

Original Papers

Comparative Study of Paired Serum and Cerebrospinal Fluid Samples from Neurocysticercosis Patients for the Detection of Specific Antibody to *Taenia solium* Immunodiagnostic Antigen

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Abstract: Neurocysticercosis (NCC) is an important disease of the central nervous system caused by infection with *Taenia solium* metacestodes. In addition to the clinical findings and the imaging analysis, the results of immunological tests are informative for the diagnosis of NCC. To compare the usefulness of serum and cerebrospinal fluid (CSF) samples for antibody detection, paired serum and CSF samples from patients with NCC and other neurological diseases were examined by an enzyme-linked immunosorbent assay with low-molecular-weight antigens purified from *T. solium* cyst fluid in a blinded fashion. The sensitivity of both serum and CSF samples was 25.0% in inactive NCC cases (n = 4) and 90.9% in active NCC cases (n = 33), and the specificity of serum and CSF was 100% and 95.8%, respectively. When the serum and CSF samples were combined, the sensitivity in active NCC cases became 100%. There was no difference in test performance between serum and CSF samples. Based on these results, we recommend the detection of specific antibodies in serum for the diagnosis of active NCC because of the ease of collection. When the antibody test is negative, however, CSF should be used to confirm NCC and to rule out other medical disorders of the central nervous system. Antibody detection test using only serum or CSF has a limited diagnostic value and cannot be recommended for the diagnosis of suspected inactive NCC cases.

Key words: *Taenia solium*, neurocysticercosis, immunodiagnosis, antibody detection, serum, cerebrospinal fluid, glycoproteins

INTRODUCTION

Cysticercosis (CC), caused by the larval stage of *Taenia solium*, is a serious human parasitic disease noted mainly in developing countries in Latin America, Africa and Asia. In addition, globalization increases the number of CC cases even in developed countries [1–4]. Humans are accidentally infected with *T. solium* by ingestion of eggs excreted with the feces of carriers harboring the adult tapeworm. Oncospheres hatched from eggs in the small intestine of humans migrate into the skeletal muscle, subcutaneous tissue, eyes or central nervous system (CNS) and develop into cysticerci. Neurocysticercosis (NCC) caused by the infection of the CNS by cysticerci is a major cause of epilepsy and mortality in developing countries [5].

The diagnosis of NCC is mainly based on clinical criteria and on the results of neuroimaging techniques such as

computed tomography (CT) and magnetic resonance imaging (MRI) [5, 6]. In some cases, however, these imaging techniques may not provide a definitive diagnosis because of unclear or atypical images. Therefore, immunological tests are considered important methods to confirm clinical findings, to facilitate diagnosis by providing information on parasites in the case of unclear images, and to conduct surveys in endemic areas where imaging techniques are not readily available [7, 8].

As immunological tests, the two methods of antibody detection [9–12] and antigen detection [13–16] have been recognized, and serum and cerebrospinal fluid (CSF) samples are mainly utilized as diagnostic specimens. For antigen detection, it has been shown and accepted that the use of CSF was suitable [17]. For antibody detection, however, some studies have suggested that the use of CSF was more sensitive than the use of serum [10, 11], while other studies

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have suggested that there was no difference in diagnostic performance between CSF and serum [12, 17]. Therefore, we still need to determine which diagnostic specimen, serum or CSF, should be used, because a consensus has not yet been obtained.

Previously, we reported the cysticercosis-specific diagnostic antigens, also known as low-molecular-weight antigens (LMWAgS), for antibody detection [18]. LMWAgS consist of glycoproteins which range in molecular size from 10 to 26 kDa under reducing condition and which have been purified and characterized by also other methods such as affinity chromatography using lentil lectin [9] or monoclonal antibody [19], trichloroacetic acid/acetone mixture precipitation [20], and cation-exchange chromatography [21]. Furthermore, we demonstrated that LMWAgS had a high specificity and sensitivity for cysticercosis by both immunoblot and enzyme-linked immunosorbent assay (ELISA) using serum samples from patients with cysticercosis, echinococcosis, schistosomiasis, sparganosis, paragonimiasis, clonorchiasis, filariasis and so on [18]. In this study, we investigated whether there is a difference in antibody detection performance between serum and CSF using paired serum/CSF samples and LMWAgS.

METHODS

Serum and CSF samples

A total of 61 paired serum and CSF samples from 37 NCC patients and 24 patients with other neurological diseases (OND) from Brazil were studied in a blinded fashion. NCC patients included cisternal NCC ($n = 6$), parenchymal NCC ($n = 23$), parenchymal and cisternal NCC ($n = 2$, cysts in the parenchyma of one patient were calcified), parenchymal and ventricular NCC ($n = 2$), spinal NCC ($n = 1$), ventricular NCC ($n = 2$) and racemose NCC ($n = 1$). Among these patients, nine had a single cyst and four were inactive cases according to the criterion described previously [22]. In brief, an active case is a patient with viable cysts and/or transitional cysts, while an inactive case is a patient with calcified dead cysts. OND consisted of acquired immune deficiency syndrome ($n = 1$), aseptic meningitis ($n = 1$), Behcet's diseases ($n = 1$), Bickerstaff's encephalitis ($n = 1$), cerebral low-grade glioma ($n = 2$), cerebral venous thrombosis ($n = 1$), CNS vasculitis ($n = 1$), CNS cryptococcosis ($n = 1$), Fabry's disease ($n = 1$), Guillain-Barré syndrome ($n = 1$), idiopathic intracranial hypertension ($n = 1$), lateral amyotrophic sclerosis and peripheral neuropathy ($n = 1$), leukemia ($n = 2$, meningeal lymphoblastic leukemia and acute lymphoblastic leukemia with intrathecal chemotherapy), multiple cranial nerve (III, V, VII and VIII nerves) dysfunction with proba-

ble diagnosis of neuroborreliosis ($n = 1$), multiple sclerosis ($n = 2$), neurosyphilis ($n = 2$), normal pressure hydrocephalus ($n = 1$), peripheral neuropathy ($n = 1$), spinal histoplasmosis ($n = 1$) and subarachnoid hemorrhage ($n = 1$).

The patients were informed about the study, and ethical approval was obtained from the University of São Paulo. Inclusion criteria for NCC cases were i) diagnosis of NCC (definitive or probable diagnoses) based on the criteria described by Del Brutto et al. [23], ii) cases followed for at least six months, iii) submission to a minimum of one MRI scan, iv) agreement on terms of consent in the study, while the exclusion criteria for NCC were absence of the minimum NCC diagnosis criteria established by Del Brutto et al. [23]. Out of 37 NCC cases used in this study, 22 were definitive NCC and 15 were probable NCC. Inclusion criteria for OND cases were i) any clinical manifestation, ii) submission to imaging analysis of CNS (CT or preferentially MRI) at least once, iii) no suspicion of NCC by imaging analysis of CNS, iv) agreement on terms of consent in the study. None of the patients had undergone immunological tests before this study.

Preparation of LMWAgS

LMWAgS was prepared from cyst fluid of *T. solium* isolated from naturally infected pigs in Ecuador by preparative isoelectric-focusing electrophoresis [18]. Briefly, 20 ml of cyst fluid dialyzed against 5 mM Tris-HCl buffer (pH 7.4) was mixed with 20 ml of 5 mM Tris-HCl buffer (pH 7.4) and 3 ml of ampholyte (Bio-Rad), and then subjected to preparative isoelectric focusing electrophoresis using a Rotofor cell (Bio-Rad). After electrophoresis, fractions containing antigen, pH range of 9.4–9.8, were recovered and kept at -20°C until use.

ELISA

ELISA plates (Nalge Nunc International) were coated with $0.1\ \mu\text{g}$ of purified LMWAgS. The wells were blocked with $300\ \mu\text{l}$ of blocking solution (1% casein in PBS) at 37°C for 1–2 hr. After the wells had been washed twice with PBS containing 0.1% Tween 20 (PBST), $100\ \mu\text{l}$ of serum samples diluted 1:100 or CSF samples diluted 1:10 in blocking solution were added and incubated at 37°C for 1 hr. The wells then were washed five times with PBST, incubated with $100\ \mu\text{l}$ of peroxidase-conjugated protein G (Invitrogen) capable of recognizing all human IgG subclasses at 37°C for 1 hr, and washed five times with PBST. After incubation with $100\ \mu\text{l}$ of substrate (0.4 mM 2,2'-azino-di-[3-ethyl-benzthiazoline sulfonate] in 0.1 M citric acid buffer, pH 4.7) for 30 min at room temperature, the absorbance at 405 nm with a reference wavelength of 490

nm was measured. Samples showing values greater than the mean value plus five standard deviations for 34 negative control serum samples and 34 negative control CSF samples, collected from healthy people not showing any clinical features of NCC were considered to be positive. Negative control serum and CSF samples used for the calculation of each cutoff value were not paired samples. The cutoff values for serum and CSF samples were 0.067 and 0.044, respectively.

Immunoblot analysis (IB)

LMWAgS were treated with a SDS sample buffer (62.5 mM Tris-HCl, pH 6.8, 2.0% SDS, 50 mM dithiothreitol and 10.0% glycerol) at 100°C for 5 min and separated in a 15.0% polyacrylamide gel. The separated proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane sheet (Millipore). The sheet was blocked with blocking solution and probed with serum samples diluted 1:20 or CSF samples diluted 1:5 in blocking solution followed by peroxidase-conjugated recombinant protein G. 4-Chloro-1-naphthol (KPL) was used for color development. If any one band was detected, it was judged to be positive.

Statistical analyses

The proportion of serum and CSF samples with positive results was compared by the binomial test, and Spearman’s rank test was used for the correlation analysis of absorbance results from serum and CSF samples.

RESULTS

In a blinded fashion, a total of 61 paired serum and CSF samples from 37 patients with NCC and 24 patients with OND were examined to detect specific antibodies to LMWAgS purified from *T. solium* cyst fluids by ELISA (Fig. 1, Table 1). Absorbance values obtained with serum and CSF samples correlated closely (Fig. 2, Spearman’s rank test; $R_s = 0.791$, $P = 0.0000002$). Six serum and seven CSF ELISA-positive samples with an absorbance value less than or equal to 0.220 were selected and analyzed by IB (Table S1). Although four CSF samples could not be

analyzed due to the insufficient amount of specimen, the results obtained by IB were consistent with those of ELISA except for two serum samples with absorbance values of 0.091 and 0.110, close to the cutoff absorbance value of 0.067 (Table 2). In this study, we performed statistical analyses on diagnostic performance between serum and CSF samples based on the results of ELISA because it was not possible to test all samples by IB and because the results of ELISA were provided by objective absorbance values. In one of four inactive NCC cases, both serum and CSF were antibody-positive, resulting in 25% sensitivity. In 27 of 33 active NCC cases, both serum and CSF samples were positive, while only serum samples and only CSF samples were positive in three cases each. Out of the six cases showing discrepant results between serum and CSF samples, two cases were patients with serum IB-negative as mentioned above and five cases were patients with a single cyst. The discrepancy in results between serum and CSF samples did not seem to be related to the location of parasite infection (Table 2). As a result, the

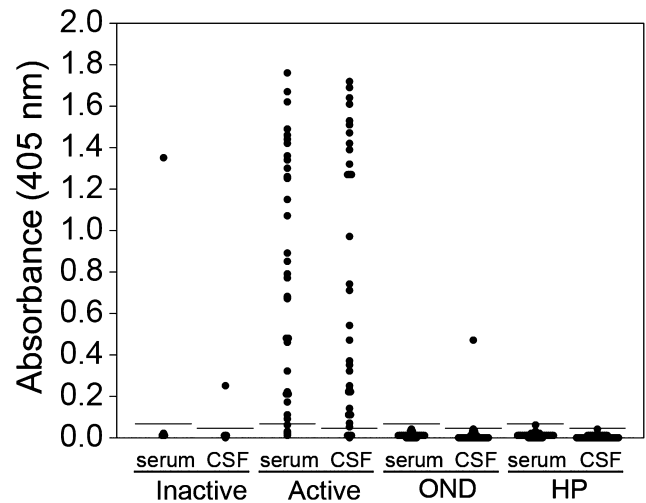


Fig. 1. Results of ELISA with LMWAgS using serum and CSF samples from four patients with inactive NCC (Inactive), 33 with active NCC (Active), 24 with other neurological diseases (OND), and 34 healthy persons (HP). The horizontal lines show the cut-off values (0.067 for serum sample and 0.044 for CSF sample).

Table 1. Results of ELISA with serum and CSF samples

Diseases category	No. of examined	Serum No. Positive (%)	CSF No. Positive (%)
Neurocysticercosis			
Inactive	4	1 (25.0)	1 (25.0)
Active	33	30 (90.9)	30 (90.9)
Other neurological diseases	24	0	1 (4.2)
Healthy controls	34	0	0

sensitivity in active NCC cases was 90.9% for both serum and CSF samples. There was no statistical difference in diagnostic performance between serum and CSF samples (Table 3). When the serum and CSF samples were combined, the sensitivity in active NCC cases became 100%. In all OND cases, both serum and CSF samples were negative except one case in which CSF was positive (Fig. 1). This case was a patient with cerebral venous thrombosis. IB analysis confirmed that this ELISA-positive CSF sample contained specific antibodies against LMWAgS (Fig. 3), indicating that the case might have been overlooked by the imaging analysis.

DISCUSSION

Using a total of 61 paired serum and CSF samples from 37 NCC patients and 24 OND patients, we showed that there was no difference between serum and CSF in the detection of specific antibodies against LMWAgS, diagnostic antigens for NCC. A previous study demonstrated that local immunity in CNS may be stimulated by antigens released from *T. solium* cyst and may enhance intra-blood-brain barrier IgG synthesis [24]. This *de novo* IgG synthesis makes the IgG level in CSF increase, in addition to IgG produced as a result of the peripheral immunity and filtrated from blood [25]. Therefore, the findings suggest that CSF is a suitable specimen for antibody detection of

NCC with a higher probability. In fact, studies carried out by ELISA with a crude *T. solium* metacestode antigen [11] and with a crude *Taenia crassiceps* cyst fluid antigen or a

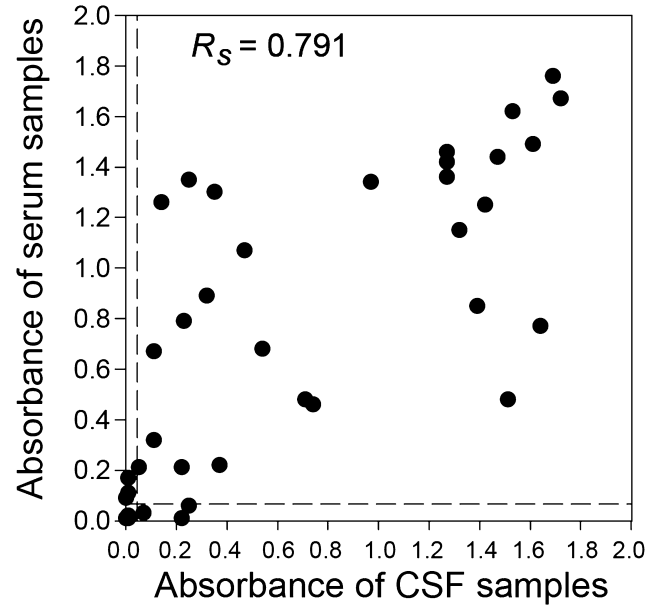


Fig. 2. Scatter graph showing the correlation between absorbance values of serum and CSF samples from 38 NCC patients in the ELISA. Spearman's rank correlation coefficient (R_s) was 0.791 ($P = 0.0000002$).

Table 2. Data of six cases showing discrepancy in ELISA results between serum and CSF

case	location of cyst	lesion number	Absorbance*		Immunoblot	
			serum	CSF	serum	CSF
1	parenchymal	multiple	0.091	0.002	Neg	nd
2	parenchymal	single	0.110	0.007	Neg	nd
3	parenchymal	single	0.166	0.010	Pos	nd
4	parenchymal	single	0.005	0.220	nd	Pos
5	cisternal	single	0.055	0.248	nd	nd
6	ventricular	single	0.031	0.073	nd	Pos

*Cutoff values for serum and CSF were 0.067 and 0.044, respectively. Neg = negative; Pos = positive; nd = not done

Table 3. Comparison of results by sera with those by CSFs from NCC patients

NCC	ELISA with CSF	ELISA with serum		
		No. positive (%)	No. negative (%)	Total (%)
Inactive	No. (%) positive	1 (25.0)	0	1 (25.0)
	No. (%) negative	0	3 (75.0)	3 (75.0)
	Total (%)	1 (25.0)	3 (75.0)	4 (100.0)
Active	No. (%) positive	27 (81.8)	3 (9.1)	30 (90.9)
	No. (%) negative	3 (9.1)	0	3 (9.1)
	Total (%)	30 (90.9)	3 (9.1)	33 (100.0)

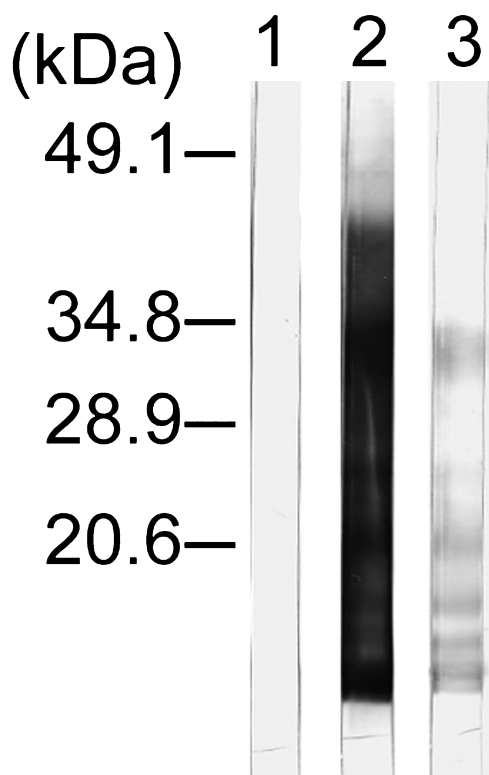


Fig. 3. Results of IB using CSF samples. Lane 1, negative control CSF; lane 2, positive control CSF; lane 3, ELISA-positive CSF in OND. Molecular size markers are indicated on the left.

crude *T. solium* metacestode antigen [10] have suggested that CSF is more sensitive than serum. However, other studies implementing enzyme-linked immunotransfer blot (EITB) with lentil-lectin-purified glycoproteins (LLGP) [17] and ELISA with saline-extracts or SDS-extracts of *T. crassiceps* metacestode [12] have demonstrated no difference in diagnostic performance between serum and CSF samples, an observation similar to our results. These varied results might be due to the antigens used in each study. Especially when CSF sample is more sensitive than serum sample, a high cut-off value resulting from non-specific reactions of serum due to impurities in the antigens might hamper the detection of weak positive serum samples by ELISA, whereas the CSF with lower protein content than serum might not produce a higher non-specific background. In this study, because we used highly purified antigens devoid of components to which serum samples react non-specifically, it was possible to detect weak positive samples due to the reliably low cut-off value. In other words, there may be no difference in detection performance between serum and CSF samples if purified antigens without components to which serum reacts non-

specifically are used.

Although antibody detection is a useful way to determine the infection of *T. solium*, it has several issues such as the possibility that patients who were exposed to failed infection become positive [26] and the very slow decline in antibody titers after the removal of the parasite by curative surgical treatment or the elimination of the parasite by immune responses and/or antiparasite chemotherapy [27]. In other words, antibody levels do not always reflect the parasite activity in patients. Antigen detection in CSF seems to be a suitable way to overcome these problems because it reflects the activity of the infected parasite [16, 17, 28]. However, the collection of CSF forces a burden on the patient because CSF is usually obtained by the invasive and painful procedure of lumbar puncture. In contrast, blood samples can be collected easily by venipuncture with minimal risks even in the field, which means that serum is a convenient material for both clinical and epidemiological work. If the diagnosis of active NCC is considered, the detection of specific antibodies against LMWAgS in serum can help to confirm NCC. However, if antibody detection test with serum is negative, CSF should be obtained and used in the antibody detection test to rule out other medical disorders that affect the CNS. In view of the current available data, antibody detection test using only serum or CSF has a limited diagnostic value and cannot be recommended for the diagnosis of suspected inactive NCC cases.

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CONFLICT OF INTERESTS

All the authors declare that they have no conflict of interests.

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