Cerebrospinal fluid viral load and biomarkers of neuronal and glial cells in Ramsay Hunt syndrome

Johan Lindström1*, Anna Grahn1, Henrik Zetterberg2,3,4, Marie Studahl1

1Department of Infectious Diseases, Institute of Biomedicine 2Department of Psychiatry and Neurochemistry, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, 3Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden, 4Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, UK

Running title: Cerebrospinal fluid biomarkers in facial nerve palsy caused by varicella zoster virus

Keywords:

Varicella-zoster virus (VZV), Central nervous system (CNS), NFL protein, GFAP, facial paralysis

Proposed journal section: Clinical and translational neuroscience

Pages: 20

Figures: 4

Tables: 1

Equations: 0

Abstract word count: 250

Manuscript word count (excluding references): 3146

Manuscript word count (including references): 4479
Correspondence:

*Johan Lindström, MD

Department of Infectious Diseases

Sahlgrenska University Hospital, Östra

Diagnosvägen 21

SE-416 85 Göteborg

E mail: johan.l.lindstrom@vgregion.se

Phone: +46 31 3439361

Fax: +46 31 847813

E-mail and addresses of co-authors:

Anna Grahn, MD, PhD

E mail: anna.m.grahn@vgregion.se

1Department of Infectious Diseases

Sahlgrenska University Hospital, Östra

Diagnosvägen 21

SE-416 85 Göteborg

Henrik Zetterberg
E mail: henrik.zetterberg@clinchem.gu.se

2 Department of Psychiatry and Neurochemistry
Sahlgrenska University Hospital, Mölndal
SE-431 80 Göteborg

Marie Studahl
E mail: marie.studahl@infect.gu.se

1 Department of Infectious Diseases
Sahlgrenska University Hospital, Östra
Diagnosvägen 21
SE- 416 85 Göteborg
Abstract

Reactivation of varicella zoster virus (VZV) can manifest with facial palsy diagnosed as Ramsay Hunt Syndrome (RHS) or Ramsay Hunt Syndrome zoster sine herpete (RHS-ZSH). These syndromes are associated with poor prognosis despite treatment with antivirals and corticosteroids. Concentrations of biomarkers such as neurofilament protein (NFL), S-100β protein and glial fibrillary acidic protein (GFAp) have previously been measured in cerebrospinal fluid (CSF) to assess neuronal damage and glial pathology.

We employed immunochemical methods to measure concentrations of NFL, S-100β protein and GFAp in CSF from patients with RHS (n=15) and RHS-ZSH (n=13) diagnosed by detection of VZV DNA in the CSF by quantitative PCR, and compared with a control group (n=52). The biomarker concentrations were correlated to CSF viral load and outcome measured by House-Brackmann score.

NFL and GFAp concentrations were increased compared with controls (\( p < 0.01 \) and \( p < 0.05 \)), while S-100β levels were decreased. This pattern was more pronounced in patients with RHS compared to the patients with RHS-ZSH (NS and \( p < 0.05 \)). The amount of viral DNA in CSF correlated with increased GFAp (\( p < 0.01 \)) and NFL (\( p < 0.01 \)). No correlations were found between biomarker concentrations and patient outcome.

Patients with facial palsy caused by VZV had biochemical signs of neuronal damage and astrogliosis. High amounts of viral DNA may be associated with the degree of damage on neuronal and astroglial cells. Prospective studies are warranted to elucidate the association of elevated biomarkers in the CSF and outcome assessed by more sensitive tests.


**Introduction**

Reactivation of varicella zoster virus (VZV) can manifest as a variety of central nervous system (CNS) syndromes with a risk of serious and even fatal complications (Gilden, 2004). Facial nerve palsy caused by reactivation of VZV from the geniculate ganglion (Wackym, 1997), is commonly accompanied by a typical vesicular rash in the ipsilateral ear, and is named Ramsay Hunt syndrome (RHS). The blisters may also be localised in the hard palate and/or on the anterior two thirds of the tongue. Associated symptoms are vestibulocochlear dysfunctions such as vertigo, hearing loss, and tinnitus (Sweeney & Gilden, 2001). However, reactivation of VZV with neurological symptoms may also occur without the presence of rash, zoster sine herpete (ZSH). Improved diagnostic methods including PCR has facilitated the differentiation between Ramsay Hunt syndrome zoster sine herpete (RHS-ZSH) and other causes of facial nerve palsy such as Bell’s palsy (Gilden *et al.*, 1994). Detection of VZV DNA by PCR and/or antibody-detection in the cerebrospinal fluid (CSF) are first-line methods for diagnosis (Gilden *et al.*, 2010).

The prognosis of RHS has been shown to be worse than in Bell’s palsy. Without treatment only one in ten patients with total facial paralysis recover, and in patients with subtotal facial paralysis approximately two thirds recover (Devriese & Moesker, 1988). Antiviral treatment is available with aciclovir, and is recommended in RHS in conjunction with corticosteroids (Gnann, 2002). Overall, despite treatment, only three in five patients recover, and with multiple cranial neuropathy, only approximately a third, as compared to more than four in five patients with Bell’s palsy (Shim *et al.*, 2011; Ryu *et al.*, 2012).

The potential association between neurological damage, severity of symptoms in RHS and outcome is poorly investigated, and the neuropathology involved remains unclear. Concentrations of biochemical markers in the CSF can be measured to assess neuronal damage, glial pathology and
inflammation. Neurofilament light protein (NFL) is a component of the axonal neurofilament core and a marker of neuron pathology (Rosengren et al., 1996; Norgren et al., 2003). S-100β protein and glial fibrillary acidic protein (GFAP) both reflect astroglial cell leakage (Rothermundt et al., 2003; Petzold, 2015). CSF biomarkers have been used to estimate severity of neuronal damage and prognosis in various CNS diseases (Aurell et al., 1991; Nylen et al., 2002; Teunissen et al., 2009), including viral infections such as herpes simplex encephalitis (HSE) and tick-borne encephalitis (TBE) (Studahl et al., 2000). Only one recent study has been made on VZV infections of the CNS, where the elevated levels of NFL and GFAP in the CSF suggested neuronal damage and astrogliosis (Grahn et al., 2013). In that study, only 3 patients with facial nerve palsy were included. CSF viral load as a biomarker, measured through quantitative real-time PCR, has hitherto been investigated in two studies on VZV CNS syndromes (Aberle et al., 2005; Persson et al., 2009). However, these studies only included a total of 20 patients with cranial nerve palsy and they were not specifically investigated.

Our aim was to study the CSF levels and patterns of the biochemical markers NFL, GFAP, S-100β as well as viral load in patients with facial nerve palsy caused by VZV compared to controls, and relate the results to clinical severity and outcome.

**Materials and methods**

**Patients**

Patients were retrospectively identified through CSF samples sent to the Virological Laboratory of Sahlgrenska University Hospital during 2002-2013 with detectable VZV DNA by quantitative in-house PCR. Patients with facial nerve palsy were identified through their medical records. Patients with other coexisting CNS diseases were excluded.
Additional information such as demographics, CSF sampling in relation to neurological symptoms, antiviral and/or corticosteroid treatment, laboratory workups and associated symptoms such as blisters, vertigo, hearing loss and pain at presentation and at follow-up were obtained.

The Medical Ethics Committee at Gothenburg University approved the study and informed consent was obtained from patients prior to their inclusion in the study.

Twenty-eight patients with VZV DNA in CSF were included (14 female and 14 male; median age 60 years, range 17–93). Patient characteristics and clinical data are shown in Table 1. Blisters were identified in 15 patients, fulfilling the strict definition of RHS, leaving 13 patients with RHS-ZSH. Blisters presented at a median of 0 days (concurrent with onset of neurological symptoms) (range -5—11). For analytical purposes all patients included were considered one group regardless of blisters unless otherwise specified. Associated symptoms were prevalent, consisting in localized pain or headache \((n = 28)\). Many patients had signs of multiplex mononeuritis, such as vestibular neuritis and vertigo \((n = 17)\), auditory nerve involvement and hearing loss \((n = 17)\) 10\(^{th}\) nerve dysfunction and dysphagia \((n = 2)\), abducens paresis and diplopia \((n = 1)\), and possibly brachial plexus involvement with upper limb paresis \((n = 2)\). Additionally, ten patients presented with a temperature above 38°C and two elderly patients presented with confusion but otherwise not fulfilling previously presented criteria of encephalitis (Persson et al., 2009). Magnetic resonance imaging (MRI) had been performed in 10 patients and in two of them an inflamed facial nerve was indicated. Recent signs of ischemia were not seen in any of the radiological examinations.

Treatment with aciclovir was given to 27/28 patients, and was administrated intravenously \((n = 11)\), intravenously with oral aciclovir or valaciclovir as follow-up \((n = 10)\), or as oral treatment exclusively \((n = 5)\). One patient was given aciclovir according to medical records without specification of treatment regimen. Aciclovir treatment was initiated concurrent with or after lumbar puncture in 19/27 treated patients (median 0; range -3—8), and treatment was given with a
median duration of 10.5 days (range 7—26.5). The patient with the longest treatment duration was rehospitalized due to severe vertigo and restarted on intravenous treatment during her oral follow-up. Adjuvant therapy with corticosteroids was given in 17 patients.

Since the timing of lumbar puncture is considered relevant in regard to the biomarkers measured (Rosengren et al., 1996; Studahl et al., 2000), patients where further subgrouped into two groups: CSF sampling within 7 days of onset of neurological symptoms \( (n = 22) \) or from day 8-15 \( (n = 5) \).

Patients were also subgrouped based on whether treatment was initiated before or after CSF sampling, and whether they presented with blisters or not. Analyses were performed both on subgroups and all included patients as presented below.

**Control subjects**

Non-infectious subjects with headache or psychoneurotic symptoms undergoing lumbar puncture were used as controls. Exclusion criteria were concomitant CNS disease or CNS disease identified within one year after sampling, elevated lymphocytes or protein levels in the CSF, increased C-reactive protein concentrations in blood, or pathological findings at neurological examination. The control subjects were age-matched to the patients. 52 control patients were included (30 female and 22 male; median age 60 years, range 16–87).

**CSF samples and analyses**

Patient CSF samples had been analysed for VZV DNA with quantitative in-house PCR previously described (Persson et al., 2009) as part of diagnostic procedure of symptoms. In the original publication referred to (Persson et al., 2009), the probe sequence was incorrectly described and the correct probe should be VZVgB_P CGCGGTCCCAAGTCCCTGGA (Persson et al.). Patient CSF samples were stored at -70 °C or -20 °C, and CSF samples from control subjects were stored at -70 °C as part of a biobank.

In this study, all CSF samples were analysed on one occasion using one batch of reagents for the biomarkers GFAp, NFL and S-100β by board-certified laboratory technicians according to
procedures accredited by the Swedish Board of Accreditation and Conformity Assessment (SWEDAC). GFAp concentrations were measured using a previously described in house ELISA (Rosengren et al., 1994). NFL concentrations were measured using a commercially available sandwich ELISA (Uman Diagnostics, Umeå, Sweden) (Norgren et al., 2003). CSF concentrations of S-100β were measured using the Modular system and the S100β reagent kit (Roche Diagnostics, Basel, Switzerland). Intra-assay coefficients of variation were ≤ 10%.

### Severity and outcome measures

In an effort to determine disease severity, extent of facial nerve palsy was retrospectively graded using the House-Brackmann facial nerve grading system, a scale from 1 to 6 where 1 is normal function and 6 is total lack of movement in corresponding facial muscles (House & Brackmann, 1985). The highest score served as baseline, and score at the latest follow-up within three months was used as outcome when applicable. A House-Brackmann score of 1-2 was considered good outcome and 3-6 poor outcomes.

### Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software, San Diego, USA). Non-parametric statistical methods were used: Mann-Whitney U test for group comparisons and Spearman’s rank test for correlations. P values of < 0.05 were considered significant.

### Results

#### Biomarkers

**NFL**

NFL concentrations in CSF were increased in patients with VZV facial palsy (n = 27) (median 911 ng/l; IQR 484–1620) when compared to the control group (521 ng/l; 337–767) (p = 0.008) (Fig. 1a). Moreover, the NFL concentrations were significantly increased in patients with blisters, and thus RHS, compared to the control group (n = 14) (median 929 ng/l; IQR 512–1668) (p = 0.0046),
but patients with RHS-ZSH did not have an increase in NFL concentrations \((n = 13)\) (median 508 ng/l; IQR 395–1685). The difference in NFL concentrations in patients with RHS compared to RHS-ZSH was not statistically significant \((p = 0.4583)\). In further subgroup analysis, there was a tendency towards increasing NFL concentrations in samples drawn after 7 days after onset of neurological symptoms compared to patients with samples within 7 days (748 ng/l; 225–2824 and 1620 ng/l; 327–7032 respectively) \((p = 0.1465)\) (Fig. 1b). In one patient, NFL could not be analysed due to low sample volume.

**GFAp**

A modest increase in GFAp concentrations was shown when all patients \((n = 28)\) (877 ng/l; 617–1650) were compared to controls (698 ng/l; 420–1082) \((p = 0.0403)\) (Fig. 2). When subgrouping and compared to controls, the increase was significant in patients with RHS \((n = 15)\) (975 ng/l; 648–3888) \((p = 0.0028)\), but not in patients with RHS-ZSH \((n = 13)\) (747 ng/l; 388–1033) \((p = 0.9001)\). When compared, RHS had higher concentrations of GFAp compared to RHS-ZSH \((p = 0.0278)\).

**S-100β**

Concentrations of S-100β were decreased in CSF from patients \((n = 22)\) (0.22 ng/ml; 0.15–0.27) when compared to controls (0.37 ng/ml; 0.27–0.44) \((p = 0.0002)\), but no further differences were found after subgrouping. In six patients, low sample volume did not allow for analysis of S-100β.

**Viral load**

VZV DNA concentrations in the CSF of all patients (median 4,683 VZV DNA copies/ml; range 50–1,148,154) correlated with the concentrations of NFL (Spearman \(r = 0.51; p < 0.01\)), GFAp (Spearman \(r = 0.54; p < 0.01\)) and S-100β (Spearman \(r = 0.59; p < 0.01\)) as shown in Fig. 3. There was no statistically significant difference in viral load between RHS (median 2239 copies/ml; IQR 479–57544) and RHS-ZSH (median 5000 copies/ml; IQR 231–33995) \((p = 0.6175)\). Diminishing viral loads over time could not be shown in this material, but a non-significant tendency for lower
viral load was seen between treatment started before (median 794 copies/ml; IQR 79-25861) and after (12359; 1546-52944) ($p = 0.072$) CSF sampling.

**Biomarkers and symptoms, severity and outcome**

No correlations could be shown between biomarkers, including viral load, and severity of symptoms such as facial palsy measured by House Brackmann score, pain, vertigo or hearing impairment at peak values or latest follow-up within three months of CSF sampling. When patients were subgrouped based on good vs. poor outcome, no significant differences in biomarker concentrations could be shown.

**Discussion**

In this first study on biomarkers in RHS and RHS-ZSH, we have identified a pattern of increased NFL, increased GFAP and decreased S-100β in CSF, interpreted as signs of neuronal damage and astrogliosis (Grahn et al., 2013). The increase in NFL reflects axonal damage, while the increase in GFAP in combination with a decreased concentration of S-100β is consistent with results in previous studies on neuroborreliosis (Dotevall et al., 1999) and TBE (Studahl et al., 2000), suggesting astrogliosis rather than damage to the astroglial cell membrane. In addition, these results are in line with a recent study on CNS biomarkers of various VZV CNS manifestations. In that study, increased CSF concentrations of NFL and GFAP were most pronounced in patients with encephalitis, but no increase in biomarker concentrations was shown in patients with peripheral nerve symptoms, and only three of the patients included had facial palsy. In our study, the biomarker elevations were modest but significant, and possibly reflecting the pathological process being more localized as compared to encephalitis, but nonetheless indicative of neuronal damage and astrogliosis.

The neuropathogenesis in RHS is largely unknown. Hunt originally theorized that the inflammation was localized to the geniculate ganglion, and that the close proximity of the geniculate ganglion to the vestibulocochlear nerve within the bony facial canal was associated with
development of the vestibulocochlear symptoms. Increasing amounts of evidence contradict this theory. Histopathological findings are scarce, and show variability. In other clinical variants of herpes zoster, autopsy findings include both necrosis of the dorsal root ganglia in the spinal cord, when infection occurred close to death and, fibrotic dorsal root ganglia in patients with infection months to years before (Head et al., 1997). In contrast, necrosis of the geniculate ganglion has not been found in RHS patients post mortem. Instead, the few post mortem studies performed have shown various findings including none to mild inflammation of the geniculate ganglion, perivascular inflammation, demyelination and axonal loss along the facial nerve (Denny-Brown et al., 1944; Guldberg-Moller et al., 1959; Blackley et al., 1967; Aleksic et al., 1973). In addition, MRI examinations have revealed abnormalities localized not only to the inner ear and vestibulocochlear nerve, but also to the facial nerve (Brandle et al., 1996; Chung et al., 2015). The intratemporal facial nerve including the geniculate ganglion seems to swell in the acute phase of RHS, which has been observed during subtotal decompression surgery (Honda et al., 2002).

Moreover, presence of VZV DNA has been found in the facial nerve sheath and middle ear mucosa as well as vesicles and CSF (Murakami et al., 1998). Taken together, evidence supports polyneuropathy with viral spread throughout the affected nerves, which may possibly lead to further damage on neurons and astroglial cells as opposed to a localized geniculate ganglionitis.

The increased CSF concentrations of GFAP in patients with blisters, and thus RHS, compared to patients with RHS-ZSH could indicate a difference in the magnitude of inflammatory response, or even a difference in the pathogenesis, taken into consideration that it has been previously shown that patients with typical RHS have a lower recovery rate compared to patients with RHS-ZSH (Lee et al., 2012). In addition, NFL concentrations were significantly elevated in patients with RHS compared to controls, but not in patients with RHS-ZSH. Although lacking a statistically significant difference in NFL concentrations between the groups with and without blisters, neuronal damage was only shown in the group with blisters, raising further questions on the neuropathogenesis in RHS and RHS-ZSH respectively.
Vasculopathy has been suggested in pathogenesis of other VZV CNS manifestations such as encephalitis (Gilden, 2002; Gilden et al., 2009), and multifocal vasculopathy following RHS has been described in one HIV patient (Ortiz et al., 2008). Transaxonal spread of VZV to vasa vasorum of cranial nerves may produce infarction, leading to cranial nerve polynueuritis (Sweeney & Gilden, 2001). Reactive astrogliosis has previously been described in ischemic events (Clark et al., 1993; Pekny & Nilsson, 2005), and as the pattern of biomarkers in our study indicates astrogliosis, we cannot exclude vasculopathy as a possible neuropathological pathway also in patients with facial palsy caused by VZV. In our study there was no evidence of patients suffering from infarction. However, only 10 out of 28 patients had an MRI performed.

CSF viral load correlated with NFL and GFAP concentrations, indicating that CSF viral load may be used to assess the level of neuronal damage in patients within a well-defined group such as facial nerve paralysis caused by VZV. On the other hand, we did not find a significant correlation between increased concentrations of NFL, GFAP or CSF viral load and severity of initial symptoms or outcome. Additionally, no differences in concentrations of biomarkers were seen between the patients when they were subgrouped into poor versus good outcomes. In our previous study on biomarkers and VZV infections in the CNS, NFL and GFAP concentrations were higher in patients with more severe CNS manifestations (Grahn et al., 2013), and in another study, viral load in CSF was shown to correlate with clinical severity (Aberle et al., 2005). However, several limitations of the present study may have prevented us from replicating such results. Firstly, variation in timing of CSF samplings in relation to symptoms is likely one important factor. In addition, increasing NFL concentrations during the course of the disease has previously been shown in CNS infections (17) and this phenomenon was also indicated in our study. Secondly, the relatively small size of our study in combination with modest elevation of biomarkers impedes statistical significance. Thirdly, the insensitivity and variability measuring symptoms retrospectively through assessment of medical journals is problematic. The House-Brackmann facial nerve grading scale has been retrospectively used before (Leong & Tristram, 2013), but has obvious limitations when used this way. A
prospective study including more sensitive methods such as Facial Nerve Grading System 2.0 (Vrabec et al., 2009), audiometry and caloric testing, and sensitive neuroradiological examinations in combination with biomarker sampling would probably add more information.

As mentioned, antiviral treatment against VZV is available with aciclovir. The combination of aciclovir and corticosteroids is considered preferable in Ramsay Hunt syndrome (de Ru & van Benthem, 2011) although two Cochrane reviews reported insufficient evidence through lack of randomized trials (Uscategui et al., 2008a; b). Early treatment seems to be of significance (Murakami et al., 1997; Kinishi et al., 2001), with reported complete recovery as high as 90% (Kinishi et al., 2001). Our results, indicating neuronal destruction and astrogliosis already at onset of symptoms, emphasize the importance of both early diagnostics and initiation of treatment in patients with RHS.

In conclusion, this study demonstrates that patients with acute peripheral facial palsy caused by VZV have increased CSF concentrations of NFL and GFAp in combination with a decrease in S-100β compared to a control group, indicating neuronal damage and astrogliosis. The amount of viral DNA in CSF correlated with increased biomarkers and may be associated with the degree of damage on neurons and astroglial cells. Further studies are needed to address the relevance of vasculopathy, and correlations on biomarkers and outcome should be studied prospectively using more sensitive outcome measures in conjunction with sensitive neuroradiological examinations.

**Acknowledgements**

This study was supported by the LUA-ALF foundation of the Sahlgrenska Academy at University of Gothenburg (ALFGBG-431361, ALFGBG-508411 and ALFGBG-441051).

**Conflicts of interest**

Authors declare no conflicts of interests.
References


Uscategui, T., Doree, C., Chamberlain Ian, J. & Burton Martin, J. (2008b) Corticosteroids as adjuvant to antiviral treatment in Ramsay Hunt syndrome (herpes zoster oticus with facial palsy) in adults *Cochrane Database of Systematic Reviews*. John Wiley & Sons, Ltd.
