

Case Report

Negative images of mycobacteria revisited in rapid on-site evaluation

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Abstract

Rapid on-site evaluation (ROSE) with routine fine-needle aspiration cytology (FNAC) is a simple, economical, highly accurate tool in the diagnosis of tuberculous lesions. The sample collected can be used for ancillary studies, i.e., Ziehl–Neelsen stain, Cartridge-based nuclei acid amplification testing (CB-NAAT) and bacterial culture for confirmation of the tubercular species. We report a case of a 31-year-old male who presented with left supraclavicular lymphadenopathy. ROSE with FNAC helped in the diagnosis of the patient. FNAC is a rapid minimally invasive method for the early detection of tubercular lesions and helps in proper treatment.

Keywords: Fine-needle aspiration, mycobacteria, negative image, rapid on-site stain, Ziehl–Neelsen stain

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INTRODUCTION

Cervical lymphadenopathy is the most common presentation of extrapulmonary form of tuberculosis (TB).^[1] The incidence of TB burden in India accounts for 2.69 million cases and 0.45 million deaths every year.^[2] Fine-needle aspiration cytology (FNAC) is the basic component of the standard diagnostic algorithm for the evaluation of cervical lymphadenopathy. Implementation of ROSE will be an add-on to the FNAC procedure for triage of patients, reducing the turnaround time and for the collection of samples for ancillary studies.^[3]

Negatively stained ghost images of mycobacteria or “footprint” give an important clue in the diagnosis of TB.^[4,5] Mycobacterial culture being a gold standard for the detection of mycobacterial TB with a disadvantage of time taken for the report.^[6] We report this case highlighting the

identification of negative images in the ROSE method for the 1st ever publication as per our search from literature.

CASE REPORT

A 31-year-old male patient presented to our surgical outpatient department with complaints of left-sided neck swelling and fever for 3 months with a history of approximately 5 kg weight loss in the last 1 month, and occasional episodes of cough were also present [Figure 1a]. He was a web designer by profession. He had no history of contact with any TB patient. He had no history of tobacco chewing or gutkha intake. On examination, the patient had left cervical lymphadenopathy. The lymph node was well defined, soft, tender, and cystic in consistency with tense overlying skin. There was no other palpable lymph node apart from the left supraclavicular level IV node and his oral cavity was normal.

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Ultrasound of the lymph node swelling revealed well defined, hetero-echoic, oval shape, soft tissue swelling measuring 4 cm × 1.6 cm in the left supraclavicular region. His chest X-ray revealed left upper and mid zones consolidations with prominent hilar coalescing opacities-likely to be infectious etiology [Figure 1b]. His serological markers for human immunodeficiency virus (HIV), hepatitis C, and hepatitis B were negative in the lateral flow card method as per the patient. The complete hemogram was in normal range with erythrocyte sedimentation rate being 52 mm in 1st hour. The patient was advised to undergo FNAC to identify the cause of the lesion.

FNAC was done using 23G needle and 5 ml syringe. We aspirated 4 ml of necrotic pus-like sample. The size of the lesion reduced slightly post FNAC.

The aspirate was smeared into 4 slides. Using 1% aqueous toluidine blue stain we did the on-site screening of the cytosmear. The smear revealed numerous epithelioid

granulomas, lymphocytes, neutrophils, and macrophages studded with negatively stained long slender, straight to curved, colorless rods in a prominent dirty blue necrotic background [Figure 2]. Also seen were negatively stained droplet like images [Figure 3]. The air-dried smears were stained for Giemsa and Ziehl–Neelsen (ZN) stain and alcohol fixed smears were stained for Papanicolaou stain. The smears were predominantly necrotic with the presence of lymphocytes, neutrophils, occasional plasma cells, macrophages, and epithelioid cell granuloma. Few Langhans type giant cells were also seen. Giemsa stain also showed the above-mentioned type of negative images. ZN stain revealed numerous acid-fast bacilli. Periodic acid Schiff (PAS) stain was negative for any fungal elements, with internal control of neutrophils [Figure 4a-e].

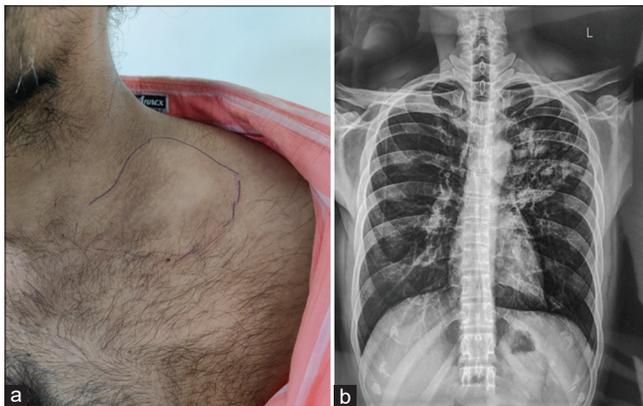


Figure 1: (a and b) Patient with left supraclavicular lymphadenopathy. Chest X-ray showing the hilar prominence

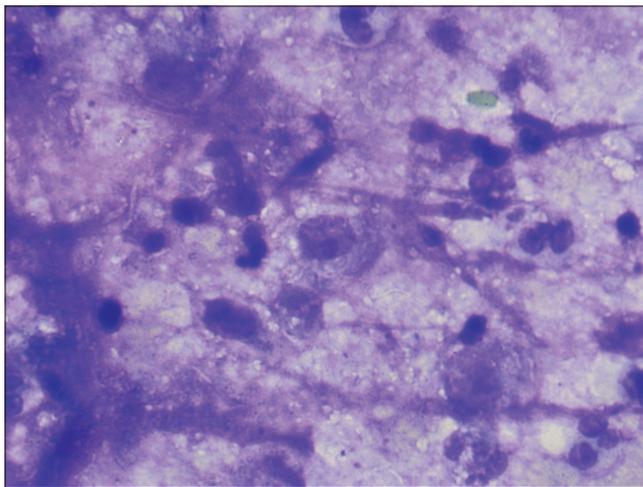


Figure 3: Cytosmears showing dense necrotic background with lymphocytes, neutrophils and macrophage with numerous negative stained images and droplet like structures (Tol blue, ×100)

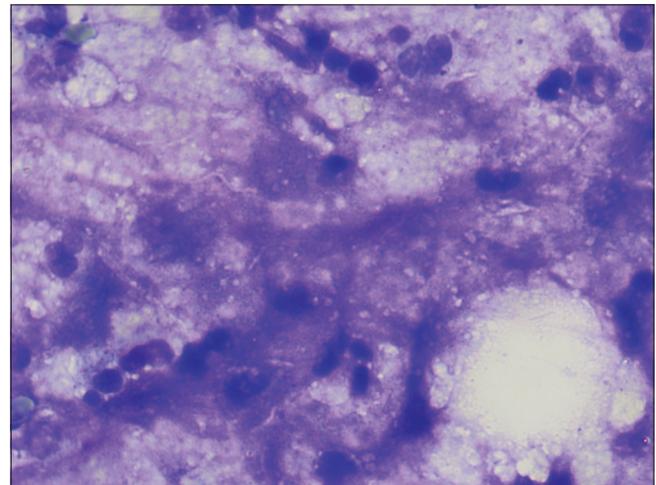


Figure 2: Cytosmears showing dense necrotic background with numerous negative stained images and droplet like structures (Tol blue, ×100)

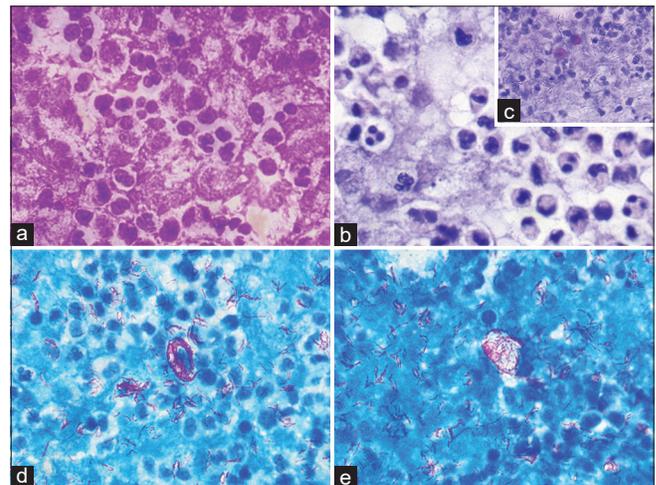


Figure 4: (a-c) Cytosmears showing numerous inflammatory cells comprising of lymphocytes, neutrophils with necrotic background. PAS stain for fungal elements is negative (inset). (a) Giemsa, ×100 and b,c: Pap, ×100 (d and e) acid fast bacilli highlighted by the bright red linear beaded appearance (ZN stain, ×100)

The sample was also sent for cartridge-based nucleic acid amplification testing (CB-NAAT) and was positive for the mycobacterium TB. He was then referred to DOTS (Directly observed treatment-short course) center for further management with category 2 anti-tubercular regimen. He is currently under follow-up of 2 months with symptoms subsided and reduction in the size of the lymph node.

DISCUSSION

India accounts for 27% of the global tubercular burden, with an incidence of TB cases being 2.69 million and multidrug resistance in almost 1.3 million of the population in India as per the 2018 World Health Organisation. TB has superseded its medieval cousins, plague and cholera, etc.^[7]

The most common site to be affected by TB in humans is the lungs, however, isolated extrapulmonary organ involvement is also noted, the common sites include lymph nodes, bones, renal, genital tract, brain, and meninges.^[6] Cervical lymphadenopathy can be seen in 20%–40% of extrapulmonary TB. Management of these lesions is very difficult due to the possible malignancy in the differential diagnosis.^[8]

Dudgeon and Patrick in 1927 had 1st diagnosed TB lymphadenitis using FNAC.^[9] Fine needle aspiration with Rapid on-site evaluation is a simple, quick, reliable, inexpensive investigative tool for identifying the etiology of cervical lymphadenopathy.^[3-5]

Negative images in cytology were 1st reported in 1986 by Solis *et al.*^[10] However, we are reporting the 1st case report of negative images in the ROSE using toluidine blue stain. *Mycobacterium avium* intracellulare (MAI) can show infected histiocytes termed as “pseudo-Gaucher” cells because of the striated appearance of cytoplasm with the needle-like inclusions, resembling Gaucher’s cells. The negative images/ghost appearance/“footprint”^[5] is due to a large amount of lipids present in the cell wall of mycobacteria rendering them impermeable of dyes in routine Romanowsky stains.^[4,11]

Cytology of TB lymphadenitis can have various patterns: a)-epithelioid granuloma without necrosis with significant lymphocytes, b)-epithelioid granuloma with necrosis with appreciable giant cells, c)-necrosis without epithelioid granuloma with neutrophilic infiltrate, and high acid-fast bacilli load.^[9,12]

Peripheral lymphadenopathy can be due to various etiological factors i.e., infective conditions (reactive and TB),

MAI in HIV-positive patients, sarcoidosis, leprosy, mycosis, silicone granuloma, non-Hodgkin lymphoma, squamous cell carcinoma, etc. The distinction from TB can be made on clinical examination, subtle cytomorphological features, and ancillary tests (ZN stain, PAS stain, CB-NAAT, and culture).^[9-12] Our patient was seronegative which also highlights the fact that clinical and cytological approach is needed in any suspected case of lymphadenopathy.

Management of TB lymphadenitis includes the institution of anti-tubercular drugs based on drug sensitivity testing results for 8 months (2 months of intensive course HRZE and 6 months of HRE).^[1,7]

CONCLUSION

FNAC with ROSE is a useful tool in diagnosing tubercular lymphadenitis. The procedure can help in collecting samples for ancillary tests and further molecular analysis. Cytopathologists should be aware of the possible differentials of negative images in routine FNACs. Patients are usually exempted from the biopsy of lymph node and cytological diagnosis helps in the early institution of treatment.

Availability of data and materials

All the data regarding the findings are available within the manuscript.

Consent for publication

Written consent for the publication and any additional related information was taken from the patient.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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