NEUROCYSTICERCOSIS: CLINICAL MANIFESTATION, NEUROIMAGING, SEROLOGY AND MOLECULAR CONFIRMATION OF HISTOPATHOLOGIC SPECIMENS

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Abstract. Diagnosis of neurocysticercosis (NCC) is usually based on neuroimaging and/or immunological analysis of cerebrospinal fluid (CSF) and/or serum samples for detection of specific antibodies against *T. solium* antigens. Additional confirmative diagnosis may be possible by morphological and molecular confirmation of resected histopathologic specimens. The majority of NCC cases do not always show typical neuroimaging figures with invaginated scolex. So, serology using highly specific antigens of *T. solium*, either semi-purified native or recombinant antigens, is essential for confirming NCC cases. There is some debate about the usefulness of CSF and serum for immunodiagnosis. When NCC cases with a solitary cyst or with calcified lesions are examined, serology is not always sensitive to differentiating such cases. Malignant brain tumor is most commonly suspected in Japan and is often treated surgically as an urgent task, if the clinicians have no experience of NCC cases. Only histopathological specimens are expected to show direct evidence of *T. solium* cysticercosis. Morphology is not always sufficient for identification of the *Taenia* species, even if the majority of cysticerci in the human brain are expected to be *T. solium*. Crucial confirmation is based on molecular identification. In this review, these four issues are briefly summarized.

INTRODUCTION

Neurocysticercosis (NCC) is expected to be one of the most potentially lethal helminthic diseases worldwide (Garcia *et al*, 1991, 1997, 2005; Tsang and Wilson, 1995; Craig and Pawlowski, 2002; Ito *et al*, 2003, 2004, 2006a; Schantz, 2006). One of the important advances in the diagnosis of NCC is the establishment of highly reliable serodiagnosis using *Taenia solium* antigens (Gottstein *et al*, 1986; Parkhouse and Harrison, 1987; Tsang *et al*, 1989; Richards and Schantz 1991; Ito *et al*, 1998; Chung *et al*, 1999; Sako *et al*, 2000). Serology (ELISA) using semi-purified native (Ito *et al*, 1998) and recombinant chimeric antigens (Sako *et al*, 2000) is now highly reliable for screening and confirmation of NCC cases. Such serology, by both ELISA and immunoblot, is applicable not only to humans but also to pigs and even dogs in highly endemic areas in Asia and the Pacific (Ito *et al*, 2002a; Sato *et al*, 2003; Margono *et al*, 2005; reviewed by Ito and Craig, 2003; Schantz, 2005). As far as we know, ELISA using recombinant chimeric antigens is the most reliable and sensitive, although there is some debate about the importance of glycosilation (Scheel *et al*, 2005; Lee *et al*, 2005; Sako *et al*, 2000, 2006). In this review, such serological work for detection of NCC and for epidemiological studies as well as clinical manifestation and neuroimaging will be summarized. Although such serology is highly specific and sensitive, there is a problem as to how to detect NCC with a solitary cyst or NCC with calcified lesions. There are no scientifically reliable data on the sensitivity of any serological tools for detection of solitary NCC cases, either by antibodies or antigens. So far, approximately 30-60% of NCC with a solitary cyst may be positive by detection of antibodies. This may be due to the quality of the diagnostic antigens. The best score is approximately 83% in Latin America (Wilson *et al*, 1991; Ohsaki *et al*, 1999; Ito *et al*, 1999a; Proaño-Narvaez *et al*, 2002). Detection of circulating antigens is expected to be useful for monitoring of prognosis of NCC cases after confirmation of NCC by other methods (Garcia *et al*, 2002; Ito and Craig 2003, 2004; Dorny *et al*, 2004). Serology for detection of antibodies is suitable for detection of active NCC cases (Ito *et al*, 1998; Chung *et al*, 1999; Sako *et al*, 2000).

NCC is very rare and not indigenous to Japan. Therefore, when clinicians suspect malignant brain tumors, they often choose urgent surgery. In such cases, histopathological specimens are available for morphological and molecular confirmation and are important for identification of the causative species. Such cases confirmed by mitochondrial DNA analysis

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are also summarized (Yamasaki *et al*, 2006a, b).

DISTRIBUTION OF TAENIASIS/ CYSTICERCOSIS

Fig 1 illustrates the geographic distribution of *T. solium*. Approximately 50 million people are infected with the taeniasis/cysticercosis complex and more than 50,000 die from cysticercosis every year (the International Task Force for Disease Eradication, 1993), while there was no information on *T. solium* from Africa (Boa *et al*, 1995). Therefore, such data are clearly underestimated. At the World Health Assembly 2003, *T. solium* cysticercosis was considered to be one of the neglected infectious diseases that are expected to be eradicable in the future. At WHO's small meeting in September 2005, it was included in such neglected infectious diseases as echinococcosis, rabies, brucellosis, anthrax, and African sleeping sickness (WHO/DFID-AHP, 2005).

CLINICAL MANIFESTATIONS OF NCC

There is no pathognomonic clinical feature or a typical NCC syndrome. It is due to 1) the type of cysticerci, either vesicular cyst or acephalic, racemose form, 2) the developmental stage of the parasite, 3) the number of cysticerci, 4) the location of cysticerci, either intraparenchymal (rather benign) or extraparenchymal (rather serious), and 5) the balance of the defense mechanisms between the host and the parasite. Most clinical manifestations become evident when the parasite is damaged and dying.

When NCC is intraparenchymal, it is associated with a good prognosis. Frequently, patients with few intraparenchymal cysts remain asymptomatic, although some develop seizures. On the other hand, in patients with massive cerebral infection, uncontrolled seizures and cognitive deficiency may develop. Seizures are widely reported to be the most common manifestation, occurring in up to 70% of patients (Garcia *et al*, 2005; Takayanagui and Odashima, 2006), and NCC is considered the main cause of late-onset epilepsy in endemic areas.

When cysticerci lodge within the ventricular system, life-threatening acute intracranial hypertension, secondary to hydrocephalus, may develop. It is directly related to obstruction of the flow of CSF by the cyst or by inflammatory reaction of the ependyma. Although the cysts may be found anywhere within the ventricular system, the fourth ventricle is most commonly involved.

Cysts in the subarachnoid space may invade the Sylvian fissure and grow to large, reaching several centimeters in diameter (giant cysts), causing intracranial hypertension with hemiparesis, partial seizure, or other focal neurological signs. Subarachnoid cysts may also invade the basal cisterns; initially the growing membranes resemble a bunch of grapes, hence this form of the disease is called "racemose" cysticercosis. It is associated with an

Fig 1- Geographic distribution of *T. solium* in the world (modified from Schantz, 2002).

intense inflammatory reaction, fibrosis and progressive thickening of the leptomeninges at the base of the brain. In approximately 50-60% of cases, the CSF circulation is obstructed, resulting in hydrocephalus and progressive intracranial hypertension and mortality in > 20% of cases. Signs of meningitis, cranial nerve palsy, chiasmatic syndrome, and cerebral infarcts secondary to vasculitis, may also develop. When hydrocephalus secondary to cysticercotic meningitis is present, the mortality rate is high (50%) and most patients die within 2 years after CSF shunting. Therefore, ventricular and basal cisternal locations are considered to be malignant forms of NCC.

NEUROIMAGING STUDIES AND LABORATORY STUDIES FOR DETECTION OF SPECIFIC ANTIBODIES IN CSF OR SERUM SAMPLES

Neuroimaging diagnosis of *T. solium* cysticercosis in humans and pigs

Early in infection, a viable cyst appears as a spherical lesion on computerized tomography (CT), and as a CSF-like signal on magnetic resonance imaging (MRI). Both CT and MRI can show the invaginated scolex (Fig 2-a). In the degenerative phase, the cyst shows a ring-like or a nodular contrast enhancement (Fig 2-b), with or without perilesional edema. The final stage is observed when the cyst dies and a process of mineralization and resorption takes place, resulting in calcified nodules (Fig 2-c).

Since the cyst membrane is thin and the fluid is isodense within the CSF, non-inflamed extra-parenchymal (ventricular or subarachnoid) cysticerci are usually not visible on CT and may only reveal subtle, indirect findings on MRI scan.

MRI is more sensitive than CT scan for the diagnosis of NCC, since it improves recognition of perilesional edema and degenerative changes of the parasite, as well as small cysts or those located inside the ventricles, brain stem, cerebellum and the racemose vesicles at the level of the posterior fossae and basal cisterns (Fig 2-d). However, CT scans are more sensitive for the detection of calcifications. Similar observation on vesicular NCC has been reported in

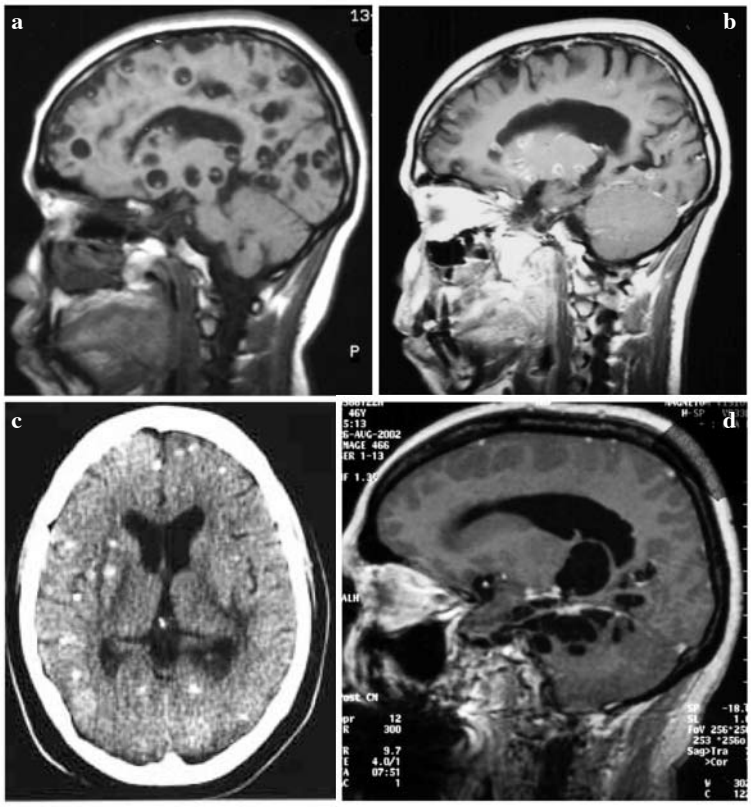

Fig 2- NCC cases of vesicular form [active (a), transitional (b), and inactive (calcified) (c) cases] and acephalic, racemose form (d).

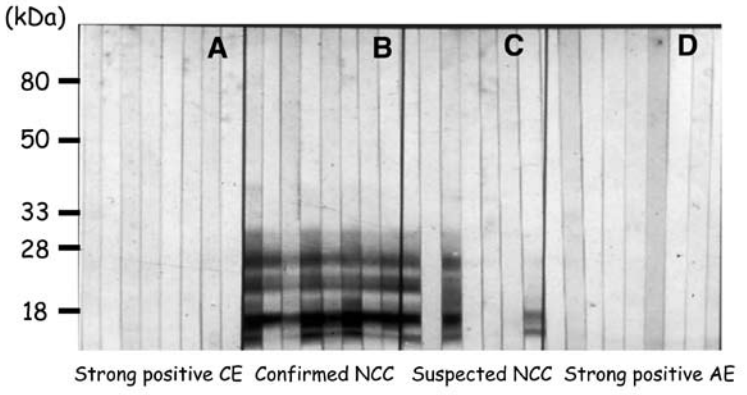

Fig 3- Immunoblots using native antigens purified by preparative isoelectric focusing (modified from Ito *et al,* 1998). Panels A and D are confirmed CE and AE cases with high titers against both crude and purified antigens of *Echinococcus granulosus* and *E. multilocularis*, respectively. Panel B shows 8 NCC cases confirmed at CDC, USA. Panel C shows 3 NCC cases with other suspected cases in Japan.

pigs (Prasad *et al*, 2002).

Serodiagnosis of *T. solium* cysticercosis in humans, pigs, and dogs

Fig 3 illustrates the immunoblot results using native *T. solium* antigens purified by preparative isoelectric focusing (modified from Ito *et al*, 1998). In such a serology, it is scientifically crucial to use confirmed serum samples with high titers for homologous parasite species. Echinococcosis of both cystic (CE) and alveolar (AE) types is the highly cross-reactive helminthic disease. Panel C show several NCC cases suspected in Japan by clinicians with no experience of NCC cases. Only three cases were confirmed serologically as NCC at first, and confirmed to be NCC later. Fig 4 illustrates ELISA results using both native and recombinant chimeric (Ag1V1/Ag2) antigens (Sako *et al*, 2000). Both native and recombinant antigens showed very similar high sensitivity and specificity in both ELISA and immunoblot. More than 94% of confirmed active NCC cases were serologically confirmed positive using *T. solium* recombinant antigens, whereas approximately 93% of them were also confirmed using native *T. solium* antigens.

There is some debate as to whether it is better to use CSF or serum samples for serodiagnosis of NCC. It has under debate and been speculated to be due to the localization of the cysts either in parenchyma or in the subaracnoid space. Most recent work using the chimeric recombinant antigens (modified from Sako *et al*, 2000) has revealed that both CSF and serum can show very similar high sensitivity for detection of active NCC cases (Takayanagui *et al*, unpublished). Therefore, confirmation of these data might dispense

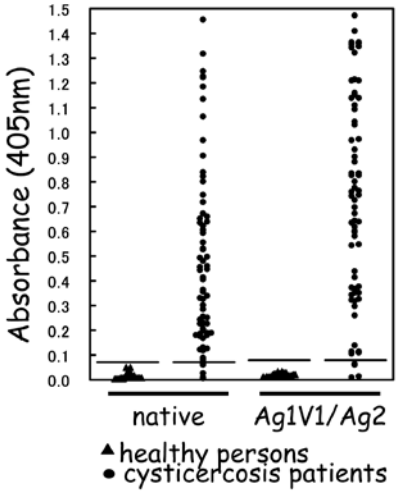

Fig 4- ELISA results using native and recombinant chimeric (Ag1V1/Ag2) antigens of *T. solium* (modified from Sako *et al*, 2000).

with CSF for immunological confirmation. Such debate on the controversial results for sensitivity may be based on the quality of antigen used. CSF might be useful for detection of species-specific DNA.

Highly specific antigens, either native *T. solium* or recombinant *T. solium*, are applicable for detection of specific antibodies at primary screenings and confirmation of animals, either pigs or dogs, as well (Ito *et al*, 1999b; 2002a, 2004, 2006).

There are two strategies for the detection of NCC in human and animal cases. One is detection of specific antibodies against specific antigens. The gold standard for detection of antibodies is the use of *T. solium* antigens (Tsang *et al*, 1989), either

native or recombinant (reviewed by Ito, 2002; Ito and Craig 2003). All other tools using antigens from heterologous species, including *T. crassiceps* (Espindola *et al*, 2005), are based on cross-reactions and are highly cross-reactive and often have difficulty in evaluating such tools under blind tests. The other is detection of circulating antigens in NCC cases. This tool uses monoclonal antibody to some component of *T. saginata*, but so far *T. solium* is not available. Therefore, it is expected to be rather useful for monitoring of prognosis after NCC cases have been confirmed by other tools, including detection of antibodies. It is strongly recommended that a new tool be developed for detecting circulating antigens using specific antibodies to *T. solium* (Ito and Craig, 2004).

MOLECULAR DIAGNOSIS OF CYSTICERCOSIS IN HUMANS, PIGS AND DOGS

Based on the sequence data of the full-length mitochondrial DNA of *T. solium* (Nakao *et al*, 2002), multiplex PCR has been developed to differentiate 3 human *Taenia* species (*T. solium, T. saginata, T. asiatica*) (Fig 5) (Yamasaki *et al*, 2004, reviewed by Yamasaki *et al*, 2006a, b). It can differentiate the two genotypes of *T. solium*: the Asian type, and the American/African type (Okamoto *et al*, 2001; Ito *et al*, 2002b; Nakao *et al*, 2002). It is well known that the clinical manifestation of cysticercosis in Asia and the Pacific often includes NCC and subcutaneous cysticercosis (SCC), whereas in America and Africa it mainly includes NCC without SCC. However, NCC without SCC in Asia, and NCC with SCC in Africa, are also often observed and reported. Therefore, such a concept might be due to bias from a small number of cases. So, we should be careful about the differentiation of clinical manifestations in the world. Although there is no evidence of the correlation of genotyping and clinical manifestations, such genotyping is informative for tracing back to where NCC patients were exposed to *T. solium* eggs. Microsatellite DNA studies may be more useful for such polymorphism of *T. solium* in the world and for tracing cases in more detail (Campbell *et al*, 2006). Such studies may be interesting for elucidating the molecular evolution of *T. solium* (Nakao *et al*, 2002).

When we checked local residents living in endemic areas and find SCC, > 70% of were confirmed cysticercosis cases (Wandra *et al*, 2003; Ito *et al*, 2004; Margono *et al*, 2006). Therefore, molecular and parasitological confirmation of resected SCC is very important.

Specimens for DNA analysis are egg(s), metacestodes, adult tapeworms (proglottids) from taeniasis- or cysticercosis-confirmed cases, nodules resected from suspected cysticercosis cases, and feces of taeniasis either confirmed by parasitological observation of expelled proglotids or copro-ELISA positive (Wandra *et al*, 2006).

WHEN AND WHY DO WE NEED MOLECULAR CONFIRMATION OF NCC CASES?

When we have typical neuroimaging figures showing a single cyst or multiple cysts with invaginated scolex, it may be sufficient to diagnose NCC clinically. However, such cases are expected to be only around 10% of NCC cases, either symptomatic or asymptomatic. The diagnosis of cysticercosis is clear for us when specific antibodies are detectable, but, when we cannot detect any specific antibody responses, we can say nothing without referring to other information, especially from neuroimaging. Furthermore, even immunocompetent NCC patients might be antibody-negative due to the negative response, anergy. Moreover, NCC patients with a

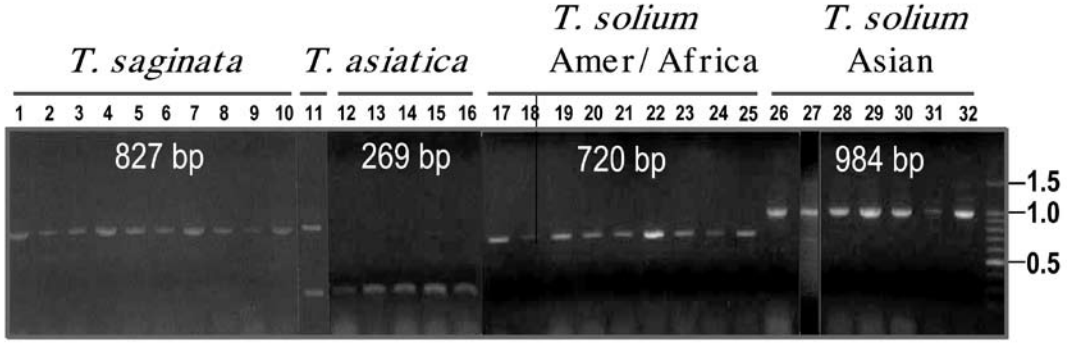

Fig 5- Differential diagnosis of human *Taenia* by multiplex PCR (modified from Yamasaki *et al*, 2004). Line 11 was mixtured eggs of *T. saginata* and *T. asiatica* from Yunnan, China.

solitary cyst or calcified lesions may be sero-negative. When we examine such cases showing no antibody responses, what we have to do? Detection of highly specific circulating antigens (not yet available) or specific DNA in CSF, which might be developed in the near future, may be alternative tools and are expected to be more reliable. As *T. solium* cysticercosis is not indigenous in Japan, no clinician has sufficient knowledge on this disease except the few who have had experience diagnosing NCC cases. Even when clinicians suspect NCC cases, if the cases are sero-negative and show no typical neuroimaging, they often choose surgery for presumably malignant brain tumor.

Racemose form of *T. solium* NCC

Fig 2-d illustrates the racemose form of *T. solium*. The mechanism of occurrence of this atypical and severe form of NCC is not totally understood. However, such cases might be more common in immunocompromized patients (reviewed by Yamasaki *et al*, 2006b).

Histopathological specimen without hooklets or even with hooklets

How do we evaluate such specimens? Is it an unexpected new case of other taeniid species without hooklet or artifact of *T. solium* showing no hooklets? Even if such specimens are showing hooklets, are they really *T. solium* cysticerci? We have to keep in mind that there might be some other zoonotic metacestodiasis due to other taeniid species that are usually uncommon in humans, such as *T. ovis*, *T. serialis*, *T. crassiceps* etc. (Yamasaki *et al*, 2006b). Such uncommon interactions might be due merely to lack of contact with such parasites by wildlife, or the immunosuppression of patients by long-term treatment with steroids, etc. In order to avoid such doubts, it is essential to perform molecular identification of such specimens.

CONCLUSIONS

Diagnostic tools for NCC cases have been briefly reviewed. The merits and the demerits of each tool were described and the recommendations are as follows:

1. Neuroimaging of active and transitional NCC cases showing typical figures of invaginated scolex in the cyst gives us confirmative information.
2. Highly specific and reliable serology to detect antibodies to *T. solium* cysticerci has been developed. No other serological tools using antigens from other species are better than this system.
3. Almost all active and transitional NCC cases with multiple cysts are detectable by antibody-ELISA or immunoblot using *T. solium*-specific antigens, either native or recombinant.
4. Calcified NCC cases are usually sero-negative. Solitary NCC cases may or may not be sero-positive.
5. Molecular identification of histopathological specimens becomes the key to identifying the causative agent.
6. Diagnosis is based on all of the items mentioned above.

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