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Clinical Report

Infections of the central nervous system of suspected viral origin: A collaborative study from Finland

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We studied 3231 patients with acute central nervous system (CNS) symptoms of suspected viral origin to elucidate the current etiologic spectrum. In 46% of the cases, a viral finding was observed. Varicella–zoster virus (VZV) was the main agent associated with encephalitis, as well as meningitis and myelitis. VZV comprised 29% of all confirmed or probable etiologic agents. Herpes simplex virus (HSV) and enteroviruses accounted 11% each, and influenza A virus 7%. VZV seems to have achieved a major role in viral infections of CNS. In encephalitis in our population, VZV is clearly more commonly associated with these neurological diseases than HSV. The increase in VZV findings may in part be a pseudophenomenon due to improved diagnostic methods, however, a true increase may have occurred and the pathogenetic mechanisms behind this should be elucidated. *Journal of NeuroVirology* (2001) **7,** 400–408.

Keywords: encephalitis; myelitis; meningitis; central nervous system; viral infection; neurologic infection

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Introduction

Herpes simplex virus (HSV) has been the most common cause of fatal nonepidemic encephalitis (Kennard and Swash, 1981; Smith, 1981). Antiviral medication is effective if started in a timely fashion (Sköldenberg *et al*, 1984; Whitley *et al*, 1986). The spectrum of HSV-associated central nervous system (CNS) diseases is wide and difficult to identify and confirm even when suspected (Koskiniemi *et al*, 1980; Kennard and Swash, 1981; Whitley *et al*, 1981; Bale, 1993; Färkkilä *et al*, 1993). In children, conventional childhood infections have been commonly associated with or been the causative agents in encephalitides (Koskiniemi *et al*, 1991). Now, many of them have been eradicated by vaccination (Koskiniemi and Vaheri, 1989; Heisler and Richmond, 1994; Peltola *et al*, 1994). Large epidemiologic studies on CNS viral diseases originate from the 1960s and 1980s (Meyer *et al*, 1960; Lennette *et al*, 1962; Beghi *et al*, 1984). Furthermore, new methods have been developed for cerebrospinal fluid (CSF) studies, warranting an update of the etiologic pattern of CNS infections.

We have studied encephalitides at the Helsinki University Hospital since the 1960s and reported a change in the etiological spectrum in the 70s and 80s (Koskiniemi *et al*, 1981; Koskiniemi and Vaheri, 1989; Koskiniemi *et al*, 1991; Färkkilä *et al*, 1997). To characterize the changing etiologic spectrum of neurologic infections in the 1990s, when new enzyme immunoassay (EIA) tests and nucleic acid detection became widely employed, we have performed a collaborative study on CNS viral infections among the population of 4 million people in Finland. Based on this study, we present the results from the most representative period, from 1995 to 1996, consisting of more than 3000 patients with CNS symptoms of suspected viral origin, and show a remarkable transformation in the etiologic pattern of viral neurologic infections.

Results

Epidemiology

The incidence of suspected viral infections of the CNS varied from 25 to 100 patients/100,000 persons-year, based on population data in different areas of the country. In 1014 patients, one third of all, the working diagnosis based on clinical findings at admission was "encephalitis" (Table 1). "Meningitis," on the other hand, was the diagnosis in 328, and "myelitis" in 45 patients. The diseases were most common in children less than 10 years of age. This was the case especially with encephalitis (Figure 1). Distribution by month did not show any special peaks.

Etiological findings

Evidence of confirmed or probable viral etiology was obtained in 659 of 3231 patients (20.4%), and possible viral etiology in 833 patients (25.8%) (Table 1). In patients with clinical encephalitis, the respective numbers were 336/1014 (33.1%) and 229/1014 (22.6%). In general, the etiological findings were clearly higher in clinically defined CNS disease entities than in cases with single symptoms or with no clinical data. In patients with clinical encephalitis or meningitis, a definitive virological finding was obtained in one third of all cases, with one fourth having a "possible" viral etiology.

Virological findings

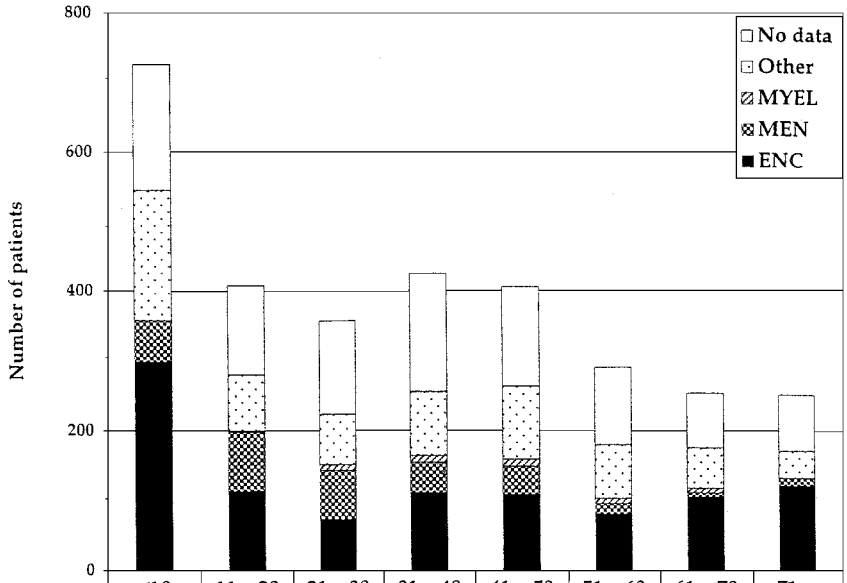
We identified 96 patients with encephalitis associated with or caused by VZV infection (Table 2). VZV was the major cause in all CNS disease entities and appeared in all age groups, although the distribution was uneven (Figure 2). In encephalitis, the number of VZV-associated cases was 3-fold higher, and in meningitis it was 2-fold higher, than HSV-associated cases (Table 2). Thirty-five patients had laboratory findings simultaneously to HSV and VZV. Both VZV and HSV cases were usually confirmed or probable, in contrast to entero and respiratory viruses in which the etiologic association often was possible, but seldom confirmed or probable. HHV-6 was studied only in selected cases; still, it accounted 5% of the viral diagnoses. The group of herpesviruses (HSV, VZV, HHV-6, EBV) accounted for 27% of all and 52% of the group of confirmed and probable etiologies in CNS viral infections (Figure 3).

Table 1 Distribution of suspected clinical diagnoses[†] of encephalitis, meningitis, and myelitis by the certainty of viral findings,[‡] confirmed, probable, and possible

	Virological diagnosis							
	Confirmed		*Probable*		*Possible*		*Summarized*	
Clinical diagnosis	*N*	%	*N*	%	*N*	%	*N*	%
Encephalitis/1014	163	16.1	173	17.1	229	22.6	565	55.7
Meningitis/328	68	20.7	28	8.5	84	25.6	180	54.9
Myelitis/45	8	17.8	4	8.9	14	31.1	26	57.8
Other symptoms/820	52	6.3	88	10.7	232	28.3	372	45.4
No data/1024	29	2.8	46	4.5	274	26.8	349	34.1
All	320	9.9%	339	10.5%	833	25.8	1492	46.2%

[†] Admission diagnosis: Encephalitis, when symptoms or signs indicating brain tissue involvement were present, i.e., focal or generalized seizures, motor or sensory pareses, depression of consciousness. Meningitis when symptoms or signs indicating meningeal, but not brain tissue involvement, were present. Myelitis when spinal cord involvement was reported, i.e., pyramidal or sensory signs on spinal level were present. If other diseases or single symptoms were present, they were included in the group Other symptoms. No clinical details were available in 1024 cases.

[‡] Viral diagnosis was regarded as 1) confirmed if virus or virus-specific nucleic acid was detected in CSF or specific intrathecal antibody production (the ratio of serum/CSF virus-specific antibodies was $\leq$20 by endpoint titers or $<$10 in EIUs, whereas other antibodies were negative in CSF), or virus-specific IgM was detected in CSF. The diagnosis was 2) probable if seroconversion or a $\geq$40 EIUs increase in specific antibody level occurred, virus-specific IgM antibody was observed in serum, or a specific viral disease appeared within 4 weeks of the CNS symptoms. Viral etiology was 3) possible if there was an extracranial viral finding or a high level of specific antibodies in serum (occurring in $<$5% of population) was observed. 4) No microbiological findings.
Total number of patients = 3231.



	≤10	11—20	21—30	31—40	41—50	51—60	61—70	71—
No data	181	128	134	169	142	111	78	81
Other	187	80	72	91	104	77	58	38
MYEL	1	2	8	9	10	7	7	1
MEN	59	84	70	44	40	15	5	11
ENC	298	114	73	111	109	81	106	121

Age group in years

Figure 1 Distribution of clinical admission diagnoses by 10-year age groups. N = number of patients in respective age group ranging from 716 in ≤10 years of age to 252 in group >70 years. See notes in Table 1.

Enteroviruses were associated with CNS infections in 14.6% of all and in 11.3% of confirmed and probable etiologies (Figure 3). In the meningitides, they represented the largest group (Table 2).

Table 2 Distribution of etiologic findings by admission diagnosis

	ENC	*MEN*	*MYEL*
Varicella-zoster virus (VZV)	96	26	4
Herpes simplex viruses (HSV)	35	13	1
HSV & VZV	13	16	2
Enteroviruses (Ent)	27	29	0
Influenza A virus (IA)	26	0	3
Herpesvirus-6 (HHV-6)*	25	0	0
Adenovirus (Ad)	13	3	0
Tick-borne encephalitis virus (TBEV)*	19	0	0
Double finding	15	0	0
Mycoplasma pneumoniae (My)	11	1	0
Parainfluenza viruses (Pa)	8	3	1
Rotavirus (Ro)	7	2	0
Epstein Barr virus (EBV)	9	1	0
Respiratory syncytial virus (RSV)	7	0	0
Vaccination (Vacc)	7	0	0
Chlamydia pneumoniae (Chl)	5	1	1
Puumala virus (NE)*	4	0	0
Influenza B virus (IB)	2	1	0

Only confirmed and probable findings are included. (*) only selected specimens were studied. ENC = encephalitis, MEN = meningitis, MYEL = myelitis, see notes in Table 1.

Influenza, adeno-, parainfluenza, and respiratory syncytial (RS) viruses comprised 28.9% of all diagnostic findings but much less, 19%, of the group of confirmed and probable etiologies (Figure 3). Notably, besides the herpes group only respiratory viruses were observed in association with myelitis (Table 2).

Identification of concomitant viral agents, other than HSV and VZV, was frequent, but the association with the neurological disease was seldom confirmed or probable (Figure 3). The number of various etiological agents was more than 15. In encephalitides, the etiological spectrum was more extensive than that of meningitides or other diseases (Table 2). In addition to the generally known agents, EBV, tick-born encephalitis virus (TBEV), RSV, and rotavirus as well as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* each accounted for ≥2% of confirmed or probable agents (Figure 3).

Diagnostic power of different methods

Most of the confirmed diagnoses, 245 of 3173 patients (7.7%), were obtained by using antibody tests in parallel on serum and CSF (Table 3). IgM specific for HSV or VZV was observed in 25 CSF samples of 422 patients. A probable CNS diagnosis was

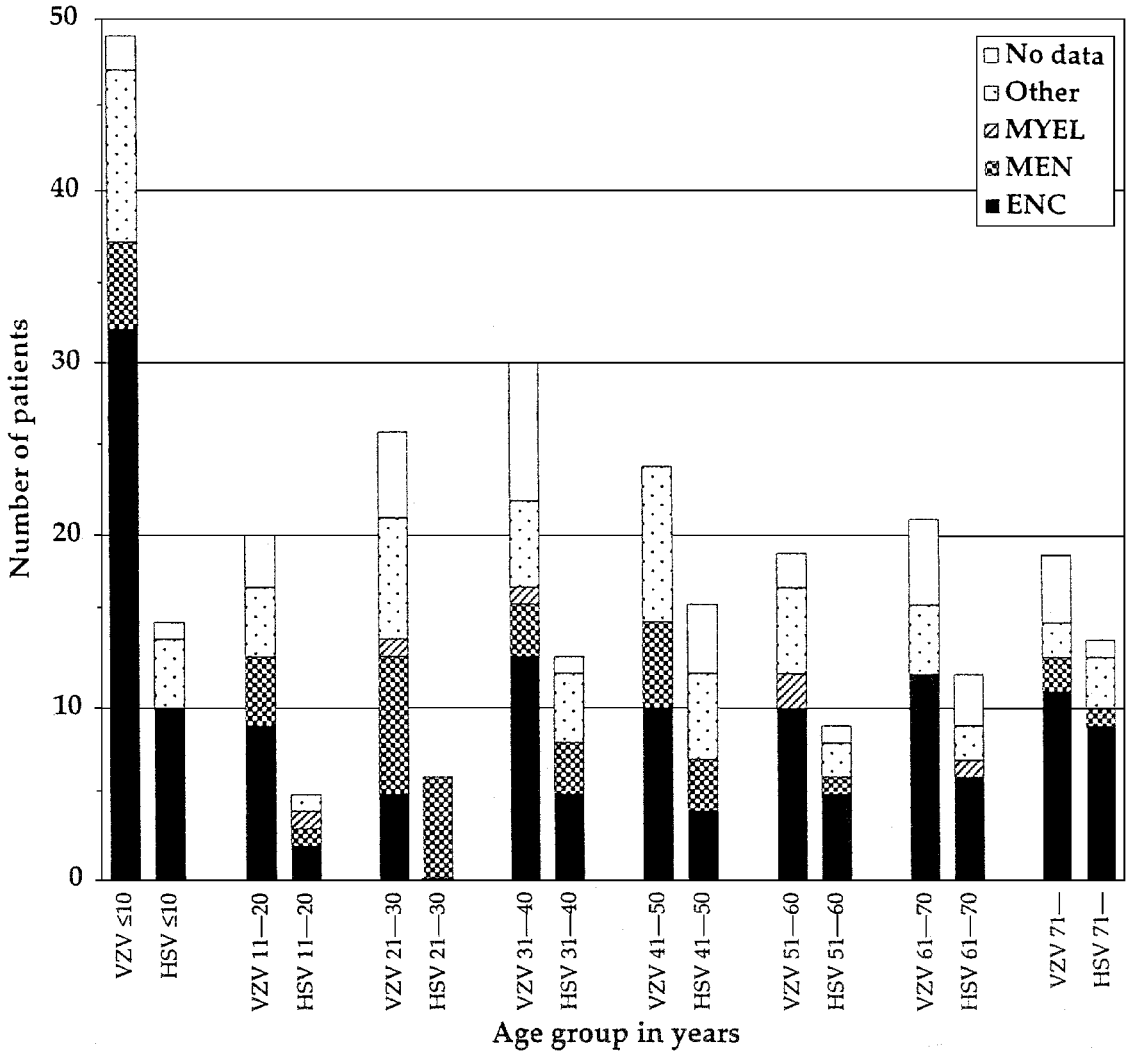


Figure 2 Distribution of patients with VZV and patients with HSV by 10-year age groups and by different clinical diagnoses. See notes in Table 1.

obtained by detecting specific IgM antibodies or IgG seroconversion in serum in one third of the selected patients.

By performing a PCR on a CSF sample, the diagnosis was obtained in 133 of 2590 patients (5.1%) for HSV-1 (32), HSV-2 (43), VZV (44) and for HHV-6 (14). Viral culture on CSF is an established diagnostic tool but seldom positive. In our series the CSF viral cultures of 15 patients were positive (1.9%) (Table 3).

Viral findings from an extracranial source, i.e., a culture on stool or a throat sample or antigen detection on a throat sample, had poor yield. In addition, those findings are only suggestive with respect to CNS infections. One hundred forty-three of the patients had had a clinical disease or received some type of vaccination within 4 weeks before their CNS symptoms began (Table 3).

Discussion

The patients studied with a suspected CNS viral infection came from throughout Finland and represented 20–40 patients/100,000 persons-year. Viral findings were obtained in nearly half of them, 5–15/100,000, which is about the same number as reported previously (Beghi *et al*, 1984; Koskiniemi *et al*, 1991; Färkkilä *et al*, 1997). In well-defined clinical entities, a proper viral diagnosis was obtained in more than one third of the cases, and when suggestive diagnoses are included, the number of viral diagnoses was more than 50%. By studying occasional samples without knowledge of the clinical problem, the diagnostic benefit was low. This finding underscores the importance of coupling both clinical and laboratory aspects to achieve a good diagnostic yield. However, these findings may

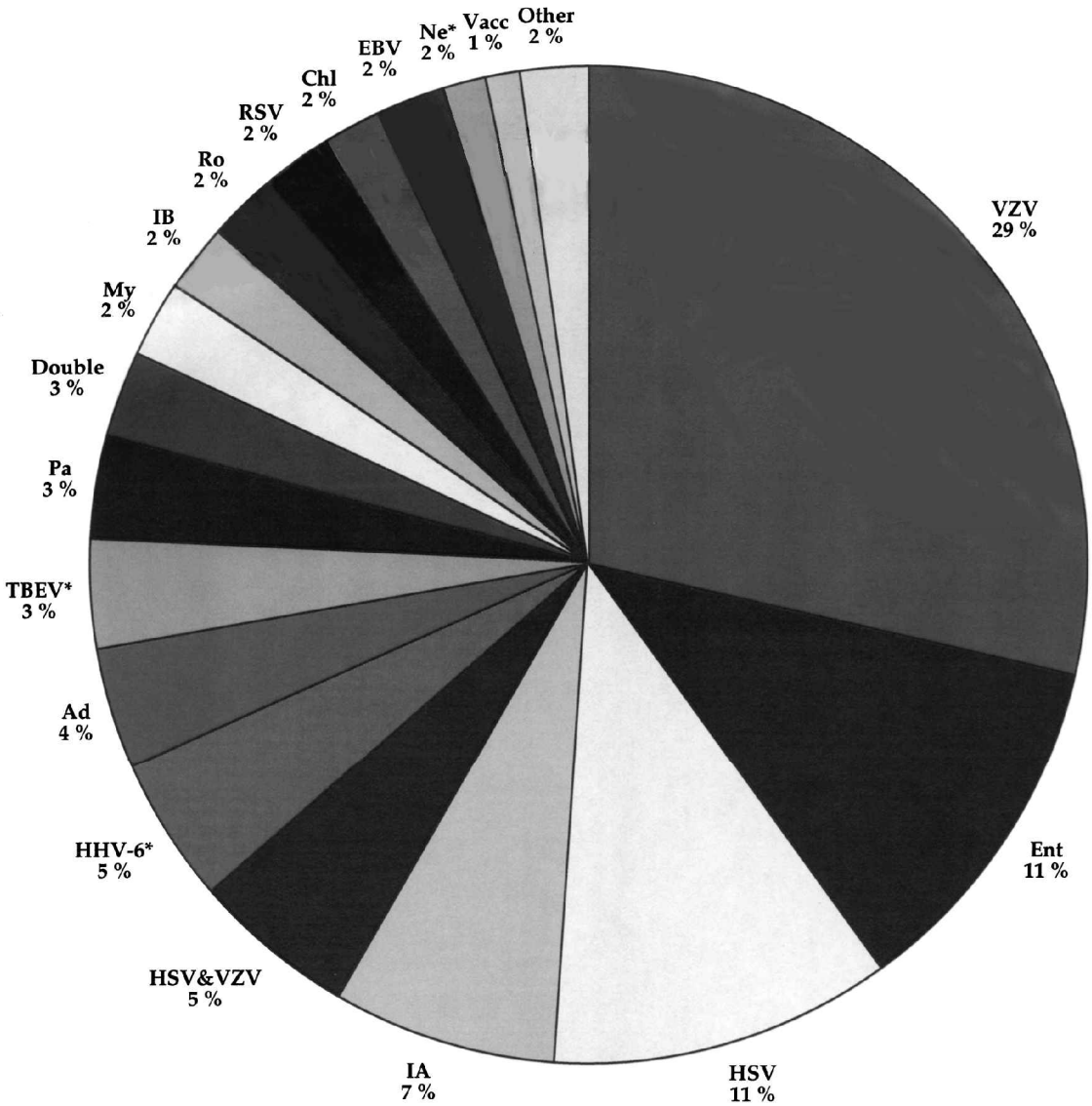


Figure 3 Occurrence of findings to different antigens. Only confirmed and probable (see notes in Table 1) diagnoses (N 659/3231 = 20%) are included. Microbes of ≥2% proportion are indicated by name and the rest are included in the group others. (*) studied in selected cases only. Abbreviations, see Table 2.

be unique to Finnish population and not widely applicable.

The major role of VZV in CNS diseases has not been previously reported (Koskiniemi *et al*, manuscript in preparation). Beghi *et al* (1984) reported VZV as an antecedent of encephalitis but not in meningitis. A review on VZV as a not-so-benign virus and VZV-associated vascular complications are reported (Jemsek *et al*, 1983; Weller, 1983; Kleinschmidt-DeMasters, 1996; Echevarria *et al*, 1997; Gilden *et al*, 2000). However, the dominant role of VZV in viral CNS infections has not been recognized before. Our series opens an important aspect of VZV-associated CNS diseases. VZV may spread from its latent state in the spinal ganglion into CNS and induce intrathecal antibody production. The virus may also float in the CSF, with or without skin manifestations.

Entero-, influenza, and adenoviruses as well as TBEV appeared to be frequent, which supports the earlier findings (Koskiniemi and Vaheri, 1982; Wahlberg *et al*, 1989; Muir and vanLoon, 1997). Respiratory viruses were as frequent as herpesviruses in this study but not as convincingly the etiological agents. Extracranial viral findings and high antibody levels in serum are merely suggestive, that is, they do not confirm the diagnosis. For enteroviruses there is a PCR test that apparently will improve diagnostics. For respiratory viruses, the PCR test for CSF studies appears problematic. The same is true for rotavirus;

Table 3 Distribution of findings by the method used

	Findings/Studied	
	N/N	%
Serological tests		
S/CSF-specific antibody ratio	245/3173	7.7
Specific IgM in CSF (HSV, VZV)	25/422*	5.9
Specific IgM in serum (HSV, VZV)	131/422*	31.0
Specific IgM in serum (TBEV)	23/77*	29.9
Seroconversion to specific IgG	89/896	9.9
PCR: CSF	133/2590	5.1
Viral culture		
CSF	15/791	1.9
Throat	39/441	8.8
Stool	56/442	12.7
Antigen detection		
Herpesviruses	11/265	4.2
Respiratory viruses	5/220	2.3
Clinical viral disease		
Laboratory confirmed	123	86.0
Without viral confirmaton	20	14.0

*Only selected specimens were studied.

the PCR test has not so far proved to be reliable for routine CSF studies. The fact that so many cases were associated with rotavirus is a new finding. In some patients, we observed even intrathecal antibody production indicating confirmed rotavirus infection in CNS. HHV-6 seems to play an important role in many CNS problems (Kondo *et al*, 1993; Suga *et al*, 1993; Ahtiluoto *et al*, 2000) and the PCR test is an excellent method to diagnose it. However, we necessarily need better serological tests to confirm the impact of HHV-6 in CNS infections.

Chlamydia pneumoniae, Puumala virus (the causative agent of nephropathia epidemica), and EBV seem to be real, although not frequent players in CNS infections. As far as therapy is concerned, *Chlamydia pneumoniae* appears to be an important agent. It should be looked for in encephalitis but also in less well-defined entities, in single symptoms such as vertigo, headache, seizures, or confusion (Koskiniemi *et al*, 1996). The same applies even to *Mycoplasma pneumoniae*. Many researchers have reported finding both these microbes in CNS infections (Koskiniemi, 1993; Socan *et al*, 1994).

In our previous study on encephalitides, including 322 adult patients from 1967–1991, HSV was three times more frequent than VZV (Rantalaiho *et al*, 2001). In this study on 1014 patients with suspected encephalitis from the period 1995–1996, the HSV/VZV ratio has literally been reversed. In fact, the number of VZV cases increased already during the last 5-year period in 1987–1991 in our adult series on encephalitis. In children, VZV had been prominent even in earlier studies (Koskiniemi *et al*, 1991, 1997), however, with the eradication of MMR-associated (measles, mumps, rubella) CNS infections, the impact of VZV seems to have risen further (Koskiniemi and Vaheri, 1989). Otherwise, the spectrum of CNS-associated microbes seems quite stable with only some exceptions: influenza viruses are less frequent, EBV is more frequent apparently due to improved diagnostics, and, as expected, the MMR group is no more present. HHV-6 and rotavirus are newcomers due to novel diagnostic methods.

With effective vaccination programs against the major CNS pathogens in children, an overall decline in CNS diseases had been anticipated; however, this expectation has not been realized (Koskiniemi and Vaheri, 1989; Heisler and Richmond, 1994; Peltola *et al*, 1994), raising many questions. Are there new emerging agents, have the conventional agents increased their capability to invade CNS, or have the new sensitive methods made it possible to detect more cases than hitherto? Furthermore, has our attitude toward different patient groups, e.g., older people, changed, or do we recognize and study not so well-defined symptoms better than before (Lakeman and Whitley, 1995; Färkkilä *et al*, 1997)?

PCR represents a new approach to viral infections of CNS (Rowley *et al*, 1990; Guffond *et al*, 1994; Lakeman and Whitley, 1995). The method to measure serum and CSF antibody levels in parallel had been published in the 1970s (MacCallum *et al*, 1974; Levine *et al*, 1978). The yield is most fruitful when modern sensitive and specific EIA tests convenient for CSF antibody measurements are used (Vaheri *et al*, 1982; Reiber and Lange, 1991). The serum/CSF antibody ratio is diagnostic in more cases than any other method. The findings are specific when a large panel of antigens is used to exclude damage of the blood–brain barrier, as in our study (see Methods). Specific IgM tests from CSF sample appeared promising, especially when sensitive IFA tests were used. The special benefit of IFAs is that only about 10 μl of CSF is sufficient for measurements. Another benefit is IFA's sensitivity and quickness.

The changing spectrum of CNS viral infections is a continuously ongoing phenomenon that needs to be recognized (Lennette *et al*, 1959; Meyer *et al*, 1960; Whitley, 1990). Our studies emphasize the need of a large panel of sensitive tests and close collaboration between clinical and diagnostic departments in order to achieve diagnosis in CNS infections. The mutual relationship of HSV and VZV may partly depend on the use of antivirals which are more effective against HSV than VZV. In many cases, diagnostic responses may be so slow that especially HSV-2 will never be observed after the therapy has been started. Some VZV-associated cases may actually be HSV-2 infections based on our preliminary findings regarding HSV-VZV double infections (Piiparinen *et al*, manuscript in preparation). On the other hand, diagnostic methods for VZV may have improved more than those for HSV. Still, there seems to be an obvious increase in VZV-associated infections which raises the question of prevention of VZV.

A new phase in the diagnostics of viral CNS infections seems obvious (Nahmias *et al*, 1982; Vaheri *et al*, 1982; Guffond *et al*, 1994; Tyler, 1994; Lakeman and Whitley, 1995). With use of PCR and EIA tests more diagnoses than ever before can be obtained without using invasive methods like brain biopsy. Now, in one third to one half of all CNS viral infections a reliable etiological diagnosis may be achieved. New methods for rapid diagnosis, however, are still needed. PCR test is laborious and takes often 2 days or even longer to complete. Specific IgM tests on CSF are easier to perform and could clearly improve the diagnostics of CNS infections.

Materials and methods

Patients

During the years 1995–1996, we studied samples from 3944 patients with neurologic symptoms of suspected viral etiology. The patients were treated in neurologic, pediatric, or infectious diseases wards or intensive care units of the 18 Central or University Hospitals covering 77% of population in Finland. From these hospitals the serum and CSF specimens were sent for viral studies to the Department of Virology, University of Helsinki. We excluded all newborn children, ≤28 days of age, as well as those patients in whom another etiology was found soon after admission. Thus, our series comprised 3231 patients from 1 month to 83 years of age. They had admission diagnoses of encephalitis, meningitis, myelitis, or other diseases, or single symptoms, or no clinical data at all (Table 1). The specialists of the centers participating in the study had uniform criteria to report about the clinical symptoms. The Study Group had biannual meetings for collaboration. The essential criterion to include the patient in the study was a CSF sample obtained due to suspected viral neurological disease.

Serologic tests

Antibodies to HSV-1, HSV-2, VZV, RSV (commercial antigens, Virion^{R}, Würzburg, Germany), adeno-, influenza A and B, rota-, coxsackie B5, nontyped entero-, and parainfluenza 1 viruses (antigens grown and purified as decribed) (Julkunen *et al*, 1984), and *Mycoplasma pneumoniae* (antigen gift from Dr M Kleemola, National Public Health Institute, Helsinki, Finland) were measured from serum and CSF by using EIA tests (Vaheri *et al*, 1982; Koskiniemi *et al*, 1995, 1997). HSV- and VZV-specific IgMs and EBV IgG and IgM were determined by using a commercial kit, and antibodies to HHV-6 and *Nephropathia epidemica* (*Puumala* virus) by using an in-house test. Tests positive for IgM were repeated after IgG inactivation (Gullsorp absorption). Microimmunofluorescence (MIF) test was used for IgG and IgM specific for *Chlamydia pneumoniae,* hemagglutination inhibition test for TBE IgG and EIA test for TBEV IgM.

In years 1993–1994, we used tests for viruses of the MMR group in CNS infections routinely. No cases were found and since 1995, only occasional cases were studied with negative results.

Nucleic acid detection

PCR assays for HSV-1, HSV-2, and VZV were performed on CSF samples. The primers were selected from the DNA polymerase genes for HSV-1, HSV-2, and VZV; the PCR product was detected using luminometric microplate hybridization (Vesanen *et al*, 1996, Koskiniemi *et al*, 1997).

Other studies

Antigens to HSV, VZV, and RSV, influenza A and B, parainfluenza 1 and 3, and adenoviruses were studied on throat specimens with conventional immunofluorecence (IFA) tests. Virus culture was attempted on CSF, throat and stool samples using 4 different cell lines: African GMK, Vero, human amniotic epithelial cells, and human embryonic skin fibroblasts.

Diagnostic grouping of the patients

Based on microbiological findings the patients were divided into four groups (Table 1): 1) confirmed CNS infection, 2) probable CNS, but confirmed systemic infection, 3) possible etiology, 4) no etiology.

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