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# Comparison of complementary diagnostic tests in cerebrospinal fluid and serum for neurocysticercosis

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## Abstract

The role of immunologic tests in the diagnosis of neurocysticercosis (NC) is controversial and few studies have made comparisons among them. The objective of this study was to compare immunological tests in both serum and cerebrospinal fluid (CSF) for the diagnosis of NC. We conducted a case-control study in Cuenca, Ecuador, enrolling patients with NC (N = 24) and matching them with other neurosurgical patients (N = 18). To detect cysticercal antigen, we used an HP10 antigen assay in serum and CSF (“HP10 Ag -serum -CSF”) and a commercial antigen assay in serum (apDia, “ELISA-Ag-serum”), and to detect cysticercal DNA, we used a polymerase chain reaction (PCR) assay in CSF (“PCR-CSF”). Assay sensitivities were: HP10 Ag-serum (41.7%, 95% confidence interval [CI] 22.1–63.4), HP10 Ag-CSF (87.5%, 95% CI: 67.6–97.3), ELISA-Ag-serum (62.5%, 95% CI: 40.6–81.2), and PCR-CSF

(79.2%, 95% CI: 57.9–92.9). Sensitivities were higher when limiting to participants with extraparenchymal NC. Specificity was 100% for all assays except ELISA-Ag-serum (72.2%). This preliminary study demonstrated the potential usefulness of the PCR and HP10 Ag assay in CSF, especially for extraparenchymal NC; thus, they could be considered as complementary diagnostic tools when neuroimaging is not conclusive.

Keywords: Evidence-based medicine, Infectious disease, Neurology

## 1. Introduction

The availability of valid and reliable diagnostic tests for neurocysticercosis (NC) is critical for its diagnosis and treatment, as well as for epidemiological studies to determine the disease burden. Diagnosis of NC is a challenge, especially for the subarachnoid forms of the disease [1]. Neuroimaging procedures are effective and desirable for diagnosis; however, they are frequently unavailable or inaccessible in endemic areas because of the technology required. Consequently, there is an urgent need for alternative diagnostic tools [2].

Current immunologic tests for the diagnosis of cysticercosis include antigen and antibody detection using enzyme-linked immunosorbent assay (ELISA) or enzyme-linked immunoelectrotransfer blot (EITB). Antibodies may persist for a considerable time after an infection has resolved and their detection is not necessarily proof of a viable infection nor does their presence necessarily implicate central nervous system (CNS) infestation. In contrast, the detection of secreted cysticercal antigen by ELISA has been reported to be highly sensitive and specific for the diagnosis of viable cysticerci located in the CNS and especially for cysts in the extraparenchymal location [3]. Detection of secreted metacestode glycoproteins constitutes proof of a viable infection; for example, the HP10 Ag ELISA, which uses a monoclonal antibody detecting a high molecular weight glycoprotein, has also been used for the diagnosis and follow-up of patients with extraparenchymal NC (EP-NC) [4]. High sensitivity and specificity for EP-NC of a polymerase chain reaction (PCR) assay that detects parasite DNA in cerebrospinal fluid (CSF) has also been reported [5, 6].

In recently published guidelines for the diagnosis and treatment of NC [7], just one immunologic test (antibody detection using EITB) is recommended as supportive evidence for the diagnosis of NC, in spite of the fact that the presence of antibody can occur in the absence of an active infection. Here we present the results of a comparative study of two assays to detect cysticercal antigen (the HP10 Ag-ELISA [8] and a commercial test [9, 10]), and PCR to detect cysticercal DNA, using CSF and serum.

## 2. Materials and methods

We conducted multicenter case-control study with enrollment from January 2015 to February 2016, to assess the validity of a PCR assay in CSF for the diagnosis of NC which is described elsewhere [5]. In a subset of participants, we also tested CSF samples using ELISA for HP10 Ag [8], and in serum we tested samples using an assay for HP10 Ag [8] and a commercial ELISA for *Taenia solium* antigen (ApDIA, “ELISA-Ag”) [9, 10]. NC cases were defined a probable or definitive diagnosis using validated diagnostic criteria [1], which required CT or MRI with contrast. For each NC case, a neurosurgical patient who did not fulfill the diagnostic criteria for NC was selected as a control. In this cohort, diagnoses for controls were hydrocephalus (n = 5), neoplasia (n = 5), cerebrovascular disease (n = 2), degenerative disease (n = 1), and others (n = 5). This study was approved by the ethics committee of the University of Cuenca and laboratory staff was blinded to the diagnosis of all participants.

PCR in CSF samples was done using a technique previously described [6], with the exception of using DNA from human cysticerci in addition to DNA from pig cysticerci as positive controls. Primers designed to amplify the highly repetitive element pTsol9 of the genome were used (GenBank accession no. U45987). The primers used in the PCR to amplify pTsol9 were 5'-CAGGGTGTGACGTCATGG-3' (forward primer; positions 21 to 38, 179 to 196, or 336 to 353) and 5'-GCTAGG-CAACTGGCCTCCT-3' (reverse primer; positions 122 to 140, 280 to 298, or 437 to 455).

CSF and serum samples were tested using an HP10 antigen ELISA with a previously described technique [8], with minor modifications [4], which detects a secreted glycoprotein of viable metacestodes. The cut-off for a positive result was defined as the mean optical density (450 nm) for the sample + 3 standard deviations.

Sera was tested in an IgG1 monoclonal antibody-based ELISA-Ag directed to a secretory-excretory antigen from *Taenia saginata* [9] with modifications [10], including pre-treatment of the sera by trichloroacetic acid. To facilitate comparison between different plates, all results were expressed as a ratio, calculated by dividing the optical density of each sample by the cut-off value. This cut-off was calculated using a *t*-test based on the optical densities of eight negative samples of Ecuadorian origin, as such, any value above 1 was classified as positive.

Sensitivity and specificity were computed for each of the assays, and among the subgroups, with exact 95% confidence intervals (CIs). Comparisons among the assays were made using McNemar's test. Cohen's kappa was calculated to determine inter-assay reliability. Statistical significance was determined based on a two-sided alpha of .05.

### 3. Results

The overall sample included 42 participants, with 24 NC cases and 18 controls (Table 1). Among the NC cases, 9 had exclusively parenchymal cysts and 15 had extraparenchymal cysts. The mean age was 49.0 years, with NC cases significantly younger than controls (43.4 years vs. 56.8 years;  $p = .017$ ). More than half of the sample was male (57.1%). The most common presenting NC symptom was headache (87.5%), followed by seizure (54.2%), and intracranial hypertension (21.7%). Most cases had vesicular cysts (83.3%), and of those who did, most had multiple vesicular cysts (70.0%). Less than half of cases had colloidal or nodular cysts (41.7%), and of those who did, most had a single one (80.0%). About half of participants had calcified cysts (54.2%), and of those who did, most had multiple calcified cysts (84.6%). Participants with EP-NC were significantly more likely to have vesicular

**Table 1.** Participant demographics, neurocysticercosis symptoms and imaging characteristics, and results of CSF analysis.

	Total n = 42	All NC cases n = 24	P-NC only 9 (37.5)	EP-NC 15 (62.5)	Controls n = 18	P-value for cases vs. controls	P-value for P-NC vs. EP-NC
<b>Age in years, mean (SD)</b>	49.0 (18.1)	43.3 (16.6)	38.0 (16.0)	46.4 (16.6)	56.8 (17.5)	$P = .017$	$P = .283$
<b>Sex, n (%)</b>							
Male	24 (57.1)	14 (58.3)	3 (33.3)	11 (73.3)	10 (55.6)	$P = .857$	$P = .092$
Female	18 (42.9)	10 (41.7)	6 (66.7)	4 (26.7)	8 (44.4)		
<b>NC symptoms</b>							
Headache	—	21 (87.5)	7 (77.8)	14 (93.3)	—	—	$P = .533$
Intracranial hypertension	—	5 (21.7)	7 (77.8)	6 (40.0)	—	—	$P = .105$
Seizure	—	13 (54.2)	0 (0.0)	5 (35.7)	—	—	$P = .116$
<b>NC imaging findings</b>							
Vesicular cyst(s)	—	20 (83.3)	5 (55.6)	15 (100.0)	—	—	$P = .012$
Single	—	6 (30.0)	3 (60.0)	3 (20.0)	—	—	$P = .131$
Multiple	—	14 (70.0)	2 (40.0)	12 (80.0)	—	—	
Colloidal/nodular cyst(s)	—	10 (41.7)	6 (66.7)	4 (26.7)	—	—	$P = .092$
Single	—	8 (80.0)	6 (100.0)	2 (50.0)	—	—	$P = .133$
Multiple	—	2 (20.0)	0 (0.0)	2 (50.0)	—	—	
Calcified cyst(s)	—	13 (54.2)	3 (33.3)	10 (66.7)	—	—	$P = .206$
Single	—	2 (15.4)	1 (33.3)	1 (10.0)	—	—	$P = .423$
Multiple	—	11 (84.6)	2 (66.7)	9 (90.0)	—	—	
<b>CSF analysis</b>							
Protein, >30 mg/dL	24 (60.0)	13 (56.5)	4 (44.4)	9 (64.3)	11 (64.7)	$P = .602$	$P = .417$
Cell count, >5 cells/mL	15 (37.5)	11 (47.8)	2 (22.2)	9 (64.3)	4 (23.5)	$P = .187$	$P = .089$
Glucose, <50 mg/dL	12 (30.0)	10 (43.5)	2 (22.2)	8 (57.1)	2 (11.8)	$P = .041$	$P = .197$

cysts than participants with parenchymal NC (P-NC) only (100% vs. 55.6%;  $p = .012$ ).

With regard to assay validity (Table 2), the highest sensitivity was for HP10 Ag CSF (87.5%), which was significantly higher ( $p = .001$ ) than HP10 Ag serum (41.7%), borderline significantly higher ( $p = .070$ ) than ELISA-Ag serum (62.5%), but not significantly higher ( $p = .500$ ) than PCR (79.2%) (Table 2). Similarly, the sensitivity of PCR was significantly higher than that of HP10 Ag serum ( $p = .012$ ). The sensitivity of ELISA-Ag serum was borderline significantly higher ( $p = .063$ ) than that of HP10 Ag serum. In the subgroup of participants with P-NC only, sensitivity was reduced for PCR (55.6%), ELISA-Ag serum (22.2%), HP10 Ag CSF (66.7%), and HP10 Ag serum (0.0%). The only significant difference that remained was the comparison of HP10 Ag CSF with HP10 serum ( $p = .031$ ), and the comparison of PCR with HP10 Ag serum was borderline significant ( $p = .063$ ). In participants with EP-NC, sensitivity improved for all assays: PCR (93.3%), ELISA-Ag (86.7%), HP10 Ag CSF (100.0%), HP10 Ag serum (66.7%). Although no comparisons were significant in this subgroup, the comparison of HP10 Ag CSF with HP10 serum was borderline significant ( $p = .063$ ). In the subgroup of participants with any vesicular cysts, results were similar to the overall sample. Specificity was 100.0% for PCR, HP10 Ag CSF, and HP10 serum, and was 72.2% for ELISA-Ag. Comparisons of specificity of other assays with ELISA-Ag were of borderline significance ( $p = .063$ ).

Overall, inter-assay reliability was excellent between PCR and HP10 Ag CSF (Kappa 0.90; 95% CI: 0.78, 1.00); fair between PCR and HP10 Ag serum (Kappa 0.45; 95% CI: 0.20, 0.70), ELISA-Ag and HP10 Ag CSF (Kappa 0.48; 95% CI: 0.25, 0.70), ELISA-Ag and HP10 Ag serum (Kappa 0.51; 95% CI: 0.28, 0.74); and poor between PCR and ELISA-Ag serum (Kappa 0.28; 95% CI: -0.01, 0.57) and HP10 Ag CSF and HP10 Ag serum (Kappa 0.38; 95% CI: 0.10, 0.66).

#### 4. Discussion

Overall, sensitivity was high for PCR in CSF, as previously reported [6]. HP10 detection in CSF also yielded a high sensitivity consistent with one study [4], but not consistent with the low sensitivity reported in another study [6]. Sensitivity varied by parasite location. In the subgroup of participants with P-NC only, all assays had lower sensitivity; however, the sample size of this subgroup was very small, and thus a larger study is needed to clarify these findings. Conversely, sensitivity improved for all assays among participants with EP-NC, consistent with previous reports [4, 6, 11].

The sensitivity of ELISA-Ag in serum was acceptable for EP-NC, but its specificity was lower than the other assays. A previous study, however, reported both excellent sensitivity and specificity [12]. Nevertheless, antigen detection is useful to assist in

**Table 2.** Sensitivity and specificity for different diagnostic tests for neurocysticercosis in serum and CSF, and comparisons among assays.

	Sensitivity and specificity*				P-values for comparisons					
	PCR CSF	ELISA-Ag serum	HP10 Ag CSF	HP10 Ag serum	PCR vs. ELISA-Ag	PCR vs. HP10 Ag CSF	PCR vs. HP10 Ag serum	ELISA-Ag vs. HP10 Ag CSF	ELISA-Ag vs. HP10 Ag serum	HP10 CSF vs. HP10 serum
<b>Sensitivity</b>										
All NC cases (N=24)	79.2 (57.9–92.9), n = 19	62.5 (40.6–81.2), n = 15	87.5 (67.6–97.3), n = 21	41.7 (22.1–63.4), n = 10	0.344	0.500	0.012	0.070	0.063	0.001
P-NC only (N=9)	55.6 (21.2–86.3), n = 5	22.2 (2.8–60.0), n = 2	66.7 (29.9–92.5), n = 6	0.0, n = 0	0.453	1.000	0.063	0.219	0.500	0.031
EP-NC (N=15)	93.3 (68.1–99.8), n = 14	86.7 (59.5–98.3), n = 13	100.0 (78.2–100.0), n = 15	66.7 (42.8–90.5), n = 10	1.000	1.000	0.219	0.500	0.250	0.063
Any vesicular cyst(s) (N=20)	80.0 (56.3–94.3), n = 16	75.0 (50.9–91.3), n = 15	90.0 (68.3–98.8), n = 18	50.0 (27.2–72.8), n = 10	1.000	0.500	0.070	0.375	0.063	0.008
<b>Specificity</b>										
Controls (N=18)	100.0 (81.5–100.0), n = 18	72.2 (46.5–90.3), n = 13	100.0 (81.5–100.0), n = 18	100.0 (81.5–100.0), n = 18	0.063	NA	NA	0.063	0.063	NA

NA, not applicable; unable to compute because no observations in 3 of 4 cells in  $2 \times 2$  table.

\* Each cell contains sensitivity or specificity and 95% confidence interval, and number positive for sensitivity or number negative for specificity.

diagnostic and treatment, as it can determine the presence/absence of viable cysts [12]. Detection of parasite DNA through PCR is useful, but is not necessarily proof of a viable infection, as the DNA, being highly stable, may persist long after death of the parasites. Therefore, only the detection of secreted products of viable parasite located in the CNS, using the HP10 Ag assay, for example, clearly indicates NC with viable cysts [3].

The main limitation of this study is its small sample size and thus, limited statistical power to produce precise confidence limits around estimates and compare assay validity. However, these preliminary results justify a more exhaustive study with a larger sample size, and possibly a meta-analysis that includes other studies, to understand the value of these assays in specific patient subgroups based on cyst phase, location, and burden. Although not a limitation specific to this study, a general limitation of using CSF for diagnostic purposes is that a lumbar puncture may not be possible to conduct in some low-resource settings.

In conclusion, this study demonstrated the potential usefulness of using a PCR assay to detect cysticercal DNA and ELISA for HP10 Ag, both in CSF, for diagnosing NC. These two assays may be useful for EP-NC when neuroimaging techniques are not conclusive.

## Declarations

### Author contribution statement

Matthew L. Romo, Arturo Carpio: Conceived and designed the experiments, Analyzed and interpreted the data, Wrote the paper.

R. Michael E Parkhouse, María Milagros Cortéz, Richar Rodríguez: Conceived and designed the experiments, Analyzed and interpreted the data.

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### Competing interest statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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