Brief Communication

Step AFB Technique: A Simple Method for Increasing AFB Yield in Tissues

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Abstract

The yield of acid-fast bacillus (AFB) in tissues is usually low in conventional formalin fixed tissues. We carried out a simple technique to increase the chances of AFB detection in tissue sections. AFB staining was done on the lung tissue of 100 consecutive autopsy lung specimens of pulmonary tuberculosis over a period of three years. In addition, we carried out the step AFB technique in which the AFB procedure was carried out after 10 serial re-cuts in all the cases. AFB appeared as pink/red in a blue background. Grading was done as negative, 1+, 2+ and 3+. AFB positivity was observed in 55% cases with the first tissue cut. However, step AFB technique increased the tissue AFB positivity yield to 66% (p=0.002). The remaining 34% cases were negative for both AFB and step AFB. Grade I positivity was observed in 91% cases, Grade 2 in 6% and Grade 3 in 3%. The results show a better edge for picking up AFB with step AFB technique as compared with the conventional method that could be attributed to the greater concentration of TB bacilli in deeper part of the tissue, hence decreasing the chance of obtaining false negative results. We conclude that Step AFB technique can be used as an adjunct to routine AFB technique to increase the chances of obtaining a positive result. **[Indian J Chest Dis Allied Sci 2016;58:273-274]**

Key words: Tissue, AFB, Step AFB.

Introduction

The diagnosis of tuberculosis (TB) in tissue depends on the characteristic histological findings supported with demonstration of the acid-fast bacillus (AFB) in tissue. The latter is usually a challenge due to low yield of the bacillus in tissue sections.[1,2] In this study, we tried a simple technique to increase the chances of AFB detection in tissue sections.

Methods

We studied 100 consecutive autopsy lung specimens of pulmonary TB over a period of three years. Microscopic findings of caseating and non-caseating granulomas, caseous necrosis only and suppurative inflammation were noted. Tissue AFB was carried out in all cases. For AFB staining, the smear was fixed, stained with carbol fuchsin and decolourised with acid-alcohol. The smear was then counter-stained with methylene blue. In addition, we carried out the step AFB technique in which the AFB procedure was carried out after 10 serial re-cuts in all the cases. Acid-fast bacillus appeared as pink/red in a blue background. Grading was done as negative, 1+, 2+ and 3+.

Results

Acid-fast bacillus positivity was observed in 55% cases with the first tissue cut. However, step AFB technique increased the tissue AFB positivity yield to 66% (p=0.002) (Table). The remaining 34% cases were negative for both AFB and step AFB. Grade 1 positivity was observed in 91% cases, 6% showed Grade 2 and 3% showed Grade 3 positivity.

Table. Increase in AFB positivity to 66% by using step AFB technique

	1st tissue cut	10th re-cut
AFB positivity by ZN staining	55%	66% (p=0.002)

Definition of abbreviations: AFB=Acid-fast bacillus; ZN=Ziehl-Neelsen

Discussion

The diagnosis of TB in tissue is a challenge for pathologists due to the variable presentation of TB combined with the low yield of the bacillus on conventional AFB stains. The method used for staining for AFB in tissue sections is identical to the procedure used in bacteriology. The cell wall of the AFB contains waxy lipid mycolic acid with a chain of numerous carbon atoms, the length of which predict the acid fastness. Mycolic acid which is the molecular target of AFB staining is soluble in organic solvents which are used to make paraffin embedded tissues.[1] Since mycolates are extracted into the liquid by the above method, the stainability of AFB in conventional formalin fixed tissues is less.[1] The positivity of AFB

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on Ziehl-Neelsen (ZN) stained smears ranges from 0% to 44% in various studies.[2] The practice patterns for identifying AFB on smears is not standardised. Wu *et al*[3] carried out a study on the practice patterns and variations of diagnosis of AFB on sputum smears and found that some pathologists would observe the smear at 40× objective while some would observe under oil immersion lens (100×). We believe that AFB are best identified under oil immersion lens, although time consuming, considering the low yield by conventional ZN staining.

The number of AFB seen on tissues is directly proportional to the amount of caseous necrosis.[4] In our study, maximum cases (98%) had caseous necrosis which explains the high percentage of AFB positivity. Also, in our study, we observed the increase in AFB positivity by 10% (total 66% positive) after performing the step AFB technique. The results show a better edge for picking up AFB with step AFB technique as compared with the conventional method. This could be attributed to the greater concentration of TB bacilli in deeper part of the tissue; hence, decreasing the chance of obtaining false negative results.

Various other methods have been used for identification of AFB in tissues like culture, immunohistochemistry and polymerase chain reaction.[2,5] Since conventional AFB is cost effective and a simple laboratory method, we conclude that step AFB technique can be used as an adjunct to routine AFB technique to increase the chances of obtaining a positive result.

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