Original Article

Serum levels of soluble CD163 and soluble CD14 following antibiotic therapy of patients with acute brucellosis

Huali Sun¹, Rongmeng Jiang¹, Bing Han¹, Xiufang Du², Chengjie Ma³, Yanli Xu¹, Zhihai Chen¹, Linghang Wang³, Siyuan Yang³, Xiao Lv⁴, Hong Zhao⁴, Xingwang Li¹

¹ Centre for Infectious Disease, Beijing Di Tan Hospital, Capital Medical University, Beijing, China
² Department of Infectious Disease, the Third People’s Hospital, Linfen City, Shanxi Province, China
³ The Laboratory of Infectious Diseases Centre, Beijing Di Tan Hospital, Capital Medical University, Beijing, China
⁴ Department of Laboratory Medicine, the Third People’s Hospital, Linfen City, Shanxi Province, China

Abstract

Introduction: Soluble CD163 (sCD163) and soluble CD14 (sCD14) levels, monocyte/macrophage activation markers, are elevated in patient serum during Brucella infection. The aim of this study was to measure serum sCD163 and sCD14 levels during treatment for acute brucellosis to determine whether they can be used to monitor treatment efficacy.

Methodology: Blood samples were collected from 30 patients with acute brucellosis (disease duration < 8 weeks) before and after 6 weeks of antibiotic therapy as well as from a comparison group of 28 healthy control individuals. Serum sCD163 and sCD14 levels were measured with specific, sandwich enzyme-linked immunosorbent assays. The clinical data and routine indices including C-reactive protein (CRP), erythrocyte sedimentation rates (ESR), as well as white cell counts (WBC) were also studied.

Results: Both serum sCD163 and sCD14 levels were significantly higher in patients with acute brucellosis than in healthy controls (p < 0.0001). A significant decline was observed in patients after cessation of treatment (p < 0.001), which still be significantly higher than that in healthy controls (p < 0.001). In additional, serum sCD163 levels were positively correlated with sCD14 levels; both of which were positively associated with CRP levels. However, neither sCD163 nor sCD14 levels were correlated with ESR or WBC.

Conclusions: The decline in sCD163 and sCD14 levels following antibiotic therapy may be used as a marker to assess therapeutic efficacy following treatment of acute brucellosis.

Key words: Biomarker; sCD14; sCD163; brucellosis.


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Introduction

Brucellosis is one of the most common worldwide zoonotic infections, which is particularly prevalent in developing countries. About 500,000 new human infections occur annually [1]. The disease is caused by intracellular bacteria belonging to the genus Brucella. The bacteria can survive and replicate within mononuclear phagocytic cells of the reticuloendothelial system, easily establishing a persistent infection in humans [2], and leads to the development of chronic disease with a strong impact on public health.

Currently, biomarkers to predict treatment outcome and relapse are not available in human brucellosis [3]. Activation of macrophages represents one of the earliest events in the innate defence against intracellular bacterial infection. Increasing evidence suggests an important role for circulating macrophage-derived biomarkers in blood and other biological fluids that reflect the activation of macrophage population in the clinical assessment of intracellular bacterial infections. Previous studies have shown that increased sCD163 and sCD14 levels are associated with poor outcome in patients with tuberculosis [4-9]. For leprosy and leishmaniasis, serum sCD163 levels have also been shown to be indicative of disease severity [10].

CD163 is a monocyte/macrophage-specific glycoprotein that functions as a scavenger receptor primarily by binding to haptoglobin-haemoglobin complexes to clear them out [11]. In response to inflammatory stimuli, CD163 can be shed from the macrophage surface as a soluble form of CD163 (sCD163) which has been associated with the alternatively activated M2 macrophages, and M2 macrophages have been shown to down-modulate inflammatory response [12]. CD14 is a myeloid differentiation marker found primarily on monocytes.
and macrophages. As a co-receptor for lipopolysaccharide (LPS) from Gram-negative bacteria, engagement of CD14 leads to potent cellular activation and release of proinflammatory cytokines. CD14 is shed from cells as soluble CD14 (sCD14) which have two opposite functions. sCD14 can either inhibits pro-inflammatory signalling cascade by competing with membrane CD14 for LPS binding or mediates the LPS-induced activation of non-CD14-expressing cells, including endothelial and epithelial cells [13,14].

The most crucial event in innate immunity to Brucella is the activation of macrophages [15]. Importantly, increased levels of sCD163 and sCD14 in the serum have been described in brucellosis [16-18]. However, it is unknown whether they can be used as markers of treatment efficacy during acute brucellosis. In this study, serum sCD163 and sCD14 levels were measured in patients during acute brucellosis (disease duration < 8 weeks) and as well after the completion of antibiotic treatment, to determine the prognostic usefulness of those measures.

**Methodology**

**Study population**

This study was performed from January 2016 to April 2017. Thirty patients with acute brucellosis were enrolled from the Department of Infectious Disease of the Third People’s Hospital of Linfen in Shanxi Province and Beijing Di Tan Hospital. All patients received the same antibiotic treatment regimen (ceftriaxone at 2 g/day for 2 weeks, doxycycline at 100 mg twice daily for 6 weeks, and rifampin at 600–900 mg/day for 6 weeks). A diagnosis of brucellosis was confirmed by the isolation of Brucella spp. from blood cultures and/or by serologic test according to current criteria [19]. The exclusion criteria were: antibiotic treatment during the 3 months prior to the study; evidence of auto-immune disease or cancer, heart, renal, and/or respiratory insufficiency; endocarditis; or neuro-brucellosis. Also we excluded patients who had taken immunosuppressants, immunomodulators, and/or other drugs capable of modifying the immune response. Breast-feeding women and subjects < 18 years of age were excluded. Twenty-eight healthy subjects from the same area, matched to the patients by gender and age, were recruited as controls. All healthy controls had not taken any medication for 4 weeks prior to sampling.

Written informed consent was obtained from each patient according to the Declaration of Helsinki. The Ethical Committee of Beijing Di Tan Hospital, Capital Medical University, approved this study.

**Routine Non-specific Laboratory Tests**

Serum CRP concentrations were measured in duplicate with specific ELISA kits (R&D Systems, Inc., Minneapolis, USA) and measuring range of human serum CRP was 0.11 - 4.29 mg/L. White blood cells (WBC) and erythrocyte sedimentation rate (ESR) were assayed by standard laboratory methods.

**Measurement of sCD163 and sCD14 in Serum Samples**

Blood was collected from healthy subjects and patients before antibiotic treatment and at 6 weeks after the end of treatment, and serum prepared. All serum samples were stored at −80°C until analyses. Serum sCD163 (R&D Systems, Inc., Minneapolis, USA) and sCD14 (R&D Systems, Inc., Minneapolis, USA) were measured in duplicate with specific ELISA kits according to the manufacturer’s instructions. The results were expressed as ng/mL. Minimum detectable levels of human sCD163 and sCD14 were 0.177 and 0.125 ng/mL, respectively. Range for detection was broadened by dilution of high concentration samples. Recovery of sCD163 and sCD14 for those samples were 101% – 110% and 84% – 107%, respectively. All assays were performed in a laboratory of the Infectious Diseases Centre using Gen5™ 3.0 (Bio Tek Synergy H1, Winooski, USA). A four-parameter logistic curve was used to interpolate values from a standard curve at 450 nm wavelength.

**Statistical analysis**

Statistical analysis and graphical presentation were performed with Graph Pad Prism 7.0 software (San Diego, CA, USA). Data are expressed as median values and analysed using a Wilcoxon ranked sum test for paired values (i.e. pre and post-treatment for the same subject) or Mann–Whitney U-test for between subject comparisons (i.e., patients versus healthy controls). Correlation analyses were performed using the Spearman rank correlation test. All tests were two-tailed, and p values < 0.05 were considered statistically significant.

**Results**

There were 24 males and 6 females in the study. Disease duration for all patients was no more than 8 weeks. The demographic features, complaints, physical examination and laboratory findings are shown in Tables 1 and 2. The most common complaint and sign of the patients were fever, sweating and weakness, respectively. All of the 30 patients were clinically cured, that symptoms and signs disappeared and CRP and ESR values returned to normal level. The cured
patients were followed up six months after healing and no recurrences were noted.

Twenty-eight healthy controls (24 men and 4 women) with a mean age of 46 years (range, 24-69 years) were concurrently studied. There was no statistical difference between patients and controls with regard to gender or mean age (p > 0.05). All healthy controls had a negative Rose-Bengal test. SAT (data are not shown), ESR and CRP were within normal ranges (Table 2).

Serum sCD14 and sCD163 levels in patients with acute brucellosis and in healthy controls are shown in Figure 1 and Table 2. Median sCD14 and sCD163 levels were all significantly elevated in patients with acute brucellosis when compared to healthy controls (Figure 1 A-B; p < 0.001). Thirty patients were evaluated after treatment and sCD14 and sCD163 serum levels were decreased significantly (p < 0.001), but still significantly greater than that of healthy controls (Figure 1 A-B; p < 0.001).

We further evaluated the correlation between serum sCD163 and sCD14 along with traditional inflammatory markers including CRP, ESR and WBC. The results of correlation analysis using mixture data from all cases before treatment and after treatment (n = 60) showed a significant positive correlation between sCD14 and sCD163 levels (r = 0.327, p = 0.011; Figure 2); we also found that serum sCD163 levels correlated

![Figure 1. Serum sCD14 and sCD163 levels of 30 patients with acute brucellosis (pre- and post-treatment) as well as levels for 28 healthy control individuals.](image_url)

**Table 1. Characteristics of patients with acute brucellosis.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>30</td>
</tr>
<tr>
<td>Age (years) (mean [range])</td>
<td>48 (20-69)</td>
</tr>
<tr>
<td>Sex (no. males/no. females)</td>
<td>24/6</td>
</tr>
<tr>
<td>Epidemiological characteristics</td>
<td></td>
</tr>
<tr>
<td>Rural residence</td>
<td>24 (80.0)</td>
</tr>
<tr>
<td>Urban residence</td>
<td>6 (20.0)</td>
</tr>
<tr>
<td>Transmission mechanisms</td>
<td></td>
</tr>
<tr>
<td>Direct contact with infected animals</td>
<td>26 (86.67)</td>
</tr>
<tr>
<td>Ingesting raw milk</td>
<td>2 (6.67)</td>
</tr>
<tr>
<td>Ingesting undercooked meat of sheep</td>
<td>2 (6.67)</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>25 (83.33)</td>
</tr>
<tr>
<td>Sweating</td>
<td>25 (83.33)</td>
</tr>
<tr>
<td>Weakness</td>
<td>23 (76.67)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>10 (33.33)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>16 (53.33)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>16 (53.33)</td>
</tr>
<tr>
<td>Lumbar pain</td>
<td>11 (36.67)</td>
</tr>
<tr>
<td>Testicular swelling and pain</td>
<td>1 (3.33)</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>1 (3.33)</td>
</tr>
<tr>
<td>Sacroiliitis</td>
<td>4 (13.33)</td>
</tr>
<tr>
<td>Disease duration, median days (range)</td>
<td>21 (7-56)</td>
</tr>
<tr>
<td>Diagnostic procedures</td>
<td></td>
</tr>
<tr>
<td>Positive haemoculture</td>
<td>16 (53.33)</td>
</tr>
<tr>
<td>Rose-Bengal test</td>
<td>30 (100)</td>
</tr>
<tr>
<td>Positive serum agglutination (SAT)</td>
<td>30 (100)</td>
</tr>
</tbody>
</table>

1 Unless otherwise indicated, data are provided as the number of cases (percentage of the total population studied); 2 The SAT titre ≥ 1/160; SAT: serum agglutination test.

Circulating levels of (A) sCD14 and (B) sCD163 in serum samples of brucellosis patients (pre- and post-treatment) compared to healthy controls, represented by dots. The horizontal line of (A) and (B) represents the median. A Mann–Whitney U-test for continuous variables was performed to evaluate significant difference between the controls and patients. The patients at pre- and post-treatment were evaluated by the Wilcoxon matched-pair signed-rank test for (A) and (B). sCD14: soluble CD14; sCD163: soluble CD163.
positively with serum CRP levels (r = 0.373, p = 0.003) but not ESR or WBC (Table 3), and serum sCD14 levels are correlated positively with serum CRP levels (r = 0.349, p = 0.006) but not ESR or WBC (Table 3).

**Discussion**

In this paper, we showed that serum sCD163 and sCD14 levels are increased in patients with brucellosis, which confirms previous findings that infection with *Brucella* might trigger the shedding of surface CD163 and CD14 from the monocytes/macrophages [16-18]. An additional finding was that serum sCD163 and sCD14 levels decreased with antibiotic therapy in patients with early *Brucella* infection, suggesting that serum sCD163 and sCD14 levels may be useful in monitoring treatment responses in patients with acute brucellosis.

Brucella interplay with the human immune system is critical for the development of chronic parasitism or infection clearance [15]. The host immune response to Brucella is initiated through innate immune mechanisms that recognize bacterial components and provide the necessary signals for the induction of an adaptive immune response. Macrophages are polarized cells and can be divided into M1- and M2- polarized macrophages, and M1 macrophages is capable of inducing the production of pro-inflammatory mediators such as TNF-α upon LPS stimulation, M2 macrophages induces the production of anti-inflammatory mediators such as sCD163, IL-10 and IL-4 [20-22]. sCD163 has been identified as a direct anti-inflammatory mediator to attenuate immune response to the phorbol ester by preventing proliferation and activation of T lymphocytes [23]. All of these indicated that CD163 shedding is considered to be a specific marker of activated M2 macrophages and may have potential anti-inflammatory properties.

Activation of macrophages is triggered by the specific binding between bacterial components and pattern recognition receptors, including CD14 molecules [24,25]. Study on mice model of brucellosis

![Figure 2. Line graph showing a positive correlation between serum sCD14 and sCD163 levels in patients with acute brucellosis (r = 0.327, p = 0.011, n = 60).](image-url)
showed that CD14/TLR4 complex can be activated by Brucella LPS, resulting in the production of proinflammatory cytokines (TNF-α, IL-12, IL-6) which are required for efficient clearance of the bacteria [26]. Another study also found that decreased expression of membrane CD14 by RNA interference reduces the production of TNF-α and nitric oxide in RAW264.7 cells infected with B. melitensis. The evidence has suggested that CD14 contribute to inflammatory process during Brucella infection [27]. Soluble form of CD14-(sCD14)-exerts inflammation suppression roles by interfering with membrane CD14 signalling during Brucella infection. In addition, sCD14 can function as an immunoregulator which can inhibit T lymphocyte activation [28,29] and as an acute-phase protein during infectious disease as well [30]. Thus, serum levels of these might be a candidate prognostic indicators of human brucellosis.

To our knowledge, there has been only one report in the literature about sCD163 levels in brucellosis. This report shows that serum sCD163 levels were higher than levels of healthy controls, but results did not permit the differentiation of the three forms of the disease (acute, subacute, and chronic) [18]. However, there were no reported data on the effects of antibiotic therapy on sCD163 during Brucella infection, we focus on it in our study for the first time. It was determined that increased serum sCD163 levels were shown to significantly decline in patients with acute brucellosis following antibiotic therapy by our data.

Two previous studies have investigated that serum sCD14 levels were increased significantly in human brucellosis, antibiotics did not produce a significant decline in sCD14 levels [16,17]. According to our research, there are different results with others’ research, it shows that increased serum sCD14 levels significantly decline after antibiotic therapy of six weeks in patients with acute brucellosis. In order to analyse inconsistent reasons, one may be differences in disease durations, which others’ study [16] (range 1-52 weeks) were longer than our study (range 1-8 weeks). Another reason– may be differences in medication, which our regiment was doxycycline and rifampin for 6 weeks plus ceftriaxone for 2 weeks in study, but others’ regiment was doxycycline for 6 weeks combined with streptomycin for 2 weeks or rifampin for 6 weeks. Triple antibiotic therapy (our regiment) is maybe better than dual antibiotic therapy (others’ regiment), this question need to require further investigation. Furthermore, our study suggests that early diagnosis and treatment is crucial for a better outcome.

In our study, we further revealed that serum levels of sCD163 and sCD14 significantly correlated in a positive manner with CRP, which is an index of the non-specific severity of infectious diseases [31,32], suggesting that sCD163 and sCD14 levels can be used as markers of disease severity. We also observed a positive correlation between serum sCD163 and sCD14 levels, demonstrating that these two variables may be synergists of monocyte/macrophage activation, and they involved in the occurrence and development of inflammation in brucellosis. Meanwhile, our study suggests that residual monocyte activation persists in individuals, even though the patient’s clinical signs and symptoms were effectively controlled, CRP and ESR returned to healthy control levels, and sCD14 and sCD163 levels did not return to normal. These findings are in accordance with the some studies that serum sCD163 and sCD14 levels decreased in parallel with HIV RNA levels but did not return to HIV-seronegative levels [33,34].

Although there are important discoveries revealed by our study, it still has limitations including a restricted follow-up time, a small sample size, and subgroup non-included subacute and chronic as well. In addition, there are only two hospitals involved this study, it would be more multicentre to be in participation.

Conclusions
Taken together, these data demonstrate firstly serum sCD163 and sCD14 levels were generally high during acute infection but fell gradually during recovery, which may be useful to evaluate treatment outcomes in patients with acute brucellosis. Antibiotic treatment does not normalize serum sCD163 and sCD14 levels despite clinical improvement, which may suggest that monocytes/macrophages activation is sustained in patients with brucellosis. Further elucidation of this finding would require a longitudinal study with a larger sample size.

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Corresponding author
Xingwang Li
Capital Medical University, No.8, Jingshun East Street, Di Tan Hospital, Chaoyang District 100000, Beijing, China
Tel: 010-84322235
Fax: 010-84322804
E-mail: ditanxw@163.com

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