SMGroup

SM Journal of Clinical Pathology

Research Article

Effect of Three Simple Quality Assurance Measures on the Detection of Acid-Fast Bacilli in Ziehl Neelsen Stained Smears of Aspirates from Lymph Nodes

Aditi Arora[1]*, Navjeevan Singh[2], Vinod Kumar Arora[2], Arati Bhatia[2] and Nuzhat Husain[1]

[1]*Department of Pathology, Dr. Ram Manohar Lohia Institute of Medical Sciences, India*
[2]*Department of Pathology, University College of Medical Sciences and Guru TegBahadur Hospital, India*

Article Information

Received date: Jan 20, 2017
Accepted date: Aug 02, 2017
Published date: Aug 07, 2017

***Corresponding author**

Aditi Arora, Department of Pathology, Dr. Ram Manohar Lohia Institute of Medical Sciences, India,
Tel: +91-9559158220;
Email: draditiarora87@gmail.com

Keywords Tuberculosis; Lymph node; Fine needle aspiration

Abstract

Introduction: Ziehl Neelsen (ZN) staining has been the mainstay for detection of acid fast bacilli. Multiple sputum smear screening is advised to increase the detection of pulmonary tuberculosis. A similar approach may increase detection in lymph node aspirates. This prospective, observational study was aimed to study the effect of staged approach on the detection of acid fast bacilli in ZN stained smears from fine needle aspirates of patients with suspected tuberculosis lymphadenitis.

Materials and Methods: 300 patients, in whom lymph node aspirates were performed with clinical suspicion of tuberculosis, or in whose aspirates tuberculosis was suspected on the basis of the following three reaction patterns-epithelioid cell granuloma, epithelioid cell granuloma with necrosis or necrosis only, were studied. Smears reported negative for Acid-Fast Bacilli (AFB) at routine reporting (Stage I) were rescreened by a first observer (Stage II). Negative smears were rescreened by a second independent observer (Stage III). In cases where no AFB was demonstrated, additional material, that is another smear if available (Stage IV), and/or re-aspirate from the same lesion (Stage V) were examined. Statistical analysis was done by applying Binomial Exact test at each stage.

Results: AFB positivity at stage I – 185/300; AFB positivity at stage II - 224/300 (p value = 0.001); AFB positivity at Stage III - 240/300 (p value = 0.119); AFB positivity at Stage IV - 263/300 (p value = 0.010) and AFB positivity at stage V - 273/300 (p value = 0.001)

Conclusions: A cascading staged approach improves AFB detection in aspirates from lymph nodes of patients with suspected tuberculous lymphadenitis. In AFB negative patients, yield of AFB can be increased by examining a second smear obtained from the same aspirate. Further increase in the AFB positivity can be obtained if repeat aspirates are examined for the presence of AFB. Combination of the above two has an additive incremental effect.

Introduction

Tuberculosis (TB) has been a major cause of morbidity and mortality over decades [1]. It is one of the oldest human diseases, with its history as old as mankind [2]. Although primarily thought to be a pulmonary disease, Extra Pulmonary Tuberculosis (EPTB) has become even more common since the advent of HIV [3,4]. Tuberculous lymphadenitis is the most common form of EPTB found in clinical practice [3].

For extra pulmonary TB, tissue diagnosis including bacteriologic confirmation is required. This involves the use of invasive procedures like excisional biopsy that are likely to be associated with complications [5]. Of late FNAC has become an important diagnostic tool because it is minimally invasive and frequently diagnostic [6]. The accuracy of FNAC in detection of tuberculosis has been reported to vary from 76.1% to 95.45% [7-11].

Granulomatous inflammation is a manifestation of many chronic inflammatory diseases like tuberculosis, leprosy [13,14], sarcoidosis [15], syphilis, and various mycoses. Yet in regions of high endemicity such as India, largely, granulomatous inflammations are considered to be of tuberculous origin unless proved otherwise. In this setting demonstration of AFB, although not diagnostic of infection by M. tuberculosis, improves the diagnostic accuracy.

ZN staining on FNAC smears is simple, cost effective and is one of the best techniques which can be used in developing countries [16]. Current WHO guidelines specify that the essential step in the diagnosis of pulmonary tuberculosis is examination of serial sputum specimens for AFB. Significant increment in the yield of acid fast bacilli by examination of a second sputum smear has been demonstrated. This study evaluates a similar approach to the detection rate of AFB in lymph node aspirates.

OPEN ACCESS
ISSN: 2576-778X

How to cite this article Arora A, Singh N, Arora VK, Bhatia A and Husain N. Effect of Three Simple Quality Assurance Measures on the Detection of Acid-Fast Bacilli in Ziehl Neelsen Stained Smears of Aspirates from Lymph Nodes. SM J Clin Pathol. 2017; 2(1): 1009.

Materials and Methods

This study was conducted in tertiary care hospital of North India. Ethical clearance for the study was granted by the Institutional review board. Patients having clinically suspected tuberculous lymphadenitis and smears with tissue reaction patterns consistent with tuberculous lymphadenitis (granulomatous inflammation, granulomatous inflammation with necrosis or necrosis only) were included. All those cases where an alternative diagnosis could be established and patients with history of treatment for tuberculosis were excluded. When all smears in a batch and/or day were negative, stain failure was suspected and these were also excluded.

FNA was carried out using 10 ml disposable plastic syringes and 22 gauge needles. A standard procedure was used: the overlying area was cleaned with 70% alcohol; the enlarged node was fixed and maintained in stable position by one hand. The node was entered and negative pressure was applied to the syringe. Multiple (average six) in and out passes were made by the needle without exiting the node. After removing the needle, a drop of the aspirate was placed on a clean slide. The drop was spread to make a smear by laying another slide on top of it. Smears were stained by MGG and ZN stains.

ZN stained smears were screened for AFB using a 40X objective coupled with 10X eyepieces. Slides reported AFB negative were re-screened by a dedicated second observer (AA). Cases which were still negative were screened by an experienced third observer (NS).

One hundred consecutive overlapping fields were covered before a smear was reported negative for AFB. In smears with necrosis, the entire necrotic area was covered. If a second, unstained or MGG stained slide was available in cases reported AFB negative, it was stained with ZN stain and screened. Repeat aspirates to screen for additional material were obtained in cases which were still AFB negative. Thus, a staged procedure was used.

Stage I - routine AFB screening

Stage II - screening by a dedicated first observer

Stage III - negatives of stage II re-screened by second observer.

Stage IV - negatives of stage III – additional material examined from the same aspirate.

Stage V - negatives of stage IV- screened for AFB in material obtained by repeat aspiration.

The data was analysed by applying Binomial Exact test at each stage.

Results

The study population consisted of three hundred patients of suspected tuberculous lymphadenitis. The ages ranged from 10 months to 85 years, with median age of 24 years. Most patients (64%) were young, between the age group of 10 years to 30 years. Male to female ratio was 0.7:1. The most common lymph node region involved was cervical (69.5%), followed by submandibular (9.9%), supraclavicular (8%), axillary (7.3%), and others (5.1%) including inguinal, submental, pre-auricular, infra-auricular, sublingual lymph nodes.

On microscopy, 62 cases had epithelioid cell granulomas, 131 cases showed necrosis, and 107 showed both granulomas and necrosis.

Table 1: AFB positivity at each stage along with the corresponding p-value.

Stage	Positivity	P value
Stage I	185/300 (61.6%)	-
Stage II	224/300 (74.6%)	0.001
Stage III	240/300 (80%)	0.119
Stage IV	263/300 (87.6%)	0.010
Stage V	273/300 (91%)	0.001

AFB positivity was calculated at each stage. Cumulative positivity was 91%. The data was analysed by using Binomial Exact test at each stage. Assuming the probability of detecting Acid Fast Bacilli in a smear to be 50%, the p-values were calculated at each stage (Table 1).

Discussion

Tuberculosis has been a major cause of morbidity and mortality since time immemorial. The diagnosis of tuberculous lymphadenitis has been a challenge in developing countries. Prompt and accurate diagnosis, together with effective treatment is essential elements of TB care and control. In low income countries like India, low cost, ease of performance and rapid diagnosis would be added advantages. A confirmed diagnosis of TB can only be established by isolating the M. tuberculosis complex or finding specific sequences of DNA in specimens. Demonstration of AFB, although not as specific as these tests, does improve the confidence in the diagnosis, particularly in areas with high burden of disease.

In this study, when the cases were seen during routine reporting, positivity rate of 61.6% was obtained. This was significantly lower than positivity at stage II. This may be attributable to the motivation and dedication of the observer at stage two, she being primarily responsible for carrying out the study. The causes of incremental yield at stage III were probably that the second observer was more skilled and experienced as compared to the first, or screened the slides at a time of relaxed settings. Another reason may be that the total number of slides examined at this stage was less than that by the first observer, as it was at every successive stage.

Current WHO guidelines specify that the essential step in investigation of patients of suspected pulmonary tuberculosis should be microscopic examination of serial sputum specimens for acid fast bacilli. Using a similar approach in our study we examined additional material (second smear) obtained from the same aspirate. At this stage additional twenty three cases were found positive for AFB, leading to a cumulative positivity of 87.6% and an increment of 7.6%. Thus, using the same approach as recommended for pulmonary TB in lymph node aspirates, the AFB detection rate and hence the diagnostic accuracy increased. The additional yield at stage V may be because by doing a repeat aspirate, more material is being examined; also, by the time the patient returned for a repeat aspirate, the reaction pattern probably became more specific and hence the chances of finding AFB also increased.

Over the years scientists have attempted various modifications of ZN stain, devised newer staining techniques, and nucleic acid amplification tests. However, ZN stain has stood the test of time and should remain the mainstay of the detection of AFB.

Conclusion

In AFB negative cases, yield of acid fast bacilli can be increased by screening ZN stained second smears obtained from the same aspirate. Further increase in the AFB positivity can be obtained if repeat aspirates are examined for the presence of AFB and Combination of the above two has an additive incremental effect. A staged approach for demonstration of AFB thus improves the outcome in the diagnosis of EPTB by FNA.

Acknowledgment

The authors would like to thank Saanvi Bansal for her help with literature search.

References

1. Handa U, Mundi I, Mohan S. Nodal Tuberculosis revisited: a review. J Infect Dev Ctries. 2012; 6: 6-12.
2. Jordao L, Vieira OV. Tuberculosis: New Aspects of an Old Disease. Int J Cell Biol. 2011.
3. Malik GB, Sen R. Pattern of extrapulmonary tuberculosis-an experience of twenty five years. Indian J Tuberc. 1985; 32: 148-153.
4. Reid MJA, Shah NS. Approaches to tuberculosis screening and diagnosis in people with HIV in resource limited settings. Lancet Infect Dis. 2009; 9: 173-184.
5. Lau SKP, Wei WI, Hsu C, Engzell UC. Efficacy of fine needle aspiration cytology in the diagnosis of tuberculous cervical lymphadenopathy. J Laryngol Otol. 1990; 104: 24-27.
6. Narain JP, Raviglione MC, Kochi A. HIV associated tuberculosis in developing countries: epidemiology and strategies for prevention. Tuber Lung Dis. 1992; 73: 311-321.
7. Bloch M. Comparative study of lymph node cytology in puncture and histopathology. Acta Cytol. 1967; 2: 139-142.
8. Nagpal BL, Dhar CN, Singh A, Bahi HH. Evaluation of imprint cytodiagnosis in case of lymphadenopathy. Indian J Pathol Microbiol. 1982; 25: 35-39.
9. Patra AK, Nanda BK, Mohapatra BK, Panda AK. Diagnosis of lymphadenopathy by fine needle aspiration cytology. Indian J Pathol Microbiol. 1983; 26: 273-276.
10. Dandapat MC, Panda BK, Patra AK, Acharya N. Diagnosis of tubercular lymphadenitis by fine needle aspiration cytology. Indian J Tuberc 1987; 34: 139-142.
11. Narang RK, Pradhan S, Singh RP, Chaturvedi S. Place of fine needle aspiration cytology in the diagnosis of lymphadenopathy. Indian J Tuberc 1990; 37:29-31.
12. Majeed MM, Bukhari MH. Evaluation for granulomatous inflammation on fine needle aspiration cytology using special stains. Pathol Res Int [Internet]. 2011; 8.
13. Kumar B, Pradhan A. Fine needle aspiration cytology in diagnosis of pure neuritic leprosy. Pathol Res Int. 2011.
14. Siddaraju N, Yaranal PJ. Use of fine needle aspiration cytology in leprotic lesions. A report of 4 cases. Acta Cytol. 2007; 51: 235-238.
15. Ravens AF, Sriram PVJ, Topalidis T, Hauber HP, Meyer A, Soehendra N, et al. Diagnosing sarcoidosis using endosonography- guided fine needle aspiration. Chest 2000; 118: 928-935.
16. Luna JAC. A Tuberculosis Guide for Specialist Physicians. Paris; 2003; 81.