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Clinical and serological approach to patients with brucellosis: A common diagnostic dilemma and a worldwide perspective

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ABSTRACT

According to the WHO factsheet, although approximately half a million brucellosis cases are reported annually, the true incidence is always 10–25 times higher than the reported number of cases. Therefore, we face a common yet uncommonly recognized entity of brucellosis, which highlights the importance of providing precise and understandable guidelines for physician to recognize and manage the disease. Up to now, there is no distinct and clear guideline for brucellosis diagnosis. Hence, this article presents for the first time an algorithm based on our 30 years clinical experiences for brucellosis diagnosis.

There are several serological patterns of brucellosis due to the insidious nature and serologic response of this disease. In contrast to most infectious diseases, the IgM response to brucellosis remains after the acute phase, IgG responses often fade after improvement and there is no lifelong positivity for IgG antibody. This diversity of serological pattern leads to seven clinical subtypes of the disease; three of those do not need any medical intervention. In endemic regions, this issue makes a challenging diagnostic puzzle for clinicians, which may consequently lead to national and international over- or underestimation of brucellosis incidence. On one hand, this may change the epidemiological landscape of brucellosis. On the other hand, drugs used in therapy are often accompanied by serious or sometimes irreversible side effects. Accordingly, we attempt to create a unique template to better identify these seven serological patterns and give a comprehensive insight into the diagnostic approach to brucellosis. Moreover, we describe in detail the appropriate use of wright, 2 ME, Coomb's WRIGHT, and ELISA tests.

1. Introduction

Jeffery Allen Marston, a British surgeon is the first one who suffered from Brucellosis and described it as an irregular fever [1]. The Brucellosis incidence is closely dependent on its prevalence in sheep, goats, and cattle. World Health Organization (WHO) reports the true incidence is always 10–25 times higher than reported Brucellosis cases (500,000 cases annually) [2]. Therefore, we face a common yet uncommonly recognized entity of brucellosis, which necessitates an understandable guidelines for health care workers to recognize and manage the disease.

Clinical signs and symptoms of brucellosis are not specific and the diagnosis mostly relies on incorporating clinical, epidemiological and serologic findings. Serologic tests play a fundamental role in the diagnosis of this disease. The interpretation of these tests is usually difficult, particularly in patients with chronic brucellosis, reinfection, and relapse states and in endemic areas where a high proportion of positive serology could be observed.

This study aims to Ref. [1] describe the details of immune response to Brucella bacteria [2]; outline state-of-the-art clinical and epidemiological findings of brucellosis research [3]; propose all possible presentations of brucellosis in the template of seven clinical scenarios [4]; present clinical problem solving and diagnostic decision making based on clinical and laboratory algorithms; and [5] discuss the pitfalls and controversial issues of diagnosis and treatment in brucellosis.

2. Immunologic responses in brucellosis

Although culture result has a definitive diagnostic value, the serologic tests have a major role in Brucellosis diagnosis [3]. The mechanisms behind the immune response could enhance our understanding of the diagnosis of Brucellosis in human and animals.

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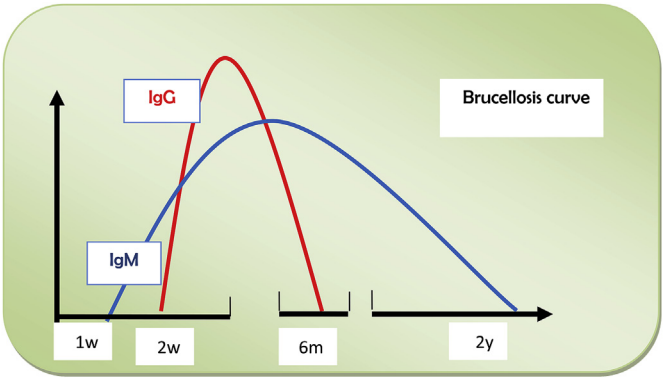

Fig. 1. The trend of antibodies response in patients with brucellosis.

Fig. 1 indicates the trend of antibody response in brucellosis. IgM antibody shows acute infection and appears about a week after the onset of disease. IgM will sharply rise and reach its peak one to three months later. In this regard, the successful or self-limited treatment of brucellosis leads to IgM decline until month 6, which disappears at the end of the first year of disease onset [4]. However, evidence on the longevity of IgM is in controversy; it has been reported that IgM could remain in the positive state in 31.9%, 24.2%, and 8.3% of patients after six months, one year and two years, respectively [5,6]. Surprisingly, in a recent study, it has been reported that IgM could be positive even after 20 years [4].

Based on IgG antibody curve, two weeks after disease onset, IgG will increase steadily until six to eight weeks and decrease sharply until the sixth months and vanish during the first year of disease onset [4]. However, if the patient undergoes inappropriate or incomplete treatment, this antibody will stay in the blood serum and reach its constant level in patients as chronic brucellosis. Studies have shown that among patients of cured brucellosis, 3.4% after six months; 1.3% after a year, and 0.26% after two years had positive IgG titer [5].

There is no association between serum titer of antibodies and disease complications; hence, a low serological titer could not be used as a diagnostic factor [7].

3. Common tests used for brucellosis diagnosis

STA: The brucellosis is diagnosed according to the slide (Huddelson) and standard tube agglutination (STA) or Wright tests [7], which measure the IgM and IgG.

Although STA showed the lowest positivity rage [8] a titer of equal or more than 1/160 with clinical symptoms or rising STA titer could be diagnostic [9]. Previous studies have shown that the titer of 1/80 and more rather than 1/160 and higher should be considered as diagnostic for Brucella [10]. It is note-worthy to mention that Brucella canis is rare in humans, but should be considered in cases with a negative STA result [11].

RBPT: As shown in Fig. 2, in the patients with clinically suspected brucellosis, Rose Bengal Plate Test (RBPT) could be performed as the first screening test. RBPT has been introduced as a very effective method to detect agglutinating and non-agglutinating antibodies due to acidic condition [6,7]. In the case of positive RBPT, standard tube agglutination test should be used. This is a simple, cost-beneficial, and general reproducible diagnostic method [8,12]. RBPT may result in false positive in 1.5% of cases [13].

2 ME: This only measures IgG antibody. Decreasing IgG is a better marker than IgM levels to show therapy success. The rapid decline in the level of IgG has been reported as an indicator of successful response to treatment [14].

COOMBS WRIGHT: In chronic Brucellosis, agglutinin IgG is replaced by non-agglutinin IgG, which can be measured only by Coombs Wright. In addition, this test can also measure IgM antibody and should be ordered in the state of negative wright test (presence of only non-agglutinin IgG) in highly suspected cases. In acute cases, STA and Coombs Wright tests must have different titer simultaneously (due to presence of both agglutinin and non-agglutinin antibodies; negative Coombs Wright and positive STA), while in chronic Brucellosis the same titer is usually expected (both tests are positive). Therefore, if only IgM antibody exists in the individual, the same titer of Wright and Coombs without positive 2 ME test may lead to misdiagnosis and over-treatment [4].

ELISA: ELISA with high sensitivity and specificity is the most efficient test for diagnosis of brucellosis [15]. It has been shown that ELISA had a sensitivity of 83.3% for IgM and 41.7% for IgG, while the combined specificity for IgG and IgM was 92.3%. Moreover, specificity of IgM, IgG, and combined ELISA tests was reported to be 73.7%, 65.0%, and 55%, respectively [16]. Although ELISA methods that detect IgG are sensitive, a study reported the low specificity with 17% rate of false negative results for this test [10]. In addition, although it is well-known that iELISA and cELISA do not have better sensitivity and specificity than RPBT in the absence of vaccination, experts believe that they have priority over other tests [12].

PCR: PCR has lower sensitivity compared to ELISA [17,18]. It has been indicated that PCR is not an appropriate test for chronic brucellosis or history of incomplete treatment due to false negative results [8]. The use of multiplex PCR with simultaneous detection of all species may be a strong alternative for confirming brucellosis [8]. A sensitivity of 94.9% and specificity of 96.5% have been reported with the combination of PCR/ELISA test [19].

Culture: The sensitivity of the blood culture is only 70.1% [19]. Culture technique often encounters several obstacles depending on the disease stage, Brucella species, culture medium, quantity of circulating bacteria, and the technique employed. These factors may contribute to a wide range of sensitivity among different studies (50%–90%) [20,21]. Positive culture can be considered as gold standard for diagnosis of brucellosis especially in those with seronegative tests [13].

4. Microbiologic, epidemiologic and clinical features of brucellosis

Up to now, eight species of Brucella have been identified: Brucella abortus (cattle), Brucella canis (dogs), Brucella melitensis (goats and sheep), Brucella neotomae (desert wood rats), Brucella ovis (sheep), and Brucella suis (pigs, reindeer, and hares) [22]. Also, two strains have been recently identified from marine mammals as: Brucella pinnipediae (seal/otter) and Brucella cetaceae (porpoise/whale) [5].

Although there are rare reports on human infection with marine Brucella [14], Brucellosis is an occupational disease which is known by different names based on endemic geographical area [5].

Human infection could occur through the consumption of unpasteurized milk, raw milk product, and raw meats [1]. Other modes of transmission include skin abrasion and inhalation of airborne animal manure particles [10]. Furthermore, recent studies have implied that sexual intercourse is a possible means of transmission [5].

Symptomatic infection among other family members is common; therefore, evaluation of family members of brucellosis patients is recommended [13]. Epidemiologic features of brucellosis vary widely from country to country. The prevalences of 1% in Turkey [23], 1.8% in India [5], 4.5% [24] in Saudi and 1.3% in Iran [13] have been reported.

Acute Brucellosis: Human brucellosis is a multi-organ disease with a wide variety of clinical manifestations. In subacute, chronic, and relapsed brucellosis, the prolonged arthralgia could be a common symptom [25]. Hepatosplenomegaly and lymphadenopathy are common findings in patients with acute brucellosis rather than chronic cases. Uncommon manifestations of brucellosis can present as chronic urinary tract infection (UTI), ulcerative colitis (UC), dermatological lesions, and thyroiditis [25,26].

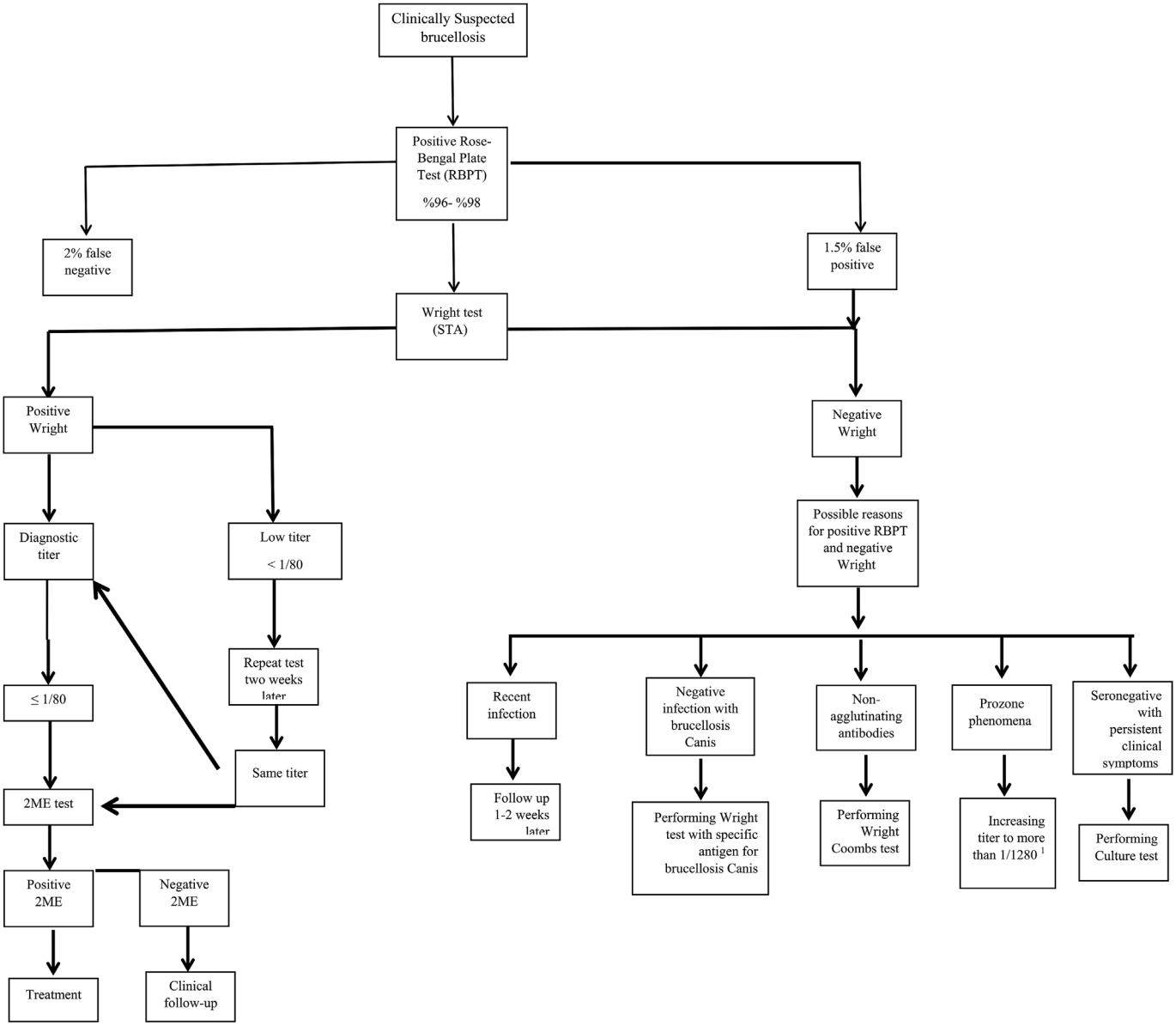

Fig. 2. Algorithm of interpretation and approach to serological tests in brucellosis patients.
1. Studies indicated that rising titer to 1/640 could be enough for elimination of prozone phenomena.

Relapse: The frequency of relapse has been reported to be 14.7%. Most relapses occur within 3–6 months after treatment. Focal disease especially osteoarticular involvement has a greater probability of treatment failure and relapse. Relapse occurs three-folds more common in cases of chronic infection with pain symptom [7,27].

Chronic Brucellosis: Since infection leads to inflammation, we expected elevation in erythrocyte sediment rate (ESR). However, it has been reported that chronic brucellosis patients do not present with high ESR, leukocytosis, fever, and/or organomegaly [21]; the mechanism behind this observation could be explained by adaptive response of body to the chronic presence of microorganism [7].

Wide spectrum of acute, relapsed, chronic, and focal presentation of Brucellosis often makes a definite clinical diagnosis difficult. Therefore, the diagnosis requires microbiological confirmation by means of isolation from blood culture or finding specific antibodies by serologic tests [3].

Based on our 30 years of experience, we found seven serological patterns in brucellosis patients. To solve this challenging diagnostic puzzle, we designed a unique algorithm of approach to brucellosis in the template of seven case scenarios.

5. Case presentations

Case 1. A 25-year-old, previously healthy male, living in a brucellosis endemic area is suffering from fever up to 38.5 °C, myalgia, joint pain, and low back pain (LBP) during the last week. He has the history of consumption of unpasteurized milk three weeks ago. Physical exam is unremarkable for this case. Wright test is positive at the titer of 1/20 and 2 ME is negative.

Case 2. A 25-year-old, previously healthy male from brucellosis endemic area who suffers from undulant fever up to 39 °C, myalgia, joint pain, and LBP during the last month. He also complains of night sweating, chills, and weight loss. He has the history of consumption of unpasteurized milk products 6 weeks ago. Physical exam reveals a soft palpable spleen. Wright and 2 ME tests are positive at the titer of 1/160 and 1/80, respectively.

Case 3. A 25-year-old male, with brucellosis infection verified 8 months ago, who was treated with rifampin and tetracycline. The

patient discontinued treatment as soon as his symptoms were relieved (after 3 weeks) due to side effect of drugs (severe esophagitis). Every three months, the patient is closely evaluated for any new clinical symptoms or change in titer of serologic tests. His chief complaints are muscle and joint pain as well as feeling depressed and sleepy. Physical exam is unremarkable. Wright and 2 ME tests are both positive at the titer of 1/320, which is equal to last follow-up titer performed based on follow-up protocol.

Case 4. During the follow-up of Case 3, he suddenly presents acute clinical symptoms (similar to Case 2). The patient had the history of incomplete treated brucellosis 18 months ago. A physical exam is unremarkable for this case except worsening LBP. Serological tests show antibody titers of 1/640 for both Wright and 2 ME. In follow-up protocol based on repeating 2 ME and Wright every three months, the last 2 ME and wright titer is 1/320 which suddenly rises to 1/640.

Case 5. In the follow-up period of Case 3, the patient develops new clinical symptoms including undulant fever up to 39 °C as well as joint and low back pain. Physical exam reveals palpable spleen. Recently, he consumed the fresh and unpasteurized milk. Wright and 2 ME are positive at the titer of 1/1280 and 1/640, respectively.

Case 6. A 35-year-old man with the history of complete treated brucellosis in the past, present with positive wright test at the titer 1/10 and negative 2 ME test. The patient has no clinical symptoms and the physical exam was normal.

Case 7. A 40-year-old healthy male veterinarian without any history of brucellosis present with positive Wright at a titer of 1/5120 and negative 2 ME. He had no clinical symptoms.

6. Case-solving

With suspected Brucellosis and lab tests, we can easily determine the clinical type and consequence management in patients by keeping the serologic pattern in mind (Fig. 1). Fig. 2 presents the laboratory approach to brucellosis patients.

To approach the suspected to Brucellosis with positive tests, we should first answer the four following questions for each patient [1]: What type of antibodies (IgG, IgM, or both) does the patient have? [2] Where is the patient located at the serology diagram? [3] Which kind of clinical subtype does the patient have? [4] What would be the subsequent decision?

6.1. Answers to questions

Case 1:

Positive Wright but negative 2 ME tests indicate only the presence of IgM, which could indicate more than 1 and less than 2 weeks have been passed since infection onset (Question 1). As can be seen from the serologic diagram, for this patient the location is before the end of the second week of disease onset (Question 2). Clinically, this case mirrored the early infection of brucellosis (Question 3); which does not require medical management and only needs clinical follow-up and repeating serologic tests for rising titer (Question 4).

Case 2:

Both Wright and 2 ME are positive (with different titer), which could highlight the presence of IgG and IgM antibodies (Question 1). The symptoms and physical exam are key findings of this case. The location in the serologic figure can be after the second week and before 6 months of symptoms onset (Question 2). The positive lab tests and acute onset of symptoms for this case indicate acute Brucellosis (Question 3). Complete standard and combination treatment is indicated (Question 4).

Case 3:

Wright and 2 ME tests are positive at the equal titer for only IgG (Question 1); the previous history of incomplete treatment plays the key role in this scenario. The serologic diagram indicates that the location of this case is after 6 months (Question 2). The diagnosis is chronic Brucellosis (Question 3) in which the IgG will remain at a constant level in patients who do not receive any medication or undergo incomplete treatment. They should undergo appropriate treatment (Question 4).

Case 4:

Wright and 2 ME are both positive at the same titer, which demonstrates that only IgG exists (Question 1). As can be seen from the serologic diagram, for this patient the location is after more than 6 months from the disease onset (Question 2). Besides, worsening of LBP in a case of untreated or partially treated brucellosis provides the main clue to make a diagnosis of relapse of chronic brucellosis in this case (Question 3). The approach closely resembles case number 2 who presented with acute infection (Question 4).

Case 5:

To answer the first and second questions, Wright and 2 ME tests are positive (at 1/1280 and 1/640 titer, respectively), which demonstrates the rising of IgG and IgM antibodies in the setting of their previous stable titer. Follow-up tests shows that the duration of disease onset is more than 6 months. The past history of brucellosis beside recent consumption of unpasteurized milk products paves the way to make a diagnosis of re-infection (with a new strain) in this patient (Question 3). This patient should be treated as an acute infection similar to Case 2 and Case 4 (Question 4)

Case 6:

Positive Wright and negative 2 ME tests indicate that only IgM plays a role in this scenario (Question 1). Fig. 3 could indicate the serologic position of this patient in which the duration varies from 1 year to 20 years (Question 2). The history of previous successful treatment in an asymptomatic patient could easily lead the physician to make a diagnosis of old brucellosis infection (Question 3). Treatment of this case is based on education and reassurance and no more medical treatment is needed (Question 4).

Case 7:

The positive Wright and negative 2 ME exhibits the high IgM response in this subject (Question 1). Number 7 in Fig. 3 shows the patient's location; i.e., subclinical infection (Question 2). Interestingly, this pattern with lack of symptoms is only seen in an individual with subclinical brucellosis (Question 3); the seropositive state usually develops without any sign and symptoms related to brucellosis in dairy farmers and their family, farm workers, and associated occupational groups such as slaughterhouse workers and veterinary surgeons. The job history is the most important point that should be kept in mind. The patient should be reassured that there is no need for treatment (Question 4).

7. Discussion

7.1. Serologic pitfall

Overall, the majority of laboratory tests do not provide suitable sensitivity for diagnosis [9]. Different factors such as age, gender, job, pregnancy, frequent contact, type of vaccination, and bacteria species could be considered as contributory factors in defining several serological patterns in brucellosis [11].

7.2. IgM challenges

1 IgM could not solely indicate the acute phase of brucellosis and other tests should also performed [4]. Although specific IgM usually is an indicator of the acute or recent infection, IgM detection in the absence of IgG may lead to the wrong diagnosis of acute infection when it is related to old infection or positive seroprevalence in an endemic area [11]. These cases have a positive titer of STA and Coombs tests but a negative 2 ME test.

2 It is worthwhile to mention that recent studies have been shown that the IgM antibody and consequently STA could become false positive in the presence of rheumatoid factor (RF). Hence, experts believe

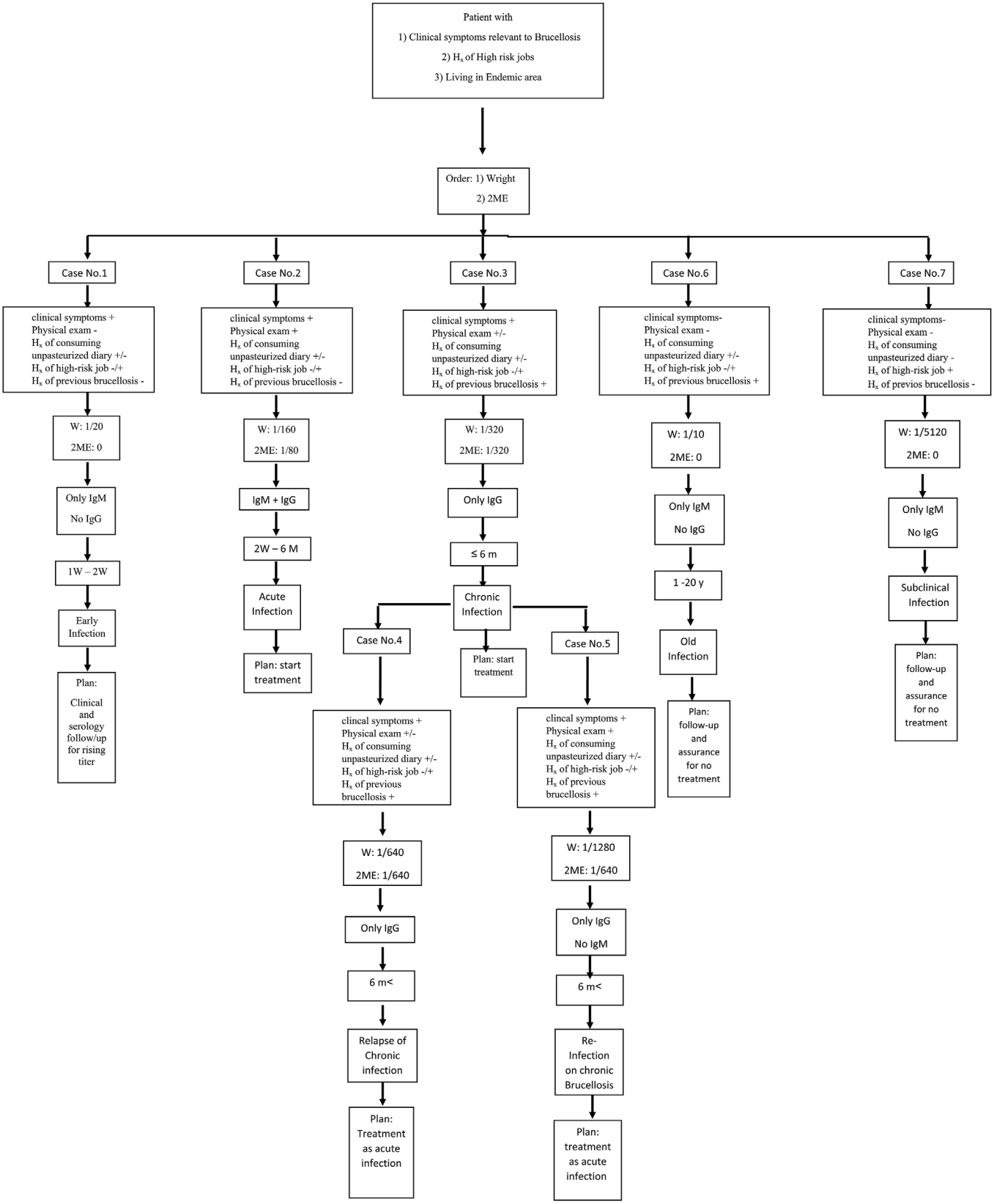

Fig. 3. Algorithm of approach to patients with various clinical and laboratory presentations of brucellosis.

that in the case of positive STA and negative 2 ME, testing for RF in the blood serum is also recommended [4].

3 The presence of cross-reactions due to the antigenic similarity of the lipopolysaccharide of the cell wall with other gram-negative bacteria such as *E-coli* O157, Tularemia, Yersinia enterocolitis, Vibrio cholera, and salmonella may lead to false positive detection of IgM antibody. In addition, other conditions such as malaria, tuberculosis, typhoid, and rheumatoid arthritis can simulate the

clinical picture of brucellosis [28].

4 IgM antibodies may be detected in acute, old, and subclinical infection of Brucellosis [4]. Consequently, a negative 2 ME test can distinguish infection from disease.

7.3. IgG challenges

1 With the progress of the disease, IgG agglutinating antibodies gradually switch to non-agglutinating antibodies [4]. In this case, STA can be false negative and Coombs Wright test should performed. This feature could provide guidance to understand and interpret Coombs Wright results.

2 Prozone phenomenon occurs when a high level of antibody exists in the serum. In other word, the excessive presence of antibody against antigen could lead to not observing agglutination. Therefore, the result of the test will be positive whilst the test is negative in the first tubes [29]. Prozone phenomenon should be suspected in the cases with negative STA but strong clinical evidence of brucellosis. A study showed that up to 11% of patients with brucellosis had no detectable levels of specific IgM and excess IgG production could be a rational explanation for such a phenomenon [5]. Higher dilution of serum will dilute the IgG and allow the positive result of IgM.

3 False negatives IgG (STA) can be observed in the first week of infection. Among those with positive STA, 2.7% of the patients had a low titer of antibodies, which is below the diagnostic test value [5] (probably has also false negative RBPT) and the diagnosis was confirmed by either follow-up for rising titer (for example early infection) or culture [3]. This can miss patients with negative STA if physicians do not follow up. Therefore, a positive STA will be expected in the following weeks and the physician should follow up with such patients.

Interpretation of serological tests is a challenging topic in the diagnosis of brucellosis. To solve this enigma, we tried to shed more light on this issue by providing an algorithm of interpretation and approach to serological tests as well as discussing the pitfalls and controversies in use of several serological methods.

Conflicts of interest

The authors have no proprietary interest in the materials presented herein.

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