High sensitivity and specificity of the C6-peptide ELISA on cerebrospinal fluid in Lyme neuroborreliosis patients

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Abstract

Lyme neuroborreliosis (LNB) is a serious but treatable disease. The diagnosis of LNB poses a challenge to clinicians, and improved tests are needed. The C6-peptide ELISA is frequently used on serum but not on cerebrospinal fluid (CSF). Data on the sensitivity of the C6-peptide ELISA in CSF in patients suffering from LNB have been conflicting. Serum–CSF pairs from 59 LNB patients, 36 Lyme non-neuroborreliosis cases, 69 infectious meningitis/encephalitis controls and 74 neurological controls were tested in a C6-peptide ELISA. With the optimal cut-off of 1.1, the sensitivity of the C6-peptide ELISA for LNB patients in CSF was 95%, and the specificity was 83% in the Lyme non-neuroborreliosis patients, 96% in the infectious controls, and 97% in the neurological controls. These results suggest that the C6-peptide ELISA has a high sensitivity and good specificity for the diagnosis of LNB patients in CSF. The C6-peptide ELISA can be used on CSF in a clinical setting to screen for LNB.

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Introduction

Lyme neuroborreliosis (LNB) is the neurological manifestation of an infection with the tick-borne spirochete *Borrelia burgdorferi* sensu lato (sl). LNB can present with many neurological signs, varying from facial nerve paralysis and Bannwarth's syndrome to a range of neurological disorders [1,2]. The diagnosis of LNB poses a challenge to clinicians. Detecting *B. burgdorferi* sl directly by culture or by PCR from cerebrospinal fluid (CSF) yields a maximum sensitivity of only about 50% [3]. A standard method for diagnosing LNB is determination of the intrathecal specific antibody index (AI), despite the fact that the sensitivity of the AI has been reported to vary from 48% to 92% [4,5].

A peptide of interest for diagnosing LNB has been the immunoreactive peptide C6, a highly conserved peptide among different *B. burgdorferi* sl [6].

C6-peptide is the sixth invariable region of the VlsE protein. The vls locus consists of 15 silent vls cassettes and the gene for the VlsE lipoprotein. By application of unidirectional recombination events, VlsE can display antigenic variation [7]. The C6-peptide has been shown to be an immunodominant peptide [8]. IgG antibodies to C6-peptide have been shown to be detectable as early as 2 weeks post-infection, and antibodies wane over time after treatment [6,9]. The sensitivity and specificity of the C6-peptide ELISA in serum have been reported to be equal, if not superior, to those of two-tier testing in North American patients [10,11]. C6-peptide serology has been shown to have high sensitivity in LNB patients, varying from 67% to 100% [12,13]. The commercially available C6-peptide ELISA has been validated only for serum samples. Data on the performance of the C6-peptide ELISA performed on CSF for the diagnosis of LNB are limited and conflicting [14–16]. The aim of this study was to determine whether a C6-peptide-based ELISA can be used on CSF samples to diagnose early and late LNB patients, using a large cohort of well-defined patients and controls.

Materials and Methods

Selection of clinical specimens and control samples

Patients and controls from the time period between January 2004 and October 2009 were identified retrospectively by use of the laboratory information management system from the Leiden University Medical Centre (Leiden), OLVG Hospital (Amsterdam), the IZORE Centre for Infectious Diseases (Leeuwarden), the Academic Medical Centre Amsterdam (Amsterdam), and the Isala clinic (Zwolle). CSF–serum pairs from 59 LNB patients were included. Criteria for diagnosing LNB patients were four of the following five: (i) detection of *B. burgdorferi* antibodies in serum; (ii) CSF pleocytosis (>5/μL); (iii) absence of other evident cause of meningitis; (iv) evidence of intrathecal production of specific *B. burgdorferi* antibodies; and (v) objective neurological complaints with favourable outcome after treatment [17]. Thirty-six CSF–serum samples were available from Lyme borreliosis (LB) patients who did not have LNB according to the applied algorithm. The LB patient group consisted of 12 recent erythema migrans (EM) patients, 21 Lyme arthritis patients, and three acrodermatitis chronica athrophicans patients. CSF and serum samples were available from 69 patients with other infectious diseases, 62 CSF–serum pairs were collected from patients with neurological inflammatory diseases, and 12 CSF–serum pairs were collected from patients with neurological complaints, including dizziness, headache and fatigue without evident diagnosis, and trauma patients (Table 1). Additional data were collected for all patient groups: age at presentation, sex, duration of illness (>6 months was classified as late LNB), and CSF findings at diagnosis (intrathecal leukocytes and erythrocytes per microlitre, percentage of mononuclear cells, glucose level, total protein, IgG, and albumin). For LNB patients, the clinical presentation, duration of complaints and report of an EM were documented.

C6-peptide ELISA

All sera and CSF samples were tested with the C6 Lyme ELISA Kit (Immunetics, Boston, MA, USA). Preliminary results showed good performance of a 1 : 5 dilution for CSF. Therefore, and for practical reasons, all CSF samples were tested in a 1 : 5 dilution with the manufacturer's protocol for serum. C6-peptide ELISA was performed on sera according to the manufacturer's protocol. The Lyme index (LI) was calculated according to the manufacturer's protocol: absorbance$_{450–650\ nm}$ sample/[(absorbance$_{450–650\ nm}$ calibrator) + 0.3]. Samples with LI values <0.9 were to be considered negative, those with LI values 0.9–1.1 equivocal, and those with LI values ≥1.1 positive for antibodies against C6-peptide in serum.

AI

All sera and CSF samples were tested with the IDEIA Lyme Neuroborreliosis kit, according to the manufacturer's protocol (Oxoid, Ely, UK). The AI was calculated as (optical density (OD)$_{CSF}$/OD$_{serum}$) × (OD$_{CSF}$ − OD$_{serum}$). The CSF was considered to contain IgG or IgM if the OD$_{CSF}$ IgG or IgM was >0.150. The AI was considered to be positive when the CSF was positive and the AI$_{IgG}$ or AI$_{IgM}$ was ≥0.3.

TABLE 1. Epidemiological characteristics of patient groups and baseline cerebrospinal fluid (CSF) leukocyte count (per μL)

	n	Male/female ratio (%)	Mean age (years) (SD)	Mean CSF leukocyte count (per μL of CSF) (SD)
Lyme neuroborreliosis	59	60/40	39 (24)	135 (159)
Lyme borreliosis	36	50/50	51 (17)	1 (1)
Infectious meningitis/encephalitis controls	69			
Treponema pallidum	12	83/17	40 (8)	40 (79)
Cryptococcus neoformans	2	50/50	52 (6)	94 (89)
Bacterial meningitis				
Streptococcus pneumoniae	2	50/50	41 (6)	337 (99)
Listeria monocytogenes	1	0/100	61	1280
Mycobacterium tuberculosis	1	0/100	4	25
Viral meningitis/encephalitis				
HIV	6	50/50	43 (8)	51 (45)
VZV	11	45/55	51 (23)	130 (173)
HSV1	6	33/67	55 (30)	46 (51)
Enterovirus	23	61/39	13 (17)	271 (381)
Parechovirus	3	0/100	0 (0)	1 (1)
TBE	2	50/50	37 (4)	59 (12)
Neurological controls	74			
Facial nerve paralysis eci	19	66/34	48 (18)	40 (145)
Multiple sclerosis	26	35/65	35 (14)	15 (17)
Polyneuritis/polyneuropathy	16	56/44	45 (17)	17 (22)
ADEM	1	0/100	21	266
Neurological non-inflammatory controls	12	25/25	47 (13)	4 (6)

ADEM, acute disseminated encephalomyelitis; HIV, human immunodeficiency virus; HSV1, herpes simplex virus 1; TBE, tick-borne encephalitis; VZV, varicella zoster virus; SD, standard deviation.