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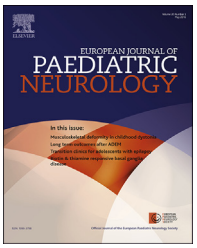

Original Article

Virological testing of cerebrospinal fluid in children aged less than 14 years with a suspected central nervous system infection: A retrospective study on 304 consecutive children from January 2012 to May 2015

Saverio G. Parisi [a,b,*], *Monica Basso* [a,b], *Claudia Del Vecchio* [a,b], *Samantha Andreis* [a], *Elisa Franchin* [a,b], *Federico Dal Bello* [a,b], *Silvana Pagni* [a,b], *Maria Angela Biasolo* [a,b], *Riccardo Manganelli* [a,b], *Luisa Barzon* [a,b], *Giorgio Palù* [a,b]

[a] *Department of Molecular Medicine, University of Padova, Via Gabelli 63, 35100 Padova, Italy*
[b] *Microbiology and Virology Unit, Padova University Hospital, Indirizzo: Via Giustiniani, 2, 35128 Padova, Italy*

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ABSTRACT

Objective: The study aimed to describe the prevalence of HSV DNA, VZV DNA, Enterovirus RNA, Parechovirus RNA, CMV DNA, EBV DNA, adenovirus DNA, HHV-6 DNA, HHV-7 DNA, HHV-8 DNA and Parvovirus B19DNA in children aged less 14 years with a suspected viral infection of the central nervous system in a clinical practice setting.

Methods: Between January 2012 and May 2015, cerebrospinal fluids from 304 children were tested with an in-house real-time PCR method.

Results: A positive PCR was detected in 64 subjects (21%): the mean number of tests performed in patients who showed a viral infection was 7.5, significantly higher ($p = 0.001$) with respect to that reported in negative samples (6.4). Enterovirus is the leading virus detected: 12 out of the 37 positive children reported were newborns (85.7% of all the newborns with a positive result). The second most frequently identified virus was HHV-7 (5 positive PCR out of 105 samples tested, 4.8%, if we excluded a child with a concomitant *S. pneumoniae* isolated), a prevalence significantly higher with respect to VZV ($p = 0.02$) and to CMV ($p = 0.04$). HHV-6 was the third most commonly identified aetiology (4.2%). All children were immunocompetent.

Significance: Only a minority of children had a specific viral aetiology identified: the rate of HHV-7 positivity suggests a routine testing of these viruses within the diagnostic algorithm in immunocompetent paediatric patients. This approach could help to define the clinical role of this herpesvirus.

* *Corresponding author*. Department of Molecular Medicine, University of Padova, Via Gabelli 63, 35100 Padova, Italy. Tel.: +39 0498272344; fax: +39 0498272355.
E-mail address: saverio.parisi@unipd.it (S.G. Parisi).

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1. Introduction

Central nervous system (CNS) infection includes a wide spectrum of illnesses, which are challenging to diagnose and manage due to the variety of aetiologies and to the similar laboratory and clinical findings regardless of the causal agent.[1] Apart from bacteria and fungi, many acute infections are caused by viruses.[2] Kadambari et al.[3] reported a significant increase in the number of meningo-encephalitis cases caused by Enterovirus (EV), Herpes simplex virus (HSV), Varicella zoster virus (VZV), Cytomegalovirus (CMV), Epstein–Barr virus (EBV) and other viruses (mumps and measles, a total of 27 cases out of 9941 laboratory-confirmed diagnoses) in the study period from 2004 to 2013. In 2013, the annual incidence per 100,000 population was 3.90. In the category of children aged less than 15 years, the highest value was reported in infants (328.7), with a progressive reduction parallel to age increase.

In clinical practice, a confirmed or probable viral aetiology is identified only in subgroups of children with suspected CNS infections, and a wide range of percentages is reported in the published studies. Positive results in 65.8% of children aged less than 1 year and 68.7% in patients with ages ranging from 1 to 15 years were described in a Spanish study including only cerebrospinal fluids (CSF) with lymphocytic pleocytosis (>5 cells/mm^3) and obtained within the first week of symptom onset.[4]

Conversely, Kleines et al.[5] reported an overall viral positive polymerase chain reaction (PCR) finding, ranging from less than 4 to about 10% (the lowest in patients aged 1–11 months, and the highest value found in the newborns) in paediatric CSF samples tested for assumed CNS infection with no other selective criteria.

The identification of a viral agent may be optimal for patient management because it can lead to avoidance of unnecessary antibiotic treatment if a bacterial aetiology can be excluded.[6] Furthermore, a widely applied standardised diagnostic algorithm would aid in elucidating the pathophysiological and prognostic role of the detection of viruses associated with severe neurological illnesses in children but not routinely tested. Among these are viruses, such as Human parechoviruses (HPeVs),[7] and Human herpesvirus 7 (HHV-7), for which detection does not necessarily indicate that the identified pathogen is the cause of the patient's disease, regardless of the immunological competence.[8] The addi of HPeV and HHV-6 testing on CSF samples found previously negative for HSV or EV led to the identification of 2.9% and 2.1% positive patients, respectively, with an increase of 14% in diagnostic efficacy.[9] On the other hand, the International Encephalitis Consortium includes only HSV, EV and HPeVs (the latter in children aged less than 3 years) as initial evaluation of a possible viral aetiology in children.

The present study investigated the incidence of HSV, VZV, HHV-6, HHV-7, HHV-8, CMV, EBV, EV, HPeVs, HAdV and parvovirus B19 (PvB19) through molecular techniques in newborns and in children aged less than who had their CSF tested in routine clinical practice for a clinically suspected CNS infection.

2. Material and methods

2.1. Study design

We conducted a retrospective study on the prevalence of detection of herpesviruses (HSV, VZV, HHV-6, HHV-7, HHV-8, CMV, EBV), EV, HPeVs, HAdV and PvB19 identified by molecular testing performed on CSF samples of children aged less than 14 years and with a suspected CNS infection. These 11 viruses were frequently included in the CSF analysis requests. A total of 101 tests, all negative, were performed to detect WNV, TBEV, measles virus, rubella virus and TOSV. They were not discussed in the work.

The Microbiology and Virology Unit of the University of Padova is a tertiary referral centre for testing, and the microbiological investigations requested were included in the routine diagnostic approach to the disease. They were at the total discre of the treating physician, both which viruses to test and total number of tests requested.

All samples selected for EV and/or HSV testing from January 1, 2012 to May 31, 2015 were included; the CSF samples were submitted from 14 hospitals of the Veneto region. In many cases, the results of Gram staining and CSF bacterial culture were not available because they were performed locally. The study was approved by the Ethical Committee for Clinical Experimentation, Padua Province (Number 50443/15). All the data analysed were part of the routine patient management, and they were anonymised prior to research use. When a patient had more than one sample available, only the first one was included in the statistical analysis, regardless of the time interval between the first and the subsequent samples.

A negligible number of tests (16 out of 2036 performed on the only or first sample, 0.8%, involving 6 patients) were requested but not performed because no sample was available. These tests were excluded from the analysis (Supplementary Table 1).

2.2. Laboratory methods

The CSF samples were stored at 4 °C and processed within 24 h of arrival. All the tests to diagnose a viral infection were performed using the protocols currently applied at the Microbiology and Virology Unit of the Padova Hospital and based on approved Clinical and Laboratory Standards Institute guidelines.[10–12]

Total nucleic acids were purified from 1 mL CSF using the NucliSENS® easyMAG® system (bioMérieux SA, Marcy l'Etoile, France). A quantitative real-time PCR detection of viral nucleic acids was performed on an ABI PRISM® 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using previously reported oligonucleotide primers (Sigma–Aldrich, St. Louis, MO, USA) and TaqMan probes® (Applied Biosystems).[13–18] Sample adequacy was tested by real-time PCR amplification of the β-globin (BGL) gene when DNA viruses were tested, and by real-time PCR amplification of a sequence of the RNase P gene in cases of RNA viruses.

During all DNA extractions and purifications, precautions were taken to reduce the risk of false-positive results. EBV-

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