

Fluid biomarkers for microglial activation and axonal injury in multiple sclerosis

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Abstract

Although it is clear that the immune system is an important disease driver in multiple sclerosis (MS), it is presently unknown what initiates the process. Infections have been mentioned as potential triggers, which is specifically dealt with in other articles of this volume. Here, I give an overview of two fluid biomarkers that reflect key elements of the MS process: microglial activation (cerebrospinal fluid [CSF] sTREM2) and axonal injury (CSF and serum/plasma neurofilament light). I review recent data on how these markers are altered in MS, how they change in relation to disease progression and treatment and, finally, how they can be used as tools in MS research.

Introduction

Multiple sclerosis (MS) is the major autoimmune disease of the brain and one of the most common causes of adult onset neurological disability. While the cause(s) of MS remains unknown, this disease is thought to be attributable to an autoimmune attack on myelin and oligodendrocytes by cells of the immune system that have entered the central nervous system (CNS). Results from genome-wide association studies, as well as the effectiveness of treatment strategies that directly target the activation or trafficking of T-cells and B-cells into the CNS, lend strong support for such a hypothesis (1). The concept that different infections may trigger such an immune reaction is explored in detail in other articles of this special volume. Here, I detail recent advances in research on biomarkers to assess microglial activation and axonal injury during MS onset, progression and remission, as well as in response to treatment.

Microglial activation in MS

Microglia are the innate immunity cells of the brain. They play important roles in normal brain development, as well as in adult brain plasticity and synaptic homeostasis, and may also respond to a wide range of CNS infections. The role of microglia in MS pathogenesis has been debated but some data suggest that microglial activation may precede T-cell infiltration and demyelination in MS lesions and aggravate axonal injury (2).

Fluid biomarkers for microglial activation

CSF concentrations of several proteins have been suggested to reflect microglial activation in the brain, but most of these are also expressed by astrocytes and it has been difficult to tease microglial and astrocytic activation apart using biomarkers. (Both processes most often occur in parallel, so it might not matter much from a practical point of view.) Nevertheless, one

cerebrospinal fluid (CSF) protein has become established as a biomarker selective for microglial activation: the secreted form of the triggering receptor expressed on myeloid cells 2 (sTREM2). TREM2 is a cell surface receptor predominantly expressed on myeloid cells, for example, monocyte-derived dendritic cells, macrophages, mast cells, osteoclasts, and microglia, but importantly not astrocytes (3).

In 2008, Piccio *et al.* first identified sTREM2 in CSF and developed an enzyme-linked immunosorbent assay (ELISA) with which they measured increased concentrations of sTREM2 in CSF samples from patients with MS, particularly so in active disease (4). Recently, researchers from Gothenburg confirmed this result and could also show that treatment with natalizumab or mitoxantrone normalised CSF sTREM2 concentrations (5).

Taken together, CSF sTREM2 seems to be a dynamic biomarker for microglial activation in MS. Plasma or serum concentrations of this protein are influenced by release of sTREM2 from extracerebral cell types, wherefore there is little hope for a reliable blood test.

Axonal injury in MS

Axonal injury is a prominent feature of MS responsible for both acute symptoms and long-term disability. Structural components in axons may even be primary targets of the immune response in MS, as there have been reports on increased prevalence of auto-antibodies against neurofilaments in MS patients.

Fluid biomarkers for axonal injury

Neurofilaments are 10-nanometer filaments in the axoplasm of neurons, where they give tensile strength to dendrites and axons. They are composed of three major proteins with

molecular masses of 200, 150 and 68 kilodaltons (kD), respectively. As the name implies, neurofilament light (NfL) is the lightest of the three components.

During the 1980s, Swedish researchers led by Professor Lars Rosengren purified neurofilaments from bovine brain and developed the first generation of polyclonal rabbit antisera specific against the individual neurofilaments (6). The most promising combination of these was developed into the first ELISA for NfL (7). Rosengren and colleagues showed that CSF NfL concentration was increased in amyotrophic lateral sclerosis (ALS), particularly so in patients with pyramidal tract involvement, and that increased concentrations also characterized Alzheimer's disease (AD), vascular dementia and normal pressure hydrocephalus, but with lower magnitude of the rise compared with that seen in ALS (7). The authors concluded that CSF NfL was a promising biomarker for axonal injury in general; a conclusion that has later been confirmed in studies examining both acute and chronic CNS disorders (6).

Monoclonal antibodies against NfL were developed and a new NfL ELISA that did not depend on exhaustible antisera was established (8). Given the high expression of NfL in large caliber myelinated axons, studies on multiple sclerosis (MS) followed. Researchers found that CSF NfL is increased in both relapsing-remitting and primary progressive MS, that CSF NfL concentration indicates ongoing axonal injury and reflects the intensity of the process, that CSF NfL concentration normalises within 6-12 months in MS patients following initiation of effective treatment and that CSF NfL thus is a promising biomarker for disease intensity and progression, as well as for treatment response (9).

NfL is detectable in serum and plasma and over the years researchers have tried to develop a reliable blood test. The two main obstacles have been (i) the low concentration of the protein in blood and (ii) the existence of heterophilic antibodies that interfere in the measurement in some people. Recently, the standard ELISA for CSF NfL was transferred onto the Single molecule array (Simoa) platform, which, together with appropriate blocking of heterophilic antibodies, allows for the ultrasensitive measurement of this protein in the blood (10, 11). Serum and plasma (there is no difference between these two matrices) NfL concentration measured by Simoa correlate strongly with CSF NF-L (10, 11). They also correlate with magnetic resonance diffusion tensor imaging parameters indicative of axonal injury in traumatic brain injury patients (12). Limited white matter axonal injury evoked by catheter insertion in MS patients who participated in a trial of a drug that was administered intrathecally also resulted in transient increases of serum NfL following catheter insertion, supporting that serum NfL is a sensitive biomarker for white matter axonal injury. This result is further corroborated by recent data on amateur boxers where a correlation of serum NfL with the number of head blows was established (13). MS patients show increased concentrations of serum NfL in a disease activity-dependent manner and serum concentrations normalise in response to disease-modifying therapy, according to two presentations at ECTRIMS 2016 (14, 15).

Taken together, the results suggest that serum and plasma NfL may be a convenient tool to detect and monitor white matter axonal damage in different phases of MS longitudinally, as well as to follow and optimise treatment.

Concluding remarks

CSF sTREM2 and CSF and serum/plasma NfL are not directly related to the pathogenesis of MS but represent novel tools with which important elements of the MS disease process can be monitored *in vivo*. sTREM2, a biomarker for microglial activation, necessitates CSF sampling, whilst NfL, a white matter axonal injury marker, can be measured in CSF or serum/plasma with highly correlated results. CSF sTREM2 is currently a research tool, whilst CSF NfL is used clinically. Serum NfL would be a very useful tool in MS clinics, not the least to monitor treatment, and intense efforts are currently ongoing to refine this test into an *in vitro* diagnostic product.

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Conflicts of interest

Dr. Zetterberg reports that he is one of the founders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

References

1. Steelman AJ. Infection as an Environmental Trigger of Multiple Sclerosis Disease Exacerbation. *Front Immunol* 2015;6:520.
2. Howell OW, Rundle JL, Garg A, Komada M, Brophy PJ, Reynolds R. Activated microglia mediate axoglial disruption that contributes to axonal injury in multiple sclerosis. *J Neuropathol Exp Neurol* 2010;69:1017-1033.
3. Lue LF, Schmitz CT, Serrano G, Sue LI, Beach TG, Walker DG. TREM2 Protein Expression Changes Correlate with Alzheimer's Disease Neurodegenerative Pathologies in Post-Mortem Temporal Cortices. *Brain Pathol* 2015;25:469-480.

4. Piccio L, Buonsanti C, Cella M, et al. Identification of soluble TREM-2 in the cerebrospinal fluid and its association with multiple sclerosis and CNS inflammation. *Brain* 2008;131:3081-3091.
5. Ohrfelt A, Axelsson M, Malmestrom C, et al. Soluble TREM-2 in cerebrospinal fluid from patients with multiple sclerosis treated with natalizumab or mitoxantrone. *Mult Scler* 2016;22:1587-1595.
6. Zetterberg H. Neurofilament Light: A Dynamic Cross-Disease Fluid Biomarker for Neurodegeneration. *Neuron* 2016;91:1-3.
7. Rosengren LE, Karlsson JE, Karlsson JO, Persson LI, Wikkelso C. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. *J Neurochem* 1996;67:2013-2018.
8. Norgren N, Rosengren L, Stigbrand T. Elevated neurofilament levels in neurological diseases. *Brain Res* 2003;987:25-31.
9. Teunissen CE, Khalil M. Neurofilaments as biomarkers in multiple sclerosis. *Mult Scler* 2012;18:552-556.
10. Gisslen M, Price RW, Andreasson U, et al. Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study. *EBioMedicine* 2016;3:135-140.
11. Kuhle J, Barro C, Andreasson U, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clin Chem Lab Med* 2016;54:1655-1661.
12. Ljungqvist J, Zetterberg H, Mitsis M, Blennow K, Skoglund T. Serum Neurofilament Light Protein as a Marker for Diffuse Axonal Injury: Results from a Case Series Study. *J Neurotrauma* 2017;34:1124-1127.
13. Shahim P, Zetterberg H, Tegner Y, Blennow K. Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports. *Neurology* 2017.
14. Novakova L, Axelsson M, Malmeström C, et al. Neurofilament light in serum: A potential biomarker for monitoring treatment efficacy in RRMS. *ECTRIMS Online Library* 2016:145814.
15. Kuhle J, Barro C, Brachat AH, et al. Blood neurofilament light chain levels are elevated in multiple sclerosis and correlate with disease activity. *ECTRIMS Online Library* 2016:147076.