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Improved Early Detection of Focal Brucellosis Complications with Anti-*Brucella* IgG

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ABSTRACT To evaluate the associations of inflammatory factors and serological test results with complicated brucellosis, we recruited 285 patients with a diagnosis of brucellosis between May 2016 and September 2019. The patients were subsequently classified into two groups according to the presence of complications. We collected demographic and clinical information and routine laboratory test results in addition to anti-*Brucella* IgG and IgM levels. Anti-*Brucella* IgG and IgM were uniformly tested using enzyme-linked immunosorbent assays (ELISAs) in this study. Among the 285 patients with brucellosis, 111 (38.95%) had complicated brucellosis. Osteoarthritis occurred more often in the subacute and chronic stages than in the acute stage ($P = 0.002$). Genital infection occurred more frequently in the acute stage than in the other stages ($P = 0.023$). Fever was not frequently observed in complicated cases ($P < 0.001$). The erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP) and anti-*Brucella* IgM and IgG levels were higher in complicated-brucellosis patients than in uncomplicated-brucellosis patients ($P < 0.001$). Anti-*Brucella* IgG, with an area under the curve of 0.885 (95% confidence interval [CI], 0.847 to 0.924), was the most robust indicator of complicated brucellosis. Positive culture, anti-*Brucella* IgM, the ESR, and CRP could be considered indicators, but their efficacy was weaker than that of IgG. In conclusion, a high ESR, high CRP, high anti-*Brucella* IgM and IgG levels, and positive culture were indicators of complicated brucellosis; among these, anti-*Brucella* IgG was the most robust biomarker.

KEYWORDS *Brucella* spp, IgG, complicated brucellosis, enzyme-linked immunosorbent assay

Brucellosis is the most common zoonosis worldwide, with 500,000 new human cases diagnosed each year (1). Human brucellosis is well controlled in developed countries, with only sporadic cases relating to travel (2). Brucellosis is endemic in most developing countries, including China (3). From 2007 to 2017, a total of 435,108 cases of human brucellosis were reported in mainland China, with an average of 3,626 cases per month (4). The heavy burden of brucellosis in China calls for effective approaches to prevent and control this disease.

The diagnosis and treatment of brucellosis is still challenging for clinicians, and recurrence is one of the most characteristic manifestations of human brucellosis (1, 5, 6). Human brucellosis is caused by *Brucella* spp., which are slow-growing, facultatively intracellular bacteria (7). Bacteria can invade multiple tissues and organs and often induce chronic infection and focal infection. As a result, brucellosis has a wide range of clinical manifestations, and physical manifestations are usually nonspecific (1, 8). Compared to patients with conventional brucellosis without complications, those with complications involving specific tissues and organs usually present with more-severe

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illness and require a longer course of medication; despite the prolonged medication course, these patients still have high brucellosis-related mortality. In addition, complications occurring at different sites require different antimicrobial agents, considering their pharmacokinetics and pharmacodynamics (9–11). Therefore, early identification of complications and the selection of appropriate medical and surgical treatments are essential to reduce treatment failure, disability, and mortality due to brucellosis.

Traditionally, the diagnosis of brucellosis-related complications was based on symptoms and signs of focal inflammation as well as laboratory and radiographic findings in involved organs (12). However, the symptoms and signs of focal brucellosis are usually insidious and unspecific, leading to underdiagnosis or misdiagnosis of this disease (13–15). To date, no single biomarker is available to detect the presence of brucellosis-related complications (16). The white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and procalcitonin (PCT) are parameters that can, to some extent, reflect the level of inflammation within the human body (17); however, these four inflammatory factors are nonspecific and can be elevated in a variety of diseases. These four markers can be used to evaluate the status of brucellosis in clinical settings. Nevertheless, their role in detecting brucellosis-related complications is underinvestigated. Detection of antibodies (including IgM and IgG) against cytosolic proteins or S-form lipopolysaccharide (S-LPS) of *Brucella* by an enzyme-linked immunosorbent assay (ELISA) has become an increasingly acceptable method for the prompt diagnosis of brucellosis (7, 18). Anti-*Brucella* antibodies are products of adaptive humoral immunity and are pathogen specific. Their concentrations may be associated with the presence of complicated brucellosis; however, this remains to be explored.

We carried out the current study to evaluate the relationship of inflammatory factors and serological test results with complicated brucellosis in order to identify a good biomarker for the detection of complications.

MATERIALS AND METHODS

The medical records of inpatients who were diagnosed with brucellosis between May 2016 and September 2019 were reviewed by two experienced infectious disease specialists. The diagnosis of brucellosis has been described in detail in previous studies (18). In brief, the diagnosis of brucellosis was based on the proper clinical context, including history (occupational exposure, consumption of raw dairy/meat products, or living in an area of endemicity), clinical presentation (fever, sweating, arthralgia, or hepatosplenomegaly), and laboratory results, as well as at least one of the following: a positive bacterial culture, a positive standard tube agglutination (STA) test result, or a positive ELISA result. The recruited patients were subsequently classified as having complicated brucellosis, defined as brucellosis with focal infection confirmed by laboratory findings and/or radiographic findings, or uncomplicated brucellosis without specific organ involvement. For example, complicated neurobrucellosis was confirmed if patients presented with symptoms and signs of encephalitis (fever, headache, neck stiffness, positive pathological reflexes, etc.) and/or had a positive *Brucella* culture, the presence of anti-*Brucella* antibodies, or a positive STA result from cerebrospinal fluid (CSF). *Brucella*-induced endocarditis was diagnosed when echocardiography confirmed valvular damage or vegetation, and infection by other pathogens was excluded. Respiratory involvement was considered when patients presented with symptoms or physical signs related to the respiratory system and/or had abnormal findings on radiologic images. Osteoarticular involvement was considered if some inflammatory signs (swelling, pain, functional disability, heat, or redness) occurred in any peripheral osteoarticular location and/or there was radiographic evidence of abnormalities. Orchitis and epididymitis were diagnosed by the presence of scrotal enlargement, swelling, pain, or tenderness and/or abnormal findings on ultrasound examination, not due to other causes. Pregnant patients, patients younger than 16 years old, and patients with immune-compromising conditions, such as tumor or anti-immune therapy, were excluded.

We collected the demographic and clinical information of brucellosis patients, including sex, age, exposure history, fever, WBC count, ESR, CRP, PCT, STA results, pathogen culture results, serum anti-*Brucella* IgG and IgM, and radiographic findings. We classified the patients into two groups according to the presence or absence of complicated or focal brucellosis as described above and compared the baseline and clinical features of the two groups. The stages of brucellosis were defined as follows: the acute stage, with a duration shorter than 8 weeks from symptom onset to admission; the subacute stage, with a duration ranging from 8 weeks to 24 weeks; and the chronic stage, with a duration longer than 24 weeks (12).

Laboratory tests other than anti-*Brucella* IgG and IgM tests were routinely performed in the hospital's laboratory, and the results were collected retrospectively. Anti-*Brucella* IgG and IgM were uniformly tested using ELISAs by a specialized technician to ensure accuracy and reduce bias. A positive STA result was defined as a titer $\geq$1:100 with a minimum of 50% agglutination. A commercial ELISA kit was used to detect anti-*Brucella* IgG and IgM (IBL International GmbH, Germany). ELISA was performed following

TABLE 1 Organs involved in complicated brucellosis

Complication	No. (%) of patients
Neurobrucellosis	6 (5.41)
Osteoarthritis	68 (61.26)
Spinal infection	50 (45.05)
Sacroiliitis	17 (15.32)
Hip synovitis	1 (0.90)
Bronchitis and/or pneumonia	17 (15.32)
Genital infection	17 (15.32)
Endocarditis	3 (2.70)

the manufacturer's protocols, and the cutoff value for both positive IgM and IgG was ≥12 U/ml. In brief, for IgG detection, patient serum was first diluted in a 1:10 ratio, and then 100 μl of diluted serum was added to each well and incubated for 1 h. After that, surplus material was washed away using a balanced salt solution; an enzyme-conjugated reagent was added; and the sample was incubated for 30 min. After another round of washing, the substrate for the enzyme was added, and the sample was incubated for 20 min. Stop buffer was added, and the optical density (OD) was measured at 450 nm. The OD values of the controls were used to construct the standard curve. The OD values of the tested samples were calculated according to the standard curve. For the detection of IgM antibodies, the procedure was similar, with an extra step of preabsorption before the procedure.

Informed consent was obtained from each participant or their authorized relatives. This study was approved by the Ethics Committee of Qilu Hospital (document KYLL-2017-714).

The chi-square test or Fisher's exact test was used to compare the frequency data. Measurement data were analyzed by the Mann-Whitney U test. The Spearman correlation test was used to determine the correlation between two continuous variables. Receiver operating characteristic (ROC) curves were constructed for measurement data to determine the optimal cutoff values for diagnosing complicated brucellosis. Additionally, the sensitivity and specificity of each parameter in distinguishing complicated brucellosis from uncomplicated brucellosis were computed, and the area under the curve (AUC) was calculated. Continuous variables were converted into categorical variables according to cutoff values obtained from the ROC analysis. Binary logistic regression was employed to analyze the risk factors for complicated brucellosis. We used SPSS 22.0 (IBM Corp., Somers, NY, USA) for the statistical analyses, and statistical significance was defined as a two-tailed P value of <0.05.

All data included in this study are available upon reasonable request from the corresponding author.

RESULTS

A total of 285 brucellosis patients were enrolled in the current study, among whom 111 (38.95%) had complicated brucellosis. In addition, we found that five anatomical systems were affected by complications, and osteoarthritis was the most common focal complication. Detailed information about the kinds of complications and the proportions of patients experiencing those complications is listed in Table 1.

Demographic and clinical information for the 285 brucellosis patients and comparisons between the complicated-brucellosis and uncomplicated-brucellosis subgroups are shown in Table 2. A total of 122 (42.81%) of 285 patients were female. The median age of the cohort was 53 years (range, 17 to 87 years), with a large proportion (68.07%) of patients younger than 60 years old. Upon admission, 142 (49.82%) patients presented with the acute stage, 90 (31.58%) patients presented with the subacute stage, and 53 (18.6%) patients presented with the chronic stage. Patients with complicated brucellosis were more likely to have subacute or chronic brucellosis than patients with uncomplicated brucellosis, but the difference was not statistically significant ($P = 0.290$). Among the complications involving different systems, osteoarthritis occurred more often in patients with the chronic stage than in patients with the acute stage ($P < 0.001$). In contrast, genital infection was more common in patients with the acute stage than in patients with the chronic stage ($P = 0.023$) (see Table S1 in the supplemental material). Among the 285 patients with brucellosis, 62 *Brucella* isolates were obtained, 1 of which was from cerebrospinal fluid, while the remaining positive specimens were from blood. The positivity rates for culture in the acute, subacute, and chronic phases were 35.92%, 11.11%, and 1.89%, respectively. ELISA demonstrated a high positivity rate of 98.95% (IgG and/or IgM positive). When IgG and IgM were analyzed separately,

TABLE 2 Demographic and clinical information on patients with brucellosis

Parameter[a]	Value[b] for patients with brucellosis			
	Total (n = 285)	Uncomplicated (n = 174)	Complicated (n = 111)	P value
Female gender	122 (42.81)	73 (41.95)	49 (44.14)	0.716
Age (yr)	53 (17–87)	52.5 (18–87)	53 (17–86)	0.867
Age of:				
<60 yr	194 (68.07)	121 (69.54)	73 (65.77)	0.505
≥60 yr	91 (31.93)	53 (30.46)	38 (34.23)	
Contact history	214 (75.09)	126 (72.41)	88 (79.28)	0.191
Stage				0.290
Acute	142 (49.82)	93 (53.45)	49 (44.14)	
Subacute	90 (31.58)	52 (29.89)	38 (34.23)	
Chronic	53 (18.60)	29 (16.67)	24 (21.62)	
Fever	259 (90.88)	169 (97.13)	90 (81.08)	<0.001
WBC ($\times 10^9$/liter)	5.55 (1.85–43)	5.54 (1.85–43)	5.56 (2–9.9)	0.741
ESR (mm/h)	41 (3–120)	38 (8–120)	48 (3–87)	<0.001
C-reactive protein (mg/liter)	21 (0.95–130.5)	17 (0.95–130.5)	29 (1.13–101.58)	<0.001
Procalcitonin (ng/liter)	0.238 (0.01–3.14)	0.245 (0.011–0.85)	0.234 (0.01–3.14)	0.444
STA	186 (65.26)	106 (60.92)	80 (72.07)	0.054
ELISA result (U/ml)				
IgM	17.54 (1.07–175.6)	12.43 (1.07–175.6)	23.85 (1.29–86.97)	<0.001
IgG	79.05 (1.26–700)	47.05 (1.26–175.8)	140.9 (32.62–700)	<0.001
Positive culture	62 (21.75)	33 (18.97)	29 (26.13)	0.153

[a]WBC, white blood cells; ESR, erythrocyte sedimentation rate; STA, standard tube agglutination test; ELISA, enzyme-linked immunosorbent assay.
[b]Values are expressed as the number (percentage) of patients for categorical variables or as the median (range) for continuous variables.

the positivity rates for IgM and IgG were 61.40% and 96.14%, respectively (Table S2). The correlation coefficients of stage with IgM and IgG were −0.352 (P <0.001) and 0.408 ($P < 0.001$), respectively.

In this study, five parameters that were significantly different between complicated and uncomplicated brucellosis were determined. Fever was observed less frequently in patients with complicated brucellosis than in those with uncomplicated brucellosis ($P < 0.001$). The ESR and the CRP and anti-*Brucella* IgM and IgG levels were higher in complicated-brucellosis patients than in uncomplicated-brucellosis patients ($P < 0.001$). In addition, STA positivity was more common in complicated-brucellosis patients than in uncomplicated-brucellosis patients, but the difference was not statistically significant ($P = 0.054$). Moreover, the positivity rate of *Brucella* culture was higher in complicated-brucellosis patients than in uncomplicated-brucellosis patients (26.13% versus 18.97%); however, the difference was not statistically significant ($P = 0.153$).

ROC curve analysis was performed (Fig. 1) to identify the optimal cutoff values of age, WBC count, ESR, CRP, PCT, and serum anti-*Brucella* IgG and IgM for detection of the occurrence of focal brucellosis. In addition, the AUCs of the ROC curves, the optimal cutoff values, and the sensitivity and specificity at the established cutoff values for each parameter are listed in Table 3. As Fig. 1 and Table 3 show, the concentration of serum anti-*Brucella* IgG, which had an AUC of 0.885 (95% confidence interval [95% CI], 0.847 to 0.924), was the most robust biomarker of complicated brucellosis, followed by the level of anti-*Brucella* IgM (AUC, 0.660 [95% CI, 0.593 to 0.727]). The AUCs of anti-*Brucella* IgG in the acute, subacute, and chronic stages were 0.858 (95% CI, 0.794 to 0.923), 0.921 (95% CI, 0.864 to 0.977), and 0.911 (95% CI, 0.837 to 0.985), respectively. The cutoff values of IgG, IgM, ESR, CRP, WBC, PCT, and age were 101.12 U/ml, 20.025 U/ml, 45.5 mm/h, 27 mg/liter, 5.325 $\times$ 10^9/liter, 0.4005 ng/liter, and 65.5 years, respectively. A logistic regression model indicated that patients with positive cultures had a higher risk of brucellosis complications than those with negative cultures (odds ratio [OR], 2.68

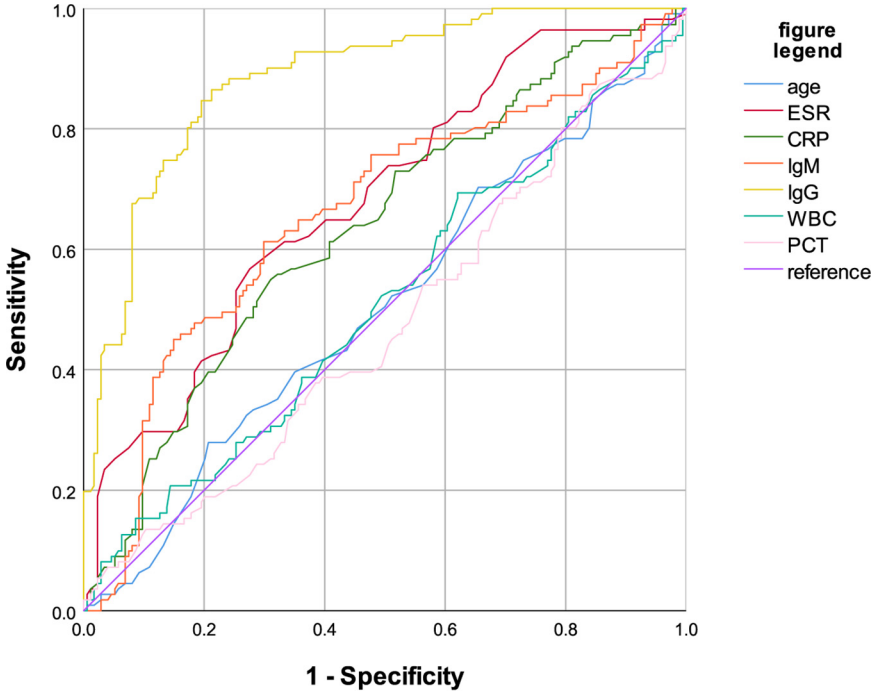

FIG 1 Receiver operating characteristic curves for seven continuous parameters. WBC, white blood cell; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; PCT, procalcitonin.

[95% CI, 1.05 to 6.86]); patients with an IgM level of >20.025 U/ml had a higher risk of brucellosis complications than those with an IgM level of ≤20.025 U/ml (OR, 4.37 [95% CI, 1.96 to 9.74]); patients with an IgG level of >101.12 U/ml had a higher risk of brucellosis complications than those with an IgG level of ≤101.12 U/ml (OR, 37.83 [95% CI, 14.90 to 96.04]); patients with an ESR of >45.5 mm/h had a higher risk of brucellosis complications than those with an ESR of ≤45.5 mm/h (OR, 6.51 [95% CI, 2.84 to 14.94]); and patients with a CRP level of >27 mg/liter had a higher risk of brucellosis complications than those with a CRP level of ≤27 mg/liter (OR, 2.68 [95% CI, 1.25 to 5.72]). Positive culture, the ESR, and CRP and IgM levels detected the occurrence of brucellosis complications, but their efficacy was weaker than that of the IgG level. PCT, WBC count, and age were not parameters indicative of complicated brucellosis. Logistic regression models including all statistically significant variables were constructed, and the results are shown in Table 4.

DISCUSSION

Complicated brucellosis, also known as focal brucellosis, is a *Brucella* sp. infection in humans that causes damage to one or more organs or systems (9, 12, 15). Distinguishing focal brucellosis from focal infection induced by other pathogens is difficult, posing a challenge in the administration of effective medication (19, 20). Therefore, it is critical

TABLE 3 Cutoff values, sensitivity, specificity, and area under the ROC curve of continuous data in predicting the presentation of complicated brucellosis

Parameter[a]	Cutoff value	Sensitivity	Specificity	AUC (95% CI)	*P* value
ELISA IgG	101.12 U/ml	0.865	0.787	0.885 (0.847–0.924)	<0.001
ELISA IgM	20.025 U/ml	0.613	0.701	0.660 (0.593–0.727)	<0.001
ESR	45.5 mm/h	0.568	0.724	0.681 (0.618–0.744)	<0.001
C-reactive protein	27 mg/liter	0.550	0.690	0.636 (0.570–0.701)	<0.001
WBC count	5.325 × 10^9/liter	0.694	0.379	0.512 (0.442–0.581)	0.741
Age	65.5 yr	0.279	0.793	0.506 (0.437–0.575)	0.867
Procalcitonin	0.4005 ng/liter	0.072	0.960	0.473 (0.404–0.542)	0.444

[a]ELISA, enzyme-linked immunosorbent assay; ESR, erythrocyte sedimentation rate; WBC, white blood cell.

TABLE 4 Logistic regression analysis including statistically significant variables

Item[a]	Exp(B)	95%CI	*P* value
Fever	0.152	0.043–0.536	0.003
Culture (+)	2.684	1.050–6.860	0.039
ELISA IgM	4.365	1.956–9.742	0.000
ELISA IgG	37.827	14.899–96.036	0.000
ESR	6.512	2.839–14.936	0.000
C-reactive protein	2.676	1.253–5.718	0.011
Total	0.050		0.000

[a]ELISA, enzyme-linked immunosorbent assay; ESR, erythrocyte sedimentation rate.

to understand the incidence and manifestations of complicated brucellosis and to identify biomarkers that can detect the occurrence of focal infection. The current study determined the incidence of complications of brucellosis and the differences in clinical features and laboratory findings between complicated and uncomplicated brucellosis. We demonstrated in this study that serum anti-*Brucella* IgG tested by ELISA could be an effective biomarker of focal brucellosis.

Previous studies have reported that osteoarthritis is one of the most common complications of brucellosis. The incidence of *Brucella*-induced arthritis ranged from 3% to 77% in different reports (9, 21). In our study, osteoarthritis was the most common complication of brucellosis, accounting for 61.26% of all complications. Spine involvement was the most frequent subtype of *Brucella*-induced osteoarthritis. Genitourinary involvement was also among the most common focal complications, with an incidence ranging from 1.1% to 25% (22). Complications involving other systems, such as the respiratory, cardiovascular, and neurological systems, were less common. The incidence of focal complications affecting these systems ranged from $<$1% to 5% (23–25). In summary, the incidence of brucellosis-associated complications in our study was in agreement with that in the existing literature.

The systemic clinical manifestations of complicated brucellosis were similar to those of uncomplicated brucellosis. According to the results of our study, a lower rate of fever was observed in the complicated- group than in the uncomplicated-brucellosis group, a result that was also validated by other researchers (12). In agreement with other reports, we also found that *Brucella*-associated osteoarthritis occurred more often in patients in the subacute or chronic stage than in patients in the acute stage (26). The differences in disease stages for other focal brucellosis complications could not be concluded due to low incidence rates. Nevertheless, the logistic regression analysis in our study found that fever and prolonged duration of illness might be negative factors for complicated brucellosis. We did not find significant differences in any other systemic clinical presentations between the complicated- and uncomplicated-brucellosis groups. Therefore, it is difficult to distinguish complicated brucellosis from uncomplicated brucellosis based on general symptoms alone.

Due to the difference in treatment regimens for complicated and uncomplicated brucellosis, it is of great importance to detect the occurrence of complications in brucellosis patients. Given the indistinguishable clinical features of focal and uncomplicated brucellosis, many researchers have explored the utility of inflammatory biomarkers and laboratory results in detecting the presence of complicated brucellosis.

Sen et al. found that the platelet-to-lymphocyte ratio (PLR) and ESR were significantly higher in complicated-brucellosis patients than in uncomplicated-brucellosis patients (*P*, 0.007 and $<$0.001, respectively) (16). They reported that the AUC for the PLR was 0.622 (95% CI, 0.538 to 0.707) for detecting complications of brucellosis (16). The neutrophil-to-lymphocyte ratio (NLR) and macrophage-to-lymphocyte ratio (MLR) were demonstrated by Balin et al. to be indicators of osteoarticular involvement (27). Nevertheless, Sen et al. concluded that the MLR and NLR were not valuable markers of complications in brucellosis patients when hematologic abnormalities were considered a complication. However, if only solid-organ involvement was regarded as a complication, and hematologic abnormalities were omitted, the ESR, mean

platelet volume (MPV), NLR, PLR, and MLR all differed significantly between complicated- and uncomplicated-brucellosis patients (*P*, 0.001, 0.011, 0.001, 0.013, and 0.040, respectively). The AUC values for the NLR and MLR were 0.649 (95% CI, 0.570 to 0.728) and 0.589 (95% CI, 0.507 to 0.671), respectively (16). In the current study, we also found that the ESR and CRP were indicative of the presence of brucellosis complications, and their performance (AUC values, 0.681 [95% CI, 0.618 to 0.744] and 0.636 [95% CI, 0.570 to 0.701], respectively) was comparable to that in other studies. In addition, the ESR and CRP were correlated with an increase in anti-*Brucella* antibodies in serum (28). Other acute-stage response agents of inflammation, such as hepcidin or adenosine deaminase, might also be used as biomarkers to diagnose brucellosis, estimate the therapeutic efficacy of treatments, and predict the recurrence of brucellosis (29, 30). Several matrix metalloproteinase family members have been reported to be helpful in indicating osteoarticular involvement (31). Despite these findings, no effective biomarkers with ROC AUCs larger than 0.800 are currently available. Universal inflammatory biomarkers might not be appropriate indicators, since their levels can be influenced by autoimmune factors or immune-compromising diseases. Our study demonstrated that anti-*Brucella* IgG, with an AUC of 0.885 (95% CI, 0.847 to 0.924), is currently the most robust biomarker for detecting the presence of complicated brucellosis. It has been suggested that IgM be tested together with IgG to avoid false-negative results in brucellosis detection (7). IgG was more robust than IgM in detecting complications. In addition, IgG was relatively stable, with strong persistence in peripheral blood, and was easily detected. Therefore, we suggest that anti-*Brucella* IgG tested by ELISA should be applied in clinical settings not only for brucellosis diagnosis but also as a biomarker for complications.

The current study validated the role of anti-*Brucella* antibodies in detecting the presence of focal brucellosis. However, there were several limitations to our study. First, the present study was retrospective, and long-term follow-up information was not obtained. Therefore, the association of anti-*Brucella* IgG/IgM with treatment efficacy was not clarified. Second, this study was carried out in a single center; thus, the results should be generalized with caution. Third, the sample size of this study was small. Despite these limitations, the present study could aid in the diagnosis of complicated brucellosis by clinical practitioners in areas of endemicity. Large-cohort and multicenter studies with long-term follow-up periods are needed to comprehensively investigate the presentation, diagnosis. and management of complicated brucellosis.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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