An Unusual Case of Brucellosis in Japan: Difficulties in the Differential Diagnosis from Pulmonary Tuberculosis


Human brucellosis is an imported zoonosis extremely uncommon in Japan. Brucellosis was found in a 38-year-old surgeon who had never been abroad; he developed intrapulmonary infiltrates and pleural thickening indistinguishable from pulmonary tuberculosis. Despite extensive antibiotic therapy for tuberculosis he developed systemic serositis. A culture of resected lung tissues grew CO₂-required coccobacilli. Polymerase chain reaction test using a specific primer pair for Brucella abortus revealed that the isolated pathogen is highly homologous to B. abortus. We emphasize that undetermined cases of human brucellosis may be present in Japan. (Internal Medicine 35: 310-314, 1996)

Key words: Brucella abortus, imported zoonosis, pulmonary disease, polymerase chain reaction

Introduction

Human brucellosis is a systemic disease that presents non-specific symptoms, and is a major health problem worldwide (1). In Japan, human brucellosis is thought to be an uncommon imported disease, of which no report has been published since 1977. Though it had sporadically occurred up to 1950, knowledge of human brucellosis among practicing physicians does not appear to be sufficient. In this report we describe the clinical and laboratory findings of human brucellosis which were difficult to distinguish from tuberculosis. We discuss the possibility of the existence of identified cases.

Case Report

A 38-year-old Japanese male surgeon was first admitted to the Sapporo Medical University Hospital on September 2, 1993, with low grade fever, night sweats, dry cough and bilateral chest pain continued for approximately three months. He had previously been in good health and has never been abroad. Laboratory data showed a white blood cell (WBC) count of 9,000/mm³, erythrocyte sedimentation rate (ESR) of 59 mm/1h and C-reactive protein (CRP) of 7.1 mg/dl. Mantoux reaction was strongly positive to human tuberculin purified protein derivative. Chest X-ray and computed tomography (CT) scan showed bilateral infiltrative shadows and pleural thickening in the upper lung fields (Fig. 1). Sputum culture revealed neither bacteria nor fungus. The polymerase chain reaction (PCR) of sputum was positive for mycobacterium tuberculosis on the 50th hospital day, whereas Ziehl-Neelsen stain and culture for acid-fast bacilli were negative. Based on these findings, a diagnosis of pulmonary tuberculosis was made at this stage. Treatment was started with daily administration of isoniazid, rifampicin and streptomycin. Two months later, his condition was improved except for the chest X-ray finding. In November 1993, streptomycin was replaced with ethanbutol.

At five months following discharge, in April 1994, he was easily fatigued, and had a low grade fever, bilateral chest pain and swelling of left axillary lymph nodes, which was histologically diagnosed as follicular hyperplasia. PCR test was negative for mycobacterium. Laboratory data showed a WBC of 9,700/mm³ with a normal differential, ESR of 85 mm/1h, CRP of 12.2 mg/dl, alkaline phosphatase of 317 IU/ml and r-glutamyl transpeptidase of 111 IU/ml. The abdominal echogram and CT findings showed no hepatosplenomegaly but mild wall thickening of the gall bladder. On the chest X-ray, the pleural thickening was slightly enhanced, with intrapulmonary infiltrative shadows adjacent to it. Despite continued antibiotic therapy...
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Figure 1. Chest radiograph and CT scan on the first admission. A) Chest radiograph shows consolidation, especially in the upper lung zones, and the dulled costophrenic angles. B) CT scan shows a pattern of ill-defined small nodules, a few of which have coalesced.

with additional administration of new quinolones and antifungal agents, chest X-ray shadows developed (Fig. 2). Sputa cultures for microorganisms were repeatedly performed, but did not reveal any pathogens. On June 26, 1994, endoscopic transthoracic pleuropulmonary biopsy was performed. Specimens were obtained from segment numbers 4 and 6 of the right lung where changes on chest CT were predominant. Histologic examination showed intraluminal polypoid fibrosis, intraluminal aggregation of histiocytes, a scattering of a few neutrophils and fibrinous materials, interstitial fibrosis with lymphocytic infiltrates, and fibrosis of the interlobular septa and pleura. There was no evidence of acid-fast bacilli or fungi.

Cultivation of the resected lung tissue was performed with brain heart infusion broth in 10% CO₂. The culture revealed growth of gram-negative coccobacilli after 6 days of incubation. This pathogen required CO₂ was catalase-positive, oxidase-positive, hydrogen sulfide-productive and fuchsinsensitive. On the basis of these bacteriologic findings, the pathogen was identified as *Brucella abortus*. Subsequently, serologic studies were performed to detect antibodies to brucella; a standard tube-agglutination test was negative (<1:20) without a prozone phenomenon, whereas a complement-fixation test were positive (62.5 IU). The lack of agglutination with brucella antigen may be due to the presence of a blocking substance (personal communication from Dr. Edward J Young, Microbiology and Immunology, Baylor College of Medicine, Texas). To confirm the serological and biological diagnosis for the pathogen, PCR test for the detection of *Brucella* spp. was performed using specific outer (B4/B5) and inner (B4/BKR-1) pairs reported by Baily et al (2). Positive results were obtained in DNA samples from the lung tissue (June 26, 1994), blood (August, 1994) and the isolated coccobacilli (Fig. 3). Nucleotide sequence of an amplified 224 bp product was found to be highly homologous (97%) to the corresponding region of *B. abortus* gene for BCSP31 reported by Mayfield et al (3) (data not shown). By antibiotic sensitivity tests, the pathogen isolated was shown to be susceptible to minocyclin, amikacin and some new quinolones, but resistant to cephalosporins. On the second admission (July 4, 1994), therefore, minocyclin and amikacin were given with rifampicin, which had been continued since the first admission. Despite continuous antibiotic therapy, by the 30th hospital day, he had a high grade fever, body weight loss of 15 kg, hepatosplenomegaly, ileus, pericarditis and meningitis with increasing inflammatory reactions. Chest X-ray findings at this stage revealed pulmonary edema and pericardial effusion (Fig. 4). Culture of drainage from intestinal juice grew gram-negative coccobacilli biologically similar to those iso-

Figure 2. Chest radiograph 4 days before endoscopic transthoracic pleuropulmonary biopsy. More consolidation is seen especially in the right middle lung zone.
Figure 3. PCR test for *B. abortus*. A) The successful detection of *B. abortus* in DNA sample from the pathogen was recognized by PCR test using a specific primer pair (B4/B5; 224bp) and the protocol reported by Baily et al (2). B) Nested amplification for the detection of *B. abortus* was performed in DNA samples from the lung tissue specimen (t) and blood (B) using a outer primer pair (B4/B5; 1st PCR, 224bp) and an inner primer pair (B4/BKR-1; 2nd PCR, 88bp). We constructed an inner primer pair, B4 as an inner sense primer and 5'-CGTTATAGCCCAATAGGCAACG-3' (BKR-1; ntd 855-877) as an inner anti-sense primer in a region of the sequence coding for BCSP31.

Figure 4. Chest radiograph 30 days after second admission. Enlarged cardiac outline and the “bat wing” distribution of predominant mid-zone shadowing spreading from the hilar regions.
The combination of doxycycline and streptomycin may be the effective, but have a high relapse rate (>20%) when used singly. Several drugs (e.g. streptomycin, gentamicin, doxycycline, minocycline, co-trimoxazole, rifampicin and ciprofloxacin) are effective, but have a high relapse rate (>20%) when used singly. The combination of doxycycline and streptomycin may be the most effective and least toxic (15, 16).

Although the incidence of brucellosis had markedly decreased in developed countries as mentioned by recent reports (12, 15, 17), it should be recalled that Brucella spp. could be imported from endemic areas. In Japan, human brucellosis may sporadically occur due to the increase in traveling abroad and to the importation of dairy products from countries endemic for brucellosis. Since serological tests for B. abortus are uncommon and specific techniques are required for cultivation, we speculate that unidentified cases of brucellosis may be present in Japan. We emphasize that, in patients with a continuous fever of unknown origin, especially in travelers returning from regions endemic for brucellosis, the serological tests, PCR for brucellosis, and prolonged cultivation for at least 35 days should be performed.

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References

