Diagnosis of chronic brucellar meningitis and meningoencephalitis: the results of the Istanbul-2 study

H. Erdem[1], S. Kilic[2], B. Sener[3], C. Acikel[4], E. Alp[5], M. Karahocagil[6], F. Yetkin[7], A. Inan[8], V. Kecik-Bosnak[9], H. C. Gul[10], S. Tekin-Koruk[11], N. Ceran[8], T. Demirdal[12], G. Yilmaz[13], A. Ulu-Kilic[5], B. Ceylan[14], A. Dogan-Celik[15], S. Nayman-Alpat[16], R. Tekin[17], A. Yalci[18], V. Turhan[1], I. Karaoglan[9], H. Yilmaz[19], B. Mete[20], A. Batirel[21], A. Ulcay[1], S. Dayan[17], A. Seza Inal[22], S. S. Ahmed[5], Z. K. Tufan[23], A. Karakas[10], B. Teker[24], M. Namiduru[9], U. Savasci[25] and G. Pappas[26,27]

1) *Department of IDCM, Gulhane Haydarpasa Training Hospital, Istanbul,* 2) *Public Health Institution of Turkey, Bacterial Zoonoses Reference Laboratory, Ankara,* 3) *Department of Microbiology and Clinical Microbiology, School of Medicine, Hacettepe University, Ankara,* 4) *Department of Public Health, Gulhane Medical Academy, Ankara,* 5) *Department of IDCM, School of Medicine, Erciyes University, Kayseri,* 6) *Department of IDCM, School of Medicine, Yüzüncü Yil University, Van,* 7) *Department of IDCM, School of Medicine, Inonu University, Malatya,* 8) *Department of IDCM, Haydarpasa Numune, Training and Research Hospital, Istanbul,* 9) *Department of IDCM, School of Medicine, Gaziantep University, Gaziantep,* 10) *Department of IDCM, Gulhane School of Medicine, Ankara,* 11) *Department of IDCM, School of Medicine, Harran University, Sanliurfa,* 12) *Department of IDCM, School of Medicine, Kocatepe University, Afyon,* 13) *Department of IDCM, School of Medicine, Karadeniz Teknik University, Trabzon,* 14) *Department of IDCM, Medical Faculty, Bezmialem Vakıf University, Istanbul,* 15) *Department of IDCM, School of Medicine, Trakya University, Edirne,* 16) *Department of IDCM, School of Medicine, Osmangazi University, Eskisehir,* 17) *Department of IDCM, School of Medicine, Dicle University, Diyarbakir,* 18) *Department of IDCM, School of Medicine, Ankara University, Ankara,* 19) *Department of IDCM, School of Medicine, Ondokuz Mayıs University, Samsun,* 20) *Department of IDCM, Cerrahpasa School of Medicine, Istanbul University, Istanbul\,* 21) *Department of IDCM, Kartal Dr Lutfi Kirdar Education and Research Hospital, Istanbul,* 22) *Department of IDCM, School of Medicine, Cukurova University, Adana,* 23) *Department of IDCM, Ankara Training and Research Hospital, Ankara,* 24) *Department of IDCM, Private Women's Hospital, Istanbul,* 25) *IDCM Service, Sarikamis Military Hospital, Kars, Turkey,* 26) *Institute of Continuing Medical Education of Ioannina, Ioannina, Greece and* 27) *Zoonoses Working Group, International Society of Chemotherapy, London, UK*

Abstract

No detailed data exist in the literature on the accurate diagnosis of chronic brucellar meningitis or meningoencephalitis. A multicentre retrospective chart review was performed at 19 health centres to determine sensitivities of the diagnostic tests. This study included 177 patients. The mean values of CSF biochemical test results were as follows: CSF protein, 330.64 ± 493.28 mg/dL; CSF/ blood-glucose ratio, 0.35 ± 0.16; CSF sodium, 140.61 ± 8.14 mMt; CSF leucocyte count, 215.99 ± 306.87. The sensitivities of the tests were as follows: serum standard tube agglutination (STA), 94%; cerebrospinal fluid (CSF) STA, 78%; serum Rose Bengal test (RBT), 96%; CSF RBT, 71%; automated blood culture, 37%; automated CSF culture, 25%; conventional CSF culture, 9%. The clinician should use every possible means to diagnose chronic neurobrucellosis. The high seropositivitiy in brucellar blood tests must facilitate the use of blood serology. Although STA should be preferred over RBT in CSF in probable neurobrucellosis other than the acute form of the disease, RBT is not as weak as expected. Moreover, automated culture systems should be applied when CSF culture is needed.

Keywords: Chronic, diagnosis, meningitis, meningoencephalitis, neurobrucellosis
Original Submission: 17 June 2012; **Revised Submission:** 15 October 2012; **Accepted:** 31 October 2012
Editor: M. Drancourt
Article published online: 4 December 2012
Clin Microbiol Infect 2013; **19:** E80–E86
10.1111/1469-0691.12092

Corresponding author: H. Erdem, GATA Haydarpaşa Asker Hastanesi Enfeksiyon Hastalıkları Servisi Uskudar, Istanbul, Turkey
E-mail: hakanerdem1969@yahoo.com

The results of the Istanbul-2 study have not been presented anywhere.

Introduction

Meningoencephalitis and meningitis were reported to have been detected in 5% of all brucellosis patients [1]. The involvement of the central nervous system (CNS) in brucel-

losis features series of clinical presentations. The clinical presentations in acute meningitis or meningoencephalitis typically resemble various forms of CNS infections, cautioning clinicians to carry out immediate intervention [2,3]. However, chronic meningitis and meningoencephalitis are major indistinct features of nuerobrucellosis with predominant neuropsychiatric changes causing deteriorated ramifications [4,5]. Despite all efforts in the treatment of the disease, 20% of cases experiences persistent sequelae [6]. Thus, accurate and timely diagnosis of the disease is extremely important in the rational management of patients with neurobrucellosis.

In the diagnosis of CNS infections, bacterial culture is the reference standard [7]. In contrast, gram-stained smears and cultures of cerebrospinal fluid (CSF) are often negative in patients with chronic neurobrucellosis, and diagnosis usually depends on the presence of specific antibodies [8,9]. Thus, alternate methods such as serological tests and molecular methods are inevitable options [10,11]. *Brucella*-specific antibodies are usually not detected in the CSF unless the central nervous system is involved. However, in neurobrucellosis, CSF contains low titres of antibodies, which can be detected by agglutination tests [8]. In addition, the data related to diagnosis of neurobrucellosis have been restricted to case reports or small case series in the literature and informative data on this subject were necessary [12–15]. Thus, the aim of this study was to determine CSF biochemical values and the sensitivities of microbiological tests, and their interrelations in chronic brucellar meningitis/meningeoencephalitis patients.

Methods

The Istanbul study was designed in two steps. The first part related to therapeutic concerns and courses of the disease, and outcomes were published elsewhere [6]. Thus, the Istanbul-2 study, targeting diagnostic issues, is presented in this paper. As a multicentre retrospective chart review, the Istanbul-2 study was performed in 19 health centres (16 university and three state training hospitals) in Turkey. In this study, adult patients treated after the year 2000 with a diagnosis of chronic brucellar meningitis or meningoencephalitis were included. The case definitions of the study were as follows [6]: (i) the presence of consistent clinical symptoms either with meningitis or meningoencephalitis for over 4 weeks to classify the case as chronic; (ii) consistency of typical CSF findings with meningitis (protein concentrations >50 mg/dL, pleocytosis over 10/mm^3 and glucose to serum glucose ratios <0.5 are accepted as abnormal [16]), (iii) positive bacterial culture or serological test results for brucellosis in blood specimens (positive Rose Bengal test (RBT) and serum tube agglutination (STA) with a titre $\geq$ 1/160) or in CSF (positive RBT or STA with any titre) or positive bone marrow culture; and (iv) absence of an alternative neurological diagnosis explaining the clinical presentations. Patients fulfilling these criteria were regarded as having brucellar meningitis or meningoencephalitis and included in the study.

The data analysis was performed with SPSS in the Windows V.15.0 program. Descriptive statistics were presented as frequencies, percentages for categorical variables and as mean $\pm$ standard deviation and median (min–max) for continuous variables. Before the analysis of titration data, logarithmic transformation (based on log10) was performed. In comparing the groups, the chi-square, Mann–Whitney *U* and *t*-tests were used. The agreements between the tests were evaluated with Kappa coefficient. κ value < 0.20 was interpreted as poor, 0.20–0.40 as fair, 0.40–0.60 as moderate, 0.60–0.80 as good and 0.80–1.00 as very good agreement [17]. In the assessment of the relations between the variables, Pearson and Spearman correlation coefficients were calculated. In comparing the sensitivity of the data, $p < 0.05$ was considered significant.

Results

This study included 177 patients (91 female and 86 male) with chronic brucellar meningitis or meningoencephalitis. The median age of the patients was 30 (14–78). The mean values of CSF biochemical test results were as follows: CSF protein, 330.64 $\pm$ 493.28 mg/dL, min–max = 25–3191 mg/dL; CSF/blood-glucose ratio, 0.35 $\pm$ 0.16, min–max = 0.03–0.82; CSF sodium, 140.61 $\pm$ 8.14, min–max = 110–153 mMt; CSF leucocyte count, 215.99 $\pm$ 306.87, min–max = 0–2700. In eight patients (4.5%), no leucocyte was detected on CSF examination. In six out of eight patients one serological test was positive; the other patient had three positive serological tests and the remaining one had four positive serological tests. In five out of eight patients CSF culture was found to be positive, and in one patient blood culture was positive. Various diagnostic tests were used in this study and the sensitivities of the microbiological tests performed for the diagnosis are presented in Table 1.

Serological tests

Serological tests such as serum-STA (n = 172), CSF-STA (n = 144), serum-RBT (n = 123), CSF-RBT (n = 106), CSF-Elisa (n = 10) and serum-Elisa (n = 11) were applied to our patients. In 100 cases, both STA and RBT tests were performed together in the CSF samples. The STA test was

TABLE 1. The sensitivities of the microbiological tests in chronic neurobrucellosis

	Total	Positive	Sensitivity %
Serum STA	172	162	94.2
CSF STA	144	113	78.5
Serum RBT	123	118	95.9
CSF RBT	106	75	70.8
Blood culture (*Bactec, BacTAlert*)	154	57	37
CSF culture (*Bactec, BacTAlert*)	87	22	25.3
CSF culture (conventional method)	52	5	9.5
CSF-Elisa (IgM)	10	8	80
CSF-Elisa (IgG)	10	8	80
Serum-Elisa (IgM)	11	7	70
Serum-Elisa (IgG)	11	10	91
BM culture (*Bactec, BacTAlert*)	8	3	37

CSF, cerebrospinal fluid; RBT, Rose-Bengal test; STA, standard tube agglutination test; BM, bone marrow.

performed both in serum and in CSF in 141 cases and RBT was performed both in serum and in CSF in 104 cases.

Among the eight serological tests applied (CSF-STA, CSF-RBT, serum-RBT, serum-STA, CSF IgM, CSF-IgG, serum IgM and Serum IgG), 32 (18.1%) patients had one, 45 (25.4%) patients had two, 23 (13.0%) patients had three, 64 (36.2%) patients had four, one (0.6%) patient had five, three (1.7%) patients had six, one (0.6%) patient had seven and three patients (1.7%) had eight with seropositivity. Five patients did not have any serological evidence. In 105 out of 110 CSF-STA positive test cases, the serum-STA test was also positive. On the other hand, in all 31 CSF-STA negative cases, serum-STA was found to be positive. In CSF-STA positive cases, CSF-RBT was negative in 15 patients. However, in two CSF-STA negative samples, CSF-RBT was found to be positive.

Comparison and agreement of serological tests. There was no significant difference between the sensitivities of CSF-RBT and CSF-STA ($p = 0.163$), and the serum RBT and the serum-STA ($p = 0.500$). Nonetheless, there were differences for either serum-RBT and CSF-RBT or serum-STA and CSF-STA ($p = 0.006$, $p < 0.001$, respectively). The agreement between RBT tests either in the serum or CSF was poor (κ, 0.137). Likewise, there was a poor agreement between serum-STA and CSF-STA tests (κ, −0.065). When CSF-STA titres were correlated with serum-STA titres ($r = 0.111$, $p = 0.261$), no concordance was detected.

Culture process

In this study, CSF culture was not obtained in 38 (21.5%) patients. Thus, 25.3% of samples were positive (22 of 87) for automated CSF culture and 8.8% of samples were positive (5 of 52) for conventional CSF culture. Among the five patients without serological evidence, three had CSF culture and two had blood culture positivity. The difference between these two culture techniques was statistically significant ($p = 0.013$). The agreement between the automated CSF and automated blood culture positivity was poor and their sensitivities did not differ statistically ($p = 0.463$, $\kappa = -0.048$). The interrelations between the culture methods are presented in Table 2.

The interrelations between laboratory tests

- *STA titres and RBT*: The mean CSF-STA titres were significantly higher in CSF-RBT positive cases (1/155.48 ($\log_{10}$: 1.88 ± 0.50), median = 1/160) than in CSF-RBT negative cases (1/21.20 ($\log_{10}$:1.26 ± 0.21), median = 1/20) ($p < 0.001$). The distribution of CSF-STA titres according to RBT results is presented in Table 3. On the other hand, the mean serum-STA titres were not significantly different in CSF-RBT positive cases (1/717.22 ($\log_{10}$:2.71 ± 0.36), median = 1/640) than in CSF-RBT negative cases (1/597.04 ($\log_{10}$:2.63 ± 0.40), median = 1/320) ($p = 0.380$).
- *Agreement of CSF culture with serology*: There was no significant difference for CSF-STA test positivity between automated CSF culture positives (*n*, 14/17; 82.4%) and negatives (*n*, 49/62; 79%) ($p = 1.000$). CSF-RBT was found to be positive in 18 of the 19 automated CSF culture positive (95%) patients and in 44 out of 46 (95%) automated CSF culture negative patients. There was a poor agreement between automated CSF culture and CSF-RBT tests (κ, −0.006). In automated CSF culture positive and negative patients there were poor agreements between positive serum-STA and CSF-STA test results (κ, −0.103 and −0.031, respectively).

TABLE 2. The interrelations between the culture methods in chronic neurobrucellosis

		Conventional CSF			Automated CSF			Blood		
Cultures		**Pos**	**Neg**	**Total**	**Pos**	**Neg**	**Total**	**Pos (%)**	**Neg (%)**	**Total**
Conventional CSF, (*n* = 52)	Pos				0 (–)	0 (–)	0	3 (60)	2 (40)	5
	Neg				1 (–)	0 (–)	1	16 (34.8)	30 (65.2)	46
Automated CSF (*n* = 87)	Pos							10 (45.5)	12 (54.5)	22
	Neg							23 (35.9)	41 (64.1)	64

Pos, positive; Neg, negative.

TABLE 3. The distribution of CSF-STA titres according to RBT and culture results

STA titres	CSF-STA (*n*)	CSF-RBT Pos (*n*)	CSF-RBT Neg (*n*)	Conventional CSF culture Pos (*n*)	Conventional CSF culture Neg (*n*)	Automated CSF culture Pos (*n*)	Automated CSF culture Neg (*n*)
≥ 1/20	26	8	14	(*n* = 2)	4	6	6
<1/20–1/40	17	15	1	(–)	4	4	4
<1/40–1/80	25	18	(–)	(–)	12	1	10
<1/80–1/160	23	16	(–)	(–)	9	2	12
<1/160–1/320	12	8	(–)	(–)	4	1	7
<1/320–1/640	4	3	(–)	(–)	(–)	(–)	4
<1/640–1/1280	1	(–)	(–)	(–)	(–)	(–)	1
<1/1280	5	1	(–)	(–)	(–)	(–)	5
Overall positives	113	69	15	2	33	14	49
Untested	33	4	2	2	10	5	4
Negative	31	2	14	1	9	3	13
Total	177	75	31	5	52	22	66

- *CSF-STA titers and CSF culture*: The CSF-STA titres were significantly lower in CSF culture positive patients ($\log_{10}$:1.50 ± 0.48 (mean titre, 1/61.75)) than in culture negative patients ($\log_{10}$:2.08 ± 0.59 (mean titer, 1/372.98)) ($p < 0.001$).
- *Serum-STA titres and CSF culture*: However, there was no significant difference for serum-STA titres between culture positive patients (1/627.37 ($\log_{10}$:2.63 ± 0.39)) and negative patients (1/675.48 ($\log_{10}$:2.68 ± 0.36)) in automated CSF culture ($p = 0.614$). Accordingly, there was no significant difference for serum-STA titres between culture positive patients (1/480.00 ($\log_{10}$:2.56 ± 0.35)) and negative patients (1/600.00 ($\log_{10}$:2.57 ± 0.43)) in conventional CSF culture ($p = 0.913$).
- *CSF-STA titres and blood culture*: The mean CSF-STA titre was 1/305.28 ($\log_{10}$:1.94 ± 0.61) in blood culture negative patients and 1/300.06 ($\log_{10}$:1.94 ± 0.64) in blood culture positive patients. The association was statistically insignificant ($p = 0.989$).
- *CSF-STA titres and biochemical tests*: There was a statistically significant, moderate, negative correlation between CSF-STA titres and CSF protein ($r = -0.241$, $p = 0.010$). When the CSF-STA titres were correlated with CSF/blood-glucose ratios ($r = 0.056$, $p = 0.558$), no significance was detected. When the CSF-STA titres were correlated with CSF leucocyte counts ($r = -0.014$, $p = 0.888$), no significance was detected.
- *The interrelations between biochemical tests*: There was a statistically significant, moderate, but inverse correlation between CSF leucocyte counts and CSF/blood-glucose ratios (n, 156; $r = -0.420$; $p < 0.001$). A statistically significant, fair and negative correlation between CSF protein levels and CSF/blood-glucose ratios was detected ($r = -0.338$, $p < 0.001$). There was a significant and fair correlation between CSF leucocyte counts and CSF protein levels ($r = 0.243$, $p = 0.002$). The interrelations between the laboratory analyses are presented in Table 4.

TABLE 4. The correlation between the laboratory data during chronic neurobrucellosis

	Serum STA titres	CSF/Blood glucose	CSF leucocyte counts
CSF-STA titres	$r = 0.111$, $p = 0.261$	$r = 0.056$, $p = 0.558$	$r = -0.014$, $p = 0.888$
CSF leucocyte Counts		$r = -0.420$, **$p < 0.001$**[a]	
CSF protein		$r = -0.338$, **$p < 0.001$**[a]	$r = 0.243$, **$p = 0.002$**[b]

[a]Statistically significant, moderate and inverse correlation.
[b]Statistically significant and moderate correlation.
Significance of bold value is $p < 0.05$.

Correlation of laboratory tests and patient demographics

The mean CSF-STA titres were not significantly different between male patients (1/220.30 ($\log_{10}$:2.34 ± 0.546) min–max = 1/8–1/2560) and female patients (1/342.42 ($\log_{10}$:2.53 ± 0.66) min–max = 1/8–1/5120) ($p = 0.196$). Accordingly, there was no significant difference between male patients (1/727.89 ($\log_{10}$:2.69 ± 0.40), min–max = 1/40–1/2560) and female patients (1/611.16 ($\log_{10}$:2.61 ± 0.40), min–max = 1/40–1/2560) for serum-STA titres ($p = 0.234$). No significant correlation was detected between the age of the patients and serum-STA titres (r,–0.1; $p = 0.205$), CSF-STA titres (r, –0.038; $p = 0.692$), CSF protein levels (r, –0.052; $p = 0.513$) and CSF/blood-glucose ratios (r, –0.010; $p = 0.903$). However, there was a weak and inverse correlation between increasing age and CSF leucocyte counts (r, –0.163; $p = 0.041$).

Discussion

Neurobrucellosis, which mainly presents as meningitis or meningoencephalitis, is a differential diagnostic challenge for clinicians. In our study, neither automated nor conventional CSF cultures were taken in one-fifth of the patients. This was probably due to the chronic and relatively silent nature of the disease compared with purulent meningitis. Furthermore, inexperienced clinicians may not consider the culture process,

but serology, for the differential diagnosis of this unexplained brain disorder, leading to the subsequent establishment of the neurobrucellosis diagnosis. On the other hand, although positive bacterial culture is the reference standard for diagnosis of infectious diseases, it has been demonstrated to be suboptimal in neurobrucellosis [18,19]. The increased efficacy of automated blood culture systems has been advocated by some authors in other forms of chronic meningitis, as in tuberculosis [20]. Accordingly, automated blood culture systems have been reported to improve the recovery speed of the *Brucella* strains from the blood [21]. The sensitivity of automated blood culture systems was reported to be 80–100% positive in brucellosis [22,23]. These systems were known to be reliable in the isolation of the pathogen within 5–7 days [24,25]. To the authors' knowledge, nothing is noted in the literature on the efficacy of automated blood culture systems in the isolation of *Brucella* species from the CSF in neurobrucellosis patients. In our study, conventional bacterial culture was reproductive in one-tenth of our cases, while automated systems yielded the pathogen from the CSF in one-fifth of the patients. Thus, the yield of automated culture was significantly higher than the conventional culture in CSF samples. According to our data, although the association between the concordant CSF and blood culture yields was poor, their sensitivities were not substantially different for automated culture systems. This poor agreement is probably related to organotropism of the infecting pathogen. In addition to blood and CSF cultures, the pathogen can be cultured from other sites of localized disease, including bone marrow. In our study, in three out of eight patients (37%), *Brucella* strains were recovered from the bone marrow specimens.

In automated CSF culture positive patients the detection rate of the disease by CSF-STA was not higher than in culture negative patients. However, the titres were significantly lower in the culture-positive group. Higher CSF-STA titres increased the quantity of antibodies in the CSF, which seems to indicate the probable clearance of the pathogen due to strong immunity, leading to high culture negativity. On the other hand, the CSF isolation of the infecting microorganisms with either automated or conventional culture methods did not alter blood STA titres. Conversely, blood culture positivity did not affect CSF-STA titres. Thus, we found that the agreement of automated CSF culture with CSF-RBT, serum-STA and CSF-STA tests was poor, indicating the different kinetics of serology and culture.

STA, RBT, ELISA, complement fixation, indirect coombs and immunecapture agglutination (Brucellacapt) methods have been used in the serological diagnosis of Brucellosis [26–28]. As culturing of Brucella takes time and is not always reliable, diagnosis is usually based on these indirect serological tests. However, because these conventional serological tests lack full sensitivity and specificity, there may be discordances between their results [29]. This was also confirmed with differences either in CSF or in blood tests in our study. Moreover, agglutination reactions become positive during the second to third week of illness [30], and thus our results show the real sensitivities of serological tests because our patients have a chronic nature of CNS disease over 4 weeks. According to our data, serological approaches appear to be the mainstays in the diagnosis of neurobrucellosis due to the relatively lower efficacy of bacterial culture. Although the CSF-STA test was not shown to be statistically superior to CSF-RBT in our data, probably due to the chronic nature of our patients, CSF-STA titres were significantly higher in CSF-RBT positive patients. However, this correlation was not established between serum-STA titres and CSF-RBT. In addition, CSF-RBT remains negative with lower CSF-STA titres. Accordingly, CSF-RBT tended to be positive when the CSF-STA titre was equal to or higher than 1/40. Only in one out of 15 patients with a CSF-STA titre lower than 1/40, was CSF-RBT found to be positive. Thus, the 1/40 STA titre seems to be the threshold for CSF-RBT positivity and CSF-RBT seems inefficient for lower titres in chronic meningitis or meningoencephalitis; however, in two CSF-STA negative patients, CSF-RBT was found to be positive. It is known that there are four IgG subclasses targeting *Brucella*, with the IgG4 subclass predominating in three-fourths of patients with chronic brucellosis, followed by IgG1, IgG2 and IgG3. The last two were detected in a quarter of the patients [31]. Basically, RBT has a predilection to detect IgG1 and in these two aforementioned CSF-STA negative and CSF-RBT positive patients, the serological profile might be attributed to this specific IgG1 positivity or, alternatively, a laboratory error might have occurred during the evaluation of the CSF-STA test in these two cases [31,32].

In our study, serum RBT and STA were found to be 95.9% and 94.2% positive and our data supported the view that blood serological tests were significantly more sensitive than CSF tests. On the other hand, the agreement between serum-STA and CSF-STA was poor and the agreement between their titres for both sites was insignificant. Accordingly, gender did not affect STA titres either in the serum or CSF. The same result was observed for agreement between CSF and serum RBTs. Moreover, positivity of CSF-RBT did not affect serum-STA titres. Thus, when the serological responses were evaluated, CSF and the blood appeared to be the two distinct sites with different kinetics.

Although the ELISA test was shown to be promising in CNS Brucellosis [13,33], the sensitivity of this method was not reported to be higher than that of conventional tests [26]. This seemed to be the case in our study. On the other hand, CSF

PCR was not applied to any of our cases, and we could not present data for PCR. However, in one study real-time PCR assay was positive in all of six neurobrucellosis cases [14]. According to these preliminary data, molecular diagnostic methods seem to be promising in the diagnosis and follow-up of neurobrucellosis [34].

The microbiological evidence of neurobrucellosis has been known to be accompanied by lymphocytic pleocytosis, elevated protein levels and hypoglycorrhachia [11,15,35]. Normal CSF values have been accepted to be <50 mg/dL of protein, a CSF-to-serum glucose ratio >0.6 and leucocyte counts <5/μL in CNS infections [19,36]. In the initial reports with small case series of neurobrucellosis, abnormalities of the cerebrospinal fluid were reported to include a pleocytosis of 10–200 leucocytes [15]. In our study, CSF leucocyte counts (215.99 ± 306.87) and CSF protein levels (330.64 ± 493.28 mg/dL) ranged over a wide spectrum and were apparently higher than expected. Moreover, although the increased CSF protein levels slightly reduced CSF-to-serum glucose ratios, leucocyte counts had a moderate effect on this issue. The pathogenesis of CSF hypoglycorrhachia is multifactorial and may include an increased glycolysis by leucocytes and bacteria and increased metabolic rate of the brain and spinal cord [36]. These data appear to indicate the effect of an inflammatory response on CSF glucose consumption. A similar correlation existed between CSF protein levels and leucocyte counts, which both increased concordantly. On the other hand, CSF leucocyte counts and CSF/blood-glucose ratios, as the indicators of inflammation and cellular metabolism, did not affect CSF-STA titres. This is probably due to the chronic nature of our neurobrucellosis patients, where STA titres were already established. However, CSF protein levels altered CSF-STA titres because Brucella immunoglobulins are one of the direct contributors to CSF protein, although the effect was moderate, indicating the presence of other sources.

It is reported that advanced age did not affect CSF leucocytes, CSF/blood-glucose ratios and CSF protein levels during the course of acute purulent meningitis in the elderly population [7]. In this chronic form of CNS disease, the results were similar, and serum or CSF-STA titres, protein levels and CSF/blood-glucose ratios were unaffected by ageing. However, there was a slight decrease in leucocyte counts with advancing age in our study.

Conclusion

Chronic brucellar meningitis and meningoencephalitis frequently have a subtle nature and cannot be diagnosed easily due to the relatively silent course of the disease [6]. Thus, the clinician should use every diagnostic modality available in the diagnosis of the disease. The high seropositivity in brucellar blood tests must facilitate the use of blood serology in a chronic CNS infection in the absence of any aetiological diagnosis. Moreover, automated culture systems should be applied when CSF culture is needed in probable neurobrucellosis patients with chronic CNS disease.

Acknowledgements

We would like to thank Professor Dr Arif Kaygusuz for his contributions in interpreting the results.

Funding

We (all of the authors) did not have any funding for the Istanbul-2 study.

Conflict of Interest

We (all of the authors) have no competing interests to declare for the Istanbul-2 study.

Transparency Declaration

None to declare.

References

1. Calik S, Gokengin D. Human brucellosis in turkey: a review of the literature between 1990 and 2009. *Turk J Med Sci* 2011; 41: 549–555.
2. Banerjee TK, Pal AK, Das S. Neurobrucellosis presenting as acute meningoencephalitis. *Neurol India* 1999; 47: 160.
3. Turel O, Sanli K, Hatipoglu N, Aydogmus C, Hatipoglu H, Siraneci R. Acute meningoencephalitis due to brucella: case report and review of neurobrucellosis in children. *Turk J Pediatr* 2010; 52: 426–429.
4. Demiroglu YZ, Turunc T, Karaca S *et al.* [neurological involvement in brucellosis; clinical classification, treatment and results]. *Mikrobiyol Bul* 2011; 45: 401–410.
5. Haji-Abdolbagi M, Rasooli-Nejad M, Jafari S, Hasibi M, Soudbakhsh A. Clinical and laboratory findings in neurobrucellosis: review of 31 cases. *Arch Iran Med* 2008; 11: 21–25.
6. Erdem H, Ulu-Kilic A, Kilic S *et al.* The efficacy and tolerability of antibiotic combinations in neurobrucellosis: results of the istanbul study. *Antimicrob Agents Chemother* 2012; 56: 1523–1528.
7. Erdem H, Kilic S, Coskun O *et al.* Community-acquired acute bacterial meningitis in the elderly in Turkey. *Clin Microbiol Infect* 2010; 16: 1223–1229.

8. Young EJ. Brucella species. In: Mandell GL, Bennett JE, Dolin R, eds. *Mandell, dolin, and bennett's principles and practice of infectious diseases*. 7th edn. Philledelphia: Churchil Livingstone, 2010; 2921–2925.
9. Shakir RA, Al-Din AS, Araj GF, Lulu AR, Mousa AR, Saadah MA. Clinical categories of neurobrucellosis. A report on 19 cases. *Brain* 1987; 110 (Pt 1): 213–223.
10. Navarro E, Segura JC, Castano MJ, Solera J. Use of real-time quantitative polymerase chain reaction to monitor the evolution of brucella melitensis DNA load during therapy and post-therapy follow-up in patients with brucellosis. *Clin Infect Dis* 2006; 42: 1266–1273.
11. Corbel MJ. *Brucellosis in humans and animals*. Geneva: World Health Organization Publications, 2006.
12. Sanchez-Sousa A, Torres C, Campello MG *et al.* Serological diagnosis of neurobrucellosis. *J Clin Pathol* 1990; 43: 79–81.
13. Araj GF. Enzyme-linked immunosorbent assay, not agglutination, is the test of choice for the diagnosis of neurobrucellosis. *Clin Infect Dis* 1997; 25: 942.
14. Colmenero JD, Queipo-Ortuno MI, Reguera JM, Baeza G, Salazar JA, Morata P. Real time polymerase chain reaction: a new powerful tool for the diagnosis of neurobrucellosis. *J Neurol Neurosurg Psychiatry* 2005; 76: 1025–1027.
15. Al Deeb SM, Yaqub BA, Sharif HS, Phadke JG. Neurobrucellosis: clinical characteristics, diagnosis, and outcome. *Neurology* 1989; 39: 498–501.
16. Tunkel RA. Approach to the patient with central nervous system infection. In: Mandell GL, Bennett JE, Dolin R, eds. *Mandell, douglas, and bennett's principles and practice of infectious diseases*, 6th edn. Philadelphia: Churchill Livingstone, 2005; 1079–1084.
17. Altman DG. *Practical statistics for medical research*. London: Chapman and Hall, 1991.
18. Bennett JE. Chronic meningitis. In: Mandell GL, Bennett JE, Dolin R, eds. *Mandell, douglas, and bennett's principles and practice of infectious diseases*. Philadelphia: Churchill-Livingstone, 2011; 1237–1242.
19. Gul HC, Erdem H, Bek S. Overview of neurobrucellosis: a pooled analysis of 187 cases. *Int J Infect Dis* 2009; 13: e339–e343.
20. Thakur R, Goyal R, Sarma S. Laboratory diagnosis of tuberculous meningitis - is there a scope for further improvement? *J Lab Physicians* 2010; 2: 21–24.
21. Mantur BG, Amarnath SK. Brucellosis in india - a review. *J Biosci* 2008; 33: 539–547.
22. Ariza J, Corredoira J, Pallares R *et al.* Characteristics of and risk factors for relapse of brucellosis in humans. *Clin Infect Dis* 1995; 20: 1241–1249.
23. Yagupsky P, Peled N, Press J, Abramson O, Abu-Rashid M. Comparison of bactec 9240 peds plus medium and isolator 1.5 microbial tube for detection of brucella melitensis from blood cultures. *J Clin Microbiol* 1997; 35: 1382–1384.
24. Bannatyne RM, Jackson MC, Memish Z. Rapid diagnosis of brucella bacteremia by using the bactec 9240 system. *J Clin Microbiol* 1997; 35: 2673–2674.
25. Ruiz J, Lorente I, Perez J, Simarro E, Martinez-Campos L. Diagnosis of brucellosis by using blood cultures. *J Clin Microbiol* 1997; 35: 2417–2418.
26. Gomez MC, Nieto JA, Rosa C *et al.* Evaluation of seven tests for diagnosis of human brucellosis in an area where the disease is endemic. *Clin Vaccine Immunol* 2008; 15: 1031–1033.
27. Araj GF, Lulu AR, Saadah MA, Mousa AM, Strannegard IL, Shakir RA. Rapid diagnosis of central nervous system brucellosis by elisa. *J Neuroimmunol* 1986; 12: 173–182.
28. Araj GF, Lulu AR, Khateeb MI, Saadah MA, Shakir RA. Elisa versus routine tests in the diagnosis of patients with systemic and neurobrucellosis. *APMIS* 1988; 96: 171–176.
29. Al Dahouk S, Tomaso H, Nockler K, Neubauer H, Frangoulidis D. Laboratory-based diagnosis of brucellosis–a review of the literature. Part i: techniques for direct detection and identification of brucella spp. *Clin Lab* 2003; 49: 487–505.
30. Wallach J. Brucellosis (undulant fever). In: Winter N, Konstant O, eds. *Interpretation of diagnostic tests*, 8th edn. Crawfordsville: Lippincott Williams & Wilkins, 2007; 916.
31. Araj GF. Profiles of brucella-specific immunoglobulin g subclasses in sera of patients with acute and chronic brucellosis. *Serodiagn Immunother Infect Dis* 1988; 2: 401–410.
32. Araj GF, Lulu AR, Khateeb MI, Haj M. Specific ige response in patients with brucellosis. *Epidemiol Infect* 1990; 105: 571–577.
33. Young EJ. Utility of the enzyme-linked immunosorbent assay for diagnosing neurobrucellosis. *Clin Infect Dis* 1998; 26: 1481.
34. Araj GF. Update on laboratory diagnosis of human brucellosis. *Int J Antimicrob Agents* 2010; 36 (Suppl 1): S12–S17.
35. Asadipooya K, Dehghanian A, Omrani GH, Abbasi F. Short-course treatment in neurobrucellosis: a study in iran. *Neurol India* 2011; 59: 101–103.
36. Tunkel AR. Approach to the patient with central nervous system infection. In: Mandell GL, Bennett JE, Dolin R, eds. *Mandell, douglas, and bennett's principles and practice of infectious diseases*. Philadelphia: Churchill-Livingstone, 2010; 1183–1188.