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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, coagulation 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 HRftELfx (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.

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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 2.59×10^9/L, neutrophil percentage (Neut%) of 92.30%, hemoglobin (Hb) level of 75.00 g/L, platelet (PLT) count of 59.00×10^9/L; alanine transaminase (ALT) 45.00 U/L; aspartate transaminase (AST) 403.20 U/L; alkaline phosphatase (ALP) 365.20

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U/L; gamma-glutamyl transpeptidase (GGT) 203.70 U/L; albumin (Alb) 29.20 g/L; total bilirubin (TBil) 46.80 umol/L; direct bilirubin (DBil)32.60 umol/L; urea nitrogen (Urea)16.04 mmol/L; creatinine (Cr) 161.81 umol/L; respectively. Her prothrombin time (PT) was 180.00 s, activated partial thromboplastin time (APTT), international normalized ratio (INR), and prothrombin activity (PTA) were undetectable; procalcitonin (PCT) 6.950 ng/mL. Electrocardiography (ECG), cardiac enzyme profile, B type natriuretic peptide (BNP), urinalysis, routine stool and occult blood tests, blood glucose, lipid, blood amylase lipase, serum electrolytes and blood gas analysis, and troponin were normal. HBsAg, HCV, HIV, syphilis, HAV, and HEV, autoantibodies, autoimmune liver disease markers, anti-neutrophil cytoplasmic antibodies, methemoglobin, carcinoembryonic antigen, and antibodies to TB, mycoplasma, or *Chlamydia pneumonia* were not detected. Coomb's, and tuberculin, and Fungal G and GM tests were all negative. Computed tomography (CT) of the head, chest, and abdomen revealed diffuse infiltration in both lungs, and bilateral pleural thickening (Figure 1). Gastroscopy revealed superficial gastritis, and the C14 breath test was negative. In fiber bronchoscopy, microscopically, relatively abundant yellow-watery secretions were observed in each bronchus bilaterally. Bacterial smear and culture of lavage fluid, fungal smear, concentrated set of bacteria antacid staining, GM test, and *Mycobacterium tuberculosis* culture and nucleic acid polymerase chain reaction (PCR) tests were all negative, and no cancer cells were seen in the exfoliative cell test. Bone marrow aspiration suggested granulocytopenia with proliferative anemia and internal and external iron mismatches. The bone marrow culture was negative.

Treatment

The patient was managed with meropenem (1 g/8 h), symptomatic and supportive treatments involving supplementation with 400 ml of fresh frozen plasma and 10 units of cold precipitation infusion daily, her symptoms did not improve after 6 days of treatment, and repeat blood test showed: WBC 1.49×10^9/L, Neut% 77.10%, Hb 63 g/L, PLT 41.00×10^9/L; liver function: ALT 31.40 U/L, AST 131.60 U/L, ALP 259.80 U/L, GGT 156.10 U/L, Alb 27.10 g/L, TBil 42.20 umol/L, DBil 20.40 umol/L; coagulation analysis: APTT 47.00 S, PT>180.00 s, INR and PTA could not be detected by the instrument. Fibrinogen: 0.65 g/L, D-dimer: 2.91 ug/ml; Renal function and electrolytes were normal. Chest CT showed diffuse infiltrates in both lungs with predominantly exudative lesions, which were exacerbated compared to previous imaging, and solid shadows similar to previous chest imaging; bilateral pleural thickening; a small amount of bilateral pleural effusion, which was a new lesion; (Figure 2). Meropenem was discontinued since the TB patient tested positive for enzyme-linked immunospot assay (ELISPOT) and metagenomic sequencing (mNGS) of *Mycobacterium tuberculosis* using bronchoscopic alveolar lavage. A diagnosis of pulmonary TB was established, and anti-TB treatment was given with HRft-ELfx (isoniazid, 0.3 g qd; rifapentine, 0.45 g biw; ethambutol, 0.75 g qd; and levofloxacin, 0.5 g qd). The patient's symptoms of oil aversion, nausea, vomiting, and weakness improved significantly; edema of the lower limbs gradually subsided; and her appetite improved significantly. After one week of anti-TB treatment, a routine blood test was repeated: WBC, 2.95×10^9/L; Neut%, 71.90%; Hb, 67.00 g/L; PLT, 50.00×10^9/L; ALT, 38.60 U/L; AST, 201.60 U/L; ALP, 340.20 U/L; GGT, 186.70 U/L; Alb, 29.60 g/L; TBil, 42.50 umol/L; DBil, 23.70 umol/L; fibrinogen, 0.54 g/L; and D-dimer, 1.61 ug/ml. Coagulation analysis revealed an APTT of 36.20 S, PT of 13.20 S, INR of 1.14, and PTA of 73.20%. Renal function and electrolytes were also normal. These findings and the patient's clinical response suggested that the anti-TB treatment was effective. After continuing the anti-TB treatment for one week, previous abnormal laboratory parameters were tested again as follows: WBC, 2.06×10^9/L; Neut%, 54.30%; Hb, 82.00 g/L; PLT, 115.00 10^9/L; ALT, 21.50 U/L; AST, 39.40 U/L; ALP, 109.60 U/L; GGT, 81.60 U/L; Alb, 34.00 g/L; TBil, 36.50 umol/L; and DBil, 26.30 umol/L. and she was discharged from the hospital.

Treatment outcome, follow-up, and prognosis

After discharge, the patient continued regular anti-TB treatment, and follow-up routine blood, liver and kidney function, and coagulation analyses returned to normal. Two months later, a chest CT scan showed that diffuse infiltrates in both lungs were significantly absorbed (Figure 3). ELfx was discontinued, and HRft consolidation anti-TB treatment was continued for four months. After treatment discontinuation, the patient was followed up for two years without recurrence.

Results and Discussion

The World Health Organization (WHO) reported a

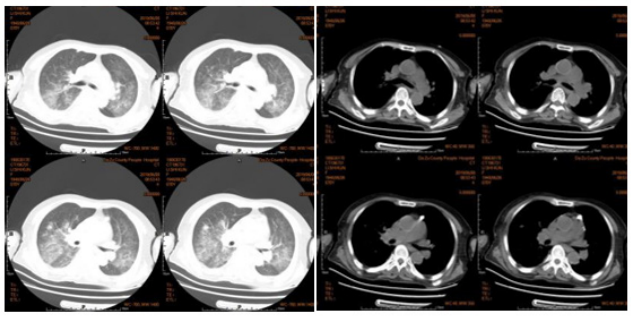

Figure 1. Chest computed tomography scan shows an increase in bronchial vascular bundles in both lungs, disorganized alignment, and diffuse large hyperdense shadows in both lungs with blurred boundaries.

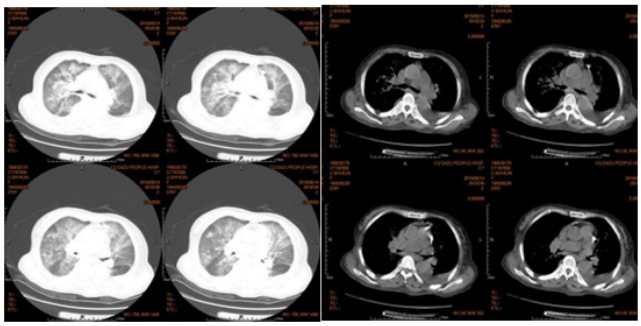

Figure 2. Chest computed tomography scans show increased bronchial vascular bundles in both lungs, disorganized alignment, diffuse large hyperdense shadows in both lungs with blurred boundaries, bilateral pleural thickening, and a small amount of bilateral pleural effusion.

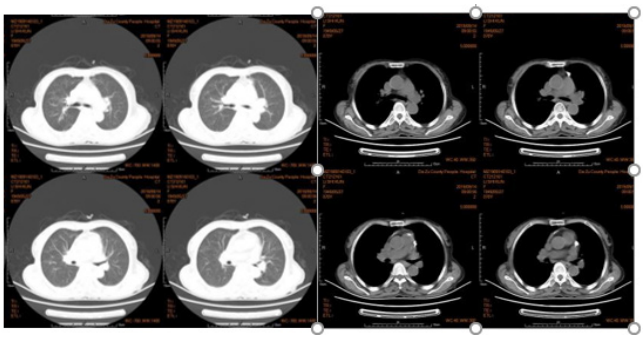

Figure 3. Chest computed tomography scans show an increase in bronchial vascular bundles in both lungs, disorganized alignment, and a diffuse flocculent and cloudy fuzzy shadow in both lungs, which was significantly absorbed compared to previous scans.

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 an HRftELfx regimen of anti-TB treatment, with the use of fluoroquinolones instead of pyrazinamide and rifapentine instead of rifampin because of the patient's heavy gastrointestinal 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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References

1. Sarınoğlu RC, Sili U, Eryuksel E, Yildizeli SO, Cimsit C, Yagci AK. Tuberculosis and COVID-19: An overlapping situation during pandemic. J Infect Dev Ctries 2020 Jul 31;14(07):721-5.
2. Acharya B, Acharya A, Gautam S, Ghimire SP, Mishra G, Parajuli N, Sapkota B. Advances in diagnosis of Tuberculosis: an update into molecular diagnosis of Mycobacterium tuberculosis. Mol Biol Rep 2020 May;47(5):4065-75.
3. Ahmed MI, Ntinginya NE, Kibiki G, Mtafya BA, Semvua H, Mpagama S, Mtabho C, Saathoff E, Held K, Loose R, Kroidl I. Phenotypic changes on Mycobacterium tuberculosis-specific CD4 T cells as surrogate markers for tuberculosis treatment efficacy. Front Immunol 2018 Sep 28;9:2247.
4. Vangara A, Gudipati M, Chan R, Do TV, Bawa O, Shyam Ganti S. Chronic Pulmonary Aspergillosis Infection in Coal Workers Pneumoconiosis With Progressive Massive Fibrosis. J Investig Med High Impact Case Rep 2022 Sep;10:23247096221127100.
5. Alrehaili M, Al-Qurashi E, Sayeed A, Muabbar H. Bronchoalveolar Lavage Fluid Caseating Granulomas in A Case of Pulmonary Tuberculosis. Am J Med Case Rep 2020 May 28;8(9):268-70.
6. Sutantoyo FF, Sugianto P. A malignant disseminated tuberculosis: concurrent intracranial tuberculosis with skipped multilevel spondylitis in a young immunocompetent patient. Rom J Neurol 2022;21(1):83.
7. Hikone M, Ainoda Y, Sakamoto N, Ohnishi K. Clinical characteristics of elderly pulmonary tuberculosis in an acute-care general hospital in Tokyo, Japan: a 12-year retrospective study. J Infect Chemother 2020 Mar 1;26(3):245-50.
8. Li-Xia W, Shi-ming C, Zhou-Lin, et al. The health industry standards of the People's Republic of China: Diagnosis for pulmonary tuberculosis:WS288-2017[J]. Chin J Infect Control. 2018;17(7):642–52.
9. Liu Y, Jiang Z, Chen H, Jing H, Cao X, Coia JE, Song Z. Description of demographic and clinical characteristics of extrapulmonary tuberculosis in Shandong, China. Hippokratia. 2020 Jan;24(1):27.
10. World Health Organization. Global tuberculosis report 2021 [R/OL]. Geneva: World Health Organization. [p. 2021-10-14]; 2021.
11. Singer-Leshinsky S. Pulmonary tuberculosis:improving diagnosis and management. JAAPA. 2016;29(2):20–5.
12. Shen-jie T. Guidelines for the diagnosis and treatment of antituberculosis drug-induced liver injury. 2019 ed. Chin J Tuberc Respir Dis. 2019;42(5):343–56.
13. Shan QIU, Na L, Yan Xiao-xia, et al. Changes and significances of hemoglutanation in patients with tuberculosis. J Lanzhou Univ (Med Sci). 2009;35(1):98–100.
14. Tojima H, Hamaoka T, Fujita A, Suzuki A. A case of pulmonary, pleural, and renal tuberculosis associated with DIC and a prolonged increase in D-dimer. Kekkaku. 1994;69(6):419–24.
15. Liang-yao GUO, Na LI, Yan Xiao-xia, et al. Several diagnostic reference standards developed at the 7th National Conference on Thrombosis and Hemostasis. Chin J Hematol. 2000;21(3):165–8.
16. Jie MIN, Ying Z, Li Xiao-qiang, et al. A case of tuberculosis combined with disseminated intravascular coagulation. Chin J Intern Med. 2006;45(12):1012.
17. Wang JY, Hsueh PR, Lee LN, et al. Mycobacterium tuberculosis inducing disseminated intravascular coagulation thrombosis and Haemostasis. Journal of the International Society on Thrombosis and Haemostasis. 2005;93(4):729–34.
18. Doan CA, Sabin FR. Local progression with spontaneous regression of tuberculosis in bone marrow of rabbits, correlated with a transitory anemia and leucopenia, after intravenous inoculation. J Exp Med. 1927;46(2):315–41.
19. Le Hô H, Barbarot N, Desrues B. Pancytopenia in disseminated tuberculosis: Think of macrophage activation syndrome. Rev Mal Respir. 2010;27(3):257–60.
20. Weintraub M, Siegman-Igra Y, Josiphov J, Rahmani R, Liron M. Histiocytic hemophagocytosis in miliary tuberculosis. Arch Intern Med. 1984;144(10):2055–6.
21. Jun F, Hong Z, Zhong Ding-rong, et al. A clinical analysis of 20 case with bone marrow granulomas. Chin J Intern Med. 2009;48(6):485–7.
22. Behzadmehr R, Rezaie-Keikhaie K. Evaluation of Active Pulmonary Tuberculosis Among Women With Diabetes. Cell Mol Biomed Rep 2022; 2(1): 56-63. doi: 10.55705/cmbr.2022.336572.1036.
23. Rezaie-Kahkhaie K, Rezaie-Keikhaie K, Rezaie-Kahkhaie L, Saravani K, Kamali A. The relationship between IL23R 1142G / A (Arg381Gln) and GM-CSF 3928 C / T (Ile117Thr) gene polymorphism in Iranian patients with tuberculosis disease. Cell Mol Biomed Rep 2021; 1(3): 113-121. doi: 10.55705/cmbr.2021.356674.1059.
24. Mohammadi M., Mehran MA, Omidi AH, Hassani MH. Clinical analysis of diagnosing a case with tuberculous peritonitis from Afghanistan. Cell Mol Biomed Rep 2021; 1(3): 122-128. doi: 10.55705/cmbr.2021.354911.1053.
25. Wenwen S, Jin G, Lin F, et al. Application value of metagenomic next-generation sequencing (mNGS) in the diagnosis of different types of tuberculosis. Chin J Tuberc Respir Dis. 2021;44(2):96–100.