

Sarcoidosis detected after COVID-19 with T-SPOT.TB positive: A case report

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Abstract. Sarcoidosis is an idiopathic multisystem disorder with unknown etiology. Due to clinical similarities among sarcoidosis, tuberculosis (TB) infection and malignant diseases (such as lymphoma, lung carcinoma and pituitary tumor), the diagnosis of sarcoidosis is challenging. The present report describes a case of sarcoidosis in a 48-year-old male with complaint of chest pain 1 month after Coronavirus disease 2019. The patient underwent whole-body ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) PET-CT imaging, which revealed multiple lymphadenopathies throughout the body without lung parenchyma involvement. Biochemical examinations such as T-SPOT.TB test and pathological examination of right supraclavicular lymph node revealed positive T-SPOT.TB but negative Ziehl-Neelsen staining. However, non-caseating epithelioid granulomas were observed in the mediastinal biopsy, indicating the diagnosis of sarcoidosis. The patient was clinically stable, and the symptom of chest pain was gradually relieved without any specific treatment. Outpatient follow-up continued every 3 months. The present case suggested a possible link between coronavirus infection and sarcoidosis, which suggests the advantages of ¹⁸F-FDG PET-CT for the detection of sarcoidosis. However, T-SPOT.TB is insufficient for differentiating between sarcoidosis and TB.

Introduction

Sarcoidosis is a systemic disorder of unknown etiology that is characterized by the presence of non-caseating epithelioid cell granulomas (1). However, recent studies have suggested that adverse autoimmune reactions and microbial organisms can serve an important role in its pathogenesis (2-4). Lung involvement including pleural, pulmonary parenchyma and trachea, is the most commonly observed presentation, followed by skin, eyes and joints (1,5). In addition, mediastinal lymph nodes are found to be affected in the majority of sarcoidosis cases (6). Sarcoidosis has a wide range of clinical phenotypes, ranging from acute to asymptomatic. However, some patients do experience severe symptoms that necessitate potent immunosuppressive treatments, including corticosteroids, methotrexate or antitumor necrosis factor- α agents. The majority of patients with sarcoidosis die from pulmonary fibrosis (1,5).

Coronavirus disease 2019 (COVID-19) is a disease induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 can induce a series of immunoinflammatory responses to disturb self-tolerance and trigger autoimmune responses (7,8). Tana *et al* (9) proposed that there may be a link between COVID-19 and sarcoidosis, since they have similar clinical manifestations and may influence each other at multiple levels, eventually affecting their clinical courses and prognosis (9).

Both sarcoidosis and tuberculosis (TB) are characterized by typical epithelioid cell granulomas (10). Previous reports have proposed the two diseases may have similarities in progression, whilst others have suggested that tuberculosis-causing mycobacterial antigens are the inciting agents in a proportion of sarcoidosis cases (11,12). The coexistence of sarcoidosis and TB infection has also been previously reported (13,14). This similarity in clinical features renders the differential diagnosis between these two diseases challenging.

The present report describes a case who presented with multiple lymphadenopathies throughout the body after COVID-19 and was T-SPOT.TB positive, but without lung parenchyma involvement. The present case report aims to suggest possible connection between COVID-19 and sarcoidosis, and provide insights to sarcoidosis diagnosis.

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Case report

A 48-year-old male presenting with chest pain for 1 week presented himself into Jinling Hospital (Nanjing, China) in January 2023. The pain was described as having no specific area, no heart burn feeling or chest pressure. It was not provoked by exertion, nor obviously relieved by rest. There were also no complaints of fever, dizziness, fainting, dyspnea or coughing. The patient had been diagnosed with SARS-CoV-2 by real time RT-PCR (RT-qPCR) testing in early December 2022 (Fig. S1), when the patient had high-grade fever (38–39°C), cough and sore throat for 4 days. He received symptomatic treatments, including cough suppressants and antipyretics, at home and recovered within 1 week. The patient had a history of hypertension for 10 years and received sustained-release felodipine tablets (5 mg/per day), with no previous history of TB. Physical examination revealed bilaterally soft and swollen cervical lymph nodes in bean size but they were not painful. All other systemic examinations and vital signs were normal.

Extensive biochemical and radiological evaluations were subsequently conducted. 12-lead ECG, coronary arteriography, echocardiography and serum markers of myocardial damage, including creatine kinase [84 U/l (normal range, 50–310 U/l)], the MB isoenzyme of creatine kinase [1.7 ng/ml (normal range, 0–3.7 ng/ml)], troponin T [0.009 ng/ml (normal range, 0–0.014 ng/ml)] and troponin I [0.03 ng/ml (normal range, <0.06 ng/ml)], were all performed and there was no evidence of cardiovascular disease. The creatine kinase level was detected using a Model 7600 Series Automatic Analyzer (Hitachi, Ltd.). The MB isoenzyme of creatine kinase level and troponin I level were detected using an AIA-2000 Automated Immunoassay Analyzer (Tosoh Bioscience). Troponin T level was detected using a cobas e601 module (Roche Diagnostics).

RT-qPCR testing for SARS-CoV-2 yielded negative results. Serum autoantibody testing revealed that the anti- β 2 glycoprotein-1 antibody level was 100.00 RU/ml (normal range, <16 RU/ml), anti- β 2 glycoprotein-1 IgG antibody level was 29.5 AU/ml (normal range, <16 AU/ml), the anti- β 2 glycoprotein-1 IgM antibody level was 56.2 AU/ml (normal range, <16 AU/ml), the anticardiolipin antibody (ACA) level was 22.8 RU/ml (normal range, <12 RU/ml) and the ACA IgM level was 26.8 MPLU/ml (normal <12 MPLU/ml). The serum level of angiotensin-converting enzyme was 25.0 U/l (normal range, <52.0 U/l). Antinuclear antibody and erythrocyte sedimentation rate revealed no significant abnormalities. Further biochemical examinations revealed positivity in T-SPOT.TB [Panel A of early secreted antigenic target 6 (ESAT6), 2 spot-forming cells (SFC); panel B of 10-kDa culture filtrate protein (CFP-10), 14 SFC; normal range for both, 0–6 SFC].

For RT-qPCR, the RNA of pharynx swab sample was extracted by using the Stream SP96 Automatic Nucleic Acid Extraction Machine (Daan Gene) and RNA Isolation Kit (cat. no. ME-0012, Shanghai ZJ Bio-Tech Co., Ltd). RT-qPCR kits for SARS-CoV-2 nucleic acid testing were obtained from Shanghai ZJ Bio-Tech Co., Ltd (Novel Coronavirus 2019-nCoV Real Time Multiplex RT-PCR Kit Detection for 3 Genes, W-RR-0479-02). RT-qPCR was performed on an Applied Biosystems 7500 real-time RT-PCR system (Thermo Fisher Scientific, Inc.) and the manufacturer's protocol was followed. The program was set as follows: pre-amplification

45°C for 10 min, 95°C for 180 sec and 45 cycles of 95°C for 15 sec and 58°C for 30 sec. The result was identified as positive if cycle threshold (Cq) value <40 (15). The T-SPOT.TB test from Oxford Immunotec (T-SPOT.TB Multi-use 8-Well Strip Plate Format. Catalogue number: TB.300) was used and we followed the manufacturer's instruction. The blood specimen was collected and extracted by using CellSep® Pro Instrument (Eureka bio, CS101) to create a standard peripheral blood mononuclear cell suspension. They were then added into specially designed plates in the kit and stimulated with TB-specific antigens, ESAT-6 and CFP10. Cells responding to these antigens would release IFN- γ , which were captured by the enzyme-labeled antibody and detection reagent to produce spots. Spots were then counted manually under a light microscope (magnification, x40).

Chest CT revealed lymphadenopathies in the bilateral hilar, mediastinum and supraclavicular areas (Fig. 1). ¹⁸F-FDG PET-CT was then performed to evaluate the whole-body condition and search for the possibility of neoplastic foci, such as lymphoma. The results demonstrated multiple lymphadenopathies in the bilateral supraclavicular regions, mediastinum, bilateral hilar and left cardio-diaphragmatic angle, with pathological uptake [maximum standardized uptake value (SUV_{max})=19.49], suggesting the possibility of malignancy. It also showed nodal abnormality in the bilateral pleura (SUV_{max}=2.55). In addition, abdominal PET-CT showed lymphadenopathies with high metabolic activity (SUV_{max}=9.98) in the retroperitoneal area and bilateral diaphragmatic angle, along with striped shape high metabolic activity of the small and large intestine (SUV_{max}=12.19), likely an inflammatory disease due to the presence (Fig. 2). Gastrointestinal endoscopy revealed intestinal polyps (data not shown), where further pathological examination indicated hyperplastic polyps to rule out cancer.

For histopathological examinations, samples of tissues from right supraclavicular lymph node and mediastinal masses were fixed in 10% neutral buffered formalin for 24 h at room temperature. The samples were then embedded in paraffin and sectioned into 4- μ m sections. For H&E staining, tissue sections were washed successively with xylene, 95% ethanol, 85% ethanol, 70% ethanol, and double distilled water, then hematoxylin stain was used for 5 min and eosin for 1 min, both at room temperature. The samples were observed using a light microscope (magnification, x200). For Ziehl-Neelsen staining, the following protocol was applied: i) For the primary stain, Carbol fuchsin was prepared by dissolving 1 g basic fuchsin in 10 ml ethanol (100%), whilst 5 g carbolic acid was dissolved in 100 ml distilled water at the same time, before these two aforementioned reagents were mixed together. A piece of filter paper was first placed on the paraffin section before the carbol fuchsin was dropped onto the paper. This was left to stain for 30 min at room temperature; ii) the section was rinsed with water, followed by 5% hydrochloric acid in ethanol for 10 sec at room temperature; and iii) the section was covered with 0.7% methylene blue for 5–10 sec at room temperature and observed with a light microscope (magnification, x200). Bacteria would be stained with bright red color.

Pathological examination was performed 3 days after the patient's admission to the hospital, right supraclavicular lymph node dissection was performed at first due to its

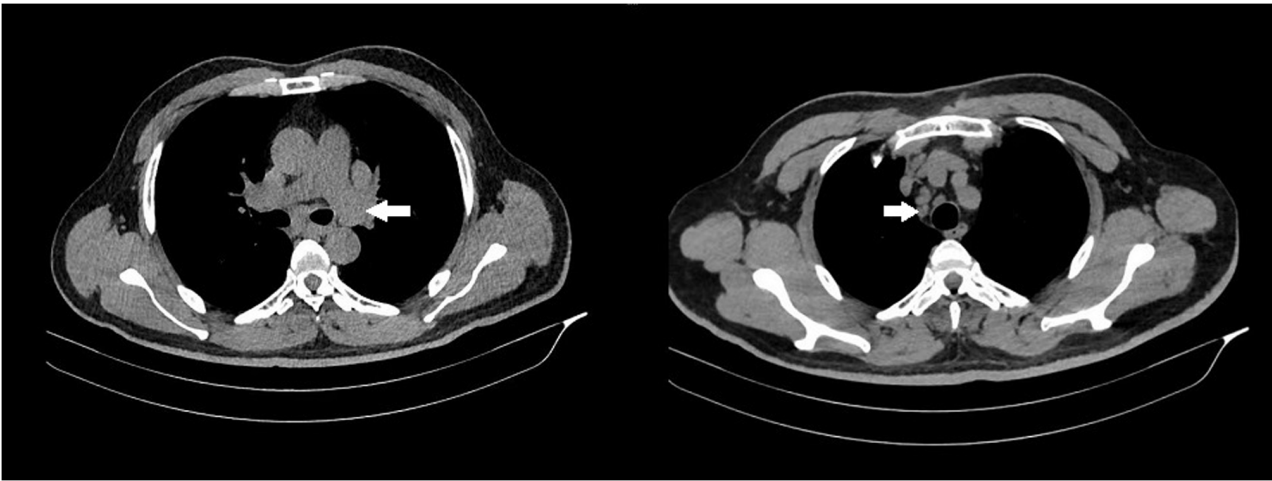


Figure 1. CT images of the chest showing bilateral hilar and mediastinal lymphadenopathy. White arrows indicate swelling lymph nodes.

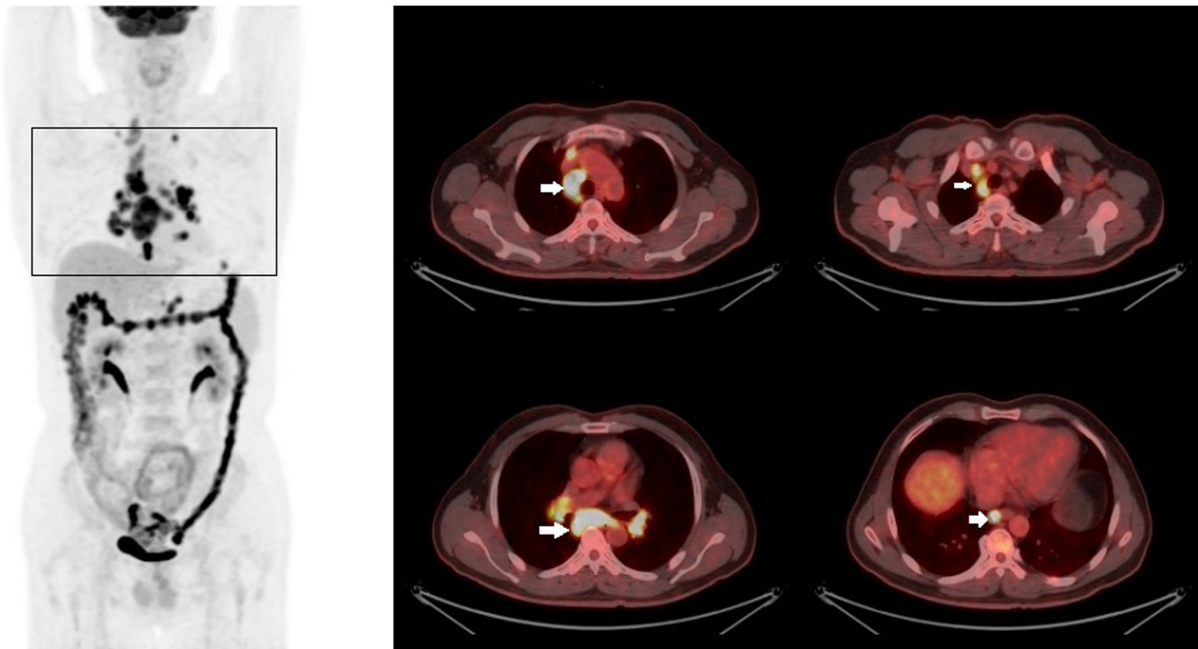


Figure 2. ^{18}F -FDG PET/CT maximum intensity projection and ^{18}F -FDG PET/CT axial slice images at diagnosis. Images showed pathological FDG uptake in the bilateral supraclavicular regions, mediastinum, bilateral hilar and left cardio-diaphragmatic angle, with pathological uptake (maximum standardized uptake value=19.49). ^{18}F -FDG, ^{18}F -fluorodeoxyglucose. White arrows indicate pathological FDG uptake regions.

approachable position, the result revealed reactive adenopathy (Fig. 3A and B). Subsequently CT-guided biopsy of the mediastinal masses was performed 1 week later, where non-caseating epithelioid granulomas and negative Ziehl-Neelsen staining were observed (Fig. 3C and D).

The patient had no cough or sputum, meaning sputum culture that is normally used for detecting *tubercle bacillus* was not available. However, the patient had no prior history of tuberculosis, no symptoms of tuberculosis and his pathological granulomas region showed negative Ziehl-Neelsen staining, which all suggested that the patient did not have active tuberculosis. Considering the clinical features, disease history and the pathological examination results of the patient, there were no concrete elements supporting cancer, therefore sarcoidosis was deemed the most likely diagnosis. The patient was

diagnosed with sarcoidosis manifesting as multiple lymphadenopathies. The patient's chest pain was gradually relieved without treatment and discharged one week after he presented, and condition of the patient remained stable at last follow-up in August 2023.

Discussion

A dysregulated immunoinflammatory response against antigens can be observed in sarcoidosis and other granuloma-forming diseases caused by acute or chronic bacterial and viral infections (16). Some patients with SARS-CoV-2 developed subcutaneous nodules with granulomatous histology similar to sarcoidosis (17-19). Subcutaneous nodules on the arms, shins, lateral thighs, glabella, submental and

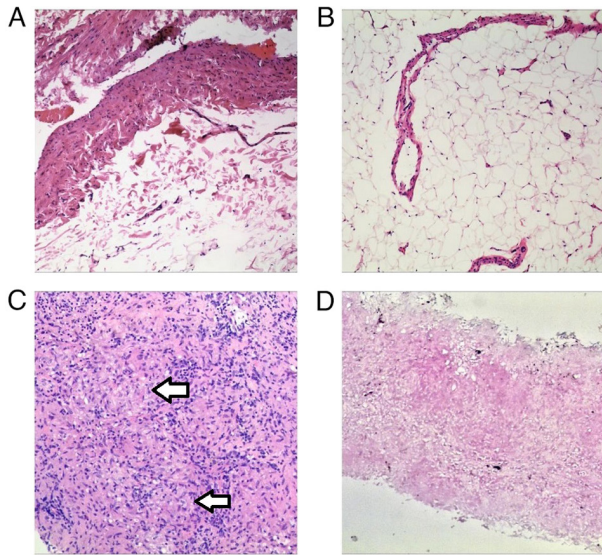


Figure 3. Histopathological examination. (A) Right supraclavicular lymph node biopsy showed fibrous tissue. H&E staining (magnification, x200). (B) Right supraclavicular lymph node biopsy showed adipose tissue. H&E staining (magnification, x200). (C) Histopathological assessment of the mediastinal masses. White arrows indicated non-necrotizing granulomas characterized by epithelioid histiocytes accompanied by lymphocytes. H&E staining (magnification, x200). (D) Ziehl-Neelsen staining of mediastinal masses was negative (magnification, x200).

bilateral pulmonary hilar lymphadenopathy have been detected in patients within 1-2 weeks of a positive RT-qPCR result for SARS-CoV-2 (20). It was hypothesized that the two diseases may share common pathological activities, including the regulation of apoptosis and immune tolerance through the programmed cell death protein-1/programmed death ligand-1 axis (21-23). In the present case, the patient was diagnosed with sarcoidosis 1 month after infection with SARS-CoV-2. Although the patient did not have chest radiography when SARS-CoV-2 was detected, on the basis of his symptoms and clinical history, it was hypothesized that there was a potential link between sarcoidosis and COVID-19.

T-SOPT:TB is a type of IFN- γ release assay (IGRA) that is widely used to identify infection by *tubercle bacillus* (24). A previous meta-analysis by Sester *et al* (20) indicated that the sensitivity of IGRAs in patients with bacteriologically confirmed tuberculosis was 80-81%, whilst the specificity was 59% (20). By contrast, in another study, the sensitivity was reported to be 87.5% whereas the specificity was 86% (25). In addition, there appeared to be a low rate of T-SPOT:TB positivity in patients with sarcoidosis regardless of the stage, where the possible mechanism may be associated with shared immune response (26). According to the WHO-consolidated guidelines on tuberculosis, Module 3: Diagnosis-Tests for tuberculosis infection (27), T-SPOT:TB can be used to test for tuberculosis infection but with exceptionally low certainty of evidence. Therefore, IGRAs alone are insufficient for the diagnosis of TB in patients with sarcoidosis and requires both pathological and microbiological evidence.

Traditional diagnostic approaches, such as CT and X-ray, are particularly useful for the detection of pulmonary sarcoidosis, but they have limits for the evaluation of extra-pulmonary involvement. ^{18}F -FDG PET-CT is widely used for

the diagnosis, staging and therapeutic assessment of malignancies and inflammatory diseases. It can reveal the anatomical localization of residual FDG uptake throughout the whole body, providing guidance for further diagnostic tests (28,29). Previous studies have demonstrated the good diagnostic performance of ^{18}F -FDG PET-CT for several inflammatory and infectious diseases, including fever of unknown origin and inflammation of unknown origin (30,31). The ^{18}F -FDG PET-CT result of the present patient revealed extrathoracic involvement that traditional CT examination could not fully detect, such that the value of FDG uptake further guided subsequent lymph node biopsy.

The diagnosis of sarcoidosis is mainly based on the exhibition of non-caseating epithelioid granulomas (5). In the present case, the pathological examination of right supraclavicular lymph node showed only reactive lymphadenopathy. Therefore, the pathological diagnosis of sarcoidosis will likely require biopsy procedures of more than one site and whether mediastinal biopsy will yield a superior pathological positive rate compared with other sites for diagnosing thoracic sarcoidosis needs further validation.

Although sarcoidosis developing after COVID-19 has been reported previously (32), the patient in the present case had a unique clinical performance other than the typical respiratory symptoms. In addition, the patient was tested T-SPOT:TB-positive, which necessitated additional procedures in delineating the diagnosis from active tuberculosis. Therefore, to the best of our knowledge, the present case was the first to document the convergence of all three diseases into discussion. In conclusion, results of the present case suggest a possible link between SARS-CoV-2 infection and sarcoidosis pathogenesis. In addition, T-SPOT:TB positivity alone will likely not be sufficient for diagnosing TB in patients with sarcoidosis. A diagnostic case with radiological examinations using ^{18}F -FDG PET-CT and multisite biopsy reported in the present account potentially provides a guide for the accurate diagnosis of sarcoidosis.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

QW and XC designed and supervised the study. XL and JG analyzed the data and images. QW and CC performed the literature review and wrote the manuscript. QW, CC and XL

confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Written informed consent was obtained from the patient for the publication of the present case.

Competing interests

The authors declare that they have no competing interests.

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