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Intrathecal synthesis of specific antibodies as a marker of herpes simplex encephalitis in patients with negative PCR

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Summary

BACKGROUND: PCR in the cerebrospinal fluid (CSF) has become the sole method used for the diagnosis of herpes simplex encephalitis (HSE). Nevertheless, PCR results may sometimes be false negative, and in this situation other techniques may be useful.

METHODS: 3 patients hospitalised for meningoencephalitis with fever showed a negative result for herpes simplex virus (HSV) PCR in their CSF. We then performed a detection of intrathecal anti-HSV immunoglobulins (IgGs) in the CSF and analysed their level in relation to those in the serum, compared to albumin.

RESULTS: We confirmed that IgG synthesis was the direct consequence of an immune system reaction in the 3 patients' CSF. These results were consistent with clinical signs and neurodiagnostic procedures. They prompted us to continue the treatment, which would have been stopped following the negative PCR results. The clinical progression was favourable for all patients.

CONCLUSIONS: PCR, which many physicians now consider the gold standard for the detection of HSV, may sometimes yield false negative results, i.e. when performed too early after the disease onset or when the viral load is too low. The method described here, although positive a few days after PCR, may prove helpful in the diagnosis of HSE for patients with negative HSV PCR in the CSF.

Key words: *encephalitis; herpes simplex virus; immunoglobulin; intrathecal; PCR; Tibbling-Link*

Introduction

PCR performed on the cerebrospinal fluid (CSF) has become the gold standard for the diagnosis of viral infections in the central nervous system (CNS). Herpes simplex virus (HSV) encephalitis (HSE) is a life-threatening disease with high mortality (approx. 70%) if not treated [1]. With a 3-week course of aciclovir the mortality decreases and is as low as 5% in the latest studies [2]. However, morbidity persists, with a wide range of behavioural changes [1, 3, 4]. In some cases, even if the clinical signs strongly suggest HSE, the PCR remains negative. In these cases, some technologies used before the era of PCR, such as the detection of intrathecal synthesis (IS) of specific immunoglobulins (Igs), can be performed [5–7]. Even if this technique is useful it seems to have been forgotten with the development of molecular biology. We briefly report 3 cases for which the diagnosis of HSE was established using Ig measurement rather than PCR.

Case reports

Three patients were hospitalised for neurological symptoms and fever, suggesting encephalitis and particularly HSE. Patient characteristics are summarised in table 1. After DNA extraction from CSF by the "QIAamp DNA Blood mini kit" (Qiagen, Germany), HSV PCR was performed using the Cepheid kit on Smartcycler (Cepheid, California, USA) and following the manufacturer recommendations. This is the protocol routinely used in our laboratory. Although HSV PCRs were negative, acyclovir (15 mg/kg tid intravenously) was prescribed for 3 weeks because the clinical signs, electroencephalogram and magnetic resonance imaging strongly suggested HSE and other aetiologies (bacteria, other viruses, etc) were ruled out. Nevertheless, this prescription was called into question and we searched for intrathecal synthesis of specific anti-HSV IgG in the CSF. For this method we measured, for each patient, albumin by immunonephelometry (Dade Behring BNII) and anti-HSV specific IgG using the quantitative α-

method Enzygnost Anti-HSV IgG (Behring, Germany) in serum and CSF sampled on the same day. The IS of specific antibodies was then evidenced by the use of Tibbling-Link calculation (table 2). With this proof of specific IS of IgG against HSV, antiviral treatment was maintained and the patients' condition improved. All patients recovered from neurological signs.

Discussion

Several studies have already reported the use of this technique for the diagnosis of HSE either when PCR was not in use [8–14] or to confirm PCR diagnosis in the initial stages of this technique [15–18]. For example, in the study by Fomsgaard et al. the diagnosis was established in 9 patients only by the detection of intrathecal antibodies, while PCR was negative. For another patient both PCR and intrathecal antibodies were positive [17]. Since the era of PCR the use of intrathecal antibodies seems to have been forgotten and we did not find relevant reports in the literature.

HSV-1 is the most frequent viral agent of encephalitis in our country [2]. Biological HSE diagnosis nowadays relies on PCR, which has become the gold standard [19, 20], and after the antiviral treatment has begun its management is based on virological results obtained with PCR. The sensitivity and specificity of PCR in this infection had been studied in comparison with brain biopsy. The results were 98% and 94% respectively in the study by Lakeman et al. [20]. Thus, after a negative result, clinicians usually stop acyclovir due to inadequate knowledge of the technique's limits.

However, for a number of reasons PCR may be negative even in patients with HSE. For example, the too short time lapse (≤ 4 days) between disease onset, which is associated with viral multiplication, and the PCR on CSF, can produce a false negative result [14, 17, 21–24]. For this reason the recommendation is to check a negative PCR 4 days later, before declaring the patient to be not suffering from HSE. Another reason could be a low viral level in the CSF, since in fact the major viral replication occurs in the brain and only a few viral particles are released in the CSF where they can be detected. A relationship between the normal neurodiagnostic studies and the percentage of negative PCR results has been demonstrated, suggesting that milder disease was less likely to be diagnosed by PCR [25]. The sensitivity of the assay used is also of concern and should be taken into account in analysing the results, since a low level of replication, according to the assay used, can result in a signal below the cut-off and may then yield a false negative result. The cut-off of various assays is approx. 10 copies [26]. In previous studies up to 25% of patients were not detected due to low replication [22, 27]. On the other hand, Sauerbrei suggested that detection of IgM in the CSF, even at a low level, may lead to neutralisation of HSV by these IgM, resulting in a negative PCR [18]. For all these reasons alternative techniques, such as detection of specific IgG IS, may be of help in the diagnosis of HSE.

Detection of IgG IS and development of formulas for its quantitative analysis were first achieved in multiple sclerosis. Afterwards, formulas were adapted to infectious agents (HSV, *Toxoplasma, Borrelia*), using the same principle. Prior to the PCR era it was one of the principal assays for HSE diagnosis [16, 20]. While PCR is positive early in the disease (from day 4), IgG secretion relies on the immune system response, and assays usually become positive 10 days after the onset of disease and then remain positive

Table 1
Patient characteristics.

	Patient 1	Patient 2	Patient 3
Sex, age	M, 54	F, 67	F, 31
Temperature	38.8°C	38°C	38.5°C
Neurological signs	Confusion Coma Seizures Personality change	Confusion Time and place Disorientation Speech disorder	Confusion Seizures Memory loss
Delay between first signs and hospitalisation and first Lumbar puncture	Less than 2 days	Less than 2 days	Less than 2 days but probably symptoms for about 10 days
First CSF	White blood cells: 89/mm3 (lymphocytes: 32%) Protein: 0.53 g/L Glucose: normal	White blood cells: 610/mm3 (lymphocytes: 96%) Protein: 1.24 g/L Glucose: normal	White blood cells: 190/mm3 (lymphocytes: 87%) Protein: 0.98 g/L Glucose: normal
EEG recording	Focal periodic discharges in right temporal area (day 1)	Periodic lateralised epileptiform discharges in left temporal area (Day 3)	Periodic lateralised epileptiform discharges in left temporal area (Day 1)
MRI	Signs of encephalitis (day 20)	Signs of encephalitis (Day 3)	Normal
PCR for HSV	Negative: day 1, 20	Negative: day 5, 10, 15, 22	Negative: day 1, 3, 7, 17
Specific Intrathecal synthesis	Positive on day 21 after any clinical signs onset.	Positive on day 10 after any clinical signs onset.	Positive on day 17 after any clinical signs onset.
Tibbling-Link index	1.14	8.65	1.40

Table 2
Results of IgG and albumin dosages for our patients.

		Patient 1	Patient 2	Patient 3
CSF	IgG	44,000 U	840,000 U	56,000 U
	Albumin	540 mg/l	776 mg/l	579 mg/l
Serum	IgG	2,541,000 U	3,927,000 U	2,541,000 U
	Albumin	35.7 g/l	31.4 g/l	36.9 g/l

in the CSF for a long period [17, 18, 28]. This explains why the sensitivity of this method is time-dependent. Indeed, Fomsgaard et al. found that, while the specificity was the same in early and late samples, sensitivity increased to 90.5% if the sample was collected more than 1 week after the onset of symptoms [17].

The formula used in this study to compare levels of anti-HSV IgGs in the CSF and in serum sampled at the same time was that developed by Tibbling and Link [29]. This formula takes into account the level of specific IgGs and albumin in the CSF and comparatively in serum, to verify that the Ig measured are only of intrathecal origin. If the index obtained with the following formula: IgG index = (IgG_{CSF} / $Albumin_{CSF}$) / (IgG_{serum}/ $Albumin_{serum}$) is over 0.7, it means that synthesis of specific IgG is intrathecal. If the result is less than 0.7, the IgGs measured in the CSF crossed from the blood.

Since IS persists for a long time this calculation may be of help in diagnosing HSE, even with a little delay, and particularly when the PCR is negative. And for patients with a highly suspect HSE and a negative PCR it is an argument for continuing the treatment. Incidentally, the combination of methods (PCR and specific IgG IS detection) is used in the algorithm proposed for the diagnosis and management of patients with suspected herpes simplex encephalitis [19, 30]. However, this technique cannot be used at the onset of the disease, because there is no secretion of Ig during the initial period. For our patients it was done on day 10, 17 and 21 respectively because this analysis is not routinely performed and is thus forgotten by clinicians. For the diagnosis of HSE this technique should be performed around day 10, after 2 negative PCR carried out on day 1 and 4 [30].

Conclusion

Despite its high sensitivity, PCR may yield false negative results. Methods such as the detection of specific intrathecally secreted immunoglobulins may be of help in establishing the diagnosis. The combination of the two techniques allows better diagnosis management with a wider time span coverage. Several authors such as Cinque, Fomsgaard or Solomon have already suggested this practice [17, 19, 30]. Even if a slight delay is necessary it seems reasonable to wait for such a result, if clinical and neurodiagnostic studies suggest HSE, before stopping an effective and well tolerated treatment.

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