

## Role of Immunological Markers in the Diagnosis, Treatment and Follow-Up of Human Neurocysticercosis

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### Abstract

Neurocysticercosis is the commonest parasitic disease of the nervous system. Its clinical manifestations are highly variable ranging from asymptomatic disease to recurrent seizures and multisystem involvement. The imaging findings of neurocysticercosis although typical are not diagnostic for the disease and can be confused lesions of tuberculomas. To circumvent the diagnostic difficulty various serological tests have been tried. Most of the early serological tests were limited by low sensitivity and specificity for the diagnosis. Recently better antibody based assays like Enzyme Linked Immuno Electro Transfer Blot (EITB) have vastly improved the specificity of diagnosis, but sensitivity esp. in single cystic lesions still remains suboptimal. Further advances in the field of serodiagnosis of neurocysticercosis, especially focusing on validation of emerging techniques of diagnosis, will be required before the can be implemented for routine use.

**Keywords:** Neurocysticercosis; Diagnosis; Serology

### Introduction

Neurocysticercosis (NCC) is the commonest parasitic disease of the human nervous system [1]. It can cause a variety of neurological manifestations, the commonest being seizures [2]. Although the mortality rate is not very high with NCC, approximately 50,000 annual NCC related deaths occur worldwide [3]. NCC is rare in Europe and major parts of North America, except for Mexico, where it is prevalent. Its prevalence is pretty low in Australia, Japan, New Zealand etc, however, in Latin America, Asia and Africa, it is an endemic disease and remains a major public health issue [4].

The disease in humans is caused by the metacystode, which develops within the central nervous system. When humans ingest the eggs of the tapeworm *Taenia solium*, they develop cysticercosis in the tissues. The eggs may come from environmental contamination e.g. through food handlers, from unwashed vegetables and fruits etc. Approximately, 2.5 million people worldwide are carriers of adult *T. solium* [5]. As per the International League Against Epilepsy (ILAE), NCC is the most common cause of acquired epilepsy in the developing world, and it is opined that the prevalence of epilepsy in the developing world is twice that of the developed countries due to the same reason [6].

### Diagnostic Modalities for NCC

Apart from clinical features and epidemiological considerations (i.e. patient hailing from an endemic area); neuro-imaging still remains the mainstay of the diagnosis of NCC.

### X-Rays of muscles and skull

These may at times show calcifications which are often cigar-shaped. X-Rays are just adjuncts to diagnostic modalities and cannot

be taken as confirmatory tests for NCC because the yield is very low. A separation of cranial sutures may be seen in the pediatric age group.

### CT scan of the head

CT scans have a high sensitivity and specificity of around 95% in the diagnosis of NCC [7]. In around 50% of the cases of NCC, calcifications are present, which are easily picked up by CT scans. The cysts appear as single or multiple rounded lesions with an eccentric nodule which represents the scolex. Ring enhancement occurs when there is inflammatory reaction or granuloma formation. Perilesional edema may as well be seen and in encephalitic varieties of NCC, there may be extensive edema. However, the role of CT scan is much inferior in the detection of ventricular and cisternal forms of NCC.

### MRI of the brain

The best modality to specifically localize the lesion and to assess the evolutionary stage of the parasite is MRI. It can detect minimal perilesional edema and can very well detect cysts located in the brain stem, cerebellum, base of the brain as well as those within the eyes and the spine [8]. The scolex appears as a mural nodule of high signal intensity on T-1 weighted MRI. In a degenerated cyst, the fluid becomes turbid and appears as high signal intensity lesion on T-1 weighted MRI. In the granulo-nodular stage, ring enhancement appears on Gadolinium injection and there is variable perilesional edema. However, calcified cysts are best seen on CT scan.

### Other non-serological tests

Apart from serological tests which have been described in greater details in the following sections, other diagnostic modalities for NCC include biopsy of the brain, skin or muscle. These are rarely needed and may be helpful in the cysts present in ocular tissues or within the extraocular muscles [9]. Ophthalmologic evaluation including fundoscopy is an essential component of the complete workup of neurocysticercosis.

Cerebrospinal Fluid (CSF) findings in neurocysticercosis have been quoted by many authors. The diagnostic clues in CSF are pleocytosis with eosinophilia, with slightly raised proteins. The gamma globulins are raised in CSF, although it is mainly of the IgG class. All these abnormalities may not be present in every case together and it may not be possible to make a diagnosis with certainty on the basis of CSF examination alone, unless we send for the special serological markers.

### Limitations of the Current Diagnostic Modalities

Whereas CT scan and MRI form the cornerstone of the diagnosis of NCC, they may often not be the decisive modalities in clinching the diagnosis. CT scans are unable to pick up ventricular and cisternal lesions. Non-enhancing cystic lesions on CT scan or MRI showing scolex constitute a very small fraction of patients of NCC. In developing countries, a large number of patients have single enhancing lesions and they pose a challenge to both radiologists as well as neurologists to differentiate these lesions from tuberculomas [10].

The clinical and imaging features of NCC and tuberculoma have a considerable overlap and it is often difficult to differentiate between these two conditions [11]. Both have a high prevalence in countries like India and to complicate the issue further, cases have been reported where both these conditions have co-existed in the same patient. This distinction is all the more important in a single lesion because a single NCC granuloma is a benign and self-limiting condition, whereas tuberculoma requires prolonged therapy with potentially toxic drugs.

### Currently Available Serological Tests for the Diagnosis of NCC

Long before the CT era, some serological tests had already been studied for NCC [12]. These included the Complement Fixation Test, Indirect Hemagglutination test, gel diffusion (including immunodiffusion, Immunoelectrophoresis, Counter-immunoelectrophoresis etc), Precipitin test, intradermal test, Indirect Immunofluorescence etc. These tests went into disrepute due to low sensitivity and specificity in human NCC.

Flisser et al. listed the possible causes of low sensitivity of these serological tests:

Existence of different serotypes of the parasite in the cysticerci populations.

The effects of anti-inflammatory drugs often administered to these patients which impart immunosuppressive effects.

Low sensitivity of the tests themselves.

Avoidance of human recognition.

Anatomic compartmentalization of antibody production in and outside of blood brain barrier [13].

To overcome these shortcomings in the serological testing of NCC, the WHO expert committee recommended the application of ELISA in 1976 [12].

### Antibody Detection Methods

Cysticercosis results in an antibody response of mainly the IgG class. Some patients however do have IgM, IgA and IgE antibodies as well [14]. The antigens used in laboratories to detect these antibodies

include either cyst fluid or crude homogenates of *T. solium cysticerci* or crude preparations of the related parasite *T. crassiceps* [15].

Although the most simple to perform as well as easily available serological test is ELISA, the most specific test developed for NCC is Enzymelinked Immuno-electro Transfer Blot (EITB). This is an immunoblot of seven cysticercus glycoproteins, purified by lentil lectin purified chromatography which has a specificity nearing around 100% and a sensitivity of around 70-90%. However, it has a much lower sensitivity with single cysts in the brain. More recently, to improve the specificity of the antibody detection, recombinant antigens have been developed. These include 10 and 14 kilodalton polypeptides that can be used in Immunoblot and ELISA. While the specificity of these antigens has been reported to be high, the sensitivity is generally lower than the native antigens.

### Antigen Detection Methods

Antigen detection is considered superior to antibody detection because antibody detection may indicate just an exposure to the infection and not always the presence of viable and established infection. Also, the antibodies may persist for a long time after the parasite has been eliminated by the immune mechanisms and/or drug therapy. In endemic areas, upto 10% or more of the population may have antibodies to *T. solium*, not necessarily reflecting a true prevalence of NCC. This remains a major concern in high endemic countries.

Considering the above limitation of antibody detection, antigen detection appears to be a better option. An added advantage of antigen detection is that it also provides a tool for serological monitoring of anti-parasite therapy. Although several assays have been developed, the most authentic ones are the monoclonal antibody based tests directed at defined parasite antigens [16]. It may be carried out either in serum or in CSF [17]. Although antigen detection in serum is considered more appropriate but because it requires a lumbar puncture, testing in serum is more commonly done. The sensitivity of antigen detecting ELISA is considered high. Even in a single viable cyst or only enhancing lesion, the sensitivity was found to be around 65% [16].

Another remarkable advantage is the low level of cross-reactivity in serum samples of different helminthic and protozoan infections [17].

### Recent Trends in the Immunological Studies of NCC

Recently, dimethyl Thiazolyl diphenyl Tetrazolium bromide (MTT) assays have been studied in the diagnosis of NCC. MTT assay is based on the cellular reduction of Tetrazolium salt by the proliferating cells and subsequent quantification of the colored product. The sensitivity, specificity and accuracy of MTT assay for the diagnosis of NCC are 87.93%, 94.68% and 91.5% respectively [18]. More recently, two peptides, HP6-3 and Ts45W-1 have been evaluated to support the clinical diagnosis of NCC [19]. These were used as antigens for the detection of the IgG4 subclass and performed better than the saline extracts.

Detection of HP10 antigen has been studied for the diagnosis and follow-up of subarachnoid and intraventricular NCC [20]. In their long term follow-up study, Fleury et al. demonstrated that HP10-Ag ELISA had a better agreement than MRI with retrospective radiological evaluation. They also demonstrated a high reproducibility of HP10-Ag ELISA between laboratories. In yet another recent study,

Castillo and coworkers have evaluated the role of urine antigen detection for the diagnosis of NCC. They used a monoclonal antibody based ELISA to detect *T. solium* antigens in urine of 87 Peruvian NCC patients [21]. Overall sensitivity of urine antigen detection for viable parasites was 92% which decreased to 62.5% in patients with a single cyst.

### Role of Serological Testing In Treatment Monitoring and Follow-up of NCC

Apart from the diagnostic significance, serological testing is also gaining popularity in the monitoring of anti-parasite therapy of NCC as well as in the long-term follow-up. In a study by Garcia et al. [22] way back in 1997, the relationship between clinical characteristics of cerebral infection (i.e. number and type of lesions) and the response on Immunoblot were analysed. In 2003, Nguekam et al. [23] in a short study, showed the significance of antigen detection ELISA in the follow-up of NCC. Patients with active NCC who received Albendazole, were followed up using CT scan and monoclonal antibody based ELISA for the detection of circulating antigen. Patients, who were cured and showed regression of the lesion on CT, also had disappearance of circulating antigens, one month after the treatment.

In 2008, Husain et al. [24] also highlighted the role of ELISA in the evaluation of therapeutic response to Albendazole in NCC in a larger sample of patients. Patients with at least one active cyst demonstrated on CT scan and a positive pre-test serum ELISA for IgG and/or IgM were treated with Albendazole and both CT scan and ELISA were repeated at 6 months. IgG ELISA, IgM ELISA and combined IgG/IgM results exhibited 81.3%, 80% and 100% specificity respectively. It was thus established that IgG ELISA was a sensitive and specific tool to assess treatment response in NCC.

In a recent study in 2012, a simple LC-MS/MS method was developed to determine the plasma and cerebrospinal fluid levels of Albendazole in NCC [25]. This method was successfully applied to determine the plasma and CSF levels of Albendazole sulfoxide and Albendazole sulfone in patients with subarachnoid NCC who received Albendazole in a dose of 30 mg/kg/day for 7 days. This method appears to be a good tool for therapeutic monitoring of NCC.

### Conclusion

The role of serology in the diagnosis of NCC has been extensively studied; however definite evidence in clinical practice has been strengthened in recent years. It is a good adjunct to diagnosis and forms an important part of the work up of ring enhancing lesions in countries where both NCC and tuberculomas are equally common. In recent years, many new serological tests have come up and have proven their efficacy over conventional methods. The antigen detection methods have performed better over antibody detection tests. Recently, the role of serology has been highlighted in the follow up and monitoring of therapy in NCC.

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