

Negative images in the fine needle aspiration cytologic diagnosis of mycobacterial infections

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Abstract

Cytologic diagnosis of mycobacterial infection has conventionally depended on the recognition of granulomatous inflammation with caseous necrosis and the identification of acid-fast bacilli with special stains. Immunocompromised patients however may not mount the expected response. Mycobacteria can be seen as negative images in fine needle aspiration cytologic smears from patients with acquired immunodeficiency syndrome. We report the cytologic findings of lymphnode aspiration from four patients where the mycobacteria were seen in the routine May Grunwald Giemsa-stained smear as unstained rod-shaped structures in the background and within histiocytes. These were confirmed to be acid-fast bacilli with the Ziehl-Neelsen stain.

Key words: Negative images, acquired immunodeficiency syndrome, mycobacterial infections, HIV infection, fine needle aspiration cytology

INTRODUCTION

A definitive and accurate diagnosis of mycobacterial infection is important because satisfactory results can be achieved with chemotherapy alone, obviating surgery. Tuberculosis is still a rampant disease world wide, more so with the advent of infection with human immunodeficiency virus (HIV) and the acquired immunodeficiency syndrome (AIDS). Disseminated tuberculosis should be considered in the differential diagnosis of lymphadenopathy in AIDS.¹ Recent reports have documented the value of cytology in the evaluation of HIV positive patients.²⁻⁴ The classical cytological feature of tuberculosis is the presence of epithelioid cell granulomas with or without caseous necrosis. Identification of acid fast bacilli is done with special stains like Ziehl-Neelsen (ZN) stain. Immunocompromised patients however may not mount the expected response and the cytopathologist must have a high index of suspicion for the presence of the organism.⁵ Negative images of mycobacteria in cytologic smears of patients with AIDS have been described.^{1,5} We report the cytologic findings of lymph nodes in four patients with HIV infection in whom mycobacteria were seen on routine May Grunwald Giemsa (MGG) stained slides as negatively-stained rod-shaped structures within histiocytes and in the background. The findings were confirmed on ZN stain that revealed numerous acid-fast bacilli.

CASE REPORTS

The clinical presentation of the four cases, that were all found to be HIV positive, are described below:

Case 1

A 54-year-old male, with a history of alcohol abuse presented with progressively enlarging swellings in the neck and low-grade fever of two months duration. On examination, he was found to be pale with multiple non-tender matted cervical lymph nodes. There was no other organomegaly.

Case 2

A 27-year-old male presented with intermittent fever and generalized weakness for 2 months. He had generalized lymphadenopathy. Lymph nodes measured 2-3 cms in size. Spleen was enlarged upto 2 cm below the costal margin.

Case 3

A 25-year-old female presented with painful swellings in the neck with fever for one month. On examination she was found to be febrile, toxic and had cervical lymphadenopathy with mild hepatosplenomegaly.

Case 4

A 24-year-old male presented with painful swellings on both sides of the neck since 2 months, for which he had received partial

treatment with antituberculous drugs. The patient was emaciated, with tender bilateral cervical lymph nodes and mild hepatomegaly.

Fine needle aspiration (FNA) cytology of the lymph nodes was performed as part of initial diagnostic work up in all the cases. Slides were stained with MGG and Papanicolaou stain. Air-dried smears were set aside for ZN stain. The smears showed scant cellularity, with mainly degenerating and viable neutrophils, lymphocytes and macrophages. The third and fourth cases showed large number of histiocytes. No granulomas, epithelioid cells, Langhan's giant cells or caseous necrosis were seen in any of the smears. Just prior to sending out reports of suppurative and non-diagnostic lymphadenopathy, a review of the MGG- stained smears showed rod-shaped unstained structures in the blue background of the stain in cases 1 and 2 (Fig 1). For cases 3 and 4, the histiocytes contained many clear linear spaces (Fig 2). The ZN stained smears of all the cases showed many acid-fast bacilli. In the first two cases the bacilli were present in the background (Fig 3). In the third case they were mainly intracytoplasmic while in the fourth they were present both within and outside the histiocytes.

A diagnosis of mycobacterial lymphadenitis was offered in all the cases, and the possibility of atypical mycobacterial infection suggested in the last two. Mycobacterial culture of lymph node aspirate was advised in order to identify the species, which unfortunately was not carried out.

DISCUSSION

Lymphadenopathy in HIV-infected individuals has a variety of causes, ranging from reactive lymphoid hyperplasia, opportunistic infections by *Mycobacterium tuberculosis*, *Mycobacterium avium complex* (MAC), *Histoplasma capsulatum*, *Cryptococcus neoformans* and malignancies like lymphoma. It is important that a diagnosis of tuberculous lymphadenitis in patients of AIDS be rapidly and reliably established.

The cytological features of tuberculous lymphadenitis can be divided into three patterns:

1. Epithelioid granulomas without necrosis
2. Epithelioid granulomas with necrosis
3. Necrotic material without granulomas, at times with marked polymorphonuclear infiltration.⁶

Demonstration of acid-fast bacilli is of paramount importance especially in the last group where granulomas are not seen. Fortunately this is the group in which the detection rate of the bacilli is high, in the range of 48.5%-77.4%.^{6,7} Immunocompromised patients do not manifest the classical response of tuberculosis and if not specifically looked for, one is likely to miss the diagnosis.^{2,4,8} Tuberculous lymphadenitis in HIV-infected individuals often shows a pattern of necrotizing lymphadenitis without granulomas or frank purulent inflammation, underscoring the importance of ZN staining and culture studies.^{2,7} Routine staining for detection of acid-fast bacilli is recommended for lymph node aspirates from all HIV positive individuals.⁴ The majority

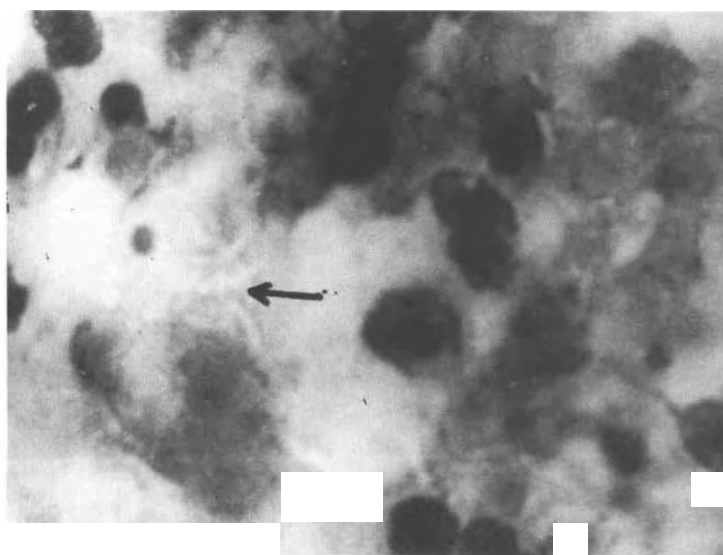


Fig 1. Negative rod shaped images of mycobacteria (arrow) seen in the background of lymph node aspiration smear, MGG X 1000.

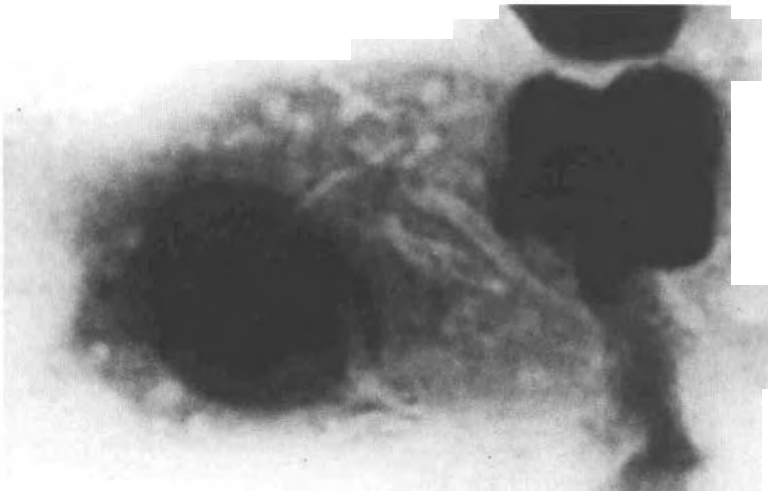


Fig 2. Negative images seen within the cytoplasm of histiocytes. MGG X 1000

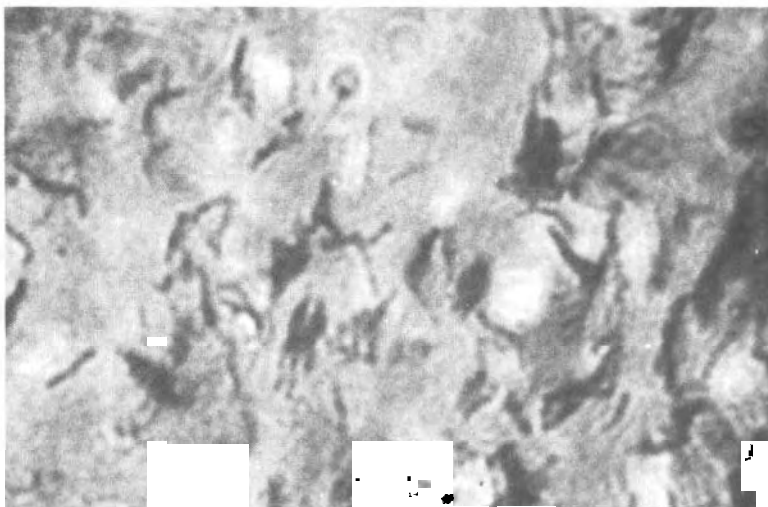


Fig 3. Numerous acid-fast bacilli seen in the aspiration smear of case 1. ZN X1000

of disseminated mycobacterial infections in immunocompromised hosts are caused by organisms belonging to MAC although *M.tuberculosis* and *M.kansasii* can also cause it.

A different cytologic and histologic picture has been described in certain cases of tuberculous lymphadenitis in AIDS.^{1,5} In FNA samples, stained with Romanowsky method, many histiocytes are seen that may resemble Gaucher-like cells. Clear, thin slightly curved unstained areas appear in these smears both in the background and within histiocytes. These are the so-called negative images of the bacilli.¹ These spaces represent mycobacteria, that usually occur in profusion in such cases.¹ Awareness of this feature may reduce the rate of underdiagnosis of tuberculosis in AIDS.

Maygarden and Flanders reported these structures in routine modified Wright stained (Diff- Quick) lymphnode aspirates of patients with AIDS.⁵ Jannotta and Sidawy, too, described these images in 10 cases of intraoperative imprint samples of lymphnodes in HIV infected individuals.¹ Six of their cases turned out to be *M. avium-intracellulare* on culture while the rest were *M. kansasii*. They also detailed the cytomorphological features in these lymph node imprints. The aspirates seen in infection due to MAC show pale blue striated histiocytes in aggregates. Necrosis is uncommon and neutrophils may be seen. Granulomas are usually absent or poorly-formed. The bacilli are short, thicker and more beaded than *M.tuberculosis*. In infection with *M.kansasii*, acute inflammation

is more commonly seen. **Necrotizing granulomas** may also be seen. The bacilli are longer with marked beading and intertwined cords have been described. It is important to differentiate between the various types of mycobacterial infection because infection with MAC is generally resistant to standard anti-tubercular regimens. Culture of the aspirated material is mandatory in order to identify atypical mycobacterial infection.^{1,2,7,8}

Such negative images of tuberculous bacilli have also been described in bone marrow aspirate smears⁹ and duodenal brush **cytology**¹⁰ of HIV infected individuals. Macrophages stuffed with unstained bacilli have been described in Romanowsky-stained FNA smears of lymph nodes⁶ and skin **nodules**¹¹ of lepromatous leprosy patients. Recently, a report described negative images of clofazimine crystals in broncho-alveolar lavage in an HIV positive patient with infection by *M. avium intracellulare*, being treated with the **drug**.¹²

In conclusion, in cases of HIV infection, an air-dried Romanowsky-stained smear of lymph node **FNA** must be examined for negative images in the cytoplasm of the histiocytes and in the background. This can help to raise the suspicion of a mycobacterial infection. It is also mandatory to stain for acid-fast bacilli in all cases. In all our four cases, initial examination of the smears revealed no significant pathology. However a closer look enabled us to spot these unstained organisms. In fact, we have now made it a routine practice to look for these images in the background and within the cytoplasm of histiocytes in all such lymph node aspirates.

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