziehl Neelsen stain would be higher. Although testing for TB is done in all HIV-positive patients, testing for HIV in patients suspected of having or diagnosed of having TB is not done routinely in Sri Lanka. There is a considerable debate on this issue right now; and hopefully in the future, testing for HIV in TB patients would be incorporated into the national policy. We had not included HIV tests in our study. However, none of the 11 patients diagnosed with TB were clinically suspected of having HIV at the time of recruitment to the study or subsequently during treatment.

Positive Ziehl Neelsen stain as the sole diagnostic criteria had a good agreement (kappa value, 0.62) with the gold standard and had a 100% positive predictive value and 83.33% negative predictive value.

There was a moderate agreement between culture and the presence of epithelioid cells together with positive Ziehl Neelsen stain. The positive predictive value was again 100%, with a lower negative predictive value. The presence of epithelioid cells alone had a good agreement (kappa value, 0.76) with the gold standard, demonstrating a higher negative predictive value compared to its positive predictive value. This is due to the fact that presence of epithelioid cells signifies only the presence of a granulomatous inflammation where tuberculosis is one possibility. A positive Ziehl Neelsen stain confirms it to be a mycobacterial infection. The presence of epithelioid cells either with caseation or positive acid-fast stain had a very good agreement (kappa value, 0.84) in the present study. It had high positive and negative predictive values. Therefore, it is the best diagnostic criterion to be used in the diagnosis of tuberculous lymphadenitis on fine needle aspirates.

The major limitation identified in the FNAC of suspected lymphadenitis was the limited volume of material that can be aspirated. This can limit the sensitivity of detection of caseation and acid-fast bacilli. However, when the lymphadenitis was associated with suppuration, the volume of material that could be aspirated was more.

CONCLUSION

Fine needle aspiration is a simple, less expensive procedure to obtain material for examination. Cytological examination together with microbiological examination of the fine needle aspirates is a reliable investigation to diagnose tuberculous lymphadenitis. The presence of epithelioid cells either with caseation or positive Ziehl Neelsen stain for acid-fast bacilli appears to be the best criterion to diagnose tuberculous lymphadenitis by fine needle aspiration. If sufficient material can be obtained, the aspirate can be cultured for use later for the selection of antituberculosis drugs whenever drug resistance is suspected.

ACKNOWLEDGMENTS

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Source of Support: Nil, **Conflict of Interest:** None declared.

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