Axonal degeneration and inflammation in acute optic neuritis

A Petzold, K Rejdak, G T Plant

Aims: To investigate whether plasma biomarkers for axonal injury and inflammation are related to loss and recovery of visual function in acute optic neuritis (ON).

Methods: Eighteen patients with ON and 14 controls were investigated in a longitudinal, prospective study. Plasma phosphorylated neurofilament heavy chain (NfHSMI35; a surrogate marker of axonal injury), nitric oxide metabolites (NOx), and citrulline (surrogate markers of inflammation) were measured.

Results: Patients with ON had higher median plasma NfHSMI35 values than controls (0.17 versus 0.05 ng/ml, p < 0.05) and higher NOx values (49 versus 35.5 μM, p < 0.001). Plasma NfHSMI35 values correlated inversely with visual acuity at presentation (R = −0.67; p = 0.01). NfHSMI35 was higher in patients with poor recovery of visual acuity than in those with good recovery (0.25 ng/ml versus 0.09 ng/ml; p < 0.05). Three of four patients with high NfHSMI35 and high NOx values experienced a poor recovery as opposed to only one of five with high NOx but normal NfHSMI35 values.

Conclusions: NfHSMI35, a surrogate marker for axonal damage, is a prognostic indicator and should be considered in the design of neuroprotective treatment strategies.

The "axonal death cascade" has been associated with nitric oxide (NO) mediated damage in vitro. However, the relation of axonal degeneration to NO release in vivo is not known. Acute optic neuritis provides a useful model to investigate whether plasma biomarkers for axonal injury and inflammation are related to loss and recovery of visual function in acute optic neuritis (ON).

Methods: Eighteen patients with ON and 14 controls were investigated in a longitudinal, prospective study. Plasma phosphorylated neurofilament heavy chain (NfHSMI35; a surrogate marker of axonal injury), nitric oxide metabolites (NOx), and citrulline (surrogate markers of inflammation) were measured.

Results: Patients with ON had higher median plasma NfHSMI35 values than controls (0.17 versus 0.05 ng/ml, p < 0.05) and higher NOx values (49 versus 35.5 μM, p < 0.001). Plasma NfHSMI35 values correlated inversely with visual acuity at presentation (R = −0.67; p = 0.01). NfHSMI35 was higher in patients with poor recovery of visual acuity than in those with good recovery (0.25 ng/ml versus 0.09 ng/ml; p < 0.05). Three of four patients with high NfHSMI35 and high NOx values experienced a poor recovery as opposed to only one of five with high NOx but normal NfHSMI35 values.

Conclusions: NfHSMI35, a surrogate marker for axonal damage, is a prognostic indicator and should be considered in the design of neuroprotective treatment strategies.

Table 1 Characteristics of patients expressed as medians (interquartile range)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Controls</th>
<th>Acute optic neuritis</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36 (30–43)</td>
<td>36 (35–46)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Male/female</td>
<td>4/10</td>
<td>9/5</td>
<td></td>
</tr>
<tr>
<td>Eye</td>
<td>N/A</td>
<td>R 8/L 6</td>
<td></td>
</tr>
<tr>
<td>RAPD</td>
<td>N/A</td>
<td>R 8/L 5</td>
<td></td>
</tr>
<tr>
<td>Visual acuity</td>
<td>1.2</td>
<td>0.2 (0.05–0.67)</td>
<td></td>
</tr>
<tr>
<td>Colour vision</td>
<td>17/17</td>
<td>0/17[0/17–7/17]</td>
<td></td>
</tr>
<tr>
<td>Disc appearance</td>
<td>Normal</td>
<td>2 swollen, 12 normal</td>
<td></td>
</tr>
<tr>
<td>Time from onset</td>
<td>N/A</td>
<td>14 days (8–26)</td>
<td></td>
</tr>
<tr>
<td>Follow up</td>
<td>N/A</td>
<td>171 days (25–189)</td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td>N/A</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Plasma NfHSMI35 (ng/ml)</td>
<td>0.005 (0.0–0.094)</td>
<td>0.17 (0.07–0.33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma NOx (μM)</td>
<td>36.2 (31–38.6)</td>
<td>48.1 (40.8–54.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma citrulline (mmol/ml)</td>
<td>83.4 (72.1–92.4)</td>
<td>67.9 (62.5–91.9)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations: MRI, magnetic resonance imaging; NA, not assessed; NfHSMI35, phosphorylated neurofilament heavy chain; NOx, nitric oxide metabolites; ON, optic neuritis; VA, visual acuity.
first visit and concentrations of the phosphorylated neurofilament heavy chain (NF 35), NOx, and citrulline were measured as described previously.14 16 17

Data analysis was performed using SAS. Because of non-Gaussian data distribution the medians and interquartile ranges are shown. All correlations were studied using Spearman’s rank correlation coefficient. Differences between groups were compared using the two sided Wilcoxon two sample test. Significances based on small numbers were checked on a categorical level using the two sided Fischer’s exact test. Trend analysis was carried out using the Mantel-Haenzel $\chi^2$ test. A 5% level of significance was used throughout.

RESULTS
The diagnosis of ON was confirmed in all 18 patients during follow up. However, in four patients the blood sample was taken over four weeks after onset of symptoms. Therefore, these patients were excluded from our analysis. The remaining 14 patients presented with a median time from onset of two weeks (table 1). At presentation, 10 of 14 of the patients had a VA of less than 0.33 (6/18, 20/60 Snellen equivalent). In total, 13 of 14 patients showed sustained improvement of their VA during follow up. A minor degree of optic atrophy was seen in four of nine patients whose VA recovered to over 0.33 (6/18). Optic nerve atrophy was more severe in those five of the patients who did not recover their VA above 0.33.

Plasma concentrations of NF 35 (fig 1 A) and NOx (fig 1B) were significantly higher in patients with ON than in controls ($p < 0.001$ and $p < 0.01$, respectively). No such difference was found for citrulline (table 1). There was a negative correlation between NF 35 and VA at the time of first presentation ($R = -0.67$; $p = 0.01$; fig 1C). One outlier with NF 35 values of 0.65 ng/ml was seen. This 48 old male patient presented with a right ON, VA was reduced to hand movement (6/60, 20/200 Snellen equivalent), colour vision to 0/17, and there was a right relative afferent pupillary deficit. This patient had suffered from an episode of myelitis two years previously. In the absence of clinical signs of myelitis, no spinal MRI was performed. After the removal of this patient from our study the correlation between NF 35 and VA remained significant ($R = -0.60$; $p < 0.05$), but the slope of the linear regression decreased from $-0.22$ to $-0.145$.

No correlation between VA and either NOx or citrulline was found at presentation. There was no correlation between NF 35, NOx, or citrulline and age or time from onset; in addition, there were no sex differences.

Plasma NF 35 concentrations at presentation were significantly higher (mean, 0.25 ng/ml) in the patients with poor recovery of visual function (0.33; 6/18, 20/60 Snellen equivalent) when compared with those whose VA recovered to above 0.33 (mean, 0.09 ng/ml; $p < 0.05$). The difference remained significant when comparing proportions of patients with NF 35 values above the cut off value (fig 1A; $p = 0.015$; two sided Fisher’s exact test). There were no differences in NOx values at presentation between those with good versus poor visual recovery at follow up (54.2 versus 44.6 μM; $p = 0.1$).

The combined association between raised NOx and NF 35 concentrations and recovery of visual function was investigated by comparing proportions of patients with high values (raised above the top value of the control group; NF 35 > 0.17 ng/ml; NOx, > 43.8 μM). There was a significant trend for increased proportion of patients experiencing a poor recovery (Mantel-Haenzel $\chi^2 = 4.6$; $p < 0.05$) across the following categories: NF 35 normal and NOx normal (none of three, 0% poor recovery), NF 35 high and NOx normal (one of five, 20% poor recovery), NF 35 high and NOx high (three of four, 75% poor recovery).

MRI results were available in 10 of 14 patients; six had an isolated ON lesion (mean NF 35, 0.12 ng/ml; mean NOx, 40.7 μM), as opposed to four with disseminated brain lesions.
Concentrations but normal NfHSMI35 values made a good group, four of five of the patients with raised NOx after six months. The results of our present study may relate to the small sample size and the relatively short period of follow up (25–189 days).

Recovery of VA in MS related ON has been attributed to resolution of the conduction block caused by acute inflammation. NO mediated conduction block also seems to be the initial mechanism in in vitro studies. Our present study and one other study failed to show a significant relation between serum NOx values and outcome. In addition, NOx and NfHSMI35 did not correlate with MRI parameters, but this could be a result of the small study population. The almost 30–40% higher values, if true, might indicate that demyelinating activity elsewhere is contributing to the worse outcome.

Citrulline is thought to be another marker of NO mediated inflammation, but it did not correlate with NOx and different differences were seen between patients with ON and controls. This finding is in keeping with one study comparing cerebrospinal fluid and serum citrulline concentrations between patients with Lewy body dementia and healthy controls. We agree with these authors that citrulline is not a good surrogate marker for NO metabolism because it is also a substrate for enzymes other than inducible nitrogen oxide synthetase.

In MS, it appears that inflammatory markers are predominately related to disease activity and the MRI lesion load but not to outcome. In addition, epidemiological data suggest that inflammation in MS has only a limited effect on the course of neurodegeneration. In placebo controlled trials and clinical experience with ON, anti-inflammatory treatment with steroids does not influence outcome in most cases. The destructive and neuroprotective aspects of inflammation have stimulated a controversial discussion.

Therefore, it is interesting that in this small group, four of five of the patients with raised NOx concentrations but normal NfHSMI35 values made a good recovery. This finding highlights the potential for selection bias in treatment trials for neuroprotective drugs by inclusion of those patients with evidence of inflammation only. Taken together, these findings support the concept that sustained loss of optic nerve function relates to axonal degeneration and that NO might contribute, at least in part, to the “axonal death cascade.”

ACKNOWLEDGEMENTS
Dr K Reidak was supported by The Foundation for Polish Science (FNP).

REFERENCES