

## Neurocysticercosis: Clinical Aspects, Immunopathology, Diagnosis, Treatment and Vaccine Development

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Received date: January 26, 2014; Accepted date: April 27, 2014; Published date: April 30, 2014

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### Abstract

Neurocysticercosis (NCC) is defined as human parasitism caused by *Taenia solium* in its larval stage, when the parasite is located at the central nervous system of the human beings. This disease represents a serious public health problem in developing countries. Neuroimaging studies are usually abnormal, but in most cases, not pathognomonic. Serological diagnosis of NCC is made by means of tests using crude antigens or semipurified fractions of *T. solium* cysticerci or the use of recombinant antigens that have improved the sensitivity and specificity of the diagnosis. Treatment of NCC is based upon the use of cystocidal drugs, namely: praziquantel and albendazole. Some vaccines to prevent porcine cysticercosis are in advanced stages of investigation, but they are not to be used in humans. Nowadays, the *T. solium* genome sequence will allow to create a rational design of new healing and preventive drugs. A vaccine for human taeniosis use could be developed in a short term. The aim of this review is to describe the most important clinical aspects of the NCC with emphasis in immunopathology and immune diagnosis.

**Keywords:** Neurocysticercosis; *Taenia solium*; Immunopathology; Serological diagnosis; Treatment; Vaccines

### Introduction

There are two types of pathologies caused by *Taenia solium* infection. The first one is taeniosis, which is caused by the adult parasite that develops exclusively in human intestine. The second one, deals with the infection caused by the larval form, named Cysticercosis (CC). Cysticerci develop in the skeletal muscle, the subcutaneous tissue, and mainly in the central nervous system (CNS), where they lead to a clinical pleomorphic disorder known as neurocysticercosis (NCC) [1-6]. This is the most frequent parasitic disease of the CNS, it also can infect the eyes (ophthalmic cysticercosis), where the larvae can lodge in the retina or in the vitreous humour [2,4,6,7].

The World Health Organization (WHO) estimated more than 2.5 million people are infected with *T. solium* around the world, and 50 thousand deaths a year can be attributed to NCC [8]. Neurocysticercosis is a reportable disease at the international level [9] due to the impact on developing countries around the world. Cysticercosis is frequent in Latin America, Africa, and Asia, where it is considered as a poverty indicator [10,11].

In Latin America, it is estimated that there are 400,000 people with symptomatic NCC [12,13] and that 18% to 50% of epilepsy cases in adult population are caused by the disease [7,14-17]. In Brazil, prevalence rates of 72 / 100 000 and 96 / 100 000 were reported from two localities of Sao Paulo State [18]. In Peru, different

seroepidemiological studies indicate a prevalence of 10-20% of CC / taeniosis in the general population and values two to three times higher in individuals with epilepsy. The main endemic areas are Sierra, North Coast and the High Jungle. NCC is present in low proportion in other zones of the country, with the area of Iquitos, apparently free of the disease [19]. In Colombia, in two rural communities of Antioquia, the prevalence of human CC was 1.2% and 2.2%. These results show that CC is endemic in this area [20]. In Venezuela, although intestinal parasites are very frequent [21-25] and tend to persist [24], very low infection rates, < 1%, of *Taenia* sp. have been reported [23,25-32]. However, these percentages might be underestimated due to the low sensitivity of examining single stool specimens. A report documents high circulating *T. solium* metacystode antigen (64.7%) and anti - parasite antibody seroprevalence of 79.4% in an Amerindian community from the Amazonas State. As the IgM was the predominant antibody class, the findings strongly suggest the recent exposure to *T. solium* of this population [10]. In three rural communities from Lara and Carabobo States, prevalences of 5.7 - 9.1% were established [11]. The detection of IgG antibodies showed that the exposure to the parasite was 4-36.5%. These findings suggest that there are CC active focal points in Venezuela with a high risk of disease transmission. In Ecuador, a high rate of seropositivity of CC among urban, individuals was found in a region endemic for *T. solium* was 12% of the case family members and 4% of the control family members by the using the enzyme-linked immunoelectrotransfer blot (EITB) assay [33]. In Guayaquil City, in NCC patients admitted in a hospital from Guayaquil, prevalences of 6.4%, 2.7%, and 3.5% were noted from 1982 to 1991, 1992 to 2001, and 2002 to 2012 respectively

[34]. In La Habana, Cuba, a very low frequency of 0.01% was found in a retrospective study of neurological patients over 25 years up to 1989 [35]. In Mexico, an incidence of 0.8 cases per 100 000 per year was reported [36]. In epidemiological studies of CC conducted in Tepetzintla, Puebla State in 2001 [37] and in Cuentepec, Morelos State in 2003 [38], the same infection rate of 9.1% was found.

In developed countries, NCC has impact on public health by the large number of immigrants coming from endemic areas. In the last years, the number of cases has increased which turns CC/NCC into an emerging pathology in some developed countries [39-43].

## Clinical Aspects

NCC is a complex disease; its symptoms depend on the number, type, size, localization and stage of development of cysticerci in the CNS and meninges, as well as on the degree of inflammatory response, and the host conditions [2,4,5,12,14]. In 70-90% of patients with NCC, the most common symptom is seizures, and in countries where the disease is endemic, there are a lot of cases of epilepsy in adults whose underlying cause is the CC [5,14,44]. When cysticerci lodge within the ventricular system the acute Intracranial Hypertension (ICH) secondary to Hydrocephalus (HC) may develop. Cysts in the subarachnoid space may invade the Sylvian fissure and grow to large sizes. The giant cysts formed; cause ICH with hemiparesis, partial seizures or other focal neurological signs. The most severe but less frequent of the NCC clinical presentation is due to the racemose cysticercus, with the presence of multilobular cysts, located in the ventricular and subarachnoid spaces, with a marked obstructive HC and arachnoiditis that may threaten the life of the patient. Racemose cysts in the basal cisterns can cause an intense inflammatory reaction, fibrosis, and progressive thickening of the leptomeninges at the base of the brain. Ventricular and basal cisternal locations are considered to be malignant forms of NCC. There is a high mortality rate (50%) when the HC secondary to cysticercotic meningitis is present. Most patients can die within two years after Cerebrospinal Fluid (CSF) shunting [44].

From a clinical standpoint has been established in the following clinical profile in HC in patients with NCC: males, 21-50 years old, ICH was observed in all patients, headache (HA), the CSF syndrome of NCC was detected in 31/47 patients (65.9%), Meningoencephalitis (ME), Psychiatric Disorders (PD). Computed Tomography (CT) scans showed cystic lesions, diffuse cerebral edema, and calcifications. Shunts were inserted in 41/47 (87.2%) patients. Evolution was satisfactory in 24 (51.1%) patients and fatal in 15 (31.9%) [45].

NCC can be active or inactive. The active form is associated with living parasites that cause arachnoiditis, HC, and cerebral stroke. In addition, patients can present a mass effect due to the presence of large cysts which can appear at spinal and intraventricular levels. Inactive NCC presents parenchymatous calcifications or meningeal fibrosis [2,4,13,17].

## Clinical and epidemiologic aspects of children and adults with NCC

The Brazilian patient presents an overall clinical and epidemiological profile for the inactive form of the NCC: men of 31-50 years old, complex partial seizures, rural origin, calcifications visible on the CT scan, the protein increase in CSF or normal values. The severity profile corresponds to the active form of the NCC: women, urban origin, 31-40 years old, typical CSF syndrome or alterations of

two or more parameters, ICH, vascular HA, and the CT scan shows vesicles or calcifications [18]. In Campina Grande and others cities of Paraíba State, 44 patients with NCC had an average age was 20.6 + 14.3 years old, with male predominance. The 86.2% came from the urban area. The initial symptom was convulsion, accompanied by a lower proportion of HA. The epileptical form predominated over the combined form. CT scans showing calcification, integral cysts, degenerating; isolated or associated. The evaluation of CSF showed alterations, pleocytose linfomonocitary predominating in 100% of the cases, and positive immunological reactions in 64.3% of them [46]. In Bocatú region, Morales et al. [47] emphasized the need to consider the NCC in the differential diagnosis of children from endemic areas with symptomatic with learning disabilities, behavioral changes, and psychomotor involution. The diagnosis of NCC in 25 children took into account epidemiological, clinical, laboratory (CSF), and CT scans. From the epidemiological point of view, predominated men, of urban origin with an age range of 1-11 (average= 8 years + 6 months). The more frequent manifestations were epileptic seizures, HA, learning disability, behavioral changes, psychomotor involution, and ICH. The neurologic examination was normal in 80% (of the 25 patients, 43.4% had remission and 47.8% had improvement) allowing infer a spontaneous resolution of NCC in this patients, without the need for cystocidal treatment.

In Peru, Maldonado et al. [48] in a case-control study realized in 41 patients with a convulsive status epilepticus demonstrated of principal clinic-epidemiologic characteristics in adults attended in a National Hospital from Lima-Peru in a period of four years. Among the most important findings are: 68.3% were male, 28.6% had age between 20 and 29 years old and 15.5% resided in endemic areas of NCC. The more frequent aetiologies were remote symptomatic secondary crisis to cranio encephalic trauma and NCC and idiopathic; 26.8% showed some intercurrent infection; while, mortality was of 7.3%. Factors associated with a convulsive status epilepticus were the abrupt interruption or suspension of drugs used for the control of convulsions ( $p=0.038$ ), chronic intake of alcohol ( $p=0.030$ ) and irregular antiepileptic treatment ( $p=0.006$ ). These authors conclude that aetiologies more frequent in the hospital studied from Lima-Peru are remote symptomatic secondary crisis to cranio encephalic trauma, neurocysticercosis and idiopathic. The irregular antiepileptic treatment constitutes a risk factor to convulsive status epilepticus.

In Ecuador, Goodman et al. [20] demonstrates a high rate of seropositivity of CC among urban, middle to upper-middle class individuals in a region endemic for *T. solium* in Ecuador. The authors compared in a case-control study realized in the city of Cuenca, the rate of seropositivity used the EITB assay among family members of patients diagnosed with NCC by CT compared with family members of controls who had a negative CT scan. That seropositivity was not related to age. No neurologic symptom proved predictive of serostatus and the only demographic variable that correlated with seropositivity was increased crowding. Positive serology in index cases did correlate with CT findings as follows: 86% of patients with active lesions, 67% with transitional lesions, and only 41% of patients with inactive lesions were positive by the EITB assay. Eighteen percent of family members with a positive EITB test result had parenchymal lesions on a subsequent CT scan.

In Cuba, the correlation between disease and epilepsy (4 of 5 cases) was assessed. The main symptoms were analyzed: the three most important, in order, were tonic-clonic convulsions, HA and motor deficit respectively. The epileptic seizures were classified as secondary

generalized partial seizures (symptomatic partial epilepsy secondary to CC). The clinical form, when the site is considered, was inactive, with intraparenchymatous and meningeal (mixed) calcifications in one case [22,49].

In Venezuela, a report of 28 cases of NCC was presented, where 75% of the patients were male, aged between 30-50 years old, 57% of which came from the Capital District and the Central States (Miranda, Carabobo, and Aragua) and 14% of the Andean States (Mérida, Táchira, and Trujillo). Among the most important symptoms were presented: IHC, seizures and focal signs. All cases with neurological symptoms had an intraventricular and / or meningeal location. Pleocytosis at the expense of lymphocytes in the CSF was observed [50].

### Immunopathology

The symptoms of the disease are a result of the granulomatous inflammatory process associated to the immune response against the parasite. In the murine model the response of the helper CD4+ T cells, Th1, seems to be decisive in the disease immunopathology and in the parasite elimination from the tissues. A marked predominance of interferon-gamma (INF- $\gamma$ ), interleukin-2 (IL-2), and minimal quantities of interleukin-4 (IL-4), were found in early evolution injuries, by using in situ hybridization on granulomas removed from the peritoneal cavity of infected mice with *T. crassiceps* cysticerci. Also, a progressive increase of IL-4 levels was observed in the late stages of the disease. This could indicate a process of low regulation of the initial Th1 response [51].

The Evans Blue (EB) vital diazo dye, very soluble in water and easily linked to albumin, has been used in pigs to determine the extent of the immune inflammation around the brain cysts in NCC. The EB dye was injected to 11 naturally infected pigs with *T. solium* cysts to identify the degree of alteration of the blood brain barrier. A total of 369 cysts were recovered from the brains and were classified according to the color of their capsules as blue or undyed. The proportion of cysts with blue capsules was significantly higher in the pigs that received treatment 48 and 120 h before the EB infusion, indicating a greater commitment of the blood-brain barrier due to therapy. This finding is particularly important because it allows to infer the commitment of the blood-brain barrier and the degree of inflammation after treatment in patients with NCC [52].

The immune response against *T. solium* antigens is responsible, on the one hand, for the infection control; on the other, for the damage or injury caused to the host tissues. In human beings, inflammatory response against viable cysticerci is minimum. However, a loss of modulating the host immune response against parasite antigens with degenerate cysticerci is observed later. In this case, an intense inflammatory response has been noted with the presence of eosinophils, plasma cells, B and T lymphocytes accumulations and macrophage phagocytosis inside the destroyed cysticerci [53-57]. The serological assays that detect IgG in serum and CSF of patients with NCC, has shown a long-term humoral response. Nonetheless, cellular response is less known [43,58-67].

Sciutto et al. [68] emphasize that the high relevance of mast cells (MC) found in the brain of NCC patients is an interesting finding. These MCs express both tryptase - chymase enzymes and were found infiltrating the meninges and the brain parenchyma around vesicular and calcified cysts and in the perivascular area of the brain blood vessels. The authors pointed out that these cells could importantly contribute to the parasite destruction by modulating the local immune

inflammation, possibly through a differential profile of cytokines secreted by the different MC protease phenotype [68-70]. It has been demonstrated that severe NCC patients exhibit a higher percent of T regulatory cells (Tregs) in CSF and the periphery. The increase in central and peripheral Tregs is accompanied by decreased in activated T cells in the peripheral proliferative response induced by cysticercal antigens and concanavalin A (ConA). The inflammatory conditions in NCC are associated with gonadal impairment in both male (low testosterone and estradiol levels) and female patients (low progesterone and estradiol levels), as with adrenal dysfunction resulting in reduced levels of androstenedione (A4) and dehydroepiandrosterone (DHEA) [68].

Lymphocytes TCD4+ / TCD8+ proportion is associated to the functionality of the immune system. This proportion seems abnormal in patients with NCC, with an increase of CD8+ T cell subpopulation suggesting that there is a diminished immune response. It is not known yet if the parasite is the cause or the effect of the immunodepression. Th1 / Th2 paradigm in NCC has not been totally elucidated yet. Th1 cytokine pattern seems to be related to protection. Whereas, Th2 cytokine pattern appears to be connected to disease outcome. High interleukin - 6 (IL-6) levels in CSF of patients with subarachnoid NCC are observed in the acute phase. Tumor necrosis factor - alpha (TNF- $\alpha$ ) has been detected in children with active NCC, unlike control groups and children with inactive NCC, where TNF- $\alpha$  has not been detected. Cytokine response to the treatment with praziquantel shows a predominance of Th1 (high IL-2 levels in patients with NCC). This is different from the Th2 pattern against *T. solium* viable cysticerci in diverse animal models [67,71-76].

High levels of eotaxin and interleukin-5 (IL-5), both eosinophil selective mediators, have been quantified in sera from patients with NCC. These cytokines are involved in the local and systemic recruitment of eosinophils. The presence of these cells as the first defensive line against parasites has been reported in porcine CC after treatment and vaccination. This suggests that eosinophils may play an important role in the degenerative phase of the infection. There are few immunohistochemical studies upon the inflammatory response against *T. solium* cysticerci in granulomas in the human CNS. Studies suggest the participation of Th1 and Th2 cytokines in granulomas derived from patients with NCC [77-82].

For therapeutic purpose, it is not convenient to interfere with the inflammatory response against the parasite in NCC. Anti-inflammatory drugs are kept for very specific cases presenting severe and persistent inflammation with diffuse encephalitis and / or basal meningitis, and ICH [76].

### Seizures, substance p, and edema perilesional in patients with calcified neurocysticercosis

Substance P (SP) is a neuropeptide within the tachykinin family produced by endothelial cells, neurons, and immunocytes such as lymphocytes and macrophages. Robinson et al. [83] established that SP is the epileptogenic agent in NCC in human and murine models. SP signaling is involved in nociception [84] and neuropathic inflammation. By immunohistochemistry on brain tissue specimens from patients with and without NCC, the SP peptide expression was readily detected within cells adjacent to parasite remnants in the 5 NCC brain biopsy specimens. In contrast, no SP+ cells were found distant from the parasite in the NCC biopsy specimens that included sufficient brain tissue. Autopsy specimens from patients without NCC

displayed no SP+ staining cells [83]. In NCC the calcified lesions are the most common cerebral finding because they accumulate in the brain and are a measure of prior infections. There is increasing evidence implicating calcified NCC in the genesis and / or maintenance of seizures and epilepsy in endemic populations [85]. Perilesional brain edema associated with calcified lesions is a recently described phenomenon [86]. Even now, symptomatic patients with perilesional edema around calcifications are commonly misdiagnosed as refractory NCC and unnecessarily treated with antihelmintics or even subject to brain biopsies. Perilesional edema was present in 34.5% of patients with only calcified lesions who presented seizures [87].

Nash et al. [88] realized the first prospective study of the incidence and clinical significance of perilesional edema around calcified *T. solium* granulomas. There are three important findings of this study: First, seizures were relatively frequent in this closely followed population. Seizures occurred in 26.36% of the cohort and 26.67% of those with a history of seizures over the course of the study, reaching an estimated 36% incidence in 5 years of follow up. Second, perilesional edema around calcified cysticerci is very common occurring in 50% of studied patients with seizures, and third, perilesional edema is strongly associated with seizures since it was only present in 8.7% of asymptomatic controls, or 3.6% of all eligible patients at baseline.

The phenomenon of perilesional edema around calcified NCC lesions suggests a unique and specific underlying pathophysiology of seizures in this subpopulation. Because much of the injury and disease due to CC are secondary to inflammation, the most plausible hypothesis is that edema represents an inflammatory response to calcified granulomas [88].

Fujita et al. [89] suggest that perilesional edema episodes are due to the persistence of low grade inflammation driven by the presence, intermittent recognition of residual *T. solium* antigens or periodic loss of suppression of the inflammation at the calcified focus. Measures to inhibit inflammation in addition to anti-seizure treatment might be helpful in control of epilepsy due to this phenomenon. The Translocator Protein (TSPO), using Positron Emission Tomography (PET) and the selective ligand 11C-PBR28 can be a useful measure of the effectiveness of the medication.

Overall these results [83,85-89], report the better up to day characterization of mechanisms that help significantly to the understanding of the immunopathology of the NCC

### **Evasion strategies of the immune response by taenia solium**

To survive, the parasite develops diverse immune- evasion and immune depression mechanisms: lodging in immunologically privileged sites, like eyes or brain; masking of the immune response by covering itself with host's antibodies; production of molecules that suppress or divert the immune response; and finally, processes of mutagenesis. Some of these mechanisms also include inactivation of the complement system, decrease of the number of lymphocytes, liberation of enzymes with a great lytic potential to the host's tissues and production and liberation of inflammatory response inhibitors [90]. Also, the parasite presents molecular mimicry with host's proteins, which makes the identification by the specific and innate immune system of animal hosts more difficult [51].

In NCC, more than 95% of the patients exhibit humoral response against carbohydrate - based antigens, and *T. solium* tegument is rich in glycoconjugates (GCs). This tegument consists in a sincytio

organized in two areas: an anucleate area named distal cytoplasm, and another nucleate area known as proximal cytoplasm. By means of GCs differential labelling, in which hydrazides and lectins were labelled with fluorochromes, it was established that either fast or persistent liberation of tegumental GCs play a key role in the parasite immunomodulation, including the immune evasion and the long - term evolution with inflammatory consequences, which were observed in patients with NCC. The external surface of the parasite reacts to the host environmental changes. Moreover, high antigenicity of *T. solium* GCs can play a role in hypersensitivity responses, and as a last resort, in the disease symptoms [12,91-94].

## **Diagnosis**

### **Serological diagnosis**

The diagnosis of NCC is based upon clinical features, epidemiologic aspects, neuroimaging studies, laboratory analysis of the CSF and antibody detection in the serum [44]. Antigens used with diagnostic purposes include *T. solium* and *T. crassiceps* extracts, preparations or fractions. From them, vesicular fluid, external membrane or total extracts are taken. They show a good sensitivity, but a low specificity (especially from the total extracts), because of occurrence of cross reactions and thereby generate false positive results (because they are complex mixtures of highly conserved antigenic epitopes) with other etiological agents of various diseases. Similarly, false negative results may be observed with the serological diagnosis which limits its application because of the unreliability of the results [95,96]. It is suggested to include at least two serological tests, as a criterion to establish the NCC diagnosis [97] the use of ELISA as a screening criterion, and western blot as a confirmatory test [98,99].

A western blot assay approved by WHO and the Pan American Health Organization (PAHO) uses as antigens a partially purified antigenic preparation of glycoproteins (50, 39-42, 24, 21, 18, 14 and 13 kDa) obtained by means of lectins (Lectil - lectins) affinity chromatography designated as LLGP-EITB, from *T. solium* cysticerci. This assay exhibits a sensitivity of 98% and a specificity of 100% in patients with two or more viable brain lesions as demonstrated by neuroimaging studies [100].

Elliot et al. [101] demonstrated that there was no significant difference in seropositivity to *T. solium* by LLGP-EITB between those individuals with epilepsy (5%) and controls (4.9%) in the Momo subdivision of Ngie, Cameroon. The detection of antibodies against *T. solium* by LLGP-EITB in blood samples reflects a history of contact with the parasite rather than a real infection by metacestodes of *T. solium*. However, one of the major limitations of this study was the inability to define focal epilepsy using Electroencephalography (EEG) given the absence of this resource in rural Cameroon. It is notoriously difficult to select out focal epilepsy based upon seizure semiology alone in patients with a history of generalized epilepsy because of the secondarily generalized epilepsy.

Antigens prepared from *T. crassiceps*, instead of *T. solium*, are used in several assays: ELISA with a sensitivity and specificity of 99%, western blot with a reliability of 96%, and complement fixation reaction with a specificity and sensitivity of 93%. The use of antigens taken from *T. crassiceps* is favourable because they are easily obtained from BALB/c mice at the laboratory and there is no risk of human infection. On the other hand, a great number of shared antigens from both *Taenia* species cysticerci almost identical are observed [97-99].

## Recombinant antigens and synthetic peptides

The development and implementation of molecular biology tools have allowed the production of fusion proteins in *T. solium*. One of the systems with efficient performance has been the coupling of proteins and peptides to the Glutathione-S-Transferase (GST) from *Schistosoma japonicum*, designated as NC-3 and NC-9, with a potential use in the NCC diagnosis. Starting from an ADNc expression library taken from the lambda ZAP II vector, and using serum probes from people with a clinical and serological NCC diagnosis, cloning, sequencing, and expressing these proteins was attained, and then they were purified by affinity chromatography to glutathione. A well characterized battery of serum samples was examined by ELISA, and NC-3 antigen; a sensitivity of 96.3% and a specificity of 91.5% were observed. However, a low sensitivity of 33.3% was noted for NC-9 antigen [102].

Sako et al. [103,104] obtained new *T. solium* fusion proteins to thioredoxin and to hexa-histidine tag or "his tag", denominated as Ag1, Ag1V1, Ag2, Ag2V1 (Ag2V1 could not be expressed in heterologous expression system). Likewise, the chimeric protein Ag1V1 / Ag2 could be expressed exhibiting 100% specificity and 89.7% sensibility by ELISA. The four obtained clones showed a 53 - 94% similarity at the amino-acid level, with a hydrophobic region (import signal sequence), which indicates that it probably deals with secretory proteins located in the cysticercus' vesicular fluid. Some of these proteins have cysteine residues involved in the linking disulphide bonds, indispensable for conformational structures needed for the proteins recognition by the antibodies. That is to say, it deals with conformational epitopes, according to the results obtained under native conditions (ELISA) or under denaturing conditions (immunoblot). Another important point discussed by these authors, is the risk that implies using a heterologous expression system, because antigen's sensitivity could be lost, as occurred with Ag1 protein. According to the sequence analysis, three N-glycosylation sites were identified at the 22, 59, and 82 positions. As *E. coli* does not carry out post-translational modifications of the N-glycosylation type, reactivities of the sera from patients with NCC were very low by ELISA. This is partially due to the lack of carbohydrates in the antigen structure; or, according to Sako et al. [103], due to the presence of conformational epitopes in the structure of Ag1 (immunoblot - obtained results). As Ag1V1 and Ag2 are the best choices as potential diagnostic antigens, PCR technique was used to design a chimera (Ag1V1 / Ag2 hybrid) which is an antigen useful for the diagnosis of NCC by ELISA and western blot.

By chemical synthesis, nine peptides that belong to a low molecular mass (8 kDa) antigen family and have several cysteine residues in their structure were obtained. Hancock et al. [104], to standardize ELISA for the diagnosis of NCC, noted that an antigen designated as TsRS1 was 100% sensitive and 100% specific against an evaluated battery of serum samples. Whereas, Ts18 var 1 and Ts18 var 3 exhibited a sensitivity of 97% and a specificity of 100% by ELISA. With the goal of avoiding false negative results, due to the problems that depend on strains or single parasites, these authors recommend to include a group of even

four recombinant or synthetic antigens to be able to establish a reliable diagnosis of NCC by ELISA.

In three Venezuelan endemic regions, five synthetic peptides (HP6-3, Ts45W-1, Ts45W-5, Ts45S-10 and TEG-1) derived from *T. solium* oncosphere molecules were evaluated for the serological diagnosis of CC and NCC caused by *T. solium*. The positivity showed was not connected to active infection. Therefore, assays with these peptides will be potentially used only for epidemiological studies and those to determine the population exposure to the parasite [41].

The use of proteomics tools have been used to obtain recombinant antigens for diagnosis of NCC. Soluble proteins from *T. solium* cysticerci were separated by two-dimensional electrophoresis and blotted onto nitrocellulose membranes. Subtracted hybridization was performed with serum samples obtained from patients with NCC and from a NCC - negative control group. Six antigenic proteins were identified and sequenced by liquid chromatography - mass spectrometry [105]. Tsol-p27 was previously identified as a diagnostic candidate in a study conducted in Nicaragua, Central America [106]. Nhancupe et al. [105] evaluated Tsol-p27 and the antigen cC1 as potential recombinant diagnostic reagents, and also investigated the localization and partial function of Tsol-p27. Immunoblotting demonstrated that Tsol-p27 was recognized by all 10 serum samples from NCC-positive individuals, whereas cC1 was identified by only five of the 10 positive sera. None of the antigens were recognized by negative control sera. Despite the limited number of serum samples evaluated in this study, the results suggest that Tsol-p27 can be a suitable candidate for diagnosis of human NCC.

The Tsol-p27 amino acid sequence was found to be homologous to the sequences of three immunogenic proteins: P-29 of *Echinococcus granulosus*, as previously described [106]; antigen 6 of *E. multilocularis* (Genbank accession No. AAB61984); antigen 5 of *E. granulosus* (Genbank accession No. ADG37650). Protein family analysis indicated that Tsol-p27 is homologous to a group of molecules, cd07594, that include a bin / amphiphysin / rvs (BAR) domain of endophilin - B. The endophilin-B proteins are found in at least 11 different eukaryotic organisms, and they are involved in regulation of intracellular transport, lipid binding, and curvature sensing modules. Given the homology between Tsol-p27 and the *E. granulosus* protein P-29, there will most likely be cross-reactivity between those two proteins in patients who are infected with both *T. solium* and *E. granulosus*. However, since the clinical manifestations of NCC and echinococcosis differ, it should not be difficult to distinguish between the two diseases in order to make a correct diagnosis [105].

Just for educational purposes, the authors of this study estimated the sensitivity and specificity values of the antigen cC1 by immunoblot, despite the limited number of samples used: 10 derived from patients with NCC, 10 clinically healthy individuals [105]. In Table 1, some of the recombinant antigens or synthetic peptides used as diagnostic markers of CC and NCC by ELISA or western blot are summarized.

Number of access to Gen Bank	Antigen	Sensitivity (%)	Specificity (%)	Reference
X95983	HP6	85	86.6	41

AJ430567	Ts45W	85	98.6	41
AJ430566	Ts45S	85	93	41
X97000	TEG	81	81	41
AJO12669	NC-3	96.3	91.5	102
AJ012670	NC-9	33.3	85.6	102
AF356341	TsRS1	100	100	104
AF350070	Ts18 var 1	97	100	104
AF098075	Ts18 var 3	97	100	104
AF082828	Ts18	91	100	104
AF356335	Ts14	84	100	104
AF356331	Ts18 var 4	84	100	104
AF356345	Ts18 var 8	76	100	104
AF356343	TsRS2 var 1	73	100	104
AAD34598.1/ <i>T. solium</i>	cC1	50	100	105
AEF14021.1/ <i>T. solium</i>	Tsolp-27	76.4	95.6	107
Q7YZT0/ <i>T. solium</i>	TsolHSP36	61.9	86.1	107

**Table 1:** Recombinant Antigens and Synthetic Peptides Used In the Diagnosis of Cysticercosis and Neurocysticercosis

## Treatment

In the last 25 years, generating or favoring reduction of the number or volume of cystic and granulomatous lesions, has been attributed to cystocidal drugs (praziquantel and albendazole) generally associated to corticosteroids. As NCC is a pleomorphic disease, to establish a unique treatment pattern for all the patients is not possible. The general patient status (nourishment, immunologic condition), number, and location of the parasites are determining factors for the chemotherapeutic management [5].

Toxicological and pharmacokinetic studies carried out on humans with cystocidal agents have highlighted their fast absorption, and often little side - effects. Cystocidal therapy efficacy is followed by the reduction of the number and size of the cysticerci and correction of the ventricular dilation, observed through CT scans, and Magnetic Resonance Imaging (MRI), and by clinical evaluation, that conduces to the elimination of the corticosteroids or anticonvulsants. The risk of relevant side effects in patients as a response to cystocidal drugs is probably related to the total number, size, and location of the parasites in the CNS, as well as to the degree of inflammatory response. Surgical treatment is indicated for lesions causing progressive neurological damage, ICH or HC. The most frequent surgical intervention consists in locating a ventricular derivation to divert CSF up to the peritoneal cavity in order to control the HC. Single intraventricular cysticerci can be surgically removed, with the help of endoscopic aspiration [2,5,58,107-113].

## Parenchymatous NCC

Patients with calcifications must not receive cystocidal therapy. When these appear along with convulsive crisis, the administration of

antiepileptic drugs is necessary. Optimum duration of the treatment is not defined, since diverse studies have shown several relapses after suspending the drugs; even when these have controlled crisis for two years or more [6,17,108,114].

Patients with viable cysts must receive cystocidal therapy. Most of the randomized studies (some of them with double - blind design), that evaluate the effectiveness of praziquantel and albendazole against placebos, have demonstrated that these drugs are useful for both patients with hypercaptant annular lesions and those with cystic lesions. After 15 days of treatment, praziquantel produces the extinction of 60%-70% of parenchymatous cysticerci. Albendazole destroys 80% of the parenchymatous cysts, and it has shown through diverse comparative studies to be superior to praziquantel, for its higher cysts destroying percentage. Besides, it has a lower cost [6,115-119].

Patients with cysticercotic encephalitis must not receive cystocidal therapy, because it could exacerbate cerebral edemas, and increase the intracranial pressure. Patients with HC and parenchymatous cysts can receive cystocidal drugs, once the HC has been solved [6,120].

## Subarachnoid NCC

Small subarachnoid and parenchymatous cysts located at the bottom of the cortical sulci must receive cystocidal treatment. Patients with HC secondary to cysticercotic arachnoiditis must be subjected to a shunt system insertion. However, a drawback regarding these cases is the highly frequency of valvular dysfunction, which is responsible for the high mortality rate registered in this disease form. In these cases, long - term administration of prednisone reduces the risk of valvular dysfunction [6,108,121].

## The racemose cysticerci

This presentation is a large parasite structure composed of a conglomerate of size varying vesicles without scolices are present only in the basal subarachnoid space (BSS) or in the ventricular system; probably, because they have here room to grow in, while in the parenchyma or in other tissues the pressure prevents their development [68]. In meningeal - racemose forms, a consensus about the treatment with repeated cystocidal and corticosteroid drugs seems to exist. Nevertheless, some authors do not agree. Some works have shown a reduction of the cysts size or the meningitic process. Other groups have evaluated prolonged therapies with cystocidal drugs and corticosteroids even for months [108,122].

Del Brutto et al. [122] treated four patients, who had giant subarachnoid cysticerci, with albendazole at daily doses of 15 mg/kg of body weight for 8 days. A marked clinical improvement in every case was noted and CT studies showed that all cysts disappeared 3 months after treatment. These results evidence clinical and parasitological cure of the racemose form of NCC.

## Intraventricular NCC

Depending on their size and location, ventricular cysticerci can be surgically treated or resected by direct excision or endoscopic aspiration. As the cyst could migrate within the ventricular cavities during the time between the diagnosis and the surgery, neuroimaging studies should be carried out immediately before the surgery in order to confirm its location [6,109].

## Spinal cysticercosis

Cysts located in the spinal cord parenchyma are usually surgically resected to confirm the diagnosis. Isolated cases of intermedullary cysts successfully treated with albendazole and dexamethasone have been published. Leptomeningeal cysts can also be surgically resected, especially when they appear isolated or when they are grouped into a sole spinal cord. These cystocidal drugs have not been experimented on these kinds of lesions [6,123].

## Searching of new drugs

Searching new chemotherapeutic targets for the treatment of NCC and other types of CC, should be based on exploiting structural differences between enzymes involved in *T. solium* intermediary metabolism and those of the vertebrate host. Thus, a drug must meet a series of characteristics: to produce a non - competitive inhibition, to be effective against all the parasite's evolutive stages (especially against *T. solium* cysticerci), to produce a radical parasitologic cure (cystocidal effect), to be effective against the whole NCC clinical spectrum, to be orally given, to go through the hematoencephalic barrier, to be efficient at low concentrations, to be well - tolerated by the patients. Up till now, albendazole and praziquantel keep on being the best choices for NCC and CC therapy, taking into account the experience of the practitioner in the disease management and monitoring, the clinical presentation and the current consensus guidelines [6,109,114].

## Development of a Vaccine

One of the alternatives to control taeniosis and cysticercosis caused by *T. solium* is pig vaccination. Several studies on mice, cattles, and sheeps demonstrate that it is possible to gain protection against

cysticercosis by means of vaccination [124-126]. An approximation to obtain vaccines against cysticercosis consisted in testing total extracts from different *Taenia* species, such as, *T. crassiceps* [127].

Among the antigens used with vaccination purposes, those obtained from oncospheres present advantages in terms of protection percentages, unlike those antigens derived from cysticerci or cestodes. Likewise, oncosphere derived antigens used as vaccines against porcine cysticercosis, show an effectiveness, irrespective from the geographical area, as demonstrated for four independent studies from Mexico, Peru, and Cameroon, by using the same antigens, Quil A adjuvants, doses, immunization patterns, and orally given challenge assays with *T. solium* eggs [128-132]. One of the major advantages derived from these trials is the possibility to obtain a universal vaccine able to confer protection, no matter the different types of strains found in Africa and Latin America, where this disease is still a serious public health problem [130-134].

The use of recombinant antigens with vaccinating purposes has allowed characterizing potential targets in different species of *Taenia solium*, *T. crassiceps*, and *T. ovis* which exhibit variable levels of protection against porcine cysticercosis [73,132-138].

In 1999, Toledo et al. [139], a group of Mexican researchers, designed peptides based on KETc7 recombinant antigen, which is a polypeptide rich in proline amino - acid, obtaining three linear peptides denominated as GK-1, GK-2 y GK-3; among them, only GK-1 induced sterile protection (total parasite elimination) against *T. crassiceps* in 40 to 70% of male BALB/c mice. This 18- amino- acid peptide contains at least a B - cell epitope. Anti-GK1 antibodies recognize epitopes found in all the parasite's evolutive stages: egg, cysticercus, and adult parasite. Cytokine response against this peptide showed a Th1 / Th2 balance with both levels of INF- $\gamma$  and IL-4.

Toledo et al. [139,140], based on chemical synthesis, obtain the complete sequence of linear amino - acids of the KETc1 and KETc2 antigens, and a protection of 52.7 to 100% was shown in the murine model of NCC. Analysis of the sequences and experimental data, obtained through immunofluorescence evinced B - cell and T - cell epitopes. As a consequence of the immune response against all the parasite's evolutive stages, these authors conclude that GK1, KETc1 and KETc2 are good candidates to be a vaccine against NCC. Nevertheless, the conformation of these chemical peptides is one of its drawbacks, because it directly attempts against immunogenicity. There is the need of including haptens (GK2 and GK3), and proteolytic degradation susceptibility [139,141].

One of the great advances in the field of swine cysticercosis vaccine development was achieved in 2004; which the use of the recombinant antigens denominated TSOL18 and TSOL45-1A, derived from oncospheres and fused to GTS. By means of glutathione affinity chromatography, Flisser et al., [131] reported that these two proteins were purified and immunized pigs, reaching almost a total protection level (99.5%) by using TSOL18 as antigen. Until today, this protection level remains as the highest ever obtained against a challenge of 5,000 - 40,000 *T. solium* eggs in pigs. These findings and other obtained by using oncosphere derived antigens, suggested that a vaccine candidate to be tested on humans could be expected in a short term [130,142,143].

## New targets

Similarly to bacteria, protozoans and helminths such as *Fasciola hepatica* and *S. mansoni*, *T. solium* can interact with proteins of the

fibrinolytic system, such as plasminogen and plasmin. This can happen because the parasite whether uses host's proteins to invade tissues or takes them to avoid being recognized by the immune system. Characterization of receptors that join plasminogen / plasmin in *Taenia* sp. offers a potential vaccine target, which has not been validated for cysticercosis yet. Similar works are being developed in malaria [144] and leishmaniasis [145].

*T. solium* calreticulin has been cloned; it is a protein located in tegumental and subtegumental level and is involved in the embryogenesis, oogenesis and spermiogenesis processes. Since it represents a clue function for this cestode, it could be an excellent candidate for taeniosis vaccine [146,147]. Recombinant functional *T. solium* calreticulin (rTsCRT) confers different degrees of protection in the experimental model of intestinal taeniosis in hamsters [148]. The cytokine response in the intestinal mucosa of hamsters infected with *T. solium* suggest that tapeworms induce a mixed Th1 / Th2 response (INF- $\gamma$ , IL-13, IL-4) by in situ hybridization in intestinal biopsies, with a polarization toward Th2 at 2 weeks post infection, which may influence the expulsion of worms [149]. In BALB/c mice, the oral immunization with rTsCRT, elicited high fecal IgA and the production of IL-4 and IL-5 by mesenteric lymph node cells after in vitro stimulation with rTsCRT, indicating a Th2 response, a common characteristic of protective immunity against helminths and, consequently, a desirable hallmark in the search for a human taeniosis vaccine [150].

The fatty acids binding proteins (FABP) identified in *Taenia solium* (TsFABP1), could be a target to develop vaccines against CC / NCC. Protection assays carried out in a murine model of CC showed that subcutaneous immunization with TsFABP1 resulted in about 45% reduction of parasite load against an intraperitoneal challenge with *T. crassiceps* cysts. This reduction in parasite load correlated with the level of cellular and humoral immune responses against TsFABP1, as determined in spleen lymphocyte proliferation and ELISA testing [151]. Similar works have been performed in *S. mansoni* [152].

## Conclusions

The human NCC should be considered as a "complex disease". Seizures are widely reported to be the most common symptoms, occurring in 70 - 90% of patients, while NCC is considered to be the main cause of late - onset epilepsy in endemic areas. The immune inflammation depends of host's factors, the number of parasites, and also the role of innate and specific responses against antigens cysticerci. This process involves mast cells, Tregs, pro-inflammatory and anti-inflammatory cytokines, in both peripheral and CNS, and low levels of A4 and DHEA in male and females. The diagnosis of NCC is based upon neuroimaging studies (CT, MRI), laboratory analysis of the CSF, antibody detection in the serum, and epidemiological and clinical features. In terms of serological diagnosis, the lectin-lentins affinity chromatography (LLPG) is approved by WHO and PAHO. Albendazole and praziquantel are the drugs most commonly used for the treatment; however, by obtaining the nuclear genomic sequence of *T. solium* [153], new drugs may be obtained for the purpose of prevention, cure, and immunotherapy. Results of protection assays with the use as vaccine rTsCRT infer that could be a candidate for use in humans.

## Acknowledgements

This paper was achieved with the sponsorship of the Fondo Nacional de Ciencia, Tecnología e Innovación FONACIT (National Fund for Science, Technology and Innovation), within the Misión Ciencia framework

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