Diagnosis of neuroschistosomiasis by antibody specificity index and semi-quantitative real-time PCR from cerebrospinal fluid and serum

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We describe the case of a 16-year-old German male expatriate from Ghana who presented with obstipation, dysuria, dysaesthesia of the gluteal region and the lower limbs, bilateral plantar hypaesthesia and paraesthesia without pareses. A serum-cerebrospinal fluid (CSF) Schistosoma spp. specific antibody specificity index of 3.1 was considered highly suggestive of intrathecal synthesis of anti-Schistosoma spp. specific antibodies, although standardization of this procedure has not previously been described. Diagnosis was confirmed by detection of Schistosoma DNA in CSF by semi-quantitative real-time PCR at 100-fold concentration compared with serum. Accordingly the two diagnostic procedures, which have not previously been applied for routine diagnosis, appear to be useful for the diagnosis of neuroschistosomiasis. Clinical symptoms resolved following anthelmintic and anti-inflammatory therapy.

Introduction

Neurological involvement represents a severe but rare clinical complication of schistosomiasis. Diagnosis of neuroschistosomiasis is challenging and diagnostic standards have not yet been established. Diagnostic approaches include the identification of ova excretion in faeces or urine or in biotptic material from the urinary bladder or rectum in combination with immunological testing (Pollner et al., 1994; Liu et al., 2006; Jaureguiberry et al., 2007). Here we describe the diagnosis of a case of neuroschistosomiasis based on comparative semi-quantitative PCR in cerebrospinal fluid (CSF) and serum and an indicative antibody specificity index.

Case report

A 16-year-old native German male adolescent returned from Ghana with a 4 week history of obstipation, dysuria and dysaesthesia of the gluteal region and the lower limbs, the last described as a feeling of 'pins and needles' in the tissue. He had been living in Accra with his expatriate parents for 2 years. The family had been swimming repeatedly in the Volta river.

Physical examination revealed bilateral plantar hypeaesthesia and paraesthesia without pareses. Magnetic resonance imaging (MRI) of the spine revealed an enlarged thoracic and lumbar cord and conus medullaris, and a patchy cord enhancement (Fig. 1a, b).

Blood analysis demonstrated a normal leukocyte count of 9.4 × 10⁹ leukocytes l⁻¹ (reference range 4.4–11.3 × 10⁹ leukocytes l⁻¹) with 10% eosinophils. Total IgE was elevated to 159 IU ml⁻¹ (reference value <100 IU ml⁻¹). The CSF showed lymphocytic pleocytosis with 74 cells µl⁻¹ (reference value <5 cells µl⁻¹) with 5% eosinophils, elevated protein of 924 mg l⁻¹ (reference value <500 mg l⁻¹) and raised IgG of 213 mg l⁻¹ (reference value <40 mg l⁻¹). Albumin in CSF was raised at 591 mg l⁻¹ (reference value <350 mg l⁻¹). In contrast, IgG and albumin in
serum were normal at 13.5 and 48.6 g l\(^{-1}\) (reference ranges 5.5–15.8 and 35–53 g l\(^{-1}\)), respectively. Oligoclonal bands were detected in CSF only.

High concentrations of anti-\textit{Schistosoma} antibodies were detected in serum and CSF by in-house ELISA and in-house indirect immunofluorescence testing (IFT), using cryo-cut slices of adult \textit{Schistosoma mansoni} as the antigen target. \textit{Schistosoma}-specific IFT titres were \(>1 \times 1280\) in serum and \(1 : 64\) in CSF. The CSF–serum antibody specificity index, based on sample dilutions with optical density values within the linear range of the ELISA, was calculated according to the algorithm described by Andiman (1991): (organism-specific CSF antibody) \(\times\) (IgG in serum) \(\times\) ([organism-specific serum antibody] \(-1\)) \(\times\) ([IgG in CSF] \(-1\)). Two-fold serial dilutions of both serum and CSF were tested by ELISA. The titres for which CSF and serum had identical optical density values by ELISA were identified. These ELISA titres as well as the total IgG concentrations in CSF and serum were used in the equation of Andiman (1991).

This procedure has not been described for use in \textit{Schistosoma} infections, but in line with procedures for varicella zoster infections, Lyme disease and syphilis an index of \(>1.5\) was considered pathological. The calculated value was 3.1 and thus highly suggestive of intrathecal production of specific anti-\textit{Schistosoma} antibodies.

A previously published real-time PCR (Wichmann \textit{et al.}, 2009) was used for what is believed to be the first time in CSF and demonstrated \textit{Schistosoma} spp. DNA in CSF and serum (Fig. 2). One microlitre each of serum and CSF were prepared for PCR as described previously (Wichmann \textit{et al.}, 2009, 2013). The mean threshold cycle \((C_t)\) values indicated a nearly 100-fold higher concentration of DNA in CSF \((C_t 25.5)\) compared to serum \((C_t 32.2)\) (Fig. 2). Both measured \(C_t\) values were within the linear range of the described PCR, making semi-quantitation possible.

\textit{S. mansoni} eggs were initially not detected in repeated stool and urine samples, but additional diagnostic work-up showed eggs in several rectal biopsies by eosin hematoxylin staining. In CSF, however, eggs or adult worms were never detected. Treatment was initiated with high-dose corticosteroids (1 g prednisolone per day for 3 days) to avoid an exaggerated inflammatory response. On day 4, anthelmintic therapy with 40 mg praziquantel kg\(^{-1}\) per day for 5 days was started with accompanying 1 mg prednisolone kg\(^{-1}\) per day. After treatment, neurological symptoms resolved rapidly. A follow-up MRI revealed a decrease of the cord and conus medullaris swelling and regressive contrast enhancement (Fig. 1c).

**Discussion**

The incidence of central nervous system (CNS) involvement in schistosomiasis is unclear, although studies suggest it is under-diagnosed. The true prevalence of neuroschistosomiasis is estimated to be between 1 and 5 % of all diagnosed schistosomiasis cases (Carod-Artal, 2008). In old African autopsy studies, more than 50 % of corpses with \textit{Schistosoma haematobium} in the bladder showed brain lesions and, even in unselected corpses, scattered ova of \textit{S. haematobium} and \textit{S. mansoni} were detected in more than 25 % of brains (Gelfand, 1950; Alves, 1958). Spreading of eggs into the CNS might be due to aberrant migration of adult worms or embolization events from remote locations (Liu, 1993; Wang \textit{et al.}, 2010), leading to mild to moderate impairment of the blood–brain barrier and intrathecal synthesis of anti-\textit{Schistosoma} antibodies. The host’s immune and inflammatory response against deposited eggs in CNS tissues surrounded by granulomas ultimately leads to symptomatic disease (Ferrari \textit{et al.}, 2008).

In patients with spinal schistosomiasis symptoms comprise lumbar pain, lower limb radicular pain, ascending weakness...
of the lower limbs, muscle weakness, sensory loss and bladder dysfunction; in severe cases, even progressive paraparesis (Koul et al., 2002; Wichmann et al., 2006; Carod-Artal, 2008; Li et al., 2011). The symptoms seen in our patient match this description, although pain was less prominent and the major symptom was dysesthesia of the gluteal region and lower limbs.

In spite of indicative symptoms the diagnosis of neuroschistosomiasis is rarely made, particularly because clinical symptoms of systemic schistosomiasis are often lacking (Zhou et al., 2009), as in the case described here, and Schistosoma ova are detected in stool and urine samples in fewer than 50% of neuroschistosomiasis patients (Ferrari et al., 2004). Frequent findings when imaging neuroschistosomiasis patients include enlargement of the medullary cone and the roots of the cauda equina (Ferrari et al., 2004). This is also consistent with the findings in our patient.

Typical laboratory findings in the CSF of neuroschistosomiasis patients comprise mild to moderate pleocytosis, presence of eosinophils, slight to moderate protein increase, elevated gamma globulin concentration and a positive immune assay. All five findings are present in only 20% of cases (Moreno-Carvalho et al., 2003; Tesser et al., 2005), including the case presented here.

Serological testing is one of the frequently used diagnostic criteria for neuroschistosomiasis (Ferrari et al., 1995; Magalhães-Santoset et al., 2003; Gryseels et al., 2006; Ferrari, 2010) but it is prone to cross-reactivity with other helminths, even with aetiologically relevant species such as Taenia solium (Pammenter et al., 1992). Egg-antigen immune complexes are usually detectable in the CSF of neuroschistosomiasis patients but are not routinely assessed in diagnostic testing (Ferrari et al., 2011).

Calculation of the CSF–serum antibody specificity index is an easily performed procedure that can be used to assess CNS involvement in infections. This index indicates pathogen-specific intrathecal antibody production, with a higher proportion of specific antibodies in CSF than in serum. The calculation requires test results within the linear range of measurement. The CSF–serum antibody specificity index has not yet been standardized for the diagnosis of neuroschistosomiasis. However, decades of experience with this diagnostic procedure are available for neurological involvement of varicella zoster virus infections, Lyme disease and syphilis (Andiman, 1991). In line with established procedures for these diseases, we chose an anti-Schistosoma CSF–serum antibody specificity index cut-off of >1.5. The calculated value of 3.1 in our case was highly suggestive of Schistosoma infection manifestation in the CNS. Use of this index might represent a valuable tool for the diagnosis of neuroschistosomiasis.

To further confirm the diagnosis semi-quantitative PCR for Schistosoma spp. (Wichmann et al., 2009, 2013) was performed, keeping in mind that circulating DNA in serum might contaminate CSF during ultrafiltration through the choroid plexus and due to contamination of CSF samples with blood during their acquisition. However, the confirmation of a higher titre of Schistosoma spp. specific DNA in CSF compared to serum made such contamination unlikely. Although PCR has previously been used to confirm Schistosoma DNA in brain tissue (Imai et al., 2011), the use of semi-quantification of Schistosoma DNA has not been reported for the confirmation of intrathecal Schistosoma cells.

Diagnosis of neuroschistosomiasis was further supported by the fact that clinical symptoms resolved following specific therapy. However, complete or partial recovery is only described for 60–70% of patients with Schistosoma-induced myeloradiculopathy (Ferrari et al., 2004; Li et al., 2011).

Neuroschistosomiasis should be considered in patients returning from regions where Schistosoma spp. are endemic and who have clinical symptoms of myeloradiculopathy, even if no Schistosoma eggs are detected in CSF, stool or urine. While microscopic detection of eggs in the CNS unambiguously proves neurological involvement in symptomatic patients, diagnosis in cases in which eggs are not detected is challenging.

In the case presented here both antibody specificity index and comparative semi-quantitative Schistosoma PCR in CSF and serum proved to be useful for confirming the diagnosis in addition to CSF findings, i.e. moderate pleocytosis, presence of eosinophils, slight to moderate protein increase, elevated gamma globulin concentration and a positive immune assay. However, further controlled studies with a critical number of patients are required to assess whether each of the two new parameters is sufficient to confirm neuroschistosomiasis in addition to standard parameters while – in contrast – their absence does not exclude the diagnosis. In our routine diagnostic service, we have also observed a plausible neuroschistosomiasis case with a negative CSF–serum antibody specificity index (data not shown). Likewise, high concentrations of Schistosoma DNA in serum, e.g. in early infections, might frustrate attempts to demonstrate higher specific DNA concentrations in CSF in spite of the presence of Schistosoma eggs in the CNS compartment. As positive immune assays are already elements of standard diagnostic procedures demanded for the proof of neuroschistosomiasis, CSF–serum antibody specificity index assessment will presumably be more readily available than PCR in routine laboratories.

In summary, neuroschistosomiasis should be considered in patients with neurological symptoms and potential exposure to Schistosoma infection, even if no Schistosoma eggs are detected. In the case presented here, both newly applied methods – the determination of CSF–serum antibody specificity index and quantitative real-time Schistosoma PCR from CSF and serum – proved useful to confirm the suspected diagnosis of neuroschistosomiasis.

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References


