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Clinical Observations

Cerebrospinal Fluid Neopterin in Children With Enterovirus-Related Brainstem Encephalitis

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ABSTRACT

Background: Enterovirus-A71 causes outbreaks of brainstem encephalitis, ranging from self-limited disease to acute flaccid paralysis. The aim of this study was to assess the role of cerebrospinal fluid (CSF) neopterin as a biomarker of disease severity in children with enterovirus-related brainstem encephalitis.

Methods: A descriptive, prospective cohort study was conducted from April 2016 to March 2017 in a tertiary hospital. Pediatric patients with a diagnosis of brainstem encephalitis with or without myelitis due to enterovirus infection were enrolled. The final study group comprised a convenience sample including all patients with sufficient CSF volume for neopterin determination. The major variables considered in estimating the severity were the diagnosis of encephalomyelitis, the presence of lesions and extensive lesions on brain and spinal magnetic resonance imaging (MRI), hospital stay length greater than seven days, and sequelae at day 30.

Results: Of 60 patients, CSF neopterin could be measured in 36. Median age was 26 months (interquartile range: 19 to 32). Thirty-three were diagnosed with brainstem encephalitis and three with encephalomyelitis. Enterovirus-A71 was the only identified genotype (25 of 25). CSF neopterin levels were elevated (>61 nmol/L) in 33 of 36 (92%), with a median of 347 nmol/L (interquartile range: 204 to 525). CSF neopterin was useful to distinguish patients with lesions on MRI (area under the receiver operating characteristic curve = 0.76; $P = 0.02$) and extensive lesions (area under the receiver operating characteristic curve = 0.76; $P = 0.04$).

Conclusions: This study suggests an association between CSF neopterin levels and the presence of inflammatory lesions on MRI.

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Conflicts of interest: Nothing to disclose.

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Introduction

An outbreak of brainstem encephalitis (BE) associated with enterovirus-A71 (EV-A71) infection affecting more than 100 children occurred in Catalonia (Spain) in 2016.[1] The clinical spectrum ranged from a mild disease to acute flaccid paralysis and cardiopulmonary failure. Outbreaks of EV-A71 have been associated with significant morbidity and mortality, particularly in the Asia-Pacific region, leading to the coining of the term *the poliomyelitis of the 21st century*.[2] Patient management depends on clinical signs and symptoms in most guidelines.[3] More recently, the prognostic utility of magnetic resonance imaging (MRI) for EV-A71 BE has been reported and its potential to guide treatment could be inferred.[4–6] There is limited literature on biomarkers to assess disease severity.

Neopterin is a marker of cell-mediated immunity, and its levels correlate with the severity of several central nervous system infections but have not been reported in EV-A71 BE.[7–9] Neopterin is formed in monocytes, macrophages, dendritic cells, and endothelial cells enhancing the cytotoxic potential. Neopterin production is induced by interferon-γ through the Jak2/Stat pathway and by tumor necrosis factor through the nuclear factor-κB pathway, released by T lymphocytes and natural killer cells.[10] The objective of this study was to assess the use of cerebrospinal fluid (CSF) neopterin as a biomarker of disease severity in children with EV-related BE.

Methods

Study design

A descriptive, prospective cohort study was conducted from April 2016 to March 2017 in a tertiary pediatric hospital (Hospital Sant Joan de Deu, University of Barcelona).

Inclusion criteria and definitions

Pediatric patients with a diagnosis of BE with or without myelitis (encephalomyelitis [EM]) according to the World Health Organization case definitions[3] due to EV infection were enrolled.[1] The final study group comprised a convenience sample including all patients with sufficient CSF volume for neopterin determination (Supplementary Figure 1).

The major variables considered in estimating the severity were the diagnosis of EM, the presence of lesions and extensive lesions (more than two affected areas) on brain and spinal MRI, hospital stay length more than seven days, and the persistence of neurological symptoms with significant disability (modified Rankin scale $\geq$2) at day 30.[11]

Management

Panenterovirus non-specific real-time polymerase chain reaction was performed in blood and CSF. In case of negative results, nasopharyngeal aspirates and stool samples were also tested while other etiologies were excluded.[1] EV-positive samples were sent to the EV Unit of the National Center for Microbiology for genotype identification.[1]

To measure neopterin, CSF samples were stored at −70°C with protection from light.[12] Neopterin was determined by oxidation by manganese dioxide and analysis by reverse-phase high-performance liquid chromatography.[12] CSF neopterin levels were considered elevated if greater than 61 nmol/L, according to our previously established cutoff value for immune inflammatory conditions.[13] Other inflammatory markers were analyzed for comparative purposes (CSF white blood cell count and protein; blood white blood cell, lymphocyte, and neutrophil counts; and serum C-reactive protein).

Brain and spinal MRI were performed in most of the children, except in those who were recovering before performing the MRI. The images were interpreted by neuroradiologists who were not aware of the laboratory results. The number of central nervous system areas involved was calculated by classifying high-intensity lesions in T2-weighted sequences according to their location in the supratentorial space, brainstem, cerebellum, cervicodorsal spine, and lumbar spine.

Treatment consisted of supportive care and consideration of intravenous immunoglobulin with or without methylprednisolone in severe cases.[1] Lumbar puncture was performed before treatment administration.

Statistics

Dichotomous variables were compared using chi-square test or Fisher's exact test. Mann-Whitney U test was used to compare biomarker levels between groups. Receiver operating characteristic (ROC) curve analysis was used to determine the diagnostic accuracy of CSF neopterin to detect lesions on MRI. The significance level considered was <0.05. Statistical analysis was performed with SPSS v22.0 software (IBM Corp., Armonk, NY, USA).

Ethics statement

The study was approved by the institutional ethics committee. Parents of all participants provided informed consent before patient enrollment.

Results

Description of the cohort

Of 60 patients, eight were excluded because the lumbar puncture was performed in another center and 16 were excluded owing to insufficient CSF volume for neopterin determination (Supplementary Figure 1). Excluded patients were comparable regarding age, sex, and lesions on MRI (data not shown). Thus CSF neopterin levels were measured in 36 patients. Median age was 26 months (interquartile range: 19 to 32), and 21 of 36 (58%) were male. Thirty-three (92%) were diagnosed with BE and three (8%) with EM (Supplementary Table 1). The three patients with EM developed paresis with a marked weakness especially in the neck and shoulder regions (two of them with severe autonomic nervous system dysfunction). No immunodeficiency was detected in those patients.

EV was genotyped in 27 of 36; EV-A71 was the most frequently identified type (25 of 27). In the other two patients, an EV belonging to species A was found, but further identification could not be performed. The group of patients in whom EV-A71 could be genotyped was comparable with the rest of the cohort (Supplementary Table 1).

MRI was abnormal in 18 of 28 (64%) patients (Supplementary Table 2). An analysis of the variables associated with abnormal MRI can be found in Supplementary Table 3. CSF neopterin levels were elevated in 33 of 36 (92%), with a median of 347 nmol/L (interquartile range: 204 to 525).

Variables associated with a more severe disease

Higher CSF neopterin levels correlated with the presence of lesions on MRI (see Table). The area under the receiver operating characteristic curve (AUC) was 0.76 (95% confidence interval: 0.56 to 0.96; $P = 0.027$). The cutoff value of more than 94 nmol/L had the

TABLE.
Inflammatory Markers in Children With Enterovirus-Related Brainstem Encephalitis or Encephalomyelitis

Severity Variables	N	CSF Neopterin, nmol/L*	CSF WBC Count, Cells*10^3/μL*	CSF Protein, mg/dL*	Blood WBC Count, Cells*10^3/μL*	Blood Lymphocyte Count, Cells*10^3/μL*	Blood Neutrophil Count, Cells*10^3/μL*	Serum C-Reactive Protein, mg/L*
Lesions on brain and spine MRI								
Yes	18	371 (213-637)	165 (80-380)	42 (33-52)	12,750 (11,950-16,175)	4000 (2475-4700)	8650 (6625-11,125)	6 (3-12)
No	10	172 (53-371)	153 (617-94)	47 (38-65)	12,050 (8925-15,150)	3450 (2150-5325)	6200 (4575-8625)	3 (1-15)
		$P = 0.027$	$P = 0.943$	$P = 0.270$	$P = 0.172$	$P = 0.792$	$P = 0.084$	$P = 0.487$
Extensive lesions on brain and spinal MRI (>2 areas)								
Yes	7	438 (189-899)	185 (50-220)	44 (35-45)	12,600 (11,600-17,400)	4700 (2600-4700)	8700 (5000-11,000)	6 (2-12)
No	21	286 (99-495)	145 (100-420)	46 (33-61)	12,600 (10,750-15,200)	3200 (2100-4900)	7200 (5650-9550)	5 (2-12)
		$P = 0.048$	$P = 0.671$	$P = 0.426$	$P = 0.633$	$P = 0.264$	$P = 0.614$	$P = 0.750$
Length of hospital stay > 7 d								
Yes	16	341 (204-559)	175 (108-348)	45 (37-47)	12,650 (11,875-15,975)	4500 (2475-5325)	8400 (5775-10,400)	5 (2-9)
No	20	358 (200-480)	103 (43-203)	39 (29-57)	12,100 (9075-13,250)	3800 (2750-5075)	6450 (4575-8250)	3 (2-12)
		$P = 0.679$	$P = 0.108$	$P = 0.671$	$P = 0.108$	$P = 0.762$	$P = 0.161$	$P = 0.962$
NRL symptoms ≥ 30 d†								
Yes	3	438	50	45	17400	4700	9600	6
No	33	334 (174-529)	145 (63-295)	40 (31-55)	12,200 (9550-14,600)	3800 (2450-5350)	7100 (4550-8900)	5 (2-10)
		$P = 0.317$	$P = 0.097$	$P = 0.841$	$P = 0.052$	$P = 0.242$	$P = 0.241$	$P = 0.442$

Abbreviations:
CSF = Cerebrospinal fluid
NRL = Neurological
MRI = Magnetic resonance imaging
WBC = White blood cell
WHO = World Health Organization
Differences were considered significant at $P < 0.05$ and these values are listed in bold type.
* Median (p25 to p75).
† The three patients with clinical diagnosis of encephalomyelitis following the WHO definition were the same three patients with persistence of neurological symptoms with significant disability at day 30.

highest average of sensitivity (100%) and specificity (50%). The positive predictive value was 78%, and the negative predictive value was 100%.

Higher CSF neopterin levels were also observed in children with extensive lesions on MRI (see Table). The area under the receiver operating characteristic curve was 0.76 (95% confidence interval:

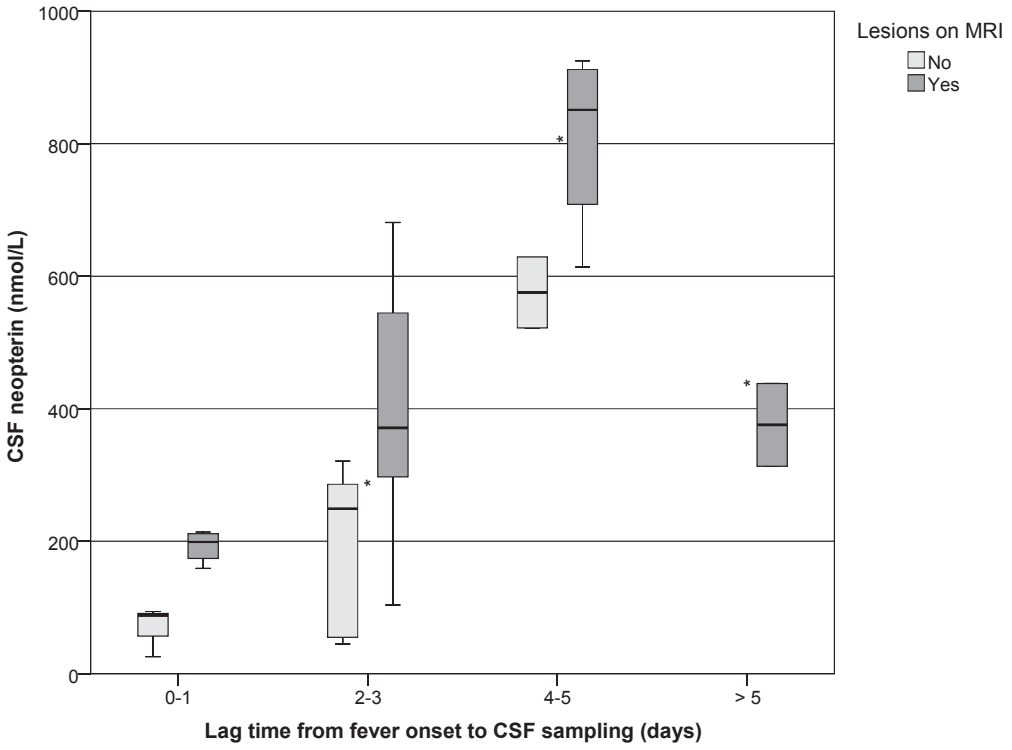

FIGURE. Graphic representation of CSF neopterin (nmol/L) and lag time from fever onset to CSF sampling (days), in patients with or without lesions on MRI. *Patients with encephalomyelitis and persistent neurological symptoms at day 30.

0.55 to 0.96; $P = 0.047$). With a cutoff value of greater than 360 nmol/L, the sensitivity was 71%, the specificity was 70%, the positive predictive value was 46%, and the negative predictive value was 88%.

The three patients with EM had significant disability at day 30 (and persistent lesions on MRI at 12-month follow-up). Median CSF neopterin was higher in this group of patients, but the differences were not statistically significant (Table).

Concerning potential confounding variables, no significant association was found between CSF neopterin levels and sex or age (data not shown). CSF neopterin levels tended to peak on day four to five from fever onset and then tapered off (Fig). The lag time from fever onset to CSF sampling did not correlate with the presence of lesions or extensive lesions on brain and spinal MRI ($P = 0.28$).

No other inflammatory marker was associated with the severity variables (Table).

Discussion

This study reveals that CSF neopterin levels are elevated in children with EV-related BE or EM and are higher in those with MRI lesions. Lower values of CSF neopterin had been previously described in EV meningitis or encephalitis.[7,14]

A correlation between CSF neopterin levels and CSF viral load has been described in several viral infections.[8] In the case of EV-A71 BE, no correlation with CSF viral load is expected, as it is rarely detected in CSF.[1] Several observations support the etiologic role of EV-A71 in BE or EM, including the observation of neuronal degeneration, necrosis, and neuronophagia, together with the identification of viral antigens and ribonucleic acid almost exclusively on neurons in fatal cases with EM.[15] Besides, EV-A71 is known to activate many inflammatory pathways that play a role in limiting viral replication and also have a deleterious effect on the brain.[16] We hypothesize that viral infection induces cell-mediated immunity, activation, and neopterin synthesis, which is a pathway linked to neurovirulence.

This study demonstrated an association between CSF neopterin levels and the presence and extent of MRI lesions; it is a promising finding because according to a recent meta-analysis of outcomes following EV-A71 BE, a normal MRI may be a good prognostic sign (lower cumulative incidence of sequelae or death).[4] Moreover, many authors have reported a statistically significant association between multiple area involvement on MRI and disease severity,[5,6] and hence the presence of lesions on MRI is useful not only for diagnosis but also for prognosis. Because of equipment availability and the need for sedation in children, MRI is not always feasible or is performed days after disease onset.

The main limitations of this study are the small sample size, the potential bias of a single-center study, and the potential confounding effect of lag time from fever onset to CSF sampling, which has been illustrated in Fig. Twenty-four patients were excluded because CSF was unavailable or insufficient, but these patients were comparable regarding epidemiologic and clinical variables. No conclusions regarding the utility of neopterin as a biomarker of neurological sequelae could be drawn as only three patients had a significant disability at day 30. Because CSF neopterin levels are generally performed in reference laboratories, the inability to obtain timely results can limit their clinical utility.

To conclude, this study suggests an association between CSF neopterin levels and the presence of inflammatory lesions on MRI. This finding must be further validated in larger cohorts, which might allow deeper insights into the role of CSF neopterin as a biomarker of disease severity.

Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pediatrneurol.2019.01.024.

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