Catlab Informa

Bulletin No. 158

New plasma biomarkers for multiple sclerosis: neurofilament light chain (NfL) and GFAP protein.

Multiple sclerosis (MS) is an inflammatory, demyelinating and degenerative disease of the central nervous system (CNS), characterised by a heterogeneous clinical presentation and an uncertain course. The primary therapeutic goal is the achievement of NEDA-3 (no evidence of disease activity) status, as the prevention of clinical relapses and subclinical activity is crucial to halting cumulative neuroaxonal damage and disability progression.

New prognostic and monitoring biomarkers

In recent years, the identification of new biomarkers has transformed the monitoring of MS. These allow for more objective assessment of disease activity and the efficacy of disease-modifying treatments (DMTs) than clinical evaluation alone. The ability of these markers to predict future relapses and detect subclinical activity enables clinicians to intensify treatment early or to select patients who may benefit from high-efficacy therapies from the time of diagnosis when presenting with a more aggressive profile. Thus, these biomarkers act as crucial prognostic indicators for improving treatment personalisation.

Among the panel of new biomarkers, neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) stand out:

- **Neurofilament light chain (NfL):** These are proteins specific to the axonal cytoskeleton, present in both the central nervous system (CNS) and the peripheral nervous system. Their primary function is to maintain structural stability, axonal homeostasis and polarisation. With axonal damage or degeneration, neurofilaments are released into the extracellular space and pass into the cerebrospinal fluid (CSF) and blood. They are direct indicators of neuronal damage and their elevation correlates with inflammatory activity (relapses and new lesions on MRI) and disability progression
- **Glial fibrillary acidic protein (GFAP):** This is an intermediate filament protein expressed specifically by astrocytes, which comprise approximately 20% of human brain cells. These cells perform essential functions in the CNS, such as maintaining the blood-brain barrier, regulating neurotransmitter levels and responding to injury. Elevated concentrations of GFAP

reflect astrocyte activation (reactive astrogliosis) and loss of CNS integrity. In MS, GFAP has been closely associated with progressive phenotypes and progression independent of relapse activity (PIRA).

Implementation in clinical routine: from CSF to plasma

Historically, these biomarkers were detected in CSF, but the transition to plasma/serum measurement has been made possible by ultrasensitive technologies such as SIMOA (Single Molecule Array) and automated systems such as Lumipulse G (Fujirebio). Plasma measurement allows easy implementation in daily routine, as it is obtained through a conventional blood draw, representing a minimally invasive and repeatable alternative that avoids the complications of lumbar puncture.

Analytical methodology at Catlab

An important challenge is that there is currently no universal international calibrator for these biomarkers, which means that results obtained by different methods (such as SIMOA or Lumipulse) are not directly comparable with one another. At Catlab, the automated Lumipulse G analyser (Fujirebio) is used for the quantification of NfL and GFAP in plasma, a system based on a chemiluminescence immunoassay (CLIA). The literature indicates that the Lumipulse demonstrates excellent precision (CV <5%) and a strong correlation with the reference method SIMOA. In the case of NfL, the Lumipulse tends to yield slightly higher values than SIMOA.

Analytical considerations

To ensure the reliability of results, the following pre-analytical requirements are recommended:

- Sample collection preferably in a fasting state.
- Use of EDTA tubes for plasma.
- Centrifugation within 3 hours of collection and transfer of plasma to a polypropylene tube to prevent adhesion of the proteins of interest to the tube.
- Storage at -80°C if analysis is not immediate.

Interpretation of results

It is essential to bear in mind that these biomarkers are influenced by physiological factors: values increase with age, are affected by IMC (due to the volume of distribution) and/or by renal

function. Normal values must be established based on these parameters to avoid erroneous interpretations.

To harmonise results and adjust them to individual characteristics, the z-score is used. Calculators such as the one available on the online platform of Dr Kuhle's research group at the MS Centre of the University Hospital of Basel (Switzerland), allow the user to enter the method used, the obtained value, the patient's age and BMI to obtain a normalised value (z-score) per patient against a database of healthy controls adjusted for age and BMI:

For NfL: https://shiny.dkfbasel.ch/baselnflreference

For GFAP: https://shiny.dkfbasel.ch/baselgfapreference

The interpretation of NfL results according to the **z-score** is as follows:

Z-score	Percentile	Clinical Interpretation
< 0.84	<80	Levels within the normal range.
≥0.84 - ≤1.5	80 - 93.3	Slightly elevated levels; suggest borderline activity.
> 1.5 - ≤ 2.0	93.3 – 97.7	**Pathological.** Associated with a 3-fold increased risk of clinical or MRI activity.
> 2.0	> 97.7	**Pathological**. Markedly elevated levels; very high risk of disability progression.

For the longitudinal monitoring of NfL, the Reference Change Value (RCV) is used. The RCV allows determination of whether the % change (absolute value) between the values of two successive measurements in the same patient is significantly greater than biological variability (according to the biological variation value from the EFML database) and analytical variability (according to the intrinsic variation of the analyser), thus indicating true disease activity:

% increase in variation (2nd sample > 1st sample) (absolute value)

If the % is > RCV: significant increase compared to the previous value.

If the % is ≤ RCV: *non*-significant increase compared to the previous value.

% decrease in variation (2nd sample < 1st sample) (absolute value):

If the % > RCV: significant decrease compared to the previous value.

If the % ≤ RCV: non-significant decrease compared to the previous value.

It should be noted that, unlike NfL which can show acute peaks, GFAP does not usually vary significantly in an acute manner over time, being more stable as a marker of chronic progression.

Recommendations for testing

It is recommended to carry out the determination of these biomarkers in the following contexts and frequencies:

- **At diagnosis:** To establish a baseline value and an initial prognostic profile. High NfL levels predict faster conversion from CIS (clinically isolated syndrome) to MS.
- **Treatment monitoring:** Measurement at 3–6 months after starting or changing a DMT to confirm therapeutic response (normalisation of the *z-score*).
- **Stable patients (NEDA-3):** Testing every 3–6 months to detect subclinical activity.
- **Post-activity:** 3–4 months after a relapse or new MRI lesion to establish a new baseline.
- **Cases of uncertainty:** To confirm activity in suspected pseudorelapses or in progressive forms that are difficult to assess clinically.

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