Serological diagnosis of neurobrucellosis

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Abstract

The presence of antibodies was determined in the serum and cerebrospinal fluid in six patients with neurobrucellosis using the Rose Bengal test, the microdilution agglutination test, and the Coombs' test. Four of the patients were followed up for more than three months. The Rose Bengal test and the microagglutination test were positive in cerebrospinal fluid in five of the six cases at some stage. The Coombs' test was positive in cerebrospinal fluid in every patient and in one was the only positive serological test. Cerebrospinal fluid positivity is not excluded by low titres or negative results of antibodies in the serum for any of the three methods. A Coombs' test or some equivalent must always be made on the cerebrospinal fluid to diagnose neurobrucellosis.

In the Mediterranean area human brucellosis is a common infectious disease. This zoonosis is an endemic infection affecting varying levels of society. The incidence of cases varies¹: in 1984 Spain reported 8698 cases (22.3 cases for 100000 inhabitants) and apparently has a higher morbidity than France (320 cases from January to October 1983), Portugal (154 cases), Greece (487 cases) and Italy (1639 cases).²

Neurobrucellosis is a neurological complication, first reported by Hughes in 1897, which is directly attributable to the presence of *Brucella* or its products in the central nervous system. This neurological complication can arise at different moments in the evolution of brucellosis. In most cases diagnosis is made between two and 12 months after onset of symptoms. The reported incidence of neurobrucellosis varies between 0.5% to 25% of all brucellosis.³⁻⁵ This large variation is attributable to the lack of standardisation of diagnostic criteria used by different authors. According to standard criteria,^{6,7} meningeal brucellosis was considered when at least one of the following criteria was present: isolation of *Brucella* from cerebrospinal fluid; presence in cerebrospinal fluid of anti-*Brucella* antibodies at any titre obtained with the microagglutination, Coombs' or Rose Bengal tests; and abnormalities in cerebrospinal fluid in the presence of confirmed brucellosis of one or more of the following variables: number of cells (> 10 cells/mm³; protein concentration (> 0.45 g/l); or glucose concentration of < 4 g/l or 40% of the simultaneous glycaemia. For many authors meningeal disease in neurobrucellosis is present in most cases.^{3,8-10}

Methods

The presence of antibodies in the serum and the cerebrospinal fluid of six patients diagnosed as having neurobrucellosis was determined. The patients, four men and two women, ranged in age from 17 to 60 years (mean 43 years). Major neurological signs or symptoms were as follows: in case 1, headache, dysarthria, vomiting, low level of consciousness, and right sided hemiparesia; in case 2, headache, low level of consciousness, anisocoria, meningeal signs, left sided hemiparesia and seventh cranial nerve paresia; in case 3, headache, low level of consciousness, left sixth cranial nerve paresia and diminished myotactic reflexes and weakness in lower extremities; in case 4, progressive paraparesis, low back pain, loss of sphincter control and severe hearing loss; in case 5, bilateral deafness, symmetrical hyperreflexia of upper and lower extremities; in case 6, low level of consciousness, stiff neck, and low pressure hydrocephalus. In all cases neurobrucellosis was diagnosed by referring to: (i) the previous clinical state being compatible with acute brucellosis—this condition was recorded at two, three, seven, nine, 13 and 22 months before the diagnosis of neurobrucellosis; (ii) epidemiological antecedents such as ingestion of dairy products of doubtful origin or working contact with infected animals; (iii) positive Rose Bengal test and Coombs' test in serum; (iv) presence of anti-*Brucella* antibodies in cerebrospinal fluid; (v) and $115-310$ cells mm³ in cerebrospinal fluid, with a protein concentration ranging from 0.70 to 3.40 g/l and a glucose concentration lower than 3.5 g/l. All patients had been previously treated with rifampin and doxycycline for at least one month, and at the time of the diagnosis of neurobrucellosis blood and cerebrospinal fluid cultures were negative except in case 6. The patients were followed up for one month (one case), two months (one case), and three or more months (four cases).

The Rose Bengal test was performed according to the method of Brinley Morgan *et al* (Bio-Mérieux).¹¹ The Rose Bengal antigen is a concentrated suspension of *Brucella abortus* 99 (Weybridge strain) inactivated by heat and phenol (0.5%) in acid buffer (lactate, pH = 3.65) stained with Rose Bengal. This technique indicates concentrations of 25 IU/ml titrated against WHO serum. In the microagglutination test (MAG) the *Brucella abortus* antigen (Knickerbocker Laboratories) was used. The test was performed on U-shaped microtitre plates. Two-fold serum dilutions (1/20-1/2560) were made with saline solution as diluent, and the incubation time was for 18-24

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Accepted for publication
30 August 1989

Serological follow up of cerebrospinal fluid and serum samples

Case No	Cerebrospinal fluid				Serum			
	Date	Rose Bengal test	MAG	Coombs'	Date	Rose Bengal test	MAG	Coombs'
1 M/60 years	23-3-84	+	1:40	1:160				
	26-3-84	+	1:40	1:320	26-3-84	+	1:80	1:1280
	9-4-84	+	1:40	1:320	2-4-84	+	1:160	1:2560
	18-4-84	+	1:40	1:320				
	25-4-84	+	1:40	1:160				
	2-5-84	+	1:40	1:160				
	9-5-84	ND	1:40	1:160				
	23-5-84	+	1:20	1:160	23-5-84	+	1:80	1:2560
	6-7-84	+	—	1:80				
	1-5-84	—	—	1:320	12-4-84	+	—	1:1280
2 M/28 years	10-5-84	—	—	1:640	30-4-84	+	—	1:1280
	24-5-84	—	—	1:320	10-5-84	+	—	1:1280
	30-5-84	—	—	1:640	24-5-84	+	—	1:2560
	2-8-84	—	—	ND	3-8-84	—	—	1:2560
					2-10-84	ND	—	1:80
3 F/22 years	14-2-84	+	1:80	1:80				
	28-3-84	+	1:80	1:80	11-3-84	+	1:20	1:160
	3-4-84	+	1:40	1:80				
	12-4-84	+	1:80	1:160				
	18-5-84	+	1:40	1:80				
	23-5-84	+	1:40	1:80	23-5-84	—	—	1:40
	6-6-84	+	1:40	1:80	6-6-84	—	—	1:40
	7-7-84	+	1:40	1:40	13-9-84	—	—	1:40
	2-10-84	+	1:20	1:40				
	25-7-85	+	1:20	1:40				
4 F/63 years	5-10-84	+	1:80	1:640	26-9-84	+	1:320	1:1024
	15-10-84	+	1:40	ND	15-10-84	+	1:160	1:640
	24-10-84	+	1:40	1:640	25-10-84	+	1:80	1:320
	12-11-84	ND	ND	1:640	12-11-84	+	1:160	1:640
	22-11-84	+	1:40	1:320	22-11-84	+	1:80	1:320
	8-1-85	+	1:40	1:320	8-1-85	+	1:80	1:160
5 M/22 years	6-12-84	+	1:160	1:2560	23-11-84	+	1:160	1:640
	17-1-85	+	1:80	1:640	10-12-84	+	1:320	1:640
	23-1-86	+	1:40	1:320	17-1-85	+	1:640	1:640
6 M/17 years	6-2-86	+	1:40	1:160	31-1-86	+	1:320	1:5120
	16-2-86	+	1:20	1:160	6-2-86	+	1:320	1:2560
					18-2-86	+	1:320	1:2560

MAG: microdilution agglutination test

hours at 37°C. A positive reaction was indicated by a mat or carpet of cells covering the bottom of the well, and a negative test was one in which the cells were settled forming a compact button or ring at the centre of the well. Positive and negative controls were performed in all cases. The Coombs' test was performed in the same way as the MAG, but the two-fold dilution of the sera ranged from 1/20–1/20 480. The plates were incubated for two hours at 37°C and centrifuged at 3000 rpm for five minutes. After three washes with saline solution human anti-globulin was added and incubated for 18–24 hours at 37°C. The interpretation of the positive and negative results was the same as that in the MAG. Forty eight sera from healthy blood donors and 40 cerebrospinal fluid specimens from non-infected patients, mainly obtained by the department of neurosurgery, were also studied for comparison.

Results

Results of the numbers of antibodies found in the serum are summarised in the table. In the six cases studied the Rose Bengal test was positive at the start of the study but the MAG was positive in only four cases. Only three of the cases were followed up for more than three months and at the end of this period the Rose Bengal test was positive in only one. The two negative Rose Bengal tests were MAG negative from the start. The patient with a positive Rose Bengal test maintained positivity after three months but had a progressively decreasing MAG titre (1/320 to 1/80). The Coombs' test titres were raised from the beginning in five of

the six cases. A progressive decrease in the number of antibodies was observed in patients with a follow up of at least three months. All 48 control sera tested gave negative results (< 1/20 for the MAG and the Coombs' tests and no agglutination with undiluted serum in the Rose Bengal test).

The Rose Bengal test was positive in cerebrospinal fluid in five out of six cases. This positive reaction was maintained throughout the observation period in all cases. The patient with repeated negative Rose Bengal tests also had negative MAGs. The MAG test was positive in the other five cases and the positive reaction was maintained in four of them during follow up, although the titres tended to decrease during this time. The Coombs' test was positive in cerebrospinal fluid in all cases including the case with negative results in the Rose Bengal and MAG tests. Titres tended to decrease during follow up. The Coombs' test titres obtained in cerebrospinal fluid were generally lower than those obtained in serum. All 40 control cerebrospinal fluids tested gave negative results with all tests.

Discussion

The diagnosis of neurobrucellosis is possible in most cases through the detection of antibodies in cerebrospinal fluid. The data that we obtained show the importance of using more than one test. The Rose Bengal test and the MAG are agglutination tests which readily detect IgM immunoglobulins. In active brucellosis high titres of IgM antibodies are present. These decrease progressively after

treatment. The IgG non-agglutinating immunoglobulins can be shown readily by the Coombs' test and remain persistently high,^{12 13} which was consistent with our findings. In case 2 the MAG test, both in cerebrospinal fluid and serum, was negative while the Coombs' test was positive, with cerebrospinal fluid and serum titres of 1/320–1/640 and of 1/80–1/2560, respectively, throughout serological follow up. False negative cerebrospinal fluid agglutination tests were described by Pedro Pons in 1972³ in a patient with neurobrucellosis who had a positive cerebrospinal fluid Coombs' test (1/160–1/320). Foz underlined the high specificity of the Coombs' test and also reported the presence of false negative results using the MAG technique in cerebrospinal fluid.¹⁴ Dupont reported two cases of neurobrucellosis with a negative MAG in cerebrospinal fluid and positive complement fixation.¹⁵ In case 3 we detected the presence of agglutinating antibodies in cerebrospinal fluid, even when the serum was negative. Olmer *et al* considered this finding to be relatively common,¹⁶ a conclusion with which we only partially agree as in our other five cases positive agglutination was observed both in cerebrospinal fluid and serum. In case 3 the Coombs' test in cerebrospinal fluid was also strongly positive while the serum titres remained very low. The Rose Bengal test was positive both in cerebrospinal fluid and serum from five of our six patients. Buades *et al* considered the Rose Bengal test to be consistently positive in serum in systemic brucellosis and also positive in cerebrospinal fluid in neurobrucellosis.¹⁷ This may not always be true as we obtained a negative Rose Bengal test in cerebrospinal fluid in case 2. It remained negative throughout three months of follow up, while the serum was positive, becoming negative only after four months.

Preliminary results on the comparative efficacy of the ELISA (Ingenasa, SA; using purified lipopolysaccharide of *Brucella melitensis*) suggests that it will not give substantially better results than the Coombs' test.

To conclude, we would point out the follow-

ing: (i) the anti-*Brucella* Coombs' test in cerebrospinal fluid is of great value in the diagnosis of neurobrucellosis. In our study it was the only positive test in case 2; (ii) in neurobrucellosis the rate of positive reactions obtained with the Rose Bengal test in cerebrospinal fluid seems to be similar to that of the MAG test and inferior to that obtained by the Coombs' test; and (iii) the serological study of both serum and cerebrospinal fluid is essential for the diagnosis of neurobrucellosis.

We thank Dr J Gobernado Serrano of the neurology department, Ramon y Cajal Hospital, for his help in providing data on the patients in this study.

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