Enzyme-linked immunospot assay and metagenomic sequencing of *Mycobacterium tuberculosis*, coagulopathy symptoms, and pancytopenia testing for characterization of pulmonary tuberculosis: case report and literature review

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**ABSTRACT**

Pulmonary Tuberculosis (TB) is common in China, but tuberculosis with coagulation disorders and pancytopenia have rarely been reported in the past. In this report presented, a 70-year-old female was admitted to the hospital with poor appetite, dark urine, nausea, vomiting, fatigue, and bilateral lower limb edema; chest CT suggested diffuse infectious lesions in both lungs, coagulopathy dysfunction, and complete pancytopenia, which was initially considered to be caused by severe infection. However, the patient's symptoms did not improve by potent empiric antibiotics treatment, and a repeat chest CT showed that the lung lesions deteriorated more than before, and coagulation disorders and pancytopenia did not improve. Finally, the TB patient tested positive for enzyme-linked immunospot assay (ELISPOT) and metagenomic sequencing (mNGS) of *Mycobacterium tuberculosis* (MTB) using bronchoscopic alveolar lavage. So ati-TB was initiated with HRfELfx (isoniazid, 0.3 g qd; rifapentine, 0.45 g biw; ethambutol, 0.75 g qd; and levofloxacin, 0.5 g qd) regimen. Eventually, the patient's clinical symptoms improved significantly, the pulmonary lesions were absorbed, and the coagulation function and blood cell count returned to normal, which achieved a satisfactory treatment effect.

**Introduction**

Tuberculosis (TB) is common, and in many cases fatal, infectious disease. This disease is caused by various species of mycobacteria, usually *Mycobacterium tuberculosis* (MTB). Pulmonary TB is an infectious respiratory disease caused by MTB (1-3). A typical patient with pulmonary TB may have respiratory symptoms such as cough, sputum, and hemoptysis and constitutional symptoms such as night sweats, fatigue, loss of appetite, and weight loss (4-6). However, a significant proportion of TB patients do not have these specific clinical manifestations or even obvious symptoms, which remains a challenge for early detection and prompt treatment (7-9). Herein, we report a rare case of non-miliary pulmonary TB with coagulation disorders and pancytopenia.

**Materials and Methods**

**General information**

A 70-year-old female was admitted to our hospital because of poor appetite and dark urine for over 20 days, along with nausea, vomiting, fatigue, and bilateral lower limb edema. She denied having soy-sauce-colored urine, chills, shivers, fever, hot flashes, night sweats, cough, sputum, hemoptysis, chest pain, chest tightness, shortness of breath, abdominal distension, and abdominal pain. She was transferred to our hospital due to deterioration of “bacterial pneumonia” (no imaging data and previous case record available) coupled with abnormal liver function, albeit under the treatment of ceftriaxone (2 g, once daily), moxifloxacin (0.4 g, once daily) plus liver protectants for seven days. The patient's medical history was unremarkable. She denied a history of TB or other contagious diseases in her family. Her vaccination status was unknown. On examination, her vital signs were normal: well nourished; clear mind; anemic appearance; slight jaundice on sclerae; no petechiae or petechiae on the skin or mucosa; no liver palm or spider nevus; no enlargement of superficial lymph nodes; cardiac arrhythmia but without murmur; lungs negative; the abdomen was soft with no tenderness; the liver and spleen were not palpable; no tenderness over the kidney region; free of mobile dullness; and no pitting edema in the lower extremities.

**Laboratory examination**

On admission, routine blood tests were as follows: white blood cell (WBC) count of 2.59×10⁹/L, neutrophil percentage (Neut%) of 92.30%, hemoglobin (Hb) level of 75.00 g/L, platelet (PLT) count of 59.00×10⁹/L; alanine transaminase (ALT) 45.00 U/L; aspartate transaminase (AST) 403.20 U/L; alkaline phosphatase (ALP) 365.20

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Chest computed tomography scan shows an increase in bronchial vascular bundles in both lungs, disorganized alignment, and diffuse large hyperdense shadows in both lungs with blurred boundaries.

**Figure 1.** Chest computed tomography scan shows an increase in bronchial vascular bundles in both lungs, disorganized alignment, and diffuse large hyperdense shadows in both lungs with blurred boundaries.

**Figure 2.** Chest computed tomography scans show increased bronchial vascular bundles in both lungs, disorganized alignment, diffuse large hyperdense shadows in both lungs with blurred boundaries, bilateral pleural thickening, and a small amount of bilateral pleural effusion.
Chest computed tomography scans show an increase in bronchial vascular bundles in both lungs, disorganized alignment, and a diffuse flocculent and cloudy fuzzy shadow in both lungs, which was significantly absorbed compared to previous scans.

Figure 3. Chest computed tomography scans show an increase in bronchial vascular bundles in both lungs, disorganized alignment, and a diffuse flocculent and cloudy fuzzy shadow in both lungs, which was significantly absorbed compared to previous scans.

decreasing prevalence of TB patients worldwide in 2020; however, TB prevention and control in China is not optimistic, with an estimated 842,000 new TB cases in 2020, an increase of 0.9 million from 2019, ranking second globally (10). Missed or delayed diagnosis and treatment can lead to the spread and aggravation of TB, further increasing its incidence and creating a vicious cycle. Early and accurate diagnosis and timely treatment can significantly reduce the spread of TB and decrease its morbidity and mortality (11). In the new version of the diagnostic criteria for pulmonary TB released in 2017, the criteria for confirming the diagnosis of pulmonary TB were expanded from bacteriologically positive to pathogenically positive (including bacteriology and molecular biology methods), and the clinical diagnostic criteria for pulmonary TB were clarified (8). Therefore, "early treatment" of pulmonary TB is based on evidence. Nevertheless, quite a few atypical TB patients are yet to be diagnosed in time.

In this case, the patient presented with abnormal coagulation function and pancytopenia, although a chest CT scan suggested severe pulmonary infectious lesions (Figure 1) without any specific etiological characteristics. Blood, bone marrow, and alveolar lavage cultures did not reveal any pathogens. Potent broad-spectrum antibiotics were empirically administered, but the patient’s condition was not controlled. The patient was later confirmed through mNGS to be infected with *M. tuberculosis* and switched to *M. bovis* instead of rifampin because of the patient’s heavy gas- trointestinal symptoms and hepatic impairment (12). This treatment showed remarkable efficacy after one week, and at subsequent follow-ups, the patient's laboratory parameters gradually returned to normal and lung lesions were significantly absorbed and improved (Figure 3).

Few studies have reported coagulation dysfunction in patients with TB, and its pathogenesis is unclear. It is speculated that this may be due to complement activation by endotoxins, metabolites, and breakdown products of *M. tuberculosis* after infection, causing inflammatory cells to accumulate and release enzymes and peroxides that damage capillaries or act directly on capillaries to release histamine and prostaglandins which damage capillaries, resulting in severe damage to vascular endothelial cells (13). Disseminated intravascular coagulation (DIC) in combination with miliary pulmonary TB has been reported (14), and the symptoms of TB intoxication are more severe in these patients. However, in this case, her PT, APTT, and INR were significantly prolonged at the time of admission, and fibrinogen levels were reduced without bleeding tendency and shock manifestations; thus, the diagnostic criteria for DIC were not met, and the possibility of pre-DIC was considered (15). Pre-DIC refers to the presence of certain risks of DIC along with abnormalities in coagulation and fibrinolytic function but does not yet meet the diagnostic criteria for DIC (16). Wang et al. (17) reported in a retrospective study that early anti-TB treatment can significantly improve the survival rate of patients with TB combined with DIC. Similarly, our case of atypical TB with abnormal coagulopathy and pancytopenia was successfully treated with anti-TB treatment, suggesting the necessity of timely detection and appropriate treatment strategy to prevent the progression to DIC.

The earliest report on TB-induced pancytopenia can be traced back to the animal experiments of Doan and Sabin (18) in 1927. In their study, 80 rabbits were inoculated with *M. bovis*, and all animals showed miliary TB changes in the bone marrow, and peripheral blood showed a dramatic decrease in platelet, granulocyte, and white blood cell counts and anemia. Subsequently, an increasing number of reports on combined pancytopenia in miliary TB began to appear abroad, and more attention was paid to this rare hematologic manifestation of TB. Although more cases have been reported, the pathogenesis is still unclear but is likely due to hypersplenism, maturation arrest, histiocytosis, hemophagocytic syndrome, infiltration of the bone marrow by caseous or non-caseous tuberculous granulomas that cause fibrosis (19), and indiscriminate phagocytosis of blood cells by histiocytes in the bone marrow (20). The patient in this case was not diagnosed with miliary TB, as previously reported, but still showed signs of pancytopenia and a significant response to anti-TB therapy. Hypersplenism is unlikely in this case because her spleen was not enlarged and could not explain the severe allohemocytopenia, and the bone marrow aspiration examination did not support hypersplenism. The diagnosis of hemophagocytic syndrome was also not well established, as the patient had a normal temperature and triglyceride levels, mildly elevated ferritin levels, and no hemophagocytes in the bone marrow. Unfortunately, no histopathological examination of the bone marrow was performed in this patient, and although it has been reported that approximately 0.38% (20/5217) of patients with TB show evidence of bone marrow granuloma (21), bone marrow biopsy is still recommended in such patients with pancytopenia to clarify whether the disease is due to invasion of the bone marrow by tuberculous granuloma.

Based on the literature, the sensitivity, specificity, and positive predictive value of mNGS for the diagnosis of TB are significantly higher than those of smear and culture methods (22-25), and it is an efficient diagnostic method for difficult and critical cases.

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