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Original Article

Clinical features and serum profile of inflammatory biomarkers in patients with brucellosis

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Abstract

Introduction: Brucellosis is a zoonosis caused by *Brucella*, a highly infectious Gram-negative coccobacillus that has been isolated from a variety of mammals. *Brucella melitensis* is considered the most common cause of human brucellosis. The aim of this retrospective study was to evaluate the diagnostic value of serum parameters that relate to *Brucella melitensis* infection.

Methodology: This investigation retrospectively analyzed the clinical laboratory tests and symptoms of brucellosis, which was confirmed by microbiological and serological methods. A total of 36 patients (31 males, 5 females; mean age 49.17 ± 13.56 years) admitted with brucellosis were included in the study over a three-year period between 2012 and 2015 in Peking University People's Hospital (PKUPH).

Results: A statistically significant increase was observed in C-reactive protein and erythrocyte sedimentation rate in patients with low titers of serum antibody when compared with those with high titers. No difference was observed between the two groups with respect to the other serum parameters such as procalcitonin or white blood cell count. Two blood culture systems also yielded different results.

Conclusions: In this study, we demonstrated that culture can be improved by using multiple blood culture systems to isolate *Brucella melitensis*. We also found the different role of inflammatory markers play during the process of brucellosis. The present study may be a helpful reference in the diagnosis of brucellosis.

Key words: brucellosis; *Brucella melitensis*; serum agglutination assay.

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Introduction

Brucellosis is a re-emerging bacterial zoonosis that is broadly distributed across the world. The symptoms and clinical signs most commonly reported are fever, fatigue, malaise, sweats, headaches, myalgia, and arthralgia. Fever and arthralgia are the most common presenting symptoms. Cardiovascular disease is one of complications of brucellosis, which is caused by *Brucella* spp. and involves multiple systems [1,2]. *Brucella* spp. endocarditis (BE) is an uncommon infection of the cardiovascular system, but it is one of the most challenging complications of brucellosis [3,4]. *Brucella melitensis* is highly virulent and associated with a severe clinical course, particularly endocarditis. The first-line treatment is most often broad-spectrum antibiotics; after positive culture, the therapy is modified and continued during brucellosis and after the procedure. The treatment of choice for *Brucella* spp. infections has typically been doxycycline. A combination of minocycline and other antimicrobials are the recommended treatment regimens at Peking University People's Hospital (PKUPH).

Due to easy dissemination, multiple routes of infection, and high environmental contamination and morbidity rates, *Brucella* spp. are considered a serious health hazard to populations living in areas where the disease is endemic or near a pasturing area. *Brucella* spp. are aerobic, Gram-negative coccobacilli found in healthy animals, particularly cattle, swine, goat, and sheep, as well as wild animals. Goat, sheep, and cattle have the highest carriage rates in China. *Brucella* spp. usually infect people through contaminated meat and direct contact with the infected animals [5]. Generally, in patients with risk factors such as consumption of contaminated foods and occupational contact, *Brucella* spp. should be considered as a potential pathogen.

Human brucellosis is usually confirmed by microbiological serological methods. The isolation of *Brucella* spp. from blood also is one of the diagnostic methods of choice for brucellosis. When the culture is found negative, investigation of classic serologic tests and antibodies is important in the diagnosis of brucellosis. Blood culture is a specific method to confirm the infection of *Brucella* spp.; however, blood culture is cumbersome and time-consuming, and thus a

serum agglutination assay together with other serum indicators may be an alternative that can be comparatively easily used. However, there are still some factors that may cause a false-negative result, such as the presence of a blocking antibody. The main purpose of this work was to evaluate the roles of classic serological methods and some serum markers of bacteremia in the populations studied, in order to analyze the role of these serum parameters in diagnosing brucellosis.

C-reactive protein (CRP) is a special type of protein produced in the liver that is present during episodes of acute inflammation or infection. Serum procalcitonin (PCT) level is also helpful for ruling out infection in patients with chronic inflammatory diseases and for distinguishing infections from systemic inflammatory diseases in the initial evaluation of patients presenting with acute fever [6]. The PCT level seems to be a reliable tool for predicting bacteremia in patients.

This retrospective study presents an evaluation of data from our diagnostic laboratory on brucellosis from the years 2012–2015. Here, we characterize the clinical features and laboratory profiles of *Brucella* spp. infection in brucellosis patients. The study's aims were to determine the clinical and laboratory features of brucellosis, and to evaluate the risk factors associated with brucellosis.

Methodology

Study population

The documents of 36 patients with *Brucella* spp. bloodstream infection who had been hospitalized in PKUPH, between 2012 and 2015, were retrospectively reviewed. A definitive diagnosis of brucellosis is based on the isolation of *Brucella* spp. from blood. The PKUPH's laboratory information system was searched for all patients with positive blood cultures, and patients' documents were reviewed to summarize clinical data. The patients were grouped by the serum antibody titers: brucellosis patients with low serum IgG titers (< 1:160) and those with high titers (≥ 1:160).

Microbiological studies

Bacterial isolation was conducted using standard blood culturing techniques (BacT/ALERT, bioMérieux Inc., Durham, USA; BACTEC FX, Becton Dickinson, Franklin, USA). When the blood culture was positive for oxidase, catalase and urease and also for the presence of Gram-negative bacilli/coccobacilli able to grew on blood agar, further characterization was performed using biochemical methods. Serology is frequently preferred because of the high risk of laboratory infections associated with culture of *Brucella* spp. Serum agglutination was used as the main laboratory parameter to monitor the course of brucellosis patients who were included in this study. Serologic evaluation was performed using a *Brucella* spp. agglutination test (Huma Tex Febrile Antigens, Wiesbaden, Germany). A titer of 1/160 or higher was considered strongly suggestive of acute infection. The serum agglutination test (SAT) results were classified as negative, 1/160 positive, 1/320 positive, 1/640 positive, or 1/1,280 positive. The associations between SAT titer and other serum parameters were examined.

Laboratory analysis

Inflammatory markers most frequently measured in the clinical assessment of brucellosis provide useful information. CRP, PCT, and erythrocyte sedimentation rate (ESR) were measured within 48 hours of the first positive blood culture draw. ESR and white blood cell (WBC) count are classic laboratory markers of inflammation applied to monitor the progress of infection. A group of serum parameters that usually relates to tissue cell damaging were examined. These parameters, which include alanine transaminase (ALT), lactate dehydrogenase (LDH), aspartate transaminase (AST), creatine kinase (CK), and α-hydroxybutyrate dehydrogenase (α-HBDH) were examined at the same time as the initial blood culture.

Statistical analysis

Descriptive statistics including means, frequencies, and percentages were used to summarize the data. Continuous variables were expressed as means ± standard deviation. Student's t-test was used to test for the significance of differences between the means. Chi-squared (Fisher's exact test in the case of a small sample) was used to compare groups of categorical variables. Two-tailed P values were calculated; a level of 0.05 was considered statistically significant. All data were analyzed with SAS 9.02 (SAS, Cary, USA).

Results

This study was a retrospective analysis of the laboratory data and clinical features of 36 brucellosis cases. Clinical data from patients with a brucellosis diagnosis were analyzed comprehensively. The median age of the patients was 49 years (range: 18–77), and 31 (86%) were male. Fever and arthralgia were the most common symptoms. Fever and arthralgia were present in 58.3% and 50% of patients, respectively, whereas splenomegaly was present in 11.1% of the patients. The primary mode of transmission was by direct contact

Table 1. Demographic and clinical characteristics of 38 patients with brucellosis.

Characteristic	N	%
Age, years [median (range)]	49.17 (18–77)	
Gender		
Male	31	86.1
Female	5	13.9
Diagnosis on admission		
Fever (outpatient)*	11	30.6
Infection department	9	25
Rheumatology & immunology	2	5.6
Cardiovascular disease	6	16.7
Orthopedic surgical oncology	1	2.8
Pulmonology clinics	3	8.3
Emergency department	3	8.3
Gastroenterology clinics	1	2.8
Risk factor		
Pastoral residents	11	30.6
Animal keeper	10	27.8
Consumption of mutton	5	13.9
Veterinarian doctor	4	11.1
Others	6	16.7
Clinical represents		
Fever	21	58.3
Arthralgia	18	50
Weakness	12	33.3
Hepatosplenomegaly	4	11.1
Testicular pain**	2	6.5

*Outpatients were admitted after diagnosis were made; **Only male patients.

with infected animals and consumption of contaminated meat (Table 1).

The diagnosis of brucellosis is usually confirmed by isolation of the organism from blood. One study reported a mean detection time of 51.2 ± 8.2 hours using BacT/ALERT standard aerobic bottles [7]. Another investigation showed the mean detection time of *Brucella* spp. using BacT/ALERT system to be 2.5 days [8]. Table 2 shows the time that was required by each of the different culture techniques in the present study to detect *Brucella* spp. This study showed that the detection time of BacT/ALERT and BACTEC FX were 69.76 ± 6.56 and 94.38 ± 18.3, respectively. Blood culture result varies depending on the progress of brucellosis. In this retrospective study, 36 patients underwent testing for antibody by SAT. Of the 36 patients tested, 19 (43%) were SAT positive for *Brucella* spp.

CRP, ESR, and WBC count are classic laboratory markers of inflammation used to monitor the progress of infection. In the laboratory, the mean WBC count was 5.18×10^9/L, mean ESR was 28.85 ± 24.52 mm/h, CRP was 39.08 ± 33.05 mg/L, and procalcitonin was 0.238 ± 0.416 ng/mL. CRP is an acute-phase reactant protein synthesized by the liver within six hours of the onset of an infectious process [9]. CRP is a sensitive and widely used acute-phase sepsis marker [10]. CRP measurements appear to be useful for monitoring patient response to therapy after the primary diagnosis of brucellosis, and for monitoring both endocarditis patients after *Brucella* spp. infection and patients with serious brucellosis. A multicenter study revealed mild to moderate increases in ESR and CRP in brucellosis patients [11]. The present study showed that CRP and ESR levels were significantly higher in brucellosis

Table 2. Patients' laboratory results.

Laboratory findings	Value
TTP (BACTEC FX)	94.38 ± 18.3
TTP (BacT/ALERT)	69.76 ± 6.56
CRP (0–10) mg/L	40.61 ± 33.05
ALT (7–40) U/L	50.83 ± 27.22
AST (13–35) U/L	60.67 ± 37.50
LDH (109–245) U/L	340.91 ± 222.31
α-HBDH (72–182) U/L	276.52 ± 192.42
CK (43–165) U/L	86.70 ± 70.19
ESR (0–20) mm/h	31.24 ± 24.52
PCT	2.12 ± 6.67
WBC (3.5–9.5)	5.32 ± 2.46
Leukocyte differential count	54.55 ± 16.71

TTP: time to positive; CRP: C-reactive protein; ALT: alanine transaminase; AST: aspartate transaminase; LDH: lactate dehydrogenase; α-HBDH: α-hydroxybutyrate dehydrogenase; CK: creatine kinase; ESR: erythrocyte sedimentation rate; PCT: procalcitonin; WBC: white blood cell count.

Table 3. Comparison of characteristics of patients with different serum agglutination levels.

	IgG ≥ 1:160	IgG < 1:160	p
ALT (U/L)	54.47 ± 28.72	40.42 ± 26.43	0.16
AST (U/L)	60.59 ± 39.08	56.38 ± 35.5	0.752
LDH (U/L)	358.1 ± 188.6	368.1 ± 255.1	0.838
CK (U/L)	87.18 ± 81.34	74.87 ± 52.81	0.619
α-HBDH (U/L)	323.4 ± 173.8	303.7 ± 211.2	0.783
WBC	4.74 ± 1.99	5.67 ± 2.84	0.271
Leukocyte differential count	49.79 ± 15.11	61.98 ± 16.01	0.027*
ESR (u n)	19 ± 15.51	40.33 ± 28.16	0.033*
CRP (u n)	24.23 ± 26.69	59.23 ± 29.81	0.002*
PCT (u n)	0.111 ± 0.023	0.365 ± 0.62	0.306

ALT: alanine transaminase; AST: aspartate transaminase; LDH: lactate dehydrogenase; CK: creatine kinase; α-HBDH: α-hydroxybutyrate dehydrogenase; WBC: white blood cell count; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; PCT: procalcitonin.

patients with low serum IgG titers (< 1:160) than in those with high titers (≥ 1:160) (p = 0.002 and 0.033, respectively). The present study showed that PCT levels measured in brucellosis patients with higher titers were lower than in those with low titers, although there were no statistically significant differences (Table 3).

With respect to inflammatory markers measured in patients with brucellosis, the WBC count was not found to be significantly elevated (5.18 × 10^9/L), with around 56% neutrophils. In the present study, there was no significant increase in the WBC count. The mean CRP levels were high in the whole study population (39.08 ± 33.05 mg/L). However, in 22% of patients, the CRP levels were low (< 10 mg/mL). ESR levels were significantly elevated in 47.2% of patients, unlike CRP, which was elevated in 77.8% of patients (Table 4).

However, both ESR and CRP levels were markedly elevated in half of the patients, whereas the procalcitonin levels were rarely elevated. The level of ALT was higher in 10 (27.8%) both SAT-positive and SAT-negative brucellosis patients, and AST level was increased in 11 (30.6%) SAT-positive brucellosis and 8 (22.2%) SAT-negative brucellosis patients. High levels of LDH were detected in 12 (33.3%) SAT-positive

Table 4. TTP, CRP and ESR associated with serum agglutination test.

Variable	IgG ≥ 1:160		IgG < 1:160		p
	No. of patients	% of patients	No. of patients	% of patients	
TTP					
BacT/ALERT	4	11.1	15	41.7	< 0.001*
BACTEC FX	15	41.7	2	5.6	
CRP					
≤ 10 mg/L	5	13.9	3	8.3	0.6951
> 10 mg/L	14	38.9	14	38.9	
ESR					
< 20	12	33.3	5	13.9	0.0543
> 20	7	19.4	12	33.3	
ALT					
≤ 40	9	25	7	19.4	0.7486
> 40	10	27.8	10	27.8	
AST					
≤ 40	8	22.2	9	25	0.7388
> 40	11	30.6	8	22.2	
LDH					
≤ 245	7	19.4	9	25	0.5027
> 245	12	33.3	8	22.2	
CK					
≤ 164	15	41.7	14	38.9	1.0000
> 165	2	5.6	3	8.3	
α-HBDH					
≤ 182	7	19.4	8	22.2	0.736
> 182	12	33.3	9	25	

TTP: time to positive; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; ALT: alanine transaminase; AST: aspartate transaminase; LDH: lactate dehydrogenase; CK: creatine kinase; α-HBDH: α-hydroxybutyrate dehydrogenase.

brucellosis and 8 (22.2%) SAT-negative brucellosis patients, and the level of CK was higher in 2 (5.6%) SAT-positive brucellosis patients and 3 (8.3%) SAT-negative brucellosis patients. A total of 12 (33.3%) SAT-positive brucellosis and 9 (25%) SAT-negative brucellosis patients exhibited elevated levels of α-HBDH (Table 4).

Discussion

Brucellosis is a zoonosis caused by *Brucella* spp., highly infectious Gram-negative coccobacilli that have been isolated from many mammals, including humans. Due to easy dissemination, multiple routes of infection, high environmental contamination, and their potential application in bioterrorism, *Brucella* spp. are considered to be a category B select agent by the Centers for Disease Control (CDC) [12]. Consistent with other studies, the present investigation found that consumption of contaminated foods and occupational contact remain the main sources of infection. Fever and arthritis are the most common signs.

Brucella melitensis is a Gram-negative, non-motile coccobacillus (or short rods) belonging to the *Brucella* family and is considered the principal cause of human brucellosis [13]. The diagnosis of brucellosis is usually based on clinical evaluation and laboratory results. Generally, the diagnosis of brucellosis needs to be confirmed by a serum agglutination test or isolation of the organism from blood or other sterile samples [14,15]. Though blood culture is the gold standard in the diagnosis of bacteremia, more than three days are required for the final results to be available, and the sensitivity is low. The diagnosis of brucellosis based on blood culture may be challenging because a relatively low sensitivity is associated with the disease progress. There is different performance among different blood culture systems [7,16]. Both BacT/ALERT and BACTEC FX blood culture systems are widely used to recover *Brucella* spp. However, the time to positive (TTP) in the BacT/ALERT blood culture was more than 20 hours faster than that for the BACTEC FX system. When the culture result is negative, investigation of classic serologic tests and antibodies occupy an important role in the diagnosis of brucellosis. A serological test is often considered a key indicator in the diagnosis of brucellosis; however, IgG begins to form after the onset of disease, in three weeks, and peaks in two months. Moreover, immune response can be reduced, giving false-negative results from standard agglutination assays in patients who have a long period of disease evolution. A previous study demonstrated that the antibodies increase more significantly in acute brucellosis than that in chronic brucellosis patients [17]. Therefore, serological tests also failed to screen some brucellosis cases. There were eight cases with a positive blood culture but with negative SAT in this investigation. Both blood culture and serologic test are critical indicators for diagnosing brucellosis effectively. With respect to the correlation between the culture media and SAT, BacT/ALERT recovered more isolates in patients with a low SAT titer, and the BACTEC FX systems performed well in patients with a high SAT titer. In view of this, the use of a variety of culture systems could benefit finding pathogens earlier compared to using a single culture system during the whole progression of brucellosis.

The early assessment of the risk of *Brucella* spp. infection in patients presenting with fever relies on a combination of information derived from clinical examination and laboratory parameters, such as CRP level, ESR, WBC count, and leukocyte differential count. Although these parameters lack specificity for early diagnosis of *Brucella* spp. infections, they are still helpful to indicate a consideration of brucellosis. In this study, we wanted to evaluate the relationship of a number of serum parameters to deduce the diagnosis of *Brucella* spp. infection in patients admitted to the hospital for suspected brucellosis. CRP has been demonstrated to be associated with brucellosis and might be used to determine the activity of acute brucellosis [18]. CRP level alone is not a good predictive factor for *Brucella* spp. infection, although nearly 80% of the patients with *Brucella* spp. infection had CRP > 10 mg/L upon admission. ESR, WBC count, and CRP values changed with brucellosis were used for evaluating whether there was a correlation between the severity of brucellosis and a higher titer in a *Brucella* spp. SAT test. We found that there were significant differences among patients grouped by different titers in terms of ESR, CRP, and leukocyte differential count (Table 3).

The early assessment of the risk of *Brucella* spp. bacteremia in patients presenting with arthralgia, fever, and having risk factor(s) brucellosis depends on a combination of information derived from clinical examination and laboratory parameters, such as CRP level, ESR, and WBC count. Brucellosis is routinely overlooked, misdiagnosed, or at best diagnosed incidentally; therefore, physicians must become aware of and consider brucellosis in their differential diagnosis of febrile diseases [19]. The present study investigated 36 brucellosis cases with different clinic profiles that were helpful in making a differential diagnosis of brucellosis. Serologic parameters play a

crucial role in the diagnosis of brucellosis because of the uncertain culture and antibodies results that are correlated with the stage of the disease. Early diagnosis and treatment is crucial in the management of brucellosis, and therefore serum markers, including CRP and ESR, should be considered. Blood culture is the method of choice, but specimens need to be obtained early, and cultures often need long periods of incubation. The sensitivity of serological tests is often considered as a key indicator while diagnosing brucellosis.

Generally, the present study demonstrated the different performance of blood culture systems; BacT/ALERT recovered more isolates in patients with a low SAT titer and the BACTEC FX systems performed well in patients with a high SAT titer. We also found there were significant differences among patients grouped by different SAT titer in terms of ESR, CRP, and leukocyte differential count. In addition, plenty of comprehensive clinical information and laboratory data were included in the manuscript, which will be helpful in the diagnosis of brucellosis.

Conclusions

The present study reported the different performance of BacT/ALERT and BACTEC FX blood culture systems in patients with different SAT titers. Significant differences were found among patients grouped by different titers in terms of ESR, CRP, and leukocyte differential count. With respect to the different progress of brucellosis, the combined use of both culture systems and serum parameters could help to improve proficiency in diagnosis of brucellosis.

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