

sICAM-1 IN MENINGOENCEPHALITIS DUE TO *ANGIOSTRONGYLUS CANTONENSIS*

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ABSTRACT-Introduction: *Angiostrongylus cantonensis* meningoencephalitis is an emergent disease in the Americas. **Method:** Twelve children suffering from eosinophilic meningoencephalitis due to this parasite aged between 6-10 years were studied. Cerebrospinal fluid (CSF) and serum samples were taken simultaneously in the first diagnostic puncture at admission. **Results:** All cases showed typical findings on the routine CSF and serum analysis: increased CSF total protein, increased Q (CSF/serum) albumin accompanied by eosinophilia in CSF. No intrathecal synthesis of immunoglobulins was found. Mean serum and CSF sICAM-1 values were 337.4 and 3.97 ng/mL. Qalbumin and QsICAM-1 mean values were 4.1 and 6.2 respectively. In 50% of the patients an increased brain-derived fraction of sICAM-1 was found. **Conclusion:** It may be suggested that a dynamic of the sICAM-1 brain derived fraction is perhaps associated to the immune response in the evolution of the disease. sICAM-1 may be an agent in negative feedback for eosinophils passage through the blood-CSF barrier into the inflammatory brain response.

KEY WORDS: *Angiostrongylus cantonensis*, albumin, sICAM-1, blood-cerebrospinal fluid barrier, eosinophils, meningoencephalitis.

sICAM-1 en meningoencefalitis por *Angiostrongylus cantonensis*.

RESUMEN-Introducción: La meningoencefalitis por *Angiostrongylus cantonensis* es una enfermedad emergente en las Américas. **Método:** Doce niños con meningoencefalitis eosinofílica por *Angiostrongylus cantonensis* con edades entre 6 y 10 años fueron estudiados. Se tomaron muestras simultáneas de suero y líquido cefalorraquídeo (LCR) en la primera punción lumbar diagnóstica. **Resultados:** Todos los casos evidenciaron hallazgos típicos en los análisis de rutina del LCR y suero: incremento de proteínas totales, aumento de la razón albúmina Q (LCR/suero) acompañado de eosinofilia en LCR. No se encontró síntesis intratecal de inmunoglobulinas. Los valores medios de sICAM-1 en suero y LCR fueron de 337,4 y 3,97 ng/mL respectivamente. Los valores medios de Q albúmina y Q sICAM-1 fueron de 4,1 y 6,2 respectivamente. En el 50% de los pacientes se encontró un incremento de la fracción de sICAM-1 derivado del cerebro. **Conclusión:** Se puede sugerir que la dinámica de la fracción sICAM-1 derivada del cerebro ocurre quizás asociada a la respuesta inmune frente a la enfermedad. sICAM-1 puede ser un agente de retroalimentación negativa para el paso de eosinófilos de la sangre a través de la barrera sangre-LCR en el cerebro inflamado.

PALABRAS CLAVES: *Angiostrongylus cantonensis*, albúmina, sICAM-1, barrera sangre/líquido cefalorraquídeo, eosinófilos, meningoencefalitis.

Eosinophilic meningitis (EM) is a distinct clinical entity that may have both infectious and noninfectious causes. Worldwide, infection with the helminthic parasite, *Angiostrongylus cantonensis*, is the most common infectious etiology^{1,2}.

Angiostrongylus cantonensis is a parasite that infects rats as principal hosts and then several species of land snails as the intermediate hosts. Modes of transmission include ingestion by man of raw fish,

snails and fresh leafy vegetables contaminated by snails slime trails containing larvae. The parasite worms are neurotrophic in man and the diagnosis should be considered in any adult or child, in endemic areas or areas with suitable intermediate host that suffers from severe unremitting headache, paresthesias or a cranial nerve palsy.

Eosinophilia in cerebrospinal fluid (CSF) suggests the diagnosis³.

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In 1981, Cuba was the first country to report this disease in the Americas⁴.

Major immunoglobulins intrathecal response against this parasite⁵ and the IgG subclasses intrathecal response, have been studied⁶. Intercellular adhesion molecule-1 (ICAM-1) is expressed on cell of central nervous system (CNS) in normal conditions⁷. 30% of sICAM-1 in normal CSF is brain-derived, and this brain-derived fraction is increased during CNS inflammation⁸. Increased levels of sICAM-1 have been found in viral and bacterial meningoencephalitis^{9,10}.

There is no information about the role of sICAM-1 in eosinophilic meningoencephalitis produced by *Angiostrongylus cantonensis*. Herein we studied the intrathecal release of sICAM-1 into CSF in patients with this disease.

METHOD

Patient – Twelve children suffering from EM due to *Angiostrongylus cantonensis* aged between 6-10 years and a control group were studied. This later group was formed of nine subjects without any organic brain disorder and three patients with Guillain-Barre syndrome. Lumbar puncture was performed on the day of admission for symptoms of EM.

Routine CSF/serum analysis – Routine CSF/serum analysis was performed in all cases according to the protocol described earlier¹⁰. CSF samples contaminated with blood were not included in the study. For measurements of sICAM-1, aliquots of CSF and serum were frozen and kept at -20°C for further analysis in groups of 10-12 samples.

sICAM-1 in CSF and serum was analyzed by a sandwich ELISA methods (R&D Systems Europe, UK). The sensitivity of the ELISA was determined by serial dilution of the standards in the sample diluents (both included in the kit). The lowest concentration distinguishable from the blank was 0.35 ng/mL. Thus, the assay was found sensitive enough to determine sICAM-1 concentration in all samples. Additionally, results obtained with two assays (R&D Systems Europe, UK, and Bender MedSystems, Austria) were compared and found to be essentially equal. A positive control serum is included in the assays and it was measured in twice during each run. The day-by-day coefficient of variation (inter-assays imprecision) was 5.5%. Undiluted CSF samples were used for the assays and serum was diluted in a ratio of 1:20. All steps of the procedure followed the manufacturer's instruction. Absorbance was measured with an automatic

ELISA reader (SLT Lab instruments, Germany) using an evaluation program (easy-fit) from the same manufacturer.

RESULTS

Routine CSF/serum analysis – All cases of EM due to *Angiostrongylus cantonensis* showed typical findings on the routine CSF/serum analysis⁵; increased CSF total protein, increased Q albumin all accompanied by eosinophils in CSF. No intrathecal immunoglobulin responses were found in the first diagnosis lumbar puncture.

sICAM-1 in CSF/serum: sICAM-1 quotient $Q_{(sICAM)}$ and Q_{alb} – Table presents sICAM and albumin values and its Q values. The patients from the control group exhibit no sICAM-1 value over the discrimination line, i.e, no increased brain-derived fraction.

Figure shows the relationship between $Q_{(sICAM)}$ and Q_{alb} for all pediatric patients with EM. Six patients were above the discriminatory curve separating subjects with release of sICAM and no release sICAM as reported previously⁸.

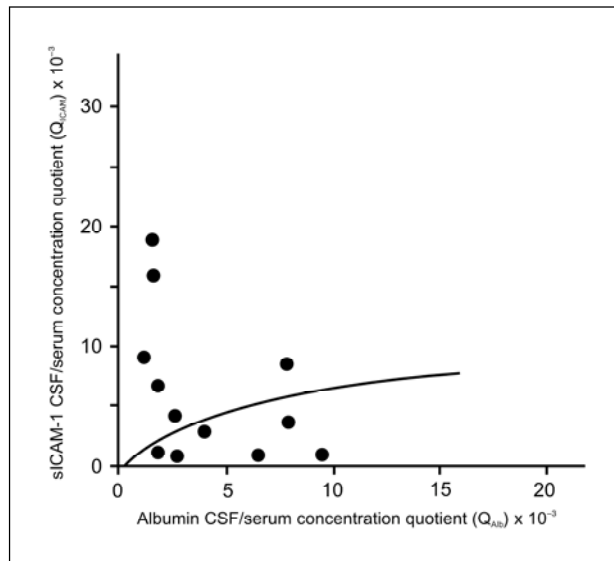


Figure. Relationship between albumin and sICAM-1 CSF/serum concentration quotients ($Q_{sICAM-1}$ and Q_{Alb}) respectively in patients with eosinophilic meningoencephalitis due to *Angiostrongylus cantonensis*. Six patients samples are above the upper discrimination curve in the diagnostic lumbar puncture and four patient has an increased Q_{Alb} ($Q_{Alb} > 5$) according to their age.

Table. Mean values of Q Albumin and sICAM-1.

Group	n	$Q_{Albumin}^*$ (mean)	sICAM-1 (mean)		
			Serum	CSF	Q (ICAM)
Eosinophilic meningitis	12	4.1	337.4	1.97	6.2

* $Q_{Albumin}$ (Serum albumin/CSF albumin).

DISCUSSION

Here we report an increased CSF concentration of sICAM-1 by CSF/serum quotient Q_{ICAM} in 50% of EM patients. We think that our result can be explained by increased brain derived fraction of sICAM-1 levels in those patients during CNS inflammation. The other 50% of the patients indicated that the brain-activated endothelium was not capable of releasing sICAM-1 into CSF.

This observation split off our sample in two subgroups: one which has an increased brain-derived fraction of sICAM-1 and one which does not. It may suggest that exists a dynamic in the production of sICAM-1 brain-derived fraction. All the samples were taken during the diagnostic lumbar puncture.

Previous reports in pediatric patients shows that in the first diagnostic lumbar puncture there is no intrathecal synthesis of immunoglobulins and a dysfunction of the blood-CSF barrier displayed by an increased $Q_{Albumin}$ ^{5,6} was observed. Eight days later, the general picture was generally changing and there were two or three classes of major immunoglobulin synthesis accompanied by a recovery of the blood-CSF brain function and an IgG₁ and IgG₂ subclass response⁶.

Perhaps sICAM-1 could be used as an early marker for some subpopulation of patients who might have an immune response in the second week after the beginning of symptoms. In our opinion further studies should be done in order to test this hypothesis a follow-up of patients with EM due to *Angiostrongylus cantonensis* should be conducted measuring sICAM-1 levels several weeks after the diagnostic lumbar puncture.

Recent observations¹¹ also advocate further research to determine whether or not sICAM-1 is a possible biomarker for various forms of meningeal infection in combination with other inflammatory mediators in children with meningitis.

The infiltration of eosinophils from peripheral blood into CSF requires prior endothelial-eosinophils

interactions that are mediated by such cell surface proteins as adhesion molecules. sICAM is responsible for strong attachment and transendothelial migration of eosinophils¹².

An increased expression of ICAM-1 as well as its soluble form sICAM-1 should be produced in the sequence and timing of the infiltration of eosinophils into CSF during the early phase of the disease. More eosinophil cells were observed in the second lumbar puncture^{5,6} than the diagnostic puncture. So, an increase of blood and brain-derived sICAM-1 levels in CSF should be expected in a follow up study. In a previous study¹⁰ of patients with neuroborreliosis an increase of the brain-derived fraction was observed on the 6th day after admission to the hospital in comparison with the first diagnostic lumbar puncture.

The role of sICAM-1 may be an agent in negative feedback for eosinophilic passage through the blood-CSF barrier to inflammatory brain¹².

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