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Clinical application of viral cerebrospinal fluid PCR testing for diagnosis of central nervous system disorders: a retrospective 11-year experience ☆

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ABSTRACT

The cerebrospinal fluid (CSF) polymerase chain reaction (PCR) is the gold standard to detect cerebral viral activity. As positive findings do not prove an impact on the neurological disorder, data interpretation is difficult. To better assess the impact of positive CSF PCR findings in different neurological diseases and to identify coherences facilitating CSF PCR data interpretation, we performed this retrospective analysis of CSF PCR data of 481 pediatric and 2604 adult patients, including herpes simplex virus (HSV), varicella zoster virus (VZV), Epstein–Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), and enteroviruses (EV). Nucleic acid of EBV was detected in 1.6% (39/2449), of VZV in 1.3% (34/2624), of HSV in 1.24% (37/2994), of EV in 0.4% (10/2364), of HHV-6 in 0.17% (4/2417), and of CMV in 0.2% (5/2514) of the patients. Newborns and elderly people showed highest infection rates. HSV, VZV, and EV prevailed in typical infectious central nervous system (CNS) diseases; EBV, in further inflammatory neurological diseases; HSV and EBV, in immunocompromised patients; and EBV, HSV, and HHV-6, in further non-inflammatory neurological diseases. Analysis of successive PCR studies revealed delayed viral detection for EBV (6/147) and HSV (1/217), respectively. Rapid viral clearance was typical for HSV, VZV, CMV, and EV infections, although the maximum duration of viral detection was 15 days for HSV and 12 days for VZV, respectively. This suggests that the detection of HSV, VZV, CMV, and EV strongly indicates symptomatic viral CNS disease. Secondary viral reactivation mostly underlies positive EBV and HHV-6 findings. Their detection does not rule out clinical impact but recommends searching for additional underlying conditions.

1. Introduction

The polymerase chain reaction (PCR) is the most important method to detect viral infections of the central nervous system (CNS) (DeBiasi et al., 2002; Häusler et al., 2003). Its widespread availability has markedly improved virological routine diagnostics but also generates new problems related to data interpretation. Whereas detection of viral DNA or RNA indicates viral activity, this viral activity does not necessarily cause the current symptoms. All herpes viruses, for example, persist lifelong following primary infection. During persistence, they are either in the state of latency or in the state of reactivation (Goodrum et al., 2012; Thorley-Lawson et al., 2013). Even in clinical CNS disorders typically associated with a distinct viral infection, the detection of viral activity does not exclude an alternative etiology, which may have secondarily reactivated the detected virus. Interpretation of positive cerebrospinal fluid (CSF) PCR findings is even more difficult in patients suffering from a CNS disease not typically associated with viral infection (Kleines et al., 2011; Sunden et al., 2011). Among these patients, the Epstein–Barr virus (EBV) seems to be the predominant virus, although herpes simplex viruses (HSV), varicella zoster viruses (VZV), cytomegaloviruses (CMV), and enteroviruses (EV) have also been detected at low frequencies. This raises the question how frequent viral activity in different clinical conditions might be, whether this viral activity really contributes to these diseases, whether it acts as a disease modifier, or whether there is no clinical impact at all. In the literature, a large number of case reports describe the detection of various viruses in many different clinical conditions (Carron et al., 2004; Sas et al., 2009; Vucic et al., 2005). Studies, in contrast, addressing the most important neurotropic viruses in a large cohort of patients with a divergent spectrum of clinical conditions are rare, are frequently restricted to few clinical

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diagnoses, or do not distinguish between different neurological conditions (Benjamin et al., 2013; Boaretti et al., 2008; Child et al., 2012; Franzen-Rohl et al., 2008; Gaeta et al., 2009; Hosseininasab et al., 2011; Hukkanen and Vuorinen, 2002; Kanerva et al., 2008; Vidal et al., 2011; Wada et al., 2009).

To better assess the extent of viral activity in different CNS diseases and to identify coherences facilitating data interpretation, we performed this retrospective analysis on CSF PCR data obtained during an 11-year period, focusing on HSV, VZV, EBV, CMV, human herpesvirus 6 (HHV-6), and EV as well as on viral kinetics.

2. Patients and methods

2.1. Patients

Data obtained from 16,496 PCR investigations on 513 pediatric and 2904 adult patient samples, which were derived from 3085 in-house patients (481 children, age 2 days–17 years; 2604 adults, age 18–97 years), were available for retrospective analysis. These samples represent all CSF samples that had been sent to the virology lab due to assumed CNS infection between the years 2001 and 2012. Successive CSF analyses had been performed in 280 patients.

PCR data of patients with positive PCR results were associated with the respective International Statistical Classification of Diseases and Related Health Problems-related diagnoses finally attributed to the patient by the clinician at discharge from the hospital. Based on these diagnoses, the patients were assigned to 4 major clinical groups: patients with symptoms typical for CNS infections, patients with further inflammatory CNS diseases, patients with impaired immunocompetence, and patients with further neurological diseases (Table 2). Patients for whom successive specimens were available were counted only once in the subsequent analyses. Stratification of patients according to virus and age is displayed in Table 1.

2.2. Laboratory methods

Quantitative PCRs were performed using in-house real-time PCR protocols on the Lightcycler instrument 1.2 (Mannheim, Germany) as published previously (Häusler et al., 2002; Häusler et al., 2003; Schaade et al., 2000). The lower detection limits were 500 geq/mL (EBV), 200 geq/mL (CMV), 500 geq/mL (HHV-6), 1000 geq/mL (HSV1/2), 250 geq/mL (VZV), and 500 geq/mL (EV), respectively. The lower limits of detection were established by testing of a dilution series of standard material from interlaboratory tests in 10 parallel assays. Interlaboratory test material was provided by INSTAND e.V., a non-profit organization providing interlaboratory tests, or by German national virological reference laboratories. Details of the CMV assay used in this study have been published previously (Schaade et al., 2000). Determination of CSF protein concentrations and cell counts was done by the clinical chemistry laboratory of the University Hospital Aachen using standard procedures.

Table 1
Stratification of enrolled patients according to investigated virus and age.

Age	CMV	EV	EBV	HHV-6	HSV	VZV
Newborn	25	16	16	16	39	20
1–11 months	35	25	29	24	62	28
1–5 years	48	42	52	64	76	57
6–17 years	221	211	219	209	276	232
18–40 years	628	608	616	624	705	656
41–60 years	729	697	717	697	838	762
61–80 years	718	789	708	694	868	768
>80 years	110	87	92	89	130	101

2.3. Statistical methods

Because of the data structure, a descriptive approach for data analysis was chosen. For comparison of the impact of detection of neurotropic viruses for a certain age group, cumulative rates of detection were determined by summing up of the individual rates of detection for each virus in the respective age group (Fig. 1).

3. Results

3.1. Prevalence of positive viral CSF PCR findings relative to the age of the patients

Including patients positive for 1 distinct virus (n = 121) and patients positive for several viruses (n = 4), viral nucleic acid was detected in 125 of the 3085 patients. Hereby, EBV DNA was detected in 39/2449 (1.6%); VZV-DNA, in 34/2624 (1.3%); HSV1/2 DNA, in 37/2994 (1.24%); EV RNA, in 10/2364 (0.4%); HHV-6 DNA, in 4/2417 (0.17%); and CMV DNA, in 5/2514 patients (0.2%), respectively. Patients tested repeatedly positive in successive investigations were counted only once.

Highest cumulative detection rates were seen among newborns and above 80 years of age (Fig. 1). In adults, the prevalence tends to increase by age.

Whereas positive EBV CSF PCR findings were detected from early infancy with maximum prevalence in young children, positive findings for HSV1/2 and HHV-6 were recorded from school age only. EV were detected in 1 newborn and in the middle aged. CMV was detected in 1 newborn and in patients of 41–80 years of age, respectively. Positive VZV CSF PCR findings were recorded in children from 1 to 5 years of age and in adults with an increase by age.

3.2. Cerebral viral activity in distinct clinical diseases

EV was the only entity exclusively associated with clinical manifestations typically linked to infections (Table 2). HSV1/2 and VZV were predominantly associated with neurological diseases typically associated with infectious agents, whereas EBV and CMV were predominantly detected in other patient groups (Table 2). The spectrum of infections among the different clinical groups was similar in children and adults.

We evaluated the association of positive CSF PCR findings with different clinical presentations covered by each of these 4 major patient groups, again focusing on patients with positive PCR results for only 1 distinct virus.

Among PCR-positive patients with clinical diseases typically linked to CNS infections, HSV1/2 was the major cause of encephalitis (Table 3). The predominant manifestation of VZV infection was the combination of a neurological deficit with zoster. EV and HSV1/2 were the most frequent causes of aseptic meningitis, followed by VZV, EBV, and HHV-6. EBV, VZV, and HSV1/2 were also detected among patients with vestibular neuritis. The only patient with EV encephalitis was a neonate showing epileptic seizures as first encephalitic symptom.

EBV-DNA was found in 35 of the 121 patients (29%) showing a positive CSF PCR result for 1 distinct virus. However, only 7 of the 70 PCR-positive patients (10%) with clinical conditions typical for infectious diseases were tested positive for EBV. In contrast, HSV1/2-DNA, VZV-DNA, and EV-RNA were found in 37 (31%), 33 (27%), and 9 (7%) of 121 patients with positive PCR findings and in 27 (39%), 25 (36%), and 9 (13%) of the 70 PCR-positive patients with typical clinical conditions, respectively.

Among PCR-positive patients with further inflammatory CNS disorders, only EBV, HSV1/2, and VZV were detected (Table 4). Here, EBV dominated with 12 of 15 viral detections.

PCR-positive patients with underlying immunodeficiency conditions showed positive CSF PCR findings for CMV, EBV, HSV1/2, VZV, and HHV-6, respectively. EBV was predominant among patients with HIV infection, whereas HSV1/2 prevailed in patients with neoplasms (Table 5).

EBV was also dominant among PCR-positive patients with neurological disorders not typically linked to inflammatory processes (Table 6). Among these patients, brain infarction was the most frequent clinical presentation.

3.3. Patients tested positive for multiple viruses

Four patients showed positive CSF PCR findings for more than 1 virus. All of them proved positive for EBV and 1 additional viral agent (Table 7). Only 2 of them showed impaired immunocompetence, which was due to HIV infection.

3.4. CSF PCR findings in case of repeated CSF PCR studies

In clinical settings, repeated CSF PCR testing can be suggested if a viral CNS disorder is assumed despite of a negative primary PCR result or to prove the treatment response.

Repeated testing was done in 280 of 3085 patients (9.1%). Sixty of these 280 patients (21.4%) showed at least 1 positive CSF PCR result. Commonly, a positive result