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Research brief

A lateral flow assay (LFA) for the rapid detection of extraparenchymal neurocysticercosis using cerebrospinal fluid

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HIGHLIGHTS

- Neurocysticercosis (NCC) is responsible for *c.*30% of preventable cases of epilepsy and 2.8 million DALY's.
- NCC is a 'silent' infection, characterised by late onset of neurological symptoms. Early diagnosis aids patient management.
- The HP10 Ag-ELISA, using cerebrospinal fluid (CSF), detects viable NCC and importantly extraparenchymally (EP) located NCC.
- A novel rapid Lateral Flow Assay (HP10-LFA) was positive for 34/34 CSF samples from viable EP-NCC cases, controls were -ve.
- The HP10-LFA was positive for 34/34 CSF samples from cases of active (viable) extraparenchymal NCC and negative for a total of 46 negative control CSF samples.
- This novel HP10-LFA should thus facilitate diagnosis, treatment and follow-up of NCC cases at hospital/clinic level.

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ABSTRACT

A lateral flow assay (LFA) for the diagnosis and monitoring of extraparenchymal neurocysticercosis, has been developed. The assay is based on the use of the monoclonal antibody HP10, and when applied to cerebrospinal fluid, correctly identified 34 cases of active extraparenchymal neurocysticercosis, but was negative with 26 samples from treated and cured neurocysticercosis patients and with 20 samples from unrelated neurological diseases. There was complete agreement between the HP10 Ag-ELISA results and the HP10-LFA. The HP10-LFA thus has utility for diagnosis and treatment of extraparenchymal neurocysticercosis, frequently a more dangerous form of the infection.

1. Introduction

Neglected Tropical diseases (NTD's), now recognized by the World Health Organization as a serious global health issue, include most of the common parasitic diseases (WHO report, 2016). Due to their disproportionate prevalence in the poorest of the world's population, their control and elimination have received low priority. Many of these diseases, for example; malaria, echinococosis, *Taenia solium* cysticercosis, and toxoplasmosis have a neurological

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involvement (Carpio et al., 2016), and this aspect of their clinical and economic impact has received even less attention. Typically, parasite infections of the nervous system are “silent”, without the classical neurological symptoms (e.g. headache, epilepsy, coma) appearing long after the initial invasion of the brain and, importantly, when considerable, sometimes irreversible, damage has occurred (Carpio et al., 2016). Thus, early and reliable confirmatory diagnosis of these parasite infections, subsequent to clinical and radiological examination, is an essential tool in the control and treatment of parasites invading the nervous system.

This is particularly true in the case of neurocysticercosis (NCC), typically caused by invasion of the central nervous system and eyes by the metacestode of *Taenia solium*, and considered to be one of the most important NTD's with neurological impact (Carpio et al., 2016). Imaging studies (Computed Tomography (CT) scan and Magnetic Resonance Imaging (MRI)) are considered, by neurologists, as the “gold standard” for NCC diagnosis. Moreover, reliable diagnosis of extraparenchymal NCC requires MRI special sequences (FIESTA), not yet universally available (Carrillo Mezo et al., 2015). Although, these tools permit the diagnosis in most of the cases, particularly when parasites are located in the parenchyma (Fleury et al., 2011, 2013), unfortunately, patients' access to CT, and MRI, in endemic countries is frequently limited or financially impossible. As a result, there has been considerable investigation into the development of serological and molecular biological procedures for the diagnosis of NCC (Wilkins, 2013). In addition to the commercially available western blot (EITB) and antibody tests (WHO, 2015, 2016), a variety of recombinant metacestode antigens and synthetic peptides has been tested as targets for detection of antibodies (Deckers and Dorny, 2010; Ferrer et al., 2007). Antibody detection, however, is not definitive proof of a current, infection with living metacestodes, as antibodies can persist for months, even years, after elimination of the parasite. As an alternative to the antibody assays, diagnosis of active cysticercosis through the detection of molecules actively secreted by viable metacestodes and found in the cerebrospinal fluid (CSF) and serum of NCC patients, has been evaluated for diagnosis and follow-up of NCC (Harrison et al., 1989; Brandt et al., 1992; Garcia et al., 1998, 2000, 2002; Nguekam et al., 2003; Rodriguez et al., 2009; Fleury et al., 2013). In systematic investigations, testing paired serum and CSF samples from clinically characterized NCC patients, the HP10 Ag-ELISA, which detects the viable metacestode surface/secreted HP10 antigen (Harrison et al., 1989) was demonstrated to function well for extraparenchymal NCC, but was often low or negative for parenchymal NCC (Bobes et al., 2006; Fleury et al., 2007, 2013).

From the practical point of view, however, extraparenchymal NCC often has a much more threatening pathogenesis than parenchymal (Bobes et al., 2006; Fleury et al., 2007, 2011, 2013) and, importantly, imaging studies are less sensitive for the detection of extraparenchymal NCC (Graeff-Teixeira et al., 2009; Fleury et al., 2011). A particularly useful application of the HP10 Ag-ELISA is the follow-up of extraparenchymal NCC patients after albendazole/praziquantel treatment as it helps determine the efficacy of cysticidal treatment. Thus effective drug treatment is clearly established by the decreased parasite antigen levels in CSF, whereas ineffective treatment, which occurs in a significant number of cases, is revealed by the continued presence of the secreted parasite product in the CSF or serum (Garcia et al., 2000; Cárdenas et al., 2010).

In this communication we report the successful adaptation of the HP10 Ag-ELISA to a lateral flow assay format (HP10 Ag-LFA), using CSF samples from clinically and imagenologically characterized patients, and so provide a preliminary validation of its application for the clinically important management of extraparenchymal NCC.

2. Materials and methods

2.1. Samples

Samples of CSF were collected by lumbar puncture from patients attending the Instituto Nacional de Neurologia y Neurocirugía (INNN), Mexico City. The study was approved by and carried out under the guidelines of the Ethical Committee of the INNN, D.F., México. All patients provided written informed consent for the collection of samples and subsequent analysis. Diagnosis of viable extraparenchymal NCC was based on clinical manifestations (intracranial hypertension, focal deficit, or affection of cranial nerves and intracranial hypertension), imaging studies (MRI with FIESTA sequences showing cystic images located in subarachnoid cisterns or ventricular system) and cytochemical analysis of CSF showing an increased cellular count (>30 cells/mm^3) and protein concentrations (>80 mg/ml). CSF samples from inactive NCC were from patients who had previously presented active extraparenchymal NCC, were treated with the cysticidal drug albendazole, and who were demonstrated to lack cysts by MRI 3–7 months after their treatment. In addition, their neurological symptoms had improved (eg. headache, epilepsy, coma) and the CSF samples used to evaluate the HP10 Ag-LFA were normal (cells<6/mm^3 and protein concentration <40 mg/ml). A total of 20 control CSF samples were collected from patients with other neurological diseases. Their diagnosis, in addition to negative MRI studies for NCC, were as follows: multiple sclerosis (10), optical neuritis (2), myelomalacia (2), non-NCC related epilepsy (1), headache (1), tumor (1), subdural hematoma (1). Diagnosis of NCC was considered negative based on a combination of MRI results, CSF cytochemical analysis and negativity of HP10. All MRI studies were interpreted in a double-blind manner by a certified neuroradiologist with extensive experience in NCC diagnosis.

2.2. Lateral flow ASSAY methodology (HP10-LFA)

All the CSF samples were assayed by ELISA for the presence of the HP10 secreted metacestode antigen (Harrison et al., 1989; Fleury et al., 2013) and tested in the current report in the HP10 Ag-LFA, immunochromatographic system.

In brief, the LFA test to detect the HP10 antigen was based on the use of different coloured carboxyl-modified latex microspheres, which were covalently linked to the specific target proteins or antibodies. Specifically, for the test line, the purified HP10 monoclonal antibody was conjugated to red latex particles using EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride) and NHS (N-Hydroxysuccinimide) to activate the surface of the beads (Hemanson, 2013). For the control line, blue latex particles were similarly coated with the control protein. Next, the red test and blue control latex particles were diluted in Tris/HCl 25 mM pH 9.5 buffer containing humidity preservatives and blocking agents (3.0% (w/v) bovine serum albumin, 1.5% (w/v) casein, 0.35% (w/v) sucrose, 1% (w/v) Tween 20, 0.095% (w/v) sodium azide) and the mixture dispensed onto the rayon conjugate pad, using a Matrix 1600 dispenser (Kinematic Automation, Inc.). For the test capture line, the HP10 MAb was diluted at 0.6 mg/ml in buffer Tris/HCl 20 mM pH 7.5, containing 5.0% (w/v) sucrose and 0.095% (w/v) sodium azide as preservative. The anti-control protein IgG monoclonal antibody used as the control line capture reagent was diluted at 1 mg/ml in the same buffer. Both test and control capture reagents were dispensed in two parallel lines on nitrocellulose membrane at 1 μl/cm. Finally, a master card was assembled on a plastic backing with adhesive (Lohmann, The Bonding Engineers) and nitrocellulose membrane (Millipore, Ltd) as follows; the conjugated pad (Operon, Ltd), sample pad (GFA/D, Operon, Ltd) and absorbent pad