Axonal degeneration and inflammation in acute optic neuritis

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Abstract

This longitudinal study of acute optic neuritis (ON) investigated whether plasma biomarkers for axonal injury and inflammation could be related to loss and recovery of visual function.

Eighteen patients with ON and 14 control patients were included into this prospective study. Plasma NfH^{SMI35} (a surrogate for axonal injury), NO_x and citrulline (surrogates for inflammation) were measured by ELISA.

Patients with ON had higher median plasma NfH^{SMI35} levels when compared to controls (0.17 ng/mL *versus* 0.005 ng/mL, $p<0.05$) and higher NO_x levels (49 μM *versus* 35.5, $p<0.001$). Plasma NfH^{SMI35} levels correlated inversely with visual acuity at presentation (R=-0.67, p=0.01). NfH^{SMI35} was higher in patients with poor (0.25 ng/mL) when compared to those with good recovery of visual acuity (0.09 ng/mL, $p<0.05$). Seventy–five percent of patients with high NfH^{SMI35} and high NO_x levels experienced a poor recovery as opposed to only 20% with high NO_x but normal NfH^{SMI35} levels.

NfH^{SMI35} a surrogate marker for axonal damage is a prognostic indicator and should be considered in the design of neuroprotective treatment strategies.

Key words: Surrogate marker, NfH^{SMI35} , NO_x, citrulline, ON, axonal loss

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The “axonal death cascade” (1) has been associated to nitric oxide (NO) mediated damage in vitro (2). The relation of axonal degeneration *in vivo* to NO release is not known. Acute optic neuritis provides a useful model because of the defined onset and the anatomically restricted area of neuronal damage (3; 4). Increased levels of the NO metabolites nitrite (NO^{2-}) and nitrate (NO^{3-}) were found in the cerebrospinal fluid and serum of patients with optic neuritis (5; 6). Axonal loss appears to be linked to the acquisition of permanent deficit (7; 2; 8; 9; 10; 11; 12). However the role of inflammation in demyelinating disease is less well defined and there is accumulating evidence that immune-mediated inflammation possesses neuroprotective properties (1; 13).

This study aimed to investigate the relationship between loss and recovery of optic nerve function with surrogate markers for inflammation (citrulline and the NO metabolites $NO2^{-}$ and $NO3^{-}$ (14; 15; 2; 16)) and for axonal injury (neurofilaments (17)) in patients with acute optic neuritis.

1 Patients and Methods

Patients presenting with symptoms suggestive of acute unilateral optic neuritis (n=18) and an age-matched healthy control group (n=14) were included in this prospective study (Table 1). Inclusion criteria were: progressive loss of vision over a few days, decreased visual acuity (Snellen chart), decreased colour vision (Ishihara colour plates) and relative afferent pupillary defect (3). Because visual acuity (VA) was in most cases severely affected the visual fields (VF) were usually assessed by confrontation. As this is not a quantitative method this precluded the use of perimetry in the quantitative assessment. Exclusion criteria were: any other macula or retinal pathology, contemporaneous symptoms suggestive demyelination elseswere or lack of spontaneous recovery. The study was approved by the local ethics committee. Recovery of VA and colour vision was assessed at each visit and once recovery plateaued no further follow-up was arranged. The last recorded VA was taken as the final outcome. Brain and optic nerve MR imaging was performed using a 1.5 Tesla system (Siemens AG, Erlangen, Germany) and consisted of an axial T1 and T2 weighted spin echo MR imaging (3; 5). Plasma was taken at the first visit and levels of the phosphorylated neurofilament heavy chain (NfH^{SMI35}), NO_x and citrulline were measured as described (17; 16; 14).

Data analysis was performed using SAS. Because of non-Gaussian data distribution the median and interquartile range are shown. All correlations were studied using Spearman 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 using the Mantel-Haenzel (M-Hχ^2) test. A 5 % level of significance was used throughout.

2 Results

The diagnosis of optic neuritis was confirmed in all 18 patients during follow-up. However in 4 patients the blood sample was taken over 4 weeks after onset of symptoms. These patients were therefore excluded from analysis. The remaining 14 patients presented with a median time from onset of 2 weeks (Table 1). At presentation 10/14 (71%) of the patients had a VA of less then 0.33 (6/18, 20/60 Snellen equivalent). In total 13/14 (93%) of patients showed sustained improvement of their VA during follow-up. A minor degree of optic atrophy was observed in 44% of patients whose VA recovered to over 0.33 (6/18). Optic nerve atrophy was more severe in those 36% of patients who did not recover their VA above 0.33.

Plasma levels of NfH^{SMI35} (Figure 1 A) and NO metabolites (NO_x) (Figure 1 B) were significantly higher in ON patients than in controls ($p<0.001$, $p<0.01$, respectively). No such difference was found for citrulline (Table 1). There was a negative correlation between NfH^{SMI35} and VA at the time of first presentation (R=-0.67, p=0.01, Figure 1 C). One outlier with NfH^{SMI35} levels of 0.65 ng/mL was observed. 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 RAPD. This patient had suffered from an episode of myelitis 2 years ago. In the absence of any clinical signs of myelitis no spinal MRI was performed. After the removal of this patient from the study the correlation between NfH^{SMI35} 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 NO_x or citrulline was found at presentation. There was no correlation for either NfH^{SMI35} , NO_x or citrulline with age, time from onset and there was no gender difference.

Plasma NfH^{SMI35} levels at presentation were significantly higher (0.25 ng/mL) in the 36% of the patients with poor recovery of visual function (0.33, 6/18, 20/60 Snellen equivalent) when compared to those whose VA recovered to above 0.33 (0.09 ng/mL, $p<0.05$). Significance remained comparing proportions of patients with NfH^{SMI35} levels above cut–off (Figure 1 A, p=0.015, two–sided Fisher's exact test). There was no difference for the NO_x levels at presentation between those with good versus poor visual recovery at

follow-up (54.2 versus 44.6 μM, p=0.1).

The combined relationship of elevated NO_x and NfH^{SMI35} levels with recovery of visual function was investigated by comparing proportions of patients with high levels (elevated above the top value of the control group; $NfH^{SMI35} > 0.17$ ng/mL, $NO_x > 43.8$ μM). There was a significant trend for increased proportion of patients experiencing a poor recovery (M-Hχ^2=4.6, p$<$0.05) across the following categories: NfH normal and NO_x normal (0/3, 0% poor recovery), NfH normal and NO_x high (1/5, 20% poor recovery), NfH high and NO_x normal (1/2, 50% poor recovery), NfH high and NO_x high (3/4, 75% poor recovery).

MRI was available in 10 of 14 patients, 6/10 had an isolated ON lesion (NfH^{SMI35} 0.12 ng/mL, NO_x 40.7 μM) as opposed to 40% with disseminated brain lesions (NfH^{SMI35} 0.17 ng/mL, NO_x 52.9 μM). Although NfH^{SMI35} and NO_x seemed to be marginally higher in patients with disseminated brain lesions this did not reach statistical significance.

3 Discussion

This is the first study to measure biomarkers for axonal injury (NfH^{SMI35}) and inflammation (NO_x, citrulline) in the plasma of patients with acute optic neuritis. The main findings were that NfH^{SMI35} levels were higher in ON than in control patients, were inversely correlated to visual acuity at presentation and were of prognostic significance. This suggests the presence of axonal damage in the acute phase. The results are in line with clinical, MRI and electrophysiological evidence for optic nerve fibre loss following a single episode of ON and insidiously in MS (18; 19; 20).

Recovery of the VA to $\geq$ 6/18 (20/60) was achieved by less patients (36%) compared to VA $\geq$ 20/50 (88%) in the optic neuritis treatment trial (ONTT) after 5 years follow–up (4). Bradley and Whitty (1967) found that recovery of VA to $\geq$ 20/30 was achieved by 50% after 1 month and 75% after 6 months (21). The result of the present study may relate to the small sample in this study and the relatively short period of follow up (25–189 days).

Recovery of VA in MS related ON has been attributed to resolution of conduction block caused by acute inflammation (19; 20). NO mediated conduction block also seems to be the initial mechanism in *in vitro* studies (2; 22; 23). The present and one other study (5) failed to show a significant relationship between serum NO_x levels and outcome. Additionally NO_x and NfH^{SMI35} did not correlate with MRI parameters, but this could be due to the small study population. The almost 30-40% higher values, if true, might in-

dicate demyelinating activity elsewhere contributing to the worse outcome.

Citrulline was thought to be a second marker for NO mediated inflammation, but it did not correlate with NO_x and no difference between ON and control patients could be shown. This finding is in keeping with one study comparing CSF and serum citrulline levels between patients with Lewy body dementia and healthy controls (18). We agree with these authors that citrulline is not a good surrogate for NO metabolism because it is a substrate for enzymes other than iNOS.

In MS it appears that inflammatory markers were predominately related to disease activity and the MRI lesion load but not outcome (5; 24). In addition epidemiological data suggested that inflammation in MS only has a limited effect on the course of neurodegeneration (25). In placebo–controlled trials and clinical experience with ON anti-inflammatory treatment with steroids does not influence outcome in the majority of cases (3). The destructive and neuroprotective aspects of inflammation have stimulated a controversial discussion (1; 13; 26; 25; 5; 27). It is therefore interesting that in this small group 4/5 (80%) of patients with elevated NO_x levels but normal NfH^{SMI35} levels 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 at least in part contribute to the “axonal death cascade” (1; 2; 8; 9; 7).

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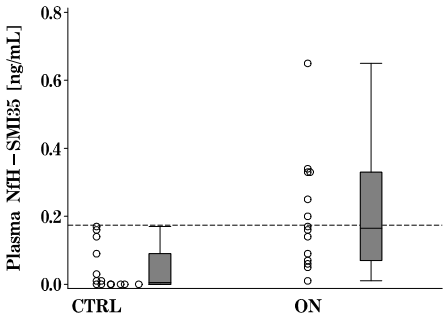
Table 1: *Characteristics of patients expressed as medians (interquartile range). MRI = magnetic resonance imaging, na = not assessed, RAPD = relative afferent pupillary deficit, VFD = visual field defect.*

Feature	Controls	Acute optic neuritis	Significance
Age (years)	36 (30–43)	36 (35-46)	
Male: Female	4:10	9:5	$p<0.05$
Eye	N/A	R 8 : L 6	
RAPD	N/A	R 8 : L 5	
Visual acuity	1.2	0.2 (0.05 – 0.67)	
Colour vision	17/17	0/17 (0/17 – 7/17)	
Disc appearance	Normal	2 swollen, 12 normal	
Time from onset	N/A	14 days (8 – 26)	
Follow–up	N/A	171 days (25 – 189)	
MRI	N/A	10	
Plasma NfH^{SMI35} (ng/mL)	0.005 (0.0 – 0.094)	0.17 (0.07 – 0.33)	$p<0.001$
Plasma NO_x (μM)	36.2 (31.2 – 38.6)	48.1 (40.8 – 54.2)	$p<0.01$
Plasma Citrulline (nmol/mL)	83.4 (72.1 – 92.4)	67.9 (62.5 – 91.9)	N.S.

(A)



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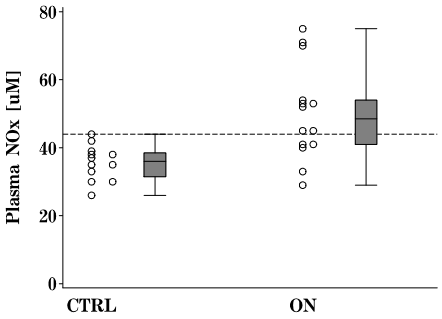

(C)

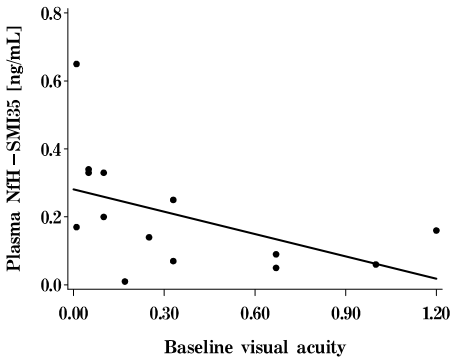

Figure 1. *(A) Plasma NfH^{SMI35} (ng/mL) levels and (B) NO_x (μM) levels in controls (CTRL) and patients with acute optic neuritis (ON). The median (50%), box (25%-75%) and whisker (0%-100%) are shown next to the individual values (open circle). (C) Plasma NfH^{SMI35} correlates with visual acuity (VA) at time of sampling (R=-0.66, p=0.01).*