

Brucellosis with erythema nodosum-like manifestations diagnosed by isolated positivity of the ELISA test for anti-Brucella IgM

Brucellosi con manifestazioni simil-eritema nodoso ed isolata positività del test ELISA per IgM anti-Brucella: descrizione di un caso e revisione della letteratura

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INTRODUCTION

Positive blood cultures give the best confirmation of diagnosis when brucellosis is suspected. However, because of the difficulty of growing *Brucella* in culture and of the very frequent early use of broad-spectrum antibiotics for fevers of any nature, the frequency of positive blood cultures during brucellosis ranges from 15% to 70% of cases [1]. Therefore, when brucellosis is suspected, often the clinician must rely on the results of serologic testing to confirm the diagnosis.

The serum agglutination test (SAT) has a high sensitivity in acute and sub-acute forms of brucellosis: positivity is around 90%, and the percentage rises if the incomplete antibodies are sought by carrying out SAT in the presence of human anti-globulins (Coombs' test) [2]. Besides SAT, a number of other methods have been proposed to identify anti-*Brucella* antibodies (complement fixation test, immunoenzymatic, immunofluorescent and radioimmunologic methods) [reviewed in 3]. Among these the immunoenzymatic test (ELISA) is considered by some authors as more sensitive than SAT, and by others as having a comparable sensitivity to SAT [4-9].

Brucellosis is endemic in the Mediterranean area. In Italy 813 cases of brucellosis were notified in 2002, the vast majority of them (742 cases) from southern Italy. Currently, in our area SAT is still the routine test for the diagnosis of brucellosis in clinical practice, and the ELISA test for *Brucella* is only a supplementary test. This paper describes the case of a patient with brucellosis presenting as fever and erythema nodosum, who had negative SAT and for whom the diagnosis of brucellosis was based on the isolated positivity of anti-*Brucella* IgM by ELISA test.

CASE REPORT

The patient is a 35-year-old female who lives in a rural area in the province of Benevento (southern Italy). The patient is an agricultural labourer who also has abattoir duties and regularly consumes her homemade milk products and fresh cheese. The illness started with fever, which was undulant in time: fever presented with a maximum body temperature of about 40° C, preceded at times by chills and followed by profuse sweats. After about 45 days from the onset of symptoms, the patient noticed 3-4

red, painful nodules of 1-2 cm in diameter on the front of her legs. At this point the patient saw a doctor who started antibiotic treatment with ciprofloxacin 400 mg IV, teicoplanin 200 mg IM and fluconazole 100 mg IV. This treatment was administered for 2 weeks, and then ampicillin 12 g IV and gentamicin 160mg IM were administered for 9 additional days. As fever persisted the patient was admitted to our Department. On admission, the general conditions were good. Physical examination showed several erythematous nodules on the front and back of the thighs and legs, some of which were painful to the touch (Figure 1a). Nothing else was noted at the physical examination. The routine hematochemical tests were normal, except ESR (60 mm/h) and ALT (values varying between 0.4 and 2.4 x upper normal limit). The rheumatic tests (anti-nuclear Ab, rheumatoid factor, ASO), the anti-*Treponema pallidum* (TPHA, FTA ABS), anti-Salmonella (Vidal test) and anti-Yersinia (HA) serological tests, and the tuberculin skin test, were all neg-

ative. Chest X-ray, upper abdomen and thyroid US scan, abdomen and thoracic CT scan were negative. Several urine cultures were sterile and cultures of the faeces were negative for pathogens. Twelve blood cultures (VITAL-AER) were negative, even when incubated for 21 days. SAT for *Brucella* was negative (titre <1:40) on three occasions. ELISA test for anti-*Brucella* antibodies (*Brucella* ELISA IgG/IgM, Schiapparelli Diagnostics, Milano, Italy), carried out at the same times as SAT, were negative for IgG antibodies, but showed a presence of specific IgM antibodies on all three occasions. Biopsy of one of the nodules showed mainly a dermal infiltrate with perivascular and periadnexial arrangement of lymphocytes, histiocytes and occasional eosinophilic granu-



Figure 1 - a) Erythema nodosum-like lesions on the thighs and legs of a 35-year-old female with undulant fever. **b)** Complete disappearance of the lesions after 15 days of anti-*Brucella* therapy (the site of biopsy of one of the nodules is visible on the right leg).

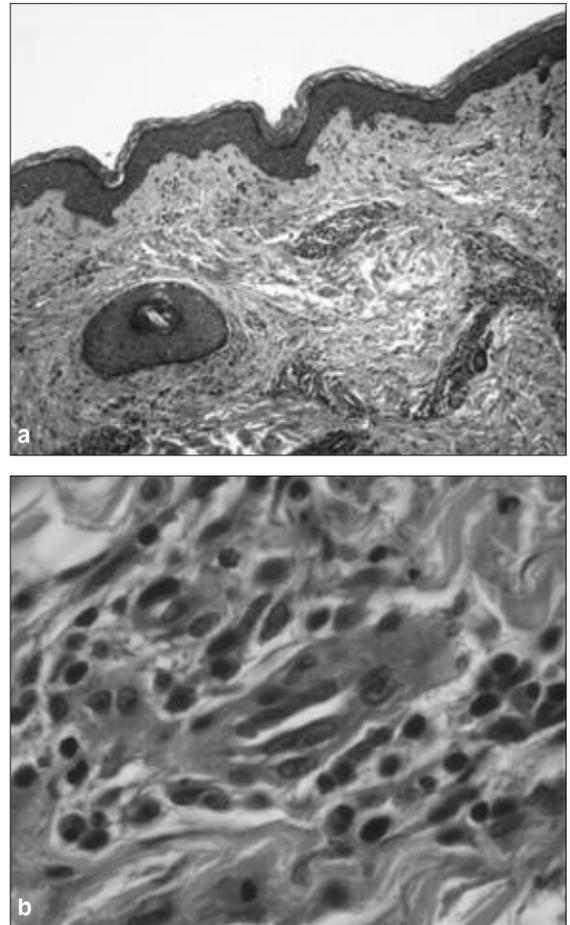


Figure 2 - a) Histological section (5 µm) from formalin-fixed, paraffin-embedded biopsy sample (1.5 cm) of nodule, showing perivascular and dermal infiltrate (haematoxylin-eosin, 100x); **b)** The same at higher magnification (400x), showing lymphocytes and some eosinophilic granulocytes.

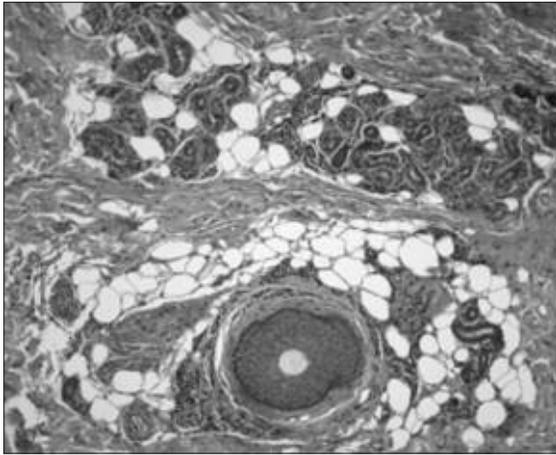


Figure 3 - Histological section (5 µm) from formalin-fixed, paraffin-embedded biopsy sample (1.5 cm) of nodule, showing focal perivascular infiltrate and fibrosis in subcutaneous tissues (haematoxylin eosin, 250x).

locytes (Figure 2). Similar focal perivascular infiltrates were also detected in subcutaneous tissues (Figure 3). The vessels of small diameter displayed a marked swelling of endothelial cells. Areas of edema and moderate fibrosis were also observed in dermal and subcutaneous tissues. No considerable alterations were identified in the epidermis. A culture of the skin biopsy material was negative for *Brucella*. After 20 days of close follow-up without treatment, during which period the registration of the temperature confirmed the undulant nature of the fever, the patient was treated with rifampin 450 mg x 2 IV, doxycycline 100 mg x 2 PO and streptomycin 1g IM. The patient became afebrile after 3 weeks of treatment. The skin lesions regressed completely after 15 days (Figure 1b). After 2 months of normal temperature the IgM test was still slightly positive; no other serum samples were available to test for anti-*Brucella* IgM after this time.

■ DISCUSSION

Although *Brucella* was not isolated from the blood and the SAT for *Brucella* was always negative, there are several elements that indicate that this case was indeed a case of brucellosis. First, the employment and lifestyle of the patient that frequently exposed her to the risk of acquiring *Brucella* by different routes and, second, the clinical picture with the presence of undulant fever and erythema nodosum-like

skin manifestations. Skin lesions are seen in about 5% of patients with brucellosis [1]. In the large case study of Ariza, skin manifestations were observed in 27 of 436 (6%) cases of brucellosis: they presented as a scattered papulo-nodular rash, erythema nodosum-like lesions, widespread hemorrhagic lesions or diffuse maculo-papular rash [10].

Erythema nodosum-like lesions have been described for some time as possible symptoms of brucellosis, at times the only sign of brucellosis [11-15]. A further point in favour of a diagnosis of brucellosis is the response of the patient's fever and skin lesions to the most active anti-*Brucella* drugs. Before the admission to our Department, the patient had been treated with ciprofloxacin and gentamicin, which are potentially active against *Brucella*, without resolution of the symptoms. However, ciprofloxacin is effective *in vitro* against the strains of *Brucella spp*, but has shown poor results *in vivo* [16]. Furthermore, both drugs are effective when used in association with tetracyclines or rifampin, but are much less active when used alone.

The isolated positivity in the anti-*Brucella* IgM test, with negative SAT and negative ELISA for anti-*Brucella* IgG, has been reported in the literature. Gazapo reported that of 46 patients with brucellosis diagnosed from blood culture, 3 patients (6,5%) did not show detectable amounts of IgG antibodies to *Brucella*, while having a good production of IgM antibodies to *Brucella* [17]. Araj reported that out of 133 serum samples from patients who had a history or clinical evidence suggestive of brucellosis, but who were culture-negative and SAT-negative, 38 (28.6%) were ELISA positive, and in particular IgM alone was present in 5 patients (3.8%) [4]. In our geographical area, which is endemic for brucellosis, ELISA is currently considered a second line test that can give a certain diagnosis in cases when classic serology is doubtful. The case we have described and a review of the literature urge us to conclude that the ELISA test should be used routinely when brucellosis is suspected.

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Key words: Brucellosis, IgM anti-*Brucella*, ELISA, Erythema nodosum, Diagnosis

SUMMARY

Brucellosis is endemic in the Mediterranean area. In spite of the false negative results, the standard agglutination test remains the routine test for the diagnosis of brucellosis in southern Italy. We present a case of a patient with undulant fever and erythema nodosum-like skin lesions, with negative serum agglutination test, but isolated

positivity of the ELISA test for anti-Brucella IgM. A diagnosis of brucellosis for this patient was supported by the anamnestic and clinical data, and by the response to therapy. This case and a review of the literature urge us to consider the ELISA test indispensable for the serological diagnosis of brucellosis.

RIASSUNTO

La brucellosi è una malattia endemica nel bacino del Mediterraneo. Nonostante l'elevata frequenza di risultati falsamente negativi, la classica reazione di sieroaagglutinazione rimane il test di routine per la diagnosi di brucellosi nella nostra area geografica (Italia meridionale).

In questo lavoro viene descritto il caso di una paziente con febbre ondulante e lesioni cutanee simil-eritema no-

doso, con negatività del test di sieroaagglutinazione ed isolata positività del test ELISA per la ricerca delle IgM anti-Brucella. La diagnosi di brucellosi in questa paziente era avvalorata dai dati clinici ed anamnestici, e dalla risposta alla terapia.

Questo caso e la revisione della letteratura spinge a considerare il test ELISA indispensabile per la diagnosi sierologica di brucellosi.

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