Novel Biomarkers in Multiple Sclerosis

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A Biomarkers for Multiple Sclerosis

- WHY?
- WHAT?
- WHERE?
- HOW?
- WHICH?
One of the biggest challenges in therapeutic decision making for MS is effective stratification (or personalization) of treatment in the face of an uncertain prognosis.
A major objective at the time of the initial diagnosis is to arrest the disease at the early inflammatory stage, with the hope that this will also delay disease progression and minimize future disability—a concept that has yet to be proven clinically.

Treatment decisions

Treatment decisions based on the risk to benefit ratios of each DMA are further complicated by:

1. Inherent disease heterogeneity.
2. Different MS subtypes and the rates of progression.
3. The variety of clinical presentations (spinal cord, cerebellar, optic neuritis, cognition, fatigue, etc.).
4. The differences in pathological subtypes, implying different disease mechanisms.
5. The heterogeneity of MS is further reflected by the unpredictable efficacy of DMAs, which varies from patient to patient.

• At present, the clinical parameters that are used to assess disease activity and therapeutic efficacy depend on relapses rates, MRI outcomes, and changes in disability scores.

• These assessments have limited sensitivity with respect to subclinical disease activity, especially when related to gray matter changes and spinal cord disease.

• Thus, there is a need for sensitive, specific, and relatively inexpensive biomarkers that can detect disease activity and serve as surrogate markers for assessing therapeutic efficacy.

• Identification and validation of predictive biomarkers of therapeutic response are urgently needed to help guide optimal treatment management strategies in patients with MS.

• Ultimately, accurate and sensitive biomarkers of subclinical disease activity will provide neurologists with more objective tools.

• In addition to MRI, to better assess and predict therapeutic outcomes in individual patients with MS.
A Biomarkers for Multiple Sclerosis

- WHY?
- WHAT?
- WHERE?
- HOW?
- WHICH?
“A measurable proteins, lipids, or reflect a disease-related or drug-related process”.
A Biomarkers for Multiple Sclerosis

- WHY?
- WHAT?
- WHERE?
- HOW?
- WHICH?
1- Blood

Advantages:

1. Blood collection is a minimally invasive procedure performed.

2. Sampling can be carried out in large cohorts of patients, as well as in healthy controls.

3. Can easily be repeated for use in longitudinal studies.
1- Blood

Disadvantages:

1. They may lack sensitivity in monitoring disease processes in the CNS, particularly with respect to monitoring progressive disease and the effect of therapeutics aimed at neuroprotection and remyelination.
2. Kidney function, liver function, and concomitant infections can influence the levels measured, as well as the time from collection to process.
3. Diurnal variation
2- CSF

Advantages:

1. Ideally suited to monitoring CNS disease activity because of its close proximity to sites of disease pathology.

2. With the understanding that CSF sampling may be necessary during clinical trials testing neuroprotective agents, the majority of patients with MS who have been polled indicated a willingness to undergo lumbar puncture procedures in order to participate.

Advantages:

1. Ideally suited to monitoring **CNS** disease activity because of its close proximity to sites of disease pathology.

2. The levels of a CSF biomarker cannot be influenced by liver or kidney function.
Disadvantages:

1. The invasiveness of the collecting method narrows the potential of multiple measurements.

2. Circadian fluctuation in CSF’s production rate dictates the necessity of standardizing the time of performing a lumbar puncture (*It is hypothesized, that CSF collection via lumbar puncture is done in morning hours, after night fasting*)
3- Urine

Advantages:

- It is the easiest material to collect, even in a 24-hour basis, overcoming the obstacles of fluctuations previously mentioned.
3- Urine

Disadvantages:

1. Bacterial colonization of the urinary tract though can distort the measurements.

2. MS patients with bladder dysfunction may regulate the amount of the fluids taken in a daily basis, affecting the quantity of produced urine.
4- Tears

Advantages:

- There have been previous efforts in measuring OligoClonal Bands (OCBs) in tears, with results comparable to those of CSF.

5- Saliva

- Saliva has served as a means of specifying soluble Human Leucocyte Antigens (HLAs) type II.

There are though considerable difficulties in correlating MRI findings with disability progression. Certain novel ambitious techniques promise to overcome all these problems.
A Biomarkers for Multiple Sclerosis

- WHY?
- WHAT?
- WHERE?
- HOW?
- WHICH?
1. Enzyme-Linked Immunosorbent Assay (ELISA)  
2. Immunofluorescence  
3. Flow Cytometry  
4. Polymerase Chain Reaction (PCR)  
5. Western Blotting  
6. The Nephelometry  
7. Isoelectric Focusing  
8. “-Oomics” Technologies
## Agenda

### A Biomarkers for Multiple Sclerosis

- **WHY?**
- **WHAT?**
- **WHERE?**
- **HOW?**
- **WHICH?**
GENETIC/IMMUNOGENETIC:

• Biomarkers specified via genomics and immunogenetic techniques.

LABORATORY:

• All other biomarkers that can be measured in body fluids.

IMAGING:

• Biomarkers provided by imaging techniques.
A. GENETIC AND IMMUNOGENETIC BIOMARKERS
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A. GENETIC AND IMMUNOGENETIC BIOMARKERS
1. HLA and Therapeutical Choice

- HLA-DRB1*0401,0408, and 1601 alleles correlate with greater risk of developing neutralizing antibodies against interferon beta (IFN-β), resulting in poor therapeutical outcome.

2. Non-MHC Polymorphisms Attributing Genetic Risk

- Various genome-wide studies revealed many non-MHC single nucleotide polymorphisms as candidates for genetic burden augmentation in MS.

- Polymorphisms of the IL2RA and IL7RA regions seem as the most promising at the moment.

3. TOB-1

- TOB-1 gene has a role against T-cell multiplication, keeping autoreactive cells in a dormant state.
- Its degreased expression leads towards a more intense immune response (higher percentage of Th1 and Th17 cells and lower percentage of T-regulatory cells).

4. Apolipoprotein E (ApoE)

- ApoE is a protein regulating lipid homeostasis, located mostly in astrocytes.
- Carrying allele of ApoE seems to attribute greater risk of developing mental disorders in MS patients.

B. LABORATORY BIOMARKERS
B. LABORATORY BIOMARKERS
Which Biomarker?

I. Biomarkers of Immunological Activation
II. Biomarkers of Neuroprotection
III. Biomarkers of BBB disruption
IV. Biomarkers of demyelination
V. Biomarkers of Oxidative Stress
VI. Biomarkers of Axonal Damage
VII. Biomarkers of Glial Activation Dysfunction
VIII. Biomarkers of Remyelination Repair
IX. Biomarkers of Therapeutic Response
X. Prognostic Biomarkers
XI. Emerging biomarkers
I. Biomarkers of Immunological Activation

1. OCB IgG in CSF:

- Positive OCB IgG in the CSF of patients with CIS was found to duplicate the risk of progression in CDMS in a 4-year observation period.

- Additional studies provide more evidence for OCB IgG being a relevant factor for conversion to CDMS.

2. OCB IgM in CSF

- Some researchers consider them as a bad prognostic biomarker, correlating with disability progression both qualitatively and quantitatively (IgM index).

- OCB IgM against certain myelin lipids may declare a more aggressive disease course.


I. Biomarkers of Immunological Activation

3. Kappa Free (KFLC) and Lambda Free Light Chains (LFLC) in CSF

- KFLC high CSF levels have been repeatedly reported in MS. In comparison to OCB IgG, slightly higher sensitivity with slightly lower specificity has been found.
- KFLC high CSF levels are considered as highly predictive for CIS conversion to CDMS.
- LFLC also represent a sensitive indicator of intrathecal synthesis in inflammatory CNS disorders.

I. Biomarkers of Immunological Activation

4. Measles-Rubella-Zoster Endothecal Reaction (MRZ Reaction)

- MRZ IgG reaction in CSF displays, compared to OCB IgG, a higher specificity for MS diagnosis and higher prognostic value of progression from CIS to CDMS [48].

- Moreover, MRZ reaction indicates a primarily B-cell mediated immune response, guiding therapeutical choice towards a relevant immunomodulating agent [49].


1. Biomarkers of Immunological Activation

5. Epstein-Barr Virus (EBV) Reaction

- Cepok et al. reported a high percentage of IgG antibodies against protein epitopes BRRF2 and EBNA-1 of the virus, in the serum and CSF samples from MS patients [50].

- EBV antibodies are considered as indicative of higher inflammatory activity and early disease onset [53].


I. Biomarkers of Immunological Activation

6. Chemokines

- Chemokine CXCL13 mobilizes B-cells and T-helper cells towards active demyelinating lesions by interacting with CXCR5 receptor.
- Consistent correlation of CXCL13 CSF levels with CSF B-cells, plasmablasts, and intrathecal Ig synthesis has been reported.
- High levels of CXCL13 have been found in patients with CIS and CDMS.


I. Biomarkers of Immunological Activation

7. Cytokines

- IFN-γ and TNF-a are the main products of Th1 immune response.

- IL-6 serves as linking arm between B-cell and T-cell immune response as well as a Th-17 response triggering factor. IL-6 serum levels were found to correlate significantly with the relapse frequency in female MS patients and age at onset for all MS patients.

Moreover, studying IL-1 levels in mice led to the conclusion that any imbalance in the IL-1 signalling (increased or decreased) may lead to CNS demyelination.

IL-10 is considered as the main anti-inflammatory cytokine. Recent research implicates single nucleotide polymorphisms at the −592 position of the IL-10 gene to the regulation of CNS autoimmunity.


Flow cytometric analysis revealed that B-cells and monocytes from MS patients overexpress IL-15, and that stimulation of CD8(+) T-cells with the latter cytokine enhances their ability to kill glial cells and enter the BBB.

IL-15 was found elevated in the sera and CSF of MS patients, in comparison with ONDs.


I. Biomarkers of Immunological Activation

8. Adhesion Molecules

- Proinflammatory cytokines cause a rise in CSF expression of sICAMs. High levels of ICAM-1 molecule correlate positively with higher disease activity.

- Higher CSF levels of sICAM-1 and sVCAM-1 were reported in NMO patients, in comparison with MS patients, suggesting that the BBB in NMO displays more severe alterations.


Finally, laminin 411, which is situated within the vascular endothelium, interacts with adhesion molecule CD146, allowing Th17 cells to overcome the BBB.
Osteopontin is a macrophage derived phosphoprotein which enhances IFN-γ and IL-12 levels and diminishes the levels of neuroprotective IL-10.

Serum and CSF osteopontin levels are upregulated during an MS relapse, but this is also the case for many other inflammatory disorders.

1. Biomarkers of Immunological Activation

10. Fetuin-A

- Fetuin-A (alpha2 Hermans Schmid glycoprotein) is a calcium-regulating surface glycoprotein. Protein’s coding m-RNA is overexpressed in MS patients’ CNS, resulting in its high concentrations in active demyelinating lesions.

- Fetuin-A seems to antagonize anti-inflammatory TGF-β1. Good responders in Natalizumab treatment present a reduction in Fetuin-A CSF levels.

II. Biomarkers of Neuroprotection

1. Vascular Endothelial Growth Factor-A (VEGF-A)

- VEGF-A is a factor of angiogenesis with neuroprotective properties. Diminished m-RNA expression of VEGF-A in serum monocytes of patients with SPMS compared to RRMS patients has been reported.

- VEGF-A could serve as biomarker of progression from RRMS to SPMS.

II. Biomarkers of Neuroprotection

2. Vitamin D

• Vitamin D suppresses Th1 immune response and enables the production of many neurotrophic factors. 25-Hydroxyvitamin D levels in untreated MS patients correlate inversely with radiologic disease activity.

• Recently, a vitamin D response element (VDRE) was recognized close to the HLA-DRB1*1501 coding area, with the aid of genomics.

II. Biomarkers of Neuroprotection

2. Vitamin D

• Vitamin D displays an inhibitory role in MS, also at a genetic level, by interacting with VDRE.

• Interestingly, Stewart et al. recently concluded that part of IFN-β therapeutic effects during MS relapses may be attributed to greater production of Vitamin D.


III. Biomarkers of BBB disruption

1. Matrix Metalloproteinase Proteins (MMPs)

- Serum and CSF MMPs levels are constantly elevated during MS relapse. MMP-9 levels have been found elevated in patients with RRMS.

- CSF CCR2(+)CCR5(+)CCR6(−) T-cell population expresses high levels of MMP-9 during relapse.


III. Biomarkers of BBB disruption

2. Ninjurin-1

• The degree of expression of the protein Ninjurin-1 by endothelial cells of the BBB and myeloid antigen-presenting cells (APCs) plays an important role in the transmigration and localization of the latter inside CNS, as it was made obvious by proteomic screen of human BBB cells.

• Ninjurin-1 was found up-regulated in active demyelinating lesions.

III. Biomarkers of BBB disruption

3. sICAM-1

- CSF sICAM-1 levels from NMO patients were found to correlate adequately with other measures of BBB disruption, like the albumin quotient and the gadolinium-enhanced lesions in MRI.

III. Biomarkers of BBB disruption

4. Endothelin System

- The term refers to an endothelial proteinic system that plays role in the transmigration of monocytes through the BBB.

- Major components of this system are the proteins endothelin-1, endothelin type B receptor, and endothelin-converting enzyme-1.

IV. Biomarkers of demyelination

1. Myelin Basic Protein (MBP)

- And its fragments are found in large quantities in the CSF of most MS patients during a relapse (80%).

IV. Biomarkers of demyelination

2. αB-Crystalline

- αB-Crystalline is a heat-shock protein which forms aggregates during stress.
- Its mechanism of action encompasses activation of IL-17, IL-10, IL-13, TNF, and chemokines CCL5 and CCL1.

V. Biomarkers of Oxidative Stress

- In MS, inflammation, demyelination, and neurodegeneration can increase the level of metabolic and oxidative stress, which in turn likely contribute to disease progression.

- Biomarkers indicative of oxidative stress pathway activity would help quantify the impact of oxidative stress on disease progression in MS.

V. Biomarkers of Oxidative Stress

1. Nitric Oxide (NO)

- High serum and CSF levels of NO in inflammatory neurological disorders were reported.
- Higher CSF concentrations were further correlated with higher disability progression rates in MS.

V. Biomarkers of Oxidative Stress

2. Reactive Oxygen Species (ROS)

- ROS damage oligodendrocytes and myelin through radical mediated oxidation.
- Myelin cholesterol breaks down to 7-ketocholesterol, whose levels in the CSF of MS patients have been reported to be elevated.

3. The isoprostane 8-iso-prostaglandin F2a (8-iso-PGF2a)

- Increased levels of 8-iso-PGF2a have been detected in urine and plasma from patients with MS.
- Highly elevated CSF 8-iso-PGF2a levels were observed in 31% of patients with SPMS, identifying a subset of patients with progressive MS that exhibited quantifiable evidence of oxidative stress.


VI. Biomarkers of Axonal Damage

1. Neurofilaments (NFs)

- Neurofilaments are major axonal cytoskeleton proteins consisting of three subunits (light chain/NF-L, intermediate chain/NF-M, and heavy chain/NF-H). NF-L CSF levels in MS patients are considerably higher compared to healthy controls.

- On the other hand, NF-H chains seem to correlate better with disease progression, with significant elevation recorded only in progressive disease forms.


VI. Biomarkers of Axonal Damage

2. Tau Protein

- Tau is a cytoskeleton protein whose basic responsibility is microtubular stabilization. High CSF levels in MS patients have been reported.

- Simultaneous elevation in Tau and NF-H values in CSF, in patients with CIS, has a 70% predictive value of conversion to CDMS, which is superior to the predictive value of MRI.

VI. Biomarkers of Axonal Damage

3. Microtubules

- Microtubules represent a major structural cytoskeleton component, consisting of two subunits, A- and B-tubulin.

- Elevated CSF tubulin and actin values have been reported in progressive disease forms, correlating well with disability measured by EDSS.

VI. Biomarkers of Axonal Damage

4. Amyloid-β (1–42)

- In Alzheimer’s disease, amyloid-β (1–42) accumulates in extracellular insoluble plaques, resulting in reduced CSF levels.
- CSF reduction can be also observed in MS patients, in correlation with greater risk for cognitive decline.

VI. Biomarkers of Axonal Damage

5. 14-3-3 Protein

- Apart from Creutzfeldt-Jacobs disease, elevated CSF values have been reported in 10%–30% of patients with RRMS, but its potential utility as a biomarker for MS seems limited for the time being.

VI. Biomarkers of Axonal Damage

6. N-AcetyloAspartate (NAA)

- NAA is an aminoacid, highly expressed in neurons, which transfers actively water molecules extracellularly against concentration gradient.

- Spectroscopy techniques revealed decreased NAA values in MS lesions, but also in NAWM, in conventional MRI. CSF-NAA reduction correlates adequately with disability progression.

- On the contrary, serum and CSF NAA levels were significantly higher in RRMS patients, in comparison to healthy donors and NMO patients. Subsequently, NAA could be helpful in differential diagnosis between MS and NMO.

VII. Biomarkers of Glial Activation Dysfunction

1. S100B Protein

- S100B is a calcium-binding protein, primarily expressed in astrocytes, whose CSF elevated values have been previously correlated with cerebral injury. There are reports of CSF elevation in RRMS patients, but overall data remain inconclusive.

2. Glial Fibrillary Acidic Protein (GFAP)

- GFAP is a structural protein of the astrocytes whose CSF levels increase in association with gliosis-astrocytosis. High CSF values have been found in SPMS patients, but rarely in RRMS patients, and seem to correlate well with disability progression.

VIII. Biomarkers of Remyelination Repair

1. Neuronal Cell Adhesion Molecule (N-CAM)

- Constant CSF elevation of N-CAM has been repeatedly reported immediately after MS relapse, in adequate correlation with clinical improvement. N-CAM is assumed to have a key role in remyelination process. The exact pathway still remains unclear.

2. Brain-Derived Neurotrophic Factor (BDNF)

- Lower CSF-BNDF levels in SPMS patients comparatively to RRMS patients have been reported. Low BDNF levels are considered to contribute in demyelination and axonal damage progress [128]. BDNF increased production was observed in Glatiramer Acetate responders, correlating well with clinical improvement.
3. **Soluble Molecule Nogo-A**

- Nogo-A is a CNS myelin component that inhibits axonal repair. Its presence in MS patients CSF constitutes a bad prognostic marker of axonal repair.
- Nogo-A is adequately specific for MS, as it could not be isolated in other autoimmune or infectious neurological disorders.

1. Neutralizing Antibodies

- Many initial responders to IFNb can develop NAbs to the drug 4–6 months after beginning the therapy, affecting the efficacy of the drug.

- The incidence of Nab development is dependent on the type of IFNb, as well as the route of administration, ranging from 4 % incidence with intramuscular IFNb-1a to up to 47 % incidence with subcutaneous IFNb-1b.

• Although natalizumab is humanized, it is also immunogenic. Like IFNb NAbs, NAbs against natalizumab can also develop early during treatment, within 6 months.
2. CSF fetuin-A

- Is emerging as candidate biomarkers for accurate and timely determination of the therapeutic efficacy of natalizumab.

IX. Biomarkers of Therapeutic Response

3. Circulating CD49d expression

- Emerging as candidate biomarkers for accurate and timely determination of the therapeutic efficacy of natalizumab.

IX. Biomarkers of Therapeutic Response

4. sICAM-1 and sE-Selectin

- CSF levels reduction of sICAM-1 and sE-Selectin may potentially serve as biomarkers of therapeutical efficacy after cladribine treatment.

Chitinase 3-like 1 (CHI3L1)

- is a chitin-binding protein, which lacks enzymatic activity and is known to play a role in chronic inflammation and tissue injury.

- Multiple studies have identified elevated CSF CHI3L1 levels in patients with MS as the result of an unbiased proteomic screen of CSF samples.
X. Prognostic Biomarkers

Chitinase 3-like 1 (CHI3L1)

- In a study of patients with CIS, elevated CSF CHI3L1 levels were associated with a risk of conversion to clinically definite MS.

- This study suggested that CSF CHI3L1 may have potential use as a prognostic biomarker in MS, although elevated CSF CHI3L1 levels were not specific to MS.

XI. Emerging Biomarker Categories

A. Transcriptomic Signatures
B. Circulating MicroRNAs
C. Exosomes/Microvesicles
D. Antigen Arrays
C. IMAGING BIOMARKERS
C. IMAGING BIOMARKERS
1. Optical Coherence Tomography (OCT)

• OCT is a noninvasive technique using emission of infrared light through the pupil and detection of its reflection from the retina.
• Retinal nerve fiber layer (RNFL) thickness can then be estimated. RNFL thinning can be used as a reliable biomarker of axonal loss, correlating adequately with brain atrophy measures.
• RNFL thickness can serve as biomarker of disease progression and neuroprotection by a certain therapeutical agent.

2. Magnetic Resonance Imaging (MRI)

- The most important MRI biomarkers for MS are the following:
  
  i. **T1 lesions with contrast enhancement**: biomarkers of acute neuroinflammation. Although they are considered as the gold standard for BBB disruption imaging, recent research claims that the same diagnosis can be inferred in many cases by combination of T1, T2, and T2-weighted FLAIR images characteristics alone.

  ii. **Hyperintense T2-weighted lesions**: reflecting a combination of mechanisms like inflammation, demyelination, axonal damage and edema. Their diagnostic value is high, but they correlate moderately with disability.
iii. **Hypointense T1-weighted lesions (black holes):** considered as satisfactory biomarkers of axonal damage. Their correlation with disability remains debatable.

iv. **Whole brain atrophy biomarkers:** the most widely used measure is the brain parenchymal fraction. Brain atrophy worsening rates are higher in untreated MS patients (0.5%–1% annualized decrease) in comparison with healthy controls (0.1%–0.3%). Brain atrophy worsening rate at initial diagnosis has been proposed as prognostic biomarker of disability eight years afterwards.
2. Magnetic Resonance Imaging (MRI)

v. **Gray matter atrophy biomarkers**: recently acquired knowledge suggests gray matter demyelination, axonal damage, and atrophy in MS. Double inversion recovery imaging techniques display gray matter atrophy in all MS stages and types, with higher worsening rates in SPMS patients. Higher worsening rates of gray matter atrophy in CIS patients correlate well with rapid conversion to RRMS.


2. Magnetic Resonance Imaging (MRI)

vi. **Spinal cord atrophy biomarkers:** upper cervical cord area (UCCA) measuring techniques display atrophy most apparently in progressive MS forms, correlating well with disability progression. UCCA atrophy presence in early disease stages in RRMS patients is a bad prognostic biomarker of future disability.

3. Contrast Magnetization Transfer Ratio (MTR)

- It is a novel MRI technique based on proton interaction between free water and macromolecules. In the absence of axonal loss, acute MRI lesions that show recovery display also increase in MTR.
- Optic nerve MTR decrease after optic neuritis shows good correlation with RNFL thickness in OCT (Section 7.1) and with reduction of amplitude in visual evoked potentials, suggesting that MTR is primarily an axonal damage biomarker.
- Nevertheless, reliable assessment of treatment effects on remyelination has been reported.
4. Diffusion Weighted Imaging (DWI) and Diffusion Tensor Imaging (DTI)

- DWI is based on mobility and spatial distribution of water molecules, while DTI measures movement in several directions in space. DTI technique provides two different measures, mean diffusivity (MD) and fractional anisotropy (FA).
- MD increases and FA decreases in hyperintense T2-weighted lesions. Similar alterations can be recorded in NAWM areas in conventional MRI, as well as in normal appearing gray matter (NAGM) areas, especially in progressive disease forms.

4. Diffusion Weighted Imaging (DWI) and Diffusion Tensor Imaging (DTI)

- Corpus callosum DTI abnormalities are present in early MS stages, even when lesions in conventional MRI are still absent.
- MD alterations precede visible in conventional MRI BBB injury by at least 5 months, being thus a reliable predictive biomarker for MS relapse.
- Corpus callosum DTI abnormalities in SPMS patients constitute a bad prognostic biomarker of future disability.

5. Magnetic Resonance Spectroscopy (MRS)

- MRS is a novel imaging method for assessment of pathobiochemical disease processes. The following substances spectroscopic measurements are of particular value in MS:
  
i. NAA: biomarker of neuronal and axonal integrity. NAA showed a progressive decline pattern in a two-year MRS followup of patients with RRMS.
  
ii. Choline: biomarker of myelin loss;
  
iii. Myoinositol and creatine: biomarkers of gliosis
  

5. Magnetic Resonance Spectroscopy (MRS)

- Early spectroscopic changes represent a bad prognostic factor of future disability. Spectroscopic findings suggest that white matter abnormalities in RRMS are more prominent than grey matter abnormalities where the injury is less diffuse.
- Diffusion tensor spectroscopy (DTS), a technique combining properties of DTI and MRS, seems promising in better distinguishing axonopathy, demyelination, inflammation, edema, and gliosis.
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Modern PET tracers have the ability to bind in proteins that show upregulation in activated microglia, making possible an early visualization of NAWM and NAGM disorders, even before contrast enhancement in conventional MRI. At present, the use of PET in MS remains experimental.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Description</th>
<th>Utility in MS</th>
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<tbody>
<tr>
<td>NAbs</td>
<td>NAbs to IFNβ and natalizumab</td>
<td>Serum NAb testing is used to support lack of response to IFNβ or natalizumab</td>
</tr>
<tr>
<td>Fetuin-A</td>
<td>Secreted glycoprotein elevated in CSF of patients with MS; fetuin-A expression is associated with MS-specific brain pathology</td>
<td>CSF biomarker of subclinical disease activity and therapeutic response to natalizumab</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Matrix protein with pleiotropic functions, including pro-inflammatory cytokine; secreted by activated immune cells and abundantly expressed in MS lesions</td>
<td>CSF biomarker of disease activity, intrathecal inflammation, and therapeutic response to natalizumab</td>
</tr>
<tr>
<td>8-iso-PGF₂α</td>
<td>Isoprostane byproduct of lipid peroxidation and a readout of oxidative stress; CSF 8-iso-PGF₂α levels are elevated in a subset of patients with MS</td>
<td>CSF biomarker of oxidative stress, with possible predictive value for therapeutics targeting oxidative pathways</td>
</tr>
<tr>
<td>CXCL13</td>
<td>B-cell chemokine elevated in CSF of patients with MS, indicative of humoral responses.</td>
<td>CSF biomarker of intrathecal B-cell response; potential biomarker of therapeutic response to rituximab and natalizumab</td>
</tr>
<tr>
<td>NFL/NFH</td>
<td>Axonal proteins elevated in CSF as a result of axonal injury</td>
<td>CSF NFH is a possible biomarker of accumulated axonal damage in progressive MS; CSF NFL is a possible biomarker of reduced axonal damage after natalizumab or rituximab</td>
</tr>
<tr>
<td>CHI3L1</td>
<td>Chitinase 3-like protein elevated in CSF of patients with CIS who convert to RRMS; expressed by microglia and astrocytes in brains of patients with MS</td>
<td>Prognostic CSF biomarker of conversion from CIS to RRMS; possible biomarker of therapeutic response to natalizumab</td>
</tr>
</tbody>
</table>

8-iso-PGF₂α, 8-iso-prostaglandin F₂α, CHI3L1 chitinase 3-like 1, CIS clinically isolated syndrome, CSF cerebrospinal fluid, CXCL13 chemokine (C-X-C motif) ligand 13, IFN interferon, NAbs neutralizing antibodies, NFH neurofilament heavy, NFL neurofilament light, RRMS relapsing–remitting multiple sclerosis