CHAPTER 9
CSF NEURAL CELL ADHESION MOLECULE IN RELATION TO 3T MRI-METRICS IN EARLY MULTIPLE SCLEROSIS

Michael Khalil\textsuperscript{1,2}, Christian Enzinger\textsuperscript{1,3}, Christian Langkammer\textsuperscript{1}, Stefan Ropele\textsuperscript{1}, Arthur Mader\textsuperscript{1}, Mirja Wallner-Blazek\textsuperscript{1}, Gerhard Bachmaier\textsuperscript{4}, Juan-Jose Archelos\textsuperscript{1}, Siegrid Fuchs\textsuperscript{1}, Marinus A. Blankenstein\textsuperscript{2}, Franz Fazekas\textsuperscript{1} and Charlotte E. Teunissen\textsuperscript{2}

\textsuperscript{1} Department of Neurology, Medical University of Graz, Austria, \textsuperscript{2} NUBIN, Department of Clinical Chemistry, VU University Medical Center Amsterdam, The Netherlands, \textsuperscript{3} Division of Neuroradiology, Department of Radiology, Medical University of Graz, Austria, \textsuperscript{4} Institute for Medical Informatics, Statistics and Documentation, Medical University Graz, Austria

ABSTRACT

BACKGROUND: Neural cell adhesion molecule (NCAM) plays an important role in neural repair and regeneration. Although reduced NCAM levels have been described in cerebrospinal fluid (CSF) of multiple sclerosis (MS), the clinical and morphologic correlates of CSF NCAM are still unclear.

OBJECTIVE: To investigate the association of CSF NCAM with clinical and MRI measures of disease stage, activity and progression in MS.

METHODS: We analyzed CSF NCAM in 85 patients (CIS N=66; MS N=19). Patients underwent clinical detailed examination and 3T MRI. A follow-up MRI was obtained from 32 patients. CSF samples from 17 patients with other neurological diseases served as controls.

RESULTS: Significantly lower CSF NCAM levels were present in MS compared to CIS (p=0.05) and in clinically active compared to non-active patients (p<0.05). CSF NCAM levels of both CIS and MS groups were not significantly different from controls. Higher CSF NCAM levels correlated with brain tissue loss over time (r=-0.542, p<0.005), but not with the change in T2 lesion-load.

CONCLUSION: Our study provides evidence for altered CSF NCAM levels in MS dependent on disease stage and activity. CSF NCAM may increase upon regenerative processes, which however may limit brain tissue damage for only a short interval.
INTRODUCTION

Neural cell adhesion molecule (NCAM) is a glycoprotein that belongs to the immunoglobulin superfamily (Berezin and Bock. 2010, Massaro. 2002, Secher. 2010). It is suggested to play an important role in neuronal repair and regeneration, including axonal outgrowth, cell-cell adhesion and synaptic plasticity (Gerrow and El-Husseini. 2006, Hinsby, et al. 2004, Massaro. 2002). Apart from membrane bound NCAM, soluble forms exist, which can be detected in brain homogenates, neuronal cell culture supernatants, in blood and cerebrospinal fluid (CSF) (Bock, et al. 1987, Secher. 2010). These are believed to result from neuronal cell secretion, enzymatic cleavage of the extracellular domain of NCAM or from detached NCAM-containing membrane fragments (Secher. 2010). The biological role of soluble NCAM isoforms is not yet completely clear (Ronn, et al. 1998, Secher. 2010), but includes modulation of neurite outgrowth and interference with NCAM-dependent cell adhesion (Secher. 2010).

Altered CSF NCAM levels have been described in a variety of neurological diseases (Gnanapavan, et al. 2010). Several studies provide evidence that CSF NCAM levels are reduced in multiple sclerosis (MS) (Gnanapavan, et al. 2010, Massaro. 2002), with lowest levels in more advanced stages of the disease (Gnanapavan, et al. 2010). CSF NCAM levels have also been shown to continuously increase over a short-term follow-up of 5 weeks in acute MS patients who clinically improved after treatment, suggesting that NCAM may be involved in recovery processes of the brain (Massaro. 2002).

Up to now, no information exists on the relation of CSF NCAM to morphologic brain changes in MS as indicated by MRI. This would be important to investigate if this marker is indeed related to brain tissue damage. We therefore aimed to explore the role of CSF NCAM in relation to clinical and MRI parameters in patients with clinically isolated syndromes (CIS) and MS in comparison to control patients with other neurological diseases of non-inflammatory aetiology.

SUBJECTS AND METHODS

We included 85 consecutive patients with a CIS suggestive of MS (Miller, et al. 2008) or a diagnosis of MS (Polman, et al. 2011) from our MS outpatient clinic (Table 1). Patients underwent diagnostic lumbar puncture for CSF analysis, detailed clinical examination and brain MRI at 3T. Assessment of demographical and clinical data included age, age at disease onset, disease duration, expanded disability status scale (Kurtzke. 1983), occurrence of relapses and MS therapy. A relapse was defined as the appearance or reappearance of at least one neurological symptom or the worsening of an old symptom attributed to MS that lasted for at least 24 hours and which was preceded by a relatively stable or improving neurological state of at least 30 days (SCHUMACKER, et al. 1965). Except for one MS patient treated with interferon beta during CSF collection, no patient included in this study received any MS specific treatment. Patients whose CSF was taken during a clinical exacerbation of the disease were defined as active and clinically stable patients were defined as non-active.

Clinical follow-up data were available from 45 CIS patients with a median follow up time of 2.3 (IQR 1.4-3.4) years. During this period 17 patients converted to MS.

We included 17 patients with other non-inflammatory neuropsychiatric diseases as controls (Table 1). The control group consisted of 10 patients with headache, 2 with facial...
 CHAPTER 9

Cerebrospinal fluid (CSF) analyses

After diagnostic lumbar puncture, excess of CSF was stored at -80 °C until use in agreement with BioMS guidelines (Teunissen, et al. 2009). All CSF analyses were performed by trained technicians or biochemists blinded to clinical information. Routine CSF parameters are given in table 2. Soluble CSF NCAM levels were determined by a commercial Luminex test kit (MILLIPLEX™ MAP, Millipore, Billerica, MA, USA).

Magnetic resonance imaging (MRI)

Eighty patients underwent MRI of the brain at 3 Tesla (Siemens Tim Trio, Siemens Healthcare, Erlangen, Germany) using a phased-array head coil with 12 receiver elements and a consistent imaging protocol as described previously (Khalil, et al. 2009, Khalil, et al. 2011). The median time interval between lumbar puncture and MRI was 3.0 (IQR 0.7-6.2) months. In 32 patients (28 CIS, 4 MS) a follow-up MRI was performed (median follow up 1.0 (IQR 1.0-1.1) years.

All image analyses were performed by trained and experienced technicians and MR readers, blinded to clinical information.

Brain tissue volume and lesion load were measured as previously described (Enzinger, et al. 2004, Khalil, et al. 2009, Khalil, et al. 2011). In short, separate estimates of volumes of gray matter, white matter, cortical gray matter and ventricular CSF, normalized for subject head

### Table 1. Clinical and demographical and magnetic resonance imaging data

<table>
<thead>
<tr>
<th>N</th>
<th>CIS</th>
<th>MS</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% female</td>
<td>71.2</td>
<td>78.9</td>
<td>64.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age at LP (years)</td>
<td>33.5 (9.9)</td>
<td>32.1 (6.9)</td>
<td>35.4 (14.0)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age at disease onset (years)</td>
<td>33.4 (9.9)</td>
<td>28.4 (7.5)</td>
<td>NA</td>
<td>0.05</td>
</tr>
<tr>
<td>Disease duration at LP (months) *</td>
<td>0.3 (0.2-1.1)</td>
<td>34.7 (14.6-49.8)</td>
<td>NA</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EDSS at LP *</td>
<td>1.0 (0.0-2.0)</td>
<td>1.3 (0.5-2.3)</td>
<td>NA</td>
<td>n.s.</td>
</tr>
<tr>
<td>Annual relapse rate *</td>
<td>NA</td>
<td>0.8 (0.4-1.7)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Magnetic resonance imaging N**

| Normalized volume [cm³] | 61 | 19 |

| Brain | 1566.9 (94.1) | 1537.4 (76.7) | NA | n.s. |
| Gray matter | 796.5 (58.6) | 788.3 (43.3) | NA | n.s. |
| Cortex | 658.0 (52.1) | 642.9 (39.0) | NA | n.s. |
| Ventricle * | 751.5 (76.5) | 749.1 (43.8) | NA | n.s. |

| 12 lesion load [cm³] * | 19 (0.7-6.7) | 3.2 (1.1-7.6) | NA | n.s. |

CIS, clinically isolated syndrome; MS, multiple sclerosis; N, number of patients / controls; LP, lumbar puncture; EDSS, Expanded Disability Status Scale; n.s., not significant; NA, not applicable. Values are given as mean (SD) or as *median (interquartile range).
Table 2. Cerebrospinal fluid parameters

<table>
<thead>
<tr>
<th></th>
<th>CIS</th>
<th>MS</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>66</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Cell count N/µl (Ref. ≤ 4)</td>
<td>8.0 (4.0-14.0)</td>
<td>9.0 (6.0-18.0)</td>
<td>2.0 (1.0-2.0)</td>
</tr>
<tr>
<td>CSF protein mg/dl (Ref. ≤ 45)</td>
<td>34.5 (29.0-44.0)</td>
<td>37.0 (31.0-52.0)</td>
<td>28.0 (22.0-34.0)</td>
</tr>
<tr>
<td>IgG index (Ref. &lt;0.7)</td>
<td>0.8 (0.6-1.2)</td>
<td>0.9 (0.8-1.4)</td>
<td>0.5 (0.5-0.5)</td>
</tr>
<tr>
<td>IgA index</td>
<td>0.3 (0.3-0.5)</td>
<td>0.4 (0.3-0.5)</td>
<td>0.3 (0.3-0.3)</td>
</tr>
<tr>
<td>IgM index</td>
<td>0.2 (0.1-0.3)</td>
<td>0.2 (0.1-0.3)</td>
<td>0.1 (0.1-0.1)</td>
</tr>
<tr>
<td>OCB positive N (%)</td>
<td>62 (93.9)</td>
<td>18 (94.7)</td>
<td>ND</td>
</tr>
<tr>
<td>NCAM [ng/ml]</td>
<td>183.6 (135.5-212.0)</td>
<td>147.1 (121.3-177.4)</td>
<td>166.9 (139.4-214.3)</td>
</tr>
</tbody>
</table>

a Cell count: white cell count in CSF
b p-value <0.001 compared to controls
c p-value <0.01 compared to controls
d p-value <0.05 compared to controls
e p-value =0.05 compared to MS

CIS, clinically isolated syndrome; MS, multiple sclerosis; N, number of patients / controls; OCB, oligoclonal bands; ND, not done; values are given as median (interquartile range) or as * number of patients / controls.

RESULTS

Group differences in CSF NCAM levels and correlations with demographical data

CSF NCAM levels were significantly lower in MS compared to CIS patients (p=0.05). NCAM levels of both patient groups, however, were not significantly different from that of controls whose mean NCAM level was between the patient groups (Figure 1). CSF NCAM levels were also significantly lower in patients whose CSF had been taken during an acute clinical exacerbation compared to clinically non-active patients (p<0.05) (Figure 2). CSF NCAM levels in control patients increased in an age dependent manner (r=0.593; p<0.05). Such a relationship was not present in CIS and MS patients. There were no significant gender differences in CSF NCAM levels.
CSF NCAM levels in correlation with clinical data and biochemical variables

Considering all patients CSF NCAM correlated with total CSF protein levels ($r=0.316$, $p<0.005$) and the albumin quotient ($r=0.258$, $p<0.01$). Correlations were not significant with regard to CSF white cell count and the immunoglobulin indices for IgG, IgM or IgA. CSF NCAM levels did not correlate with disease duration and the EDSS score at time of lumbar puncture. In MS, we did not find any correlation of CSF NCAM with the annualised relapse rate. Considering only CIS patients with a clinically follow-up of at least one year, no significant differences of CSF NCAM levels emerged comparing patients who converted to MS with non-converters.
Correlations of CSF NCAM with MRI parameters

Levels of CSF NCAM correlated with tissue loss over time regarding normalized volumes for whole brain ($r=-0.542$, $p<0.005$), whole gray matter ($r=-0.460$, $p=0.01$) and cortex ($r=-0.573$, $p<0.005$). This was also true when considering only CIS patients. Figure 3 displays the correlation of CSF NCAM with the change in cortical volume over time regarding CIS and MS patients. CSF NCAM levels did not correlate with baseline MRI measures, including normalized whole and regional brain volumes and T2 lesion load. No significant correlations of CSF NCAM levels were present regarding the change of normalized white matter, ventricular CSF volume and in T2 lesion load.

DISCUSSION

Our study provides evidence for reduced CSF NCAM levels in patients with a diagnosis of MS compared to CIS, which is inline with previous reports (Gnanapavan, et al. 2010, Massaro, 1998). In a recently described ELISA method for CSF NCAM detection 51 MS patients were analyzed and compared to other neuropsychiatric inflammatory, non-inflammatory diseases and healthy controls (Gnanapavan, et al. 2010). Compared to controls significant reduced CSF NCAM levels were present in multiple sclerosis, Alzheimer’s disease and meningitis, indicating that that the low NCAM levels might represent increased neuronal damage or a reduced capacity for endogenous repair mechanisms (Gnanapavan, et al. 2010).

In our study on MS patients, CSF NCAM levels were also lower compared to controls, although this difference did not reach statistical significance. We here investigated early MS patients, which could explain the lack of pronounced group differences between MS and control patients.

It is not yet understood why CSF NCAM levels are reduced in patients with MS (Massaro, 2002). NCAM is a glycoprotein that is part of the immunoglobulin superfamily (Berezin, et al. 2000). It plays a central role in regenerative and reparative processes, including cell/cell contacts, axonal outgrowth and synaptic plasticity (Berezin, et al. 2000, Hinsby, et al. 2004, Walmod, et al. 2004). Apart from membrane bound isoforms of NCAM, its soluble forms have been described in body fluids and culture media from neurons, exerting various effects, including the promotion of neurite outgrowth (Nybroe, et al. 1989, Secher. 2010).

![Figure 3. Correlation of CSF NCAM levels with the change in normalized cortex volume over time. Higher CSF NCAM levels correlated with increased reduction in normalized cortex volume over time ($r=-0.573$, $p<0.005$).](image)
A major finding of our study was that higher CSF NCAM levels correlated with MRI measures for brain tissue loss over time regarding normalized volumes for whole brain, gray matter and cortex, but not with the change in T2 lesion load. At a first glance, this finding seems counter-intuitive, since previous studies associated higher CSF NCAM levels with clinical improvement (Massaro. 1998, Massaro. 2002). In that study, patients were followed for up to 5 weeks and serial CSF samples were analysed. Whereas higher CSF NCAM levels may be associated with clinical improvement over short-term, our results demonstrate that over a longer period, e.g. one year, higher CSF NCAM levels could be associated with brain atrophy, in particular the gray matter. Following an immunological attack, CSF NCAM could increase upon regenerative processes, which however may limit brain tissue damage for only a short interval. Indirectly, however, this could be evidence for a more active disease process. Our notion of higher CSF NCAM levels in patients in whom CSF was sampled close to a clinical attack compared to sampling during clinically inactive disease would support this hypothesis. Another explanation for our findings could be that higher CSF NCAM levels may also exert deleterious effects. From in vitro and in vivo studies evidence exists, that high levels of soluble NCAM may indeed lead to impaired synaptic connectivity, possibly via formation of toxic aggregates (Pillai-Nair, et al. 2005, Secher. 2010). CSF NCAM levels did not correlate with the change of normalized white matter volumes. This can be explained by the fact that white matter volume loss is rarely seen in early phases of MS, in contrast to gray matter atrophy (Chard, et al. 2002, Dalton, et al. 2004).

In CIS patients CSF NCAM levels showed a wide range from very low to high values. However, we could not find any significant correlation with baseline MRI findings, including T2 lesion load. Patients with acute clinical exacerbations at the time of lumbar puncture had significantly lower CSF NCAM levels than patients clinically inactive at that time. This is in contrast to a previous report who analysed 30 MS and found lower CSF NCAM concentrations in those with non-acute MS (Massaro. 1998).

Contrarily to a recently published study (Gnanapavan, et al. 2010) we found a correlation of CSF NCAM with total CSF protein levels and the albumin quotient. The source of soluble NCAM is mainly the central nervous system, although increased levels were also described in serum of cancer patients, indicating disease progression (Jaques, et al. 1993, Zoltowska, et al. 2001). Thus we cannot completely exclude that CSF NCAM concentrations could partially result from passive transfer of serum NCAM into the CSF. Another interesting finding of our study was that CSF NCAM levels correlated with increasing age in control patients, which was not present in CIS and MS patients. CSF NCAM levels may rise in parallel to age dependent axonal degeneration. This association however may be disrupted in pathophysiologic conditions with increased tissue damage, e.g. in CIS and MS. CSF NCAM levels did not correlate with clinical data, including conversion from CIS to MS, disease duration, EDSS score and the annualised relapse rate when considering MS patients only.

CSF NCAM concentrations have been reported to underlie a temporal dynamic following an acute clinical exacerbation in MS (Massaro. 1998), showing a steady increase in patients who clinically improved upon treatment of the attack during a follow-up period of 5 weeks (Massaro. 1998). In this respect time point of lumbar puncture in regard to a relapse may thus be critical when relating CSF NCAM concentrations to clinical data.

Our study has also limitations. One factor, which has to be taken into account, is that our MS cohort compared to CIS was rather small, reducing effect size of statistical analyses. Future studies should also include more advanced MS.
Altogether, our study provides evidence for altered CSF NCAM levels in MS dependent on disease stage and activity. The association of higher CSF NCAM levels with brain tissue loss over time suggests an interrelation of regenerative and destructive mechanisms. Further studies are required to investigate the relation of CSF NCAM levels with non-conventional MRI techniques, including magnetization transfer imaging for refined quantitative analyses.

ACKNOWLEDGEMENT

We thank Mrs. Sisi Durieux-Lu for excellent technical assistance. Austrian Science Fund (FWF) [J2992-B09] is acknowledged for support of MK.

REFERENCES


