CARE Checklist – 2016: Information for writing a case report

Topic	Item	Checklist item description	Line/Page
Title,	1	Case study of an individual with concurrent chest wall tuberculosis and triple-negative essential thrombocythemia	**Line 1 / Page 1**
Key Words	2	Platelet; Thrombocythemia; Triple-negative; Chest wall tuberculosis;SOCS3 gene; JAK-STAT pathway.	**Lines14-15/ Page 1**
Abstract	3	Essential thrombocythemia (ET) is a well-known myeloproliferative neoplasm (MPN) disorder resulting from genetic mutations in one or more common oncogenes, such as JAK2, MPL, and CALR. However, negative genetic markers, which cause triple-negative diseases, may occur in the presence of other, less common mutation types. Cases of comorbid diseases, such as thrombocytosis with chest wall tuberculosis are rare; furthermore, a relationship between the two diseases is rare. In this paper, we report the case of a 23-year-old male patient with a chest wall mass and an extremely high platelet count, which remained extremely high even after surgical excision of the chest wall mass, chest wall tuberculosis diagnosis, and anti-tuberculosis combinatory drug therapy. Bone marrow biopsy and further related tests supported the diagnosis of triple-negative essential thrombocytosis. Bone marrow was negative for BCR-ABL fusion gene, JAK2V617F, JAK2(EXON12), MPL, and CALR genes; MPN/acute myelogenous/myelodysplastic syndrome leukemia mutation screening of 34 genes was negative, and clinical bioinformatics analysis initially identified 100 molecules that were highly correlated with a possible disease. Suppressor of cytokine signaling (SOCS3) may be a novel MPN biomarker found from this analysis. In conclusion, concurrent triple-negative essential thrombocythemia and chest wall tuberculosis is uncommon, and its pathogenesis requires further research.	**Lines 17-35 / Page1-2**
Introduction	4	Essential thrombocythemia is a clonal myeloproliferative neoplasm (MPN) originating from hematopoietic stem cells. It is characterized abnormal megakaryocyte proliferation in the bone marrow and persistent thrombocytosis in the peripheral blood, which is accompanied by abnormal platelet morphology and function. Some patients may be asymptomatic; the most common clinical manifestations in symptomatic patients are thrombosis and bleeding. In some patients, the disease can transform into primary myelofibrosis (PMF), acute myeloid leukemia (AML), or polycythemia vera (PV). In 2008, 44 the World Health Organization (WHO) classified essential thrombocythemia (ET), PV, and PMF as myeloproliferative tumors with a negative BCR-ABL fusion gene . In approximately 80 – 90% of patients with ET, disease-defining mutations such as JAK2 V617F, mutations in MPL exon 10, and CALR exon 9 mutations, are found in a mutually 48 exclusive manner. Any of these gene mutations induces the constitutive activation of MPL, 49 the thrombopoietin (TPO) receptor, and its downstream molecules, leading to the clonal expansion of hematopoietic stem cells and the cell-autonomous expansion of megakaryocytes; thus, causing thrombocytosis. Negative disease-defining mutations in JAK2, MPL, and CALR, are observed in 10–15% of patients with ET and are defined as triple-negative essential thrombocythemia (TN-ET). Patients with TN-ET lack known mutations, and the mechanism behind it is unclear. Cases of concurrent chest wall tuberculosis and thrombocytosis have rarely been reported in China or abroad. Here, we report a case of chest wall tuberculosis combined with TN-ET.	**Lines 36-56 / Page2-3**
Timeline	5	A 23-year-old male patient was admitted to a local hospital in July 2017.After 8 months,he was diagnosed with TN-ET	
Patient Information	6	A 23-year-old male patient was admitted to a local hospital in July 2017. He had a headache and low-grade fever for two days, with bodily	

temperature fluctuating around 36.8°C. Hematological analysis showed a high platelet (PLT) count of 1503×109/L. Subsequently, the patient visited our hematology department for further investigation. The patient underwent right axillary lipoma resection in 2012 and had no history of thrombosis or bleeding and no significant weight loss. He had no risk factors for coronary artery disease, and no thrombocytosis was observed in the family. **Lines 57-64 / Page 3**

Physical Exam **7** Upon examination, the patient looked well, with no signs of jaundice or cyanosis. His blood pressure, temperature, pulse, respiratory rate, and oxygen saturation were 129/70 mmHg, 37.3°C, 85 beats per minute, 20 breaths per minute, and 100% on room air, respectively. No bleeding spots or ecchymosis was observed, and the peripheral superficial lymph nodes were not enlarged. Additionally, no pressure pain was observed in the sternum. The liver was not palpable and the spleen was palpable 2 cm below the ribs, with medium quality and no pressure pain. There was no edema in either lower limb.Initial investigations showed a hemoglobin level, white blood cell count, platelet count, and neutrophil count of 140 g/L, 18.38x10/uL, 2064x10/μL, and 13.82x10/uL. respectively. A normal blood sedimentation rate of 4 mm/h and normal C-reactive protein of 1.22 mg/L were observed. Additionally, he tested negative on serum tuberculosis (TB) antibodies. The TB-infected T-cell spot assay A result was 97 SFCs/2.5*PBMC and the TB-infected T-cell spot assay B result was 53 SFCs/2.5*PBMC. Prothrombin time (PT) was 14.1s. Computed tomography (CT) of the chest suggested a submural soft tissue density shadow in the left lower chest wall, a flocculent hyperdensity shadow in the upper lobe of both lungs, a small nodular shadow in the upper lobe of the right lung, and multiple cord shadows in the left lung. Abdominal ultrasonography showed that the spleen was 130x68 mm, and the width of the splenic vein was 8.9 mm; the liver, gallbladder, pancreas, and both kidneys showed no significant abnormalities. Vascular ultrasonography of the lower extremity suggested cloudy echogenicity in the lumen of the bilateral intermuscular veins of the lower leg, and no significant abnormalities were observed in the bilateral femoral and bilateral popliteal veins.Bone marrow imaging: 1. Good sampling, smear, and staining, granular (++) oil (−); 2. Bone marrow proliferation was evidently active. Granulocytes accounted for 72%, while red lineage accounted for 13.5%. Granulocytes were red with a lineage ratio of 5.3:1; 3. Increased granulocyte ratio, eosinophils were clearly observed, reduced red lineage ratio, and no obvious abnormalities in morphology were observed. A total of approximately 47 megakaryocytes were seen in the whole glass slide, and platelets were mostly distributed in piles and patches (Figure 3). Blood examination revealed increased white blood cell count, increased granulocyte ratio, no significant abnormalities in granulocyte and mature red blood cell morphology, and no nucleated red blood cells in the count of 100 white blood cells. Platelets were distributed in piles and observed more frequently. Bone marrow biopsy pathological diagnosis of the right posterior superior iliac spine bone marrow biopsy tissue: small amount of bone marrow tissue, hematopoietic area of about 70%, large size, multifoliation, and the presence of other various lineages of cells is consistent with megakaryocytosis (Figure 4). Immunohistochemical staining: CD42b (+++), CD71 (+), MPO (+), CD34 (-), CD3 (±), and CD20 (±). Characteristic staining: reticular fibers (+) and Masson (-). Bone marrow negative for BCR-ABL fusion gene, JAK2 V617F, JAK2 (exon 12), MPL, and CALR genes; MPN/AML/MDS-leukemia mutation gene screening (34 genes: ASXL1, BCOR, BCORL1, CALR, CBL, CSF3R, DNMT3A, ETV6 EZH2, IDH1, IDH2, JAK2, KRAS, MPL, NRAS, PIGA, RUNX1, SETBP1, SF3B1, SH2B3, SRSF2, TET2, TP53, U2AF1, ZRSR2, CEBPA, and FLT3) was negative; Clinical bioinformatics analysis (GeneCan®, Beijing Jinyou Qikang Technology Co. Ltd.) (Table 1) initially identified a total of 100 molecules that were highly correlated with a possible disease.
Lines 65-107 / Page 4-5

Diagnostic **8a** Preliminary diagnosis was ET and a left chest wall mass, and possible chest wall TB.

Assessment	**8b**	The patient's platelet count was extremely high and required surgical treatment.
Interventions	**9**	The patient's platelet count was extremely high and required surgical treatment. Thus, 1.0g/d hydroxyurea was orally administered to lower the platelet count. He was transferred to the Department of Thoracic Surgery and underwent chest wall tumor resection under general anesthesia. There was an irregularly bordered, surface-rich, encapsulated mass on the surface of the chest wall, measuring 43x34x65 mm. The process proceeded smoothly, with approximately 20 ml of bleeding. The pathological findings of the swelling were reported as TB with massive caseous necrosis. The diagnosis of chest wall tuberculosis was further confirmed, and the patient was subsequently administered isoniazid, rifampin, ethambutol, and pyrazinamide as anti-TB treatments, during which the platelet count remained extremely high after repeated hematological analysis. Subsequently, platelet-lowering treatment was performed using 2.0g/d hydroxyurea. After completing 8 months of regular anti-TB treatment, the chest CT and hematology analysis were re-checked, and it was observed that the lung lesions had disappeared. **Lines123-135 / Page6-7**
Follow-up and	**10a**	The PLT count continued to be >1000×109/L. Subcutaneous injection of recombinant human interferon a2b at 3 million u, thrice a week, was performed, and the amount of hydroxyurea was adjusted according to the platelet and white blood cell levels, ranging from 0.5–1.0g/d. The patient's PLT count fluctuated from 537–1260×109/L. The patient was very young and had a high chance of long-term survival. Thus, hydroxyurea was discontinued and replaced by weekly injections of 180 ug polyethylene glycol a-2b (pegaptan), after which the patient's PLT count fluctuated from 440–520×109/L. **Lines 135-142 / Page 7**
Discussion	**11a**	Thrombocytopenia occurs with elevated platelet count. The main types are essential (primary) and reactive (secondary) thrombocythemia. ET is an MPN that results from abnormal dysregulation of platelet production from bone marrow progenitor cells and is associated with mutated genes such as JAK2. Secondary thrombocytosis, also known as reactive thrombocytosis, is defined as an abnormally high platelet count due to an underlying event, disease, or the use of certain medications. Reactive causes of thrombocytosis include transient reactive thrombocytosis (such as acute blood loss and acute infection) or persistent reactive thrombocytosis (including iron deficiency, azoospermia, cancer, and chronic inflammatory or infectious diseases), such as TB [8]. TB is a chronic infectious disease caused by Mycobacterium tuberculosis (MTB), with pulmonary tuberculosis (PTB) being the most common. It can also spread throughout the body via lymphatic and hematogenous dissemination, leading to extrapulmonary tuberculosis (EPTB) [9]. Extrapulmonary infections from TB can affect any organ, and the most common extrapulmonary sites of infection are the lymph nodes, pleura, and bone and joint areas. Chest wall TB is a rare form of tuberculosis, with an incidence of 1–5% in osteoarticular site TB, and 0.1% in all forms of TB. Chest wall TB most commonly presents as a solitary lesion without fluctuating or local inflammatory signs, and nonspecific clinical signs, such as irritated cough, pleuritic discomfort, weight loss, or night sweats. Diagnosis is based on sampling, preferably surgical biopsy, because of the low sensitivity of fine needle aspiration (PAAF) and the low presence of Mycobacterium tuberculosis in the biopsy sample. Most authors advocate combined medical and surgical treatment to reduce the recurrence rate of chest wall TB [10,11]. In this case study, the patient underwent surgical excision of the chest wall mass after CT, and puncture biopsy results did not exclude TB. Histopathological analysis led to the diagnosis of TB with massive caseous necrosis. The clinical manifestations of most TBs include systemic and respiratory symptoms, such as fever, night sweats, weight loss, as well as thrombocytosis. Although many reports have described TB-associated hematologic abnormalities (including thrombocytosis) as a reactive rise in platelet count due to TB causes, the mechanisms are unclear and may be related to factors such as TB activity, increased thrombopoietin, and cytokines. Additionally, there are also reports mentioning IL-6 synthesis and release after phagocytosis of Mycobacterium tuberculosis by macrophages in primary TB, which subsequently activates inflammatory cells and causes systemic effects, such as induction of acute phase reactants and thrombocytosis [12]. The association between TB and thrombocytosis is rare, but reactive thrombocytosis is common in patients with active TB. Nonetheless, there are few cases of thrombocytosis with platelet counts >1000×109/L. A case of TB peritonitis with thrombocytosis was reported in 1974 [13] in a 20-year-old female patient who presented with marked thrombocytosis (platelet count > 1372 x109/L) and a 2-month history of generalized abdominal pain and swelling. Upon examination, she was febrile (with a body temperature of 38°C) and emaciated. Her abdomen appeared doughy, and signs of ascites were observed; however, there was no hepatomegaly or splenomegaly. Furthermore, her chest x-ray was normal. During peritoneoscopy, the visceral and parietal peritoneum was found to be studded with tubercles, and nodule biopsy confirmed the presence of caseous tuberculosis. A bone marrow aspiration specimen showed a myeloid reaction with an increased number of megakaryocytes and normal morphology. After treatment with streptomycin, isoniazid, and ethambutol, the platelet counts gradually decreased to 1152×109/L, 1034×109/L, and 579×109/L at 3, 4, and 8 weeks, respectively. Thus, there may be a correlation between thrombocytosis and TB in this patient. In contrast, the patient in this case study recovered well from TB when regular quadruple anti-TB treatment was administered after mass resection and interferon and hydroxyurea platelet-lowering therapy was applied at the same time. However, the platelet count always decreased insignificantly; thus, thrombocytosis in the patient in this case study may not be correlated with TB.

The WHO 2016 version of the classification criteria for myeloid neoplasms and acute leukemia includes three driver mutations, JAK2, CALR, and MPL, as the main diagnostic criteria for MPN [14]. In addition to these driver mutations, several recent studies have identified the presence of other genetic mutations in MPN, such as ASXL1, EZH2, IDH1, IDH2, and SRSF2, as high-risk mutations (HMR), and have shown an association with poor prognosis in MPN [5, 15, 16]. In addition, there is a subset of patients who are negative for BCR/ABL, JAK2/V617, MPL, and CARL genes, but ET cannot be excluded due to limited genetic testing and can be diagnosed as triple negative thrombocythemia (TN-ET) according to the diagnostic criteria. In 2016, WHO designated the diagnostic criteria [14]. ET can be diagnosed by meeting four main criteria or the first three main criteria and secondary criteria. The main criteria were as follows: 1. Consistent PLT count ⩾ 450 × 109/L; 2. Bone marrow biopsy showing highly proliferated megakaryocytes with an increased number of mature megakaryocytes with large cytosomes and lobulated nuclei, no significant proliferation or leftward shift of granular and red lineages, and minimal and mild (Grade 1) increase in reticulocytes; 3. Failure to meet the diagnosis of MDS, BCR-ABL+CML, PV, PMF, and other diagnostic criteria for myeloid neoplasms; 4. The presence of JAK2, CALR or MPL gene mutations. The secondary criterion was evidence of clonal markers or unresponsive thrombocytosis. In this case, biopsy histopathology confirmed the diagnosis of chest wall tuberculosis after chest wall mass resection, and the PLT count persisted >450×10^9/L even after 8 months of regular anti-tuberculosis drug treatment with the 1HRZE regimen. A bone marrow smear and biopsy suggested megakaryocytosis, large size, multi-lobularity, platelets in piles and patchy distribution, and multiplicity, with no significant hyperplasia or left shift of granular and red lineages. Absence of morbid hematopoiesis, primitive naïve cells, etc., did not meet the diagnostic criteria for CML, PV, PMF, MDS, or other myeloid neoplasms; were negative for BCR/ABL, JAK2V617F, CALR, and MPL fusion genes. Individuals who met the first three diagnostic and secondary criteria of the main criteria were diagnosed with TN-ET. Compared to JAK2 mutated cases, TN-ET patients were younger and had lower hemoglobin levels and lower thrombotic incidence [17, 18]. This patient was 23 years old at the time of initial diagnosis and had a very low thrombosis risk (no history of thrombosis, aged less 60 years, and no JAK2 mutation).

JAK2V617F is by far the most common mutation in BCR-ABL1-negative MPN [19], with 95% of PV patients, 65% of PMF patients, and 55% of ET patients having JAK2V617F mutations, in addition to the more commonly mutated MPL and CARL genes. ET patients have higher JAK-STAT3 activity, and JAK2V617F-negative ET may have mutations in other genes of the JAK-STAT pathway. JAK2 V617F is found in most patients with myeloproliferative neoplasms, and tyrosine phosphorylates SOCS3 and escapes its repression. In addition, the JAK2 exon 12 mutant described in the V617F-negative MPN disease set also stabilizes tyrosine phosphorylated SOCS3 [20, 21]. It has been reported that differentially expressed miRNAs between JAK2V617F-positive and -negative patients could explain the activation of the JAK/STAT pathway in the absence of V617F mutation. The miRNA alone, in combination with the methylation process, could explain the downregulation of SOCS1 and SOCS3 in ET-JAK2V617F-negative patients, and thus participate in the activation of the JAK2 pathway [22]. Bioinformatic analysis of this patient, who was JAK2V617F negative, detected changes in the expression of the downregulated gene SOCS3. Thus, suppressor of cytokine signaling (SOCS 3), a negative regulator of erythropoietin receptor (EPOR) and receptor-associated JAK2 kinase, may be a novel MPN biomarker and a potential mechanism for mutant JAK2 kinase to overcome SOCS3 inhibition [20]..

Discussion / Page 9-14

Patient Perspective	**12**	Hydroxyurea was discontinued and replaced by weekly injections of 180 ug polyethylene glycol a-2b (pegaptan), after which the patient's PLT count fluctuated from 440–520×109/L.	**Lines 139-142 / Page 7**
Informed Consent	**13**	Written informed consent for this case report has been obtained from the patient.	**Lines 275-276 / Page 14**
Additional Information	**14**	Acknowledgments :This study was financially supported by the Hebei Province 2020 Medical Science Research Project Plan (20200727).	
		Disclosure :The authors report no conflicts of interest in this work.	**Lines 277-281 / Page 15**