

Neurofilament light chain in the assessment of patients with multiple sclerosis

Neurofilamento de cadeia leve na avaliação de pacientes com esclerose múltipla

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ABSTRACT

Multiple sclerosis (MS) is an autoimmune, inflammatory, and degenerative disease of the central nervous system. Axonal degeneration is triggered by inflammation and is the pathological substrate of progressive disability in patients with MS. Therapeutic interventions can reduce inflammatory activity, thus delaying neurodegeneration and the progression of disability. Disease activity and neurodegeneration are assessed mainly through clinical evaluation and magnetic resonance imaging. These measures lack sensitivity and accuracy, so new biomarkers are necessary. Several markers have been studied and to date the most promising is neurofilament light (NfL), a component of the axonal cytoskeleton, which is released into cerebrospinal fluid (CSF) following axonal damage. In the present study, we review the current knowledge about CSF NfL determination in MS, clinically isolated syndrome, and radiologically isolated syndrome, and critically discuss how CSF NfL measurement may contribute to therapeutic decision-making in these patients.

Keywords: Multiple sclerosis; cerebrospinal fluid; neurofilament proteins; demyelinating disorders; enzyme-linked immunosorbent assay

RESUMO

A esclerose múltipla (EM) é uma doença autoimune, inflamatória e degenerativa do sistema nervoso central. A degeneração axonal é deflagrada pelo processo inflamatório e é o substrato patológico da incapacidade na EM. As intervenções terapêuticas reduzem a inflamação retardando a neurodegeneração e a progressão da incapacidade. A neurodegeneração é avaliada pelo quadro clínico e pela ressonância magnética. Estas mensurações não suficientemente acuradas, havendo necessidade de novos biomarcadores. Diversos biomarcadores têm sido estudados e, até o presente, o mais promissor é o neurofilamento de cadeia leve (NfL). O mesmo é um componente do citoesqueleto que é liberado no líquido cefalorraquidiano após injúria axonal. No presente estudo nós revisamos o conhecimento atual acerca do NfL na EM, síndrome clínica isolada e síndrome radiológica isolada, discutindo criticamente como a determinação deste biomarcador pode contribuir na tomada de decisões clínicas.

Palavras-chave: Esclerose múltipla; líquido cefalorraquidiano; proteínas de neurofilamento; doenças desmielinizantes; ensaio de imunoadsorção enzimática.

Neurofilaments (Nf) are the most important components of the axonal cytoskeleton in neurons. They comprise three subunits: light (NfL), medium (NfM), and heavy (NfH), with 68–70 kDa, 145–160 kDa, and 200–220 kDa, respectively. They provide structural support to neurons and regulate axon diameter. The Nfs are released in significant quantity following axonal damage or neuronal degeneration. In these situations, Nfs are released into the interstitial fluid and into the cerebrospinal fluid (CSF)¹. It has been suggested that Nf measurement in CSF, particularly the NfL subunit, is a surrogate marker of axonal damage. Many studies have shown elevated

CSF levels of NfL in a wide variety of neurological disorders in which axonal degeneration occurs².

Multiple sclerosis (MS) is an autoimmune disease with inflammation, demyelination, and axonal degeneration, leading to an accumulation of brain tissue damage over the years³. In recent years, there has been an increase in the number of available therapies for MS, allowing a more personalized approach according to the peculiarities of the disease in each patient. The assessment of disease activity is one of the parameters to guide the therapeutic interventions. It is reasonable to consider prescribing higher efficacy drugs when greater

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disease activity exists. The current criteria to assess disease activity still lack sensitivity, especially in the early stages of the disease. Another parameter to be considered in therapeutic decisions is the ability to predict MS clinical progression. The rate of axonal degeneration is correlated with clinical progression, but it is still difficult to measure this parameter in clinical practice^{4,5}. The third parameter that can contribute to the individualization of therapeutic decisions is the assessment of the therapeutic response. Therapeutic response evaluation is based on the classical occurrence of clinical relapses, clinical progression, and the appearance of new lesions seen on magnetic resonance imaging (MRI) during a given treatment. New parameters such as gray matter disease activity and brain atrophy have also been evaluated but, overall, these clinical and radiologic parameters are still imprecise⁶.

The need for new parameters to assess disease activity, neurodegeneration, and therapeutic response has led to the search for new radiology and laboratory MS biomarkers. In this context, the measurement of Nfs has emerged as a potential biomarker for MS. In the present review, we critically discuss the practical aspects of Nf assessment, its potential clinical utility in MS, clinically isolated syndrome (CIS), and radiologically isolated syndrome (RIS).

PRACTICAL ASPECTS

Diagnostic assays for Nf measurement were made possible through the development of highly-specific monoclonal antibodies against Nf epitopes. Most of the reports have been based on results obtained with a commercially-available ELISA that uses two highly-specific, noncompeting monoclonal antibodies to quantify soluble NfL in CSF samples⁷.

The NfL has a greater discriminatory power than NfH in patients with CIS and in patients with different MS forms⁸. This explains why most recent studies have evaluated NfL and not the other Nf subunits. The NfL ELISA allows fast quantification of CSF NfL, requiring a small sample volume of 50 μ L. The NfL ELISA has shown good pre-analytical stability, without alterations in NfL concentrations after samples had been kept for days at room temperature and after repeated thawing. The standard curve of CSF NfL ranges from 100-10,000 pg/mL, with high reproducibility⁷.

Cerebrospinal fluid NfL concentration increases three- to ten-fold after clinical relapses, reaching its peak at two weeks after the beginning of symptoms of a new clinical relapse. Its levels remain elevated for at least 15 weeks after the onset of the symptoms of a clinical relapse and return to baseline levels after this period^{9,10}. The NfL levels are especially increased in spinal cord relapses, reflecting large-fiber axon lesions¹¹. Therefore, for prognostic evaluation of the disease, it is recommended to wait a period of at least 15 weeks after the last clinical relapse to obtain a CSF or serum sample for NfL determination. It is possible that subclinical lesions also

result in a transitory increase in CSF NfL; however, there is no accurate information in this regard.

Serum NfL determination has been studied in MS with new generation immunoassays¹². Blood analysis is obviously preferable as it does not require lumbar puncture. Although a statistically significant correlation between serum and CSF NfL levels exists¹³, the NfL is found in higher concentrations in CSF when compared to serum. In paired serum and CSF samples, the serum concentration is about 100 times smaller than in the CSF concentration. Conventional ELISA is not sensitive enough to detect the small concentrations of serum NfL. A more sophisticated and expensive method is required for serum NfL determination. Most recent studies have employed the Simoa (single molecule array) platform with a homebrew kit (Quanterix Corp, Boston, MA)¹³.

EVALUATION OF NFL IN MS DIAGNOSIS

The ability of CSF NfL to predict an MS diagnosis has been studied. A recent meta-analysis identified 10 studies comparing CSF NfL between MS patients and controls¹⁴. In all the individual studies of this meta-analysis there was a significant difference between NfL concentrations in MS populations when compared with control groups. However, there was a great variability in the NfL levels of MS patients between different studies¹⁵. This suggests that there was a great methodological variation in the measurement of this biomarker between the different studies. It also suggests there was a great variability in the MS populations included in these studies. Overall, these variations point to a lack of a reproducible cut-off that can be adopted in clinical practice.

Four studies demonstrated higher serum NfL levels in MS patients when compared with controls¹⁵. Although all these studies found statistically significant differences in NfL levels between patients and controls, the differences were quite small, making it impossible to establish an accurate cut-off.

Therefore, neither CSF NfL nor serum NfL seem to be useful for the diagnosis of MS due to the important overlap of the results between patients and controls, without the establishment of an accurate cut-off. In addition, since the NfL is a marker of axonal degeneration, it may be increased in other diseases with a differential diagnosis for MS; for instance, neuromyelitis optica spectrum disorder¹⁶. Future comparative studies are still necessary to establish if serum or CSF NfL can discriminate between MS and other potentially confounding diseases but, at this point, NfL can not be considered a good candidate for an MS diagnosis biomarker.

EVALUATION OF DISEASE ACTIVITY

The assessment of disease activity is usually based on the frequency of clinical relapses and the appearance of new

hyperintense and gadolinium-enhancing lesions on MRI¹⁷. Several studies have sought the correlation between NfL and clinical and MRI activity, to evaluate its ability to assess disease activity.

As mentioned above, the CSF NfL concentration increases after clinical relapses. The CSF NfL levels are higher in patients with clinical relapse than in patients in clinical remission¹⁸. A significant correlation between the clinical relapse rate and CSF NfL has been shown¹⁹. Besides the correlation with the clinical activity of the disease, several studies have assessed the correlation between NfL levels and disease activity measured by MRI. In a study of 44 patients with MS, the NfL was higher in patients with gadolinium-enhancing MRI lesions when compared with controls, and with MS patients without gadolinium-enhancing lesions¹⁹. In the same study, patients with new MRI T2 lesions had higher NfL levels than patients without recent T2 lesions¹⁹. Higher CSF NfL levels were associated with higher risk of appearance of new MRI T1 and T2 lesions^{19,20}. In a study including 74 patients with MS, the baseline CSF NfL predicted the appearance of new MRI T2 lesions in consecutive MRI examinations²¹.

The correlation between CSF NfL and disability progression has also been studied. A correlation between CSF NfL levels and disability worsening as measured by the Expanded Disability Status Scale (EDSS)¹¹ has been shown. A long-term follow-up study in 44 patients with MS showed that patients whose EDSS scores progressed after five years of follow-up had significantly higher mean baseline NfL levels than those whose scores did not progress (944 ng/L vs 246 mg/L). In the same study, patients who converted to secondary progressive MS had significantly higher baseline NfL levels when compared with patients who did not convert after five years of clinical follow-up²².

A recent study evaluated serum and CSF NfL correlation with no evidence of disease activity (NEDA-3). The definition of NEDA-3 includes (i) absence of clinical relapses, (ii) absence of disability worsening, defined as ≥ 1 -point increase in the EDSS score; and (iii) absence of radiological activity, seen on MRI as gadolinium-enhancing lesions or new/enlarged T2-hyperintense lesions⁶. Forty-one MS patients and 22 healthy controls were assessed by serum and CSF NfL assay. Baseline CSF NfL significantly correlated with NEDA status after four follow-up years. Baseline CSF NfL showed a stronger correlation with disease activity measured by clinical and MRI parameters, but this same correlation was not found for serum NfL²³.

These data suggest that an association between NfL levels and disease activity exists. The number of new T2 lesions, gadolinium-enhancing lesions, clinical relapses, and disability accumulation are effective measures of disease activity and can reliably be used to guide therapeutic decisions. Whether NfL determination can contribute in this sense or replace some of the traditional parameters is not yet

established. There are no studies comparing the performance of NfL with clinical and MRI parameters. Although the NfL level seems to be a good predictor of future disease activity to be used along with clinical and radiological data, its ability to individually measure disease activity and assess prognosis still need to be better defined.

EVALUATION OF NEURODEGENERATION

Axonal degeneration accumulates over the course of the disease and is the primary cause of sustained neurological disability in MS patients. The evaluation of axonal degeneration is still a major challenge in MS and it has been based mainly on MRI findings, particularly brain volume measures, which lack reproducibility²⁴⁻²⁷. The ability of optical coherence tomography to disclose neurodegeneration in MS has also been evaluated, but its use in clinical practice is still debatable²⁸. Measuring an intracellular cytoskeletal protein such as NfL seems to be a comprehensive way to assess the extent of axonal damage within the central nervous system².

One study evaluated NfL and brain atrophy in 25 patients treated with natalizumab. Patients were evaluated, before natalizumab treatment, with CSF assays and MRI and were followed with MRI for three years. There was a mean brain volume reduction after three years. Patients with higher baseline NfL had greater reduction in brain volume, suggesting that the baseline CSF NfL level predicts brain atrophy development²⁹. Other studies sought correlations between serum NfL and brain neurodegeneration measured by brain atrophy. A prospective study in 257 MS patients showed that higher baseline serum NfL predicted a progressive decrease in brain volume. It was shown that an increase in serum NfL of 10 ng/L was associated with an additional reduction in brain volume of 0.17% after two years³⁰. A similar finding was shown in another study with 74 MS patients, in which a higher baseline serum NfL showed a high prediction accuracy for reduced brain volume after two years²¹.

These studies suggest that higher NfL concentrations are predictive of brain atrophy and the progression of the neurodegenerative process. However, some caution should be adopted in the interpretation of these findings. As discussed above, NfL levels fluctuate according to the disease activity. The disease activity is, by itself, a predictor of future neurodegeneration and brain atrophy. In fact, it has been shown that the presence of baseline gadolinium-enhancing lesions is associated with a higher percentage of future brain volume reduction³¹. Gadolinium-enhancing lesions, in turn, are associated with higher NfL levels. Thus, it remains to be established whether the baseline NfL levels can independently predict neurodegeneration or if it is the elevated NfL levels reflecting inflammatory activity that predicts brain atrophy. It is also not known whether NfL is comparatively a

better marker than other predictors of neurodegeneration, notably MRI. These issues are important to better clarify the role of NfL as a neurodegeneration marker in MS.

EVALUATION OF THERAPEUTIC RESPONSE

Given the greater availability of therapeutic options currently available for the treatment of MS, it is important to determine the variables to be considered for choosing any treatment and for switching drugs. Early substitution of a treatment to which the patient is not responding may contribute to improving the long-term prognosis. Most of the algorithms guiding drug switching are based on the occurrence of clinical relapses, clinical progression, and the appearance of new lesions in MRI. Some new parameters such as gray matter disease activity and brain atrophy have also been evaluated. However, the parameters to assess the effectiveness of treatment are still imprecise and may take too long to reveal a therapeutic failure⁶.

There is increasing evidence that NfL levels are reduced after effective MS treatment. This has been demonstrated in patients receiving different types of treatments. One study showed a significant reduction of 51% in CSF NfL levels after 12-24 months of treatment with mitoxantrone in 35 patients with MS. This reduction was verified mainly in previously-untreated patients and in patients with baseline MRI enhancing lesions³². The CSF NfL levels were also tested in MS patients treated with natalizumab³³. Natalizumab treatment over 6-12 months significantly reduced the mean NfL level from 1,300 ng/l to 400 ng/l³³. In this study, natalizumab treatment reduced NfL levels to similar values obtained in the CSF of healthy controls. This significant reduction occurred regardless of previous disease-modifying treatments or previous activity of the disease. Significant reduction in CSF NfL levels was reported in 43 MS patients receiving fingolimod for 4-12 months, and those previously treated with first-line drugs^{34,35}.

The reduction of NfL with the treatment seems to differ according to the efficacy of the drug used. A recent study showed higher CSF NfL levels in patients treated with first-line therapy compared with natalizumab. In this study, 33 patients were using interferon beta and 19 were on natalizumab for at least one year¹⁹. Patients with breakthrough disease on first-line therapies showed reduced NfL levels after switching to fingolimod, and this reduction correlated with reduction in relapse rates and MRI measures³⁵. In the same study, fingolimod was not associated with a change in NfL levels in patients previously treated with natalizumab³⁰. A follow-up study in 286 MS patients showed that patients had significant reduction in serum and CSF NfL levels when escalated from drugs with lower efficacy to drugs with higher efficacy¹³. In the same study, there was no change in NfL levels when there was a change between drugs with a similar level of efficacy¹³. Another study showed no reduction in NfL

levels from baseline with vitamin D supplementation, a treatment with no proven efficacy³⁶.

These data suggest that the MS treatment appears to be associated with reduced levels of NfL in blood and CSF. This reduction is apparently greater with the more effective treatments. To date, it is not yet known whether this reduction is a more effective parameter than neuroimaging parameters to measure the therapeutic response. It is also not yet known by how much the NfL concentration must be reduced to indicate a good therapeutic efficacy. Until these questions are answered, it would be premature to propose monitoring NfL levels as an individual indicator of the need for medication change. The current data suggest that the absence of NfL reduction with treatment could be another potential indicator of lack of an adequate therapeutic response.

EVALUATION OF PATIENTS WITH CIS AND RIS

There is accumulating evidence that the NfL baseline level may correlate with the risk of development of MS in CIS and RIS patients. One study showed that CIS patients with NfL levels above 900 ng/L had a higher risk of developing MS over a two-year follow-up period³⁷. In the group of patients who evolved from CIS to MS, the mean CSF NfL level was 1,555 ng/L and in the group who remained as CIS after two years, the mean CSF NfL level was 499 ng/L ($p < 0.0001$). Another study showed that the CSF NfL level was an independent predictor of clinical conversion to CIS and to MS in patients with RIS³⁸. The RIS patients with CSF NfL levels above 619 ng/L had a significantly higher risk of developing CIS and a significantly higher risk of developing MS than RIS patients with lower NfL levels. However, other studies including CIS patients did not find higher CSF NfL levels in patients converting to MS compared with patients who remained as CIS and did not convert to MS³⁹⁻⁴¹. It is possible that these discrepancies may be attributable to different sample sizes between studies. Also, the lack of uniformity in baseline characteristics of the CIS patients may have contributed to the different findings.

One study showed a correlation between gray matter damage measures and CSF NfL levels in patients with CIS⁴², suggesting that NfL levels may be an indicator of very early neurodegenerative changes in CIS patients. Another study did not find a correlation between CSF NfL and volumes of the whole brain, gray matter, white matter, and cortical gray matter³⁹. However, this study found a significant correlation between higher NfL levels and changes in brain volume over one year.

Therefore, the current data suggest that the NfL is a potential marker of early neurodegenerative process and is associated with higher risk of conversion to MS of patients with RIS and CIS. It is possible that RIS patients with a high baseline NfL should be more closely monitored. Also, it is

possible that CIS patients with a high baseline NfL should be considered high risk CIS patients. However, the CSF and serum NfL cut-offs indicating high risk RIS to CIS and CIS to MS conversion are not well established. Taking into account some discrepant results, further studies are still needed to establish precisely the role of NfL levels in the clinical evaluation of RIS and CIS patients.

CONCLUSION

The existing data allow us some practical considerations about the use of NfL levels in the evaluation of MS patients:

1) NfL is not a good diagnostic marker for MS. The overlapping of concentration values between patients and controls does not allow the establishment of a cut-off with good levels of sensitivity and specificity.

2) NfL may be an indicator of disease activity and may be a prognostic marker, but its ability in this regard has not yet been compared with clinical and neuroimaging parameters. The CSF NfL seems to predict and reflect disease activity better than serum NfL.

3) NfL possibly indicates present neurodegeneration and possibly predicts future brain atrophy.

4) NfL seems to be an indicator of treatment efficacy and may be used in the future, along with MRI, as an additional parameter to be taken into account when making therapeutic decisions. It is possible that both CSF and serum NfL are useful for treatment follow-up.

5) NfL may contribute to assess the risk of future MS development in patients with RIS and CIS⁴³, but the cut-off concentrations for high-risk RIS and CIS still need to be established.

Some points need to be better defined:

1) There are still no clear recommendations how often control determinations should be carried-out for disease monitoring.

2) More uniform standardizations of pre-analytical and analytical procedures are still required.

3) Better determinations of the cut-offs and the reference values in different clinical situations and disease forms are still needed.

The determination of NfL has several potential contributions in the evaluation of MS patients. Future large studies, in different MS populations, with different disease forms and with different types of treatment, would contribute to better defining of the clinical value of this biomarker in the management of MS patients.

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