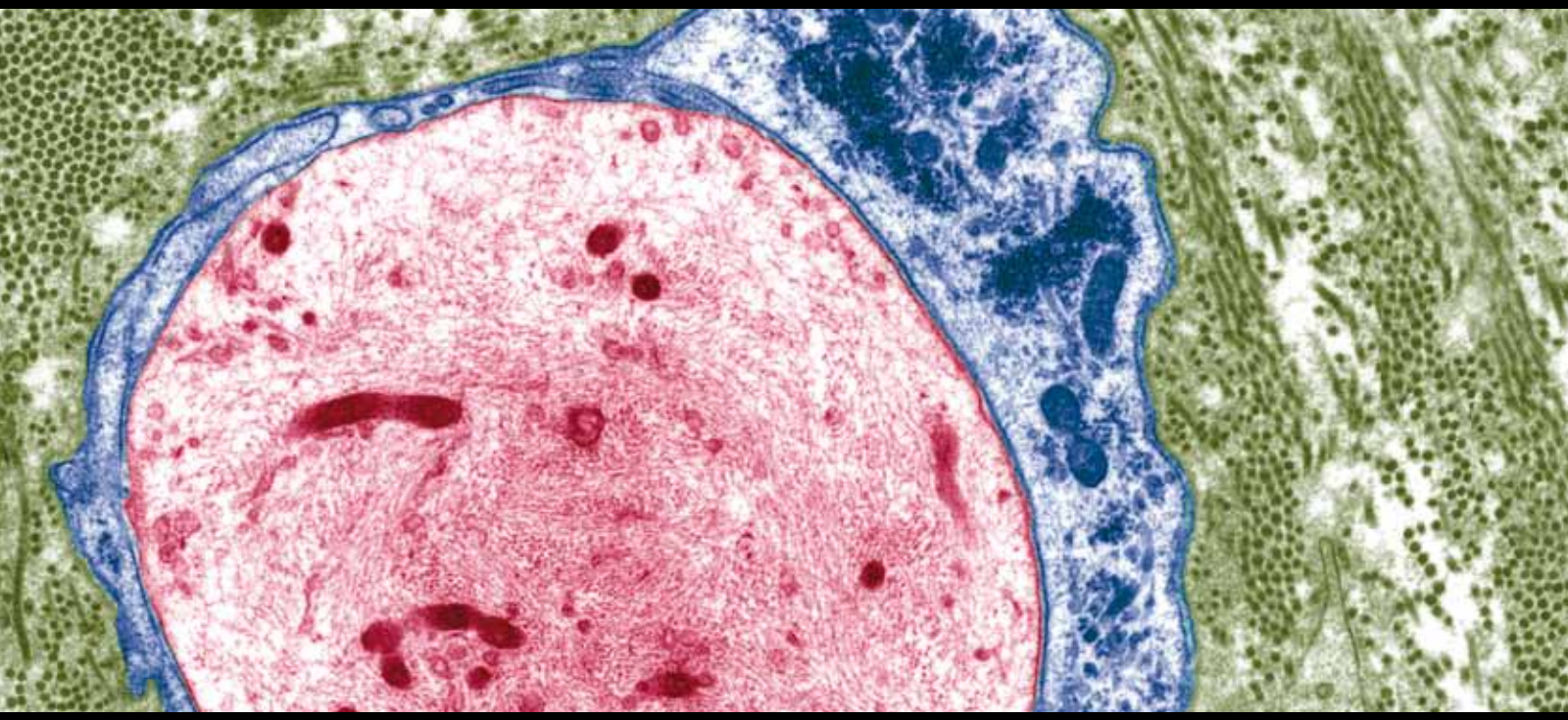
Multiple Sclerosis International

Neurodegeneration in MS and NMO: The Eye and the Blood

Guest Editors: Axel Petzold, Jeroen J. G. Geurts, Ichiro Nakashima, Helmut Butzkueven, and Robert Weissert



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Editorial Board

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Editorial

Neurodegeneration in MS and NMO: The Eye and the Blood

Axel Petzold,[1] Jeroen J. G. Geurts,[2] Ichiro Nakashima,[3] Helmut Butzkueven,[4] and Robert Weissert[5]

[1] *UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK*
[2] *VU University Amsterdam, 1081 HV Amsterdam, The Netherlands*
[3] *Tohoku University, Aoba-ku 980-8576, Japan*
[4] *Department of Medicine, University of Melbourne, Parkville 3010, Australia*
[5] *Department of Neurology, University of Regensburg, Universitaetsstrasse 84, 93053 Regensburg, Germany*

Correspondence should be addressed to Axel Petzold, a.petzold@ion.ucl.ac.uk

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Not surprisingly, patients perceive a considerable reduction of quality of life following impairment of their visual function. What perhaps few of us in the field of multiple sclerosis realized is that patients rank loss of visual function second only to loss of mobility [1]. Fittingly, this first of a series of special issues comprises three reviews on the visual system in multiple sclerosis and related disorders. From Canada, we have F. Costello telling us that the saying "the patient does not see anything, nor does the doctor" may become history with the broader application of a rapidly evolving technology, optical coherence tomography (OCT) in "*Evaluating the use of optical coherence tomography in optic neuritis.*" The data discussed in this review provides compelling evidence that loss of retinal axons can be quantified with high accuracy. Further extending this new technology, S. Noval et al., from Spain, conclude that OCT may "represent an objective outcome measure" for treatment trials in "*Optical coherence tomography in multiple sclerosis and neuromyelitis optica*: an update" Indeed, several therapeutic trials in MS utilizing OCT as a secondary outcome measure are in advanced planning stages. Given the ease and speed of the examination, OCT could become a valuable tool for the MS community. Given the anatomical constraints of the human nervous system, the technique is unlikely to pose a challenge to firmly established magnetic resonance imaging (MRI) of the brain. So those of us who spend hours sleeping as control subjects in ever-increasing magnetic fields may continue collecting colored ear plugs. In fact, the comprehensive review on MRI of the visual pathways by C. Pfueller and F. Paul (Germany) tells why these two methods are complementary (in "*Imaging the visual pathway in neuromyelitis optica*"). Nonetheless, assessment of retinal nerve fibre layer thickness by OCT is a readily accessible measure, unlike cerebral volume, and one could imagine using this quantitative marker for routine monitoring of MS progression in the clinic in the near future.

The focus on the elusive cause of MS has shifted in this special issue to possible mechanisms driving neurodegeneration, the major cause for sustained disability. From Italy we have B. Tavazzi et al. presenting biomarker data which suggests that oxidative damage hangs as a Damocles sword over the impaired energy metabolism in axons damaged in multiple sclerosis in "*Serum metabolic profile in multiple sclerosis patients.*" One perennially popular candidate out of Pandora's box of oxidative damage is iron. In a beautiful translational review M. Khalil et al. from Austria summarize the evidence linking iron to neurodegeneration in MS (in "*Iron and neurodegeneration in multiple sclerosis*"). Neurodegeneration in MS is a process which can be captured in time by using specific biomarkers as illustrated by two comprehensive reviews by I. Dujmovic (Serbia) and M. M. Gresle et al. (Australia) ("*Cerebrospinal fluid and blood biomarkers of neuroaxonal damage in multiple sclerosis*" and "*Neurofilament proteins as body fluid biomarkers of neurodegeneration in multiple sclerosis*"). The lecture of these reviews brings to the readers mind why it is so important to have biomarker research standardized, reflected in a restated and updated consensus paper on this issue by C. Teunissen et al. from The Netherlands (titled "*Consensus guidelines for CSF and blood biobanking for CNS biomarker studies*"). Would it not be nice if we could measure neurodegeneration from a patient's

blood sample similar to what cardiologists can do with troponin? M. J. Eikelenboom et al. also from The Netherlands remind us that our experience with cerebrospinal fluid data (in "*Cerebrospinal fluid and blood biomarkers of neuroaxonal damage in multiple sclerosis*" and "*Neurofilament proteins as body fluid biomarkers of neurodegeneration in multiple sclerosis*") cannot readily be extrapolated to the blood during the chronic phase of the disease in "*Blood and CSF biomarker dynamics in multiple sclerosis: implications for data interpretation.*" Unfortunately during the progressive phase of multiple sclerosis, there is no general convincing evidence for the efficacy of disease-modifying drugs, and experimental options such as repeated intrathecal steroids are still under investigation as M. Abu-Mugheisib et al. from Germany summarize (in "*Repeated intrathecal triamcinolone acetonide administration in progressive multiple sclerosis*: a review"). In this context the anecdotal observation by G. T. Plant et al. from London that steroid treatment in the hyperacute phase of optic neuritis prevents loss of vision is remarkable (in "*Hyperacute corticosteroid treatment of optic neuritis at the onset of pain may prevent visual loss: a case series*"). The eight patients reported in this paper appear as a David compared to the large Goliath-like dataset of the Optic Neuritis Treatment Trial (ONTT) which demonstrated that steroids did not change the outcome of visual function. Nevertheless, small patient numbers might be sufficient if the disease biology is clear-cut: James Lind only investigated twelve sailors to find the cause and treatment for scurvy [2]. The careful examination of patients with acute optic neuritis by F. Costello et al. using OCT in "*Exploring the association between retinal nerve fiber layer thickness and initial magnetic resonance imaging findings in patients with acute optic neuritis*" may just provide the outcome measure needed for a future treatment trial in this direction.

Acknowledgments

It seems timely to express our gratitude to the referees who carefully revised the 19 papers submitted to this first special issue of a new journal. Their constructive comments permitted us to accept 68% of the submissions. We extend our thanks to the Editorial Office, particularly Mrs Miada Elsharkawy, who made it possible to draw together such an international group of authors to what is the first open access journal on multiple sclerosis. We support this activity in the hope that the here published information will reach scientists and clinicians around the world free of charge.

Axel Petzold
Jeroen J. G. Geurts
Ichiro Nakashima
Helmut Butzkueven
Robert Weissert

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Review Article

Neurofilament Proteins as Body Fluid Biomarkers of Neurodegeneration in Multiple Sclerosis

Melissa M. Gresle,[1,2] Helmut Butzkueven,[1,2] and Gerry Shaw[3,4]

[1] *Department of Medicine, University of Melbourne, Parkville, Victoria 3010, Australia*
[2] *Florey Neuroscience Institutes, Howard Florey Institute, Parkville, Victoria 3010, Australia*
[3] *Department of Neuroscience, McKnight Brain Institute, University of Florida College of Medicine, Gainesville, FL 32610, USA*
[4] *EnCor Biotechnology Inc., Suite 40, 4949 SW 41st Boulevard, Gainesville, FL 32608, USA*

Correspondence should be addressed to Melissa M. Gresle, mgresle@unimelb.edu.au

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Biomarkers of axonal degeneration have the potential to improve our capacity to predict and monitor neurological outcome in multiple sclerosis (MS) patients. Neurofilament proteins, one of the major proteins expressed within neurons and axons, have been detected in cerebrospinal fluid and blood samples from MS patients and are now being actively investigated for their utility as prognostic indicators of disease progression in MS. In this paper, we summarize the current literature on neurofilament structure, assembly, and degeneration and discuss their potential utility as biomarkers for monitoring neurological decline in MS. We also discuss the need to further develop sensitive methods for assaying neurofilaments in blood to improve clinical applicability.

1. Introduction

Multiple Sclerosis (MS) is a chronic, debilitating neurological disease with an unknown aetiology. The pathology of this disease is complex and heterogeneous but is typically characterized by the presence of multifocal demyelinated plaques, inflammation, and axonal injury [1]. The functional consequences of this pathology can include visual disturbances, fatigue, depression, weakness, numbness, and cognitive impairment. The earliest symptoms typically begin in young adulthood [2], a time when the diagnosis of an unpredictable, chronic neurological disease with significant and often progressive disability is particularly devastating and unexpected.

Increasingly, axonal injury is recognized as the main pathological correlate of progressive neurological disability in MS [3, 4]. Historically, this axonal damage was thought to be restricted to chronically demyelinated lesions, caused by trophic factor deprivation [5] or maladaptive responses in chronically demyelinated axons [6]. Several histological and imaging studies have now demonstrated, however, that axonal damage may also occur in association with inflammation in acute grey and white matter lesions, and also more diffusely in normal-appearing white matter [7–9].

At present, surrogate markers for axonal damage are not routinely used to monitor disease activity in MS patients. The most commonly used diagnostic and monitoring tool for MS is magnetic resonance imaging, utilizing T_2-weigheted imaging and Gadolinium- (Gd-) enhanced T_1-weighted imaging [10]. These measures, however, lack pathological specificity, which is likely to contribute to the poor association between conventional MRI measures and disability in MS patients [11, 12]. To address this need, several studies have now focussed on the detection of neuronal/axonal proteins in CSF or blood, as biomarkers of axonal degeneration. These studies are based on the concept that degenerating axons release their contents into the surrounding extracellular space, and that some of these axonal components might be abundant and stable enough to be detectable with appropriate assays. The detection of such components would provide a convenient means to assess the presence and degree of axonal degeneration in MS, and this information could be useful for predicting and monitoring the progression of the disease, and for assessing the efficacy of therapeutic strategies that are aimed at preventing axonal loss.

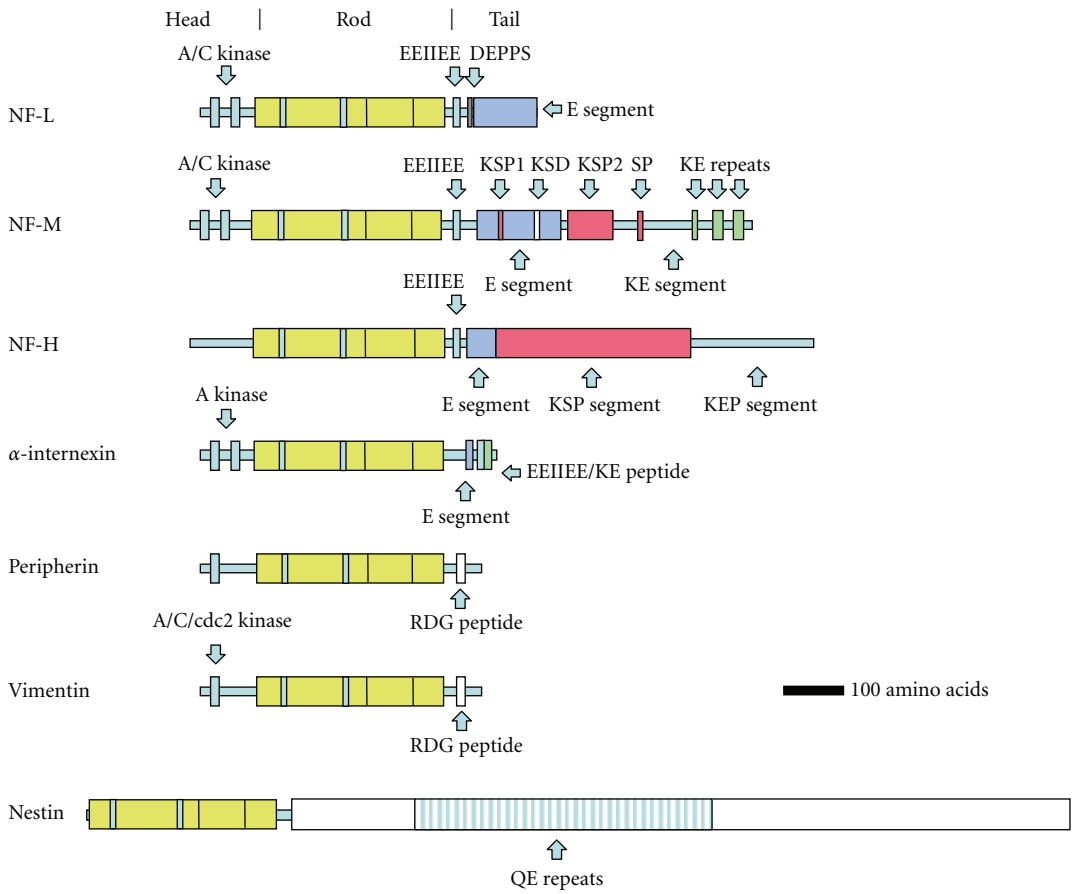

Figure 1: Diagram of the subunit proteins of neurofilaments. NF-L, NF-M, NF-H, and α-internexin can be regarded as the major subunits of adult NFs though NFs may also contain peripherin, vimentin, and nestin in certain locations, developmental stages, and possibly damage or disease states. Phosphorylation sites for protein kinase A (A kinase), protein kinase C (C kinase), and cdc2 kinases (cdc2 kinase) have been characterized in the globular "head" regions of certain of these molecules as indicated. The regions indicated by KSP, SP, KSD, and DEPPS are known serine phosphorylation sites in the "tail" regions. EEIIEE, KE repeats, Tail a, E segment, KEP segment, KE segment, RGD, and QE repeats each refer to specific kinds of sequence motif. For further details, see Shaw 1998 [13].

2. Introduction to Neurofilament Proteins

Neurofilaments (NFs) are the major structural proteins of neurons. They are most abundant in larger neurons and are heavily concentrated in axons, in particular long projection axons. The subunits of NFs belong to the intermediate (IF) family of proteins, which are characterized by a structurally conserved α-helical coiled-coil "rod" region which forms the backbone of the filament, with variable N and C terminal extensions (Figure 1). The IF subunits of vertebrates are divided into five classes based on protein characteristics, expression pattern, and intron placement. IF subunits Class I and II include the epithelial keratins; the class III IFs include vimentin, desmin, and glial fibrillary acidic protein (GFAP), while the major neurofilament subunits NF-Light (NF-L), NF-medium (NF-M), NF-Heavy (NF-H), α-internexin, and nestin form IF class IV. Class V IFs are the lamin proteins of the nuclear matrix. The expression profile of Class IV proteins is limited to the nervous system, with the exception of nestin, which may be found in stem cells throughout the body. All 5 Class IV genes share a distinct intron pattern from that seen in other IF genes, indicating a close evolutionary relationship. The class IV subunits NF-H and NF-M have unusually long and complex C-terminal "tail" regions which are responsible for the wispy spacers seen by electron microscopy and the wider spacing of NFs compared to other IFs. NF-L, NF-M, NF-H, and α-internexin are all abundant proteins of the nervous systems of adult mammals, while nestin is expressed early in development and is normally downregulated in the adult. One class III IF protein, peripherin, is found copolymerized with NF-L, NF-M, NF-H, and α-internexin in the NF of some neurons in significant amounts, particularly in the peripheral nervous system. Finally, a few apparently unusual neurons in the adult express another Class III protein, vimentin. Neuroblasts express this protein, but it is generally downregulated as development proceeds. However, in many damage and disease states, cells will re-express proteins which were downregulated developmentally, so that expression of any of the 7 subunits shown in Figure 1 could be associated with specific damage or disease states. The various proteins which may be included in NFs are known to be phosphorylated,

Table 1: Summary of studies assessing the utility of neurofilaments as biomarkers of axonal damage in patients with multiple sclerosis (MS) and clinically isolated syndrome (CIS).

Biomarker	Fluid	Study design	Observations	Associations with clinical measures	Ref.
NF-L	CSF	RRMS ($n = 60$); LP on trial initiation and 2 yr	78% patients showed ↑ at 0 or 2 yrs. Associated with recent relapse. No relation with age, gender, or disease duration.	EDSS 0 yr ($r^2 = 0.27,\ P < .05$); 2 yr ($r^2 = 0.35,\ P < .01$)	[16]
NF-L	CSF	RRMS ($n = 41$), SPMS ($n = 25$), healthy ($n = 50$)	↑ mean level in all MS subtypes. Highest during acute relapse.	EDSS (ns)	[17]
NF-L	CSF	CIS ($n = 38$), RRMS ($n = 42$), SPMS ($n = 28$), PPMS ($n = 6$); diagnostic LP	MS/CIS > controls. ↑ NF-L associated with relapse, T2 lesion number, Gd enhancing lesions. ↑ in CIS that convert to RRMS.	EDSS ($r = 0.192, P < .05$)	[18]
NF-L	CSF	RRMS ($n = 16$), SP/PP MS ($n = 18$)	MS > controls	EDSS ($r = 0.41, P < .05$)	[23]
NF-L	CSF	RRMS ($n = 47$)	MS > controls	—	[24]
NF-L	CSF	CDMS ($n = 47$)	MS > controls	—	[25]
NF-L	CSF	RRMS ($n = 65$), SPMS ($n = 10$), PPMS ($n = 20$); LP on initiation. Followup at 5 and 14 yrs.	↑ NF-L associated with recent relapse; associated with 3-fold ↑ in risk of developing high MSSS; more likely to convert from RRMS to SPMS.	MSSS 14 yr ($r = 0.3, P < .01$)	[26, 27]
NF-H	CSF	CIS ($n = 38$), RRMS ($n = 92$), SPMS ($n = 28$), PPMS ($n = 6$); diagnostic LP	MS > controls. SP/PPMS > RRMS. ↑ levels at CIS do not predict conversion to RRMS. ↑ with relapse. Correlated to age.	EDSS ($r = 0.253,\ P < .01$)	[18]
NF-H	CSF	RRMS ($n = 11$), SP/PPMS ($n = 23$); LP on trial initiation and 3 yr	SP/PPMS > RRMS.	EDSS 3 yr (ns trend)	[19]
NF-H	CSF	CIS ($n = 52$), RRMS ($n = 38$)	CIS > controls. ↑ acute relapse.	Correlated with EDSS for CIS and RRMS.	[28]
NF-H	Plasma	RRMS ($n = 30$)	Median levels RRMS > controls	—	[20]

Abbreviations: NF-L: neurofilament light; NF-H: neurofilament heavy; CSF: cerebrospinal fluid; RRMS: relapsing-remitting MS; SPMS: secondary progressive MS; PPMS: primary progressive MS; CDMS: clinically definite MS; LP: lumbar puncture; Gd: gadolinium; EDSS: expanded disability status scale; MSSS: multiple sclerosis severity scale; NS: nonsignificant; Ref: reference.

glycosylated, and modified on many sites and contain many interesting protein sequence motifs, details of which are discussed in previous publications [13, 14].

It is noteworthy that for a typical large projection neuron, the volume of the axon may exceed that of the cell body by a factor of a thousand or more and that NFs can occupy more than 90% of the axonal cross section. The function of NFs appears to be to provide axons with mechanical strength and to control axonal volume. Apparently, in order to meet these requirements, NF subunits have a very long half-life and are resistant to endogenous proteases. It is therefore reasonable to assume that large amounts of NFs, their subunits, breakdown products, and associated proteins would be released following axonal loss in MS and in other damage and disease states, and that their abundance and stability might make them relatively easy to detect. There has therefore been much interest in the use of these proteins as potential biomarkers of damage, disease, and progression in a variety of neurological states, including MS [15].

3. Neurofilaments As Biomarkers of Axonal Degeneration in MS

Several studies have now demonstrated the presence of NF peptides in the cerebrospinal fluid (CSF) of MS patients (summarized in Table 1). Most commonly, CSF levels of these proteins have been assessed using enzyme-linked immunosorbent assays (ELISAs) [16–20]; however, Western blotting and dot blotting have also been utilized on occasion [21–23].

3.1. NF-L. The potential use of the NF protein subunits as surrogate markers of axonal degeneration in MS was first explored by Lycke et al. [16], who developed an NF-L ELISA in house using an affinity purified chicken NF-L antibody. In this study, CSF levels of NF-L protein were measured in 60 patients with clinically definite relapsing-remitting MS (RR-MS). The CSF samples were collected from patients on initiation of the trial, and then 2 years later. It was

demonstrated that CSF NF-L levels were increased on at least one occasion in 78% of cases, and that these levels were moderately associated with disability. Levels of NF-L were also found to be higher in patients who had suffered a relapse within 3 months of sampling, indicating a temporal correlation between acute inflammatory activity and neurodegeneration in this disease. An interesting observation to arise from this study was that sequential CSF NF-L samples were not persistently elevated in all patients, highlighting the dynamic nature of MS-associated axonal degeneration.

Increases in intrathecal NF-L were subsequently confirmed by several groups, for both RR-MS and progressive MS cases [17, 23–25]. In accordance with original observations made byLyke et al. [16], a small prospective study of 13 RR-MS patients with recent relapse confirmed that NF-L levels peak during acute relapse and decline within 3 months [17]. In this same study, CSF NF-L levels were also assessed in a larger group of 66 patients with clinically definite MS for associations with disability as assessed by expanded disability status scale (EDSS) and neurologic symptoms. The level of NF-L in CSF was not found to be associated with neurological disability outcome measures.

In a more recent set of retrospective studies, NF-L levels were measured from CSF samples collected at diagnostic lumbar puncture in 99 patients with clinically definite MS, to evaluate whether NF-L levels at diagnosis could be used to predict more rapidly progressing disease. Of these, 94 patients had comprehensive clinical data available to conduct association studies between CSF NF-L levels at diagnosis, and disease severity at 5 years [26] and 14 years [27]. It was found that elevated NF-L levels were associated with a 3-fold increase in the risk of developing severe MS, as estimated by bivariate and multivariate logistic regression analysis, particularly among cases with RR-MS and cases with a recent relapse. Further, approximately 60% of patients with high CSF NF-L levels (>386 ng/mL) converted from RR-MS to secondary progressive MS (SP-MS) within the 14-year followup period compared to 30% of patients with moderate or low levels (<386 ng/mL). These studies suggest that high CSF NF-L levels, assessed in early MS, are potentially predictive of more rapid disease progression over time. High NF-L levels in early MS may also be useful for predicting conversion to progressive disease. In accordance with these studies, it has also been reported that CSF NF-L levels are higher, on average, in clinically isolated syndrome (CIS) cases that convert to RR-MS within 3 years compared to nonconverters [18]. Collectively, this recent work supports the use of NF-L CSF levels as a prognostic indicator of disease course in MS patients.

3.2. NF-H. The high-molecular-weight NF subunit, NF-H, has also been a focus of biomarker studies. Compared with assessments of CSF NF-L levels, however, fewer studies have been conducted to evaluate NF-H as a biomarker of neurodegeneration in MS. The axonal form of NF-H is heavily phosphorylated [21], is resistant to proteolysis [29], and is very immunogenic, which allows it to be sensitively detected using appropriate immunological assays [30]. The phosphorylated region of this axonal form of NF-H (here referred to as pNF-H) is also very unusual, comprising ~50 tandem repeat lysine-serine-proline (KSP) containing peptides, the serine of each being a phosphorylation site [13, 31]. These unusual properties make pNF-H an ideal target for immunological detection, since it is stable upon release from neurons and can be captured and detected with exceptionally high avidity due to its exotic multiepitope nature. Since pNF-H is only found in axons, its detection in CSF, blood or other bodily fluids points unambiguously to release of this protein from axons.

The first ELISA method described for NF-H made use of the commercial SMI35 monoclonal antibody in the capture role [32]. SMI35, available from Covance (Princeton, NJ) is specific for the axonal, heavily phosphorylated form of NF-H, namely pNF-H. This ELISA was subsequently utilized in a 3-year followup study conducted to evaluate CSF levels of pNF-H in RR-MS and progressive MS cases. In contrast to observations for NF-L, the median pNF-H levels were found to be highest for patients with progressive disease [19]. There was also some evidence to support an association between pNF-H level and EDSS at followup, and also a potential propensity for patients with high pNF-H levels at baseline to exhibit progression in disease EDSS at followup. These analyses did not, however, reach statistical significance, most likely due to the limited sample size. In support of these observations, CSF pNF-H levels were shown to be weakly associated with EDSS ($R = 0.253$, $P < .009$) in a larger cohort of patients comprising CIS ($n = 38$), RR-MS ($n = 42$), SP-MS ($n = 28$), and primary progressive MS (PP-MS, $n = 6$) cases [18]. In this study, the average CSF pNF-H levels were higher in patients with all subtypes of MS relative to samples taken from controls. Interestingly, CSF pNF-H levels were, on average, 1.5-fold higher for SP-MS and PP-MS cases relative to RR-MS cases. Further, in contrast to observations for NF-L, levels of CSF pNF-H in CIS cases did not predict conversion to clinically definite MS. These studies suggest the pNF-H may be more useful as a measure of ongoing neurodegenerative activity in MS patients, which would make this protein a potential candidate for use as a surrogate marker for assessment of treatments aimed at reducing axonal injury. A retrospective study of 30 patients has already been conducted to determine whether plasma pNF-H levels could be used to measure responsiveness to interferon-β treatment. Although it was found that plasma pNF-H levels tended to be higher in patients who did not respond well to Interferon-β treatment, this did not reach statistical significance, possibly due to the small sample size. Hence, additional studies are required to assess the utility of blood or CSF pNF-H levels as an indicator of disease activity and, potentially, therapeutic efficacy.

3.3. NF-M and α-Internexin. The other major neurofilament subunits of the mature nervous system, NF-M and α-internexin, are presumably, like NF-L and NF-H, released during axonal degradation. Some evidence suggests that α-internexin is a particularly unstable protein, difficult to isolate biochemically because it is readily degraded [33], and

therefore the intact form of this protein is unlikely to be a suitable biomarker. On the other hand, the NF-M appears to be intermediate in resistance to proteases compared to NF-L and NF-H but has not, to date, been studied as a potential biomarker [34].

4. Axonal Injury Biomarker Panels Utilising Neurofilament Proteins

The use of multiple axonal injury biomarkers in combination panels in CSF has been explored as a method to gain additional power to explore disease prognosis and activity. The premise behind these studies is that individual axonal biomarkers may only reflect particular aspects of disease activity or may be released at different stages in the degenerative process and are therefore likely to be less informative in isolation than in combination.

Studies by Brettschneider et al. [28] measured CSF levels of the axonal cytoskeletal proteins microtubule-associated protein tau and pNF-H in the same MS patients to ascertain whether this could improve the sensitivity of predicting disability progression relative to conventional MRI methods. Interestingly, when utilized alone, changes in CSF pNF-H or tau levels did not increase the sensitivity for predicting conversion to clinically definite MS in patients with CIS at 48-month followup. However, by testing CSF for either tau or pNF-H, it was possible to improve the sensitivity and specificity of these biomarkers relative to MRI. Interestingly, higher CSF levels of pNF-H, but not tau, were found to be associated with higher EDSS in both CIS and RR-MS, which may indicate that NF-H proteins are more sensitive indicators of axonal damage that is associated with physically disabling symptoms of MS.

Teunissen et al. [18] have also evaluated the utility of combinations of axonal biomarkers for monitoring disease activity in MS patients. In their study, levels of neuron-specific amino acid n-acetyl aspartate (NAA), NF-L, pNF-H, and tau were measured in the same CSF samples and were then assessed for associations with disease subtype, disability, and MRI outcome measures. Each of these proteins showed specific patterns of change over the course of the disease, with the highest average NF-L levels observed in RR-MS patients. Conversely, average NAA and pNF-H levels were highest in patients with progressive disease. Levels of tau were not significantly changed between the groups. It was proposed that these differences could reflect variability in the dynamics of release for these axonal proteins during degenerative processes. However, both of these observations need validation in independent cohorts.

5. Neurofilament Protein Subunits As Blood Biomarkers of Axonal Degeneration in MS

Despite these promising observations, the clinical utility of CSF biomarkers is limited by the lack of acceptability, invasiveness, and risks posed by repeated lumbar puncture. Hence, blood biomarkers of axonal degeneration could provide a major advance in the paraclinical monitoring of disease activity in MS. The development of methods for measuring biomarkers in serum is, however, complicated by the potential influence of the blood brain barrier on the dynamics of release of these proteins into blood and the much higher protein concentration and complexity of blood as compared to CSF. In addition, proteins released into blood may be subjected to proteolysis and may bind to blood components that are actively cleared from the blood. However, blood brain barrier compromise appears to be an early feature of the MS disease process [35], and presumably, potential axonal biomarker proteins released into the extracellular space in lesions would have ready access into blood from sites of active MS pathology. Such proteins could then be detected with assays of sufficient affinity and avidity to work effectively in the concentrated and complex protein context of blood, plasma, and serum.

Relatively few studies have evaluated blood biomarkers as potential measures of neurodegeneration in MS. Thus far, a suitable method for detecting NF-L in blood has not been identified. The pNF-H protein, however, has been detected in blood samples, using two independently developed ELISA methods. The SMI35-based pNF-H ELISA was used to show that in a small group of 30 RR-MS patients, median plasma pNF-H levels were elevated relative to healthy controls [20]. This same method was used to demonstrate that in patients with acute optic neuritis, elevated plasma NF-H levels were associated with poor recovery of visual acuity and were also inversely correlated with visual acuity at presentation [36].

One of the present authors (G. Shaw) has also published a pNF-H assay which used an affinity purified chicken polyclonal antibody in that capture role (Cα-pNF-H ELISA) [37], which has become commercially available from EnCor Biotechnology Inc. (Gainesville, FL), from BioVendor (Modrice, Czech Republic) and from Millipore (Billerica, MA). This assay has been used on a set of human CSF samples which included MS samples and shown to produce signals very similar to the SMI35-based assay [38]. A second generation pNF-H assay, using a novel monoclonal pNF-H capture antibody screened originally by ability to capture pNF-H from concentrated protein solutions, has also been described and is marketed by EnCor Biotechnology (Mα-pNF-H ELISA) [39]. These ELISA methods have been used to measure pNF-H in serum in several neurological conditions including aneurysmal subarachnoid haemorrhage [40], amyotrophic lateral sclerosis [39], Leber's hereditary optic neuropathy [41], and neonatal hypoxic-ischemic encephalopathy [42, 43], but no publication has as yet described the use of this assay on plasma or serum samples from MS patients.

We have shown, however, that the Cα-pNF-H ELISA is a powerful serum marker of spinal cord axon loss in mice with experimental autoimmune encephalomyelitis (EAE), an experimental model of MS. In this model, serum pNF-H levels are associated with axonal loss ($r = -0.80$, $P < .001$) and disability ($r = 0.75$, $P < .001$), providing evidence that serum pNF-H levels accurately measure axon loss during inflammatory neurodegenerative changes in the CNS [44]. Interestingly, a drug known to ameliorate the EAE phenotype in these mice also greatly reduced the blood

pNF-H levels. This suggests that serum pNF-H levels might be used in animal models to discover novel MS drugs and, if established in humans, to monitor the effectiveness of therapies in MS patients. In preliminary studies, we have also used the Mα-pNF-H ELISA to show that serum pNF-H levels are elevated in around 10% of RRMS patients who show more rapidly progressing disease, suggesting that previously reported changes in CSF pNF-H levels in association with disease progression in MS could also be reflected in serum. Importantly, we have also been able to confirm the presence of NF-H peptides in these samples by MALDI-TOF mass spectrometry methodology, further validating the likely specificity of this ELISA methodology for detecting NF-H peptides [45].

6. Conclusion

The detection of NF subunits holds considerable promise as a means to monitor axonal loss in in MS patients. Work is being focused on the development of yet more specific and sensitive assays for NF proteins of utility both in CSF and blood. It will also be important to understand how these proteins are released from axons degenerating as a result of the MS disease process. While pNF-H released into the CSF of aneurysmal subarachnoid hemorrhage patients is mostly intact and unproteolyzed [40], we do not currently know what form is present in the blood of these patients, or in fact, any other group of patients. This raises the interesting possibility that NF subunits that are released with damage may be processed in disease-specific ways, and that these products might be detectable with refined assays of appropriate specificity. If this is correct, it may eventually be possible to detect axonal loss specifically due to the MS disease process. Future studies should therefore aim to develop more sensitive methods for measuring the various NF subunits in CSF, plasma, or serum to ascertain whether these biomarkers will be useful in the clinic for predicting MS onset, and for monitoring MS progression and response to therapy.

Disclosure

G. Shaw holds equity in EnCor Biotechnology Inc., a company that commercializes antibodies for some of the ELISA methods that are discussed in this paper, and may benefit by receiving royalties or equity growth.

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Review Article

Cerebrospinal Fluid and Blood Biomarkers of Neuroaxonal Damage in Multiple Sclerosis

Irena Dujmovic

Neurology Clinic, Clinical Center of Serbia, Dr Subotica 6, P.O. Box 12, 11129 Belgrade 102, Serbia

Correspondence should be addressed to Irena Dujmovic, irdujm@eunet.rs

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Following emerging evidence that neurodegenerative processes in multiple sclerosis (MS) are present from its early stages, an intensive scientific interest has been directed to biomarkers of neuro-axonal damage in body fluids of MS patients. Recent research has introduced new candidate biomarkers but also elucidated pathogenetic and clinical relevance of the well-known ones. This paper reviews the existing data on blood and cerebrospinal fluid biomarkers of neuroaxonal damage in MS and highlights their relation to clinical parameters, as well as their potential predictive value to estimate future disease course, disability, and treatment response. Strategies for future research in this field are suggested.

1. Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by unpredictable clinical relapses and remissions and/or by progression of disability over time [1]. Relapses are considered to be the clinical expression of acute inflammation in the CNS, whereas progression reflects chronic demyelination, gliosis, and axonal loss [2]. Although axonal/neuronal damage has been recognized in MS for more than a century [3], a refocused interest on the role of axonal pathology and neurodegeneration as the cause of permanent neurological disability in MS patients appeared since the 1990s [4–9]. The development of new immunostaining protocols and new magnetic resonance imaging (MRI) techniques has enabled earlier detection of more subtle changes in diffuse neuroaxonal pathology not only within focal white matter [6, 10] and gray matter lesions [11–13], but also within normal appearing white matter (NAWM) [14–16] and normal appearing gray matter in MS [14, 15]. Current evidence suggests that axonal loss occurs at an early stage of MS [6, 17], but because of CNS compensatory mechanisms it remains clinically silent until a threshold level of axonal loss is achieved and the functional reserve capacity is exhausted [9, 18]. Subsequent progressive axonal loss underlies a continuous and irreversible neurological decline [19], causing a transition from initially relapsing remitting (RR) to the secondary-progressive (SP) MS [7, 9].

Since inflammation correlates only poorly with disability and the loss of neurons and axons may be subject to biochemical monitoring [20], biochemical markers of neuroaxonal degeneration gain increasing importance. Such biomarkers could provide tools for development and evaluation of new therapeutic strategies [21] and might serve as prescreening tools and/or cross-sectional surrogate endpoints in MS clinical trials [22, 23], more importantly in those testing potentially axon-protective compounds [24]. Additionally, the assessment of neuroaxonal biomarkers could help in better understanding of MS pathogenesis and identification of disease subtypes [22], as well as in routine patient management for (1) prediction of conversion to MS after a first clinical episode, (2) early prediction of disease course and future disability, (3) selection of patients for individually tailored treatments, and (4) monitoring of disease activity and individual treatment response [23, 25–27]. However, it is unlikely that a single biomarker could serve for any of these aims due to the extreme complexity of the pathogenetic processes which cause tissue damage and neuroaxonal loss in MS [26].

Recent research has introduced new candidate biomarkers but also elucidated pathogenetic and clinical relevance

of the well-known ones. This paper reviews the existing data on blood and cerebrospinal fluid (CSF) biomarkers of neuroaxonal damage in MS in the light of their clinical relevance and suggests strategies for future research in this field.

2. Mechanisms of Neuroaxonal Damage in MS

The mechanisms leading to axonal damage in MS are essentially not well elucidated [21]. However, challenging some clinical [28–30], neuroradiological [31], and neuropathological [11, 32] observations that neurodegeneration in MS might progress independently from or even precede the inflammation, recent neuropathological reports confirmed the positive correlation between axonal pathology and the degree of inflammation even in cases with progressive MS [33, 34]. This further supports the scenario in which a variety of effectors from the inflammatory microenvironment could injure axons, such as direct attack by autoreactive antibodies [35–37] or cytotoxic $CD8^+$T-cells [36, 38, 39], invading macrophages, proteolytic enzymes, cytokines, nitric oxide [39–41] and free radicals [42, 43], defects in calcium homeostasis [21], glutamate-mediated excitotoxicity [43, 44], an increased axonal energy demand [45], and mitochondrial injury and failure [45, 46] (Figure 1). Axonal damage could also be secondary to acute or chronic demyelination [6, 47], damaging changes in sodium channel distribution [2, 48], and disruption of axonal/glial interactions [49, 50] as well as related to the lack of myelin-derived trophic deprivation and/or impaired axonal regeneration by axon growth inhibitory molecules including those from myelin debris recently called myelin-associated inhibitory factors (MAIFs) such as myelin-associated glycoprotein, oligodendrocyte myelin glycoprotein, Nogo-A, semaphorin 4D/CD100, and ephrin B3 [51, 52] (Figure 1).

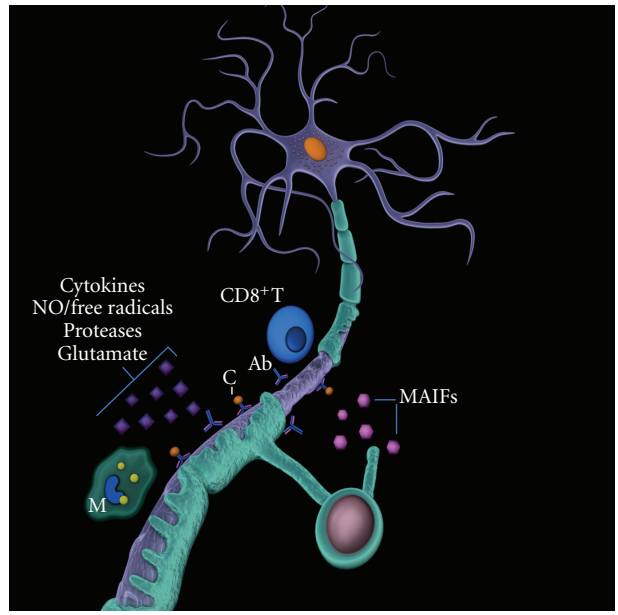

Figure 1: The mechanisms of neuroaxonal damage in multiple sclerosis. Legend: NO: nitric oxide; M: macrophage; C: complement; Ab: antibody; $CD8^+$T: $CD8^+$T-lymphocyte; MAIFs: myelin-associated inhibitory factors.

3. Biomarkers of Neuroaxonal Damage in Multiple Sclerosis

Following damaging processes, molecules released from neuronal cytoplasm, membrane, or nucleus are released into the extracellular CNS compartment (Figure 2) and their further metabolic, transport, and reuptake mechanisms, drainage pathways or other interactions with the CNS tissue, as well as the degree of tissue destruction would determine the level of these substances in CSF and blood [21].

CSF analysis is more pathology specific as it provides information from the body fluid that is most closely associated to the disease process [53], but sometimes substances measured in lumbar-sac-CSF are not necessarily completely representative of brain pathology [20]. However, there is a need for new biomarkers in more easily accessible body fluids such as peripheral blood [53], since substances produced within the CNS and found in the blood could also be representative of the ongoing CNS pathology [20].

CSF and/or blood levels of biomarkers associated with neuroaxonal injury in patients with MS and clinically isolated syndrome suggestive of MS (CIS) are summarized in Tables 1, 3, 4 and 5 and their relation to clinical parameters in Table 2.

3.1. Neurofilaments. Neurofilaments (NFs) are cytoskeletal proteins which play a role in stabilizing axons, determining axon diameter and participate in axonal transport [54, 55]. As NFs are found exclusively within neurons, their detection in blood or CSF therefore reflects neuronal and axonal damage [56]. Mammalian NFs consist of different subunits: NF-light chain (NF-L), which serves as a backbone for NF-intermediate chain (NF-M) and a heavy chain (NF-H) to copolymerise [21]. The most abundant, the smallest and most soluble NF subunit is NF-L but is susceptible to proteases [57]. On the other hand, HF-H is a larger molecule and more resistant to proteases if phosphorylated [58]. Phosphorylated parts of NF molecules are mostly abundant within HF-M and NF-H subunits [59], and the state of phosphorylation influences axonal diameter [60]. The highly phosphorylated NF-H are normally found only in axonal NFs and this marker is thought to indicate axonal injury and/or degeneration [56], whereas NF-L constitutes only a minor part of the neuronal cell body and dendrites relative to axons [61]. NF-H phosphorylation may increase during the progressive phase of MS [62]. Due to the lower molecular weight of NF-L and/or its lower phosphorylation rate, NF-L could diffuse earlier from the parenchyma into CSF than NF-H, but also could be degraded quicker [54]. Although changes in the blood-brain barrier (BBB) might influence the CSF NF-L concentration, the degree of such influence was considered to be negligible [61].

Table 1: Neurofilament subunits in the cerebrospinal fluid (CSF) and blood of patients with multiple sclerosis (MS) and/or clinically isolated syndrome suggestive of MS (CIS).

Biomarker (subtype)	Body fluid	Immunoassay	Number of patients	Main findings	REF
NF-L *(cytoplasmic)*	CSF	ELISA	60 MS (RR)	RR ↑↑ HCo	[61]
	CSF	Dot-blot	35 MS (RR/SP/PP)	MS ↑↑ OIND or NIND, PP/SP ↑↑ RR	[63]
	CSF	ELISA	66 MS (RR/SP)	RR/SP ↑↑ HCo	[64]
	CSF	ELISA	99 MS (RR/SP/PP/PR)	RR/SP/PP ↑↑ NHCo, SP the highest	[65]
	CSF	ELISA	51 MS (RR/SP/PP)	not detected	[66]
	CSF	ELISA	47 MS	MS ↑↑ healthy siblings or HCo	[67]
	CSF	ELISA	76 MS (RR/SP/PP) + 38 CIS	CIS+MS ↑↑ NIND+OIND + NHCo	[54]
	CSF	ELISA	5 MS (RR)	MS ↑↑ NHCo	[68]
NF-M *(cytoplasmic)*	CSF/PL/SE	not investigated	—	—	—
NF-H *(cytoplasmic)*	CSF	ELISA	38 MS (RR) + 52 CIS	NF-H^{SMI35} in CIS ↑↑ NHCo	[25]
	CSF	ELISA	41 ON	NF-H^{SMI34} and NF-$H^{SMI34/SMI35}$ in ON ↑↑ OND	[69]
	CSF	ELISA	51 MS (RR/SP/PP)	NF-H^{SMI35} RR ↔ SP ↔ PP	[66]
	CSF	ELISA	34 MS (RR/SP/PP)	NF-H^{SMI34} in PP/SP ↑↑ RR, NF-H^{SMI35} in SP/PP ↑↑ RR	[62]
	CSF	ECL	95 MS	MS ↑↑ NHCo	[23]
	CSF	ELISA	24 MS (RR/SP/PP)	NF-H^{SMI35} in SP ↑ RR	[70]
	PL	ELISA	18 ON	NF-H^{SMI35} in ON ↑↑ HCo	[71]
	CSF	ELISA	34 MS (RR)	NF-H^{SMI35} in MS ↑↑ NHCo	[72]
	CSF	ELISA	76 MS (RR/SP/PP) + 38 CIS	NF-H^{SMI35} in MS+CIS ↑↑ NHCo + NIND + OIND	[54]

REF: reference; NF-L: neurofilament-light chain; NF-M: neurofilament-intermediate chain; NF-H: neurofilament-heavy chain; PL: plasma; SE: serum; ELISA: Enzyme-Linked Immunosorbent Assay; ECL: Electrochemiluminescence-based solid-phase sandwich immunoassay; RR: relapsing-remitting MS; SP: secondary-progressive MS; PP: primary-progressive MS; ON: optic neuritis; NF-H^{SMI35}: NF-H phosphorylated form; NF-H^{SMI34}: NF-H hyperphosphorylated form; ↑↑ significantly higher than; ↑ higher than; ↔ no difference between; HCo: healthy controls; OIND: other inflammatory neurological diseases; NIND: noninflammatory neurological disorders; NHCo: neurologically healthy controls; OND: other neurological diseases.

Healthy individuals have no NF-L in their CSF, whereas most people with neurological disorders, such as amyotrophic lateral sclerosis, stroke, MS and Alzheimer's disease, can have elevated levels [73]. Several studies have shown the increase of the CSF NF-L levels in MS or CIS patients (Table 1), in the latter more so in those who converted to MS within 3 years [54]. On the other hand, CSF NF-L was detectable at low concentrations [74], or even undetectable in some other studies albeit the assay was similar to that used by others [66]. CSF NF-L levels were reported to be increased during acute relapses [54, 64], in patients with enhancing MRI lesions [54], as well as in patients with higher relapse rate [61] (Table 2) and were also shown to have a peak during the first two months after the start of the previous exacerbation and to gradually decrease thereafter to a low level [61]. A correlation between NF-L with Expanded Disability Status Scale (EDSS) score as a disability measure was found in some studies [54, 63]. Norgren et al. [65] reported a significant correlation between CSF NF-L levels and progression index over 10 years whereas in a recent study the risk for high *Multiple Sclerosis* Severity Score (*MSSS*) at long-term follow-up after 14 years was increased threefold for cases with high NF-L levels [75]. Conversion from RRMS to SPMS was more likely in cases with high CSF NF-L levels when compared with those with undetectable or intermediate NF-L levels [75] (Table 2). Other authors could not demonstrate any correlation with disability measures [64, 66]. In some studies CSF NF-L concentration did not correlate with gender or age [61, 64, 66, 67] or disease duration [64, 65], but in some reports CSF NF-L levels were found to increase with age [68]. Blood NF-L levels have not been reported to date (Table 1).

NF-M subunit has not been analysed so far in body fluids of MS patients.

In patients with optic neuritis (ON), the levels of NF-H phosphorylated form (NF-H^{SMI35}) in plasma [71], or its hyperphosphorylated form in CSF (NF-H^{SMI34}) [69], as well as CSF NF-H^{SMI35} levels in CIS patients [25], were found to be significantly higher compared to controls (Table 1). Significantly higher CSF NF-H levels in MS patients than in control subjects (Table 1) were also reported in several recent studies (Table 1) [23, 54, 72], with higher [70], or significantly higher [54, 62] levels in patients with a progressive course. Opposite to these findings, no difference in CSF levels of this biomarker was found between RR, SP and primary progressive (PP) MS patients by Eikelenboom et al. [66]. In some studies, CSF NF-H levels correlated significantly with EDSS score both in CIS [25] and MS patients [54, 62].

Table 2: Biomarkers of neuroaxonal damage in patients with multiple sclerosis (MS) and/or clinically isolated syndrome suggestive of MS (CIS) in relation to clinical parameters.

Biomarker	Correlation with disability*	Correlation with disease activity*	Prediction of CIS conversion to CDMS*	Prediction of future disease course*	Prediction of future disability*	Prediction of treatment response*
NF-L	4 (308) + 2 (117) −	4 (339) +	1 (38) +	1 (95) +	3 (308) +	—
Anti-NF-L	3 (180) + 2 (181) −	—	—	—	—	—
Anti-NF-M	1 (47) + 1 (49) −	—	—	—	—	—
NF-H	5 (256) + 2 (81) −	3 (254) + 1 (30) −	1 (52) +	1 (34) +	3 (86) +	1 (30)** + 1 (32) +
Anti-NF-H	1 (67) + 1 (51) −	—	—	—	—	—
Tubulin	1 (35) +	—	—	—	—	—
Antitubulin	1 (67) + 2 (81) −	—	—	—	—	—
Actin	1 (35) +	—	—	—	—	—
Tau	1 (90) + 4 (218) −	3 (179) + 1 (90) −	1 (52)** + 1 (53) −	1 (32) +	1 (32) + 1 (53) −	—
Amyloid β42	1 (21) −	1(21) +	—	—	—	—
BACE1	—	—	—	—	1 (100) −	—
NAA	2 (160) +	—	—	—	—	—
Apo-E	—	—	—	—	—	—
NSE	1 (64) + 2 (87) −	—	—	—	—	—
GAP-43	1 (49)** +	—	—	—	—	—
24S-OH-chol	1 (118) +	2 (206) +	—	—	—	—
14-3-3	2 (82) +	1 (38) +	2 (123) +	—	2 (101) +	—

*Number of positive (+) or negative (−) studies with total number of patients included (in brackets); **a tendency; CDMS: clinically definite MS; NF-L: neurofilament-light chain; Anti-NF-L: antibodies to NF-L; Anti-NF-M: antibodies to neurofilament-intermediate chain; NF-H: neurofilament-heavy chain; Anti-NF-H: antibodies to NF-H; BACE1: β-site amyloid precursor protein-cleaving enzyme 1; NAA: N-acetylaspartate; Apo-E: apolipoprotein-E; NSE: neuron-specific enolase; GAP-43: growth-associated protein 43; 24S-OH-chol: 24S-hydroxycholesterol.

CSF NF-H levels also significantly correlated with the ambulation index and the nine-hole-peg test scores [62], as well as with the MSSS [76]. In the latter study the degree of NF phosphorylation (ratio, hyperphosphorylated versus phosphorylated NF-H) was 8-fold higher in severely versus mildly disabled patients [76], whereas no correlation of NF-H levels with EDSS was found in some other studies in CSF [66] or plasma [77]. The highest CSF NF-H levels were found during relapses [25, 54] or correlated with MRI lesion enhancement [78], but Petzold et al. found no correlation of plasma HF-H^{SMI35} with the relapse rate [77]. In the study of Brettschneider et al., the sensitivity for predicting conversion to clinically definite (CD) MS after CIS was generally low, but could be increased by combining MRI with CSF NF-H criteria [25]. Additionally, a tendency towards a higher RRMS to SPMS conversion rate over 3 years in patients with high CSF NF-H levels was also shown [62]. Moreover, Petzold et al. [71] found significantly higher plasma NF-H levels in ON patients with poor recovery of visual acuity than in those with good recovery. In the study of Lim et al., in which 8/18 patients in the ON trial and 15/32 subjects in the MS attack trial were treated with oral methylprednisolone, in the MS attack trial group, CSF NF-H^{SMI34} and NF-H^{SMI35} measured at week 3 and CSF NF-H^{SMI34} levels from baseline to week 3 were predictive of clinical outcome at week 8 and 52 [78]. In the study of Rejdak et al., CSF NF-H levels inversely correlated with the EDSS recovery grade over a short-term follow-up of 6–8 weeks [72]. Moreover, in 30 RRMS patients, plasma NF-H^{SMI35} levels were higher, albeit nonsignificantly, in nonresponders than in responders to IFN beta1-a or 1-b over 1 year of treatment [77]. A correlation of CSF NF-H levels with age was found in CIS or MS patients in some studies [54], but in the others no age influence [62, 66] or a correlation with disease duration was found [66, 72].

3.2. Antineurofilament Antibodies. Axonal damage and subsequent exposure of NFs could lead to antibody generation in a T-cell-dependent secondary immune response to a foreign antigen [85]. Cytoskeletal and myelin debris, released by

Table 3: Parameters of humoral and cellular response to markers of neuroaxonal damage in the cerebrospinal fluid (CSF) and blood of patients with multiple sclerosis (MS) and clinically isolated syndrome suggestive of MS (CIS).

Biomarker	Body fluid	Immunoassay	Number of patients	Main findings	REF
Anti-NF-L	CSF/SE	ELISA (IgG)	67 MS (RR/SP/PP)	CSF/SE index in PP or SP ↑↑ RR or OIND/NIND/NHCo	[74]
	CSF/SE	ELISA (IgM, IgG)	58 MS (RR/SP/PP) + 8 CIS	specific IgG-index in MS ↑↑ CD	[79]
	CSF/SE	ELISA (IgG)	51 MS (RR/SP/PP)	CSF/SE index correlated with brain atrophy, RR ↔ SP ↔ PP	[66]
	CSF/SE	ELISA (IgG)	130 MS (RR/SP/PP)	serum antibody levels in PP ↑↑ OND or HCo	[80]
Anti-NF-M	CSF/SE	ELISA (IgG)	47 MS (RR/SP/PP)	significant correlation with anti-NF-L and antitubulin IgG in serum and CSF	[81]
	CSF/SE	ELISA (IgG, IgM)	49 MS (RR/SP/PP)	IgM and IgG specific indices in MS subgroups ↑↑ CD or CN	[82]
Anti-NF-H	CSF/SE	ELISA (IgG)	51 MS (RR/SP/PP)	RR ↔ SP ↔ PP	[66]
	CSF/SE	ELISA (IgG)	67 MS (RR/SP/PP)	MS ↔ OIND/NIND/NHCo	[74]
Antitubulin	CSF/SE	ELISA (IgG)	67 MS (RR/SP/PP)	MS ↔ OIND/NIND/NHCo	[74]
	CSF/SE	ELISA (IgG)	47 MS (RR/SP/PP)	significant correlation with anti-NF-L and anti-NF-M IgG in serum and CSF	[81]
	CSF/SE	ELISA (IgG)	29 MS (RR/SP/PP) + 5CIS	CSF levels in MS+CIS ↑↑ CN	[83]
Anti-NSE T-cell response	PBMC	T-cell Proliferation Assay	35 MS	prevalence of response in MS ↑↑ HCo	[84]

REF: reference; Anti-NF-L: antibodies to neurofilament-light chain; anti-NF-M: antibodies to neurofilament-intermediate chain; anti-NF-H: antibodies to neurofilament-heavy chain; NSE: neuron-specific enolase; SE: serum; PBMC: peripheral blood mononuclear cells; ELISA: Enzyme-Linked Immunosorbent Assay; IgG: immunoglobulin G, IgM: immunoglobulin M; RR: relapsing-remitting MS; SP: secondary-progressive MS; PP: primary-progressive MS; ↑↑ significantly higher than; ↔ no difference between; OIND: other inflammatory neurological diseases; NIND: noninflammatory neurological disorders; NHCo: neurologically healthy controls; CD: miscellaneous neurological diseases; OND: other neurological diseases; HCo: healthy controls, CN: normal controls (vertigo, headache, psychogenic syndrome, and fatigue).

neurons, are removed by macrophages which may be able to reach the peripheral lymph nodes [86]. Additionally, anti-NF-antibodies could be induced from exposure to exogenous agents, possibly virus-derived peptides and subseqently may cross-react with neuronal antigens [87].

Autoimmune responses to neuronal antigens might contribute to axonal damage and irreversible disability in MS [12], but could also be an epiphenomenon [79]. In the latter study, intrathecal immunoglobulin (Ig) G and IgM anti-NF-L synthesis did not differ between MS subgroups (RR, SP, or PP) or between CIS, MS patients, or healthy controls [79] (Table 3). On the other hand, the intrathecal anti-NF-L IgG was shown to correlate with MRI parameters of cerebral atrophy [66] and NF-L-autoimmunity has been also recently reported to be pathogenic in mice [12]. Additionally, anti-NF-L-IgG levels in serum were found to be significantly increased in PPMS patients compared to other neurological diseases or healthy controls [80] and in some studies a specific CSF/serum anti-NF-L IgG index correlated with EDSS or MSSS scores [74, 81] (Table 2). In some reports, anti-NF-L levels did not correlate with age or disease duration or EDSS score [66, 80], although some other authors showed a correlation between both anti-NF-L IgG index and CSF anti-NF-L IgG and duration of symptoms before lumbar puncture [74].

Anti-NF-M antibody response was analyzed in MS in a few studies. In a study of Bartoš et al., the extent of anti-NF-M antibody levels did not correspond to any individualized clinical profiles of MS patients although the intrathecal production of IgM and IgG anti-NF-M was significantly increased in all MS subgroups compared to patients with other diseases or healthy controls [82] (Table 3). In the latter study [82], the intrathecal IgG and IgM anti-NF-M synthesis in MS patients was unrelated to gender, age, disease duration, and EDSS score, but Fialová et al. [81] found a correlation between anti-NF-M IgG intrathecal synthesis and disability.

Anti-NF-H IgG in CSF/serum was found to be similar in MS patients and controls [74] and between RR, SP, and PPMS patients [66] (Table 3). The intrathecal production of anti-NF-H IgG correlated with some parameters of brain

atrophy such as the parenchymal fraction [74]. The CSF anti-NF-H levels correlated with the duration of disease before lumbar puncture and EDSS score in the study of Silber et al. [74], but no correlation with EDSS was shown by others [66].

3.3. Tubulin and Antitubulin Antibodies. In addition to NFs, the other major component of the axonal cytoskeleton is the microtubule, which mainly consists of α and β tubulin subunits [21], but also of microtubule-associated proteins (MAPs) such as MAP2 and tau [88]. Tubulin comprises as much as 20% of the cellular protein in brain [89] and is mainly responsible for axonal migration and longitudinal growth as well as for providing the conduit for fast axonal transport [90]. CSF tubulin levels were found to be increased in progressive MS (SP+PP) patients as compared to RRMS or controls [63] (Table 4). Antitubulin antibodies were also investigated and in some studies CSF levels of these antibodies were shown to be increased in MS patients [83], a finding which was not shown by others [74]. CSF tubulin levels [63], but also CSF antibodies to tubulin and the CSF/serum antitubulin index correlated significantly with EDSS in one study [74], whereas no similar correlations with disability were found in two other studies [81, 83] (Table 2). No correlations of antitubulin antibodies were found with age or disease duration [74, 83].

3.4. Actin. Actin is the major component of the microfilaments [21]. CSF actin levels were found to be significantly elevated in MS patients than in the control group, with higher levels in progressive MS cases and correlated with the EDSS scores [63] (Table 4). Anti-actin antibodies were not separately investigated in MS patients to date and could also be detected in normal sera [91].

3.5. Gelsolin. Gelsolin is an actin-binding protein that regulates actin organization [92] and is expressed in neurons in addition to the other cells [93]. Additionally, its secreted isoform could be found in the circulation [94] and belongs to the extracellular actin scavenger system [95]. Following some preliminary results showing low blood and CSF gelsolin concentration in 4 MS patients [92], Kulakowska et al. recently reported significantly lower plasma gelsolin levels in MS samples than in controls, whereas there was no difference in its CSF levels between the two groups [96] (Table 4).

3.6. Tau Protein. Tau is an axonal cytoskeletal protein that is involved in microtubule assembly and stabilisation [97] and therefore is essential for the efficient axonal transport [98]. Abnormal phosphorylation of tau can lead to the formation of potentially neurotoxic insoluble tau aggregates that have been shown to be characteristic features of common neurodegenerative diseases [97, 99, 100]. Pathological studies demonstrated the association of abnormally phosphorylated tau (p-tau) protein with SPMS and PPMS [101, 102] but also the absence of insoluble tau fraction in early MS, thus indicating the possibility that insoluble tau accumulates with disease progression [100, 102].

Total-tau (t-tau) protein and/or p-tau have been investigated in a respectable number of studies performed in CIS [25, 54, 103–106] and MS patients (Table 4) and the reported results are quite contradictory. Tau protein could be detectable in serum, but in concentrations that are ten times lower than in CSF [21]. CSF t-tau levels in CIS have been reported to be higher than in controls [25], but other authors found no difference in t-tau or p-tau compared to controls [104–106] (Table 4). In some MS studies, CSF levels of t-tau [103, 107–110] and p-tau [109] were reported to be significantly higher in MS patients than in controls, whereas some other authors could not confirm these differences [54, 104, 111]. T-tau and p-tau were also investigated in childhood RRMS cases in which their CSF levels were similar to controls [112]. A positive correlation of CSF t-tau levels was shown with EDSS in RRMS and CIS patients [25] and with the progression index over 3 years in early RRMS [113], but in some other studies no correlation with disability in CIS or MS was demonstrated for the CSF [103, 104, 110] or serum and CSF levels [105] (Table 2). Two studies have indicated an increased CSF-tau release in clinically active disease states [109, 114], in one study there was a significant elevation of CSF t-tau among patients with gadolinium-enhancing brain MRI lesions [103], but the latter finding was not confirmed afterwards [25]. However, the relation of CSF tau levels with the extent of intrathecal inflammation in MS was also supported by findings of Bartosik-Psujek and Archelos who showed a significant positive correlation between CSF t-tau levels and IgG index [108]. As shown by Brettschneider et al., the sensitivity and specificity of CSF tau levels for predicting CIS conversion to CDMS was generally low, but could be increased by combining with MRI parameters or with NF-H^{SMI35} levels [25]. Gajofatto et al. [115] could not show a significant predictive value of CSF t-tau in patients with acute myelitis either for conversion to MS or for disability after a median followup of 6.2 years, but in the study of Martínez-Yélamos et al. [113], CSF-t-tau was the only independent variable to predict time to the next relapse (Table 2). Interestingly, phosphorylation of tau and axonal pathology were significantly reduced when EAE rats were treated with prednisolone [116], but similar findings in MS were not reported. Although CSF t-tau levels were found associated with age in the control group in the study of Bartosik-Psujek and Archelos [108], no correlation of CSF t-tau/p-tau levels with age or disease duration was found in the majority of other studies [104, 106, 107, 110, 111, 117].

3.7. Amyloid-Precursor Protein and Related Molecules. Amyloid precursor protein (APP) probably works as a cargo receptor for binding proteins during axonal transport [21], but could have some other important neural functions including those in memory processes [118]. APP is transported by a fast anterograde axonal transport [119] and subtle changes in axonal transport or axonal transection could lead to APP deposits in the axon that are easily detectable by immunocytochemistry [120]. Based on immunopathological findings, it was suggested that APP accumulation could be a sensitive marker of MS disease

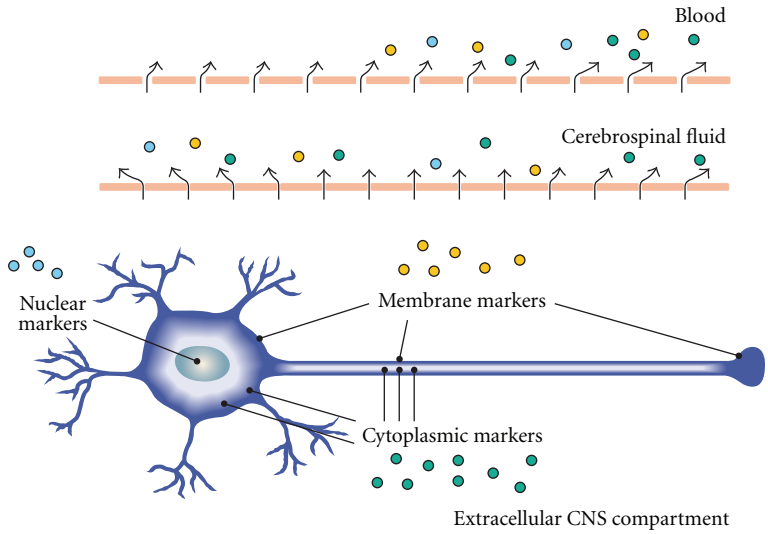

Figure 2: Membrane, cytoplasmic, and nuclear markers of neuroaxonal damage in the central nervous system (CNS) and their dynamics within three compartments (extracellular space, cerebrospinal fluid, and blood).

progression [121], but also a potential marker of acute or active MS [10]. APP is cleaved by an integral membrane aspartyl protease (β-site APP-cleaving enzyme 1, BACE1), which results in the release of N-terminal β-cleaved soluble APP (β-sAPP). The C-terminal fragment is further processed by γ-secretase to yield the amyloid beta (Aβ42) and the APP intracellular domain [122]. APP can also undergo α-secretase-mediated cleavage, which results in the release of the solubile α-sAPP [123].

In the recent study of Mattsson et al. [122], CSF levels of α-sAPP, β-sAPP, and Aβ42 were significantly lower in MS patients than in controls and patients with ongoing or recent MS exacerbation had lower α-sAPP levels than stable MS patients. CSF BACE1 activity was slightly reduced in patients with MS compared to controls and patients with SPMS tended to have lower BACE1 activity than patients with RRMS [122]. Baseline BACE1 activity did not predict change in EDSS score after 10 years (Table 2) but low BACE1 activity was associated with prolonged MS disease duration and disease severity. In contrast to controls, a reduction in BACE1 activity over 10 years was seen only in RRMS, whereas patients with SPMS displayed constantly low BACE1 activity levels [122]. Additionally, two molecules, Bri2 and Bri2-23 have been shown to interact with APP and regulate Aβ42 cleavage and aggregation *in vivo* [124, 125]. Recent findings revealed that CSF levels of Bri2-23, a peptide cleaved from Bri2, may be a biomarker of cerebellar/cognition dysfunction in progressive MS patients in which CSF Bri2-23 levels have been recently shown to be decreased [125]. In a recent study of Mori et al. [126], CSF Aβ42 levels were significantly lower in cognitively impaired MS patients and were inversely correlated with MRI parameters of disease activity. On the contrary, Vališ et al. [106] found a significantly higher CSF Aβ42 levels in MS patients compared to the control group (Table 4). No study has followed the effect of treatment on CSF-APP-derived proteins although some preliminary results in 16 nondemented, non-MS patients indicated a significant decrease in CSF Aβ42 levels following corticosteroid treatment [127]. CSF Aβ42 levels were not shown to correlate with age or disease duration [122, 126].

3.8. N-Acetylaspartate. N-Acetylaspartate (NAA) is the amino acid synthesized and almost exclusively localized in neurons [128] and is one of the most abundant molecules in the CNS [129]. Postmortem studies of spinal cords from MS patients related lower tissue concentrations of NAA to the lower axonal volume [8]. Several functions of NAA molecule in the CNS have been hypothesized, such as (a) an osmolite to remove water from neurons, (b) an acetate contributor in myelin sheath synthesis, (c) a mitochondrial energy source, (d) a precursor for *N*-acetylaspartyl glutamate, and (e) a ligand for certain metabotropic glutamate receptors [130]. Brain proton MR spectroscopy (MRS) allows *in vivo* examination of axonal integrity by quantifying the resonance intensity of NAA [131]. Previous proton MRS studies have found the reduced NAA levels in MS lesions, the surrounding NAWM and cortical grey matter [132], and a decline in global MRS-NAA levels was also demonstrated in benign MS [133]. A decrease in relative NAA levels by proton MRS was found in patients with CIS in CNS gray matter [134] and WM lesions [135]. Other studies have shown that NAA decrease in lesions and NAWM is related to clinical disability and progressive brain atrophy [136–138] and was indicated to be a feature of progression [16]. Moreover, some preliminary MRS findings have shown a beneficial effect of glatiramer acetate on increase of relative NAA peaks in MS lesions and NAWM over 2 years [131], whereas relative NAA peaks had become significantly higher in the interferon beta-1b -treated MS patients following 1 year of treatment [139].

In the study of Jasperse et al. [141], CSF concentrations of NAA correlated with EDSS and MS Functional Composite

Table 4: Cytoplasmic, non-neurofilament biomarkers of neuroaxonal damage in the cerebrospinal fluid (CSF) and blood of patients with multiple sclerosis (MS) and/or clinically isolated syndrome suggestive of MS (CIS).

Biomarker	Body fluid	Immunoassay	Number of patients	Main findings	REF
Tubulin	CSF	Dot-blot	35 MS (RR/SP/PP)	MS ↑↑ OIND or NIND, PP/SP ↑↑ RR	[63]
Actin	CSF	Dot-blot	35 MS (RR/SP/PP)	MS ↑↑ OIND or NIND, PP/SP ↑↑ RR	[63]
Gelsolin	CSF/PL	Western blot	56 MS	PL levels in MS ↓↓ Co*, CSF levels in MS ↔ Co*	[96]
Tau	CSF	ELISA (t-t)	38 MS (RR) + 52 CIS	CIS ↑↑ NHCo	[25]
	CSF	ELISA (t-t)	45 MS (RR/SP/PP)	MS ↑↑ OIND + NIND, SP ↔ RR ↔ PP	[110]
	CSF	ELISA (t-t, p-t)	42 RRMS + 18 CIS	t-tau and p-tau in MS+CIS↑↑ NHCo, t-tau in CIS ↑↑ NHCo	[109]
	CSF	ELISA (t-t)	38 MS (RR/SP/PP) + 12 CIS	MS+CIS↔ NHCo	[104]
	CSF	ELISA (t-t)	76 MS (RR/SP/PP) + 38 CIS	MS/CIS ↔ NHCo	[54]
	CSF	ELISA (t-t)	114 MS (RR/SP/PP)	MS ↑↑ NIND	[108]
	CSF	ELISA (t-t)	52 MS (RR/SP/PP) + 50 CIS	MS+CIS ↑↑ NHCo, the highest in CIS	[103]
	CSF	ELISA (t-t)	20 MS (RR/progressive MS)	MS ↔ NHCo	[111]
	CSF	ELISA (t-t)	36 MS (RR/SP/PP)	MS ↑↑ NHCo	[107]
	CSF	ELISA (t-t, p-t)	25 RRMS	MS ↔ OIND or NIND	[112]
	CSF/SE	ELISA (t-t, p-t)	21 CIS	CIS ↔ Co**	[105]
	CSF	ELISA (t-t, p-t)	14 MS + 9 CIS	MS ↔ CIS ↔ NHCo	[106]
	CSF	ELISA (t-t)	43 MS (RR/SP/PP/PR) + 20 CIS	MS ↔ CIS ↔ NHCo+OND	[140]
Amyloid β42	CSF/SE	ELISA	21 CIS	CIS ↔ Co**	[105]
Amyloid β42	CSF	ELISA	14 MS + 9 CIS	MS ↑↑NHCo	[106]
Amyloid β42	CSF	xMAP	100 MS (RR/SP/PP)	MS ↓↓ NHCo	[122]
α-sAPP	CSF	Multiplex Assay	100 MS (RR/SP/PP)	MS ↓↓ NHCo	[122]
β-sAPP	CSF	Multiplex Assay	100 MS (RR/SP/PP)	MS ↓↓ NHCo	[122]
Bri2-23	CSF	SELDI-TOF	40 MS (SP/PP)	MS ↓ OND	[125]
NAA	CSF	GC-MS	76 MS (RR/SP/PP) + 38 CIS	SP ↓↓ RR/CIS, CIS ↔ RR ↔ NHCo	[54]
	CSF	GC-MS	46 MS (RR/SP/PP)	MS ↔ OND, SP ↓↓ RR	[141]
NSE	CSF	Luminescence	66 MS (RR/SP)	MS ↔ HCo	[64]
	SE	RIA	21 MS	levels within normal range	[142]
	PL	Luminescence	64 MS (RR/SP/PP)	progressive MS ↓ RR	[143]
	CSF/SE	ELISA	21 CIS	CIS ↓ Co**	[105]
	CSF	Immunoluminometry	33MS (RR/SP)	RR ↔ SP	[144]

REF: reference; APP: amyloid-precursor protein; NAA: N-acetylaspartate; NSE: neuron-specific enolase; SE: serum; PL: plasma; ELISA: Enzyme-Linked Immunosorbent Assay; t-t: total tau protein, p-t: abnormally phosphorylated tau protein; xMAP: xMap Bead-based immunoassay; SELDI-TOF: Surface-Enhanced Laser Desorption/Ionization Time-Of-Flight; GC-MS: stable isotope dilution gas chromatography-mass spectrometry; RIA: radioimmunoassay; RR: relapsing-remitting MS; SP: secondary-progressive MS; PP: primary-progressive MS; ↑↑ significantly higher than; ↓↓ significantly lower than; ↓ lower than; ↔ no difference between; OIND: other inflammatory neurological diseases; NIND: noninflammatory neurological disorders; Co: controls with *idiopathic headache, Bell's palsy and ischialgia or **idiopathic headache and migraine; NHCo: neurologically healthy controls; OND: other neurological diseases; HCo: healthy controls.

(MSFC) (Table 2), although these levels were similar to controls both in MS [141] and early MS patients [54]. CSF NAA levels were shown to be lower in SPMS patients compared to RR and PPMS cases [54, 141] (Table 4). Teunissen et al. reported a decrease in CSF NAA levels as the disease progressed, therefore possibly reflecting the accumulation of axonal degeneration in a later MS stage [54]. One study has shown CSF NAA concentrations to correlate with age [54].

3.9. Apolipoprotein E. Apolipoprotein E (Apo-E) is mostly produced by astrocytes in the CNS, but it is also found in neurons [21]. There are three different alleles of the human Apo-E gene coding for the three isoforms: $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$ [145]. Apo-E is generally involved in lipid transport and cholesterol homeostasis [21]. However, its functions within the CNS are not so clear and might include immunomodulation, a protective role against oxidative stress [146], a role in maintaining the BBB integrity [147], a role in myelin lipid metabolism [148], and a potential role in neurorepair [21]. As there is a limited permeability of Apo- E across the BBB, Apo- E changes in CSF might dominantly result from a reduction of its local synthesis and secretion by brain tissue [146, 149].

Several studies have indicated that Apo-E $\varepsilon 4$ allele might be associated with MS, although evidence is still not sufficient enough [146, 150]. Although plausible [151], the association between Apo-E and MS course and disease severity remains controversial [152]. Apo-E genotypes seem not to influence the development of MS, but Apo-E $\varepsilon 4$ allele might predispose carriers with MS to a faster disease progression [153, 154]. In line with this, lower levels of NAA in MS patients with the Apo-E $\varepsilon 4$ allele have been demonstrated by MRS [155]. It has been recently suggested that Apo-E polymorphism may interact with cigarette smoking in promoting MS progression [156]. Although some authors have shown an association between Apo-E $\varepsilon 4$ and cognitive impairment in MS patients [157], the others could not confirm this finding [158].

However, it was suggested that the decreased CSF Apo-E concentration in MS patients occurs independent of the Apo-E genotype [159]. In one study the plasma concentration of Apo-E was significantly lower in MS patients than in healthy controls although its CSF concentrations were similar in these two groups [148]. Rifai et al. have shown higher CSF/serum Apo-E index in RRMS in remission compared to controls [160], Chiasserini et al. demonstrated an increased CSF expression of an Apo-E isoform in RRMS compared to CIS patients or controls [161], whereas Gaillard et al. [159] found lower concentrations of both CSF Apo-E and intrathecal Apo-E in MS patients than in controls (Table 5). No correlation of Apo-E in serum or CSF with age or clinical course was found [159].

3.10. Neuron-Specific Enolase. Enolase is a glycolytic enzyme (2-phospho-D-glycerate hydrolase), which exists in three isoforms: α-enolase, which is ubiquitous, β-enolase, which is predominant in muscle, and γ-enolase (neuron-specific enolase, NSE), which is found in neurons, glial, and neuroendocrine cells [84]. NSE was shown to be an indicator of the acute neuro-destructive disorders [162], but its levels are usually normal in MS patients [162] and no clear difference in the NSE levels has been observed between MS patients and controls in CSF [64] or serum [142], or between patients with RR and SPMS in CSF [144] (Table 4). NSE concentration in CSF and serum was shown to be decreased in CIS patients when compared to the control group, potentially indicating a reduced neuronal metabolic activity at the early stage of the disease [105]. Interestingly, Forooghian et al. demonstrated a higher T-cell responses against NSE in the *peripheral blood* mononuclear cells of MS patients than in controls [84]. One study which followed CSF NSE levels in MS patients treated with intrathecal triamcinolone administration did not show any changes in its levels following treatment [163]. A recent study examined plasma NSE levels in MS patients over a five-year period and found a strong inverse relationship between serum NSE levels and disease progression [143] as expressed through EDSS and MSSS score changes, thus potentially reflecting a reduced metabolic activity secondary to axonal loss. In two other studies no correlation of CSF [64, 105] or serum [105] NSE levels were found with EDSS (Table 2). Age-related changes of NSE in CSF with an increase of 1% per year have been reported [164], but in some other studies CSF NSE levels were independent of gender, age [64, 165], and disease duration [64].

3.11. Growth-Associated Protein 43. Growth-associated protein 43 (GAP-43), also known as B-50 or neuromodulin, is a marker associated with growth cones, synaptic plasticity, and synaptic regeneration [166]. It is a calmodulin-binding protein being attached to the cytoplasmic site of the axonal membrane, involved in neurotransmitter release [167] which also stimulates neurite outgrowth [166]. A decreased GAP-43 expression was found in postmortem white matter MS lesions, independent of the lesion stage, whereas increased or unaltered expression was detected in remyelinated lesions and was found unchanged in grey matter lesions [167].

In a recent study, CSF GAP-43 levels were comparable among RR/SP and PPMS subtypes and controls and GAP-43 was not detected in serum [167] (Table 5). A tendency towards a negative correlation between CSF GAP-43 levels and EDSS was found, but CSF GAP 43 levels positively correlated with MRI measures of atrophy [167] (Table 2). Moreover, a positive correlation was observed between CSF NAA and GAP-43 levels [141]. No significant correlation was reported between CSF GAP-43 levels and age, gender, and disease duration [167].

3.12. 24S-Hydroxycholesterol. Cholesterol plays a crucial structural role in the brain [168] being the main lipid of neuronal membranes [21]. For maintenance of brain cholesterol homeostasis [168], cholesterol is converted into its more polar metabolite cerebrosterol (24S-hydroxycholesterol, 24S-OH-chol) by the CNS-specific cytochrome P450 enzyme CYP46 [169]. Virtually all of the cerebrosterol in the

Table 5: Membrane and nuclear biomarkers of neuroaxonal damage in the cerebrospinal fluid (CSF) and blood of patients with multiple sclerosis (MS) and/or clinically isolated syndrome suggestive of MS (CIS).

Biomarker (subtype)	Body fluid	Immunoassay	Number of patients	Main findings	REF
BACE1 *(membrane)*	CSF	Enzymatic	100 MS (RR/SP/PP)	MS slightly↓NHCo	[122]
Apo-E *(membrane)*	CSF/SE	Immunofluorometry	34 MS	CSF levels in MS ↓↓ NHCo	[159]
	CSF	2DE-MS	10 RRMS + 11 CIS	one isoform expression in RR ↑↑ CIS or NHCo	[161]
	CSF/SE	Immunoturbidimetry	33 MS (RR)	CSF/serum index in MS in remission ↑↑ NHCo	[160]
GAP-43 *(membrane)*	CSF/SE	xMap Bead –based	49 MS (RR/SP/PP)	CSF in RR ↔ SP ↔ PP ↔ OIND + NIND + HCo, not detected in serum	[167]
	CSF	xMap Bead –based	44 MS (RR/SP/PP)	significant positive correlation with NAA levels	[141]
24S-OH-chol *(membrane)*	PL	IDMS	46 MS (RR/PP)	negative correlation with T2 lesion volume	[169]
	PL	IDMS	11 MS	MS ↔ HCo	[170]
	CSF/PL	IDMS	118 MS (RR/SP/PP)	PL levels in older MS ↓↓ HCo	[171]
	SE	IDMS	60 MS (RR/SP/PP)	PP or older RR ↓ HCo	[172]
	CSF/PL	IDMS	88 MS	PL levels in MS ↔ Co	[173]
Protein 14-3-3 *(cytoplasmic, nuclear, membrane)*	CSF	Western blot	22 MS (RR/SP/PP) + 15 ATM	detected in about 8% RR/ATM patients	[174]
	CSF	Immunoblot	38 CIS	detected in 13% CIS patients	[175]
	CSF/SE	Immunoblot	21 CIS	detected in 1/21 patient	[105]
	CSF	ELISA	114 MS (RR/SP/PP)	detected in 22% MS patients, not detected in HCo	[108]
	CSF	Immunoblot	43 MS (RR/SP/PP/PR) + 20 CIS	detected in 38% CIS/MS, similar in MS subgroups	[140]
	CSF	Immunoblot	85 CIS	detected in 8.2 % CIS patients	[176]

REF: reference; BACE1: β-site amyloid precursor protein-cleaving enzyme 1; Apo-E: Apolipoprotein-E; GAP-43: growth-associated protein-43; 24S-OH-chol: 24S-hydroxycholesterol; SE: serum; PL: plasma; Enzymatic: Enzymatic solution-based assay; 2DE-MS: two-dimensional electrophoresis-mass spectrometry; IDMS: isotope-dilution mass spectrometry; ELISA: Enzyme-Linked Immunosorbent Assay; RR: relapsing-remitting MS; SP: secondary-progressive MS; PP: primary-progressive MS; ATM: acute transverse myelitis; NAA: N-acetylaspartate; ↓ lower than; ↓↓ significantly lower than; ↑↑ significantly higher than; ↔ no difference between; NHCo: neurologically healthy controls; OIND: other inflammatory neurological diseases; NIND: noninflammatory neurological disorders; HCo: healthy controls; Co: controls with idiopathic headache.

peripheral circulation is CNS derived and its blood levels are assumed to reflect the relation between cholesterol CNS production caused by demyelination or neurodegeneration and hepatic clearance [170, 177, 178]. The level of 24S-OH-chol highly correlated with total cholesterol and the ratio between 24S-OH-chol and cholesterol is assumed to be a better marker for the cerebral production than the absolute cerebrosterol concentration [171]. The majority of daily efflux of this oxysterol from the brain to the circulation apparently occurs as a direct transport across the BBB and less than 1% of the total flux of 24S-OH-chol from the brain occurs via CSF [168], which might cause the lack of correlation between CSF and plasma levels of this metabolite [171].

The higher CSF levels of 24S-OH-chol were shown in patients with gadolinium-enhancing MRI lesions, indicating the pronounced release of the 24S-OH-chol from damaged cells during CNS inflammation [171, 173] (Table 2). Moreover, patients with a defective BBB were found to have markedly increased absolute levels of 24-OH-chol in CSF [179]. Karrenbauer et al. [169] demonstrated a negative correlation between the cerebrosterol/cholesterol ratio in plasma and volume of T_2-weighted MRI lesions, whereas a significant inverse relation between the EDSS score and plasma cholesterol-related levels of 24S-OH-chol was found in the other study [171]. Teunissen et al. [172] showed the reduction in serum 24S-OH-chol concentrations to be most pronounced in the PP clinical subtype (Table 5). Leoni et al. found a tendency to increased plasma levels of 24S-OH-chol in younger patients with high levels in the 3rd and 4th decades of life, and significantly lower levels in older MS patients aged 51–70 years than in healthy age-matched controls [171]. There seems to be no gender influence on plasma levels of 24S-OH-chol or the ratio

between cerebrosterol/cholesterol [171] and no correlation of the latter with disease duration was reported to date [172].

3.13. Protein 14-3-3. 14-3-3 family proteins are ubiquitous, highly conserved proteins with the highest concentrations in brain [162, 180] and within CNS are constitutively expressed in neurons and glia both in cytoplasmic and nuclear regions [181] with small amounts bound to synaptic membranes [162]. A growing body of evidence indicates that it might act as a novel type of molecular chaperone which interacts with key molecules involved in cell differentiation, proliferation, and transformation [182], and recent data suggested its antiapoptotic effects [183, 184]. The detection of 14-3-3 protein in the CSF is highly sensitive for *in vivo* diagnosis of Creutzfeldt-Jakob disease [185], but this protein, in the CSF, could be also detected in some other prion-unrelated conditions associated with CNS tissue damage [175, 181, 186].

The 14-3-3 protein is more frequently detectable in the CSF of MS or CIS patients than in controls although in such cases it is present in a small subgroup of patients [108, 174]. However, in the study of Colucci et al. [140] it was more frequently positive than previously reported (Table 5). The detection of the 14-3-3 protein in the CSF of CIS patients was shown to be an independent predictor of short-term conversion to CDMS [175, 176] (Table 2). Moreover, the 14-3-3 positive group had a significantly higher relapse rate and a higher frequency of patients with EDSS ≥ 2.0 after a median followup of 33.4 months [176], which confirmed previous results by the same authors [175]. In some studies, 14-3-3 protein positivity in MS patients was associated with a more severe disability [140, 187] and the rate of disease progression during a mean of 10-month clinical followup [140], but was also shown to be a potential predictor of permanent neurological disability after an episode of the acute transverse myelitis [188]. However, the latter was not shown in two other studies [115, 174]. The presence of 14-3-3 reactivity was not shown to prevail in MS clinical subgroups [140] and seems not to correlate with age [108] or disease duration [140].

3.14. Proteomics Research. Recently, a rapid development of proteomic approaches refocused biomarker research interest to the use of novel methods in the discovery of potential MS-specific biomarkers in biological fluids and especially in the CSF [189, 190]. Among the wide range of proteins that have been found to be exclusively present in the CSF of MS patients [125, 189, 191–194], only some of them are expressed on neurons (contactin-1, neurofascin, neurotrimin, and chromogranins/secretogranins) [193–195]. It was recently shown that contactin-2 was recognised by both autoantibodies and Th1/Th17 T-cells in MS patients [36, 37] and neurofascin-specific autoantibodies were identified in MS patients [196]. However, there is a range of neuroaxonal proteins which still need to be studied in CIS/MS patients although some of them have been investigated in animal models [197].

4. Summary and Future Directives

Based on the majority of available results, the increased levels of the CSF NF-L or NF-H seem to be present even at early MS phases, a scenario which continues during the entire course of the disease and correlates with different measures of disability; the increased levels of these markers seem to be more pronounced in active disease states and have a potential value in an effort to predict conversion to CDMS after a first CIS episode, estimate future progression and disability, but their value for the prediction of treatment response still has to be investigated, most importantly in early MS patients.

Although the presence of anti-NF antibodies could, in part, be an epiphenomenon of the disease, the elevated levels of these antibodies in progressive disease and correlations with disease duration and disability indicate a rise in antibodies induced by axonal destruction, but also a possible pathogenic role of these antibodies in promoting axonal damage and disease progression. This indicates that serum and/or CSF anti-NF-L, NF-M, and NF-H antibodies might be a potential a marker of CNS tissue damage in MS, but their potential predictive value for the future disease course, disability, disease progression, and treatment response needs to be investigated.

CSF levels of actin and tubulin seem to be elevated in progressive MS and correlate with disability, but their levels in early MS patients, as well as the potential predictive value have been underreported to date.

It is possible that elevated CSF t-tau levels are present from early MS phases and increase in clinically/MRI active disease phases; although its potential correlation with ongoing disease progression has been indicated, the reports related to this molecule so far have been quite contradictory and its validity as a biomarker needs to be further studied both in blood and CSF.

CSF and blood levels of APP-derived proteins seem not to be reliable markers of disease activity or progression since their levels are largely dependent on complex regulatory metabolic processes which could be highly variable in a complex and heterogenous disease such as MS.

A correlation of CSF NAA levels with disability measures even in CIS patients suggests the potential clinical relevance of this molecule as a biomarker that should be further investigated.

NSE and Apo-E levels in CSF/blood are not consistently abnormal in MS patients and their relation to neuroaxonal damage is complicated since the expression of both molecules is not limited to neurons.

CSF/blood levels of GAP-43 were investigated to date in a paucity of studies and some preliminary results might indicate the need for further investigations of this molecule as of the potential biomarker of disease progression and disability.

Serum 24S-OH-chol levels seem to be as reliable as levels in CSF to estimate neuronal membrane status. Some reports indicated its correlation with disability and MS disease activity and thus the validity of this molecule as a biomarker should be further investigated.

The 14-3-3 family proteins could be potentially related to CIS conversion to MS, disability, and its progression, but this still has to be further confirmed. Astrocyte-derived 14-3-3 protein could complicate the relation of CSF/blood levels of 14-3-3 protein only to neuroaxonal status in MS.

Additionally, it would be desirable to systematically compare the proteome profiles of MS subgroups at a defined disease stages and in large cohorts in order to identify proteins which are consistently present in the CSF at a certain disease phase in a given subgroup, a task which is still facing a lot of obstacles.

So far, the abovementioned markers have been investigated in the light of their significance to reflect the presence and the extent of neuroaxonal damage in CIS/MS patients. Since each of them could be related to different structural levels of neuroaxonal loss of integrity, the combined evaluation of these markers could be more informative on the ongoing neurodegenerative process [54]. Moreover, the relevance of the single biomarker has to be judged in the light of disease stage and/or disease activity since biomarker levels could show temporal dynamics that correlates with the dynamics of the MS natural course [54, 198].

The results of the biomarker studies could have been influenced by small study sizes, cross-sectional designs, and insufficient followup to allow meaningful conclusions [199]. Biomarker studies in MS neurodegeneration have been conducted in a variable patient population, varying from a few [68] to over a hundred patients included [108, 171]. Moreover, followup in most of these studies was up to three years [54, 62, 113, 140, 175, 176] which could allow only tentative conclusions on biomarker's long-term prognostic significance. The differences in preanalytical processes and different assay sensitivities could also cause contradictory results in biomarker studies [199]. The comparable results were shown in several studies that used the same NF-H Enzyme-Linked Immunosorbent Assay (ELISA) method [62, 70–72]. However, a poor interlaboratory coefficient of variation in a recent multicenter NF-L ELISA validation study has been shown, mainly due to the lack of preparation of accurate and consistent protein standards [200]. Therefore, a standardization of body fluid sampling and storage [201], as well as the use of the standardized and validated assay procedures [23, 200], are needed.

Since none of the potential CSF/blood biomarkers studied so far fulfils all necessary criteria for a surrogate biomarker [22] there is an ongoing need for further biomarker studies, especially those aiming to predict future disease course, disability, and/or treatment response at the early MS stage.

Conflict of Interests

There is no conflict of interests to declare.

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Research Article

Blood and CSF Biomarker Dynamics in Multiple Sclerosis: Implications for Data Interpretation

M. J. Eikelenboom,[1] B. M. J. Uitdehaag,[1] and A. Petzold[1,2]

[1] *Department of Neurology, MS Center Amsterdam, VU University Medical Center, Amsterdam, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands*
[2] *Department of Neuroinflammation, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK*

Correspondence should be addressed to A. Petzold, a.petzold@ion.ucl.ac.uk

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Background. Disability in multiple sclerosis (MS) is related to neuroaxonal degeneration. A reliable blood biomarker for neuroaxonal degeneration is needed. *Objectives*. To explore the relationship between cerebrospinal fluid (CSF) and serum concentrations of a protein biomarker for neuroaxonal degeneration, the neurofilaments heavy chain (NfH). *Methods*. An exploratory cross-sectional ($n = 51$) and longitudinal ($n = 34$) study on cerebrospinal fluid (CSF) and serum NfH phosphoform levels in patients with MS. The expanded disability status scale (EDSS), CSF, and serum levels of NfH-SMI34 and NfH-SMI35 were quantified at baseline. Disability progression was assessed at 3-year followup. *Results*. At baseline, patients with primary progressive MS (PPMS, EDSS 6) and secondary progressive MS (SPMS, EDSS 6) were more disabled compared to patients with relapsing remitting MS (RRMS, EDSS 2, $P < .0001$). Serum and CSF NfH phosphoform levels were not correlated. Baseline serum levels of the NfH-SMI34 were significantly ($P < .05$) higher in patients with PPMS (2.05 ng/mL) compared to SPMS (0.03 ng/mL) and RRMS (1.56 ng/mL). In SPMS higher serum than CSF NfH-SMI34 levels predicted disability progression from baseline (ΔEDSS 2, $P < .05$). In RRMS higher CSF than serum NfH-SMI35 levels predicted disability progression (ΔEDSS 2, $P < .05$). *Conclusion*. Serum and CSF NfH-SMI34 and NfH-SMI35 levels did not correlate with each other in MS. The quantitative relationship of CSF and serum NfH levels suggests that neuroaxonal degeneration of the central nervous system is the likely cause for disability progression in RRMS. In more severely disabled patients with PP/SPMS, subtle pathology of the peripheral nervous system cannot be excluded as an alternative source for blood NfH levels. Therefore, the interpretation of blood protein biomarker data in diseases of the central nervous system (CNS) should consider the possibility that pathology of the peripheral nervous system (PNS) may influence the results.

1. Introduction

In multiple sclerosis, irreversible disability progression is anatomically associated with neuroaxonal degeneration [1–3]. Using cerebral microdialysis, it was shown that as a result of neuroaxonal degeneration, protein biomarkers were released into the extracellular fluid (ECF) [4, 5]. Once released into the ECF of the brain, these brain-specific proteins diffuse into the cerebrospinal fluid (CSF) [6]. A protein biomarker specific for neuroaxonal degeneration are neurofilaments [7, 8]. Of the various neurofilament proteins (Nf), the light (NfL) and heavy (NfH) chains were successfully quantified from the CSF and found to be of prognostic value in patients with MS (reviewed in [7, 8] and newer references [9–12]). The Nf proteins diffuse from the CSF into the blood stream from where different NfH phosphoforms have been quantified by different groups [13–15]. Because of the relative ease of blood sampling compared to a spinal tap, it is highly desirable to have a reliable blood biomarker for neuroaxonal degeneration.

There is a need to better understand the relationship between CSF and serum protein biomarkers for neuroaxonal degeneration. The situation in the CSF is relatively straightforward, because any increase can with a considerable degree of confidence be associated with damage to the brain. Interpretation of serum data is more complex. One

potentially confounding issue is that Nf are also expressed in the peripheral nervous system [7].

The relationship between CSF and serum NfH levels in MS is not known. In this study, we hypothesized that the well-recognized neuroaxonal degeneration of the central nervous system would lead to a higher concentration in the CSF compared to the serum. To test this hypothesis, we quantified NfH heavy chain phosphoforms from the serum and CSF in a cohort of MS patients we have been published on before [16].

2. Methods

2.1. Patients. This study was approved by the local ethics committee, and informed written consent was obtained from the patients. All patients with MS were from a previously published Dutch cohort [17] and were classified into having relapsing remitting MS (RRMS, $n = 21$), secondary progressive MS (SPMS, $n = 22$), or primary progressive MS (PPMS, $n = 9$) according to published criteria [18]. Blood and CSF samples were taken at the same time. Matched aliquots of CSF and serum samples were coded and stored in polypropylene tubes as described [19].

2.2. Clinical Assessment. Disability was recorded on the expanded disability status scale score (EDSS) [20]. Progression of disability was calculated over the 3-year interval as ΔEDSS = followup EDSS − baseline-EDSS. Significant disability progression was defined as worsening on the EDSS scale by at least 1 point for an EDSS < 5.5 or at least 0.5 point for an EDSS ≥ 5.5.

2.3. Neurofilament Analysis. CSF and serum Nf levels were measured using a sensitive sandwich ELISA which allows to quantify various NfH phosphoforms by exchanging the capturing monoclonal antibodies [21]. This ELISA gives the best analytical performance for the monoclonal antibodies SMI34 and SMI35 (originally from Sternberger Monoclonals, now sold through Covance). Adhering to a previously proposed nomenclature NfH captured by SMI34 is indicated as NfH^{SMI34} and NfH captured by SMI35 as NfH^{SMI35}. The precise binding epitopes of these antibodies are not known. Binding of SMI34 is phosphate dependent which is correlated with but not identical to the degree of NfH phosphorylation. SMI35 binds more specifically to phosphorylated NfH. Nonmeasurable samples were reported as 0 ng/mL.

2.4. Data Analysis. Because of non-Gaussian data distribution the median and interquartile range (IQR) are shown. Nonparametric statistics were used for comparison throughout. We used general linear models for comparison of three variables. The concentration of NfH was compared between CSF and serum for each patient individually. If the concentration of NfH was higher in the CSF compared to the matched serum sample, this was indicated as "C > S", otherwise as "S ≥ C". The relationship between higher CSF or serum NfH levels with clinical progression on the EDSS was analyzed using the Kruskall-Wallis test. Correlation analyzes were performed using Spearman's R. The Bonferroni method was used to correct for multiple correlations. All statistical analyzes were performed in SAS (version 9.1).

3. Results

3.1. Baseline. The demographic data of the MS patients is summarized in Table 1. At baseline, the groups differed in their demographic data for age ($F_{2,48} = 4.87$, $P < .05$), EDSS ($F_{2,48} = 19{,}26$, $P < .0001$), and disease duration ($F_{2,48} = 7.33$, $P < .01$). None of the biomarkers was correlated with age, age at onset, disease duration, or the EDSS in the MS groups (data not shown).

The only biomarker distinguishing the MS groups were serum NfH^{SMI34} levels ($F_{2,48} = 3.63$, $P < .05$). Significance was missed for CSF NfH^{SMI34} ($P = .62$), CSF NfH^{SMI35} ($P = .59$), and serum NfH^{SMI35} ($P = .83$).

There was no correlation between either the CSF and serum NfH^{SMI35} or NfH^{SMI34} concentration in any of the MS groups (data not shown). The post hoc analysis showed that serum NfH^{SMI34} levels were higher in PPMS patients compared to SPMS patients ($P = .0128$).

The concentration for NfH^{SMI35} was higher in the CSF compared to the serum in 8/9 (89%) of PPMS, 18/22 (82%) of SPMS, and 14/20 (70%) of RRMS patients. Surprisingly, for NfH^{SMI34} the CSF concentration was higher in 2/9 (22%) of PPMS, 11/22 (50%) of SPMS, and 5/20 (25%) of RRMS patients.

3.2. 3-Year Followup. The dropout rate for the followup clinical assessment was 4/9 (44%) for PPMS, 4/22 (18%) for SPMS, and 9/20 (45%) for RRMS patients.

At 3-year followup most of the MS patients had progressed clinically on the EDSS scale (Table 2). At followup, there was a difference between the MS groups for the EDSS ($F_{2,31} = 9.63$, $P < .001$) and also for the individual progression on the EDSS ($F_{2,31} = 3.69$, $P < .05$).

Tables 3–5 summarizes the demographic data of the MS patients according to their individual CSF and serum NfH^{SMI35} and NfH^{SMI34} levels.

The low number of patients with PPMS at followup ($n = 5$) precluded any meaningful statistical analyzes. Disability progression (ΔEDSS) appeared to be associated with higher CSF than serum levels for NfH^{SMI35} and higher serum than CSF levels for NfH^{SMI34} (Table 3).

In patients with SPMS, NfH^{SMI34} predicted disability progression (ΔEDSS) from baseline if higher in the serum compared to the CSF ($P = .0358$, Table 4, Figure 2).

In patients with RRMS, NfH^{SMI35} predicted disability progression (ΔEDSS) from baseline if higher in the CSF compared to the serum ($P = .0298$, Table 5, Figure 1).

4. Discussion

The main finding at baseline was that the concentration of NfH phosphoforms was not correlated between matched CSF and serum samples. Unexpectedly, a proportion of

Table 1: Patient characteristics at baseline. The median (range) is shown.

Characteristic	PPMS	SPMS	RRMS
Number	9	22	20
Age	52 (46–55)	46 (29–65)	40 (27–55)
Gender (female : male)	6 : 3	11 : 11	11 : 9
Disease duration (years)	16 (6–27)	18 (6–35)	8 (4–13)
EDSS	6 (2–8)	6 (4–7)	2 (1–8)
CSF NfH^{SMI34} (ng/mL)	0.01 (0–0.05)	0.11 (0–0.04)	0.01 (0–0.04)
CSF NfH^{SMI35} (ng/mL)	0.10 (0.01–0.15)	0.04 (0.01–0.24)	0.08 (0–1.39)
Serum NfH^{SMI34} (ng/mL)	2.05 (0–3.08)	0.03 (0.19–2.44)	1.56 (0–2.05)
Serum NfH^{SMI35} (ng/mL)	0 (0–0.49)	0 (0–0.16)	0 (0–0.27)

Table 2: Patient characteristics at 3-year followup. The median (range) are shown.

Characteristic	PPMS	SPMS	RRMS
Number	5	18	11
Gender (female : male)	2 : 3	9 : 9	4 : 7
EDSS	7 (4–8)	6 (3–8)	4 (0–5)
ΔEDSS	0 (−1–1.0)	0 (−2–3)	2 (−1–4)

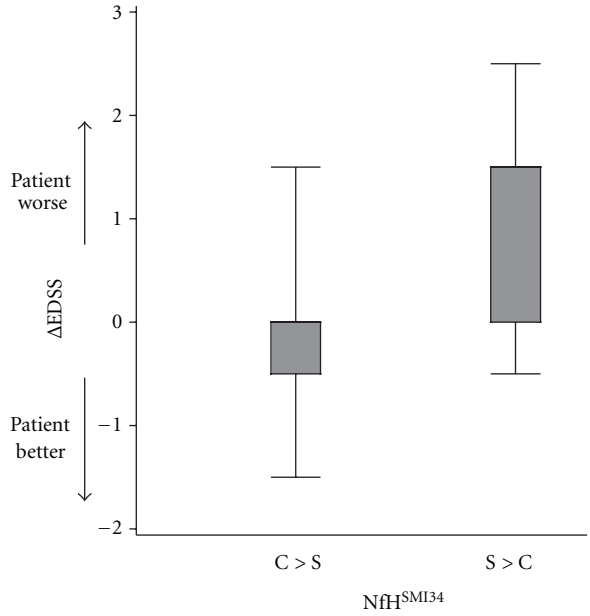

Figure 1: Disability progression in patients with RRMS is associated with higher CSF NfH^{SMI35} levels compared to the corresponding serum concentrations ($P = .0298$) likely indicating neuroaxonal degeneration of the central nervous system. The median (thick horizontal bar), IQR (boxes), and range (whiskers) are shown.

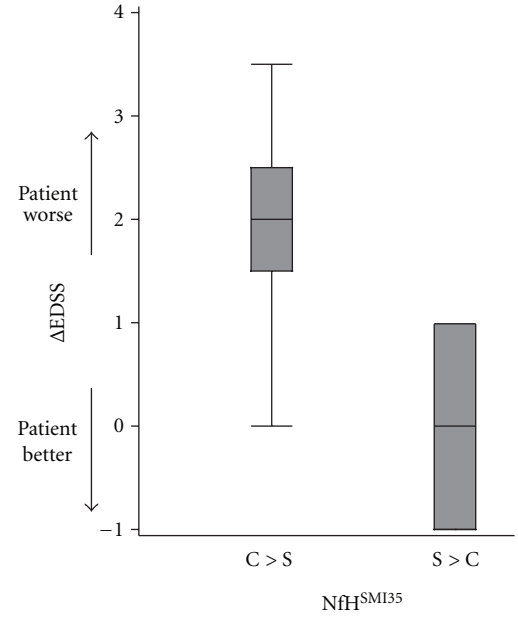

Figure 2: Disability progression in patients with SPMS is associated with higher serum NfH^{SMI34} levels compared to CSF data ($P = .0358$) suggesting that some degree of damage to the peripheral nervous system may exist in these patients.

patients with MS had higher concentration of NfH phosphoforms in the serum compared to the CSF which was considerable for NfH^{SMI34} (50–88%). Furthermore the absolute concentration of serum NfH^{SMI34} was highest in patients with PPMS (Table 1). This is consistent with the notion that neurodegeneration may be more severe and predominating over inflammation in PPMS [22].

At baseline, there were also demographic differences between patients with RRMS, SPMS and PPMS. Patients with PPMS, and SPMS tended to be older and have a longer disease duration compared to patients with RRMS. These demographic differences did not appear to be related to CSF or blood NfH phosphoform levels, because no correlations were found. This is consistent with other NfH studies on CSF [23] and blood samples [13, 24] based on the same ELISA technique. The present study is underpowered to reveal weak correlations which remain statistical possible. Examining a larger cohort with aid of a newer and more

Table 3: Primary progressive MS. The patients were classified according to the relationship of CSF and blood NfH levels. If the concentration was higher in the CSF compared to the blood, this was indicated by "C > S" and "S ≥ C" otherwise. The data is presented for each of the two NfH phosphoforms quantified (NfH^{SMI34} and NfH^{SMI35}).

Characteristic	NfH^{SMI35}		NfH^{SMI34}	
	C > S	S ≥ C	C > S	S ≥ C
Number	4	1	2	3
Age	51 (46–55)	52	48 (46–49)	54 (52–54)
Gender (female : male)	1 : 3	1 : 0	0 : 2	2 : 1
Disease duration (years)	13 (8–20)	17	14 (8–20)	16 (10–17)
EDSS	5 (3–8)	7	7 (7–8)	3 (3–7)
ΔEDSS	1 (−1–1)	0	0 (−1–0)	1 (0–1)

Table 4: Secondary progressive MS. The patients were classified according to the relationship of CSF and blood NfH levels. If the concentration was higher in the CSF compared to the blood, this was indicated by "C > S" and "S ≥ C" otherwise. The data is presented for each of the two NfH phosphoforms quantified (NfH^{SMI34} and NfH^{SMI35}). $^{\star}P < .05$.

Characteristic	NfH^{SMI35}		NfH^{SMI34}	
	C > S	S ≥ C	C > S	S ≥ C
Number	15	3	11	7
Age	45 (29–55)	57 (32–65)	49 (32–65)	44 (29–50)
Gender (female : male)	6 : 9	3 : 0	7 : 4	2 : 5
Disease duration (years)	16 (6–28)	22 (15–24)	19 (6–28)	16 (7–22)
EDSS	5 (1–8)	7 (6–8)	6 (3–8)	3 (1–7)
ΔEDSS	0 (−1–3)	0 (−2–0)	0 (−1.5–1.5)	2 (−1–3)*

sensitive ECL-based technique compared to our ELISA, age was found to correlated with NfH^{SMI35} levels [12].

Another weakness of this study was the high dropout of patients from baseline to followup. This was likely due to the requirement of a second spinal tap from this community rather than hospital-based cohort of MS patients [16, 25].

At 3-year followup, most patients had progressed on the EDSS, but some did improve (Table 2). Sustained progression on the EDSS was highest for patients with RRMS. Consistent with previous data on this [16] and other cohorts [9–12] of patients with MS, high CSF NfH^{SMI35} levels were of prognostic value (Figure 1). Because in these patients the concentration of CSF NfH^{SMI35} levels was higher compared to the matched serum (Table 5), it is suggested that the NfH^{SMI35} measured was of central origin.

The followup data on patients with PPMS and SPMS showed a prognostic value for higher concentration of NfH^{SMI34} in the serum compared to the CSF. This was significant for patients with SPMS (Figure 2). Among the number of different explanations, we tentatively suggest that it may be possible that the source for serum NfH^{SMI34} could at least in part originate from the peripheral nervous system. There is some clinical literature supporting this idea. Subtle alterations on routine electrophysiological measurements in patients with MS are found by some [26] but not by others [27]. More sophisticated measurements using nerve excitability measures [28] show changes in the motor nerve recovery cycle, providing indirect evidence for Na^+/K^+ ATPase pump dysfunction [29–31], a feature of MS pathology [32]. In addition, teased fibre studies from sural nerve biopsies in MS patients showed more disorganized axonal cytoskeleton (Figure 6 in [33]) similar to what is seen in the brain [34, 35]. In view of this data, we speculate that subtle neuroaxonal degeneration of the peripheral nervous system may be present in more severely disabled PPMS and SPMS patients.

An important limitation of our study is that in absence of specific tests [26–28], there is no direct evidence for damage to the peripheral nervous system. Therefore, this hypothesis will need to be investigated prospectively to be substantiated or defeated. We think this is important in order to ensure that attempts to find a blood-based biomarker for central neuroaxonal degeneration in MS are not contaminated by possible pathology of the peripheral nervous system.

It is noted that the results for NfH^{SMI34} are different to NfH^{SMI35}. For NfH^{SMI34}, serum levels were frequently higher than CSF levels compared to NfH^{SMI35}. Trapp et al. reported changes of NfH phosphorylation, particularly dephosphorylation of demyelinated axons in the MS brain [1]. As MS progresses from RR to SP disease, the burden of altered NfH phosphorylation increases [34–36]. Because NfH^{SMI34} binds to a wider range of NfH phosphoforms than NfH^{SMI35}, it may be that serum NfH^{SMI34} levels are more sensitive in detecting axonal damage in MS than serum NfH^{SMI35} levels. This argumentation would be consistent with the finding that a higher concentration of NfH^{SMI34} in the serum compared to the CSF was predictive of disease progression in SPMS.

Could inflammation-related impairment of the blood brain barrier (BBB) function in MS explain higher blood than CSF NfH levels? We do not think so. Historically, the concept of the BBB originated from the observation that

Table 5: Relapsing remitting MS. The patients were classified according to the relationship of CSF and blood NfH levels. If the concentration was higher in the CSF compared to the blood this was indicated by "C > S" and "S ≥ C" otherwise. The data is presented for each of the two NfH phosphoforms quantified (NfH^{SMI34} and NfH^{SMI35}). $^{\star}P < .05$.

Characteristic	NfH^{SMI35}		NfH^{SMI34}	
	C > S	S ≥ C	C > S	S ≥ C
Number	8	3	5	6
Age	41 (34–53)	38 (29–40)	40 (34–53)	39 (29–48)
Gender (female : male)	4 : 4	0 : 3	2 : 3	2 : 4
Disease duration (years)	10 (4–19)	3 (1–10)	8 (4–13)	9 (1–19)
EDSS	2 (0–4)	2 (1–2)	1 (1–4)	2 (0–2)
ΔEDSS	2 (0–4)*	0 (−1–1)	2 (0–3)	1 (−1–4)

certain compounds did not diffuse freely into the central nervous system (CNS), but they would lead to dramatic symptoms if injected intracerebrally, intraventricularly, or intrathecally [37]. For over a century, research on the BBB has focused on diffusion of compounds *into* the brain. It is now well established that assessment of BBB integrity requires quantification of compounds on both sides of the barrier (reviewed in [38]). In fact, two barriers need to be considered: the morphologically defined BBB and the functionally defined blood CSF barrier (BCB) [6, 38, 39]. Large molecules (e.g., IgM with a molecular weight of 800 kD) can pass the barriers in very small quantities (e.g., IgM serum : IgM CSF = 3000 : 1). Smaller molecules pass through the barriers more easily because of molecular size-dependent diffusion (QAlb = 1 : 200; QIgG = 1 : 500). Starling's principle applies, and an increase of QAlb can be caused by a reduced CSF flow rate without any leakage in the morphological structures [6, 38]. The concept of using a biomarker for parenchymal brain damage (e.g., NfH) for a "BBB leakage" model is incompatible with Starling's principle and the well-established physiology of the BBB/BCB function. Brain-derived proteins in blood can indicate brain damage, as consistently reported by a number of groups, but "leakage" of the BBB/BCB is not a precondition of increased blood concentrations.

Could the localization of MS lesion formation in the CNS influence whether products of damage are predominantly released into CSF or blood? This certainly is a possibility. The CSF flow dynamics are such that biomarkers released from cortical pathology are likely to diffuse through the cortical arachnoid villi into the blood stream and only a fraction may reach the lumbar CSF [40]. In contrast, pathology of the spinal cord is more likely to be reflected in lumbar CSF. Neurofilaments are one of the few CNS protein biomarkers with a higher lumbar spinal CSF concentration compared to ventricular CSF [41]. The likely reason for this anatomical: there is a rostrocaudal gradient of the parenchymal Nf protein concentration with the lowest concentration in cortical neurons and the highest concentration in spinal cord axons [34, 42, 43]. It could, therefore, be that a small amount of spinal cord damage may mask more extensive cortical damage if investigated from lumbar CSF alone. Conversely, one may hypothesize that blood NfH levels may be better suited for investigating cortical pathology. This hypothesis is tempting, because of the relative ease to obtain serial blood samples as opposed to CSF samples. Precisely for testing, this hypothesis it will be important to ensure that there is no data contamination by pathology of the PNS as tentatively suggested by the present study.

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Research Article

Serum Metabolic Profile in Multiple Sclerosis Patients

Barbara Tavazzi,[1] Anna Paola Batocchi,[2] Angela Maria Amorini,[1] Viviana Nociti,[2] Serafina D'Urso,[3] Salvatore Longo,[3] Stefano Gullotta,[3] Marika Picardi,[1] and Giuseppe Lazzarino[3]

[1] *Institute of Biochemistry and Clinical Biochemistry, Catholic University of Rome, 00168 Rome, Italy*
[2] *Institute of Neurology, Catholic University of Rome, 00168 Rome, Italy*
[3] *Department of Biology, Geology and Environmental Sciences, Division of Biochemistry and Molecular Biology, University of Catania, 95125 Catania, Italy*

Correspondence should be addressed to Giuseppe Lazzarino, lazzarig@unict.it

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Multiple sclerosis (MS) is a progressive demyelinating process considered as an autoimmune disease, although the causes of this pathology have not been yet fully established. Similarly to other neurodegenerations, MS is characterized by a series of biochemical changes affecting to different extent neuronal functions; great attention has been given to oxidative/nitrosative stress and to alterations in mitochondrial functions. According to previous data, MS patients show significant changes in the circulating concentrations of different metabolites, although it is still unclear whether uric acid undergoes to decrease, increase, or no change under this pathological condition. In this study, we report the serum metabolic profile in terms of purines, pyrimidines, creatinine, malondialdehyde, ascorbic acid, nitrite, and nitrate in a group of 170 MS patients. The results show increase in circulating uric acid and other oxypurines (hypoxanthine and xanthine), as well as in uridine and β-pseudouridine. The concomitant increase in circulating creatinine, malondialdehyde, nitrite, and nitrate, and decrease in ascorbic acid, demonstrates that MS induces alteration in energy metabolism and in oxidants/antioxidants balance that can be monitored in serum of MS patients.

1. Introduction

Multiple sclerosis (MS) is a progressive, invalidating pathological state, the exact etiology of which is still uncertain [1]. It is considered as an autoimmune disease although the reasons for the autoimmune demyelinization are far to be clear [2]. At the molecular level, MS is characterized by a series of biochemical changes affecting neuronal functions [3], some of which are in common with other neurodegenerations such as Alzheimer's [4] and Parkinson's diseases [5]. Particularly, one of these common features is the neuronal imbalance in oxidants/antioxidants, with reactive oxygen species (ROS) and reactive nitrogen species (RNS) as the excess oxidants [6, 7] and uric acid as the putative defective antioxidant [8–11]. Recently, mitochondrial malfunctioning has been indicated to play a central role in the overall derangement of brain metabolism observed in MS [12]. The consequences of mitochondrial perturbation are critical for the correct functioning of the electron transport chain coupled to oxidative phosphorylation and, hence, for the maintenance of the cell energy homeostasis. Furthermore, the abundant literature has linked mitochondrial dysfunction with ROS overflow [13]. If the imbalance in energy production and consumption is operative, that is, the amount of ATP produced does not satisfy the cell energy demand, it is unavoidable that the purine nucleotide degradation pathway is activated. This provokes an increased generation of nucleosides (adenosine, guanosine, and inosine) and oxypurines (hypoxanthine, xanthine, and uric acid), which can freely cross the cell membrane being released in part in the extracellular space. In the brain tissue, this phenomenon contributes to the significant increase of these compounds in the cerebrospinal fluid (CSF) observed under different pathological states [14, 15], including MS [16].

Permeable metabolites generated in excess by transient or chronic dysfunction of brain metabolism are sooner or

later found into the blood stream, potentially contributing to a significant raise over their respective circulating physiological levels [17, 18]. Therefore, several low-molecular-weight compounds can be good candidate as potential blood biomarkers of neurodegeneration. According to the present knowledge, it is conceivable that compounds deriving from ROS and RNS overproduction and metabolites generated by altered energy metabolism might be detected in excess in blood samples from MS patients, possibly being valid predictors of the disease evolution. This logical cause-effect link has been proven for several compounds related to ROS and RNS overproduction, so that increase in circulating nitric oxide (NO) products [19, 20] and increase in lipid peroxidation products [21] have been found in plasma/serum of MS patients. Surprisingly, this does not seem to apply to products deriving from the imbalance of energy metabolism since several studies indicated significant decrease in plasma/serum concentrations of uric acid (the end product of purine nucleotide catabolism) in MS patients [22–25]. The rather improbable explanation for this fact is that brain uric acid, acting as a potent NO scavenger, is oxidized in consequence of the increased NO generation. The final result would be a significant decrease in uric acid circulating levels. In contrast to these results, a number of clinical studies have indicated either no change [26, 27] or increase [28, 29] in plasma/serum uric acid of MS patients, thereby rendering unclear whether this compound is modified under this pathological condition. Recently, we reported a concomitant increase in the plasma and CSF concentrations not only of uric acid but also of other oxypurines and nucleosides in a cohort of MS patients [16, 30].

To reinforce our previous results, we here report the metabolic profile in terms of purines, pyrimidines, creatinine, malondialdehyde (MDA), ascorbic acid, nitrite, and nitrate determined in a group of 170 MS patients. Concentrations of the various metabolites were compared with those recorded in a group of 163 healthy controls. In order to have indications on the potential clinical utility of the routine metabolic profiling of MS patients, metabolite changes were analyzed for a correlation with the severity of the disease and MS subtypes.

2. Materials and Methods

2.1. Selection and Clinical Evaluation of the Patients. One hundred and seventy MS patients were included in this study. They were assessed clinically at the Institute of Neurology of the "Policlinico Gemelli" of the Catholic University of Rome, using the Extended Disability Status Scale score (EDSS) [31]. Patients were classified into relapsing remitting (RR), secondary progressive (SP), or primary progressive (PP), according to what described elsewhere [32]. The control group consisted of 163 healthy subjects, matched for age and gender, and recruited among the personnel of the two Universities undergoing the annual health checkup. All selected subjects had no acute or chronic pathologies. The study was approved by the local Ethic Committee. Written informed consents were obtained.

2.2. Preparation of Samples for the Serum Metabolic Profiling. In both patients and controls, peripheral venous blood samples were collected from the antecubital vein into VACUETTE polypropylene tubes containing serum separator and clot activator (Greiner-Bio One GmbH, Kremsmunster, Austria). After 40 minutes at room temperature, samples were centrifuged at $1890 \times g$ for 10 min to separate sera. Aliquots were first diluted with doubly-distilled water (1 : 2, v : v) and then deproteinized by ultrafiltration, according to a procedure described in detail elsewhere [33]. The deproteinized ultrafiltrate fluid was used to quantify the metabolites of interest using a single, ion-pairing, high-performance liquid chromatographic (HPLC) analysis which allows the simultaneous isocratic separation of creatinine, purines (hypoxantine, xanthine, uric acid, inosine, guanosine), pyrimidines (uracil, β-pseudouridine, thymine, uridine, thymidine, orotic acid), ascorbic acid, MDA, nitrite, and nitrate [33].

Deproteinized samples were loaded (200 μL) onto a Hypersil C-18, 250 × 4.6 mm, 5 μm particle size column, provided with its own guard column (ThermoFisher Italia, Rodano, Milan, Italy). The chromatographic column was connected to an HPLC apparatus consisting of a SpectraSystem P4000 pump system and a highly-sensitive UV6000LP diode array detector (ThermoFisher Italia, Rodano, Milan, Italy), equipped with a 5 cm light path flow cell and set up between 200 and 300 nm wavelength. Data acquisition and analysis were performed by a PC using the ChromQuest software package provided by the HPLC manufacturer. Assignment and calculation of the compounds of interest in chromatographic runs of biological fluid extracts were carried out at 206 (nitrite and nitrate), 234 (creatinine), or 260 (purines, pyrimidines, ascorbic acid, MDA) nm wavelengths by comparing retention times, absorption spectra, and areas of peaks with those of peaks of chromatographic runs of freshly prepared ultrapure standard mixtures with known concentrations.

2.3. Statistical Analysis. All variables were skewed and, therefore, were log-transformed to approach Gaussian distribution before application of parametric tests. Differences between controls and MS patients were assessed by the Student's t-test for unpaired observations. Due to the different number of subjects, differences among subgroups of MS patients on EDSS or on clinical MS subtypes (RR, SP, PP) were assessed by the Kruskal-Wallis one-way ANOVA by ranks. A value of $P < .05$ was considered significant.

3. Results

The characteristics of both the MS patients and the control group are summarized in Table 1. The clinical classification indicated that 66.5% of the patients were RR, 25.3% were SP, and 8.2% only were PP.

3.1. Serum Metabolic Profile of MS Patients: Purines, Pyrimidines, and Creatinine. Data referring to the circulating levels of the different metabolites under evaluation in controls and

Table 1: Clinical features of MS patients and controls.

	Controls	MS patients
Number of patients	163	170
Female : male	106 : 57	115 : 55
Average age at onset	NA	31.77 ± 11.72
Average age at assessment	43.45 ± 3.21	45.27 ± 6.80
Duration of pathology (years)	NA	13.5 ± 5.22
RR	NA	113
SP	NA	43
PP	NA	14
Average EDSS	NA	3.26 ± 2.29

NA: not available.
RR: relapsing-remitting MS; SP: secondary progressive MS; PP: primary progressive MS; EDSS: expanded disability scale score.

Table 2: Concentration of circulating creatinine, pyrimidine (β-pseudouridine and uridine), oxypurines (hypoxanthine, xanthine and uric acid) malondialdehyde (MDA), nitrite and nitrate (NO_2 + NO_3), and ascorbic acid determined by HPLC in serum samples of healthy controls and MS patients.

	Controls ($n = 163$)	MS patients ($n = 170$)
Creatinine	56.87 ± 17.98	71.10 ± 19.27[a]
Uracile	1.97 ± 0.90	2.11 ± 1.04
β-pseudouridine	3.03 ± 1.24	4.67 ± 1.71[a]
Uridine	4.83 ± 2.19	7.20 ± 1.82[a]
Hypoxanthine	4.19 ± 1.58	12.30 ± 4.84[a]
Xanthine	1.44 ± 0.96	4.03 ± 2.20[a]
Uric acid	258.08 ± 50.39	299.88 ± 70.17[a]
MDA	0.005 ± 0.004	0.84 ± 0.54[a]
$NO_2 + NO_3$	69.06 ± 29.04	107.94 ± 43.87[a]
Ascorbic acid	57.52 ± 14.81	37.36 ± 10.95[a]

Values are means ± standard deviations and are expressed in μmol/L serum.
[a]significantly different from controls ($P < .001$).

MS patients are reported in Table 2. With respect to values in controls, the HPLC analysis of serum oxypurines evidenced a 2.94 ± 1.14-fold increase (mean ± standard deviation) in the value of hypoxanthine ($P < .001$), a 2.80 ± 1.53-fold increase (mean ± standard deviation) in the value of xanthine ($P < .001$) and a 1.16 ± 0.27-fold increase (mean ± standard deviation) in the value of uric acid ($P < .001$). When considering the sum of circulating oxypurines in MS patients (316.20 ± 72.21 μmol/L serum; mean ± standard deviation), a 1.21 ± 0.28-fold increase (mean ± standard deviation) with respect to controls (261.16 ± 48.89 μmol/L serum; mean ± standard deviation; $P < .001$) was observed. These values are illustrated in the scatter plot of Figure 1(a); in the same figure, levels of serum creatinine in controls and MS patients are also reported (Figure 1(b)). Similarly to what observed for oxypurines, value of circulating creatinine in MS patients (71.10 ± 19.27 μmol/L serum; mean ± standard deviation) was 1.25 ± 0.34 times higher (mean ± standard deviation) than that recorded in controls (56.87 ± 17.98 μmol/L serum; mean ± standard deviation; $P < .001$). When MS patients were divided on the basis of the disability, no one of the aforementioned metabolites correlated with increasing EDSS. Differently, the classification of the patients into three subgroups on the basis of the MS subtypes (Figure 2) showed that RR patients had significantly different values of creatinine, uric acid, and sum of oxypurines in comparison to both SP ($P < .001$) and PP patients ($P < .001$).

Among the pyrimidine compounds, uracil, β-pseudouridine, and uridine were always detectable in all serum samples analyzed using this HPLC method. Uracil concentration in serum of controls (1.97 ± 0.90 μmol/L serum; mean ± standard deviation) did not differ from that measured in MS patients (2.11 ± 1.04 μmol/L serum; mean ± standard deviation). Viceversa, Figure 3 illustrates that circulating uridine (a) and β-pseudouridine (b) were significantly different in MS patients (7.20 ± 1.81 and 4.67 ± 1.71 μmol/L serum, resp.; means ± standard deviations) and controls (4.83 ± 2.19 and 3.03 ± 1.23 μmol/L serum, resp.; means ± standard deviations). Uridine and β-pseudouridine did not correlate with increasing EDSS, nor they showed significant differences in the subgroups of patients divided on MS subtypes.

3.2. Serum Metabolic Profile of MS Patients: Oxidants and Antioxidants. Figure 4 reports concentrations of circulating MDA (a), as an index of lipid peroxidation, and of nitrite + nitrate (b), generated from nitric oxide decomposition, in controls and MS patients. MDA in serum of MS patients (0.84 ± 0.53 μmol/L serum; mean ± standard deviation) showed a tremendous 210 ± 132-fold increase (mean ± standard deviation) in comparison with the concentration measured in serum of controls (0.004 ± 0.003 μmol/L serum; mean ± standard deviation). Serum nitrite + nitrate in MS patients (mean ± standard deviation = 107.94 ± 43.87 μmol/L serum) was 1.56 ± 0.63-fold higher (mean ± standard deviation) than the circulating value of these two nitrogen anions measured in controls (69.05 ± 29.04 μmol/L serum; mean ± standard deviation).

Data in Figure 5 show that the serum concentration of ascorbic acid in MS patients (37.36 ± 10.95 μmol/L serum; mean ± standard deviation) was 1.54 ± 0.45 times lower (mean ± standard deviation) than that recorded in controls (57.52 ± 14.81 μmol/L serum; mean ± standard deviation), thereby indicating a decrease in this circulating antioxidant as a consequence of the increased oxidative/nitrosative stress occurring in MS. It is worth recalling that MDA, nitrite + nitrate, and ascorbic acid did not correlate with increasing EDSS, nor they showed significant differences in the subgroups of patients divided on MS subtypes.

4. Discussion

Data reported in the present study confirm our previous findings obtained in a smaller group of MS patients [16, 30] and indicate alterations of circulating compounds related to energy metabolism, oxidative/nitrosative stresses, and antioxidant status occurring in multiple sclerosis.

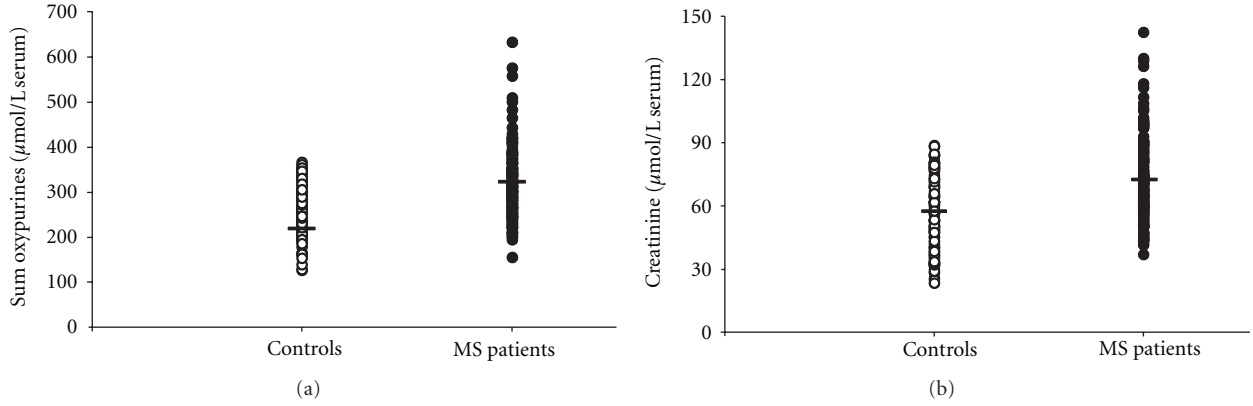

Figure 1: Scatter plot showing the sum of oxypurines (uric acid + hypoxanthine + xanthine) (a) and creatinine (b) recorded in serum of 163 healthy controls and 170 MS patients. Horizontal bars indicate the mean values calculated in the two groups.

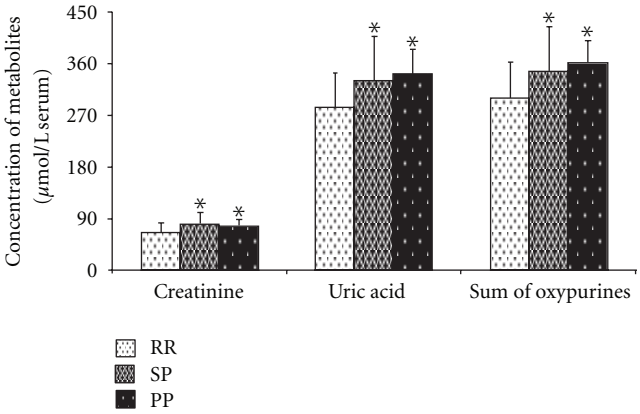

Figure 2: Bar graph showing the mean values of creatinine, uric acid, and sum of oxypurines (uric acid + hypoxanthine + xanthine) in the 170 MS patients divided on the basis of the clinical MS subtype. RR: relapsing remitting; SP: secondary progressive; PP: primary progressive. Standard deviations are indicated by vertical bars. Asterisk = significantly different from RR ($P < .01$).

Most of the studies suggest that circulating concentrations of uric acid, which metabolically derives from catabolism of phosphorylated purines (ATP and GTP) and also from nucleic acid degradation, are decreased in MS patients [22–25]. However, a meaningful body of the literature contrasts this evidence, indicating that MS patients have levels of plasma/serum uric acid comparable or higher than those recorded in controls [26–29]. In the present cohort of 170 MS patients, confirming our previous observations [16, 30], we again found higher concentrations in serum uric acid than those recorded in 163 age- and gender-matched healthy controls (Table 2). This increase in serum uric acid was accompanied by an almost three times raise in both hypoxanthine and xanthine, thus rendering more evident the overall net increase in circulating oxypurines associated with MS (Figure 1(a)). Furthermore, although none of the aforementioned parameters correlated with EDSS, either uric acid or the sum of oxypurines did correlate with the MS clinical subtypes, with the RR subgroup showing lower values than those found in both the SP and the PP subgroups (Figure 2).

The three oxypurines considered are mainly produced along the cascade of purine nucleotide degradation, when energy metabolism does not satisfy the cell/tissue ATP demand [34, 35]. Hence, it is conceivable to affirm that MS patients might suffer from imbalance between energy production and consumption. Since the machinery to ensure adequate ATP production is localized in mitochondria, the recent data showing neuronal mitochondrial malfunctioning in MS [12] corroborate the hypothesis that the increase in circulating oxypurines in our patients is the direct consequence of altered mitochondrial functions. Certainly, our results do not support the notion sustained by various studies which affirm that MS patients have lower plasma/serum uric acid than controls [22–25]. In particular, since MS patients suffer from increased oxidative/nitrosative stress [6, 7], it has been suggested that decrease of circulating uric acid in MS is due to the potent uric acid scavenging activity towards peroxynitrite [9]. Together with the results on the full profile of serum purine compounds, our data evidenced a decline in circulating antioxidant defenses of MS patients, in terms of ascorbic acid and not of uric acid decrease (Figure 5). Ascorbic acid, a hydrophilic low-molecular-weight antioxidant, is not synthesized by the human body and adequate amount should, therefore, be assumed with the diet to allow a reasonable distribution by the blood stream to the different tissues. The brain has a specific transporter for ascorbic acid devoted to permit that this compound can cross the blood brain barrier and is accumulated within the cerebral cells, against a concentration gradient [36, 37]. Through this facilitated transport mechanism, cerebral ascorbic acid reaches the concentration of about 2300 nmoL/g wet weight (corresponding to about 2500 μmol/L brain water) and is the second most abundant, water-soluble, brain antioxidant [38, 39]. Ascorbic acid has the same affinity for peroxynitrite than that of uric acid [9], but in the brain it is about 1000 times more concentrated than uric acid [16, 40]. Even if cerebral uric acid had a role as

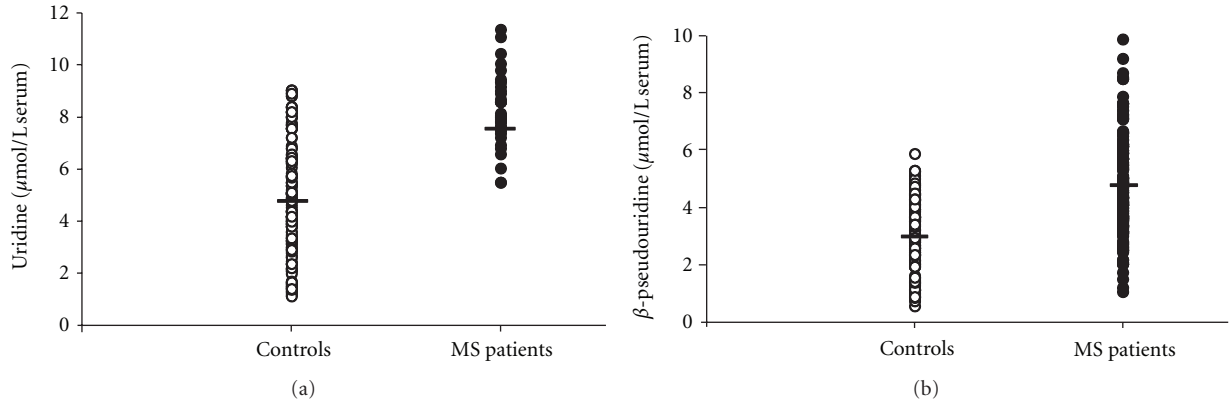

Figure 3: Scatter plot showing the concentrations of uridine (a) and β-pseudouridine (b) recorded in serum of 163 controls healthy and 170 MS patients. Horizontal bars indicate the mean values calculated in the two groups.

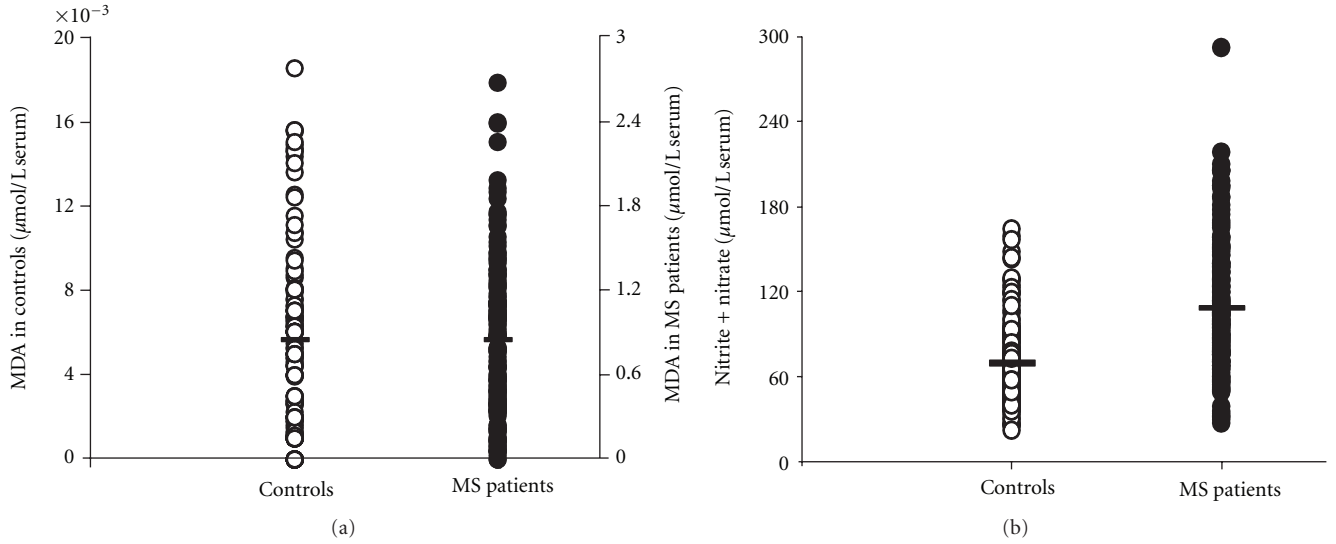

Figure 4: Scatter plot showing the concentrations of MDA (a) and sum of nitrite and nitrate (b) recorded in serum 163 controls healthy and 170 MS patients. Horizontal bars indicate the mean values calculated in the two groups.

an antioxidant, it appears evident that in the case of increased oxidative/nitrosative stress a decrease in brain ascorbic acid rather than in uric acid would certainly occur. Cerebral uric acid would be oxidized only when the concentration ratio ascorbic acid/uric acid in the brain were in favor of uric acid. According to the present results, our cohort of MS patients, in consequence of increased oxidative/nitrosative stress (Figure 4), showed a 35% decrease in circulating ascorbic acid. Such a decrease might render less efficient the mechanism of its cerebral accumulation and to reduce, in turn, the brain antioxidant capacity. If the decrease in serum ascorbic acid was hypothetically mirrored by an equal decrease in the brain tissue, cerebral ascorbic acid would then be 1400–1500 nmoL/g wet weight, that is, still 700 times higher than brain uric acid [16, 40]. Therefore, it appears that even in conditions of increased oxidative/nitrosative stress, there are not the biochemical presuppositions to sustain a role of uric acid as a valid brain tissue antioxidant, nor to imagine that MS might provoke its decrease in serum.

The evidence of impaired energy metabolism in our MS patients was also supported by data referring to circulating uridine, the value of which was 1.5 times higher than that found in controls (Figure 3(a)). According to previous observations [41], the increase in plasma uridine can be considered as an indirect indicator of tissue energy crisis. In fact, in conditions of metabolic energy imbalance in humans, it has clearly been demonstrated a close association between myocardial ATP exhaustion and the increase either in circulating purines (hypoxanthine, xanthine, uric acid), or in circulating uridine [42]. This reinforces the concept that changes in plasma uridine reflect changes in cell/tissue energy metabolism. The overall conclusion, when analyzing results of circulating purines and pyrimidines, is that MS patients suffer indeed from energy deficit, probably in

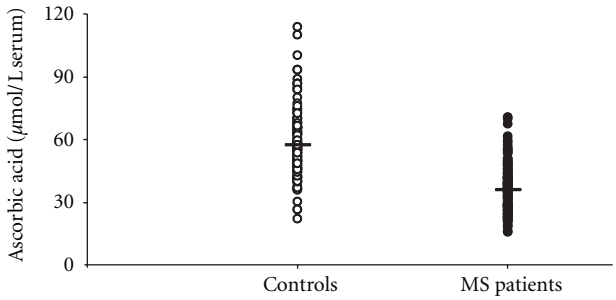

Figure 5: Scatter plot showing the concentration of ascorbic acid recorded in serum 163 healthy controls and 170 MS patients. Horizontal bars indicate the mean values calculated in the two groups.

consequence of altered mitochondrial functions [12, 43, 44]. Since MS patients are at risk of a number of intercurrent systemic inflammatory or noninflammatory conditions [45, 46], it cannot be excluded a significant extracerebral contribution in the overall serum increase of these metabolites. In addition, the muscular involvement in MS [47, 48], possibly caused by a metabolic imbalance of myocytes and also recently evidenced by an increased cost of walking in patients with mild disability [49], might further contribute to exacerbate alterations in the serum metabolic profile of these patients. Data indicating higher serum creatinine in MS patients than in controls (Figure 1(b)) strongly reinforce this concept.

In our MS patients, a significant increase in serum β-pseudouridine was also observed. Since this modified pyrimidine is exclusively found in transfer and ribosomal RNAs, its increase in body fluids is generally considered as an index of increased rate of RNAs turnover, due to increased rate of protein synthesis [50]. Since in experimental autoimmune encephalomyelitis (EAE) protein synthesis has been shown to increase 4-fold over the basal level [51], it may be hypothesized that this phenomenon is responsible for the increase in serum β-pseudouridine in MS patients.

In this study, the most dramatic change associated with MS occurred to serum MDA (Figure 4(a)). This compound, originating from the irreversible decomposition of peroxidized polyunsaturated fatty acids of membrane phospholipids, is considered a reliable indicator of increased oxidative stress [52, 53], if properly assayed. In MS patients, the 210-fold increase of MDA over the value recorded in controls is the clear evidence that reactive oxygen species-mediated lipid peroxidation is operative under this pathological condition. Since we also found a significant increase in nitrite + nitrate in serum of MS patients (Figure 4(b)), we can conclude that these patients are exposed to the concomitant oxidative/nitrosative stress, stating that the sum of these two nitrogen anions is considered as an index of NO generation [54, 55]. This implies an elevated risk of producing the highly oxidizing radical peroxynitrite $ONOO^{\cdot}$ with serious consequences for the brain tissue integrity.

The main limitations of this study are that changes in serum metabolites failed to correlate with EDSS, probably because of a low number of subjects in several patient subgroups. Even the differences recorded for some metabolites when patients were divided into the three clinical MS subtypes failed to discriminate SP from PP, most likely because of the limited number of PP patients. Recruitment of additional MS patients is in progress.

5. Conclusions

In conclusion, our results on the serum metabolic profile in MS clearly indicate that these patients suffer from a profound purine and pyrimidine dysmetabolism, potentially due to altered mitochondrial functions. This causes the increase in circulating uric acid, hypoxanthine, xanthine, creatinine, β-pseudouridine, and uridine, with creatinine, uric acid, and sum of oxypurines being in correlation with the clinical MS subtypes. The clear evidence of concomitant oxidative/nitrosative stress suggests that possible therapeutic approaches aimed to improve cerebral mitochondrial functions and neuronal energy state, as well as to increase the brain antioxidant defenses, might ameliorate the status of MS patients.

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Review Article

Consensus Guidelines for CSF and Blood Biobanking for CNS Biomarker Studies

Charlotte E. Teunissen,[1] Hayrettin Tumani,[2] Jeffrey L. Bennett,[3] Frode S. Berven,[4] Lou Brundin,[5] Manuel Comabella,[6] Diego Franciotta,[7] Jette L. Federiksen,[8] John O. Fleming,[9] Roberto Furlan,[10] Rogier Q. Hintzen,[11] Steve G. Hughes,[12] Connie R. Jimenez,[13] Michael H. Johnson,[14] Joep Killestein,[15] Eva Krasulova,[16] Jens Kuhle,[17] Maria-Chiara Magnone,[18] Axel Petzold,[19] Cecilia Rajda,[20] Konrad Rejdak,[21, 22] Hollie K. Schmidt,[23] Vincent van Pesch,[24] Emmanuelle Waubant,[25] Christian Wolf,[26] Florian Deisenhammer,[27] Gavin Giovannoni,[28] and Bernhard Hemmer[29]

[1] *Department of Clinical Chemistry, Neurological Laboratory and Biobank, VU University Medical Center, FdG, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands*
[2] *University of Ulm, Ulm 89081, Germany*
[3] *Departments of Neurology & Ophthalmology, University of Colorado Denver, Aurora, USA*
[4] *Proteomics Unit (PROBE), Department of Biomedicine, University of Bergen, Bergen 5020, Norway*
[5] *Division Neurology, Department Clin Neuroscience, Karolinska University Hospital, Stockholm, Sweden*
[6] *Neurology, Centre d'Esclerosi Múltiple de Catalunya, CEM-Cat, Unitat de Neuroimmunologia Clínica, Hospital Universitari Vall d'Hebron, Barcelona, Spain*
[7] *Laboratory of Neuroimmunology, IRCCS, "C. Mondino Neurological Institute", Pavia, Italy*
[8] *Neurology, Glostrup Hospital, University of Copenhagen, Glostrup 2600, Denmark*
[9] *Department of Neurology, University of Wisconsin, Madison 53705, WI, USA*
[10] *Clinical Neuroimmunology Unit, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, 20132 Milan, Italy*
[11] *Neurology, Erasmus MC, Rotterdam, The Netherlands*
[12] *Clinical Developement Department, Isis Pharmaceuticals, Carlsbad, California 92024, USA*
[13] *OncoProteomics Laboratory, Department of Medical Oncology, VU University Medical Center, 1007 MB Amsterdam, The Netherlands*
[14] *Department of Neurology, Leeds Teaching Hospitals NHS Trust, Leeds LS1 3EX, UK*
[15] *Department of Neurology and Center of Clinical Neuroscience, 1st Faculty of Medicine and General University Hospital, Charles University, 128 21 Prague, Czech Republic*
[16] *Department of Neurology, VU University Medical Center, 1007 MB Amsterdam, The Netherlands*
[17] *Neurology and Clinical Neuroimmunology, University Hospital, University of Basel, Basel 4031, Switzerland*
[18] *F. Hoffmann- La Roche Pharma, Basel, Switzerland*
[19] *Department of Neuroimmunology, UCL Institute of Neurology, Queen Square, wc1n 3bg London, UK*
[20] *Department of Neurology, University of Szeged, Szeged, Hungary*
[21] *Department of Neurology, Medical University of Lublin, Lublin, Poland*
[22] *Department of Experimental Pharmacology, Medical Research Center, Warsaw, MA 02451, Poland*
[23] *Accelerated Cure Project for Multiple Sclerosis, Waltham, MA, USA*
[24] *Neurology Department UCL, Université Catholique de Louvain, Brussels CA94117, Belgium*
[25] *Clinical Development, Lycalis sprl, 1180 Brussels, Belgium*
[26] *Clinical Development, UCB Pharma S.A., 6020 Braine l'Alleud, Belgium*
[27] *Department of Clinical Neurology, Innsbruck Medical University, Innsbruck E1 2AT, Austria*
[28] *Queen Mary University of London, Neuroscience & Trauma Centre, Blizard Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, London, UK*
[29] *Deptartment of Neurology, Klinikum rechts der Isar, Technische Universität, Munich 81245, Germany*

Correspondence should be addressed to Charlotte E. Teunissen, c.teunissen@vumc.nl

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There is a long history of research into body fluid biomarkers in neurodegenerative and neuroinflammatory diseases. However, only a few biomarkers in cerebrospinal fluid (CSF) are being used in clinical practice. Anti-aquaporin-4 antibodies in serum are currently useful for the diagnosis of neuromyelitis optica (NMO), but we could expect novel CSF biomarkers that help define prognosis and response to treatment for this disease. One of the most critical factors in biomarker research is the inadequate powering of studies performed by single centers. Collaboration between investigators is needed to establish large biobanks of well-defined samples. A key issue in collaboration is to establish standardized protocols for biobanking to ensure that the statistical power gained by increasing the numbers of CSF samples is not compromised by pre-analytical factors. Here, consensus guidelines for CSF collection and biobanking are presented, based on the guidelines that have been published by the BioMS-eu network for CSF biomarker research. We focussed on CSF collection procedures, pre-analytical factors and high quality clinical and paraclinical information. Importantly, the biobanking protocols are applicable for CSF biobanks for research targeting any neurological disease.

1. Introduction: The Need for Collaborative Biobanking and Biomarker Studies

NMO can be diagnosed based on a blood-derived biomarker, that is antibodies against aquaporin-4, a channel protein present on astrocytes, extensively discussed in other contributions in this special issue. The presence of antibodies against aquaporin-4 has been proven as one of the most successful results of biomarker studies, and is supportive for the idea that central nervous system (CNS) abnormalities are reflected in changes in body fluids. It also proofs the autoimmune component of this disorder and of pathologies that are related to the NMO spectrum disorders, such as longitudinally extensive transverse myelitis.

Determination of serum anti-aquaporin-4 antibody levels is a mainstay in the diagnosis of NMO, but the discovery of such disease-specific antibodies is relatively recent [1], and, therefore, further studies in body fluids are warranted. One case report suggested that NMO-immunoglobulin G (IgG), the NMO-associated antibodies that are reactive to cerebellar tissue [1], can be absent in serum, but present in CSF [2]. However, another study on a relative large cohort of patients showed that testing CSF does not increase diagnostic sensitivity [3]. Another recently identified candidate biomarker for NMO is glial fibrillary acid protein (GFAP). Takano and colleagues observed that the analysis of CSF glial fibrillary acid protein is useful in the differential diagnosis between NMO and multiple sclerosis or acute demyelinating encephalomyelitis, and that its CSF levels at disease onset correlated with expanded disability score scale (EDSS) in NMO [4]. However, studies on larger cohorts are needed before drawing definite conclusions. Taken together, no biomarkers are available yet for prognosis or therapy response in NMO and in NMO-related disorders. Therefore, biomarker studies on CSF are ongoing.

One important flaw in several previously performed biomarker studies in CNS diseases has been the lack of large cohorts to sufficiently power the study. This is especially an issue for such a rare disease as NMO, where a single center will not be able to collect a large cohort within a reasonable time frame. The need for collaboration was the reason for biomarker researchers in Multiple Sclerosis to start a network (BioMS-eu, http://www.bioms.eu/). The aim of this collaboration is to obtain well-proven, high-quality biomarkers, which will be achieved by sharing patient samples, standardization, and improvement of procedures important in the research area. One of the most urgent prerequisites for collaboration was felt to be standardization of biobanking protocols. Therefore, a consensus-meeting was organised and the result was collection and biobanking guidelines, which the network developed and published in 2009 [5]. There are currently major efforts worldwide to professionalize biobanks and the collection and biobanking guidelines established by consensus among 26 groups participating in BioMS-eu (http://www.bioms.eu/) is a major achievement in the CNS biomarker field [5]. One year after publication of the guidelines, over 90% of the BioMS-eu laboratories had already adapted their procedures in agreement with the guidelines. A great use of the guidelines is the applicability for any neurological disease, including NMO, and that it provides guidelines for setting up a novel biobank. Furthermore, it will greatly facilitate biomarker studies in the CNS biomarker research area. In the concensus discussions, we have sought a balance between practicality and scientific rationale, and the background of each decision is provided. Before the consensus, it was clear that large differences were present between collection protocols, highlighting the need to address these differences (Figure 1 and Table 1). In the current paper, we include only the items and their rationale from the original paper that are relevant for biobanking for NMO. Other modifications from the original protocol is an adaptation of item 1 (samples should be pooled if multiple collection tubes are used for one patient), and the inclusion of an item addressing transportation (item 20) and information of some more physiological confounders (item 25).

We would like to stress that researchers adhere to these protocols for optimal collaboration in the field of CSF biomarker research. We suggest using Tables 2 and 3 as a checklist for CSF biomarker research and recommend that future studies of CSF biomarker take these issues into account. In discovery-based biomarker research, all these items should be considered carefully before initiating a study. Some procedures may not be possible in everyday clinical practice (e.g., processing within one hour), but less stringent requirements will suffice for specific research questions.

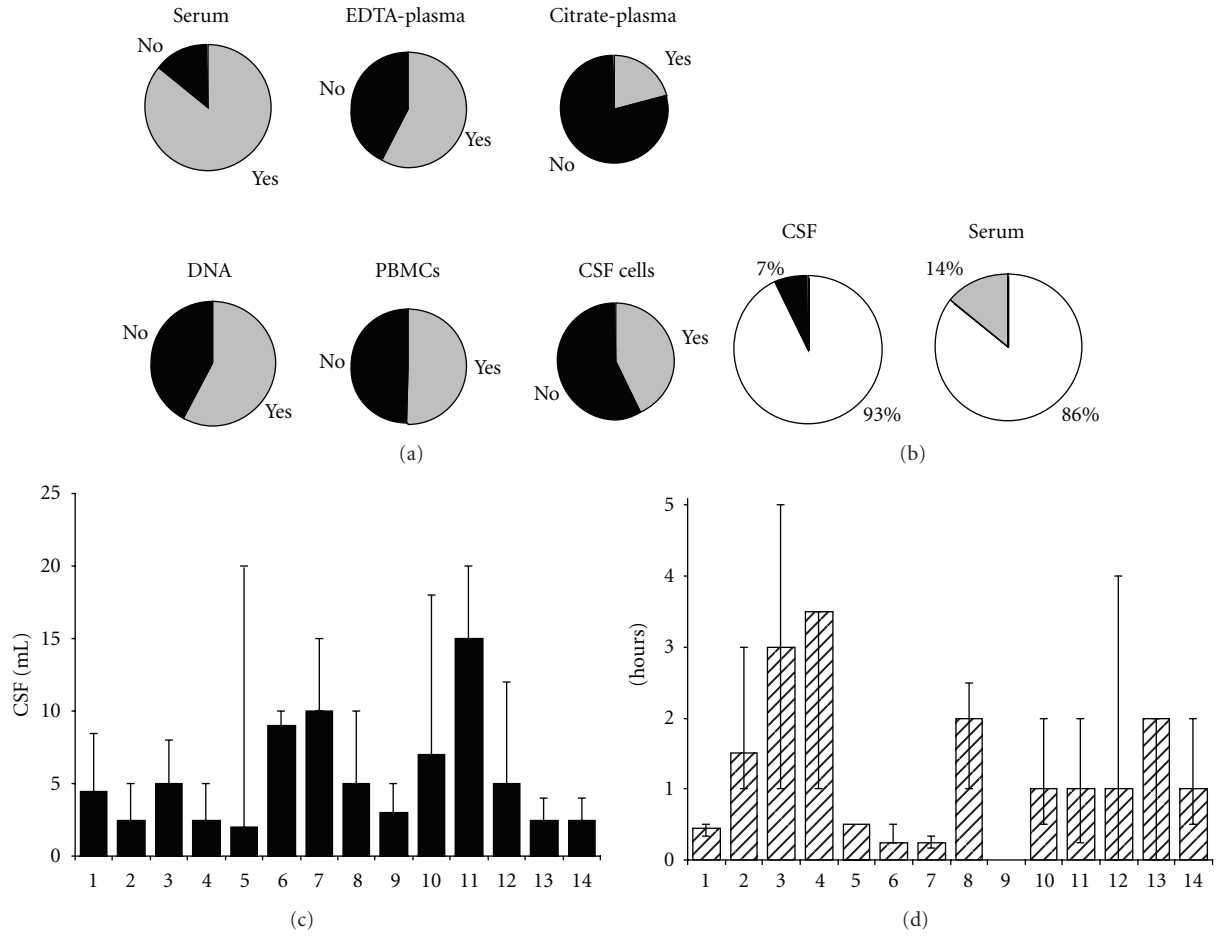

Figure 1: Results of inventory of collection procedures among 14 European centers with CSF biobanks for MS research in 2006. (a) Other body fluids that are collected simultaneously with CSF. Filled area indicates "yes"; open areas: not collected. (b) Storage temperature of CSF and serum. Open area: −80; closed area: −20; grey area: not collected. (c) Average volume of CSF that is collected per patient per CSF withdrawal. Bars indicate the average and ranges of volume per center. (d) Time-delay between CSF withdrawal, spinning and storage into the freezer. Bars indicate the average and ranges of time per center.

Table 1: Results of inventory on collection protocols among 14 MS Biomarker Research Centers.

Procedure of CSF withdrawal	Previous status among European CSF centers
Type of needle:	71% atraumatic, 21% traumatic, 8% both
Time of the day of withdrawal (important for markers that are sensitive for circadian rhythm)	71% no specific day/time of withdrawal, 29% in the afternoon only
Temperature until storage	57% room temperature, 43% at 4°C
Type of tube:	50% Sarstedt, 29% Eppendorf, 21% other
aliquoting:	Range from 0.2 mL to 2 mL
(1) surveillance of freezers	Present at 93% of the centers
(2) several freezers to split the samples (backup)	Present at 14% of the centers

Therefore, careful documentation of these issues is crucial to facilitate retrieval of appropriate samples dictated by specific study aims. As indicated before, the procedures for withdrawal and storage of CSF (Table 2) are broadly applicable for any neurological disease.

Besides methodological issues, ethical approval is a crucial prerequisite for collaboration between international or national centers. The signed informed consent should include a statement that exchange of samples between (international) centers is allowed. Furthermore, to bring a biomarker to clinical practice, one may need patents and the involvement of industrial partners could be needed, who have the infrastructure for large-scale production, quality control procedures, and to reach as many laboratories as

Table 2: Guidelines for procedure of CSF withdrawal.

Item no.	Procedure	Ideal situation
		(A) Collection procedures
(1)	Preferred volume	At least 12 mL. First 1-2 mL for basic CSF assessment (item 26). Last 10 mL for biobanking. Record volume taken and fraction used for biobanking, if applicable.
(2)	Location	Vertebral body L3–L5
(3)	If bloody	Do not process further. Criteria for bloody: more than 500 red blood cells/μL. Record number of blood cells in diagnostic samples.
(4)	Type of needle	Atraumatic
(5)	Type of collection tube	Polypropylene tubes, screw cap, volume >10 mL.
(6)	Time of day of withdrawal and storage	Preferably standardized within each center allowing for intercenter differences in local logistics. Record date and time of collection.
(7)	Other body fluids that should be collected simultaneously	Serum
(8)	Other body fluids that should be collected simultaneously	Plasma: EDTA (preferred over citrate).
		(B) Processing for storage
(9)	Storage temperature until freezing	Room temperature before, during, and after spinning.
(10)	Spinning conditions	Serum: 2,000 g, 10 min at room temperature. CSF: 400 g, 10 min at room temperature/2,000 g if no cells are to be preserved.
(11)	Time delay between withdrawal and spinning and freezing	Optimal for CSF: 1-2 hours Optimal for serum: 30–60 min. Thus doing "*both body fluids simultaneously*": ideally within one hour. After spinning, samples must be aliquoted and frozen immediately for storage at -80^{o}C.
(12)	Type of tube for aliquoting	Small polypropylene tubes (1 to 2 mL) with screw caps. Record manufacturer.
(13)	Aliquoting	A minimum of two aliquots is recommended. The advised research sample volume of 10 mL should be enough for >10 aliquots.
(14)	Volume of aliquots	Minimum 0.1 mL. Depending on total volume of tube: 0.2, 0.5, and 1 mL. Preferably, the tubes are filled up to 75%.
(15)	Coding	Unique codes. Freezing-proof labels. Ideally barcodes to facilitate searching, to aid in blinding the analysis and to protect the privacy of patients.
		(C) Storage conditions and administration
(16)	Freezing temperature	-80^{o}C
(17)	Additional items on sample collection protocols that must be recorded	Location of samples
(18)	Additional items on sample collection protocols that must be recorded	Surveillance of freezers
(19)	Additional items on sample collection protocols that must be recorded	Splitting of samples over two or more freezers
(20)	Transport conditions	Always on dry-ice, sufficient volume of dry-ice for minimal 3 days of transport. Initiated on Mondays. Avoid high temperatures for thawing and mix thoroughly.

possible. For large-scale validation studies, patient samples are of course also needed, and it will be wise to consider this possibility at the start of biobank formation and, if ethical laws permit and the option is perceived to be important, indicate the possibility for industrial cooperation in the patient information and consent.

Lastly, researchers should be willing to share their samples and information for the benefit of the whole, that is,

Table 3: Guidelines for patient information requirement in databases of MS patients.

Item no.	(D) Patient information requirement in databases
	(a) Basic demographics
(21)	(1) date of birth (age if date of birth is not available)
(22)	(2) Gender
(23)	(3) Ethnicity
(24)	(4) Use of drugs, at sampling and year before sampling.
(25)	(5) Actual nonneuronal infections, fasting or nonfasting, pregnancy.
(26)	(6) Basic CSF analysis (CSF cell count, differential cytology, erythrocyte count, oligoclonal IgG bands (which is at least two bands by definition), albumin ratio, total protein (if albumin is not measured), and IgG index)
	(7) Record the methods of routine analysis
(27)	(8) The data in the CSF database should be in English and use Standardized International Units

obtaining reliable biomarkers that can be used for patient care and cure.

2. Guidelines for CSF Biobanking for Biomarker Research, Rationale, and Details

2.1. Procedure of CSF Collection (Table 2)

(A) Collection Procedures:

Item 1 Volume of Withdrawal of at Least 12 mL. The CSF volume taken can influence the concentration of biomarkers. Most molecules and cell numbers have a rostrocaudal concentration gradient [6, 7]. If a small volume is taken, the CSF will reflect the composition of the lumbar dural sac, whereas large volumes may reflect the rostral spinal or even ventricular CSF. Therefore, if biomarker concentrations in a sample from a puncture of 2 mL are compared to that in a puncture of 15 mL, this can lead to erroneous results. Also collecting different portions of the CSF for biobanking (e.g., initial and final volumes of the puncture) may introduce errors. Thus, a standard volume of CSF should be collected during lumbar puncture, the first 2 mL can be used for basic CSF analysis (item 26), and the remainder of the sample should be pooled before spinning and aliquoting. At least, the procedure must be recorded. The volume of collected CSF does not correlate with the risk of postlumbar puncture headache [8, 9].

Item 2 Location of Puncture: Vertebral Body L3–L5. Usually, diagnostic CSF is obtained by lumbar puncture. Because of the increasing gradient in protein concentration from ventricular to lumbar CSF [10], the site of CSF withdrawal must be recorded. When CSF is taken from other locations such as the cervical cisterns or from the lateral ventricles (e.g., ventricular drainage), this should be documented.

Item 3 Removal of Bloody CSF Samples. A traumatic tap causing blood contamination of CSF occurs in about 14–20% of standard lumbar punctures [11]. For biomarkers that have high serum concentrations, such as coagulation factors, blood contamination can lead to false positive results. In addition, blood proteins lead to suppressed matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-TOF/MS) proteomics patterns in CSF. This suppression by blood proteins is, however, highly reduced after removal of the blood cells by centrifugation prior to initial freezing [12, 13]. Recording of erythrocyte count is essential to select CSF samples appropriate for these measurements. CSF samples with an erythrocyte count above 500/μL should not be used for biomarker studies.

Item 4 Use of Atraumatic Needle (Sprotte or Whitacre Needle). There is no evidence that the type of lumbar puncture needle influences biomarker concentrations. However, atraumatic needles are best tolerated by patients, and are associated with a lower risk for postlumbar puncture headache, that is about 12% for a needle size of 20–22 G compared to about 70% for a needle size of 16–19 G [14, 15].

Item 5 Use of Polypropylene Collection Tubes. There are several reports showing that the type of collection tube influences biomarker outcomes, for example, total tau proteins and amyloid β peptides [16]. Therefore, standardization is important. We propose to use polypropylene tubes, with their low protein binding potential, for collecting CSF. No additives should be used. Glass tubes should be avoided, due to safety reasons for personnel. When multiple tubes are used, the total volume should be mixed after centrifugation to avoid gradient effects.

Item 6 Time of the Day of Withdrawal. For biomarkers that are influenced by circadian rhythm, time of withdrawal is important [17]. Since it is often difficult to accomplish standardization of withdrawal time in everyday clinical practice, documentation is necessary to select the appropriate samples to minimize the effect of this variable.

Items 7 and 8 Serum, Plasma, and DNA Linked to the CSF Sample. It is important to collect matched serum and/or

plasma samples for evaluation of CSF biomarkers because the concentration of the marker in blood often influences that in CSF [18]. Further, serum/plasma pairs are essential to study the intrathecal origin of a biomarker and its CNS specificity. Furthermore, the presence of CNS markers in serum/plasma may aid in disease monitoring. Vacuum tubes that use EDTA (in dried format) are preferred over those that use citrate (in solution) because if tubes containing a standard volume of citrate are filled incompletely, the final biomarker concentration is diluted unequally compared to other samples. Depending on the type of biomarkers and methods of study, we recommend collecting both serum and plasma [19]; for some methods, plasma is preferred over serum and vice versa. Serum/plasma samples should not be haemolysed. We advise to perform a blood draw using vacuum systems, since tourniquet use is related to additional confounding factors in the preanalytic phase include tourniquet time and posture [20]. Furthermore, instructions of the supplier should be followed, such as mixing.

Lastly, DNA collection expands the possibilities for studying the phenotypes and genotypes within individuals. A protocol for storage and handling of DNA can be found in the supplementary files (E-Appendix 1).

(B) Processing for Storage:

Item 9 Storage at Room Temperature Until Spinning and Aliquoting. For CSF, there are no data available yet that support a preference for leaving the samples at room temperature or at 4°C until processing. For serum/plasma preprocessing temperature is more crucial. To avoid platelet activation [21], serum/plasma samples should be kept at room temperature before centrifugation. Therefore, processing at room temperature for both serum/plasma and CSF, including during and after spinning, is suitable for most studies. Relatively few systematic studies have been performed on this issue. We would recommend exploratory studies to define the effect of temperature on specific biomarkers.

Item 10 Standardized Spinning Conditions. We propose to adhere to a standardized spinning protocol of 400 g for 10 minutes at room temperature when fragile cells need to be preserved for RNA of cell isolation, and otherwise at 2,000 g. For serum/plasma, we propose to spin at 2,000 g for 10 min at room temperature. Standardization of spinning temperature and speed may be important for some biomarkers, although no studies have addressed these specific preanalytical variables for CSF. For plasma and serum, temperature of processing is known to be critical for specific biomarkers [22]. After centrifugation, the supernatant must be aliquoted and stored immediately. If this is not done, the processing time should be documented.

Item 11 Standardization of Time-Delay between Withdrawal, Spinning, and Freezing. Studies of the effects of preanalytical variables by MALDI-TOF/MS proteomics (proteins/peptides <20 kD) have shown that the time between sampling and storage is more crucial for specific serum proteins or peptides than for CSF, [12, 13, 23]. For CSF, it was observed that processing within two hours does not lead to artefactual results [12, 13]. For serum, it was observed that small differences in processing time (~10–30 min) can result in changes in the protein profile [19]. Some biomarkers, such as antibodies or specific cytokines, are not very sensitive to sampling and storage conditions [24]. For practical reasons, and in view of the standard of 30–60 min clotting time for serum, we recommend a time delay of 1.5 hours (±30 min) for both matrices. When CSF cells are to be preserved, processing as soon as possible is to be advised as cell numbers decrease quickly. However, in most of the centers, processing of the body fluid samples within one hour is not common practice. Therefore, documentation of time of withdrawal and storage is required in order to select uniform samples. For newly discovered biomarkers, these preanalytical variables should be evaluated.

Item 12 Use of Small Polypropylene Tubes for Aliquoting. Due to the same rationale as for CSF withdrawal (item 5), we recommend that polypropylene tubes should be used for aliquoting and storage. Furthermore, vials with screw caps should be used for a secure sealing. The proposed tube size is 0.25, 0.5, and 1 mL.

Item 13 Aliquoting. Freeze/thaw cycles can influence biomarker concentrations [25]. For example, one-time freezing of CSF samples can lead to a highly significant loss of amyloid β(1-42) which is decreased a further 20% after three more thawing cycles [26, 27]. By contrast, no effects on CSF proteome profiles obtained by MALDI-TOF/MS have been observed after up to four freeze/thaw cycles [13].

In principle, repeated freeze/thawing of samples should be avoided, as data addressing this topic are available for only a few biomarkers and the response to freeze/thaw cycles of new biomarkers is not known. Thus, splitting the pooled sample in multiple small aliquots is optimal, and possible freeze/thaw cycles should be recorded.

Item 14 Volumes of Aliquots of 0.2, 0.5, and 1 mL. Small aliquot volumes are optimal to avoid freeze/thawing and to avoid waste of CSF. Tubes should be filled up to 75% to prevent freeze-drying within the tube, which will affect the concentration of biomarkers, although it may only be a problem if the seal of the cryogenic tubes are not airtight. This issue has not been formally studied and is not referred to in related standard operating procedures [28].

Item 15 Coding and Use of Freezing-Proof Labels. Unique codes are necessary to track samples and pair with clinical data. Ideally barcodes should be used to facilitate searching, to aid in blinding the analysis, and to protect the privacy of patients. It is important to have center-unique codes, to track data retrospectively. Labels must be water and frost (−80°C) resistant.

(C) Storage and Administration of Samples (Table 2, Lower Part):

Item 16 Freezing Temperature of −80°C. Proteins may not be stable at −20°C for years. In one study, the effect of storing CSF at −20°C and −80°C on cystatin C, an abundant CSF protein, was investigated. Cleavage of this protein occurred in all samples stored at −20°C but not in samples stored at −80°C [29]. Apart from the cystatin C truncation, changes in the low molecular weight polypeptide profile due to CSF sample storage at −20°C for three months appeared to be minimal [12, 13]. Oligoclonal bands in CSF may be recovered after several years of storage at −20°C indicating a high stability of immunoglobulins. Nevertheless, self-defrosting freezers must not be used. No data are available showing the benefit of storage of CSF or serum in liquid nitrogen. As this is expensive and not practical for CSF biobanking, there is no basis yet to recommend storage in liquid nitrogen.

Taken together, we recommend that samples are stored at −80°C to ensure long-term stability of biomarkers.

Item 17 Location of Samples. To enable easy tracking and fast relocation of samples, storage information should include freezer location, freezer identification, and sample location within freezer.

Items 18 and 19: Surveillance of Freezers and Splitting of Samples. Freezers should be alarm controlled and a sample rescue plan established and documented. All freezers must be registered in a freezer log file. Ideally, daily temperature logs should be available for all freezers. Aliquots of samples should be distributed among different freezers, although not absolutely needed if good surveillance is in place. An empty, an empty back-up freezer should be available.

Item 20: Transport Conditions and Thawing before Use. Transport of frozen samples should always be performed on dry-ice, and the volume should be sufficient for transport for minimal 3 days. Preferably, transports are initiated on Monday for the samples to arrive within the same week. Once the samples have arrived and are ready for experiments, excessive thawing temperatures (such as 37°C) are to be avoided to prevent protein degradation. Furthermore, inadequate agitation can cause salt and protein gradients to form in thawed samples.

(D) Patient Information Requirement in Database:

Items 21-22 Basic Demographics, such as Age and Gender. Information on the age at sampling is needed to allow comparability to age-matched reference values, since many proteins show age-dependent changes, for example, albumin or IgG [30]. Ideally, date of birth and date of sampling are recorded. Gender has to be provided due to variability of markers influenced by hormones.

Item 23 Ethnicity. Reference ranges of biomarkers can be influenced by the genetic status [31]. For example, a recent study observed a higher IgG index in African Americans than in Caucasians, unrelated to socioeconomic status [32]. Criteria for race and ethnicity are available via the website of the National Institutes of Health [33].

Item 24 Treatment at Sampling and Year before Sampling. It is well known that commonly used drugs for treatment of MS, including immunomodulatory agents and use of methylprednisolone for treatment or prevention of relapses, have an influence on expression of biomarkers [34, 35]. Other treatments could likewise influence biomarker results in NMO patients. Therefore, type and duration of treatment should be documented in detail, preferably beginning at least one year before CSF collection.

Item 25 Fasting, Infections and Pregnancies. Other relevant physiological variables that can influence CSF and blood analyte levels should be recorded including fasting versus non fasting, pregnancy, and underlying nonneurological conditions such as infections [20].

Item 26 Basic CSF Analysis (Protein, Cell Counts, Erythrocytes, Etc...). To enable stratification of patients according to their CSF findings and to evaluate suitability of samples for further analysis, results of basic CSF analysis should be recorded. Primarily, the CSF profile serves for exclusion of other diseases. In addition, quantitative changes of immunological markers are likely to occur depending on disease stage, relapse activity, and medication. Inflammatory processes may influence the blood-CSF barrier function and thereby biomarker concentrations [18].

The presence of Oligoclonal IgG bands (OGB) in NMO is quite distinct from that in MS in that OGBs in MS are persistent, while they are transient in NMO [36, 37]. For example, OGBs were detected in 399 of 411 MS patients (97%) and never disappeared. In NMO, OGBs were detected in three of 11 patients (27%) and always disappeared. The sensitivity of oligoclonal IgG bands is strongly dependent on the method used. We strongly recommend isoelectric focusing followed by immunoblotting and staining for IgG [38, 39]. Preferably, the methods of all routine diagnostic procedures, including oligoclonal banding, should be documented.

Item 27 Data in the CSF Database in English. The mask on the database screen could be in the local language, but the underlying files will need to be in English. It is strongly suggested to use a commercially available program, if not a common database for networks like BioMS-eu. The database should also adhere to standardized international units.

3. Concluding Remarks

The lists provided in Table 2 can be used as an easy checklist for CSF biobanking for any CNS disease, applicable during setup of the procedures and also as a checklist for recording sample characteristics. It is expected that these standardizations will pave the way for large biomarker studies and fruitful collaborations. In the original paper, we present

guidelines for outcome measures to be included for MS biomarker studies [5]. For NMO, standardisation of outcome measures is still needed. Ultimately, these endeavors are to arrive at validated biomarker assays for diagnosis, prognosis, and treatment of CNS diseases and a potential to elucidate relevant disease mechanisms.

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Review Article

Evaluating the Use of Optical Coherence Tomography in Optic Neuritis

Fiona Costello

Departments of Clinical Neurosciences and Surgery, Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada T2N 2T9

Correspondence should be addressed to Fiona Costello, fionacostello@rogers.com

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Optic neuritis (ON) is an inflammatory optic nerve injury, which is strongly associated with multiple sclerosis (MS). Axonal damage in the optic nerve manifests as retinal nerve fiber layer (RNFL) deficits, which can be readily quantified with optical coherence tomography (OCT). The RNFL represents the most proximal region of the afferent visual pathway; and, as such, is a unique region of the central nervous system (CNS) because it lacks myelin. Changes in retinal integrity can be correlated with reliable and quantifiable visual outcomes to provide a structural-functional paradigm of CNS injury. Because the eye provides a unique "view" into the effects of CNS inflammation, the ON "system model" may provide greater understanding about disease mechanisms, which underpin disability in MS. This review addresses the applications of OCT in study of ON patients, with specific reference to the published reports to date. The future role of OCT is discussed, both in terms of the potential gains and certain challenges associated with this evolving technology.

1. Optic Neuritis: An Overview

Optic neuritis (ON) is an inflammatory optic nerve injury, which causes subacute onset vision loss in children and young adults. Much of our understanding regarding the clinical presentation of ON has been derived from the Optic Neuritis Treatment Trial (ONTT) [1]. This randomized, placebo-controlled, multicenter trial compared the visual benefits of treatment with either intravenous methylprednisolone (250 mg every 6 hours for 3 days followed by oral prednisone (1 mg/kg per day) for 11 days), oral prednisone (1 mg/kg per day for 14 days), or oral placebo (for 14 days) in 457 patients with acute ON [1]. From the ONTT, we learned that most ON patients are young (mean age 32 years) Caucasian (85%) women (77%) [1]. Over ninety percent of ON patients report pain at the onset of vision loss [1] which is often characterized as an "ache" made worse with eye movements. Vision loss is generally acute, to sub-acute in onset progressing over a period of hours to days. The severity of vision loss may range from mild (Snellen equivalent of 20/20 vision) to no light perception (NLP) [2]. Dyschromatopsia or decreased color vision is quite common [2], and this finding can help localize the diagnosis in patients with relatively mild visual acuity deficits. Patients with unilateral ON often manifest a relative afferent pupil defect, unless there is coexisting optic nerve damage in the contralateral eye [2]. Visual field defects in ON correspond to the topography of the retinal nerve fiber layer (RNFL) and may be arcuate, altitudinal, or cecocentral in shape. In cases of retrobulbar ON the fundus examination is initially normal, whereas patients with anterior ON or "papillitis" may manifest optic disc swelling [2, 3]. Atypical fundus findings in ON patients include severe optic disc edema, peripapillary hemorrhages, or retinal exudates [4]. Clinical features which are not typical of ON should prompt an investigation for other diagnoses including ischemic, compressive, infiltrative, toxic-metabolic, and inflammatory optic neuropathies.

The majority of ON patients recover vision over a period of weeks [1–3], during which time optic disc pallor may evolve as a "footprint" of the previous inflammatory injury. Yet, even in patients who recover 20/20 vision in their

affected eye, persistent visual problems (fatigue-induced vision loss; altered motion and depth perception; loss of contrast sensitivity) are common. Patients with previous ON frequently describe transient vision loss with increased body temperature, which is known as "Uhthoff's" phenomenon [2]. Because axonal damage is an early manifestation in demyelinating plaques of multiple sclerosis (MS) patients [5], persistent visual deficits after ON may be a consequence of prior demyelination and/or permanent axonal damage in the anterior visual pathway.

2. Exploring the Link between Optic Neuritis and Multiple Sclerosis

There is a strong association between ON and MS, such that approximately 20% of patients experience ON as their initial demyelinating event, and 30–70% of MS patients develop ON during the course of their disease [6, 7]. Many patients who present with ON as a clinically isolated syndrome (CIS) demonstrate evidence of disseminated central nervous system (CNS) inflammation on their baseline magnetic resonance imaging (MRI) study (50% to 70%) and harbor abnormal cerebrospinal fluid (CSF) constituents (60 to 70%), which increase their future risk of MS [2, 4, 8–11]. After 15 years, 72% of patients in the ONTT who had one or more white matter lesions on their baseline MRI developed clinically definite MS (CDMS) as compared to only 25% of patients with no MRI lesions [10].

3. Optic Neuritis: A System Model of Multiple Sclerosis?

Vision loss is both prevalent and relevant in MS patients. Because the visual system is a functionally eloquent region of the CNS, patients are apt to notice and seek help for their symptoms from a health care professional. Therefore, it is possible to establish a definite time of onset of symptoms and follow patients through the acute and convalescent phases of ON. Furthermore, visual impairment can be quantified with reliable and validated measures of visual function including high- and low-contrast visual acuity, automated perimetry, and color vision testing [12–24]. Moreover, damage to the optic nerve causes atrophy of the RNFL, which can be measured and quantified with ocular imaging techniques, such as optical coherence tomography (OCT) [12–24]. The RNFL is the most proximal region of the afferent visual pathway, and it is a unique CNS structure because it lacks myelin. Given that the back of the eye represents the front of the brain, OCT provides noninvasive means to quantify the structural effects of an inflammatory insult to the optic nerve, which can then be compared to functional outcomes, to construct a structural-functional paradigm of CNS injury. For this paradigm to gain acceptance, however, OCT needs to provide a reliable means of detecting true pathological changes in the anterior visual pathway, which can be clearly distinguished from test-retest variability inherent to the technology. Furthermore, structural changes in the anterior visual pathway captured with OCT need to show concordance with other markers of disease activity in MS. As the data from OCT studies continue to mount, there may be evidence to support the tenability of the ON system model in clinical research and, potentially, to establish a role for OCT in the care of MS patients.

4. Optical Coherence Tomography

Optical coherence tomography (OCT) is a noninvasive, ocular imaging technique that uses low-coherence interferometry to generate in vivo, high-resolution (within 10 microns), cross-sectional images of the RNFL by measuring backscatter of infrared light [7, 25–28]. Early OCT systems employed a Michelson-type interferometer with a low-coherence-length, superluminescent diode light source [7]. One arm of the interferometer directed light onto the sample and collected the backscattered signal. A second reference arm had a reflecting mirror, which was mechanically controlled to vary the time delay and measure interference. The use of a low-coherence-length light source meant that interference occurred only when the distance traveled by the light in the sample and reference arms of the interferometer are matched to within the coherence length [7]. This characteristic allowed echo delays of the light from the tissue to be measured with temporal accuracy [7]. The data were processed and displayed as a two-dimensional, false-color image [7].

5. Early OCT Studies in Optic Neuritis: Breaking New Ground

The first study to investigate the role of OCT technology in the evaluation of MS patients was reported by Parisi [12], who used an early generation of OCT to compare RNFL values between 14 MS patients with prior ON and 14 age-matched controls. The thickness of the RNFL was 46% lower in MS eyes relative to control eyes ($P < .01$) and 28% lower in ON eyes as compared to unaffected eyes of the same patient (non-NON eyes) ($P < .01$) [7, 12]. In this paper, it was not clear whether patients had recurrent ON events, which may have contributed to the robust differences in RNFL thickness between ON eyes, non-ON eyes, and control eyes. Yet, even in the absence of known ON, RNFL values were 26% lower in MS eyes as compared to control eyes, which suggested that RNFL damage occurred independent of clinically overt ON in MS patients [7, 12]. Parisi's innovative work set the stage for followup studies, which would further delineate how changes in RNFL integrity are influenced by ON and illustrate how axonal changes in the anterior visual pathway mirror global CNS damage MS patients.

In a subsequent study, Trip and colleagues [13] compared RNFL values between 25 ON patients and 15 healthy controls. Optic neuritis patients were recruited with a selection bias towards incomplete visual recovery. Retinal nerve fiber layer thickness was significantly reduced (33%) in ON eyes (68.7 μm) ($P < .001$) relative to control eyes (102.9 μm) and in ON eyes (27%) ($P < .001$) relative to non-ON eyes (94.6 μm) [13]. Retinal nerve fiber layer atrophy was associated with lower VEP amplitudes, worse logMAR visual acuity

scores, reduced visual field mean deviation, and decreased color vision in ON patients [13]. This intriguing study further expanded our understanding of OCT by showing that, in addition to RNFL thickness, macular volumes were significantly reduced after ON in patients with incomplete visual recovery. More specifically, macular volumes were 11% in lower ON eyes as compared to control eyes ($P < .001$) and 9% lower in ON eyes relative to non-ON eyes ($P < .001$) [13].

In a more recent study, Burkholder et al. [14] further explored how inner and outer macular volumes related to RNFL thickness and visual function in 530 MS patients (with and without ON) and 111 control eyes. Lower macular volumes were associated with RNFL thinning, such that a 10-μm difference in RNFL thickness corresponded to 0.20 mm^3 reduction in total macular volume [14]. Correlations between RNFL thickness and inner macular volume were significant ($r = 0.58$, $P < .001$), particularly in ON eyes relative to non-ON eyes ($r = 0.61$ versus $r = 0.50$) in MS patients [14]. These findings were significant because the ganglion cell layer comprises 34% of the total average macular thickness [14]; thus, tracking macular volumes in ON patients may help determine the temporal relation between primary neuronal cell death and axonal loss after a CNS inflammatory event.

Fisher and colleagues [20] used OCT to compare RNFL values between 90 MS patients and 36 control subjects. While median Snellen acuity equivalents were better than 20/20 in both groups, mean RNFL thickness was reduced in MS patients (92 μm) relative to controls (105 μm) ($P < .001$) with the lowest values noted in the ON eyes (85 μm) of MS patients ($P < .001$) [20]. Lower visual function scores were associated with reduced average overall RNFL thickness in MS patients, such that, for every 1-line decrease in low-contrast letter acuity or contrast sensitivity score, the mean RNFL thickness decreased by 4 μm [20]. The findings of this study suggested a role for OCT as a structural biomarker and potential secondary outcome measure in future MS clinical trials.

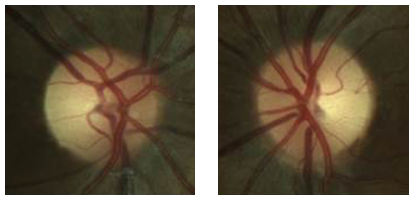
In 2006 [15], we reported the findings from 54 patients who were followed for a mean period of 13 months to determine whether the extent of RNFL thinning predicted visual recovery after acute ON. After a year, 74% of ON patients manifested significant RNFL atrophy in their affected eyes, with most RNFL loss occurring within 3 to 6 months of the ON event [15]. Average RNFL values were lower in ON eyes (78 μm) relative to non-ON eyes (100 μm) ($P < .0001$) [15]. Subclinical ON was detected in four patients during the course of the study [15]. These patients did not report a history of pain or vision loss, but ophthalmic testing showed a new visual field deficit, an abnormal visual-evoked potential (VEP) result, the evolution of optic disc pallor, and newly detected thinning of the RNFL to support the diagnosis of subclinical ON. By using regression analysis, we showed that there was a linear relationship between RNFL thickness and visual field mean deviation after ON, such that, below a "cutoff" of 75 μm, every 10 μm drop in RNFL was associated with a 6.46 dB decrease in mean sensitivity [15]. Our observations suggested that visual function may be relatively well preserved after ON until a critical threshold of axonal integrity is violated; after which, permanent vision loss is more likely to ensue.

Klistorner and colleagues [16] later evaluated 32 patients with unilateral ON and 25 control subjects with multifocal VEP (mfVEP) testing and OCT. The mean RNFL thickness in ON eyes (85 μm) was reduced by 19.2% compared with control eyes (104 μm) ($P < .0001$) [16]. There was a 39.8% reduction in the amplitude of the mfVEP in ON eyes relative to control eyes ($P < .0001$) [16]. Linear regression analysis demonstrated a strong correlation between inter-eye asymmetry values of RNFL thickness and mfVEP amplitude ($r = 0.90$, $P < .0001$). Lower RNFL values were also associated with increased mfVEP latency ($r = -0.66$, $P < .002$) [16]. In addition to demonstrating the utility of mfVEP in tracking optic nerve injury in ON patients, this study further confirmed the significant correlations between structural and functional measures of optic nerve integrity and showed that demyelination contributes to axonal loss in the anterior visual pathway.

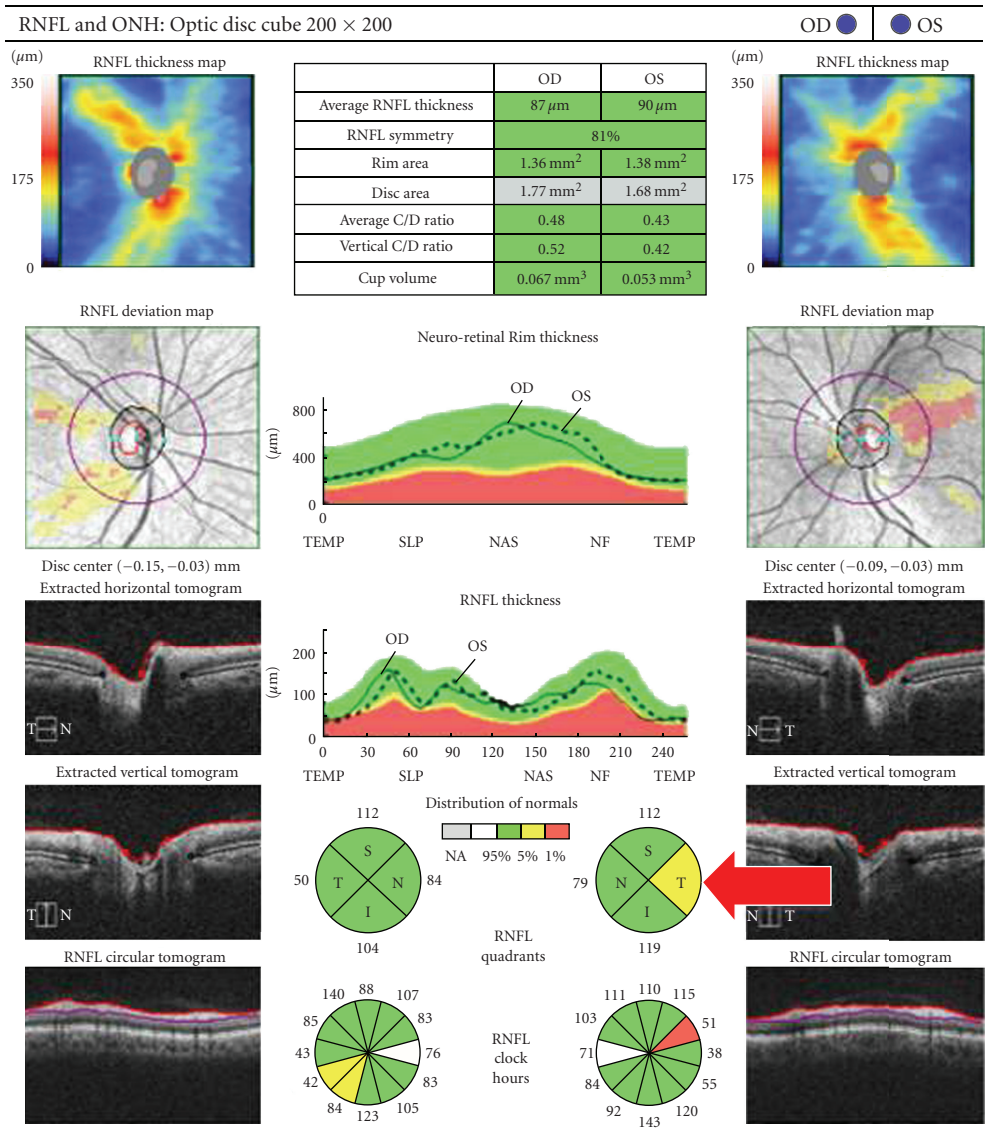
6. Retinal Nerve Fiber Layer Atrophy: The Impact of Recurrent Optic Neuritis

After an isolated ON event, RNFL values decrease by approximately 20% when patients are recruited without selection bias [7, 15–20, 24, 29]. For eyes affected by two or more ON events, however, RNFL atrophy tends to be more severe [23, 30] and the corresponding impact on visual function, more dire. In a recent study of 193 MS patients, we compared RNFL values between 29 eyes affected by two or more ON events (recurrent ON eyes), 125 eyes affected by a single ON event (single ON eyes), and 232 non-ON eyes [23]. Retinal nerve fiber layer values were significantly lower in recurrent ON eyes (64.2 μm) relative to single ON eyes (86.3 μm) ($P < .0001$) and non-ON eyes (100.1 μm) ($P < .0001$) [23]. Retinal nerve fiber layer atrophy was also significantly worse in single ON eyes as compared to non-ON eyes ($P < .0001$) [23]. Similarly, Yeh and colleagues [30] noted that average RNFL thickness decreased with increasing number of episodes of ON in pediatric patients. These findings indicate that recurrent inflammatory events have a cumulative impact and erode axonal integrity in the CNS.

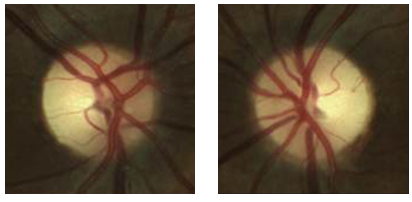
The detection of new RNFL atrophy after ON can be more challenging in patients with prior ON as compared to patients experiencing their first ON event. Robust inter-eye differences in RNFL thickness may be observed for a CIS patient presenting with unilateral ON, because the anterior visual pathway has presumably been unscathed by prior inflammation. In contrast, a patient with RRMS may manifest less apparent inter-eye differences in RNFL thickness (Figure 1) and macular volume after ON if there has been previous optic nerve damage in the contralateral eye. Similarly, a patient with a new ON event and a prior history of ON in the same eye may show little change in RNFL thickness over time, because it is difficult to detect new RNFL thinning super imposed upon preexisting RNFL atrophy. Given the inherent heterogeneity of MS cohorts and the predilection for clinical and subclinical ON in



(a) Right eye (b) Left eye

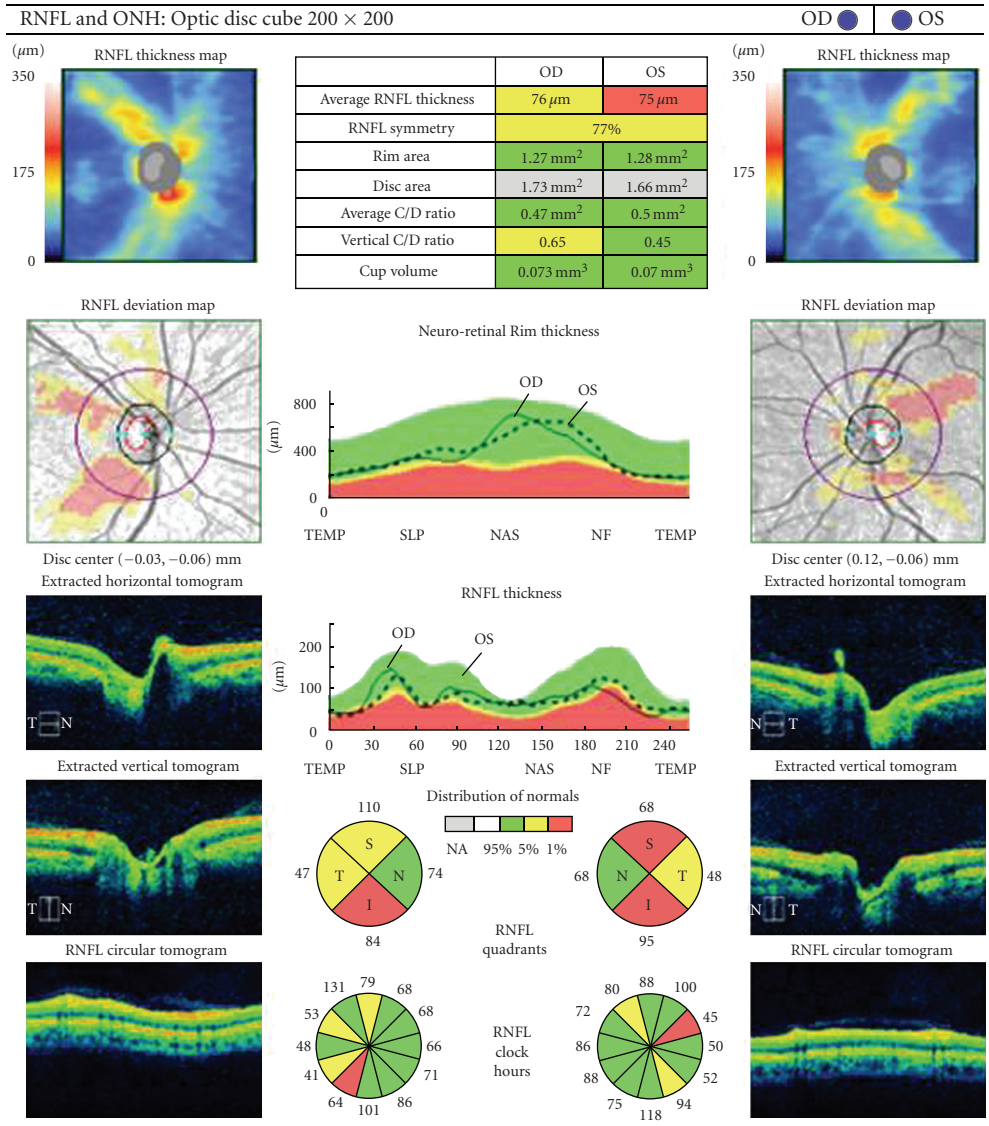


(c) Spectral Domain OCT (SD-OCT)



(d) Right eye (e) Left eye

Figure 1: Continued.



(f) Spectral Domain OCT

Figure 1: Case: A 26-year old woman with MS presented with a 2-month history of vision loss in both eyes. Best-corrected visual acuity was 20/150 in the right eye and count fingers (at 2 feet) in the left eye. There was a left relative afferent pupil defect. Fundus examination showed mild temporal pallor in the right (a) and left (b) eyes. Spectral domain OCT (c) showed that global average RNFL measurements were within normal limits in the right eye (OD) (87 μm) and the left eye (OS) (90 μm). There was relative temporal RNFL thinning in the left eye (arrow). Two and a half months later, the patient's visual acuity improved to 20/20 in both eyes, albeit with mild residual color vision deficits. There was more obvious temporal pallor in the right (d) and left (e) eyes. Repeat SD-OCT testing (f) showed progressive global average RNFL atrophy in the right (OD) (76 μm) and left (OS) eyes (75 μm).

this disease, caution is needed in the interpretation of RNFL values, particularly in cross-sectional studies.

7. Defining the Window of Axon Loss after Acute ON: Designing Future Clinical Trials

Only a few prospective studies have tried to define the time interval during which RNFL atrophy progresses after acute ON [17, 21, 24]. Establishing a potential therapeutic "window" is important for the design of future trials employing OCT as an outcome measure in ON patients. Noval and colleagues [21] followed 12 patients with acute ON and observed an initial increase in RNFL thickness, which resolved by 1.5 months. In ON eyes, they reported a 25% reduction in RNFL thickness at 6 months [21]. In 2008, we followed 78 ON patients over a mean period of 28 months and observed that the earliest significant difference in RNFL thickness between ON eyes and non-ON eyes manifested after two months, in the temporal RNFL region

(inter-eye difference = 12.5 μm, $P = .005$) [17]. In a subset of 20 patients who underwent regular OCT testing over a 12-month period, we reported that RNFL thinning progressed up to 6 months after acute ON and stabilized thereafter [17]. Similarly, in a recent longitudinal study, Henderson and colleagues [24] evaluated 23 patients with acute unilateral ON with serial OCT testing at presentation and after 3, 6, 12, and 18 months of follow up. Twelve control subjects were also imaged, on two occasions, a median of 552 days (range 350–907 days) apart [24]. Retinal nerve fiber layer values were significantly increased in ON eyes relative to non-ON eyes at baseline but then significantly decreased at all later time points [24]. Visual recovery at 12 months was not related to the extent of RNFL swelling seen acutely but was associated with the amount of RNFL loss observed in ON eyes [24]. As was noted in previous studies, the authors concluded that RNFL thinning is usually evident within 3 months of an acute ON event and that OCT-measured RNFL loss after 6 months is a tenable outcome measure for neuroprotection trials [24].

Given the small patient numbers included in the aforementioned studies, caution must again be exercised when interpreting the collective results. Our study included only 78 ON patients, and there was variability in followup across testing intervals [17]. To determine the time required for RNFL atrophy to stabilize within 12 months of an acute ON event, we tracked RNFL changes in a subset of 20 patients at regular intervals over a one-year period [17]. Our study lacked a control group, which further limited our conclusions [17]. Similarly, for the 23 ON patients and 12 control subjects studied by Henderson and colleagues there was some variability in followup [24]. It was also noteworthy that in ON eyes RNFL values ranged from 87 to 281 μm (median 117 μm, mean 133 μm) at baseline, and two patients had initial RNFL values exceeding 200 μm. Retinal nerve fiber layer values equal to or greater than 250 μm are atypical in ON, because the extent of optic disc edema tends to be relatively mild relative to other optic neuropathies associated with more severe optic disc swelling (i.e., anterior ischemic optic neuropathy). When a patient population size is limited, it is unclear how outliers impact the overall interpretation of results, even in the context of an elegantly designed study. Therefore, further controlled, prospective clinical trials involving a larger numbers of patients will be needed to firmly establish the optimal time "window" to trial new therapeutic strategies in ON patients.

8. Optical Coherence Tomography: Risk of Multiple Sclerosis after Optic Neuritis

Only two prior studies have explored the association between RNFL atrophy and future risk of MS in ON patients, and the data were largely negative [18, 31]. Previously, we compared RNFL values in ON eyes and non-ON eyes between patients who developed CDMS (42%) and those that did not develop MS 24 months after an ON event (58%) [18]. Mean RNFL values were lower in ON eyes of non-MS patients as compared to CDMS ON eyes after one year ($P = .05$) due to more severe ON events in the former [18]. Temporal RNFL values were lower in the non-ON eyes of CDMS patients, but the results were not statistically significant ($P = .13$) [18]. From our findings, we concluded that RNFL thickness did not reliably distinguish patients at higher risk of converting to CDMS after ON. Similarly, Outteryck and colleagues [31] performed OCT testing on 56 CIS patients (18 with optic neuritis and 38 without optic neuritis) and 32 control subjects, to investigate whether measures of RNFL thickness and macular volume revealed early retinal axonal loss. In this prospective case series, there was no link between RNFL and (1) MRI evidence of disseminated CNS inflammation at baseline, (2) disseminated CNS inflammation according to the revised McDonald criteria, (3) gadolinium enhancement on initial MRI, (4) multifocal CIS presentation, (5) altered visual evoked potentials, or (6) development of "McDonald-" proven MS at 6 months [31]. Furthermore, patients who developed CDMS ($n = 13$) or McDonald-criteria proven MS ($n = 23$) did not have more severe RNFL atrophy [31]. These investigators concluded that OCT does not predict conversion to MS at 6 months in CIS patients and postulated that conversion to MS after ON is more likely influenced by inflammatory events than axonal degeneration.

9. Optical Coherence Tomography Studies in Pediatric Optic Neuritis Patients

Multifocal CNS demyelination has been reported to occur in approximately 0.4 per 100,000 of the pediatric patient population [30]. Similar to adults, ON is a relatively common occurrence in pediatric patients such that 22% of children experience ON as their first demyelinating event and 35% of children who eventually develop MS experienced ON during their first clinical episode [30]. In a recent pediatric ON study, Wilejto and colleagues [32] reported unilateral optic nerve involvement in the majority (58%) of pediatric patients ($n = 36$). Visual recovery after ON was considered complete in 39 of 47 affected eyes (83%) [32]. Cranial MRI scans demonstrated white matter lesions separate from the optic nerves in 54% of children. In this study, the risk of MS was 36% at 2 years, and bilateral ON was associated with a greater future risk of MS [32]. Clinical findings extrinsic to the visual system on baseline examination and MRI evidence of white matter lesions outside the optic nerves were strongly associated with a future diagnosis of MS [32]. Yeh and colleagues [30] used OCT in a cross-sectional study of 38 consecutive children (age <18 years) who had at least one documented clinical episode of an acquired demyelinating event and two control groups, including (1) 15 normal healthy children (30 eyes) with no history of neurological or other chronic disease and (2) 5 children (10 eyes) with other nondemyelinating disorders (OND), including headache, attention deficit hyperactivity disorder, and depression [30]. In MS patients RNFL thickness was 99 μm in non-ON eyes and 83 μm in ON eyes. Children with acute disseminated encephalomyelitis (ADEM) and transverse myelitis (TM) had lower RNFL values in ON eyes (67 μm) relative to non-ON eyes (102 μm) [30]. Macular volumes were markedly lower in ON eyes of children with ADEM/TM (6.2 mm^3) and chronic relapsing inflammatory optic neuropathy (CRION)

($6.0\,mm^3$), suggesting a more widespread disease process in these clinical entities [30]. All subgroups with a clinical history of ON had lower average RNFL values (83 μm for MS patients; 67 μm for ADEM/TM patients; 89 μm for CIS patients; 50 μm for CRION patients) than controls (107 μm). Differences between children with demyelinating disease and controls and between ON and non-ON eyes were statistically significant ($P < .001$). On the basis of their findings, the investigators concluded that OCT may be a valuable tool for monitoring anterior optic pathway dysfunction in children with demyelinating diseases.

10. Optical Coherence Tomography and Neuromyelitis Optica

Neuromyelitis optica (NMO) is a severe inflammatory process of the optic nerves and spinal cord and is associated with poor clinical recovery [33–38]. There have been several studies which have explored the role of OCT in quantifying the extent of axonal damage in the anterior visual pathway secondary to NMO with a view to distinguishing the ON associated with this clinical syndrome [36–38]. Naismith [36] used OCT to study 22 subjects with NMO or NMO spectrum disorders and 47 MS patients. In ON eyes, NMO was associated with lower RNFL values compared to MS, when controlling for visual acuity (57 μm versus 67 μm; $P = .01$) or for contrast sensitivity (61 μm versus 70 μm; $P = .02$). The superior and inferior quadrants were more severely affected in NMO than MS eyes. These authors noted that the odds of falling into the NMO group increased by 8% for every 1 μm decrease in RNFL thickness [36]. Similar findings were noted by Ratchford and colleagues [37] who used OCT to study 26 NMO spectrum patients with a history of ON, 17 patients with isolated longitudinally extensive transverse myelitis (LETM) without ON, 378 patients with RRMS, and 77 healthy controls. These investigators observed significant RNFL thinning in NMO ON eyes (63.6 μm) relative to RRMS ON eyes (88.3 μm) ($P < .0001$) and control eyes (102.4 μm) ($P < .0001$). A first episode of ON was estimated to cause 24 μm more loss of RNFL thickness in NMO than RRMS eyes [37]. In a third study, Nakamura [38] evaluated 35 eyes of 18 patients with the "NMO spectrum" and 14 MS patients to determine whether RNFL thickness correlated with the clinical presentation in patients with NMO and ascertain what clinical factors lead to poor visual outcomes. Overall RNFL measurements were thinner in NMO ON eyes than MS ON eyes (64 μm versus 84 μm; $P = .0006$) especially in the superior and inferior RNFL quadrants [38]. Mean RNFL negatively correlated with the number of relapses in the NMO group. A receiver operating characteristic (ROC) analysis showed that the overall RNFL "cutoff" value for decreased visual acuity (measuring less than 20/20) was 71 μm in the NMO group [38]. The frequency of the ON relapses and the time to initiate treatment with high-dose intravenous methylprednisolone significantly affected the preservation of RNFL thickness in this study [38]. Hence, the studies to date suggest that OCT may be used to distinguish anterior visual axis involvement in NMO from ON associated with MS.

11. Conclusions and Future Directions

Recent technological innovations have introduced the era of "Fourier" or "Spectral" domain OCT (SD-OCT). In this new generation of the device, all light echoes are detected simultaneously, leading to a dramatic increase in sensitivity that enables high-speed imaging [7]. Spectral domain OCT is now commercially available and provides an axial image resolution of 5–7 μm, with imaging speeds of 25,000 axial scans per second. This imaging speed is approximately 50 times faster than the previous generations of OCT technology [7]. Retinal nerve fiber layer measurements in MS patients differ considerably between TD-OCT and SD-OCT devices, with excellent correlations between values obtained from both imaging techniques [39–43]. Recently, Bock and colleagues [39] compared SD-OCT and TD-OCT imaging techniques in 55 MS patients and reported a strong correlation (Pearson's $r = 0.926$, $P < .001$) between the two technologies. There were, however, significant differences in the absolute RNFL measurements (mean ± standard deviation 8.1 μm ± 6.2, range −12 to 23 μm), and therefore the results from the two devices were not interchangeable. The findings of this study were similar to those reported by Knight et al. [42] who compared SD-OCT and TD-OCT RNFL values in glaucomatous patients. In both studies, SD-OCT tended to measure "more thinly" than TD-OCT at higher RNFL values, whereas, for thinner RNFL values, SD-OCT measured "more thickly" than TD-OCT [39].

The reproducibility of SD-OCT retinal measurements relative to TD-OCT has recently been evaluated in MS and glaucoma patients. In a prospective study of 58 MS patients and 32 healthy controls, SD-OCT testing was performed to determine optimal intervisit, interrater, and intrarater reproducibility [41]. The authors noted excellent reproducibility of average and quadrant RNFL values, average macular thickness, and total macular volumes [41]. Leung and colleagues [40] evaluated RNFL measurement variability, diagnostic sensitivity and specificity, and the strength of the structure-function association obtained with SD-OCT versus TD-OCT in a prospective, cross-sectional study of 97 healthy controls and 83 glaucoma patients. The intra-visit repeatability of SD-OCT ranged between 5.12 and 15.02 μm and the intervisit reproducibility, between 4.31 and 22.01 μm. Overall, SD-OCT demonstrated lower measurement variability compared with TD-OCT. Finally, in a prospective observational study of 110 eyes, retinal measurements were compared between six different TD-OCT and SD-OCT devices including Stratus and Cirrus (Carl Zeiss Meditec, Inc.), Spectralis HRA + OCT (Heidelberg Engineering), RTVue-100 (Optovue Inc.), SDOCT Copernicus HR (Optopol Technology S.A.), and 3D OCT-1000 (Topcon Corporation) [43]. The standard analysis protocols for macular thickness evaluation were evaluated with each instrument. The six different devices produced measurements that differed in variance (Bartlett test, $P = .006$), and mean values (Friedman test, $P < .001$) [43]. Bland-Altman analyses showed that the limits of agreement for all the comparisons were not acceptable, and

regression analysis revealed high standard error values [43]. The findings of this study indicated that retinal thickness measurements obtained with various OCT devices were different beyond clinical practice tolerance. The differences between devices were attributed to the analysis algorithms used to set retinal inner and outer boundaries [43].

Thus, while innovations in OCT technology offer potential advantages including minimizing error and improving test-retest reliability in the evaluation of MS patients, challenges remain. With each new reincarnation of the technology, there is a disruption in the collection of longitudinal data, which is vital in the management of a chronic disease. Furthermore, as the first SD-OCT studies are beginning to emerge, it is debatable whether any of these early reports have taught us anything that we did not already know regarding the potential role for OCT in the management of ON and MS patients. As the technology continues to advance, it is imperative that the results of OCT studies prove to be clinically relevant, not simply statistically significant to meaningfully impact our understanding and treatment of disease. In the context of MS, the challenge remains to define the amount of RNFL "signal" that represents pathology, and to distinguish this from the "noise" of the technology. This task may be onerous given the heterogeneity of MS cohorts and the prevalence subclinical disease activity in this patient population. On a practical level, MS patients may develop other occult ocular conditions (i.e., age-related macular degeneration and glaucoma) that damage the retinal architecture, making the interpretation of RNFL atrophy difficult. Also, retrograde transsynaptic retinal ganglion cell degeneration due to MS lesions within the posterior optic pathways has been shown to cause RNFL atrophy [44]. Thus, in the evaluation of RNFL thickness, it may also be necessary to distinguish the effects of postgeniculate lesions from subclinical disease in the anterior visual pathway in MS patients.

In conclusion, there is increasing evidence to suggest a role for OCT in the evaluation of ON and MS patients. Optical coherence tomography may complement our existing arsenal of tools including tests of visual function, neuroimaging techniques, and electrophysiological studies and help develop a structural-functional paradigm of CNS inflammation. Furthermore, OCT may be used to capture structural changes in the anterior visual pathway, which will provide unique insights regarding pathogenic mechanisms of CNS injury and, in turn, to develop more effective therapeutic strategies for MS patients. Future longitudinal, large-scale studies will be needed to ultimately determine how this technology can be optimally implemented in a research setting, with the ultimate goal of enhancing patient care.

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Review Article

Optical Coherence Tomography in Multiple Sclerosis and Neuromyelitis Optica: An Update

Susana Noval,[1] Inés Contreras,[2] Silvia Muñoz,[3] Celia Oreja-Guevara,[4] Beatriz Manzano,[5] and Gema Rebolleda[2]

[1] *HIV Unit, Ophthalmology Department, Hospital La Paz/Autónoma University School of Medicine, IdiPAZ, Madrid, Spain*
[2] *Ophthalmology Department, Clinica Rementería, Hospital Ramón y Cajal, Alcalá de Henares University School of Medicine, Madrid, Spain*
[3] *Ophthalmology Department, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain*
[4] *HIV Unit, Neurology Department, Hospital La Paz/Autónoma University School of Medicine, IdiPAZ, Madrid, Spain*
[5] *HIV Unit, Hospital La Paz/Autónoma University School of Medicine, IdiPAZ, Madrid, Spain*

Correspondence should be addressed to Susana Noval, sunoval@gmail.com

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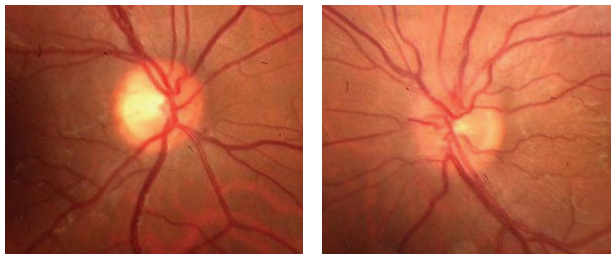
Optical coherence tomography (OCT) uses light interference patterns to produce a cross-sectional image of the retina. It is capable of measuring the unmyelinated axons of the retinal ganglionar cells as they converge on the optic disc. In a disease like multiple sclerosis (MS), in which axonal loss has been identified as an important cause of sustained disability, it may prove an invaluable tool. OCT has demonstrated that axonal loss occurs after each episode of optic neuritis and that the degree of axonal loss is correlated to visual outcomes. Furthermore, axonal loss occurs in MS even in the absence of inflammatory episodes, and the degree of this loss is correlated with the duration of the disease process, with more thinning as the disease advances and in progressive forms. Thus, OCT retinal nerve fiber layer measurements may represent an objective outcome measure with which to evaluate the effect of treatment.

1. Introduction

The optic nerve as it leaves the eye is the only tissue composed of unmyelinated axons which can be imaged directly. The retinal nerve fiber layer (RNFL) is made up of the axons of the retinal ganglionar cells that convey the visual information from the retina to the lateral geniculate nucleus; until they exit the eye, they do not acquire the protective myelin sheath. This extraordinary circumstance allows us to study the influence on isolated axons of several diseases. Ganglionar cells and their axons, besides being the main retinal component around the optic nerve (90% of retinal thickness) are also representative at the macula (30–35%).

Both time-domain and spectral-domain optical coherence tomographies (OCT) use light interference patterns to produce a tomogram, or cross-section, through the layers of the retina. From this information, the OCT software constructs a two-dimensional (time-domain, TD-OCT) or three-dimensional (spectral-domain, SD-OCT) image of the retina and the optic nerve and is capable of measuring the different layers of the retina with a margin of error of 4–6 μm. The reproducibility of RNFL and macular measures has been found to be excellent with SD-OCT in multiple sclerosis [1]. Multiple studies have provided information on the normal values for the RNFL thickness and have reported the RNFL loss that occurs after different pathologies that affect the optic nerve.

Axonal loss, in contrast to demyelination, is not reversible and is therefore an important cause of sustained disability. In patients diagnosed with multiple sclerosis, it has been demonstrated that axonal loss occurs in the early stages of the disease. This is one of the reasons that support the early use of neuroprotective drugs. Monitoring axonal loss has become a priority in multiple sclerosis and



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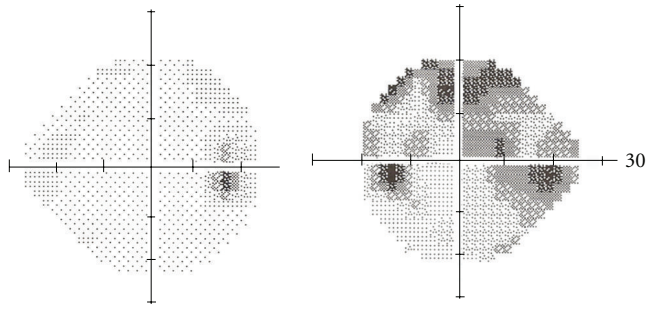

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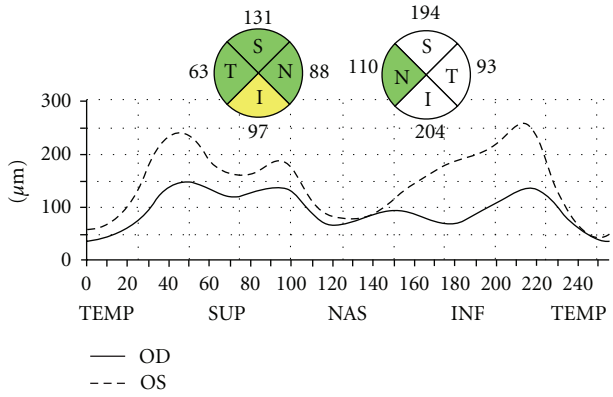


(c)

	RE	LE
VA	1	0.8
MD	+0.31 dB	−12.24 dB
Mean RNFL thickness	94.83 μm	150.29 μm

(d)

Figure 1: Fundus photograph, visual fields, and optical coherence tomography of a 30-year-old man who consulted due to ocular pain and visual loss in his left eye for two weeks. Optical coherence tomography shows an increased retinal nerve fiber layer thickness in his left eye due to optic nerve edema.

OCT as a sensitive, precise, and reproducible technique is acquiring increasing importance for both neurologists and ophthalmologists [2].

2. Isolated Acute Optic Neuritis

Optic neuritis is one of the manifestations of multiple sclerosis; it has been described as the second most frequent mode of presentation. The Optic Neuritis Treatment Trial (ONTT) has shown us that a patient diagnosed with a first episode of optic neuritis has a risk of 50% of developing multiple sclerosis in the following 15 years. The risk increases to 72% in those patients with at least one demyelinated lesion on magnetic resonance imaging (MRI) and decreases to 25% in those without lesions [3]. However, these data are not applicable to all patients, for example, in Asian populations MRI lesions are less frequently encountered [4].

2.1. Acute Changes in Anterior Optic Neuritis. Optical coherence tomography has a high sensitivity for detecting acute optic nerve oedema in anterior optic neuritis (Figure 1). Our study group performed one of the first prospective studies evaluating TD-OCT in acute optic neuritis. Twenty-three patients underwent a complete ophthalmological evaluation, including visual acuity (VA) measurement, automated static perimetry, and OCT at onset and periodically for six months. We found a statistically significant increase in initial mean RNFL thickness in anterior forms (166.30 μm, SD 34.87 μm) as compared to retrobulbar neuritis (98.60 μm, SD 21.58 μm), Doctoral thesis: "Study of optic neuritis with optical coherence tomography."

2.2. Axonal Loss. Following an initial episode of optic neuritis, OCT can detect axonal loss as a thinning of the RNFL, occurring mainly in the first three to six months (Figure 2). After this period, axonal loss stabilizes [5–7]. RNFL measurements obtained within the first eight weeks of optic neuritis may reflect the extent of optic disc and RFNL edema due to an acute bulbar or retrobulbar injury; whereas RNFL values obtained 3 or more months after ON may indicate the extent of axonal injury referable to the acute inflammatory event [7].

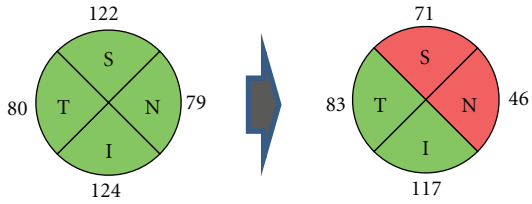
Prospective studies estimate that there is a 20 to 25% loss in the RNFL thickness when compared to the fellow unaffected eye (Table 1) [5, 6, 8]. The decrease is greater in Asian patients whose visual function is damaged more severely [4]. Most studies have been unable to demonstrate a significant thinning of the RNFL of the fellow eyes when compared to healthy controls [8–11].

Axonal loss affects diffusely the whole peripapillary RNFL, although the temporal quadrant is often the most affected. This loss may be detected as soon as two months after the event, when compared to the fellow eye and healthy controls [4, 7, 8]. Temporal thickness decreases

Table 1: Comparison of RNFL thickness (μm) between both eyes of patients who have suffered unilateral optic neuritis and healthy control subjects. Values are expressed as mean (standard deviation).

	Patients	ON eye	Fellow eye	Control
Outteryck et al.[19]	Non-MS	92.27 (12.82)	—	98.71 (9.08)
Grazioli et al. [28]	MS	81.7 (19.2)	93.6 (15.3)	—
Klistorner et al. [9]	MS and non-MS	84.5 (15. 1)	103.8 (10.8)	104.0 (9.2)
Siger et al. [22]	MS	83.92 (17.63)	91.08 (19.3)	—
Costello et al. [7]	Non-MS	86.1	101.6	—
Noval et al. (data not published)	MS and non-MS	84.95 (23.45)	103.40 (15.27)	105.5 (10.51)
Fisher et al. [10]	MS	85 (17)	96 (14)	105 (12)
Costello et al. [5]	Non-MS	77.5 (29.87)	99.8 (32.5)	—
Trip et al. [11]	MS and non-MS	68.7 (18.8)	94.6 (14.9)	102.9 (14.6)
Parisi et al. [12]	MS	59.79 (10.80)	82.73 (10.73)	111.11 (11.42)

MS: multiple sclerosis.
NON: MS healthy subjects.
ON: optic neuritis.



(a)

	RE	LE
Feb 2009	101.31 μm	109.61 μm
May 2009	79.13 μm	110.69 μm

(b)

Figure 2: Patient who developed retrobulbar neuritis in his right eye as an initial clinically isolated syndrome. In only three months, axonal loss can be detected in the eye that suffered the neuritis.

between 25 and 34% [5, 8, 12]. This reflects a predominant affectation of the papillomacular bundle, which conveys the information from the fovea, the central macular structure mainly responsible for detailed visual and color functions. Therefore, macular volume is also mildly reduced in eyes that have suffered optic neuritis [13], especially in the nasal sectors of the macula [14]. Cutoff points of 51.5 μm for the temporal RNFL thickness and 88.8 μm for the average RNFL thickness have shown the highest sensitivity (0.72 and 0.60, resp.) and specificity (0.95 and 0.97, resp.) for differencing optic neuritis eyes from control eyes [8].

The optic nerve can become pale after an episode of optic neuritis. This pallor can be diffuse or located in the temporal quadrant and reflects the RNFL loss detectable with OCT. Because the temporal quadrant of the optic disc is relatively thinner than other quadrants, even diffuse atrophy may be perceived only as temporal pallor on exam. Our study group also found a mild increase in the cup to disc ratio of approximately 0.1 to 0.2 when compared to the fellow eye, in accordance with previous clinical observations [15, 16].

2.3. Visual Prognosis. Since RNFL loss after an episode of optic neuritis stabilizes after six months, most studies that analyze the relationship between axonal loss and visual outcome are performed at or after this time point. Costello et al. found that patients with incomplete visual recovery after optic neuritis suffer a greater RNFL loss and through regression analysis obtained a threshold of RNFL thickness (75 μm) below which RNFL measurements predicted persistent visual dysfunction. This finding could be interpreted as a threshold effect whereby changes in RNFL thickness above 75 μm are associated with minimal and clinically insignificant changes in visual field threshold sensitivity [5, 7]. In Costello's study, for RNFL values below 75 μm, a 10 μm decrease in RNFL thickness predicted a decrease of 6.83 dB in visual field mean deviation scores among affected eyes [7].

Visual fields represent a subjective method of measuring visual function, which requires active collaboration and attentiveness from the patient. Visual fields usually improve after a first episode of optic neuritis to normal or near normal levels. However, OCT often reveals subclinical permanent axonal damage, which may not be reflected by subjective explorations. Thus, 60% of patients with normal visual fields in our study had abnormal RNFL thickness measurements by OCT at the six-month visit [6]. Pueyo et al. enrolled 40 patients with multiple sclerosis who had normal VA and visual fields in a prospective cohort. Although the former examinations were normal, significant differences with healthy subjects were observed in Ishihara color tests and in most RNFL measurements provided by OCT. Comparisons with the normative database showed RNFL defects in 12 eyes (30%) [17].

Low contrast letter acuity (LCLA) is being increasingly used now as a visual outcome measure in MS and ON studies. Talman et al. demonstrated that progressive RNFL thinning occurs as a function of time in MS and is associated with clinically significant visual loss by low-contrast letter acuity. They found that visual loss by the 2.5% contrast chart was

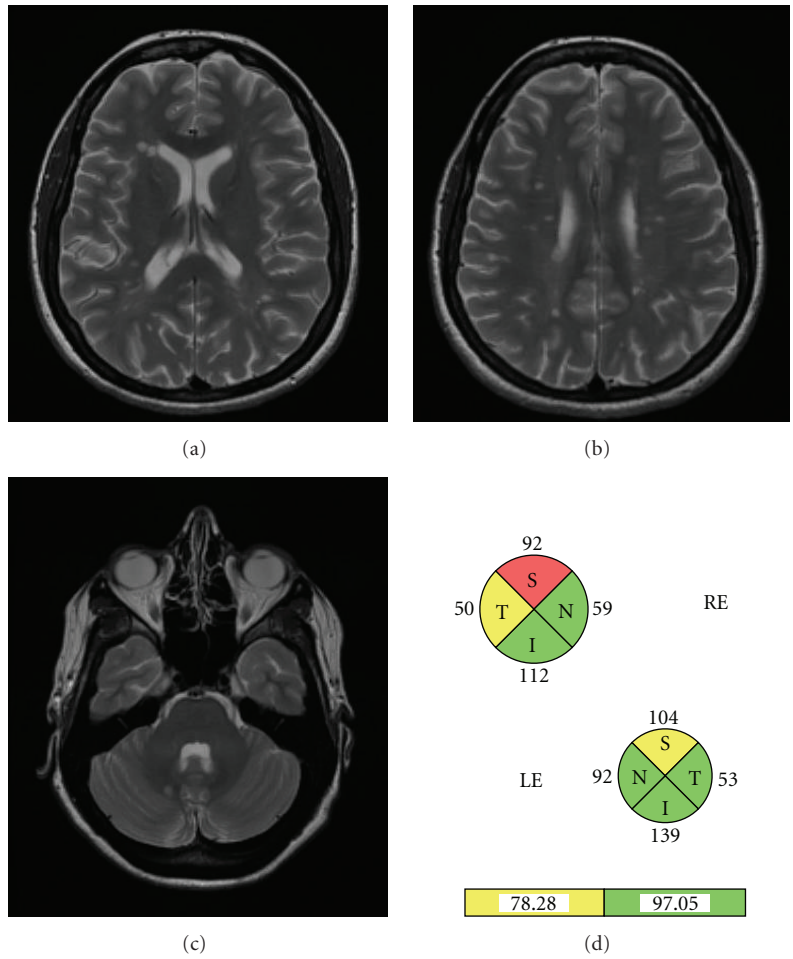


Figure 3: Patient who suffered a motor clinical isolated syndrome and fulfilled Barkhof's magnetic resonance imaging criteria. Optical coherence tomography shows a decreased retinal nerve fiber layer in both eyes (more intense in the right eye) even without a history of optic neuritis.

significantly associated with RNFL thinning. Scores from the 1.25% contrast chart, however, correlated less well with RNFL loss [18]. Fisher et al. found that lower visual function scores were associated with reduced average overall RNFL thickness. For every 1-line change in low-contrast letter acuity and in contrast sensitivity scores, RNFL thickness differences of 4 μm on average were noted, accounting for age. Spearman rank correlations between overall average RNFL thickness and visual function scores were highly significant yet modest in magnitude, suggesting that visual dysfunction may occur in some patients in the absence of (or perhaps in advance of) RNFL axonal loss (Spearman r [rs] = 0.33 and $P < .0001$ for low-contrast letter acuity, $rs = 0.31$ and $P < .0001$ for contrast sensitivity, $rs = 0.26$ and $P = .0005$ for high-contrast VA) [10].

Thus, both LCLA and RNFL thinnings are being introduced as surrogate markers for disability in MS trials. However, it should be taken into account that some patients may present with visual loss even in the presence of a preserved RNFL thickness, while on the other hand patients with decreased RNFL thickness may not present severe dysfunction if nerve fiber loss does not affect the papillomacular bundle. Furthermore, patients with visual loss adapt with time to their scotomas, so that visual function may improve as they learn to manage with their limitations.

3. Optical Coherence Tomography in Patients with Clinically Isolated Syndrome

The development of a clinically isolated syndrome (CIS) represents the earliest clinical stage of multiple sclerosis. Outteryck et al. studied 56 patients with CIS, 18 with optic neuritis, and 38 without it. Two-thirds of the patients had dissemination in space according to the Barkhof criteria. All of the patients had a normal overall RNFL thickness. However, 14 patients (25%) and 7 controls (22%) had RNFL atrophy in at least 1 quadrant, according to the OCT database (Figure 3). There was no link between atrophy in 1 or more quadrants of the RNFL and dissemination in space according to the Barkhof criteria at initial MRI, nor with multifocal presentation, alteration of visual evoked potentials, or development of multiple sclerosis after 6 months according to the

Table 2: Comparison of RNFL thickness (μm) between both eyes of patients diagnosed of multiple sclerosis who have suffered unilateral optic neuritis and healthy control subjects.

	MS with ON	MS without ON	Control
Siepman et al. [13]	72.2 (14.4)	89.5 (14.2)	—
Khanifar et al.* [14]	83.0 (14.0)	90.5 (13.2)	97
Costello et al. [23]	79.5 (18.8)	97.0 (14.3)	—
Bock et al. [27]	86.2 (16.2)	97.0 (13.1)	105.2 (9.4)
Quelly et al. [30]	78.01 (17.43)	95.24 (11.64)	—
Merle et al. [31]	80.81 (18.4)	96.7 (15.8)	106 (12.2)
Oreja-Guevara et al. [32]	76.42 (16.87)	89.45 (17.68)	—
Frohman et al. [33]	70.3 (13.4)	101.8 (6)	101.9 (8.9)
Burkholder et al. [21]	85.7 (19.0)	95.6 (14.5)	104.5 (10.7)
Spain et al. [34]	75.81	90.93	—
Siger et al. [22]	83.92 (17.63)	94.38 (15.0)	100.3 (12.1)
Pueyo et al. [35]	84.46	94.20	104.97
Zaveri et al. [36]	81.8 (19.3)	95.6 (15.0)	104.6 (10.3)
Pulicken et al. [37]	84.2 (14.7)	95.9 (14)	102.7 (11.5)
Gundogan et al. [20]	—	107.6 (16.3)	110.9 (10.3)
Cheng et al. [38]	76.12 (14.92)	96.45 (11.73)	—
Fisher et al. [10]	85 (17)	96 (14)	105 (12)

* Heidelberg Spectralis. Value for normals taken from normative database.
MS: multiple sclerosis.
ON: optic neuritis.

revised McDonald criteria [19]. However, the relationship between RNFL thinning in CIS and progression to MS is still unclear, since only long-term followup will determine if these changes are clinically relevant.

4. Multiple Sclerosis

In the absence of optic neuritis, retrograde trans-synaptic retinal ganglion cell degeneration due to multiple sclerosis lesions within the posterior optic pathways could cause RNFL loss. Progressive axonal loss could also explain the RNFL thinning found in eyes of patients with multiple sclerosis without a history of optic neuritis (Table 2) [14]. In these eyes, mean RNFL loss is milder (7.08 μm) than in eyes that have suffered optic neuritis (20.38 μm) [2]. Some studies have only found a significant difference in eyes with and without optic neuritis for the temporal quadrant [20].

On average, 10 μm differences in RNFL thickness are associated with 0.20 mm^3 reductions in total macular volume. Eyes of patients with multiple sclerosis both with and without optic neuritis showed similar degrees of total macular volume reduction [21].

A moderate correlation has been found between RNFL thickness and the time from diagnosis of multiple sclerosis [11, 14, 22, 23]. However, the correlation with neurological disability quantified by the Expanded Disability Status Scale (EDSS) is less consistent: some authors have found a significant correlation between the EDSS and RNFL thickness [15, 22, 24, 25], while others have not [17, 26]. The strongest correlation was found in relapsing remitting MS (RRMS) [23]. The differences between these studies may be due to differences in the neurological status among study populations. It has been proposed that OCT may be more optimally used in little or moderately affected patients [13, 23].

RNFL thinning is greater when multiple sclerosis patients suffer an optic neuritis [21, 24], and more pronounced in the temporal quadrant (Table 2) [27]. No differences were detected between unaffected eyes of patients with multiple sclerosis with or without an eye affected of optic neuritis [22, 27]. When the RNFL is measured in patients who have suffered optic neuritis, the thinning is greater in patients with multiple sclerosis than when it constitutes a clinical isolated syndrome, and the difference is significant for the temporal quadrant [23]. This suggests that the disease process underlying multiple sclerosis increases the damage produced by an inflammatory episode.

4.1. Macular Edema. Optical coherence tomography has become the most useful tool for the diagnosis of macular edema. This is not a common isolated manifestation; however, it could appear in multiple sclerosis patients with intermediate uveitis or as a side effect of therapies.

4.2. Recurrent Episodes of Optic Neuritis in Patients with Multiple Sclerosis. Costello et al. compared eyes with isolated optic neuritis to eyes with recurrent episodes of patients with different forms of MS and found an additional thinning when the inflammation recurs [23]. They conclude that the majority of patients may recover visual function after an isolated optic neuritis event because they do not suffer enough axonal damage to result in permanent visual impairment. Patients with severe or recurrent optic neuritis, however, may be at a greater risk of losing so many axons

Table 3: Comparison of RNFL thickness (μm) among different types of multiple sclerosis.

		RRMS	SPMS	PPMS	Control
Albrecht et al. [39]		86.91 (21.51)	70.57 (16.76)	80.45 (17.76)	103.4 (10.96)
Henderson et al. [40]	Non-ON	Not supplied	88.4 (10.9)	Not supplied	Not supplied
Pulicken et al. [37]		94.4 (14.6)	81.8 (15.6)	88.9 (13.3)	—

RRMS: recurrent remittent multiple sclerosis.
SPMS: secondary progressive multiple sclerosis.
PPMS: primary progressive multiple sclerosis.

that they fall under the threshold required for complete visual recovery, thus increasing the likelihood that they will experience persistent visual defects [23].

4.3. Relationship with MRI Findings. Magnetic resonance image findings are currently considered the most sensitive and reliable markers for assessing inflammatory and axonal pathology in patients with multiple sclerosis. Conventional techniques are designed to be largely sensitive to inflammation (T2-weighted lesions) and not specifically reflect axonal damage with only modest correlation with clinical disability [25]. Alternatively diffuse brain atrophy has been linked with disability progression in multiple sclerosis [25]. Significant associations have been shown between the RNFL thickness and several MRI findings characteristic of brain atrophy:

(i) brain parenchymal fraction (which computes the volumes of various intracranial compartments and total brain parenchyma) [22, 25, 28],

(ii) diffusion tensor imaging values [29],

(iii) gray matter [22, 24] and white matter volume [24],

(iv) increase in cerebrospinal fluid volume [25],

(v) magnetization transfer ratio [29],

(vi) T1-lesion volumen [22].

Frohman et al. studied twelve patients with multiple sclerosis and found that low contrast visual acuity, RNFL thickness, and optic nerve radius were the variables with the highest predictive value in discerning differences between healthy controls and patients. Both the radius of the affected eyes and its fractional anisotropy predicted the RNFL of the affected eye; however, the RNFL thickness was the only independent predictor of lower contrast sensitivity. T1 and T2 lesion volumes, measures of optic nerve atrophy, and measures of grey matter atrophy were related to RNFL thickness, however, they explained only about 20% of variance [29].

In multiple sclerosis patients without optic neuritis, axonal loss seems to correlate better with MRI parameters than in those that have suffered optic neuritis [22]. RNFL thickness correlates with brain atrophy more strongly in RRMS than in secondary progressive multiple sclerosis (SPMS). This might be due to a basement effect in the progressive group, in which RNFL or brain tissue may have reached their lowest levels so that further damage is almost impossible. Another possibility is that most patients with SPMS have clinical decline due to cumulative spinal cord disease rather than accumulation of brain disease [25]. Macular volume does not correlate with MRI features [25].

4.4. Types of Multiple Sclerosis. There seem to be different patterns of axonal loss among the different types of multiple sclerosis according to their clinical course (Table 3). Henderson et al. studied patients with progressive forms of multiple sclerosis, excluding those who had suffered optic neuritis in both eyes. Axonal loss was significant compared to controls, for the mean RNFL in the SPMS group and for the temporal quadrant in both progressive forms (53.6 μm, SD 13.2 μm and 63.7 μm, SD 14.6 μm, for SPMS and primary progressive (PPMS] types, resp.). The only difference found between both groups was the thinner temporal quadrant in the SPMS group [40]. These results could be explained by subclinical optic neuritis attacks suffered by SPMS patients in the remittent recurrent phase, by differences in the time from diagnosis that existed between both groups or due to distinctive preferences in the nervous system affected in each form [40].

Pulicken et al. also found RNFL thinning in the progressive forms, which was more pronounced than in patients with RRMS [37], and Siepman et al. did not find significant differences when comparing eyes of patients with PPMS and RRMS [13]. Therefore, it seems that during the progressive phase progressive axonal loss also develops, being more pronounced in the SPMS type [37].

Costello et al. studied patients who had had isolated optic neuritis (without a diagnosis of MS) and patients who had had an episode of neuritis and were already diagnosed with RRMS, SPMS, or PPMS. Optic atrophy was more severe in the secondary progressive group with more pronounced differences in the temporal quadrant. The differences among multiple sclerosis types are more difficult to appreciate in eyes without optic neuritis [23].

Total macular volumes also differed between multiple sclerosis disease subtypes, with lower values seen in SPMS [mean (SD), 6.25(0.52] mm^3) than in PPMS (mean (SD), 6.57(0.50] mm^3) [21].

4.5. Visual Prognosis. Multiple sclerosis patients have worse contrast sensitivity and visual fields if they have suffered an episode of optic neuritis, in accordance with their decreased RNFL thickness, than if they just have subclinical axonal loss [10, 32, 41].

Visual prognosis after an episode of optic neuritis is good, since approximately three out of four patients retain a visual acuity of 20/20 after 15 years [42]. Visual acuity

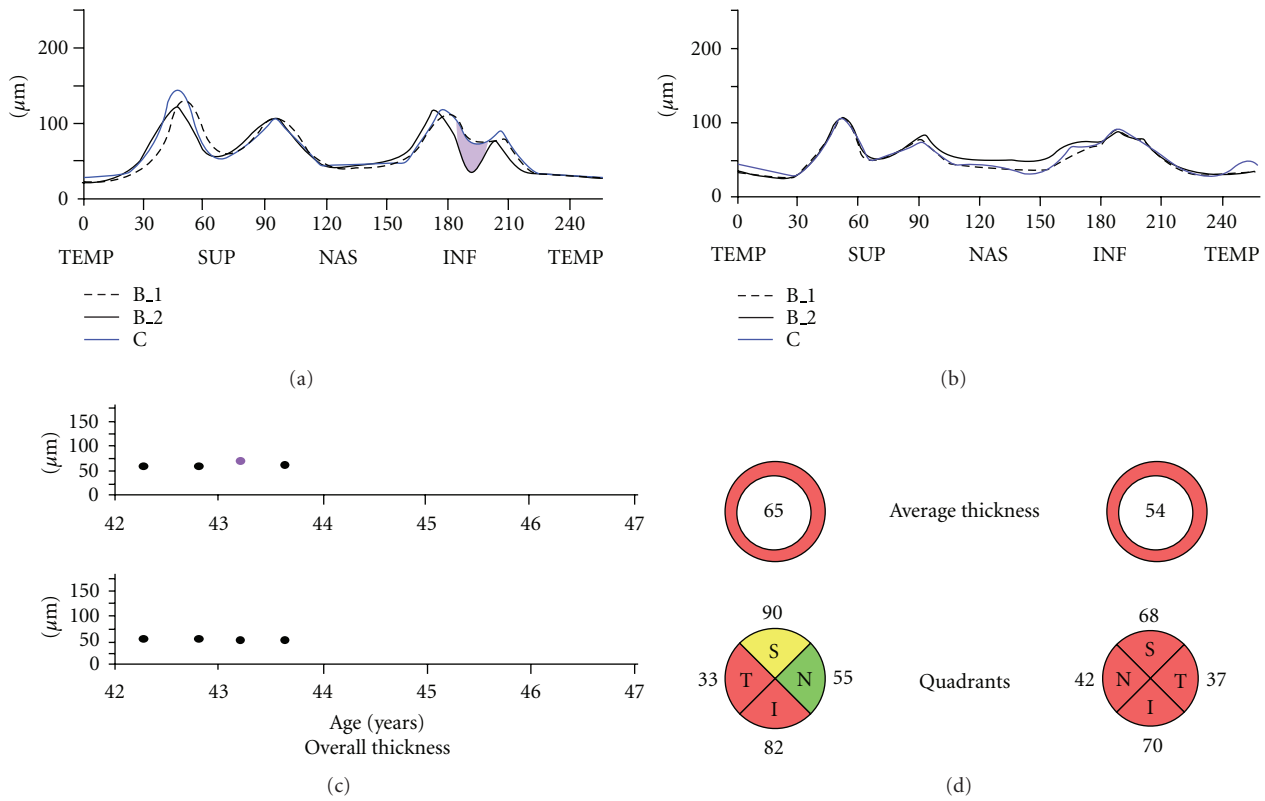


Figure 4: Patient with multiple sclerosis and severe bilateral RNFL thinning with history of optic neuritis. SD-optical coherence tomography analysis found no further loss during a year.

after an episode of neuritis is similar in patients already diagnosed with multiple sclerosis to patients in whom it is an isolated manifestation. However, visual field mean deviation, a parameter that measures light sensibility depression, is slightly lower for multiple sclerosis patients [32], probably because visual acuity is less sensitive to asses visual function in multiple sclerosis patients than other examinations, such as color vision, stereopsis, or contrast sensitivity [8, 43].

Visual acuity in patients with multiple sclerosis without optic neuritis does not differ from healthy controls. However, when tests that explore spatial (Sloan and Pelli-Robson charts) and temporal (frequency doubling technology perimetry) contrast sensitivity are employed, visual function is found to be worse in multiple sclerosis patients when compared to control subjects [31]. In their study, Merle et al. found that eyes with a previous history of optic neuritis presented an important decrease in RNFL thickness, which was correlated with the results of all the visual function tests performed, including VA [31].

Visual fields may be normal even if RNFL loss is detected: Cheng et al. found no perimetric defects in 4% patients with mean RNFL atrophy and in 18% with at least sectorial atrophy according to normative data [38]. Disagreement between RNFL thickness and visual fields is probably influenced by cognitive dysfunction or slowed reaction times during subjective field testing, which can interfere with decision-making, in patients with multiple sclerosis and also by central visual pathway damage that does not result in retrograde degeneration. However, when functional loss is worse than −10 dB, the authors concluded that it is better to use mean deviation for monitoring disease progression, because RNFL loss has almost reached a plateau at approximately 60 μm (Figure 4) [38].

Lower RNFL values have been correlated with reduced visual acuity and mean deviation. Every 10 μm decrease in RNFL correlated with a 5.8 dB decrease in visual field sensitivity and a 0.46 reduction in visual acuity for RNFL values below 75 μm [23].

4.6. Evolution. Longitudinal studies have been performed to assess changes in RNFL thickness. Talman et al. followed 299 patients with multiple sclerosis for at least six months, with a median followup of 18 months, ranging between 6 months and four and a half years. They found that each year of followup was associated on average with a 2.0 μm decreases in RFNL thickness. This rate of axonal loss was similar in patients with and without a history of optic neuritis. Eyes with visual loss by low-contrast letter acuity and VA (measured with an ETDRS chart and concerted to logMAR for analysis) had greater degrees of RNFL thinning during followup compared to those without visual changes. RNFL thinning was also associated with progressive changes in neurological impairment measured by the EDSS [18]. Sepulcre et al. found a decrease in average RNFL thickness of 4.8 μm in patients after 2 years. They found that patients with more active disease have a thinner temporal quadrant RNFL

Table 4: Comparison of RNFL thickness (μm) between multiple sclerosis and neuromyelitis optica.

	NMO ON eye	NMO fellow eye	MS ON eye	MS fellow eye	Control
Nakamura et al. [45]	63.84 (23.47)	106.36 (14.55)	84.28 (14.18)	109.45 (12.78)	—
Naismith et al. [26]	54.8 (3.7)	—	76.5 (2.4)	—	—
Merle et al. [46]	65.44 (24.19)		83.85 (24.12)		106.24 (12.46)

MS: multiple sclerosis.
NMO: neuromyelitis optica.

compared with stable patients. Patients with new relapses during followup had a thinner RNFL in the temporal quadrant than relapse-free patients by the end of the study [24]. García-Martín et al. enrolled 81 patients with a diagnosis of defined multiple sclerosis: 31 of the patients (38.3%) received no specific treatment, whereas the other 50 patients (61.7%) were treated with interferon beta. Most studied eyes (75.3%) were from patients without a history of optic neuritis. Statistically significant differences were observed between the baseline and 1-year examinations in all the RNFL thickness measurements and macular volume and in VA (logMAR), whereas perimetry results revealed no differences between treated and untreated patients. The greatest decrease was found in the average and the inferior OCT RNFL thickness (of 3%), with a baseline mean of 90.46 μm and 115.46 μm versus 85.96 μm and 109.12 μm at the 1-year followup, respectively, No correlation was found between the 1-year change in EDSS and RNFL measurements. They concluded that axonal loss in the optic nerve of patients with multiple sclerosis is far greater than that which occurs in healthy subjects, regardless of the presence of a history of optic neuritis [44].

5. Neuromyelitis Optica

Visual prognosis is much worse if optic neuritis occurs in patients with neuromyelitis optica. Only one episode is capable of producing legal blindness in almost one third of patients and only about 45% of them completely recover visual function [47]. An ischemic vascular mechanism has been proposed in its pathogenesis to explain at least partially its severity [48]. Ratchford et al. have suggested that an RNFL thickness loss after an episode of optic neuritis of more than 15 μm may be considered a marker for this disease instead of multiple sclerosis, together with the absence of a visual improvement of at least two lines [49]. Naismith et al. estimated that for every 1 μm decrease in the RNFL thickness, the odds of being diagnosed with NMO increased by 8% [26]. Several reports have confirmed that axonal loss after an episode of optic neuritis in patients with neuromyelitis optica is greater than in patients with multiple sclerosis (Table 4) [45, 46]. This difference persisted even after adjusting the RNFL for visual outcome [26]. It has also been shown that the superior and inferior quadrants are more intensely affected after NMO, so that the pattern of the RNFL loses its characteristic humps [26, 45]. Recurrent episodes of optic neuritis lead to further RNFL loss [45].

The mean RNFL thickness of the unaffected fellow eye in NMO has been found to be greater than the unaffected multiple sclerosis eyes. This sparing of the unaffected felloe eye in NMO compared to MS may be explained by a more common occurrence of subclinical optic neuritis in multiple sclerosis: axonal attrition in multiple sclerosis independent of optic neuritis or an increased predilection of multiple sclerosis lesions in the optic chiasm or tracts [26].

As in multiple sclerosis, mean RNFL is correlated with best-corrected visual acuity. Studies agree on the fact that there is a critical value of RNFL thickness below which further decreases of the RNFL lead to incomplete visual recovery. This critical value has been set at 71.41 μm [45]. Below 50–52 μm, vision drops to $\leq$20/100 [26, 46].

In a retrospective study, Nakamura et al. evaluated the effects of high dose intravenous methyl-prednisolone on the outcomes after optic neuritis in patients with neuromyelitis optica. Early treatment, especially within 3 days after onset, led to a greater probability of preserving an RNFL > 71.41 μm (the cutoff point in this study for preserving a visual acuity >20/20) [45].

Even if RNFL loss is greater in NMO as compared to MS, at each level of visual function there was a considerable overlap in OCT measures, limiting the role of OCT to differentiate the two conditions on an individual basis (Figure 5) [50].

6. Conclusions

(i) Optical coherence tomography confirms the presence of optic disc edema in anterior neuritis, reflected as a thickening of the RNFL.

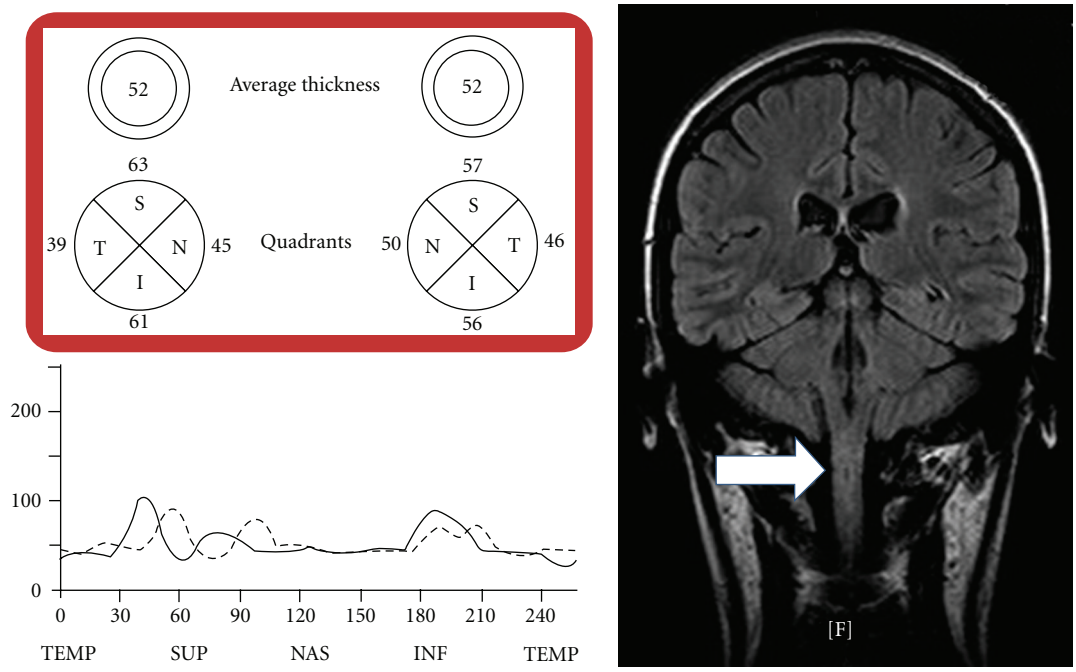
(ii) Axonal atrophy develops after optic neuritis so that six months after the event, the RNFL thickness is predictive of visual and neurological disability. However, it may be more optimally used in little or moderately disable patients.

(iii) Optical coherence tomography can detect subclinical axonal loss in patients with normal visual acuity and visual fields. Contrast sensitivity seems to be the most useful test to detect subtle visual impairment.

(iv) The temporal quadrant is the most vulnerable to the disease process.

  (a) Temporal RNFL thickness may be decreased as soon as two months after the event.

  (b) Reduced temporal thickness is often the only sign that may differentiate multiple sclerosis



	RE	LE
VA	0.1	0.66
Ishihara	0/20	0/20

(c)

Figure 5: Teenager diagnosed of neuromyelitis optica who has suffered bilateral optic neuritis as well as myelitis. Although normative data are not available, the retinal nerve fiber layer thickness measured with optical coherence tomography is extremely low.

patients from healthy subjects and between primary and secondary progressive forms.

(c) It may provide important insights regarding relapse related activity in multiple sclerosis patients.

(v) During the progressive phases of multiple sclerosis, axonal loss also occurs at the optic nerve; this axonal loss is detected by OCT as RNFL loss and is greater in the SPMS type.

(vi) When multiple sclerosis patients are followedup, an approximate decrease of $2\,\mu$m in RFNL thickness is detected per year. Progressive changes seem to correlate with changes in neurological impairment measured by the EDSS.

(vii) Brain atrophy is at least moderately correlated to RNFL thickness and multiple sclerosis patients have decreased RNFL thickness even without a history of optic neuritis. These results suggest that the RNFL thinning reflects pathology that extends beyond local injury to the optic nerve by optic neuritis. A stronger correlation with MRI results is unlikely since axons are not the only component of the brain and because brain atrophy also reflects synaptic changes, loss of myelin, gliosis, and changes in water content.

(viii) Visual prognosis is much worse if optic neuritis occurs in patients with neuromyelitis optica, which leads to a more sever thinning of the RNFL when compared to optic neuritis in multiple sclerosis patients.

(ix) OCT thus seems to be a promising outcome measure for neuroprotective trials. However, overall RNFL thickness is not always directly correlated with visual function and measuring both mean RNFL thickness and temporal RNFL thickness (usually more related to visual acuity) would require more patients to be included into the studies. Furthermore, it may be difficult to distinguish axonal loss related to age with axonal loss due to the disease process in MS. The relationship between axonal loss and OCT is not clear: future studies need to evaluate it.

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Research Article

Exploring the Association between Retinal Nerve Fiber Layer Thickness and Initial Magnetic Resonance Imaging Findings in Patients with Acute Optic Neuritis

Fiona Costello,[1] William Hodge,[2] and Y. Irene Pan[3]

[1] *Departments of Clinical Neurosciences and Surgery, Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada T2N 2T9*
[2] *Department of Ophthalmology, The University of Western Ontario, London, ON, Canada N6A 4V2*
[3] *McGill University, Montreal, QC, Canada H3a*

Correspondence should be addressed to Fiona Costello, fionacostello@rogers.com

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Background. Recent studies have shown that OCT-measured retinal nerve fiber layer (RNFL) values may represent a marker for axonal damage in the anterior visual pathway of optic neuritis (ON) and multiple sclerosis (MS) patients. The goal of this study was to determine the link between RNFL values and initial magnetic resonance imaging (MRI) evidence of central nervous system (CNS) inflammation in patients with acute ON. *Methods*. Fifty patients who experienced ON as a clinically isolated syndrome (CIS) were followed for a mean period of 34 months with OCT testing. RNFL values in affected (ON) eyes and clinically unaffected (non-ON) eyes were compared between patients with MRI evidence of white matter lesions and those with normal baseline MRI findings, over a two year period. *Findings*. Twenty-one patients (42%) developed clinically definite MS (CDMS) during the study. After two years, temporal RNFL values were thinner ($P = .07$) in ON patients with MRI lesions at baseline, but the results were not significant. *Conclusions*. There is no association between RNFL values and baseline MRI status in ON patients at risk for future CDMS over a two year period.

1. Introduction

Optic neuritis (ON) is an inflammatory optic nerve injury, which is strongly associated with multiple sclerosis (MS) [1–11]. Approximately 20% of MS patients present with ON as their first demyelinating event, and 30 to 70% of MS patients develop ON during the course of their disease [2, 7]. The longitudinal followup from the optic neuritis treatment trial [3] (ONTT) demonstrated that the initial magnetic resonance imaging (MRI) study is the most potent predictor for future MS after acute ON: such that evidence of disseminated central nervous system (CNS) inflammation on baseline MRI increases the risk for future clinically definite MS (CDMS) after acute ON [4–6]. After 15 years 72% of ON patients with one or more white matter lesions on their initial MRI developed CDMS, as compared to 25% of patients with no MRI lesions [6]. The administration of high-dose corticosteroids has been shown to delay the conversion to MS for up to two years after ON, but not thereafter [8]. Early initiation of disease-modifying therapies delays the development of MS in patients with ON and other clinically isolated syndromes (CISs) [9–11].

The evidence supporting early initiation of disease-modifying therapy in patients at risk for MS is robust [9–11], as is the need to develop effective, cost-effective strategies to capture disease activity and monitor therapeutic effects in these patients. One tool which has emerged as a potential structural marker for axonal loss in MS patients is optical coherence tomography (OCT) [7, 12–25]. This noninvasive ocular imaging technology employs low-coherence interferometry to generate high-resolution [$\leq$10 microns (μm)], cross-sectional images of the retinal nerve fiber layer (RNFL) by measuring the backscatter of infrared light [26, 27]. The RNFL contains the retinal ganglion cell axons that comprise the optic nerve; and because it lacks myelin, represents a unique region of the central nervous system. Changes in

RNFL thickness after ON have been interpreted to reflect initial axoplasmic flow stasis and subsequent attrition caused by inflammation in the anterior visual pathway. Recent studies have shown that OCT-measured RNFL values are reduced after ON [12–16, 25] and that the extent of RNFL atrophy correlates with diminished scores of visual and neurological function [12–16, 19, 21, 24, 25] MRI measures of optic nerve and brain atrophy [17–20], and disease activity in MS patients [19]. To date, an association between RNFL thickness and initial MRI status in ON patients at risk of developing MS has not been established. Thus, the primary objective of this study was to determine whether RNFL values differed between ON patients with evidence of clinically silent brain lesions on initial MRI and patients with no MRI evidence of CNS inflammation over a two-year period. Our second aim was to compare RNFL values between patients treated with high-dose corticosteroid therapy and untreated patients, to ascertain whether RNFL differences distinguished the two groups.

2. Methods

2.1. Study Design and Sampling. This was a prospective cohort study of consecutively sampled patients referred to the Ottawa Hospital Neuro-Ophthalmology Clinic for the evaluation of acute ON between January 2003 and June 2007. The study received approval by the local ethics committee at this institution, and participating patients provided informed written consent.

2.2. Inclusion and Exclusion Criteria. Fifty CIS patients who experienced a single, unilateral ON event were included in the study. Patients were diagnosed with ON if they demonstrated the following clinical features: decreased visual acuity, a visual field defect, which followed the topography of the RNFL, a relative afferent pupil defect, and a compatible fundus examination (mild or no optic disc edema and the absence of pallor at the time of the acute event). Exclusion criteria included other established causes of vision loss in the affected eye (amblyopia, glaucoma, and dense cataracts), a known diagnosis of MS or neuromyelitis optica (NMO), and inability to undergo reliable OCT testing.

Other Variables. Demographic and clinical variables including age, gender, the presence of pain, mono-focal (ON without other neurological symptoms) versus multi-focal ON (associated with neurological symptoms referable to a region of the CNS different from the anterior visual pathway) as a CIS presentation, and the initiation of disease-modifying disease (DMD) therapy were recorded. The time to baseline MRI acquisition and MRI protocols varied among patients. For this reason, we were not able to employ the revised McDonald criteria [28] to define radiological conversion to MS after ON Instead, we documented whether patients had MRI evidence of clinically silent lesions representing CNS inflammation at the time of the ON event [9]. All MRI results were interpreted by qualified neuroradiologists at the University of Ottawa. Specific MS MRI protocols were employed (1.5T GE scanners), which included (coronal, axial, and sagittal imaging) T1-weighted, T2-weighted, and FLAIR sequences, either with or without gadolinium. The treating physician employed individualized discretion regarding the decision to administer corticosteroid therapy for acute management of ON. Patients who received corticosteroid therapy were treated within two weeks of the ON event with the equivalent of 1000 mg intravenous methylprednisolone daily for three days. Disease-modifying therapies were administered to a minority (12/50) of patients during the course of this study. No patients initiated disease-modifying therapy earlier than 6 months after ON. The limited number of CIS patients who received disease-modifying agents precluded efforts to compare the effects of disease-modifying therapy on RNFL values after ON.

2.3. Clinical Assessment. Patients were followed with repeat visual and OCT testing for a minimum of 24 months. The neuroophthalmic assessment included best-corrected Snellen visual acuity, visual field analysis, and dilated ophthalmoscopy. Neurological evaluations were performed at 6-month intervals by a neurologist at the MS Clinic at the Ottawa Hospital. Patients aged greater than 45 years or those with atypical features (including incomplete recovery after ON) also underwent visual electrodiagnostic testing (including pattern visual evoked potential (VEP) and multi-focal electroretinogram studies) to exclude retinal mimics of ON. If clinically indicated, additional studies were also performed to exclude conditions that could mimic MS such as myeloproliferative disorders, NMO, syphilis, sarcoidosis, or Lyme disease.

2.4. Optical Coherence Tomography. The OCT (Stratus version 3; OCT 4 Software, Zeiss Meditech, Dublin, Calif, USA) system was used to obtain circular peripapillary scans (Fast RNFL protocol), which included a set of three 3.4 mm diameter retinal scans averaged to provide the RNFL thickness at 256 points along the circumference of the circular scan in each eye after mydriasis with 1% tropicamide. The OCT software employed an automated computerized algorithm to calculate the average thickness of the RNFL and to compare these measurements to a normative database of age-matched controls, for patients aged 18 years or older. A trained ophthalmic medical technologist performed all OCT testing and monitored scans to ensure that fixation was reliable. OCT scans with signal strength equal to or greater than 7 (out of 10) were included in the study.

2.5. Outcome Measures and Statistical Analysis. The primary outcome measures in this study were RNFL values in ON eyes and non-ON eyes, which were compared between patients with clinically silent lesions on their baseline MRI scan and patients with no baseline evidence of CNS inflammation. As a secondary outcome measure, we compared RNFL values between patients treated with high-dose corticosteroid therapy for acute ON and untreated patients. Continuous variables were first checked for normality, and then summary statistics were calculated and reported. Median and range

Table 1: Demographic and clinical characteristics among optic neuritis patients.

Demographics/characteristics	
Number of patients	50
Mean age in years (range)	34 (18–50)
Male: female[‡]	10 : 40
CIS*: CDMS[†]	29 : 21
Pain (%)	42/50 (84)
Mono-focal[§]: Multi-focal (%)	18 : 32
Abnormal MRI[#] (%)	32/50 (64)
Abnormal CSF** (%)	14/20 (70)
Corticosteroids^ (%)	25/50 (50)

[‡]Ratio of male to female patients included in the study; ratio of * clinically isolated syndrome patients to [†]clinically definite MS patients; number of patients presenting with [§]mono-focal versus multi-focal optic neuritis as a CIS at baseline; [10#]number and percentage of abnormal magnetic resonance imaging scans at presentation; [9]** number and percentage of patients with positive oligoclonal bands in their cerebrospinal fluid analysis at baseline: ^ number and percentage of patients who received treatment with the equivalent of 1000 mg intravenous methylprednisolone for three days for acute optic neuritis.

were computed for continuous variables that demonstrated a non-Gaussian distribution, including counts and percentages for categorical variables. For group comparisons, either the Student's *t*-test or the Kruskal-Wallis rank sum test was used depending on the variable's distribution attribute. The entire statistical analysis was performed using STATA (v. 9, College Station, Tex, USA).

3. Results

3.1. Demographics and Clinical Presentation. Fifty patients (100 eyes) were included in the study. The mean age was 34 years, and the mean follow-up period was 34 months (range 24–44 months). All patients were followed for a minimum of 24 months. Twenty-one patients (42%) developed CDMS during the course of the study, with a mean conversion time of 27 months. The baseline demographics and clinical data are included in Table 1.

3.2. Comparing Baseline MRI Status, Treatment with High-Dose Corticosteroids, and RNFL Thickness. ON Eyes: The presence or absence of disseminated white matter lesions on the baseline cranial MRI scan in patients was not associated with significant differences in RNFL thickness a year after ON. By the second year of followup, temporal RNFL values (49.8 μm) were thinner ($P = .07$) in patients with abnormal baseline MRI scans, but the results did not statistically significant (Table 2). RNFL values were not significantly differ between patients treated with high-dose corticosteroid therapy and untreated patients for up to two years after acute ON (Table 2). *Non-ON Eyes* RNFL values did not differ between patients with clinically silent lesions on initial MRI and patients without initial MRI evidence of CNS inflammation for up to two years (Table 3). Similarly, treatment with high-dose corticosteroid therapy, or the lack thereof, was not associated with any significant differences in RNFL thickness for non-ON eyes at year one or year two of followup (Table 3).

4. Discussion

In the current study, we observed no association between the presence of clinically silent lesions on baseline MRI and RNFL thickness in CIS patients followed for two years after an acute ON event. By the second year of followup, temporal RNFL values were thinner in ON-eyes of patients with abnormal baseline MRI scans, yet differences did not reach statistical significance ($P = .07$). One explanation for this observation is that there is discordance between OCT-measured axonal damage and MRI evidence of disseminated CNS inflammation in CIS patients. Conventional MRI protocols may reflect inflammatory activity in lieu of axonal pathology, particularly at the earliest stage of MS. Given that more robust correlations have been noted between RNFL atrophy in the anterior visual pathway and MRI measures of brain volume and atrophy [17, 19, 20], diffusion tensor imaging [29, 30], and magnetic transfer ratios [18], there may be a stronger correlation between OCT outcomes and evolving MRI protocols that are specifically aimed at capturing axonal attrition in the CNS.

There were no differences in RNFL atrophy noted between patients treated with high-dose corticosteroids for acute ON and untreated patients in this study. It is noteworthy that we did not randomize ON patients into treatment protocols. It is therefore possible that there was some bias on the part of the treating physician that influenced the decision to administer or withhold treatment, which may have affected our results, Yet, the administration of high-dose corticosteroids does not impact visual recovery after ON or future risk of MS in the long term [8]; therefore, another potential explanation for our findings is that RNFL atrophy after acute ON is not significantly impacted by acute management with corticosteroid therapy.

Only two prior reports have explored whether OCT-measured RNFL values distinguish patients at future risk of CDMS after ON, and in both studies the results were predominantly negative [31, 32] Previously, we compared RNFL values in ON eyes and non-ON eyes between patients who developed CDMS (42%) and those that did not develop MS within a minimum of 24 months after an acute ON event (58%), in the same patient cohort. [31] Mean RNFL values were reduced in ON eyes of non-MS patients as compared to CDMS ON eyes after one year ($P = .05$) due to more severe ON events in the former [31]. Temporal RNFL values were lower in the non-ON eyes of CDMS patients, but the results were not statistically significant ($P = .13$) [31]. From our findings, we concluded that RNFL thickness did not differentiate patients at higher risk of converting to CDMS after ON [31]. Similarly, Outteryck and colleagues [32] recently performed OCT testing on 56 CIS patients (18 with optic neuritis and 38 without optic neuritis) and 32 control subjects, to investigate whether RNFL thickness and macular volume revealed early retinal axonal loss. In this prospective case series there was no link between RNFL

Table 2: Comparing MRI status, corticosteroid therapy, and RNFL* values in optic-neuritis-affected eyes.

RNFL ON Eyes*	Year 1		*P* value	Year 2		*P*-value
	MRI+§ ($n = 32$)	MRI−|| ($n = 18$)		MRI+ ($n = 32$)	MRI− ($n = 18$)	
Overall (SD)¶	84.4 (17.3)	80.1 (21.1)	.45	80.9 (16.6)	85.8 (21.6)	.36
Superior	110.8 (22.3)	102.9 (31.9)	.32	106.4 (21.6)	106.6 (29.6)	.98
Inferior	106.3 (25.0)	95.8 (28.7)	.20	99.3 (29.2)	107.9 (33.0)	.35
Nasal	68.1 (19.8)	67.1 (21.7)	.87	67.3 (16.5)	70.1 (21.0)	.61
Temporal	52.4 (17.5)	53.9 (15.2)	.78	49.8 (14.2)	58.5 (18.7)	.07
	Steroid# ($n = 25$)	Untreated** ($n = 25$)		Steroid ($n = 25$)	Untreated ($n = 25$)	
Overall (SD)	81.4 (15.8)	84.3 (21.4)	.59	81.8 (18.6)	83.4 (18.7)	.77
Superior	104.7 (22.2)	111.4 (29.7)	.38	104.5 (23.5)	108.5 (25.7)	.57
Inferior	100.7 (22.2)	104.5 (30.5)	.63	102.1 (31.3)	102.8 (30.5)	.94
Nasal	70.5 (21.7)	65.2 (18.6)	.37	67.8 (18.9)	68.8 (17.6)	.85
Temporal	49.5 (14.9)	56.2 (17.7)	.16	52.3 (15.4)	53.3 (17.5)	.88

* Retinal nerve fiber layer thickness (μm); §baseline magnetic resonance imaging evidence of clinically silent inflammation [8]; ||normal baseline magnetic resonance imaging scan; ¶mean overall RNFL values (standard deviation) and mean RNFL values in the superior, inferior, nasal, and temporal quadrants; #patients treated with the equivalent of 1000 mg of intravenous methylprednisolone for acute management of optic neuritis; **patients not treated acutely for optic neuritis.

Table 3: Comparing MRI status, corticosteroid therapy, and RNFL* values in nonaffected eyes.

RNFL Non-ON Eyes*	Year 1		*P* value	Year 2		*P* value
	MRI+§ ($n = 32$)	MRI−|| ($n = 18$)		MRI+ ($n = 32$)	MRI− ($n = 18$)	
Overall (SD)¶	103.9 (10.5)	102.2 (9.4)	.56	104.1 (12.2)	104.3 (9.9)	.94
Superior	131.1 (15.6)	132.9 (13.0)	.67	134.4 (20.6)	135.3 (13.6)	.87
Inferior	134.0 (16.9)	125.4 (12.7)	.07	131.0 (17.9)	127 (14.9)	.42
Nasal	84.3 (17.0)	80.4 (17.7)	.46	84.0 (18.0)	84.7 (21.4)	.89
Temporal	66.3 (13.0)	70.0 (10.1)	.30	66.9 (13.6)	70.7 (13.8)	.34
	Steroid# ($n = 25$)	Untreated** ($n = 25$)		Steroid ($n = 25$)	Untreated ($n = 25$)	
Overall (SD)	103.6 (9.3)	103.0 (11.0)	.84	104.7 (10.5)	103.6 (12.5)	.75
Superior	130.7 (12.4)	132.8 (16.7)	.63	136.0 (14.7)	133.5 (21.4)	.63
Inferior	131.4 (14.2)	130.5 (17.8)	.85	130.2 (17.8)	129.0 (16.2)	.80
Nasal	85.8 (18.6)	80.0 (15.5)	.23	82.8 (19.8)	85.7 (18.6)	.60
Temporal	66.6 (11.2)	68.6 (13.0)	.56	69.7 (13.8)	66.8 (13.7)	.47

* Retinal nerve fiber layer thickness (μm); §Abnormal baseline magnetic resonance imaging study [8]; ||Normal baseline magnetic resonance imaging study; ¶Mean overall RNFL value (standard deviation), and mean RNFL values in the superior, inferior, nasal and temporal quadrants; #Patients treated with the equivalent of 1000 mg of intravenous methyl-prednisolone for acute management of optic neuritis; **Patients not treated acutely for optic neuritis.

atrophy and dissemination of CNS inflammatory lesions on the initial MRI, gadolinium enhancement on baseline MRI, multifocal presentation, abnormal visual evoked potentials, or the development of Mc-Donald proven MS at 6-months [32]. Furthermore-patients who developed CDMS ($n = 13$) or McDonald criteria-proven MS ($n = 23$) did not have more severe RNFL atrophy [32]. These investigators concluded that OCT does not demonstrate retinal atrophy at the earliest clinical stage of MS, nor does it predict conversion to MS at 6 months in CIS patients [32].

There were a number of shortcomings in our study, which may have impacted our results. First and foremost, our patient population was quite limited, which likely hindered the statistical power of our comparisons. Second, we used clinical criteria [33] and not radiological criteria [28] to confirm the diagnosis of MS, which may have diminished our sensitivity to detect the diagnosis of MS in some CIS patients. Third, we did not randomize ON patients into treatment protocols, and it is therefore possible that patients who received corticosteroids were treated due to some bias on the part of the treating physician. Fourth, the two-year followup period may have been insufficient to detect significant RNFL differences between ON patients with disseminated CNS inflammation on initial MRI and those without clinically silent MRI results at baseline. Therefore, further study is needed to determine the predictive value of RNFL thickness

in ON patients at risk of future MS and to establish the role of OCT in the earliest phases of this disease. Future clinical trials should include larger patient cohorts and potentially employ novel MRI strategies, which are sensitive to the early detection of axonal attrition in MS.

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Clinical Study

Hyperacute Corticosteroid Treatment of Optic Neuritis at the Onset of Pain May Prevent Visual Loss: A Case Series

G. T. Plant,[1,2,3] N. A. Sibtain,[4] and D. Thomas[2]

[1] *Department of Neuro-Ophthalmology, The National Hospital for Neurology and Neurosurgery, London WC1N 3BG, UK*
[2] *Department of Neuro-Ophthalmology, Moorfields Eye Hospital, London EC1V 2PD, UK*
[3] *The Medical Eye Unit, St Thomas' Hospital, London SE1 7EH, UK*
[4] *Department of Neuro-Radiology, King's College Hospital, London SE5 9RS, UK*

Correspondence should be addressed to G. T. Plant, plant@globalnet.co.uk

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Aim. To show that high-dose corticosteroids may prevent visual loss in patients with optic neuritis (ON) treated at the prodromal, hyperacute, phase of retrobulbar pain. *Method*. Prospective case series: patients were recruited with a history of ON associated with pain. The patients were advised to report immediately to the investigators should the pain recur in either eye. Where possible, orbital magnetic resonance imaging (MRI) was performed to confirm a recurrence of ON and treatment with high-dose corticosteroids was commenced. Visual function and the patient's subjective account were monitored. *Results*. Eight patients (including cases of MS, CRION and NMO) presented in the hyperacute phase. MRI confirmed optic nerve inflammation in 5/5. Treatment was commenced immediately, and, in all cases, no visual loss ensued. *Conclusion*. MRI can be used to confirm acute optic neuritis prior to visual loss in the hyperacute phase. We suggest that treatment with high-dose corticosteroids may abort the attack and prevent loss of vision in patients with ON who are treated at the onset of pain. This has potential implications for the management of acute ON and also for our understanding of the pathogenesis and potential therapeutic targets in the neuroinflammatory conditions associated with ON.

1. Introduction

Subacute loss of vision accompanied by pain is most commonly due to optic neuritis (ON). Demyelinating optic neuritis—as occurs in association with multiple sclerosis (MS)—is the most common cause of acute and reversible visual failure in young adults of Northern European, North American, and Australasian origin and is second only to glaucoma as the most common acquired optic nerve disorder in persons younger than 50 years old. Optic neuritis is the initial presentation in 15% to 20% of cases of MS, and 38% to 50% of patients with MS develop optic neuritis at some point during the course of their disease [1]. Ten-year follow-up data from the Optic Neuritis Treatment Trial (ONTT) suggested that the prognosis for visual recovery is generally good; however, return of visual function is almost never complete [2]. The ONTT and other studies [3] have confirmed that the use of corticosteroids in the acute phase of optic neuritis shortens the time to recovery but has no effect on the final visual outcome. The results of these treatment trials have considerably altered the practice patterns of ophthalmologists and neurologists: in particular the use of corticosteroids in MS-associated optic neuritis has declined considerably [4, 5].

Optic neuritis also occurs in patients who have no evidence of MS. In some patients, the clinical phenotype is no different but others follow a very different clinical course. The term "chronic relapsing inflammatory optic neuropathy" (CRION) has been used to distinguish a type of optic neuritis characterised by pain and visual loss in which the symptoms recur when corticosteroids are withdrawn in the same or fellow eye; this does not occur in MS-associated optic neuritis. Kidd et al. [6] presented a series of 15 patients and suggested that the degree of visual loss in CRION is more severe than in demyelinating optic neuritis in general, usually with bilateral sequential involvement of both optic

nerves. In this series, treatment with corticosteroids resulted in rapid resolution of pain and improvement in vision, with relapses when treatment was withdrawn or visual loss with abrupt steroid withdrawal. Long-term immunosuppression with agents such as azathioprine is often indicated. Some cases of isolated optic neuritis without evidence of MS may be cases of neuromyelitis optica (NMO) in whom spinal cord lesions do not occur, have not yet occurred, or have been prevented by long-term immunosuppression [7]. However, the proportion of such cases found to have the NMO-IgG antibody (anti-aquaporin-4) is low—around 5% only [8] as opposed to 70% in cases of NMO with both myelitis and optic neuritis.

Optic neuritis differs from all other MS relapses, such as those affecting the spinal cord or brainstem, in that there is in 90% of cases a period of retrobulbar pain which may precede loss of visual function. Such pain is also common in non-MS optic neuritis including CRION and NMO. This therefore provides a unique opportunity to suppress the inflammatory lesion at an earlier phase in its evolution than in any of the published trials. The following cases suggest that treatment with a course of high-dose corticosteroids in patients who present with pain *before* the onset of visual loss may abort the pathological process and completely prevent the occurrence of any visual loss and, by inference, improve the final visual outcome.

2. Methods and Materials

Patients with decreased vision from a previous episode of optic neuritis were advised to present in what we are referring to as the "hyperacute" phase, that is, at the onset of retrobulbar pain before the onset of visual loss. When logistically possible, orbital magnetic resonance imaging was carried out using short-tau inversion recovery (STIR) or T2 weighted imaging and also T1-weighted Gadolinium-enhanced images with fat suppression to confirm a recurrence of optic neuritis. Treatment with a course of high dose corticosteroids was commenced using either an oral or an intravenous regimen dependent upon what was immediately available. This study is a case series and not a controlled clinical trial. Documentation of visual function was carried out as was practicable in a clinical setting, but we also relied upon the patient's subjective account of whether or not vision deteriorated following the institution of treatment. The cases reported are eight sequential cases managed in this manner: no case treated with corticosteroids in the hyperacute phase has lost vision.

3. Results

Eight patients with decreased vision from optic neuritis presented in the hyperacute phase with recurrent retrobulbar pain before visual loss. Where performed magnetic resonance imaging confirmed optic nerve enhancement. High-dose corticosteroids treatment was commenced immediately, and, in all cases no visual loss occurred. The clinical presentation and evolution of the visual symptoms in these eight patients is detailed in the case series and figures below.

3.1. Case 1: Chronic Relapsing Inflammatory Optic Neuropathy. A 34-year-old female of Australasian ancestry, born in the UK, presented with subacute visual loss in the right eye preceded by retroocular pain. Vision deteriorated to no perception of light; vision in the unaffected (left) eye was 6/5 with normal perimetry. Magnetic resonance (MR) imaging performed 2 weeks after the onset of her symptoms showed hyperintensity on STIR images and Gadolinium enhancement of the intraorbital portion of the right optic nerve (Figures 1(e) and 1(f)). The brain appeared normal. A lumbar puncture was performed: the cerebrospinal fluid (CSF) analysis was normal apart from minimal elevation in the number of lymphocytes ($5/mm^3$). On CSF protein electrophoresis, one oligoclonal band was found in both CSF and serum and one or two further bands were found in the CSF only. Serological tests for NMO were negative. The diagnosis was made of non-MS optic neuritis.

The patient was treated with 1 g of Methyl Prednisolone intravenously for 3 days and with 60 mg of oral prednisolone for 3 weeks thereafter, at which point there had been good improvement in the right peripheral field. Her right visual acuity remained at 6/60 with a dense central scotoma, and the dose of prednisolone was steadily reduced as it was considered unlikely to improve further. Eventually, the daily dose was 7.5 mg and the signs were stable with no new symptoms. Repeat MR imaging was performed after one month at this dose and showed no persistent or recurrent enhancement. It was therefore considered reasonable to gradually reduce the corticosteroid dose further, that is, by 1 mg every two weeks. This proceeded uneventfully, and two months later the steroids were discontinued by which time the daily dose had been reduced to 1 mg.

One day after stopping the steroid treatment, the patient noticed pain on moving the *left* eye but no loss of vision. The patient had been instructed to attend immediately in this eventuality and hence presented to Eye Casualty within 48 hours where she was noted to have no change in her visual acuity, normal colour vision, and normal Goldmann perimetry. MR imaging showed STIR hyperintensity and enhancement of the left optic nerve (Figures 1(k) and 1(l)). The patient was again treated with the same corticosteroid regimen. The pain resolved within a few hours, and there was no loss of vision, not subjectively, not on acuity and not on visual field examination. Her vision remains unchanged after 6 months follow-up and long-term immunosuppression with Azathioprine has been initiated.

3.2. Case 2: Multiple-Sclerosis-Associated Optic Neuritis. A 46-year-old woman of Latin American ancestry noticed an acute onset of pain on moving her right eye followed by a decrease in her right eye vision 3 days later.

Vision on the right deteriorated to hand movements but on the left was 6/5. By the time MR imaging was performed, a month had elapsed and her right visual acuity had improved to 6/18 but she was still only able to read 2/16 Ishihara colour plates and unable to see the I2e isopter on Goldmann visual field testing. Her left visual acuity was 6/5 and Goldmann field normal. MR imaging revealed multiple lesions typical

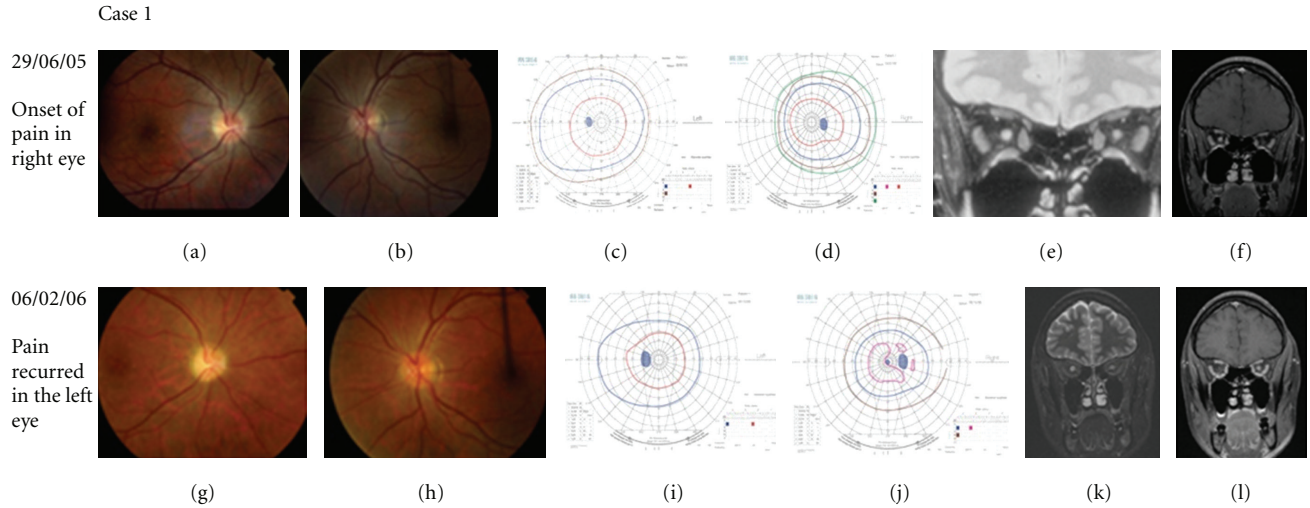


Figure 1: Disc photographs, Goldmann visual fields, and MR images of patient 1. (a)–(f) represent the initial episode in the right eye and (g)–(l) represent the recurrence in the left eye. Figure (e) is a coronal STIR image showing high signal within the right optic nerve, and Figure (f) is a coronal T1 fat saturation postgadolinium image showing associated optic nerve enhancement. (k) and (l) are coronal STIR and T1 postgadolinium images showing left optic nerve swelling and enhancement.

of multiple sclerosis in the brain and cervical spinal cord. A diagnosis of MS-associated optic neuritis was made.

The patient returned one month later with pain on moving her left eye for two days. Visual acuity remained 6/5, and Goldmann perimetry was normal (Figure 2(a)). The left optic disc appearance was also normal. The patient was treated immediately with intravenous Methylprednisolone 1 g daily for 3 days with no oral taper. The pain on eye movement, which she reported as being identical to that experienced previously disappeared immediately without there being any subjective deterioration in vision. The patient attended for review one month later by which time she had had an episode of sensory loss on her face. Her left visual function and visual field had remained unchanged on follow-up 2 months after treatment with intravenous methylprednisolone (Figure 2(c)).

3.3. Case 3: Chronic Relapsing Inflammatory Optic Neuropathy. A 27-year-old female finance officer of African-Caribbean parentage, born in the UK, developed pain on moving the left eye. This progressed over a week and at its worst was severe enough to keep her awake at night. Two weeks from the onset of the pain, the vision in her left eye became blurred and progressed to no perception of light over 3 days. Over a month, there was modest spontaneous improvement in vision to hand movements only. The optic disc was swollen, and there was a dense left relative afferent pupillary defect. MR imaging revealed STIR hyperintensity within the left optic nerve; no abnormality was detected within the brain (Figure 3). Gadolinium enhancement was not undertaken. CSF was normal and negative for oligoclonal bands. A diagnosis of non-MS optic neuritis was made. She was treated with intravenous methylprednisolone 1 g daily for 3 days followed by oral prednisolone 60 mg daily. There was modest further improvement in her vision only. The dose of prednisolone was reduced gradually and discontinued entirely after 3 months.

Two weeks later, the patient developed recurrent pain on moving her left eye, exactly as she had previously experienced. The pain continued for 3 days at which point she was seen in the clinic and treatment was restarted at a dose of 60 mg prednisolone daily. The vision did not deteriorate. A month later, she had discontinued prednisolone, and a few days later she developed pain on moving the as yet unaffected right eye. After a few days of pain, prednisolone was restarted and, once again, the pain resolved without loss of vision. No imaging was performed in these two episodes, and we are relying on the patient's subjective report of no deterioration in vision. The patient was intolerant of azathioprine and therefore commenced treatment with mycophenolate mofetil. The patient was maintained on this for 2 years but has since been lost to followup. NMO-IgG status is not known.

3.4. Case 4: Chronic Relapsing Inflammatory Optic Neuropathy in Neuromyelitis Optica. A 34-year-old African-Caribbean man, born in the UK, presented with a 2-week history of left retroorbital pain and deterioration in vision. The right visual acuity was poor due to myopia and amblyopia: a right divergent strabismus had been present since childhood. On admission, his visual acuity was perception of light only on the left and finger counting on the right. The appearance of the left fundus was normal. On the right, there was a staphyloma. MR imaging revealed no abnormality in the brain but there was enhancement of the left optic nerve, both its intracranial and intraorbital portions. The CSF was negative for oligoclonal bands. NMO-IgG was positive.

The patient was treated with intravenous methyl prednisolone followed by prolonged treatment with oral prednisolone. Two weeks later, Goldmann perimetry revealed

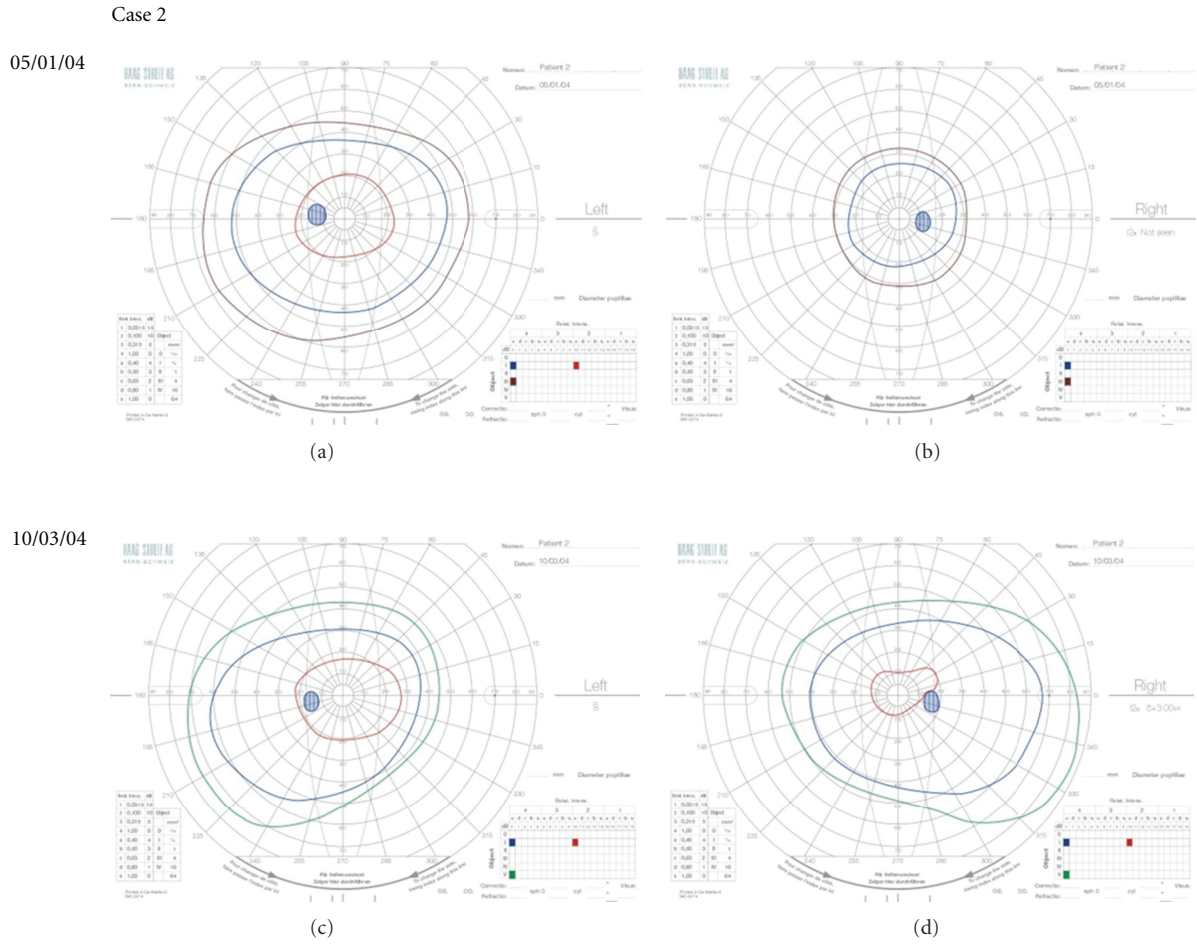


Figure 2: Goldmann visual fields performed on patient 2 at presentation of a recurrent episode of left retroorbital pain (a) and 2 months following treatment with intravenous methylprednisolone (c). The right eye had sustained an episode of optic neuritis previously (b and d).

Case 3

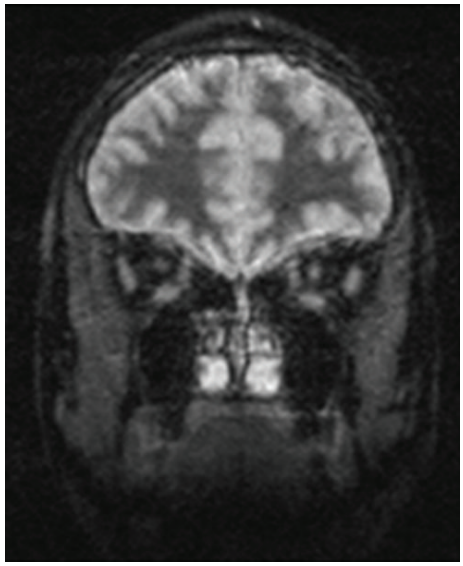


Figure 3: Coronal STIR image showing high signal within the optic nerve corresponding to the side of the pain in Case 3.

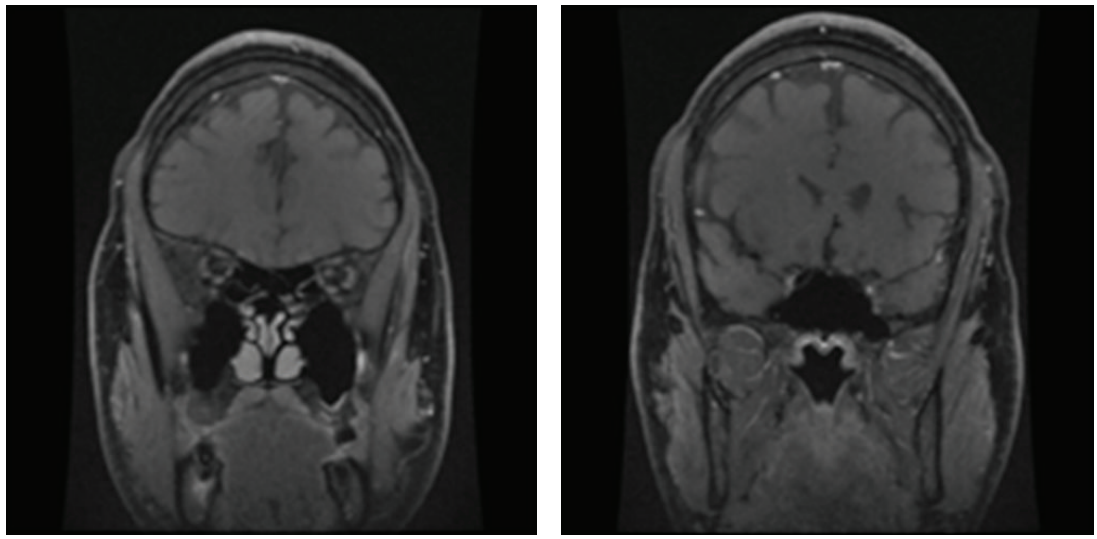
a dense central scotoma, but after a further 2 weeks, the scotoma was no longer detectable. The pain resolved and visual acuity improved to 6/9 with 12/17 Ishihara plates read correctly. The dose of prednisolone was gradually reduced.

He presented again several months later. At that point the patient was taking 20 mg of prednisolone once daily. The left retroorbital pain had recurred, but there was no subjective deterioration in vision. In fact, the visual acuity was 6/7.5, N4.5 with one error on the Ishihara plates and the Goldmann field was only mildly, generally depressed. MR imaging showed extensive enhancement of the left optic nerve both intraorbitally and intracranially (Figure 4). The patient was treated with intravenous methylprednisolone. His pain resolved, and there was no change in his vision.

He was most recently seen a year later. His vision in the left eye remained good, and he was on reducing doses of prednisolone.

3.5. Case 5: Multiple-Sclerosis-Associated Optic Neuritis. A 33-year-old white female patient presented with right optic neuritis with pain. No treatment was given, she had a poor outcome with a visual acuity of 1/60. The following year,

Case 4



(a) (b)

Figure 4: MR imaging of Case 4 showing extensive enhancement of the left optic nerve both intraorbitally (a) and intracranially (b).

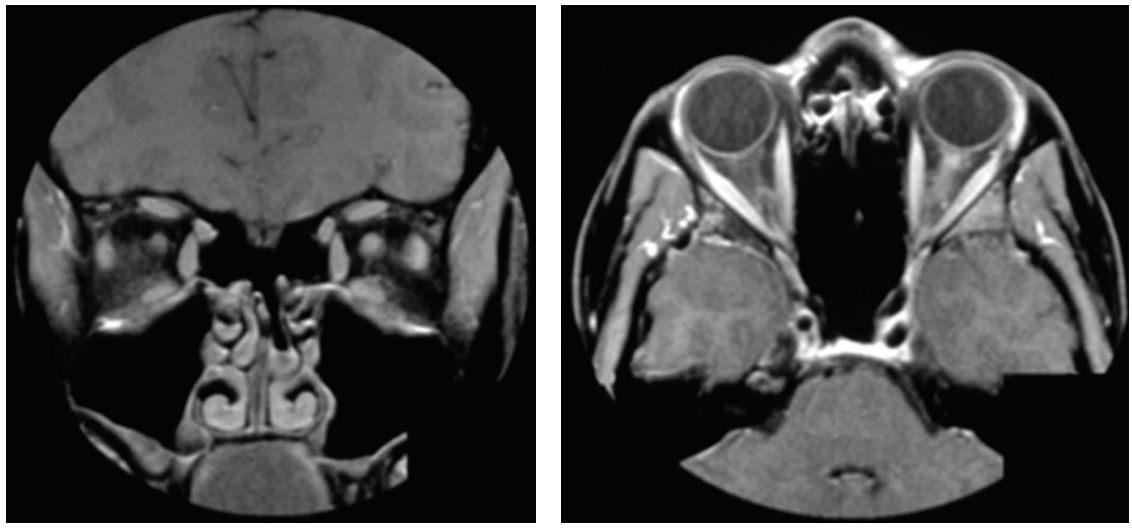
she had a spinal cord relapse and MR imaging of the brain revealed appearances typical of multiple sclerosis. A year later, she developed similar pain on moving the right eye again and presented that day for advice. There had been no change in her vision. The patient was treated with oral methylprednisolone 500 mg daily for 5 days. Over 3 days, the pain resolved. There was no subjective deterioration in her vision.

3.6. Case 6: Recurrent Isolated Optic Neuritis. A 33-year-old female presented with a typical episode of right optic neuritis with pain and good recovery of vision. Three months later, she presented with a 24-hour history of pain on moving the left eye. MR imaging of the orbits with Gadolinium enhancement confirmed acute optic neuritis (Figure 5). She was started immediately on oral prednisolone 40 mg daily, and, within 24 hours, the pain had resolved and no loss of vision occurred. The patient remained on a tapering dose of prednisolone for 3 weeks. MR imaging of the brain and spinal cord revealed no abnormalities to indicate multiple sclerosis. Further episodes of optic neuritis have occurred over 6 years of follow-up and she has had sensory symptoms in her limbs. Repeat MR imaging and CSF examination has not confirmed a diagnosis of multiple sclerosis, and all other routine blood tests including NMO IgG testing have proven normal. The patient therefore has a diagnosis of isolated recurrent optic neuritis (RION) of unknown cause.

3.7. Case 7: Chronic Relapsing Inflammatory Optic Neuropathy. A 32-year-old Moroccan lady developed symptoms of left optic neuritis, consisting of pain on eye movement and progressive visual loss. The patient was given no treatment and her vision deteriorated to no perception of light. The following year, she developed similar symptoms on the right whilst in Morocco but on this occasion she was treated with corticosteroids. Her vision returned to normal but after 2 weeks the steroids were discontinued and, her vision deteriorated again. On coming under our care her vision was counting fingers and further treatment with corticosteroids produced an improvement in vision to 6/12 with inferior field loss and 11/13 Ishihara plates seen. The patient was maintained on immunosuppression but as the dose of corticosteroids was reduced her vision deteriorated again and she was treated with a conventional course of intravenous cyclophosphamide. MR imaging showed enhancement of the right intraorbital optic nerve. There were no brain or spinal cord lesions. Oligoclonal bands were found in both CSF and serum but with fewer bands in the serum. NMO antibody was negative. She was eventually maintained on 10 mg of prednisolone daily. Two years later, she presented again with pain on movement of her right eye. There had been no subjective deterioration in vision. Urgent MR imaging of the orbits showed enhancement of the right optic nerve. The patient was given 3 days of intravenous methylprednisolone, and the dose of oral prednisolone continued at 60 mg daily. No deterioration in her vision occurred subjectively, and this was confirmed by serial measurements of visual acuity and the Ishihara test.

3.8. Case 8: Neuromyelitis Optica (NMO-IgG Negative). A 23-year-old white female was admitted with a two-week history of pain on eye movement followed by visual loss in the left eye and a one-week history of similar symptoms in the right eye. On admission, her vision was counting fingers in the right eye and no perception of light in the left. The patient also gave a one-day history of sensory symptoms in her legs and saddle area with urinary retention. There was a sensory level to pinprick at T8. The appearance of the optic

Case 6



(a) (b)

Figure 5: MR images of Case 6 showing gadolinium enhancement of the left optic nerve 24 hours after the onset of left retroorbital pain.

disc was normal. MR imaging showed T2 hyperintensity and enhancement of both optic nerves intracranially just anterior to the chiasm. The brain appeared normal. MR imaging of the spinal cord showed a T2 hyperintense lesion within the distal spinal cord extending across 3 to 4 vertebral segments. Cerebrospinal fluid examination revealed a white cell count of 59/mm^3, 78% of which were lymphocytes and the remainder polymorphs. Matched oligoclonal bands were present in CSF and serum.

The patient was treated with high-dose methylprednisolone followed by oral prednisolone and made an excellent visual recovery, and when seen three months later, her visual acuity was 6/6 in the right eye and 6/9 in the left, N5 bilaterally with no mistakes made on the Ishihara plates with either eye. There was mild bilateral temporal pallor of the optic discs. Oral prednisolone was discontinued after six-month treatment, and she remained entirely well until a year later when she developed pain on moving the left eye and MR imaging of the orbits showed enhancement of the left optic nerve/sheath complex. No loss of vision occurred, and the optic disc appearance was unchanged. She was treated immediately following the scan with high-dose intravenous methylprednisolone followed by oral prednisolone. At the time steroids were given, the visual acuities were recorded as 6/5, N5 bilaterally with full colour vision; there was no subjective deterioration in vision. Goldmann visual fields 4 months before and 1 month after this episode are shown in Figure 6. A mild spinal cord relapse occurred a week later. She subsequently discontinued all therapy and remains well. NMO antibody testing has been negative.

4. Discussion

Pain in the distribution of the first division of the trigeminal nerve and pain on eye movement are often reported by patients with acute optic neuritis [9, 10]. In the Optic Neuritis Treatment Trial (ONTT) for example, pain on eye movements was reported by 92% of participants, 87% of whom noted a worsening of the pain with eye movement [11]. However, the pathophysiological basis for this phenomenon has not been determined. Conditions such as papilloedema cause extensive optic nerve swelling and sheath enlargement but are not associated with periocular pain, suggesting that simple distension of the meninges is unlikely to be a primary source of pain in optic neuritis [12].

Lepore [13] suggested that pain on eye movement in optic neuritis occurs because of the close association of the optic nerve sheath and the sheaths of the superior and inferior recti at the orbital apex. These authors hypothesised that a mechanical irritation of the inflamed optic nerve sheath is caused by traction when the extraocular muscles contract resulting in pain on eye movement.

Fazzone et al. [14] subsequently used MR imaging in patients with acute optic neuritis and found that the incidence of pain may be dependent on the localisation of the inflammation. In this study, periorbital pain and pain on eye movement were found to be more frequent when enhancement of the orbital or retrobulbar segments of the nerve was seen and pain was twenty times more likely to be absent when there was no enhancement of the orbital segment.

Several trials have shown that treatment of acute optic neuritis with high-dose, intravenous corticosteroids followed by oral corticosteroids accelerated visual recovery but provided no long-term benefit to vision [2, 3]. A meta-analysis of trials evaluating methylprednisolone with total dose greater than 3000 mg administered intravenously supported this view [15]. It was found that the relative risk of normal visual acuity with intravenous corticosteroids compared with placebo was 1.06 (95% CI 0.89 to 1.27) at six months and 1.06 (95% CI 0.92 to 1.22) at one year. The authors

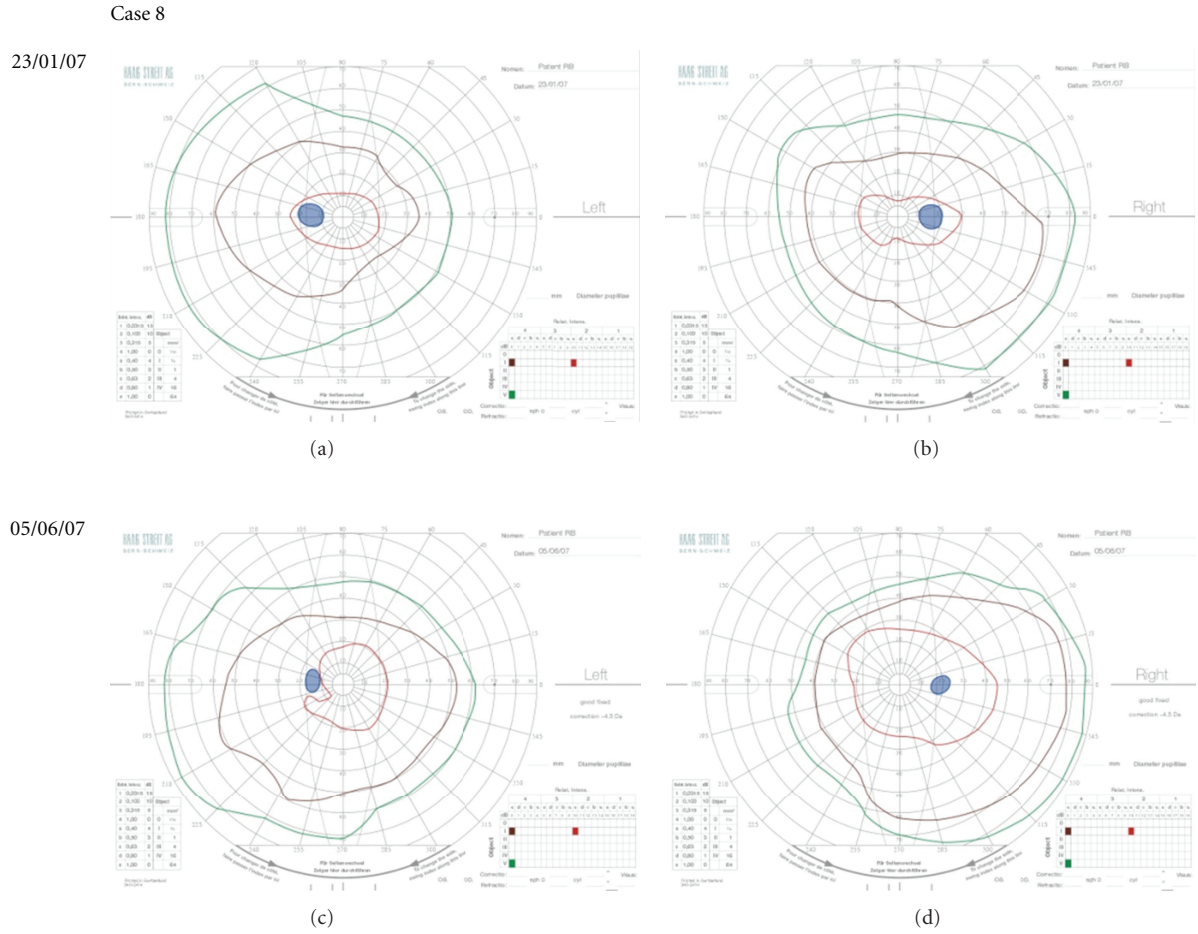


Figure 6: Goldmann visual fields showing minimal change in the left eye 1 month after a recurrent episode of optic nerve swelling (c) compared with visual fields performed 4 months before this recurrence (a). The contemporaneous right visual fields (b and d) are presented for comparison.

concluded that there was no conclusive evidence of benefit in terms of recovery to normal visual acuity, visual field, or contrast sensitivity with either intravenous or oral corticosteroids at the doses evaluated in trials included in the paper. However, these studies have concentrated exclusively on MS-associated optic neuritis and the situation is very different in NMO and cases of optic neuritis of unknown aetiology where corticosteroids may indeed improve the outcome.

The eight cases presented above show no subjective loss of vision and, when measurements have been practicable, there has been no change in visual acuity, in colour vision nor in visual field following treatment with high-dose corticosteroids despite clear imaging evidence in 5/8 cases of optic nerve inflammation.

5. Conclusions

We are proposing that patients who have had optic neuritis, whether in the context of MS or otherwise, may benefit from hyperacute treatment with corticosteroids given at the phase of retrobulbar pain in subsequent episodes. Our experience in the cases reported here is that, when treatment is given in this way, patients may not lose vision to any significant degree, perhaps not at all, thus clearly predicting a more favourable outcome than if steroids are given later or not given.

There are many potential causes of ocular pain or discomfort but if patients have experienced optic neuritis previously, they will be in a position to tell us whether the pain has the same qualities. Furthermore, MR imaging can be used to confirm acute optic neuritis as in the five cases described above in whom scans were performed. We propose a new trial with a novel protocol looking at corticosteroid treatment in the hyperacute phase preceding the onset of visual loss. It is unlikely that pain preceding optic neuritis could ever be recognised in a first, clinically isolated attack but, if patients are appropriately instructed, presentation during this phase in subsequent attacks would be feasible.

Acknowledgment

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Review Article

Repeated Intrathecal Triamcinolone Acetonide Administration in Progressive Multiple Sclerosis: A Review

Mazen Abu-Mugheisib, Reiner Benecke, and Uwe K. Zettl

Department of Neurology, University of Rostock, Gehlsheimer Straße 20, 18147 Rostock, Germany

Correspondence should be addressed to Mazen Abu-Mugheisib, mazen.abu-mugheisib@med.uni-rostock.de

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At the present time, anti-inflammatory, immunomodulatory, or immunosuppressive treatments of multiple sclerosis (MS) are mainly effective in the early phases of the disease but are of less advantage in progressive phases. Current therapeutic strategies of both primary and secondary progressive MS are rare. One alternative may be intrathecal application of triamcinolone acetonide (TCA). Number of papers deal with advantages and disadvantages of intrathecal administration in MS. Former trials lacked detailed selection of MS patients, with small sample sizes, low steroid dosages, and only a small number of intrathecal administration of short acting steroids. The present paper summarizes recent trials performed following a different treatment regime. They were conducted in patients with progressive MS suffering mainly from spinal symptoms and documented a significant improvement of EDSS and walking distance (WD). Intrathecal TCA administration is a proposal to take into account as one therapy option in patients with a progressive clinical course and predominantly spinal symptoms.

1. Introduction

Multiple sclerosis (MS) as one of the most frequent diseases of the central nervous system (CNS) in young adults often entails persistent physical and mental disability. The prevalence is assumed to be 400,000 people in the United States and approximately 2,1 million people worldwide [1]. MS is an immune-mediated demyelinating inflammatory disease. Its natural history in most patients is marked by a chronic progressive decline [2]. Mostly, MS begins with a relapsing course (relapse remitting MS, RRMS). After years, it leads to a progressive course (secondary progressive MS, SPMS) [3, 4]. Another form of MS, progressive-relapsing MS (PRMS), is defined as a progressive disease from onset with acute relapses and with periods of progression between relapses. Nearly 10% of MS patients develop primary progressive MS (PPMS), which is defined as a progressive type from onset with temporary impairment. Regarding to the pathogenesis of MS, two different approximations are common. MS is mainly characterized by multitopic inflammation and demyelination. As the disease proceeds, the role of axonal loss and gliosis increases [5].

Hence, MS pathophysiology is much more complex than assumed up to now. Consequently, one therapy with a single immune mechanism cannot fit such a complex pathogenic disease. At the present time, the anti-inflammatory, immunomodulatory, or immunosuppressive treatments are mainly effective in the early phase of the disease but are of less advantage in the progressive phase [6]. Therefore, an axon-protective therapy will be essential to reduce disease progression [7]. Current treating strategies of progressive MS are rare. Mitoxantrone is an FDA-approved therapy option for progressive phase in MS. Meanwhile, the application of glucocorticosteroids in the treatment of relapses has been accepted. There is general agreement that intravenous methylprednisolone (IVMP) administration (1000 mg daily for 3–5 days) is first-line therapy in the recovery from relapses [8–10]. Treatment with

IVMP minimizes tissue damage and assists lesion recovery in patients with RRMS [11]. IVMP recovers the blood-brain barrier (BBB) by downregulating adhesion molecule expression. Furthermore, it induces different immunological changes as inhibition of proinflammatory cytokines, lymphocyte apoptosis, and remyelination [12]. So, as of a result, their immunosuppressive and anti-inflammatory power glucocorticoids are established in the standard treatment for acute relapses. Although IVMP could reduce the duration of a relapse, no effect on the exacerbation rate or on the development of long-term disability was determined [13]. The benefit of corticosteroids in the treatment of acute relapses has been examined in clinical trials. Another double-blind, placebo-controlled, randomized trial of high-dose methylprednisolone (1 g IV daily for 5 days) was arranged in 35 patients with PPMS [14]. A statistically significant amelioration of the expanded disability status scale (EDSS) score was observed. This improvement persisted for at least 3 months. One phase II randomized controlled trial (RCT) in RRMS compared the benefit of repeated pulsed IVMP with IVMP at the same dosage but administered only for relapses. It could be demonstrated that pulsed IVMP decreased the development of T1 black holes, brain atrophy progression, and associated development of permanent disability [10]. On the other hand, pulsed application of intravenous corticosteroids is related to transient and dose-dependent side effects, such as temporary mood disorders, gastric ulcer, headache, and myalgia [15]. Chronic administration may even result in more serious side effects, such as hypertension, hyperglycemia, decline of cardiac conditions, osteoporosis and an increased incidence of fractures, hepatic steatosis, infection, cataract, and transient memory impairment [16]. Consequentially, one interesting alternative may be the intrathecal administration of triamcinolone acetonide (TCA), which has been adopted for the treatment of many other diseases. This paper reviews data on the efficacy of intrathecal steroid application in the treatment of MS. Trials were classified according to the system established by the American Academy of Neurology (AAN) [17].

2. Historical Experiences with Intrathecal Steroids in Multiple Sclerosis

A number of historical papers deal with advantages and disadvantages of intrathecal administration in MS. Since 1953, several mainly uncontrolled trials have been published. Different dosages and diverse conventional steroid compounds, that is, methylprednisolone acetate (MPA) or TCA, are mentioned. Despite the controversial discussion especially in progressive MS patients with predominantly spinal symptomatology according to some trials, positive effects could be noticed [18–20] (Table 1).

In 1953, Kamen and Erdman [23] referred treating a patient with RRMS with intrathecal hydrocortisone (HC) and intramuscular adrenocorticotropic hormone (ACTH). The patient recovered during a 6-week follow up. In a couple of open-label, uncontrolled trials between 1961 and 1963, Boines [24, 25] reported 75–80% recovery, particularly of spasticity with intrathecal MPA in 42 patients during a follow up of 12–52 weeks. Goldstein et al. [30] reported that intrathecal MPA decreased CSF γ-globulin in MS but without correlation to improvement of spasticity. In 1964, Van Buskirk et al. [26] performed an open-label, uncontrolled prospective study of intrathecal MPA in 20 patients. The treatment appears to decrease spasticity in 14 patients and consequently results in improved walking distance and bladder function. In 1970, again Goldstein et al. [27] referred in an open-label, uncontrolled trial to 38 patients treated with 4 to 8 intrathecal MPA infusions and followed up for 2 to 8 years. Neurological examinations revealed an initial improvement in 30 patients that remained stable in only 6 patients. In 1973, again Nelson et al. [28] reported in an open-label, uncontrolled prospective study on 23 patients with MS. They received intrathecal MPA infusions for acute exacerbations. A mild amelioration of EDSS was detected in 4 patients (17%). All the above-mentioned studies have to be rated as class IV evidence, only.

For the first time, Rohrbach et al. [29] performed a double-blind, randomized, controlled trial (short report). 42 distinctly chronic progressive MS patients with predominantly spinal symptoms were enrolled. One cohort was treated either with 3 or 4 intrathecal TCA injections of 80 mg. The other cohort received oral triamcinolone starting with 48 mg/d in descending dosage. In the intrathecal cohort, a better and consistent improvement in the spinal score could be observed than the other treatment arm. This study corresponds to class II evidence.

In 1992, Heun et al. [18] conducted an open-label, randomized, prospective, unblinded study on 50 MS patients with different MS forms (RRMS, PPMS, and SPMS). One group received 3 intrathecal TCA injections of 40 mg on days 1, 8, and 15; the other cohort was treated with methylprednisolone 100 mg i.v. from day 1 to 5, then in descending dose. A slender but significant improvement in disability was noticeable in both cohorts. No significant difference in the examined frequency of improved neurological symptoms or in EDSS between the two cohorts was found. The study has to be classified as class III evidence.

In conclusion, the majority of the mentioned historical trials of intrathecal steroid for MS performed in the past were uncontrolled and have to be rated as class IV evidence. Despite their lacks, the trials of Rohrbach et al. [29] and Heun et al. [18] are notable (class II/III evidence). Especially trials that conform to generally defined criteria of evidence-based medicine are missing. According to intrathecal TCA applications, there is a controversial discussion [19, 31]. Repeated lumbar punctures under double-blind design including the agreement of patients and the ethical committee are nowadays not feasible.

2.1. Risks. Intrathecal MPA therapy for MS caused transient urinary incontinence in two of 20 patients [26]. In two other reports on 61 patients, constrictive arachnoiditis in thoracic or lumbar area, aseptic meningitis, subarachnoid haemorrhage, and neurogenic bladder were described [27, 28]. Other mentioned complications were brain damage,

Table 1: Representative intrathecal steroid trials 1953–1992 [21, 22].

	Design	Patients included, MS type	Dosage and duration	Primary outcome	Results	Evidence
Kamen and Erdman, 1953 [23]	Case report	1; RR	Intrathecal HC and intramuscular ACTH; no specific data available	Recovery	Patient recovered	IV
Boines, 1961 and 1963 [24, 25]	Open-label, uncontrolled, retrospective, unblinded follow up of 12–52 weeks	42; no specific data available	40–100 mg intrathecal MPA every 2-3 weeks for a total of 6 injections, then "follow-up booster injections"	"recovery, particular of spasticity"; no specific outcome data available	"80% of patients improved/showed excellent or good results"	IV
Van Buskirk et al., 1964 [26]	Open-label, uncontrolled, prospective, unblinded	20; no specific data available	Weekly increasing doses intrathecal MPA (20–80 mg), then booster injection monthly (80–100 mg MPA); follow up 1 week-16–months	"clinical improvement"	"no effect on frequency of exacerbations, but improvement in spasticity in 14 patients"	IV
Goldstein et al., 1970 [27]	Open-label, uncontrolled, retrospective, unblinded	38; no specific data available	40–80 mg intrathecal MPA/4–8 times within 1-2 weeks; follow up 2–8 years	"improvement"	"79% improvement"	IV
Nelson et al., 1973 [28]	Open-label, uncontrolled, prospective, unblinded	23; RR, SP	40–120 mg intrathecal MPA/1–23 times within 2 months; follow up 1–84 months	EDSS CSF changes	EDSS: 4 patients (17%) improved; significant increase of CSF protein	IV
Rohrbach et al., 1988 [29]	double-blind, randomized, prospective	42, "mainly chronic progressive"	Intrathecal TCA: 80 mg/3-4 times within 14 days Oral TCA: 48 mg/d, tapering off	"spinal score"	Intrathecal TCA: "better improvement in the spinal score"	II
Heun et al., 1992 [18]	open-label, prospective, randomized, unblinded, follow up of 21 days	Intrathecal TCA: 25 Systemic MPA: 25	TCA: 40 mg on days 1, 8, and 15 MPA: 100 mg for 5 days, tapering off	EDSS AI SSEP	EDSS improved in both groups ($P < .01$); EDSS changes between both groups n.s.; AI n.s.	III

TCA: triamcinolone-acetonide acid; HC: hydrocortisone; ACTH: adrenocorticotropic hormone; MPA: methylprednisolone acetate; RR: relapsing-remitting MS; PP: primary chronic progressive MS; SP: secondary chronic progressive MS; MIX: mitoxantrone, EDSS: expanded disability status scale; WD: maximum walking distance; WT: maximum walking time; SSEP: somatosensory evoked potentials; AI: ambulation index; CSF: cerebrospinal fluid; n.s: non significant.

spinal cord lesions, and dense widespread pachymeningitis [32–34]. In spite of these reports, intrathecal steroid therapy is still advised [21, 35].

3. Recent Trials with Intrathecal TCA Administration in Progressive Multiple Sclerosis

The revival of intrathecal steroid treatment started with the positive results of a trial on intractable postherpetic neuralgia, in which 89 subjects received up to 4 intrathecal methylprednisolone administrations within 4 weeks without any serious side effects [36]. In a rapid succession, a few further open-label uncontrolled trials were performed following a different treatment regime [37–39] (Table 2).

Hoffmann et al. [37] performed an open-label, uncontrolled, prospective trial on the short-term and long-term efficacy and tolerability of repeated intrathecal TCA application. 36 patients with progressive MS (22 SPSS, 14 PPMS, EDSS < 7.5) were included. Patients did not receive steroids and were on a stable immunomodulatory drug treatment for at least 4 weeks before the start of the study. They had to show symptom progression of at least one point on the EDSS scale, in the last 2 years before study entry, but had to be stable for at least 4 weeks before inclusion. An atraumatic (Sprotte®) needle was used in order to minimize the risk of postlumbar puncture syndrome [41]. 6 injections with 40 mg TCA were administered within 3 weeks. EDSS scores significantly decreased ($P = .00065$), and the walking distance (WD) significantly increased ($P = .003$). None of the measured parameters deteriorated in any patient. Patients with an improvement in their EDSS or WD were provided to receive further treatment with one TCA application at an individual rate every 6 to 12 weeks. The follow-up treatment period amounted to 13.1 ± 6.22, 3–23 (mean ± S.D., range) months

Table 2: Representative intrathecal steroid investigations in multiple sclerosis (since 2003).

	Design	Patients included, MS type	Dosage and duration	Primary and secondary outcomes	Results	Evidence
Hoffmann et al., 2003 [37]	Open-label, prospective, uncontrolled, unblinded, short follow up	36 (SP, PP)	TCA 40 mg/6 times within 3 weeks; follow up with 40 mg every 6–12 weeks; 13.1 ± 6.22, 3–23 (mean ± SD., range) months	EDSS WD	initial phase: EDSS (initial 5.6 ± 0.93 (mean ± S.D.); end: 4.9 ± 1.0; $P < .001$). WD: (initial: 294 ± 314 m; end: 604 ± 540 m; $P < .001$) follow up: EDSS and WD remained stable	IV
Hellwig et al., 2004 [38]	Open-label, prospective, uncontrolled, unblinded, short follow up	161 (RR, SP, PP)	TCA 40 mg/6 times within 3 weeks	EDSS WD SSEP	EDSS: (initial: 6.44 ± 1.06; end: 5.47 ± 1.24): WD: (initial 158.03 ± 501.20, end: 439.38 ± 895.24). SSEP latencies: reduced for all variables ($P < .0001$)	IV
Hoffmann et al., 2006 [39]	Open-label, prospective, uncontrolled, unblinded, short follow up	27 (SP, PP)	TCA 40 mg/6 times within 3 weeks	EDSS WD WT 25-f-test CSF changes	EDSS: (initial: 5.4 ± 1.3; end: 4.9 ± 1.1; $P < .001$). WD and WT increased: $P < .001$, 25 f-test increased: $P < .01$ CSF changes n.s.	IV
Hellwig et al., 2006 [40]	open-label over a 52-week long interval, prospective, randomized, unblinded	TCA: 34 (SP, PP) MIX: 30 (SP, PP)	TCA: 40 mg every 6–12 weeks, 52 weeks MIX: initial dose: 12 mg/m^2 2nd dose: 8–10 mg/m^2 6 weeks later/then quarterly: 52 weeks	EDSS WD	TCA: EDSS decreased ($P < .001$) WD: increased ($P < .001$) MIX: EDSS, WD n.s.	III

TCA: triamcinolone-acetonide acid; RR: relapsing-remitting MS; PP: primary chronic progressive MS; SP: secondarychronic progressive MS; MIX: mitoxantrone; EDSS: expanded disability status scale; WD: maximum walking distance; WT: maximum walking time; SSEP: somatosensory evoked potentials; CSF: cerebrospinal fluid; n.s: non significant.

with 6.35 ± 3.91, 2–15 TCA administrations. The post hoc analysis demonstrated that a significant decline of EDSS and the improvement of WD occurred after first initial 6 TCA applications and then remained stable. Neither a significant impact of covariates in statistical analysis nor relevant side effects were found. This study accomplished a total of 340 lumbar punctures. A temporary increase of CSF protein above 500 mg/L and transitory increase of CSF cells (maximum cell count was 38/μL) was noticed. Nevertheless, no new clinical symptoms were caused in any subject. 5 patients developed a slight post-lumbar puncture syndrome, but they did not abandon further TCA applications. This study illustrated efficacy and safety of repeated intrathecal TCA administration in progressive MS patients with spinal symptoms. The application frequency (6 TCA injections within 3 weeks and follow-up injection every 6 to 12 weeks) was markedly higher in contrast to other previous trials. This analysis demonstrated that particularly PPMS and SPMS patients benefit from described therapy design. Although long-term data did not prove any further improvement of neurological symptoms, the amelioration reached remained robust over the following treatment period with one TCA application every 6 to 12 weeks. Nevertheless, this uncontrolled study has to be graduated as class IV evidence.

Hellwig et al. [38] performed another open-label, uncontrolled, prospective study on 161 MS patients (35 PPMS, 122 SPMS, 4 RRMS) with pronounced spinal symptoms on the impact of the administration of 40 mg of the sustained released steroid TCA. Subjects did not suffer from an acute onset of exacerbation or recent pronounced increased progression of MS symptoms. An established immune system modulating therapy was not altered. EDSS, Barthel index, WD, and somatosensory evoked potentials (SSEPs) were analysed before start and at the end of the TCA treatment [42]. The patients achieved a supplemental standardized rehabilitation therapy. Atraumatic Sprotte® needles were used to avoid post-lumbar puncture syndrome [41, 43]. Each patient received 6 applications of 40 mg TCA within 3 weeks. EDSS and Barthel indices were enhanced, WD increased,

and latencies of SSEP of the median and tibial nerves were reduced in all patients at serial evaluation ($P < .0001$ for all variables). Neither slight nor severe side effects were registered. 5 patients abandoned the study due to lumbar puncture headache.

In this uncontrolled trial, an improvement of spinal symptoms, WD, and SSEP latencies in progressive MS patients were documented, and the results from a previous trial were confirmed [37]. The electrophysiological results may mirror a certain potential of intrathecal TCA administration for demyelinating actions. Again this uncontrolled study has to be rated as class IV evidence.

3.1. Absent Hints as to Cell Injury by Repeated TCA Applications. Steroids were suspected to induce a neuronal cell injury due to brain atrophy [10, 22, 44]. Another open-label, uncontrolled, prospectice trial on short-term efficacy of repeated intrathecal TCA applications in progressive MS dealt with this aspect [39]. 27 subjects with progressive MS were included. They received similar therapy as described in previous trials [37, 38]. In addition to the mentioned clinical parameters, CSF was examined for the unspecific markers of cell injury neuron-specific enolase (NSE), Tau-protein, S 100B, and β-amyloid [45–49]. 6 TCA injections, performed every third day, reduced EDSS (initial: 5.4 ± 1.3, 3–7.5 (mean $\pm$ SD, range); end: 4.9 ± 1.1; 2.5–6.5; $P < .001$) and significantly increased WD primarily after the fourth TCA injection. These results indicated that the role of TCA administrations is undercharged in those trials without any persuasive clinical output [18, 20]. The assessed CSF marker did not significantly change within the interval of TCA treatment. This supported the statement that the sustained released steroid TCA is not toxic and causes no relevant cell injury or deterioration of neuronal cells [10, 20, 44, 50–53]. Furthermore, no serious clinical side effects appeared. This uncontrolled study has to be classified as class IV evidence.

3.1.1. Comparison of Repeated Intrathecal TCA Administration with Mitoxantrone Therapy in Patients with Progressive MS. Previous studies showed that repeated intrathecal TCA administrations generated a clear prolonged benefit in patients with progressive MS suffering from mainly spinal symptoms [37]. Mitoxantrone (MIX) application is performed similarly in progressive MS patients with a continuous, rapid worsening of symptoms [54]. In contrast to TCA administration, MIX application is a worldwide accredited therapy to diminish or abandon progression. There exists important restriction due to its cardiac toxicity. Hence, a cumulative maximal life-time dose should be respected [54–56].

Based on this consideration, Hellwig et al. [40] performed an open-label study over a 52-week-long interval and compared TCA and MIX therapy in two matched cohorts of subjects with progressive MS. Only patients with progressive MS with an EDSS ≤ 7.5 were recruited. In the MIX arm, 30 patients were included and observed over 1 year. The initial MIX dose was 12 mg/m^2. The second infusion was followed 6 weeks later and then quarterly. The MIX dose was minimized to 10 mg/m^2 and 8 mg/m^2 dependent on patients' stable condition. 34 patients were recruited in the TCA arm and treated as previously described [37]. EDSS significantly decreased and WD significantly increased ($P < .001$) after the initial 6 TCA administrations and then remained relatively constant. Neither EDSS nor WD deteriorated in any of the TCA patients. On the other hand, MIX therapy did not significantly influence EDSS ($P = .056$) or WD ($P = .12$), even though no additional decline of EDDS or WD was measured. Two patients in the MIX arm suffered from moderate nausea. An isolated and temporary increase in CSF protein (>500 mg/L) and a temporary rise of CSF cells without development of neurological symptoms in all subject was observed. 8 patients in the TCA arm suffered from post-lumbar puncture syndrome without termination of further TCA treatment. Again, the efficacy and safety of repeated intrathecal TCA administrations in progressive MS patients with predominantly spinal symptoms was approved [37, 38]. It has to be pointed out that a rate with 6 TCA applications within 3 weeks was definitively higher compared to previous trials [18]. Following this concept, especially PPMS and SPMS patients appear to improve initially and then remain stable during TCA treatment at least over one year. In contrast in this trial, MIX therapy did not improve EDSS or WD, but no significant impairment was recognized. Other trials approved a positive impact of MIX on MS symptoms especially in patients with progressive MS and superposed relapses [54, 57, 58]. The number of relapses in the year before MIX treatment started is regarded to be a predictive parameter in MIX efficacy in MS patients [59]. In this trial, mainly patients without superimposed relapses were included. Therefore, the lack of EDSS improvement could be attributed to this. In conclusion, TCA and MIX proved their efficacy in different ways. Maybe a combination of both should be investigated in progressive MS patients as it has been performed with IVMP and MIX [60]. Despite the mentioned limitations, this study has to be rated as class III evidence.

4. Conclusion

Up to now, clinical trials on patients with progressive MS demonstrated no distinct proof of a potent symptomatic treatment intended to improve or at least stabilize disability, as soon as the progressive phase of the disease stage appears. Immunomodulatory treatment minimizes the rate of MS relapses noticeably but shows no evident positive effects in patients with progressive MS [17]. So the immunomodulatory treatment is a rather preventive one. Numerous papers dealt with the efficacy of intrathecal application of different dosages of various released steroid compounds, above all methylprednisolone acetate was used. This formerly used steroids were mainly short acting cortisone derivates. Further, these steroids were administrated intrathecally less frequently. So, these trials lacked of detailed selection and clinical characterization of MS patients, with small sample sizes, low steroid dosages, and only a few intrathecal administration of mostly short-acting cortisone derivates [19, 20, 36]. Beneficial but controversialy discussed

effects were mentioned in progressive MS patients with predominantly spinal symptoms according to case reports, open-label trials, and one double-blind, controlled study (class of evidence II) with the sustained released steroid TCA [18–20]. As is known, the anti-inflammatory impact of a steroid application depends not only on the dosage but also on the duration of exposure [9, 10, 61]. Hence, the frequency of application and the utilization of a delayed released steroid derivative as TCA are recommendable.

In a rapid succession, a few further open-label uncontrolled trials were performed following a different treatment regime [37–39]. 6 injections of 40 mg of the sustained released steroid derivate TCA were administered within 3 weeks. Patients with an improvement of EDSS or WD were provided to receive further treatment with one TCA application in an individual rate every 6 to 12 weeks. All forecited more recent open label trials were performed in patients with progressive MS with mainly spinal symptoms. They documented a significant improvement of EDSS and WD, respectively. With additional administrations, a stable effect was achieved. However, the mechanism which these improvements are based on is unacquainted. One item debated was the decrease of spasticity by the long-acting steroid. But a significant decrease of antispastic scores was not essential to achieve the mentioned results in recent trials. Another point of discussion could be that intrathecal administration of a sustained released steroid circumvents the BBB and has a positive impact on the still continuing chronic inflammation process. The one thing common to all the recent examples we gave was that they had all been focused on progressive MS patients without signs of an acute exacerbation.

The before-described great number of serious side effects could not be reproduced in the recent trials. There were some raised concerns about a possible neuronal cell injury promoting effect induced by the administered steroid, with inducing brain atrophy [10, 22, 44]. The additional serial assessment of potential unspecific cell injury markers, that is, NSE or S-100, in CSF of progressive MS patients treated with repeated intrathecal TCA did not provide evidence of such a steroid associated risk [39]. Particularly, the long half-life of the applied sustained released steroids appears to be the key of the missing proof of a toxic effect. Further detailed trials with examination of selected CSF biomarkers in MS patients treated with intrathecal steroids are necessary to illuminate these interesting aspects.

In general, further trials are needed to gain more results about the utility of this therapy. All of the mentioned historical and recent studies have to be classified just as class II–IV evidence. The ideal trial design would be a randomized, placebo-controlled, double-blind one. In this case, repeated performance of intrathecal placebo application under double-blind conditions with the consent of patients and the ethical committee seems not to be realistic. Furthermore, such a design including withholding treatment causes maybe ethical qualms [62]. Contrariwise, one could claim that due to the limited evidence for efficacy of intrathecal TCA treatment, the only existing open-label, not placebo-controlled study with repeated lumbar punctures is unethical without level A evidence.

From this point of view, further trials on the potency and safety of intrathecal TCA applications are needed. A multicenter clinical study has to be established to evaluate these items and to compare systemic and intrathecal steroid treatment, initially in progressive MS patients with predominantly spinal symptoms and afterwards in patients with an acute relapse. In addition to the investigation of the long- and short-term benefits, potential risks related to the intrathecal application have to be examined in a blinded analysis. Furthermore, the potential efficacy of intrathecal TCA treatment combined with MIX in progressive MS has to be explored [60].

Anyhow, the intrathecal TCA administration has to be taken into account as one therapy option in handpicked MS patients with a slow progressive clinical course with predominantly spinal symptom features. The intrathecal TCA application should be offered by neurologists with a comprehensive experience in this special treatment. In fact, an individual risk-benefit analysis and the patient's approval are required.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Review Article

Imaging the Visual Pathway in Neuromyelitis Optica

Caspar F. Pfueller and Friedemann Paul

NeuroCure Clinical Research Center, Charité Universitätsmedizin Berlin, 10117 Berlin, Germany

Correspondence should be addressed to Caspar F. Pfueller, caspar.pfueller@charite.de

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The focus of this paper is to summarize the current knowledge on visual pathway damage in neuromyelitis optica (NMO) assessed by magnetic resonance imaging (MRI) and optical coherence tomography (OCT).

1. Introduction

Neuromyelitis optica (NMO, Devic's syndrome) is a rare inflammatory autoimmune central nervous system (CNS) disorder clinically characterised by mostly severe attacks to the optic nerves and the spinal cord [1]. For a long time, NMO was regarded as variant of multiple sclerosis (MS); however, recent increasing evidence points to a distinct pathogenesis. A milestone was the detection of a highly specific biomarker for NMO, the so-called NMO-IgG, the target antigen of which was shown to be the most abundant CNS water channel aquaporin-4 (AQP4) [2, 3]. Various assays for the detection of antibodies to AQP4 have since been developed which has facilitated the clinically relevant distinction of NMO from MS [4, 5]. Antibodies to AQP4 are detectable in 60 to 90% of NMO patients with a specificity of 91–100%. In contrast to previous beliefs, NMO is today regarded as a relapsing disease in 80–90% of patients. With the detection of AQP4 antibodies, the clinical spectrum of NMO has broadened, and currently also AQP4-positive longitudinally extensive transverse myelitis (LETM) and AQP4-positive recurrent optic neuritis are regarded as part of NMO spectrum disorders (NMOSDs) [1, 6].

Studies in MS have shown that neuroinflammation and neurodegeneration can lead to an affection of different parts of the visual pathway, including intraretinal inflammation, retinal neurodegeneration, retinal nerve axonal degeneration as well as pathological changes in the optic tract, the lateral geniculate nucleus, the optic radiation, and the visual cortex [7–11].

The aim of this paper is to give an overview on the recent research results on a similar or different involvement of the visual pathway in NMO pathology as assessed by magnetic resonance imaging (MRI) and optical coherence tomography (OCT).

2. Visual Dysfunction in NMO

In comparison to optic neuritis (ON) in MS, attacks to the optic nerve in NMO occur more often bilaterally and show a poor and incomplete remission of visual functions despite anti-inflammatory and immunosuppressive treatment. At a mean disease duration of 7.7 years after disease onset, more than half of patients with relapsing NMO are functionally blind in at least one eye [12].

3. Imaging

3.1. Magnetic Resonance Imaging

3.1.1. Spinal Cord. In case of myelitis, spinal cord MRI may reveal a characteristic feature: centrally located longitudinal lesions expanding over 3 or more vertebral segments which may be associated with cord swelling and enhancement with intravenous gadolinium administration. These findings

have—in combination with a nondiagnostic brain MRI at disease onset—a specificity of 90% for NMO [13].

3.1.2. Brain. From a clinical standpoint, abnormal brain MRI was until recently considered to argue against an NMO diagnosis. However, several publications in the past few years have highlighted that in NMO various brain lesions may be present at onset or during the course of the disease. A high percentage of NMO patients develop nonspecific brain lesions after diagnosis (60% in the series by Pittock et al. [14], 85% in a Chinese series by Li et al. [15], and in 10% of 60 patients brain lesions met the diagnostic criteria for MS [14]. Another 10% have brain lesions in periependymal regions (e.g., hypothalamus, periaqueductal brainstem) which are rich in AQP4 [15–19]. In contrast to MS, extensive and diffuse widespread white matter abnormalities have been described by various groups. Recently, also large edematous callosal lesions were reported in 4 of 22 NMO patients [20].

Additional MR techniques which are not routinely implemented in clinical practice are magnetization transfer imaging (MTI), diffusion tensor imaging (DTI), and MR spectroscopy (MRS). MTI is a technique that analyses the energy transfer from matrix bound water to free water hydrogen nuclei and thereby allows to evaluate the integrity of an anatomical structure. DTI measures the direction-dependent diffusibility restriction of water, and the resulting data can be computed to characterise fibrous structures, like the optic radiation. MRS is different from other imaging techniques, as it does not provide structural information, but information about ongoing metabolic processes by a concentration measurement of metabolites representative for energy metabolism, integrity of cell membranes, axonal or neuronal integrity and others. These methods have revealed damage to the normal-appearing gray matter but no [21–24] or only minimal abnormalities in the normal-appearing white matter [25]. In contrast, a recent DTI study by Yu and colleagues [26] reported an increased diffusivity of the corticospinal tract and the optic radiations but not of the corpus callosum and the cingulum in NMO patients compared to controls, and the authors concluded that the abnormal diffusion was restricted to the regions with connections to the optic nerves and the spinal cord, thus arguing for axonal degeneration secondary to lesions in the optic nerve and the spinal cord.

3.1.3. Visual Pathway: Optic Nerve and Optic Radiation. MR Imaging studies aimed to visualize optic nerve damage in NMO are sparse. Li et al. reported optic nerve sheath thickening in 16 of 33 patients, all of whom had symptoms of recurrent ON [15]. Wang and colleagues found optic nerve hyperintensities in 6 of 10 patients with available optic nerve MRI [19]. Enhancement of the optic nerves with intravenous gadolinium administration was detected in 4 patients in whom MRI was performed within 6 weeks of onset of acute ON. Similar observations have been made in a comparable study [27]. However, as similar findings have been described in MS optic neuritis [28], it is doubtful whether the aforementioned findings are specific features of NMO or rather indicate optic nerve inflammation irrespective of the underlying condition. Lin et al. reported the possibility to discriminate NMO from MS based on DTI data. However, no tract-specific analysis was performed here [29]. The only MRI study to date that assessed the retrogeniculate part of the visual pathway in NMO by DTI reported an increased diffusivity of the optic radiation [26]. To our knowledge, there is no bigger study on MT imaging of the visual pathway in NMO patients.

3.2. Optical Coherence Tomography. OCT is a noninvasive and reproducible technique to study unmyelinated retinal axons with a high spatial resolution *in vivo* and to quantify the thickness of the peripapillary retinal nerve fiber layer (RNFL), fovea, and macula [30]. In MS patients, OCT has been consistently shown to detect thinning of the RNFL which is most probably due to a diffuse damage of retinal axons that occurs at least in part independent of a previous or present attack of ON [31–33]. Against this background, OCT might prove as a valuable tool for the detection and monitoring of axonal damage in MS and other inflammatory CNS conditions such as NMO. Several groups have investigated anterior visual pathway damage in NMO by OCT in comparison to healthy controls or MS patients. The first published study on OCT in NMO by de Seze and colleagues [34] reported dramatically reduced average RNFL thickness in NMO patients as compared to healthy controls (77.9 μm versus 102.3 μm) and a good correlation between OCT results and both visual acuity and visual evoked potential latencies. Interestingly, among patients at high risk for NMO (recurrent ON of LETM), only those with recurrent ON had a similarly severe reduction of average RNFL thickness (74.2 μm) in contrast to those with recurrent myelitis who did not differ from controls with respect to average RNFL thickness (101.8 μm versus 102.3 μm). Another recent OCT study by Naismith and colleagues compared RNFL measurements in NMO patients to MS [35]. In accordance with the clinical experience of a more severe loss of visual function in NMO compared to MS following ON, RNFL thickness was significantly thinner in NMO patients than in MS ones after ON suggesting a more profound axon loss in the optic nerve in NMO. Similar results were obtained by Ratchford et al. [36] who estimated a first episode of ON to cause 24 μm more loss of RNFL thickness in NMO than in relapsing-remitting MS. Moreover, also macular volume was significantly reduced in NMO ON eyes both versus MS and healthy controls. Interestingly, eyes in the subgroup of patients with LETM and unaffected NMO eyes were not different from controls. The difference in RNFL thickness between ON and non-ON eyes was much greater in NMO patients than in MS ones (34.3 μm versus 9.6 μm). This may suggest that retinal axonal damage in NMO is predominantly linked to attacks of ON while in MS thinning of the RNFL has been reported—albeit to a lesser extent—also in non-ON eyes [32, 33]. Other hypothetical explanations may be a more frequent occurrence of subclinical optic neuritis in MS than in NMO, or MS lesions in the chiasm or optic tracts causing bilateral RNFL involvement [35].

It is worth to critically discuss the significance of OCT for monitoring axonal damage. On one hand the validity of OCT measurements in the past was limited by device-specific measurement variations of the time-domain tomographs in the range of a suspected effect, for example, for RNFLT changes over time. It is expected that the novel spectral-domain OCT devices provide an improved spatial resolution and a better retest-reliability in the future and thereby help to give a more precise description of damage to the visual pathway and its underlying pathogenetic correlate [37, 38].

On the other hand, there is increasing evidence that OCT measurements do not reflect axonal damage exclusively, but could also be affected by intraretinal inflammation or retinal neuronal degeneration, as described in a recent neuropathological study on postmortem analysis on postmortem MS brain tissue by Green et al. [7].

3.3. Is There an Interplay of Damage to the Anterior and the Posterior Visual Pathway? Imaging the entire visual pathway in inflammatory CNS conditions with clinical optic nerve involvement may provide the opportunity to study the relationship between damage to the anterior (optic nerves, chiasm, optic tracts) and attrition to the posterior visual pathway (lateral geniculate nucleus (LGN), optic radiations, visual cortex in the occipital lobe). It is conceivable that damage to one part of the visual pathway may cause—via antero- or retrograde transsynaptic degeneration—an alteration in the other part of the visual pathway. In the past decades, transsynaptic degeneration in the visual system has been shown in various animal models [39–43]. A recent OCT study showed significant RNFL thinning in patients with retrogeniculate lesions (congenital or acquired occipital lobe damage) [44]. In MS, a histopathological study revealed neuronal loss in the LGN [9]. Several other groups have since addressed the question of transsynaptic degeneration by different imaging techniques in MS patients *in vivo*. Sepulcre and colleagues demonstrated that atrophy of the LGN was related to the presence of lesions specifically in the optic radiations but not in the rest of the brain [10]. Ciccarelli et al. examined patients one year after an episode of ON and found reduced connectivity in the optic radiations, suggesting both transsynaptic effects and axonal loss in the optic radiations related to LGN neuronal loss [45]. Audoin et al. reported a decreased visual cortex magnetization transfer ratio in patients with optic neuritis [11]. Dasenbrock et al., in a combined DTI-OCT study in patients with MS and healthy controls, reported a significant association of optic tract diffusion abnormalities with RNFL thinning [46]. In a large cross-sectional study comprising 86 relapsing-remitting MS patients, we investigated the association of RNFL changes with brain atrophy ("brain parenchymal fraction", BPF) and n-acetyl-aspartate (NAA) concentrations as a marker of neuroaxonal integrity in the visual cortex and the normal-appearing white matter (NAWM) (Pfueller et al., in revision). We found a significant correlation of RNFL thickness with visual cortex NAA, and patients with a previous history of ON on one or both eyes had significantly lower visual cortex NAA values than patients without history of optic neuritis. In contrast, NAWM NAA did not correlate with RNFL thickness, and there was no difference in NAWM NAA between patients with and without ON. BPF also correlated with RNFL thickness, and in a multivariate analysis, both BPF and visual cortex NAA were independently associated with RNFL thickness. Our data suggest that attacks to the optic nerve in MS have a detrimental impact on parts of the visual pathway as remote as the visual cortex, further supporting the assumption of transsynaptic degeneration in the visual pathway.

In NMO, no studies have to date assessed the entire visual pathway by a combination of various imaging techniques (e.g., OCT and optic nerve MRI for the anterior visual pathway, DTI or MRS for the retrogeniculate visual pathway). It is, however, conceivable, that damage to one part of the visual pathway has an impact on the other part also in NMO, as transsynaptic degeneration has been shown in different neurological conditions as delineated above and also in ophthalmologic diseases such as glaucoma [47], (reviewed in [48]). The study by Yu and colleagues showing increased diffusivity in the optic radiations of NMO patients supports this notion [26]. Studying the entire visual pathway by a combination of OCT and different MRI techniques may provide additional insights into the interplay between damage to the anterior and the retrogeniculate part of the visual pathway, as these measurements are far less likely to be confounded by focal inflammatory and demyelinating lesions in the brain as is the case in MS. The absence of these lesions should also facilitate the volumetric analysis of different parts of the visual pathway to address the hypothesis of transsynaptical degeneration and also to assess the dynamics of degenerative processes triggered by single inflammatory events, such as acute optic neuritis. To our knowledge, this approach has not been addressed systematically in a larger cohort of NMO patients.

To summarize, the repertoire of available imaging techniques (including DTI, MTI, and MR spectroscopy) has not been used yet to its full potential; especially studies focusing on the visual pathway and its anatomical correlates are still missing. Addressing these points could help to identify significant differences between NMO and MS and give further insight into NMO-specific damage processes as well as into transsynaptical damage processes independent of their underlying condition. Of special interest will be the question, whether neurodegeneration in NMO can only occur in the context of acute or previous neuroinflammation or whether there is evidence for diffuse, chronic axonal, and neuronal degeneration independent of acute neuroinflammation, as was shown for MS.

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Review Article

Iron and Neurodegeneration in Multiple Sclerosis

Michael Khalil,[1,2] Charlotte Teunissen,[2] and Christian Langkammer[1]

[1] *Department of Neurology, Medical University of Graz, A-8036 Graz, Austria*
[2] *NUBIN, Department of Clinical Chemistry, VU University Medical Center, 1007MB Amsterdam, The Netherlands*

Correspondence should be addressed to Michael Khalil, michael.khalil@medunigraz.at

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Increased iron deposition might be implicated in multiple sclerosis (MS). Recent development of MRI enabled to determine brain iron levels in a quantitative manner, which has put more interest on studying the role of iron in MS. Evidence for abnormal iron homeostasis in MS comes also from analyses of iron and iron-related proteins in CSF and blood and postmortem MS brain sections. However, it is not yet clear if iron accumulation is implicated in MS pathology or merely reflects an epiphenomenon. Further interest has been generated by the idea of chronic cerebrospinal venous insufficiency that might be associated with brain iron accumulation due to a reduction in venous outflow, but its existence and etiologic role in MS are currently controversially debated. In future studies, combined approaches applying quantitative MRI together with CSF and serum analyses of iron and iron-related proteins in a clinical followup setting might help to elucidate the implication of iron accumulation in MS.

1. Introduction

Iron is essential for normal neuronal metabolism, including mitochondrial energy generation and myelination [1, 2]. However, excessive levels of brain iron may exert iron-induced oxidative stress and thus lead to neurodegeneration [3]. During the process of normal aging, various regions of the brain, predominantly the basal ganglia, tend to accumulate nonhemin iron, which is primarily stored in the form of ferritin [4]. Increased iron deposition has been observed in various chronic neurological disorders, including multiple sclerosis (MS) [5].

Evidence for increased iron accumulation in MS is mainly derived from magnetic resonance imaging (MRI) and histopathologic studies; however, some information exists also from analyses of iron and iron-related proteins in cerebrospinal fluid (CSF) and blood. The following review summarizes current knowledge of increased brain iron accumulation in MS derived from (2) MRI, (3) histopathologic analyses, (4) studies on CSF and blood, and (5), finally, provides an outlook on potential therapeutic interventions.

2. Magnetic Resonance Imaging

In several studies, evidence for increased iron accumulation, preferentially in deep gray matter areas of the brain, was mainly derived from the signal reduction on T2-weighted MR images [5].

First reports on a regionally signal reduction on T2-weighted brain MRI images in MS indicative of increased iron deposition were published by Drayer et al. [6] and Grimaud et al. [7].

Several studies then followed with a focus on the clinical implication of increased iron accumulation in MS. Increased deep gray matter T2 hypointensities were found to be correlated with disease duration [8, 9], physical disability [9–13], and cognitive impairment [14]. Clinical followup studies in MS revealed that baseline gray matter T2 hypointensities were associated with disability progression over time [12, 15]. Another consistent finding is that deep gray matter T2 hypointensity, suggestive of increased iron content, is correlated with brain atrophy [8, 16]. While this was evidenced in patients with definite MS, there is only little information available regarding the extent and clinical significance of increased iron deposition in patients with a clinically isolated syndrome. Ceccarelli et al. found only minor changes of signal reductions on T2-weighted images compared to healthy controls, and the extent did not predict conversion to clinically definite MS [17]. The approaches used in the studies mentioned above suffered from the methodological drawback of deducing iron concentrations

from a visual grading of the reduction of signal intensity on T2-weighted images even though more recent studies have determined the extent of T2 hypointensity in a semiquantitative manner [8, 10, 14, 16].

In recent years, methodical development of MRI enabled to assess brain iron concentrations quantitatively. In addition, quantitative iron mapping by MRI offers a more sensitive discrimination of iron levels and, therefore, is especially advantageous in longitudinal studies and monitoring of long-term disease progression.

The techniques utilized for quantitative iron mapping are mainly based on relaxation time mapping [18–20] (Figure 1) but also other approaches such as phase mapping [21, 22], magnetic field correlation [23], or direct saturation imaging [24] are applied.

Susceptibility weighted imaging (SWI), a technique that takes advantage from the full complex MR signal by combining magnitude and phase images, has gained attention as a means to assess brain iron [25, 26]. However, the complexity of the postprocessing involved in SWI renders comparative studies challenging and remains an objective of research [27]. Quantitative susceptibility mapping (QSM) is an approach using solely phase images and produces susceptibility maps which are independent of the orientation of the tissue to the main magnetic field [28, 29]. Because paramagnetic iron is considered a main determinant of brain tissue susceptibility, QSM seems especially useful to assess brain iron.

2.1. Validation of MRI Methods. Several methods have been proposed for the measurement of brain iron concentration; however, the majority of them lack validation and, therefore, the specificity and sensitivity of these techniques are not reliably known.

From theoretical considerations based on susceptibility models for brain tissue, it can be concluded that iron is a main determinant of susceptibility-induced contrast in MRI [30]. Several studies have indirectly investigated the relation of MRI parameters with iron by using the age-dependency of iron accumulation in the basal ganglia as reported in [4, 31].

Recently, high-pass filtered SWI phase images were compared to regional iron concentrations in postmortem tissue determined by synchrotron X-ray fluorescence and revealed a correlation between phase shifts and iron [32].

Other recent work acquired quantitative MRI directly after death from seven human brains and subsequently determined brain iron concentrations by using inductively coupled plasma mass spectrometry [33]. This study showed that the relaxation rates R2 and R2* can be used as sensitive and linear measures for brain iron concentration.

These quantitative MRI techniques together with a better understanding of pathophysiologic concepts of increased iron levels [1–3] have put more interest on elucidating the role of iron in MS.

In recently performed studies on quantitative brain iron levels in MS, based on R2* relaxometry at 3 Tesla, increased iron levels have been found in patients with advancing MS compared to clinically isolated syndrome [20]. Using this validated quantitative technique, higher R2* levels in basal ganglia structures reflecting higher iron content were correlated with gray matter atrophy and also with T2-lesion volume [20]. These findings are supported by earlier studies where MRI T2 hypointensities suggestive of increased brain iron, preferentially located in deep gray matter areas, were linked to physical disability and gray mater atrophy in MS [8–10, 12, 34]. Further support comes from a followup study showing that MRI T2 shortenings in deep gray matter areas at baseline are predictive of the evolution of brain atrophy [16].

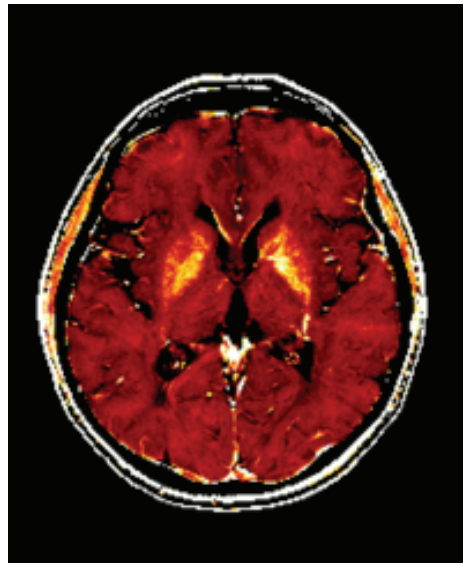

Figure 1: R2* map of a 50-year-old female MS patient. Higher iron concentrations in basal ganglia structures are reflected by brighter signal intensities.

Apart from gray matter regions with known high iron levels (putamen, globus pallidus, caudate nucleus, substantia nigra, and red nucleus) efforts were made to investigate iron levels in white matter by MRI [22, 35, 36]. Using SWI, the phase values of MS lesions were investigated and compared to adjacent white matter [36]. However, compared with chemically determined iron concentrations of postmortem studies, the iron levels within MS lesions were not substantially altered than in reference white matter structures [4, 33]. Due to the confounding impacts of iron and myelin to MRI contrast generation, disease-induced alterations of iron levels in white matter need to be treated with caution and are an objective of ongoing research [37].

Further interest on iron deposition in MS has been generated by the idea of chronic cerebrospinal venous insufficiency (CCSVI) [38] that might be associated with the accumulation of iron in the brain due to a reduction in venous outflow [39, 40]. Following this hypothesis, CCSVI is postulated to be implicated in the etiology of MS. The underlying mechanism is believed to originate from increased iron accumulation in patients due to a reduced venous blood flow caused by constrictions of cerebral veins. This then leads to extravasation of erythrocytes with subsequent iron deposition [41], subsequently triggering inflammation-dependent tissue damage [42]. However, the existence of

CCSVI as well as its etiologic role in MS are currently controversially debated [43], and there is an increasing amount of papers published now that challenge this hypothesis [44–47]. Furthermore, histopathologic studies do not provide clear evidence for extravasation of erythrocytes into lesions caused by increased intraluminal venous pressure [48–52].

3. Histopathology and Pathologic Significance of Increased Brain Iron

The normal anatomic and cellular age-dependent iron distribution within the brain, as described previously [4, 53, 54], should be considered when comparing with iron deposition in pathological conditions.

Craelius et al. described positive iron staining in MS brain sections surrounding demyelinated plaques, myelinated white matter near the lesions, and within blood vessels of gray matter near the lesion [55]. Iron deposits were also described in the putamen and the thalamus [6], in macrophages and reactive microglia [56] and in normal-appearing white matter tissue [57]. Mehindate et al. showed that heme oxygenase 1, which is involved in regulating iron metabolism, was upregulated in astrocytes of MS spinal cord tissue [58].

The exact underlying mechanism by which brain iron accumulates in MS is not fully understood. Iron transport across the blood-brain barrier is dependent on iron transport proteins, predominantly by transferring receptors expressed on brain epithelial cells [59]. Other transporters may also facilitate iron transport across the blood-brain barrier, such as the divalent metal transporter (DMT) and the lactoferrin receptor [60].

It is also not yet clear if increased brain iron deposition is implicated in MS pathology or merely reflects an epiphenomenon [3, 61]. Potential toxic iron products may arise when hydrogen peroxide is formed by superoxide dismutase, which then reacts with free or poorly liganded iron (Fenton reaction [62]). Superoxide may also react with ferric iron through the Haber-Weiss reaction, producing Fe^{2+}, which then again affects the redox cycling [1, 2] (Figure 2).

The resulting highly reactive free hydroxyl radicals ($OH^{\bullet}$) interact with molecules leading to the production of other free radicals [63]. This leads to oxidative stress-induced lipid peroxidation, mitochondrial dysfunction, increase in intracellular free-calcium concentration, and finally causing cell dysfunction and death [62–64]. Because neuronal membrane lipids are rich in highly polyunsaturated fatty acid, they are susceptible to damage caused by lipid peroxidation [62, 63]. Iron itself can initiate and amplify lipid peroxidation [62, 63]. Several naturally produced antioxidants, such as alphatocopherol, may help to reduce oxidative stress-induced tissue damage [62].

4. Cerebrospinal Fluid and Blood

Only a limited number of studies have analyzed iron and iron-related protein levels in CSF and peripheral blood of MS patients. CSF ferritin levels were shown to be elevated in patients with chronic progressive active MS [65] and in patients with SPMS compared to controls [46, 57]. Another study showed that CSF ferritin levels were lower but within normal limits in patients with optic neuritis compared to patients with other neurologic diseases [66]. Similar levels of CSF ferritin were detected in RRMS patients compared to controls [57, 67]. In a recently performed cross-sectional and longitudinal study, CSF ferritin levels did not significantly change over a time period of 3 years, which also may argue against an etiologic role for CCSVI-related parenchymal iron deposition in MS [46].

Serum soluble transferring-receptor levels were significantly increased in MS compared to controls [68, 69], while serum ferritin levels were elevated in patients with chronic active MS only [68]. Conversely, analyses of iron status in two children with recurrent episodes of tumefactive cerebral demyelination revealed decreased serum iron and ferritin and constant iron supplementation was needed to prevent an iron deficiency state in both children [70].

5. Therapeutic Implications

On basis of pathophysiologic concepts implicating iron-induced tissue damage in MS, potential therapeutic interventions, including iron chelators, and inhibitors of iron-related oxidative stress and lipid peroxidation may have beneficial effects [3, 71, 72]. Several chelators are of putative therapeutic value in neurodegenerative disorders [73].

Studies on experimental autoimmune encephalomyelitis (EAE), the animal model of MS, showed that treatment with the iron chelator desferrioxamine reduced clinical and pathologic signs of EAE [74]. Deferiprone, an orally delivered iron chelator, ameliorated signs of EAE, an inhibited T-cell function [75]. However, a clinical trial testing the iron chelating drug desferrioxamine in chronic progressive MS patients failed to demonstrate any effects on disease progression [76]. A recent observation revealed that supplementing nonanaemic iron deficiency in two children with recurrent episodes of tumefactive demyelination leads to sustained remission [70].

In the future large randomized double-blinded multicenter studies are needed to elucidate the potential use of therapies targeting oxidative stress and lipid peroxidation in patients with MS. Quantitative MRI techniques and detailed monitoring of body-fluid iron and iron-related proteins levels should be included in such study protocols.

6. Summary

In summary, increased iron deposition has been consistently reported to occur in MS, but its role in pathogenetic processes of this disease has not yet been completely clarified. Whether increased brain iron levels are also the cause or only the consequence of tissue destruction is still a matter of debate. Future longitudinal studies combining clinical disease status, quantitative MRI techniques sensitive for iron, and additional analyses of iron in CSF/serum and iron-related proteins (as well as iron regulator proteins), might help to unravel the implication of increased iron accumulation in MS. Quantitative MRI and histopathologic

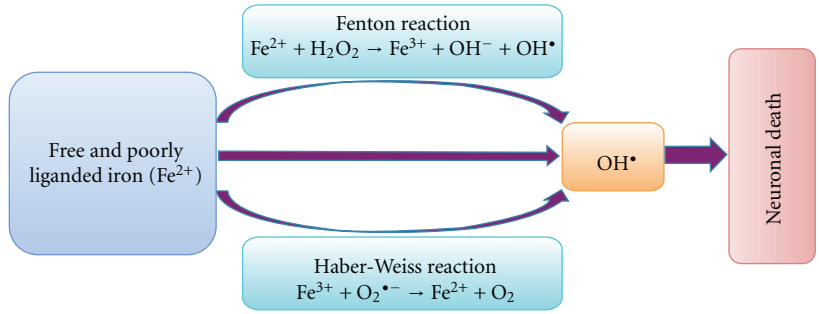

Figure 2: Generation of reactive and damaging hydroxyl radicals ($OH^\bullet$). Free Iron (Fe^{2+}) reacts trough the Fenton reaction with hydrogen peroxide, leading to the generation of very reactive and damaging hydroxyl radicals ($OH^\bullet$). Superoxide can also react with ferric iron in the Haber-Weiss reaction leading to the production of Fe^{2+}, which then again affects redox cycling. The highly reactive hydroxyl radicals lead to oxidative stress-induced lipid peroxidation, mitochondrial dysfunction, and increase in intracellular free-calcium concentration, and finally causing neuronal death.

analyses of postmortem MS brains should complement these studies.

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