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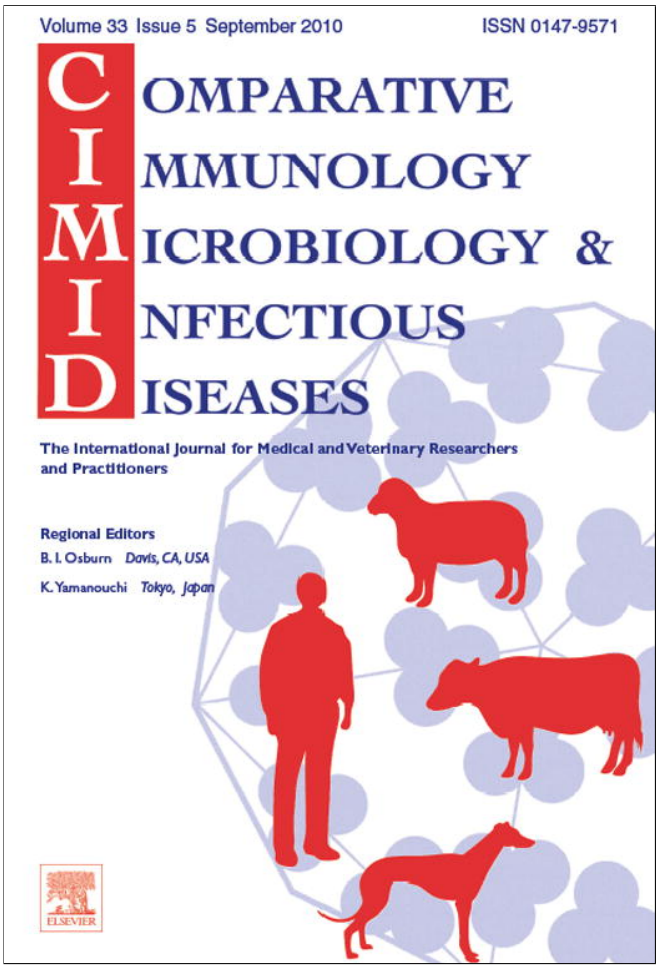

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The role of Brucellacapt test for follow-up patients with brucellosis

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Abstract

The dynamic of Brucellacapt titers was evaluated in 104 patients with brucellosis with favorable outcome and in 28 patients with persistent illness duration, during the follow-up period of 15 months. In patients with favorable outcome, a permanently decreasing tendency of Brucellacapt titers was evident. Titers $\leq$ 1/320 were noted in 27% and 90% of the patients, at the end of the 4th and 15th month, respectively. In patients with persistent disease, persistence or slow titre regression during the entire follow-up period was evident. Four and 15 months of the treatment, titers of 1/320 were registered in 4%, and 14%, respectively, and in no one less than 1/320. The evaluation of Brucellacapt titres between recovered and patients with persistent illness showed significant difference at the 3rd month after beginning of treatment. The evolution of Brucellacapt titers over time proves to be a handy indicator of brucellosis activity when combined with clinical parameters.

Keywords: Brucellosis; Evolution; Serology; Follow-up study; Immunocapture-agglutination

1. Introduction

Serological tests assaying specific antibodies to brucellar lipopolysacharide are an important tool for diagnosis and follow-up the disease. In spite of their limitations such as decreased specificity in endemic areas, low titers in patients with long disease evolution [1]

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and false positive results due to cross-reactions with many bacteria [2], their diagnostic importance remains significant, especially because cultural examinations are time consuming, not sensitive, and hazardous for laboratory infection [3]. The role of serological tests in assessing a disease outcome after the treatment sometimes is difficult to evaluate [4]. The increased antibody levels can persist for prolonged period in people who have recovered from brucellosis, thus making it difficult to differentiate serologically patients with present (active) from patients with past (inactive) brucellosis [4–6]. Determination of the Brucellosis outcome based on serological evaluation is even more complicated if after the treatment, patient continues with occupational exposure to infected animals [5].

At the same time, a definitive assessment of disease activity cannot be based only on clinical evaluation as well. A high proportion of patients report nonspecific symptoms after concluding their treatment, which makes difficult to decide whether they are really recovered [7,8]. Also there are patients who acquired an illness with symptoms similar to brucellosis during the follow-up period [9], patients who ignore their symptoms or are not appropriately followed by the physicians and patients that continue to complain of symptoms that do not exist, because of their interest in other than medical benefits (pension, change of work place, etc.). Therefore, a definitive assessment of disease activity should be made both with clinical and serological evaluation.

Several serological tests are available for diagnosis and follow-up of brucellosis such as: standard tube agglutination, complement fixation [10,11], 2-mercaptoethanol [8,12], 2-ditiothreitol [4,13], Coombs [4,8,14], etc. ELISA is currently best method for follow-up [5,15,16] but it is expensive and less available especially in endemic regions. PCR is a promising technique for diagnosis and follow-up of brucellosis, but so far, with limited clinical experience.

Brucellacapt (an immunocapture-agglutination technique), is a modification of Coombs test for detection of an incomplete or blocking IgG and IgA antibodies, with similar sensitivity and specificity in diagnosis of brucellosis, but with an advantage that is easier to be carried out [1,17,18].

Our goal was to evaluate the convenience of Brucellacapt test in determining brucellosis activity during the follow-up period.

2. Materials and methods

The study was performed during the period March 2003 and December 2006 at the Clinic for Infectious Diseases and Febrile Conditions in Skopje. It included 132 patients with brucellosis that fulfilled the following criteria: (a) the diagnosis based on clinical findings compatible with brucellosis [19], supported by detection of Brucellacapt titers $>1/320$, or demonstration of an at least fourfold rise in antibody titer in serum specimens obtained 3–4 weeks apart; (b) without previous history of brucellosis; (c) clinical and serological follow-up ≥ 15 months after the treatment; (d) without relapses during the follow-up period; (e) no contact with animals during the follow-up period.

For all patients, demographic, epidemiological data, clinical manifestations and outcome were recorded. The Brucellacapt test (Vircell SL, Granada, Spain) was performed

as specified by the manufacturer. Clinical, laboratory and serological evaluation was performed at admission, and then 2, 3, 4, 6, 9, 12 and 15 months after beginning of treatment. The treatment consisted of various antimicrobial combinations composed of two or three drugs, as shown in our previous reports [19,20]. Duration of therapy lasted at least 6 weeks.

Patients were classified in two groups: recovered and patients with persistent infection. Recovered were patients that at the end of a 45th day after initiation of the treatment did not have any symptoms or signs of infection and during the follow-up period did not relapsed. Persistent illness was defined as presence of symptoms and/or signs attributable to brucellosis during the follow-up period, despite the prolongation or repetition of the established therapeutic course. Brucellacapt titers were evaluated according to assessed outcome.

Statistical analysis was preformed using Statistical Package for Social Sciences (SPSS) version 12.0. Chi-squared test was used for qualitative and Mann–Whitney *U*-test for quantitative variables. p-Values <0.05 were considered significant.

3. Results

Demographic, epidemiological and clinical characteristics of analyzed patients corresponding to the outcome are presented in Table 1. Patients with persistent illness were older and more frequently had a focal disease. Other analyzed clinical and laboratory parameters did not show significant differences between the two groups (data not shown).

In 70% of patients with favorable outcome, Brucellacapt titers were initially $\geq$1/2560, but during the follow-up period were permanently decreasing (Table 2, Fig. 1). Titers $\leq$1/320 were registered in 27%, 50% and 66% of the patients, at the end of 4th, 6th and 9th month, respectively. Fifteen months after beginning of the treatment 90% of the patients had Brucellacapt levels $\leq$1/320 and in none was registered titer $>$1/1280. On the other hand, in patients with persistent disease Brucellacapt titers that in 90% were $\geq$1/2560 at admission, during the follow-up period showed slow regression to 1/1280–1/640 and never reached value $<$1/320. Fourth, 6th and 15th months after beginning of treatment, titers of 1/320 were registered in 4%, 7% and 14%, respectively. In this group, at the end of 12th and 15th month from the treatment there were almost no changes in Brucellacapt titers. As shown in Fig. 1, during the follow-up period of 15 months the median titers in recovered

Table 1
Demographic, epidemiological and clinical characteristics of patients with brucellosis.

Parameter	Patients with favorable outcome (n = 104)	Patients with persistent illness (n = 28)	p
Male gender, *N* (%)	79 (76)	24 (85.7)	0.269
Age in years (median, range)	32 (1–82)	51 (3–77)	0.001
Contact with animals, *N* (%)	68 (65.4)	19 (67.8)	0.806
Days of illness prior to antibiotic treatment (median, range)	21 (3–360)	30 (10–360)	0.064
Focal form, *N* (%)	56 (53.8)	25 (89.3)	0.001

Table 2
Brucellacapt titers in 132 patients with brucellosis at admission and during the follow-up period[a].

Brucellacapt test	1/5120	1/2560	1/1280	1/640	1/320	1/160	Negative	*p*
At admission	57 (54.8)/19 (67.8)	15 (14.4)/6 (21.5)	14 (13.5)/2 (7.1)	13 (12.5)/1 (3.6)	5 (4.8)/0	0/0	0/0	0.085
2 months	51 (49)/15 (53.6)	15 (14.4)/9 (32.1)	18 (17.3)/1 (3.6)	12 (11.5)/3 (10.7)	6 (5.8)/0	2 (1.9)/0	0/0	0.227
3 months	27 (26)/13 (46.4)	19 (18.3)/7 (25)	22 (21.1)/4 (14.3)	22 (21.1)/2 (7.1)	10 (9.6)/2 (7.1)	3 (2.9)/0	1 (1)/0	0.010
4 months	14 (13.5)/11 (39.3)	14 (13.5)/8 (28.6)	21 (20.2)/3 (10.7)	27 (26)/5 (17.8)	16 (15.4)/1 (3.6)	6 (5.7)/0	6 (5.7)/0	0.000
6 months	4 (3.8)/5 (17.9)	8 (7.6)/5 (17.9)	14 (13.5)/10 (35.7)	26 (25)/6 (21.5)	27 (26)/2 (7.1)	9 (8.6)/0	16 (15.4)/0	0.000
9 months	0/6 (21.5)	2 (1.9)/6 (21.5)	13 (12.5)/6 (21.5)	20 (19.2)/7 (25)	30 (28.8)/3 (10.7)	16 (15.4)/0	23 (22.2)/0	0.000
12 months	0/3 (10.7)	0/4 (14.3)	9 (8.6)/8 (28.6)	10 (9.6)/9 (32.1)	29 (27.9)/4 (14.3)	20 (19.2)/0	36 (34.6)/0	0.000
15 months	0/3 (10.7)	0/6 (21.5)	5 (4.8)/5 (17.8)	5 (4.8)/10 (35.7)	21 (20.2)/4 (14.3)	26 (25)/0	47 (45.2)/0	0.000

[a] Data are in *N* (%) of recovered/*N* (%) of patients with persistent illness.

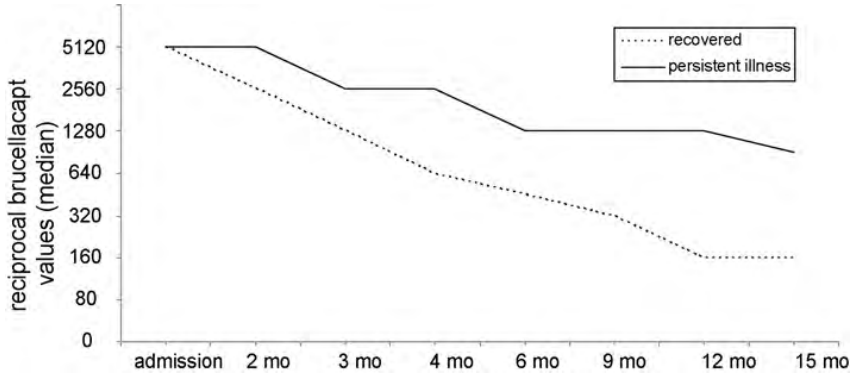

Fig. 1. Reciprocal median Brucellacapt titers during the follow-up in recovered and patients with persistent illness. Dotted line: reciprocal median Brucellacapt titers during the follow-up in recovered patients. Full line: reciprocal median Brucellacapt titers during the follow-up in patients with persistent illness.

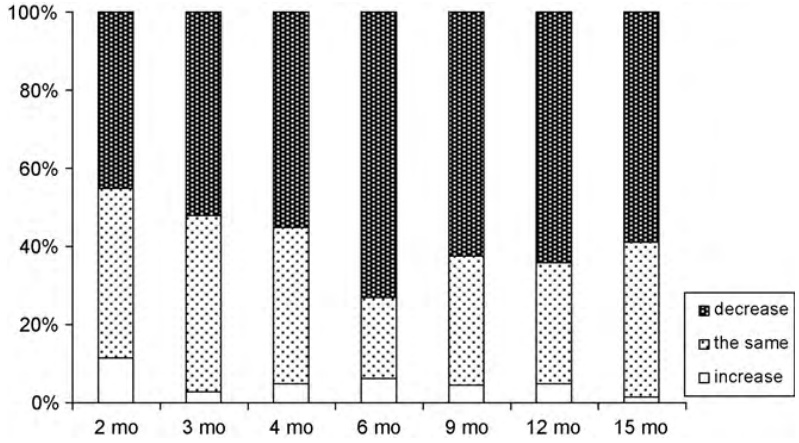

Fig. 2. Dynamic of Brucellacapt titers in the consecutive follow-up periods in the recovered patients.*Blanc bars: titer increase in the consecutive follow-up periods. Dotted bars: titer the same in the consecutive follow-up periods. Crossed lines bars: titer decrease in the consecutive follow-up periods. *Patients that reached to titer of $<1/160$ were excluded from the further comparisons.

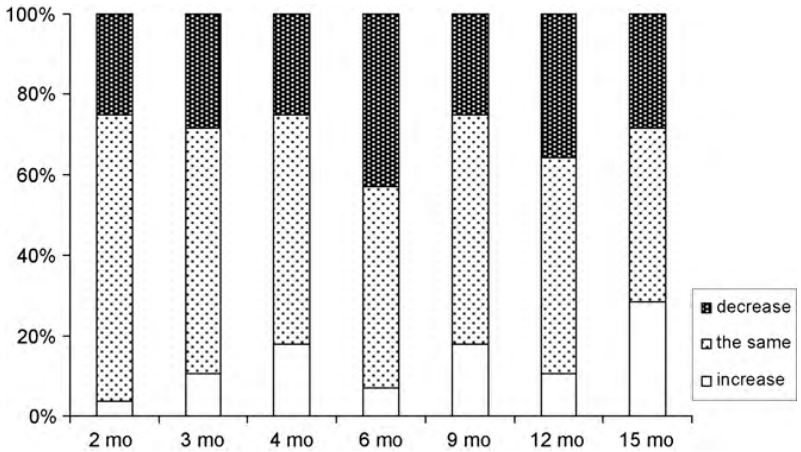

Fig. 3. Dynamic of Brucellacapt titers in the consecutive follow-up periods in patients with persistent illness. Blanc bars: titer increase in the consecutive follow-up periods. Dotted bars: titer the same in the consecutive follow-up periods. Crossed lines bars: titer decrease in the consecutive follow-up periods.

patients decreased from 1/5120 to 1/160, whereas during the same period in patients with persistent disease, the decrease was from 1/5120 to 1/960. The evaluation of Brucellacapt titers between recovered and patients with persistent illness showed statistical significant difference at the 3rd month after the beginning of treatment.

When we analyzed the dynamic of Brucellacapt titers in each patient individually, comparing actual with measured value at the previous control we found that in recovered patients, the titers predominantly were decreasing (Fig. 2), while in patients with persistent disease, maintenance of the same level of the titers was dominant (Fig. 3).

4. Discussion

The dynamic of specific antibody profile in human brucellosis is in the focus of clinical studies especially in the last few decades. In serological follow-up of the disease, most important is determination of brucella specific IgG antibodies as a marker of active infection [14,21,22]. Rapid fall of the level of brucella specific IgG antibodies is considered to be a prognostic indicator of successful therapy [8,23]. Persisting high levels of brucella specific IgG antibodies during the follow-up period is almost always present in patients with active disease [8,23,24]. In patients with relapses, raise of the titers again is most frequently notified serological parameter [4,8,25,26]. However, the persistence of high IgG titers long after the therapy could be seen in some patients with satisfactory clinical outcome, due to a constant exposure to *Brucellae* [14,21,27], focal forms [5,13], or in patients with high IgG titers at admission [13], therefore some authors are doubting in the usefulness of this class immunoglobulin as a marker of chronic infection [6,13].

Our study showed that Brucellacapt test is useful method for monitoring the evolution of Brucellosis, if titers are followed and compared in determined time intervals. 70% of recovered patients initially had Brucellacapt titers of $\geq$1/2560. During the follow-up period, rapid titer decrease was the evident characteristic in this group. In patients with persistent disease, Brucellacapt titers were initially $\geq$1/2560 almost without an exception and during the follow-up period showed slow tendency for decrease. At the end of the follow-up just few patients had titers decreasing to 1/320, and in none titers became negative.

Only scarce literature data studied the usefulness of Brucellacapt in following the patients with Brucellosis and there is no study that compares the dynamic of titers between patients with favorable and those with persistent illness. Two Spanish studies compared Coombs and Brucellacapt in recovered patients and concluded that the titers persisted positive long time after the therapy. Still, Brucellacapt decreased faster than Coombs titers [1,28]. According to the first study, Brucellacapt titers of $\geq$1/320 were reported in 100%, 67% and in 61% of the patients after 1, 3 and 6 months of the treatment. The patients had median titers of 1/5120 at the beginning of therapy, 1/640 titer was recorded 90 days latter, and 1/320 at the end of the 6th month [1]. These results corresponded to our findings. The other study demonstrated high initial median Brucellacapt titers (1/20480) and their prompt reduction to 1/640 3 months latter. However, during the next 9 months of follow-up, there was not further titer reduction, contrary to our findings that in cured patients the titers

were permanently reducing [28]. Concerning the patients with persistent illness, our observations are close to the findings of Orduna et al., which reported Brucellacapt titers of $\geq$1/1280 in four patients with persistent disease [29].

In conclusion, the follow-up of the dynamic of Brucellacapt titers is proved to be helpful amendment to clinical parameters in assessment of brucellosis activity. Rapid reduction of Brucellacapt levels, together with drawn off clinical parameters is good indicator of patient recovery. Increased values of Brucellacapt titers in asymptomatic patients should not be an indication for antibiotic therapy. Patients with persistent high Brucellacapt titers or with slow regression to intermediate levels after the treatment period necessitate continuous and careful monitoring, and decision for prolonging or repeating the treatment, should be made only if there are signs or symptoms suggestive of brucellosis.

Conflict of interest statement

No conflict declared.

Acknowledgments

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