Challenge of a false-positive acid-fast bacilli: a diagnostic conundrum

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Abstract

Infectious and autoimmune diseases are distinct entities that require opposite therapeutic approaches. However, differentiating between the two can be a challenge, especially when the histopathology misguides the clinician. We highlight the case of a 66-year-old female who presented with fever, shortness of breath and cough for three months duration. She had raised inflammatory markers, and imaging revealed bilateral cavitating nodules. Histopathology revealed necrotising granulomatous inflammation along with acid-fast bacilli on Ziehl–Neelsen staining. The patient was diagnosed with tuberculosis and started on anti-tubercular therapy. When no response was seen, a rheumatologist was consulted, and a suspected diagnosis of granulomatosis with polyangiitis was made on the basis of clinical and laboratory features. Dramatic response to steroids and a negative mycobacteria culture confirmed the diagnosis. The patient responded to a combination of steroids and cyclophosphamide. This case highlights the importance of recognising the possibility of a false-positive acid-fast bacilli report.

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Introduction

With advances in histopathological techniques, we are relying more on histopathological evidence for establishing a diagnosis. However, sometimes there are instances when histopathology should not overshadow a clinician's reasoning when in doubt. The present case report illustrates this point.

Case presentation

A 66-year-old female presented in the pulmonology clinic with complaints of low-grade fever, shortness of breath and dry cough for three months duration. She also had associated complaints of loss of appetite and malaise which caused significant weight loss over the past three months. She gave a past history of abdominal tuberculosis (TB) two years ago, for which she had been successfully treated with a nine-month course of anti-tubercular therapy (ATT). Six months prior to her presentation, she had history of bilateral ear discharge, which was treated with multiple courses of antibiotics but resulted in development of mild hearing loss in both ears. She had no known comorbidities, and her personal history did not reveal anything significant. On examination, she had a low body mass index of 15.5 with normal blood pressure and pulse rate. She was maintaining a saturation of 97% on room air. No lymph nodes were palpable on examination. Respiratory system examination was clear on auscultation and the rest of the systemic examination was unremarkable as well. Laboratory work-up revealed a Hb of 8.3g/dL, total leucocyte count of 12,300/cu mm with 85% neutrophils and a platelet count of 790,000/cu mm. She had raised inflammatory markers (erythrocyte sedimentation rate 85 mm/h, C-reactive protein 160 mg/L) and a low albumin of 1.7 g/dL. Her liver and kidney function tests and urine routine examination were within normal limits. Her chest X-ray revealed bilateral ill-defined lung opacities (Figure 1A). Contrast-enhanced computed tomography (CT) scan of the chest revealed a large cavitating nodule on the right side and a single homogenous nodule on the left side (Figure 1B). Her Mantoux test and QuantiFERON-TB Gold test results were negative. A bronchoscopy was performed and bronchoalveolar lavage fluid examination was inconclusive for gram stain and culture, acid-fast bacilli (AFB), GeneXpert *Mycobacterium tuberculosis* (MTB) and fungal staining. Transbronchial needle aspiration revealed necrotising granulomatous inflammation. Meanwhile, her serum galactomannan levels were elevated and in the range of 2.7 (reference range <0.5). Due to these findings and a past history of TB, a CT-guided lung biopsy was planned to differentiate between tubercular and

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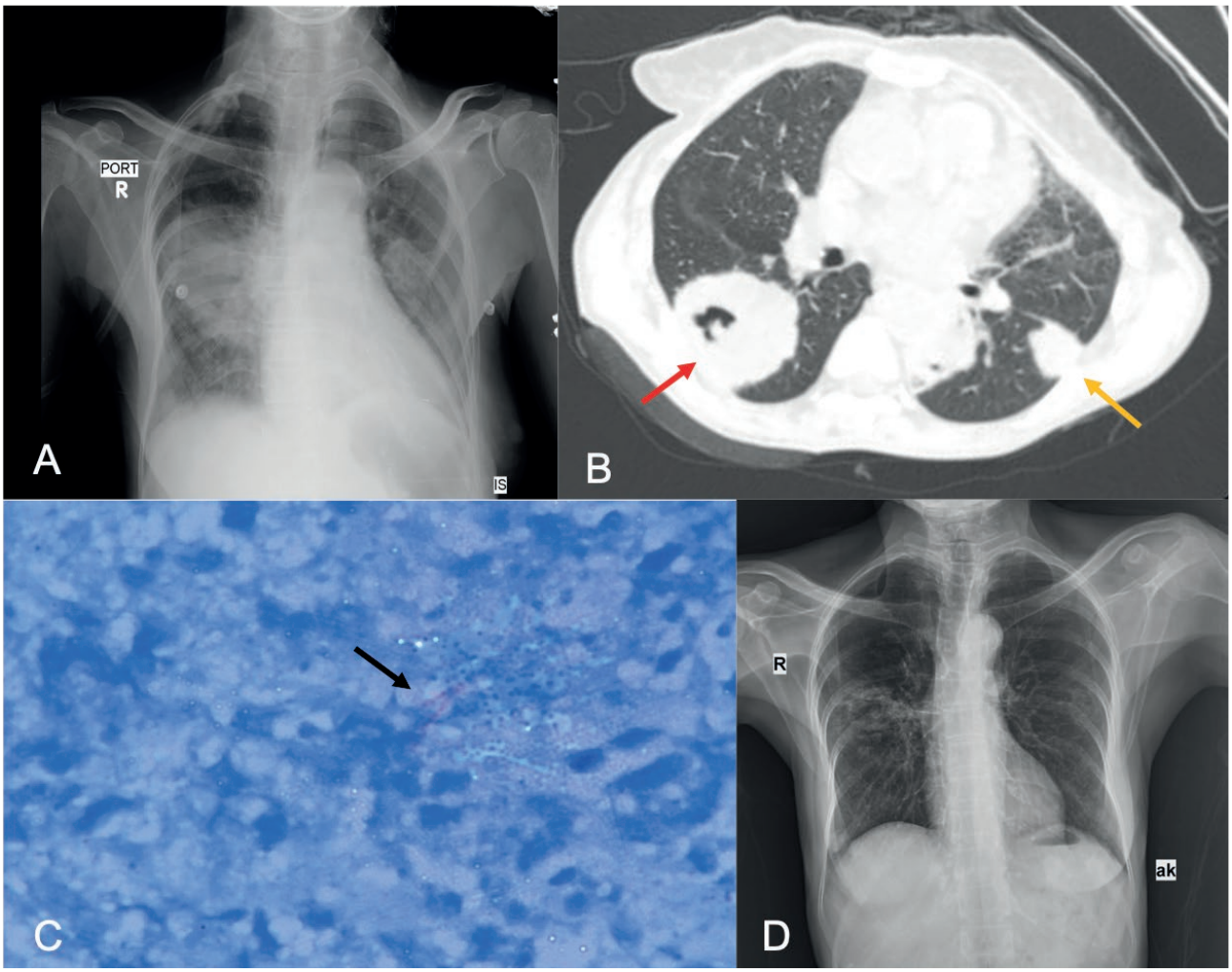
Figure 1 (A) Chest X-ray showing a large opacity involving right middle and lower zone and a soft tissue shadow near the left hilum; (B) CT scan of chest with red arrow showing cavitating lung lesion involving right middle and lower lobe and yellow arrow showing nodule in the left lower lobe; (C) histopathological section of lung tissue showing the classical beaded appearance of AFB on Ziehl–Neelson staining; (D) repeat chest X-ray, six weeks after steroid therapy showing considerable resolution of lesions.



fungal aetiology. The biopsy revealed chronic necrotising inflammation with positive AFB on Ziehl–Neelsen staining (Figure 1C). The patient was diagnosed with pulmonary TB and was discharged on four-drug ATT.

She was followed up after one month, with no improvement in her symptoms and worsening of clinical condition. Her primary physician found C-anti-neutrophil cytoplasmic antibodies (C-ANCA), which was reported positive at a titre of 1:40. A rheumatology opinion was sought at this point and anti-PR3 was found to be positive (42.2, reference range 0–3.5). On reviewing the case history (bilateral cavitating lung lesions with necrotising granulomatous inflammation on histology, history of bilateral ear discharge, leukocytosis, negative Mantoux test, raised anti-PR3 levels and poor response to ATT), a suspicion of granulomatosis with polyangiitis (GPA) was made, and a decision was taken to start the patient on prednisolone 1 mg/kg while continuing ATT. The patient showed marked improvement in her symptoms within two weeks, with normalisation of total leukocyte count and a significant decrease in her inflammatory markers. In view of dramatic response to steroids, lung biopsy was submitted for re-evaluation. Repeat evaluation revealed similar findings of necrotising granulomatous inflammation; however, the staining for AFB was negative. By this time, mycobacterial culture had also come back negative. In light of these findings, the earlier report of positive AFB was regarded as false-positive and it was decided to stop ATT and treat the patient with high dose steroids alone. Her chest X-ray showed considerable improvement within a month (Figure 1D). A diagnosis of GPA was confirmed, and she was started on oral cyclophosphamide 2 mg/kg/day. During follow-up, she developed constriction in the left upper lobe bronchus with collapse of the left upper lobe. An attempt was made at restoring the patency of bronchus with endobronchial stenting, but the procedure failed due to extensive fibrosis in the bronchus. The patient ultimately developed fibrosis of the left upper lobe with compensatory right lung hypertrophy. After completing six months of cyclophosphamide, the patient was started on rituximab as maintenance therapy. Presently, her disease remains in remission, and she is following up regularly in the Outpatient Department.

Discussion

GPA (formerly Wegener's granulomatosis) is a life-threatening vasculitis which primarily affects blood vessels of smaller calibre. It is characterised by necrotising granulomatous inflammation of upper and lower respiratory tract along with pauci-immune glomerulonephritis. GPA is a rare disease with an estimated incidence of 10 per million, whereas the incidence of TB in India is estimated to be about 1,990 per million.[1,2] In a TB endemic country like India, TB is among the most common causes of cavitary lung lesions (seen in up to 38% of cases).[3] The implications of making a correct diagnosis are huge as immunosuppression in patients with TB can lead to dissemination of the disease, whereas GPA in the absence of adequate treatment could be fatal. Occasionally, the situation can become more complicated by the fact that in some instances TB and GPA can coexist together. However, this was not the scenario in our case which is evident by the response to steroids alone.

The presence of extra-pulmonary features (ear discharge, nasal bleeding and crusting, tracheal stenosis and glomerulonephritis) generally aid the diagnosis of GPA; however, it can be difficult to distinguish GPA from TB, particularly where pulmonary involvement is the sole manifestation. This is due to the presence of overlapping clinical (fever, weight loss, cough and dyspnoea), serological (raised erythrocyte sedimentation rate and C-reactive protein), radiological (infiltrates and cavitating lung lesions) and histological features (necrotising granulomatous inflammation). Despite such overlapping features, there are several points that can help differentiate between the two entities. Leukocytosis, negative Mantoux test and QuantiFERON-TB Gold along with negative staining and culture for *M. tuberculosis* all favour a diagnosis of GPA. Demonstration of anti-neutrophil cytoplasmic antibodies (ANCA) both by immunofluorescence and enzyme-linked immunosorbent assay, especially C-ANCA along with anti-PR3, have a very high specificity (98% and 99%, respectively) for the diagnosis of GPA.[4] However, a diagnosis of GPA should not be made solely on the presence of ANCAs and should always be corroborated with the presence of other clinical and laboratory features as some case reports have described the presence of these antibodies in patients with TB as well.[5]

Radiologically, lesions in GPA tend to be bilateral and more random in distribution, whereas the lesions in TB are generally unilateral and more peribronchial in distribution with an upper lobe predilection. The presence of lymphadenopathy and effusion leans more towards a diagnosis of TB. Histologically, although it is possible to find evidence of vasculitis in both conditions, lung parenchymal lesions in GPA tend to be angiocentric. Evidence of vasculitis in the presence of normal lung parenchyma is a pathognomonic feature of systemic vasculitis.

A diagnosis of TB mostly relies on the demonstration of AFB or detection of TB bacilli by polymerase chain reaction; however, mycobacterial culture remains the gold standard. The presence of AFB has a very high specificity for the diagnosis of TB (97%); however, false-positive results of AFB have been reported in the literature.[6–9] A study was undertaken to understand the outcome of sputum samples positive for AFB but negative for mycobacterial culture.[7] Out of 447 sputum smear samples positive for AFB, 29 were negative on mycobacterial culture. Of these 29 samples, six were identified as genuine false-positives (based on clinical follow-up), whereas in the remaining samples, it was deemed to be a failure on the part of laboratory to culture mycobacteria.

False-positive AFB can be due to a number of reasons (Table 1). Several organisms other than *M. tuberculosis* are acid-fast and can give rise to false-positive results. Laboratory errors, such as the use of scratched slides, old stains and contaminated water, can also produce false-positive results. One study described a pseudo-outbreak of TB in a community hospital due to laboratory error in the processing of samples.[8] In our literature review, we found one case similar to ours where the presence of false-positive AFB was attributed to contamination by tap water and it led to the delay in treatment of the patient.[9] We, however, could not identify the cause of false-positive AFB in our case.

Table 1 Causes of false-positive acid-fast bacilli

Acid-fast organisms	All *Mycobacterium* species – *M. tuberculosis*, *Mycobacterium leprae*, *Mycobacterium smegmatis* and atypical mycobacterium
	Actinomycetes, Nocardia, Rhodococcus, Gordonia
	Endospore (bacterial spores)
	Oocysts – *Cryptosporidium parvum*, *Isospora*, *Cyclospora*
	Head of sperm
	Taenia saginata eggs, hooks of Hydatid, inclusion bodies in lead poisoning
Laboratory errors	Reuse of positive slides/scratched slides (improper storage)
	Contamination of dye/water
	Improper discolouration (with sulfuric acid)
	Transferred from oil on magnifying lens
	Artefacts (precipitation of old stains)

Conclusion

Differentiating between GPA and TB can be a challenge, especially in patients where pulmonary involvement is the major and sole manifestation of the disease. In the absence of systemic features, distinguishing between these two aetiologies mostly relies on histopathology. Although demonstration of AFB has a very high specificity for TB, this case highlights the fact that AFB can also be false-positive. Such false-positive results can be misleading and result in delayed diagnosis and treatment.

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