

# Diagnostic significance of intrathecally produced herpes simplex and varicella-zoster virus-specific antibodies in central nervous system infections

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## Summary

**Background and objectives:** The optimal strategy for the diagnosis of herpes simplex virus (HSV) and varicella-zoster virus (VZV) disease of the central nervous system is the detection of viral DNA by polymerase chain reaction assay (PCR) in cerebrospinal fluid (CSF) and the examination of intrathecal production of specific antibodies. However, in acute neurological disease caused by either HSV or VZV, dual intrathecal synthesis of HSV-1, 2- as well as VZV-specific antibodies may be detectable and thus can hamper accurate aetiological diagnosis. This paper illustrates such equivocal findings in two case reports, investigates their frequency and discusses the possible reasons.

**Methods:** Consecutive CSF/serum pairs of two patients with central nervous system (CNS) disease were tested by HSV-1-, HSV-2-, and VZV-specific PCR and by different serological assays for detection of neurotropic viruses and bacteria. Additionally, the results of microbiological investigations of 1'155 CSF/serum samples were retrospectively analyzed for coincident intrathecal antibody synthesis against HSV-1, 2 and VZV.

**Results:** Although only HSV-1 and VZV-specific DNA was detectable in the CSF of two patients with encephalitis and chronic meningitis, respectively, increasing intrathecal antibody production against both virus species could be demonstrated. Retrospective analysis of 1155 CSF/serum pairs revealed 55 (4.8%) pairs with evidence for intrathecally produced antibodies against either HSV-1, 2 (30/55) or VZV (14/55). Eleven of these 55 (20%) pairs showed intrathecal antibody-production against both virus species.

**Conclusions:** Patients with CNS infection with HSV and VZV can be diagnosed by detecting intrathecally produced virus-specific antibodies, in addition to virus-specific PCR. However, in an appreciable proportion of patients a correct diagnosis is hampered by coincidentally detected antibodies in CSF against both virus species. Possible reasons for these equivocal findings are given.

**Key words:** herpes simplex virus; varicella-zoster virus; central nervous system viral diseases; encephalitis; mixed infections

## Introduction

The diagnosis of primary or recurrent HSV and VZV infection with neurological disease depends mainly on laboratory investigation [1-7]. In patients with negative results for HSV-1, 2- or VZV-specific PCR, diagnosis can be established by demonstration of intrathecally produced virus-specific antibodies [1, 8-10]. However, both VZV- and HSV-1, 2-specific antibodies may be detectable in CSF and thus hamper correct diagnosis [1].

Two cases of CNS infections with VZV and HSV-1, respectively, are presented with intrathecally produced antibodies against both HSV-1, 2 and VZV in each patient. To estimate the frequency of such findings, we retrospectively looked for coincidental detection of intrathecally produced HSV-1, 2- and VZV-antibodies in CSF/serum samples sent to our institute for microbiological investigation over the past 6.5 years.

## Patients and methods

### Patients and specimens

Both patients were treated at the Department of Neurology, Kantonsspital St. Gallen.

Between 1996 and 2002 a total of 1155 CSF/serum pairs was submitted for investigation of intrathecally produced HSV-1, 2-Ig and VZV-specific IgG, and partly for diagnosis of CNS-infection with mumps virus, tick-borne encephalitis virus (TBE-V), measles virus, *Borrelia burgdorferi*, and *Treponema pallidum*.

### Detection of intrathecal antibody synthesis

HSV-1, 2- and VZV-specific antibodies were determined by EIA (Cobas Core Anti-HSV-1, 2 Ig, Roche Diagnostics; Enzygnost Anti-VZV/IgG, Dade Behring). After adjustment of patient's serum and CSF to the same total IgG concentration, HSV-1, 2- and VZV-specific antibody indices were calculated by means of optical density (OD) using the formula antibody index =  $OD_{CSF} / OD_{serum}$  without further correction [1]. An intrathecal antibody synthesis was assumed with an antibody index of  $\geq 2.0$ . This method was also applied to detect intrathecally produced antibodies directed against measles-, mumps-, cytomegalo-, and TBE-V.

Detection of HSV-1, 2- and VZV-specific oligoclonal IgG in CSF were carried out by antigen-mediated capillary blot technique, performed after isoelectric focusing of serum and CSF (IEF-AMI) [1, 11]. Samples were adjusted to the same total IgG level, separated by isoelectric focusing and blotted onto nylon membranes, coated

with HSV-1- or VZV- antigen (lysate of HSV-1- or VZV-infected cells, respectively) or control antigen (cell-lysate), kindly provided by Dade Behring. IgG bands were subsequently stained by enzyme immuno-assay. The presence of virus-specific oligoclonal IgG was assessed by comparing the reactivity pattern in CSF and serum [12]. Data of IEF with control antigen are not shown.

### Specificity of HSV-1, 2- and VZV-oligoclonal IgG

Since CSF and serum samples may contain HSV-1, 2-specific IgG that crossreacts to VZV antigen and vice versa [13], human sera containing either HSV-1, 2-specific Ig or VZV-specific IgG, were analysed by IEF-AMI. Binding of sera only occurred with the homologous antigen (data not shown).

Absorption tests were carried out with CSF/serum pairs of patient 1. Aliquots of undiluted CSF and serum adjusted to the same IgG concentration were absorbed with various dilutions of either HSV-1- or VZV-antigen, followed by high-speed centrifugation and analysis of supernates by IEF-AMI.

### VZV, HSV-1 and HSV-2 PCR

A PCR based on published primers and probe [14] was used for the qualitative detection of VZV-DNA in CSF. The presence of HSV-1 and HSV-2 DNA was investigated in CSF according to a previously published PCR protocol [15].

## Results

### Case reports

A 47-year old woman presented with chronic headache, progressing over a period of more than six months. She was fully orientated and febrile (38.8 °C). Cranial CT, EEG and MRI were not diagnostic. Analysis of CSF showed a pleocytosis and an elevated total protein content. Bacteriological investigations, serological assays for *Borrelia burgdorferi*, HIV-1, 2 and CSF/serum antibody indices specific for mumps virus, measles virus,

TBE-V were negative. She was diagnosed as having chronic meningitis and therapy was started with ceftriaxone and acyclovir. In CSF taken on day 4 of her hospital stay, VZV-DNA could be detected by PCR, with simultaneously positive IEF-AMI for both VZV- and HSV-1, 2-specific IgG (table 1, figure 1). She was discharged in good health after 3.5 weeks.

Absorption tests with HSV-1-antigen led to a higher extinction of HSV-1, 2-specific bands than

**Table 1**  
Laboratory results of patient 1 and 2.

Patient 1 hospital day	CSF		PCR			CSF / Se			
	cells (µl) <sup>1</sup> / protein (g/l) <sup>2</sup>	PCR			antibody index <sup>3</sup>		IEF-AMI		
		HSV-1	HSV-2	VZV	HSV-1, 2	VZV	HSV-1, 2	VZV	
1	299 / 0.96	-	-	-	-	-	-	-	
4	267 / 1.34	neg	neg	pos	neg	neg	pos	pos	
10	136 / 0.74	neg	neg	neg	6.3	neg	pos	pos	
83	- / -	neg	neg	neg	9.2	8.8	pos	pos	

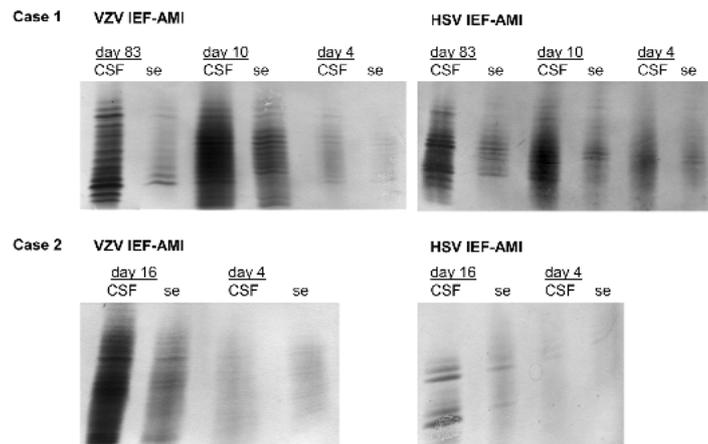
  

Patient 2 hospital day	CSF		PCR			CSF / Se			
	cells (µl) <sup>1</sup> / protein (g/l) <sup>2</sup>	PCR			antibody index <sup>3</sup>		IEF-AMI		
		HSV-1	HSV-2	VZV	HSV-1, 2	VZV	HSV-1, 2	VZV	
4	181 / 0.89	pos	neg	neg	neg	neg	pos	pos	
16	81 / 1.04	neg	neg	neg	6.3	2.3	pos	pos	

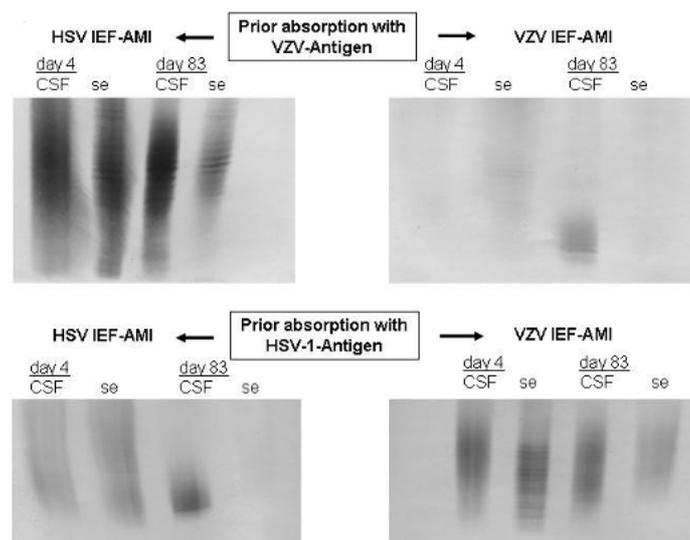
CSF = cerebrospinal fluid; IEF-AMI = isoelectric focusing and antigen-mediated capillary blot technique, 50 ng of total IgG of each CSF and serum sample was applied to agarose gels (normal: cf. text); neg = negative result; pos = positive result; Se = serum; - = not tested; <sup>1</sup> normal:  $\leq 3$  cells/µl; <sup>2</sup> normal: 0.15-0.45 g/l; <sup>3</sup> normal:  $< 2.0$

**Figure 1**

Patient 1 and 2. Results of isoelectric focusing of cerebrospinal fluid (CSF) / serum (se)-pairs, adjusted to the same total IgG level with subsequent antigen-mediated immunoblot (IEF-AMI) on to nylon membranes coated with HSV-1- or VZV- antigen; day = of patient's hospital stay.


**Figure 2**

Patient 1. Results of cross absorption tests of cerebrospinal fluid (CSF) / serum (se)-pairs prior to isoelectric focusing of cerebrospinal fluid (CSF) and serum, adjusted to the same total IgG level with subsequent antigen-mediated immunoblot (IEF-AMI) on to nylon membranes coated with HSV-1- or VZV-antigen; day = of patient's hospital stay.


**Table 2**

Laboratory results of eleven patients with coincidental intrathecal production of HSV and VZV specific antibodies.

Patient	Sex	Age	CSF / Se-antibody index <sub>1</sub>		CSF / Se-IEF-AMI		CSF PCR		Clinical diagnosis
			HSV	VZV	HSV	VZV	HSV	VZV	
1	f	21	2.0	5.0	–	–	–	–	Multiple sclerosis
2	f	79	3.0	3.2	pos	pos	–	–	Meningoradiculopathy of unclear origin
3	m	80	3.1	2.8	–	–	–	–	Monoradiculopathy in VZV infection, vascular dementia
4	f	32	2.8	3.2	pos	–	neg	–	Epileptic seizure after HSV encephalitis
5	m	54	3.5	5.1	pos	–	–	–	Polyradiculopathy of unclear origin
6	m	19	2.4	2.0	pos	–	–	–	Lymphocytic meningitis
7	f	74	2.3	12.1	pos	–	–	–	Radiculopathy on the right side in lumbar spinal stenosis
8	f	34	4.3	6.9	–	–	–	–	HIV-1-encephalopathy, cocaine abuse
9	f	73	2.2	2.6	pos	pos	neg	pos	VZV infection complicated by meningoencephalomyelitis

10–11 are represented by the two case reports

CSF = cerebrospinal fluid; IEF-AMI = isoelectric focusing and antigen-mediated capillary blot technique (normal: cf. text); neg = negative result; pos = positive result; Se = serum; – = not tested; <sup>1</sup>normal: <2.0

of VZV-specific IgG bands. By contrast, after absorption with VZV-Antigen the IEF-AMI subsequently displayed more intense and differently localized HSV-1, 2-specific IgG bands (figure 2).

The second patient, a 68-year old man developed fever with vomiting, and three days later became lethargic with anomia. Cranial CT showed slight cortical oedema, with signs of viral meningo-encephalitis in MRI on the following day. EEG revealed bitemporal slowing and periodic complexes on day 12. Analysis of CSF showed predominantly mononuclear pleocytosis and elevated total protein content. As HSV-1-specific DNA could be detected by PCR, he was diagnosed as having acute encephalitis and therapy was started with acyclovir. Serology revealed positive results by HSV-1, 2- and VZV-specific IEF-AMI, before antibody indices became positive (table 1, figure 1). Negative antibody indices were calculated for TBE-V, measles virus, mumps virus, and *Treponema pallidum*. He was transferred to a rehabilitation centre.

### Retrospective data analysis of CSF/serum pairs

Fifty five (4.8%) out of 1155 CSF/serum pairs revealed intrathecal production of herpesvirus-specific antibodies with positive antibody indices for either HSV-1, 2 (n = 30) or VZV (n = 14). Eleven of these 55 (20.0%) patients showed intrathecal production of HSV-1, 2- as well as VZV-specific antibodies. Among these eleven patients antibody indices calculated for mumps virus, measles virus, and TBE-V were negative. CSF-samples of six patients investigated by IEF-AMI contained VZV- or HSV-1, 2-specific oligoclonal IgG or both (table 2).

## Discussion

In the two cases presented here, intrathecal synthesis of both HSV-1, 2- and VZV-IgG could be demonstrated. It is noteworthy, that in both patients a virus-specific intrathecal response was detected earlier by IEF-AMI than by antibody index. Retrospectively, an appreciable portion of CSF/serum-pairs with intrathecally produced herpesvirus-specific antibodies turned out to contain antibodies against both HSV-1, 2 and VZV. Casas et al. [3] described similar findings in two patients with signs of encephalitis, who produced increasing IgG intrathecally against both HSV and VZV, but contained only HSV-1 or VZV-DNA in their CSF. Koskiniemi et al. [5] describe 35 cases of CNS infections in which dual HSV- and VZV-specific produced antibodies were detectable, with predominantly HSV type 2-specific antibodies. We were not able to test for this finding, as our serological assays make use of cross-reacting epitopes and thus cannot distinguish between HSV type 1 and type 2.

A coincident increase of antibodies against HSV-1, 2 and VZV might reflect failure to detect DNA of both viruses, e.g. due to initial negative PCR results early in the course of infection [1, 3, 17-19]. As consecutive CSF specimens of our two patients presented here were repeatedly tested for HSV-1, 2- and VZV-DNA, it is unlikely that we failed to detect HSV-1, 2 or VZV.

In multiple sclerosis patients, low serum-to-CSF antibody ratios are seen with different viruses, that may indicate an ingress of B lymphocytes in the CNS with local antibody production or a past infection of the CNS [20]. In fact, the intrathecal antibody response in HSV encephalitis is maintained at high level for years and thus, the detection of intrathecally produced antibodies against different herpesviruses might only reflect the coincidence of persistent and actual production [8, 13].

Cross-reactions in serological tests may cause detection of both VZV- and HSV-IgG in CSF [13].

In our two cases presented here, VZV-specific IgG bands displayed oligoclonal features partly different from HSV-1, 2-specific IgG bands and vice versa. Furthermore, cross-absorption experiments with CSF and serum of patient 1 revealed separately produced HSV-1, 2- and VZV-specific IgG.

Normally, the initiation of production of virus-specific IgG requires an antigen-specific interaction between B-lymphocytes and T-cells. Thereby, soluble cytokines are produced that help in activating the B-cells to produce their specific antibodies. It is proposed that such cytokines are also able to co-activate so-called innocent bystander B-cells with an unrelated specificity. It is feasible that such a mechanism during an HSV infection could lead to reactivation of pre-existing VZV-specific memory B-cells and vice versa. Partial cross-reactivity at the level of T-cell recognition of HSV and VZV could also be involved in such bystander activation. Such an explanation is however somewhat hypothetical and debated [21, 22].

In conclusion, patients with suspected CNS-infection with HSV or VZV can be diagnosed by detecting intrathecally produced virus-specific antibody, in addition to virus-specific PCR. However, in an appreciable proportion of cases, dual intrathecal antibody production against HSV and VZV can occur. This dual intrathecal antibody synthesis may even increase over time, as exemplified in our case reports and thus may hamper the correct diagnosis.

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