Abstract

Demonstration of acid fast bacilli (AFB) is essential for the definitive cytodiagnosis of tuberculosis. However, it is not seen in all cases of tuberculosis, being more commonly observed in necrotic lesions. Tuberculosis shows a spectrum of lesions that form a continuum extending from good immunity end with purely granulomatous lesions to poor immunity end with purely necrotic lesions. Each stage has distinctive microscopic picture and determines the chance of finding AFB. AFB yield was the highest (92.7%) in necrotic lesions containing eosinophilic structures (ES).

Keywords: Acid fast bacilli, eosinophilic structure, FNAC, tuberculosis

INTRODUCTION

Although tuberculosis (TB) is one of the most common diagnosis we make in our daily fine needle aspiration (FNA) reporting, it is not fully understood. The morphologic description of a tuberculous lesion has remained unchanged over many decades. As a result it is very well understood by everyone, and to discuss something different in this context is a difficult proposition. Nonetheless, given the fact that the diagnosis of TB is overlooked/missed on occasions indicates that our understanding of the disease is not yet complete. In particular, the intermediate stages in disease progression are not clearly elucidated, and a comprehensive understanding of AFB detection in TB needs elaboration.

Cytodiagnosis of TB involves demonstration of epithelioid cell granuloma, necrosis, and acid fast bacilli (AFB). While identification of granuloma and necrosis helps in its diagnosis, presence of AFB is confirmatory. Thus, sincere effort should be made to identify AFB.

AFB identification can be done by various methods such as Ziehl–Neelsen (ZN) stain,[1] fluorescent stain,[2] mycobacterial culture,[3] and nucleic acid amplification test.[4] However, ZN stain is still most widely used for AFB detection.

It is commonly known that AFB is more often encountered in necrotic lesions.[5] However, it is also known that all necrotic tuberculous lesions do not yield AFB on microscopy. Thus, if some morphological change could be identified in tuberculous smears, whose presence correlated with presence of AFB, it would save on time and manpower, besides optimizing the use of resources.

In the quest to find an answer to this problem, I came across a paper by Pandit et al.[6] where the authors described eosinophilic structures (ES) in tuberculous lesions. They reported that eosinophilic structures are degenerated acellular granulomas which are always associated with caseous necrosis. ES reveal presence of mycobacterial antigen by immunohistochemical staining and they are not seen in nontuberculous necrotic lesions.

MATERIALS AND METHODS

We performed a prospective study to assess the correlation between ES and AFB.[7] Inclusion criteria were (a) cases where lymph node aspiration yielded necrotic material, (b) treatment naïve patients, and (c) clinically and therapeutically proven cases. Exclusion criteria were (a) immunocompromised or immunosuppressed patients and (b) cases of atypical mycobacteriosis. Because ES has not been observed in any other disease, a negative control could not be set up. Three smears were made in each case and stained...
with hematoxylin and eosin (H and E), May Grünwald Giesma (MGG), and ZN stain. Slides were screened for epithelioid cell granulomas, eosinophilic structures, necrosis, and AFB in each case. Statistical analysis was done using Chi-square test with Yates correction to document the association between presence of ES and incidence of AFB detection.

**Results**

Results are shown in Table 1. AFB was most often seen in cases having cytologic picture of ES + AFB+. Significant association between the presence of ES and detection of AFB was seen, $\chi^2 = 6.422$ with 1 degrees of freedom ($P$ value = 0.0113). Graphic representation of the same data is shown in Figure 1. The near total overlap between curves for ES and AFB show that they are both present in the same phase of the disease and that there is an intimate relationship between the two.

**Discussion**

About 2–4 weeks after infection, two host responses to *M. tuberculosis* develop – (a) macrophage-activating CMI response and (b) tissue damaging response which is a delayed-type hypersensitivity reaction. Both these responses can inhibit mycobacterial growth. It is the balance between these two responses that determines the form of TB that will develop subsequently in a given individual.[8]

The macrophage activating response is a T-cell-mediated phenomenon resulting in the activation of macrophages that are capable of killing and digesting tubercle bacilli. With the development of specific immunity, large numbers of activated macrophages aggregate around the lesion’s centre and granulomatous lesions (tubercles) are formed. Thereby tubercle bacilli are effectively neutralized without causing further tissue destruction.[8]

The tissue damaging response is the result of a delayed-type hypersensitivity reaction to various bacillary antigens. Here the unactivated macrophages containing multiplying bacilli are destroyed. This response not only destroys macrophages but also destroys the involved tissue and produces early solid necrosis in the centre of the tubercle.[8]

As the disease progresses, the lesion tends to enlarge and the surrounding tissue is progressively damaged. At the centre of the lesion, caseous material liquefies. This liquefied caseous material contains large number of bacilli.[8]

It would not be unreasonable to argue that, as necrosis progresses, not only does caseous material liquefy but granulomas also degenerate to form eosinophilic structures. Thus, it can be deduced that eosinophilic structures would be present in that phase of disease when the necrotic lesions are rich in AFB. Our above study[7] substantiates this hypothesis.

Similar to leprosy, there is an immunological gradient in tuberculosis [Figure 2]. At the good immunity end are purely granulomatous lesions which do not contain any AFB. At the poor immunity end are purely necrotic lesions not containing any AFB. In between are several intermediate stages having varying cytologic pictures [Table 2]. When the immunity is good only granulomas are seen in FNA smears. As these granulomas are able to contain the tubercle bacilli, no ES, no necrosis, and no AFB are seen. Thus, the cytologic picture is G+ES−N−AFB−. As disease progresses, necrosis begins, but the granulomas are still capable of neutralizing the bacilli. No ES and no AFB are seen in this stage. Thus, the cytologic picture in this stage is G+ES−N+AFB−. With further disease progression, the granulomas begin to degenerate to form ES. Yet they are able capable of containing the infection and so AFB is not yet detected. The cytologic picture at this juncture is G+ES+N+AFB−. Further disease progression results in profound degeneration of granulomas which are now no longer

| Cytologic picture           | n  | Epithelioid cell granuloma | AFB |
|-----------------------------|----|---------------------------|-----|
| ES-AFB-                     | 13 | 13 (100.0%)               | 0   |
| ES+AFB-                     | 22 | 22 (100.0%)               | 0   |
| ES+AFB+                     | 63 | 63 (100.0%)               | 63  |
| ES-AFB+                     | 10 | 03 (30.0%)                | 10  |

Total | 108 | 66 | 73 |

Figure 1: Graphical representation of correlation between presence of ES and detection of AFB

Figure 2: Illustration of the complete spectrum of Tuberculosis
capable of containing the tubercle bacilli. Thus, the cytologic picture seen at this stage is G+ES+N+AFB+. With further downhill disease progression, degeneration of granulomas is complete and AFB proliferation is unchecked. The cytologic picture now is G−ES−N+AFB+. With further progression, the eosinophilic structures completely degenerate to give the cytologic picture of G+ES−N−AFB+. Paradoxically, the frequency of AFB detection in this stage is less compared to the previous stage. What happens is that, as necrosis progresses, the pH and pO\textsuperscript{2} of necrotic material slowly decreases. The resulting necrotic environment gradually becomes unfavorable for the growth and multiplication of tubercle bacilli. And finally when necrosis reaches its zenith, the anerobic acidic necrotic environment is entirely unfavorable for the growth of tubercle bacilli and the picture now seen is G−ES−N+AFB−. This explains why we come across necrotic lesions which do not show AFB not only on microscopy but even on culture and even on nucleic acid amplification test.

To assess our above observation, we quantified AFB detection in 236 consecutive cases of tuberculous lymphadenitis with the same inclusion and exclusion criteria that was used in the aforementioned study using the grading system recommended by IUATLD for sputum samples.\[8\] It was observed that AFB detection was much higher in presence of ES (127/137 = 92.7\%) in comparison to lesions not containing ES (35/99 = 35.3\%). It was also found that AFB was most often seen in patients with microscopic picture of G+ES+N+AFB+ (73/236 = 30.9\%) followed by G−ES−N+AFB+ (54/236 = 22.9\%) and G−ES−N+AFB+ (35/236 = 14.8\%). Also, high bacillary load, calculated using the sum of cases showing Grade1+, Grade 2+ and Grade3+, was the highest with G−ES−N+AFB+ (18/54 = 33.3\%), followed by G−ES+N+AFB+ (7/35 = 20\%) and then G+ES+N+AFB+ (13/73 = 17.8\%) [Table 3].

It is essential to understand that tuberculosis does not result in a single microscopic picture. It has a spectrum of lesions which form a continuum extending from one end with purely granulomatous lesion through degenerating granuloma, acellular eosinophilic structures, necrotic lesion with AFB to finally necrotic lesion without AFB at the other end. In a given patient, the infection will eventually move either in the direction of full containment or of disease and would determine the microscopic picture seen at a given point of time.\[9\] Thus, AFB detection would be determined by the stage of disease in which the patient presents for FNA.

**Conclusion**

It is necessary to understand that AFB is not detected in all phases of tuberculosis. The chance of finding AFB is high in (a) necrotic lesions and (b) in lesions containing eosinophilic structures. To sum it up in one sentence, the chance of finding AFB is the highest in necrotic lesions containing eosinophilic structures.

**Financial support and sponsorship**

Nil.

**Table 2: Salient features of each immunologically distinct stage of the proposed tubercular spectrum**

| G | ES | N | AFB | Interpretation |
|---|---|---|---|---|
| + | − | − | − | Granuloma only, AFB neutralized |
| + | + | + | − | ES formation begins, but AFB contained |
| + | + | + | + | Granuloma degeneration pronounced, unable to contain AFB |
| − | + | + | + | Complete granuloma degeneration, AFB rampant |
| − | + | + | − | Fully necrosed |
| − | − | − | − | Environment unfavorable for AFB |

**Table 3: Distribution of burden of acid fast bacilli in various immunologically distinct stages of the proposed tubercular spectrum (n=236)**

| Grade | Grade 0 | Grade 1+ | Grade 2+ | Grade 3+ |
|---|---|---|---|---|
| G+ES−N−AFB− | 5 | - | - | - |
| G+ES−N+AFB− | 59 | - | - | - |
| G+ES+N+AFB− | 10 | - | - | - |
| G+ES+N+AFB+ | - | 60 | 10 | 3 |
| G+ES−N+AFB+ | - | 36 | 16 | 1 |
| G−ES−N+AFB+ | - | 28 | 6 | 1 |
| G−ES−N+AFB− | - | - | - | - |
| G+ES−N−AFB− | - | - | - | - |

**Conflicts of interest**

There are no conflicts of interest.

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