

## SHORT COMMUNICATION

## Use of the paired samples (cerebrospinal fluid and serum) in immunodiagnostic of active and inactive human neurocysticercosis

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*Paired samples of cerebrospinal fluid (CSF) and serum of 30 patients – 10 with active, 10 with inactive neurocysticercosis (NCC), and 10 control subjects – were evaluated by enzyme-linked immunosorbent assay (ELISA) using two Taenia crassiceps metacestode extracts as antigen in order to detect IgG antibodies. In active NCC, high levels of IgG were detected ( $p < 0.05$ ). The CSF samples showed 80% (CI 72-88) of reactivity in the saline extract (S) and 90% (CI 84-95) in sodium dodecyl sulphate (SDS) and the serum samples were reactive in 90% (CI 84-95) and 100% (CI 98-100) in the S and SDS antigenic extracts, respectively. The use of the paired samples of CSF and serum in active NCC showed equivalent results suggesting that the serum samples could be used as a screening in those patients whose CSF puncture is counter-indicated.*

Key words: *Taenia crassiceps* - neurocysticercosis - enzyme-linked immunosorbent assay - serum - cerebrospinal fluid

Neurocysticercosis (NCC) is the most common parasitosis of the central nervous system (CNS) in humans and the infection is acquired through ingestion of eggs from *Taenia solium*. Signs and symptoms are polymorphic and non-specific, including seizures, focal neurological signs, intra-cerebral hypertension, and cognitive behavioral dysfunctions. Given the wide range of clinical presentation, diagnosis is seldom made by history and physical examination alone. Accurate diagnosis is based on the combination of clinical, epidemiologic, radiographic, and immunologic data (Del Brutto et al. 2001, Hawk et al. 2005).

NCC has been classified in active and inactive forms, according to the associated immune response and the neuroimaging studies (Sotelo et al. 1985). In the active form there are vesicular or degenerating parasites cysts circundated by a strong inflammatory reaction in the cerebral adjacent tissue, but in the inactive form the patient has only calcified lesions and the immune response is minimal (Sotelo et al. 1985, Salgado et al. 1997, Castilho 2004). The cells from inflammatory NCC patients show a predominance of a Th1 immune response upon in vitro stimulation, and a mixed Th1/Th2 cellular immune response in the non-inflammatory phase (Bueno et al. 2004).

Enzyme-linked immunosorbent assay (ELISA) has been widely employed as a useful tool for the diagnosis of NCC through the detection of *T. solium* metacestodes IgG antibodies in cerebrospinal fluid (CSF) and serum samples (Costa et al. 1982, White Jr 2000, Hawk et al 2005). The antigens of murine *Taenia crassiceps* metacestodes, have been used as effective substitutes to the *T. solium* metacestodes antigens in the detection of IgG in CSF and serum samples due to intense antigenic similarity between both parasites (Vaz et al. 1997, Barcelos et al. 2001, Bragazza et al. 2002, Ishida et al. 2003). A recombinant 10-kDa protein of *T. solium* metacestode (CyDA) was identified as being specific to active NCC and having high homology with *T. crassiceps* metacestode (Chung et al. 2002).

The aim of this current study was to evaluate the use of paired samples (CSF and serum) in immunodiagnostic of active and inactive forms of human NCC using two *T. crassiceps* metacestodes antigens. This study was approved by the Research Ethics Committee of the Federal University of Uberlândia, Brazil.

A total of 30 paired CSF and serum samples were analyzed and divided into three groups based on clinical and laboratorial data for NCC: (1) active NCC group consisted of 10 samples from patients (6 male and 4 female, mean age: 35 years) with signs and symptoms suggestive of NCC and computed tomography and/or magnetic resonance imaging results showing vesicular or degenerative lesions, with or without calcification, in the central nervous system (CNS); (2) inactive NCC group consisted of 10 samples from patients (4 male and 6 female, mean age: 31 years) with signs and symptoms compatible with NCC and imaging studies demonstrating only calcifications in the central nervous system; (3) control group consisted

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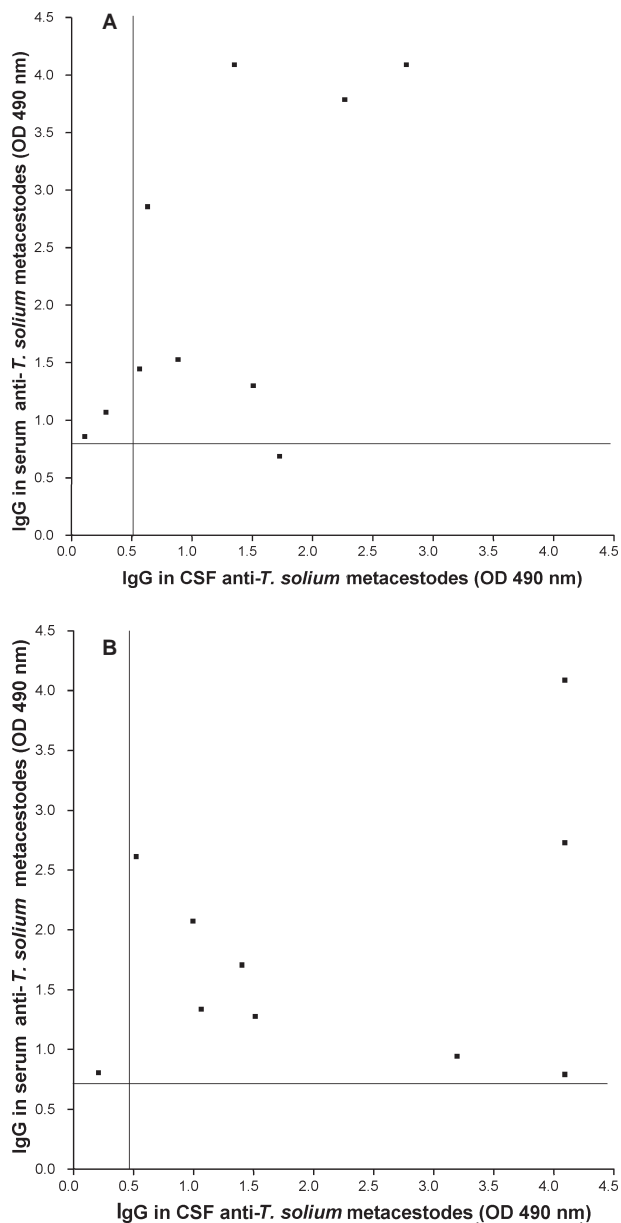
of 10 samples from patients (4 male and 6 female, mean age: 32 years) with other neurological disorders and normal imaging results.

ELISA for the detection of IgG antibodies was carried out according to Barcelos et al. (2001), using two different antigenic extracts: saline (S) and sodium dodecyl sulphate (SDS), obtained from *T. crassiceps* metacestodes, and used at a protein concentration of 10 µg/ml. CSF samples were tested undiluted and serum samples were diluted 1:200 in phosphate buffered saline (PBS, 0.1M, pH 7.2) containing 0.05% Tween 20. The conjugate goat IgG anti-human IgG-peroxidase (Fc chain specific; Sigma) was diluted at 1:2000. The cut off was established by the mean optical density (OD) obtained from three negative control samples plus two standard deviations. Statistical analysis was performed using the software Statistic for Windows (Stat soft, Inc. 1993) for the comparative analysis between two proportions, considering significance level at  $p < 0.05$ . A 95% confidence interval (CI) was stipulated.

The results of ELISA from the three patient groups are showed in the Table, the values of OD were significantly higher in the active NCC than the other two groups ( $p < 0.05$ ). The Figure shows the relationship between the values of OD in the CSF and serum samples obtained from patients with active NCC using two antigenic extracts. The results indicate that there were high correlate index using of S extract.

In the present study, we used the *T. crassiceps* heterologous cysticerci as an alternative source of antigens for the immunological diagnosis of the human NCC. In a previous ELISA study using *T. crassiceps* S and SDS antigens in the CSF samples from patients with NCC, Barcelos et al. (2001) demonstrated 85 and 87.5% of sensitivity and 100 and 97.9% of specificity, respectively. Pardini et al. (2002), in an ELISA study for detection of IgG antibodies in CSF samples in NCC, showed 100% of sensitivity and specificity, using antigen extracts obtained from the vesicular fluid of *T. crassiceps* cysticerci and from fractions purified by affinity chromatography with lectin concanavalin A and the glycoprotein antigen separated by electrophoresis.

Serology for NCC can produce false-positive results in samples of patients coming from endemic countries for cysticercosis, such as Brazil, because there is a production of specific antibodies due to previous infections that did not progress for the establishment of metacestodes or because these latter are located outside the neural tis-



Relationship between the optical density (OD) values of IgG anti-neurocysticercosis (NCC) in the no diluted cerebrospinal fluid (CSF) and diluted serum samples at 1:200 of 10 patients with the active form of NCC, using *Taenia crassiceps* metacestodes extracts by ELISA. A: saline (S); B: sodium dodecyl sulphate (SDS) — : cut off values. Pearson values  $r = 0.6150$  (S) and  $r = 0.3077$  (SDS).

TABLE  
Paired cerebrospinal fluid (CSF) and serum samples in ELISA for the detection of IgG antibodies in human eurocysticercosis (NCC) using two antigenic extracts (saline and sodium dodecyl sulphate) from *Taenia crassiceps* metacestodes

Patient	n	Saline		Serum		Sodium dodecyl sulphate		S erum	
		%	(CI)	%	(CI)	%	(CI)	%	(CI)
Active NCC	10	80	(72.0-88.0)	90	(84.0-95.0)	90	(84.0-95.0)	100	(98.0-100)
Inactive NCC	10	0		10	(4.0-16.0)	0		20	(12.0-28.0)
Control	10	0		0		0		0	

CI: confidence interval

sue (Sciutto et al. 2000). The ELISA cross-reactivity among helminthiases was found with the use of antigens (*Echinococcus granulosus* hydatid fluid, *T. solium* cysticerci saline extract, and vesicular fluid of *T. crassiceps*) belonging to phylogenetically related parasite species, by sharing same antigenic components (Ishida et al. 2003). By analyzing CSF samples of patients with NCC using excretion/secretion antigens of *T. solium* metacestodes, a significant difference between ELISA results in the detection of IgG antibodies was shown in order to distinguish the active NCC from the inactive one (Molinari et al. 2002).

The results of this study showed that patients presenting the active form of NCC showed the highest levels of specific IgG antibodies in both samples analyzed, since the immune response is maximized when the parasite goes into the degenerative phases. In the control group analyzed here none of the individuals showed reactivity.

In conclusion the used of the paired samples of CSF and serum in active NCC showed equivalent results suggesting that the serum samples could be used as a screening of those patients whose CSF puncture is contra-indicated.

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