

Negative staining of mycobacteria – A clue to the diagnosis in cytological aspirates: Two case reports

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SUMMARY

Large amounts of lipids present in the cell wall of mycobacteria render them impermeable to dyes used in routine stains. Special staining techniques like Ziehl-Neelsen (ZN), Auramine Rhodamine are needed to demonstrate them. However, they may appear as negatively stained ghosts in Leishman, Giemsa, Hematoxylin and Eosin stain (H and E) and Gram stained smears. Awareness of this feature prompts one to look for mycobacteria by special techniques, even in the absence of cytological features of tuberculous infection like epithelioid granulomas and caseous necrosis. In this paper, we aim to present two cases showing negatively stained images in the lymphnode aspirates of Human Immunodeficiency Virus (HIV) positive patients. A study has been done of two cases of HIV positive patients with cervical lymphadenopathy. Fine needle aspiration cytology (FNAC) was carried out for them from the enlarged lymph nodes which revealed purulent material. Smears of FNAC material were prepared for histopathological examination. Fixed smears were stained with Papanicolaou stain and air dried smears were stained with Giemsa and ZN-stain and studied. Smear study showed scant cellularity, cells composed of neutrophils, lymphocytes, plasma cells and macrophages. Background was necrotic. Giemsa stained smears showed, in addition to cells mentioned, negatively stained ghostly rod shaped structures in the cytoplasm of macrophages and also in the background. ZN-stain showed numerous acid fast bacilli. Both the aspirates were signed out as tuberculous lymphadenitis. In both the cases, cultures grew *Mycobacterium avium* Intracellulare. Classical cytological features of tuberculosis may not be present in immunocompromised patients and on the contrary there may be suppuration rich in neutrophils or sheets of histiocytes in tuberculosis. One may miss these cases if mycobacteria are not looked for specifically by special stains. Negatively stained ghost images of Tuberculous bacilli on different types of strains are a very helpful finding in such cases.

Key words: Acquired immunodeficiency syndrome, fine needle aspiration cytology, Giemsa Stain, Leishman stain, Mycobacterial infection, negatively stained images

Introduction

Utility of Fine needle aspiration cytology (FNAC) is well appreciated in investigating lymphadenopathy in patients suffering from Acquired Immunodeficiency syndrome (AIDS) and those who are Human Immunodeficiency Virus (HIV) positive.^[1,2] In an immunocompromised

patient, high index of suspicion is needed as there may not be epithelioid granulomas, giant cells or caseous necrosis. One helpful finding in some of these cases is the presence of negatively stained ghost images of tuberculous bacilli. This has been reported earlier in the literature.^[3-5] We report two cases with negative staining and tried to document the ghost images as the actual mycobacteria.

Case Reports

Case 1

35 year old HIV positive male patient with a CD4 count of 50, presented with bilateral cervical lymphadenopathy and fever of one month duration. There were multiple matted cervical lymphnodes on both sides. FNAC yielded purulent material.

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Case 2

20 year old HIV positive male patient with 2 month history of fever presented with right sided tender cervical lymphadenopathy. His CD4 count was 105. Aspiration yielded purulent material.

Fixed smears were stained with Papanicolaou stain and air dried smears were stained with Giemsa and ZN-stain. Smear study showed scant cellularity, cells composed of neutrophils, lymphocytes, plasma cells and macrophages. Background was necrotic. Giemsa stained smears showed, in addition to cells mentioned, negatively stained ghostly rod shaped structures in the cytoplasm of macrophages and also in the background [Figures 1 and 2].

ZN-stain showed numerous acid fast bacilli. Both the aspirates were signed out as tuberculous lymphadenitis.

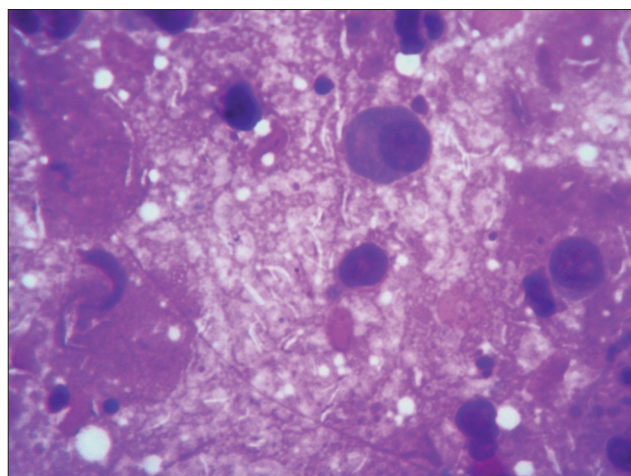


Figure 1: Histopathological examination of fine needle aspiration cytology smear of an HIV positive patient showing inflammatory cells and negative stained rods (Giemsa stain, x1000)

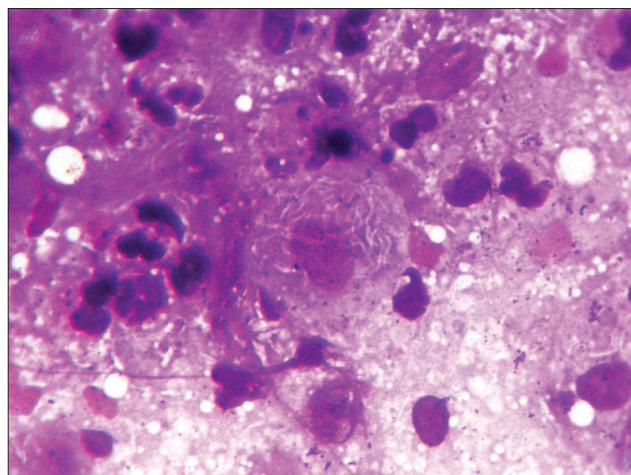


Figure 2: Histopathological examination of fine needle aspiration cytology smear of an HIV positive patient showing macrophages with many negative staining rods. Similar structures are also seen in the background (Giemsa stain, x1000)

In both the cases, cultures grew *Mycobacterium avium* Intracellular.

Discussion

Various infections like, *Mycobacterium tuberculosis*, *Mycobacterium avium* intracellular complex (MAIC), *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Toxoplasma gondii* and various neoplasms like, lymphomas, Kaposi's sarcoma can present as lymphadenopathy in HIV positive patients. Classical cytological features may not be present in immunocompromised patients and on the contrary there may be suppuration rich in neutrophils or sheets of histiocytes in tuberculosis. One may miss these cases if mycobacteria are not looked for specifically by special stains.

A different cytological pattern has been described in tuberculosis. FNAC smears stained by Romanovsky stains may show negative stained images in the cytoplasm of histiocytes and also in the background.^[4,5] Maygarten and Flanders described these structures in Diff-Quick stains. Jannotta and Sidawy, too, described these images in ten cases of intraoperative imprint samples of lymphnodes in HIV infected individuals.^[3] Six of these cases turned out to be MAIC and the rest *M. kansasii*. Garza and Barboza described them in Papanicolaou stain in duodenal brush cytology. Lepa bacilli also seem to exhibit the similar property of negative staining.^[6,7] In an immunocompromised patient, high index of suspicion is needed when typical cytological features are absent. Negatively stained ghost images of Tuberculous bacilli are a very helpful finding in such cases. This has been reported earlier in

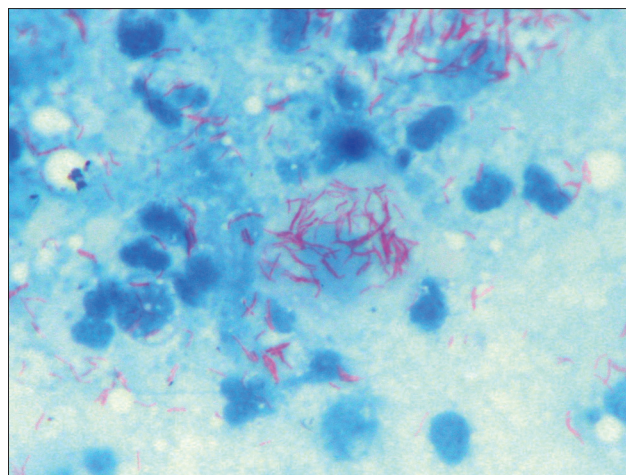


Figure 3: The area shown in the Figure 2 stained with Ziehl-Neelsen stain. Acid fast bacilli confirm to the negative stained ghost areas in the cytoplasm of macrophage and also in the background of Figure 2. (Ziehl-Neelsen stain. x1000)

the literature.^[3-5] In our cases, the figures are the well documented proof of negative stained ghost images being the actual bacilli [Figure 3]. First, we took images from the Giemsa stained slide and then the same slides were subjected to ZN stain and matched the areas. We noticed in our study that, the phenomenon of negative staining is also seen in H and E, Leishman, Gram stained smears, and the same smears can be subjected to ZN-staining without destaining to demonstrate the bacilli in negatively stained ghost areas as shown in the figures.

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