Biomarkers and Multiple Sclerosis

Biomarkers have resulted from breakthrough treatments and, in turn, provide targets for more breakthroughs.

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**Biomarkers**

The value of a biomarker is in its ability to predict or be a surrogate for a clinical state of a patient. In this review we take the approach of how various biomarkers span multiple domains to define a clinical state in patients with multiple sclerosis (MS). Viewed in this manner, the value of various biomarkers in understanding the patient with MS becomes evident.

A biomarker can be a clinical/physiological sign, enzyme, hormone, imaging modality, or a molecular marker (eg, gene or gene product) that can be objectively measured. The reader is referred to the FDA/NIH working group resource, Biomarkers, Endpoints, and Other Tools (BEST) for the various categories of biomarkers.1

One of the best recent examples that has revolutionized patient care is the discovery of aquaporin 4 antibodies that show neuromyelitis optica spectrum disorder (NMOSD) is a different entity from MS.2 Treating patients with NMOSD with disease-modifying treatments (DMTs) approved for treatment of patients with MS can have disastrous consequences and should be avoided.

Early diagnosis and treatment of patients with MS can alter disease course and slow disability progression. The need to improve diagnosis for initiation of these breakthrough treatments and to monitor patients’ responses to treatment have led to advances in biomarkers in MS. In turn, these biomarkers sometimes become new targets for more potential breakthrough treatments. The search for a reliable biomarker to predict disease progression and monitor response to therapy remains a challenge. Several biomarkers including MRI, blood and CSF markers, and optical coherence tomography (OCT) are promising in this regard.

We focus on biomarkers that are reliable tools for diagnosis and monitoring patients with MS in clinical practice (Table 1) and briefly summarize potential future biomarkers.

**Diagnostic Markers**

**Magnetic Resonance Imaging**

MR imaging is without a doubt the most useful adjunct to clinical evaluation in the diagnosis of patients with MS (Table 2). Unlike previous criteria, current McDonald criteria place emphasis on the morphology and location of the lesions than the quantity of lesions. The periventricular, infratentorial, corpus callosal, juxtacortical locations, and morphology (ovoid, linear) take precedence over numbers of T2 lesions. Presence of asymptomatic spinal cord or cortical lesions, or lesions enhancing with gadolinium-based contrast agents can be quite valuable in ruling out alternative diagnoses that can present with multifocal white matter lesions. Today, the diagnosis of MS can be made based on a single scan following an isolated clinical event.3 The diagnostic value of MRI has been further shown in patients with radiologically isolated syndrome (RIS), in which the presence of asymptomatic spinal cord lesions can be a predictor of the probability of developing the first clinical event.4

**Intrathecal Immunoglobulin G Synthesis**

Increased intrathecal immunoglobulin G (IgG) synthesis is a hallmark of MS, occurring in > 90% of patients with definite MS, identified quantitatively as an elevated IgG index or qualitatively as the presence of IgG oligoclonal bands (OCB).5 The presence of CSF-specific IgG OCB in patients with MS reflects clonal expansion of immunoglobulin-secreting B cells and plasma cells in the central nervous system (CNS). The presence of OCB in CSF also predicts conversion from clinically isolated syndrome (CIS) to definite MS.6 The new diagnostic criteria for MS include elevated IgG index and the presence of CSF OCB as evidence of dissemination in time, which allows for diagnosis of early MS in patients with a single clinical attack or CIS.3

**Biomarkers to Guide Choice of Therapy**

**John Cunningham Virus Antibody Index**

Use of natalizumab, a humanized antibody to α4 integrin,
which reduces cell traffic across the blood-brain barrier, is used to treat patients with MS. Natalizumab can be associated with progressive multifocal encephalopathy (PML), a rare but potentially fatal opportunistic brain infection caused by reactivation of John Cunningham virus (JCV). This association led to the use of a 2-step JCV serology test as a biomarker to guide the decision of whether or not to use natalizumab. Patients who are JCV-negative and have not previously been on immunosuppressants have the least likelihood of PML (1/1,000). Patients with a high JCV index, long duration of therapy, and previous immunosuppression, have the least favorable risk. This biomarker is only relevant for stratifying a patient’s risk of PML with use of natalizumab and should not be used to predict PML risk for patients using other treatments (eg, fingolimod or dimethyl fumarate). It should also be noted that JCV-negative status is not equivalent to noninfected status due to seroconversion and false negatives in some patients.

Use of the JCV index is among the best examples of biomarker used to guide choice of a specific therapy in patients with MS and is an invaluable tool in monitoring this potentially hazardous drug.

Neutralizing Antibodies
Approximately 40% of patients who take interferon β (IFN-β) generate neutralizing antibodies (NAbs) after 12 to 18 months, which may lead to reduced response. Production of NAbs is lowest with intramuscular IFN-β-1a and highest with subcutaneous IFN-β-1b. Because there is cross-reactivity to different formulations of IFN-β, a patient with NAbs should be switched to a nonIFN-β DMT.

Patients treated with natalizumab infrequently develop NAbs to natalizumab (4%-12% of patients), typically within 3 months of starting treatment. Here too, a patient should be switched to another therapy if NAbs are detected.

CD19 Lymphocyte Counts
Ocrelizumab, approved for treatment of patients with relapsing and primary-progressive MS and rituximab, used off-label for treating patients with MS, both target the CD20 antigen, found on pre-B cells, mature, and memory B cells, but not on lymphoid stem cells and plasma cells. Cells expressing CD19 have a similar expression profile as cells expressing CD20, and peripheral CD19 lymphocyte count is used as a surrogate marker to monitor treatment efficacy of ocrelizumab and rituximab. The time frame for restoration of the B-cell population that is depleted after a single course of rituximab or ocrelizumab is variable and therefore, so is the dose interval. Blood count of CD19 lymphocytes can potentially be a useful marker to guide optimal dosing interval and minimize unnecessary exposure to these extensive therapies with serious potential toxicity from long-term exposure.

Prognostic Markers
Human Leukocyte Antigen
Human leukocyte antigen (HLA) has been associated with MS for decades, however, HLA association with prognosis is more recent. Having both HLA-DRB1*15 and OCB is associated with faster disease progression. In contrast, positivity for HLA-A*02 can be associated with better clinical and MRI outcomes. A protective association of HLA-A*02:01 in African Americans, independent of the HLA-DRB1 risk alleles has been observed along with a lower frequency for HLA-DRB1*11.

Cerebrospinal Fluid Oligoclonal IgG Bands
Presence of OCB at diagnosis is associated with more severe gray-matter pathology and higher physical disability and cognitive impairment after 10 years. Levels of B-cell-related cytokines and proinflammatory immune responses in patients who have findings of OCBs support the proposed role of a compartmentalized, intrathecal B-cell response in pathogenesis of cortical lesions and OCB production.
There is a significant correlation of early breakthrough activity on MRI in the form of new T2 or gadolinium-enhancing lesions 1 year after therapy initiation with subsequent clinical progression. MRI of brain and sometimes the spinal cord, is used in clinical practice to guide therapy. Changes in MRI during therapy are probably the best indicator of how effective a DMT is for an individual patient. The goal of treatment is to achieve the state defined as no evidence of disease activity (NEDA), although this is sometimes a lofty goal in practice. NEDA is traditionally defined as a triad of no relapse, progression, or evidence of new lesions on MRI. Although NEDA may be the ideal, changing therapies based solely on MRI activity can be short-sighted especially because the value of achieving NEDA has recently been challenged because patients who fulfilled criteria for NEDA did not always fall into the desirable low-morbidity group on longitudinal study. MRI is also a very helpful surrogate biomarker of CNS tissue inflammation, injury and repair. Although it is well recognized that total disease burden in the MRI is a poor surrogate for function, it has been demonstrated that the total T2 lesion burden at the time of initial presentation and 5 years after disease onset are among the best measures of future disease severity. Total T2 volume > 1.23 mm³ at presentation is indicative of a worse prognosis at 15 years.

Markers That Show Promise (Table 3)

**Brain Atrophy**

Probably the best MRI correlate for disease progression is annual loss of brain volume, which is 0.5% to 1.5% in persons with MS compared to 0.1% to 0.5% in persons without MS. Brain volume is the sum total of many competing changes that occur during pathogenesis of MS, which include expansion during acute inflammation, loss of volume after treatment with DMTs termed pseudoatrophy, and most importantly, further loss from gliosis and neuroaxonal loss. Regional brain-volume loss may be a useful marker of specific impairments; thalamic-volume loss has been correlated with cognitive impairment, depression, and fatigue. Measurement of atrophy in routine patient care is not yet feasible, in part because of the large postprocessing needed to make such measurements meaningful.

**MR Spectroscopy**

Magnetic resonance spectroscopy allows the examination of qualitative changes in brain levels of N-acetyl aspartate (NAA) produced by neuronal mitochondria and serves as a marker for neuronal/axonal integrity and health. Similarly, myoinositol (MI) is a marker for glia, particularly astrocytes, that can be useful in estimating the degree of gliosis. In a longitudinal follow-up study it was shown that the MI/NAA metabolite ratio in normal-appearing white matter predicted progression of MS as well as the measure of brain atrophy.

### Table 2. A Partial Review of MR Imaging Correlates

<table>
<thead>
<tr>
<th>Pathology</th>
<th>MR Correlate</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>T2/T2 FLAIR lesion</td>
<td>May disappear during recovery if progression to demyelination doesn’t occur</td>
</tr>
<tr>
<td>Blood-brain barrier integrity</td>
<td>Gd enhancing lesion</td>
<td>Most T2 lesions (demyelination) are preceded by a Gd⁺ lesion</td>
</tr>
<tr>
<td>Edema</td>
<td>T2/T2 FLAIR lesion</td>
<td>Revert to normal during recovery</td>
</tr>
<tr>
<td>Demyelination</td>
<td>T2/T2 FLAIR lesion</td>
<td>Generally permanent; remyelination may occur despite not being seen on MRI</td>
</tr>
<tr>
<td>Axonal loss</td>
<td>Hypodensities (black holes) on T1</td>
<td>Hypodensities should be distinguished between transient and permanent most Gd⁺ lesions are hypointense on T1 but revert during recovery</td>
</tr>
<tr>
<td>Glial</td>
<td>Myoinositol spectroscopy</td>
<td>Good marker for astroglialis</td>
</tr>
<tr>
<td>Tissue injury/disruption of architecture</td>
<td>Magnetization transfer imaging/ratio</td>
<td>Degree of tissue destruction/disorganization is a predictor of future black hole formation</td>
</tr>
<tr>
<td>Hypoxia/energy crisis</td>
<td>Diffusion abnormality</td>
<td>Indicative of mitochondrial dysfunction</td>
</tr>
<tr>
<td>Progressive MS</td>
<td>Atrophy</td>
<td>Best metric for progressive multiple sclerosis</td>
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**Markers for Disease Progression and Response to Therapy**

**MRI Predictors of Disease Progression**

The predictive value of MRI is seen in patients with RIS, in whom lesions are a good predictor of future conversion of RIS to MS (Table 2). MRI is also a prognostic tool using brain or spinal cord atrophy as indirect markers of neurodegeneration. The presence of thalamic atrophy and increased ventricular size in patients with RIS may be associated with a risk of developing definite MS. Spinal cord gray matter atrophy correlates with disability in patients with progressive MS. Cortical lesions contribute to physical and cognitive disability in patients with MS.
Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) is an MRI modality that assesses tissue by measuring the motility of water molecules that has been proposed as a potential biomarker of neurodegeneration, demyelination, and remyelination in MS. The DTI parameters used to study tissue damage, myelin loss, and remyelination include fractional anisotropy (FA), radial diffusivity (RD), mean diffusivity (MD), and apparent diffusion coefficient (ADC). Decreased FA values seen in MS lesions and normal-appearing white and gray matter in patients with MS likely reflect axonal and cortical injury. Decrease in FA is seen in acute enhancing lesions whereas increased radial diffusivity is related to myelin loss in MS lesions.

Ultra-High Field MRI

Probably one of the greatest breakthroughs in recent times has been the ability to image brain at ultra-high fields of 7 T or 8 T. Although FDA-approved, this technology has not entered routine use yet. The signal-to-noise ratio is vastly improved as is spatial resolution but level of artifacts is high, and the spine and posterior fossa cannot be imaged. Cortical lesions of MS can be imaged at a microscopic level with 8 T MRI. The utility of imaging at 8 T may be in the ability or disease progression.

Optical Coherence Tomography

Axons in the human retinal nerve fiber layer (RNFL) are unmyelinated and can be imaged with OCT to examine changes in unmyelinated axons in the CNS of patients with MS. OCT measures of thickness of the RNFL, ganglionic cell, and inner plexiform layer (GCIP) are considered good makers of retinal ganglion axon (ie, optic nerve) integrity. Because axon loss is believed to be due to neurodegeneration and considered among the most important pathogenic mechanisms contributing to disability and disease progression in patients with MS, OCT metrics of RNFL and GCIP thickness have been proposed as an alternative diagnostic modality for assessing and quantitating neurodegeneration. The degree of RNFL thinning also correlates with MRI measures of brain atrophy and with physical and cognitive disability in patients with MS. Specifically, GCIP atrophy correlates with gray matter atrophy, especially in patients with progressive MS, reflecting underlying disease progression.

Neurofilament Light Chain

Serum and CSF levels of neurofilament light (NfL), 1 of 3 major components of axonal neurofilaments and a marker of axon degeneration, have been proposed as promising predictive markers for disease activity and disability in MS. Serum NfL levels correlate with the CSF NfL levels and predict MRI disease activity (eg, lesion volume, acute enhancing lesion, and whole brain atrophy). The baseline levels of CSF NfL are predictive of disease activity (relapses, MRI activity or disability worsening) during various stages of MS with the highest levels observed during a relapse. Serum NfL levels are elevated in relapsing-remitting MS and correlate with CSF levels and with MRI measures of disease activity including white matter-lesion volume and enhancing lesions, and DMT may lower the CSF NfL levels in patients with MS. Levels of NfL in CSF have been associated with definite diagnosis of MS in children and in adults with CIS.

Astroglial Markers

Glia fibrillary acidic protein (GFAP) is a major cytoskeleton protein of astrocytes that can be measured in the CSF, reflecting the rate of astrogliosis, and may be a potential biomarker for disease progression and the effect of DMT. Levels of GFAP seem to be a useful biomarker for highly active acute inflammation in patients with relapsing-remitting MS and those with CIS. Levels of GFAP in CSF predicted future disability in a study in which NfL levels had shown no relationship to disability or disease progression.

Microglial Markers

Chitinase-3-like protein 1 (CH3L1) is a protein expressed by microglia, macrophages, and astrocytes, all of which are implicated in the pathogenesis of MS, making CH3L1 a potential biomarker. High CSF levels of CH3L1 in patients with CIS are associated with a higher rate of conversion to clinically definite MS. The CSF levels of CH3L1 correlate with the clinical and/or radiological disease activity. Additionally, CH3L1 levels in CSF correlate with disability progression.

| TABLE 3. POTENTIAL FUTURE BIOMARKERS |
|-------------------|-------------------|
| Marker            | Metric            |
| CH3L1             | Microglial activation |
| GFAP              | Astroglia         |
| NfL               | Inflammatory disease activity, brain atrophy |
| DTI               | Inflammatory disease activity, atrophy |
| MRI               | Brain atrophy measurement |
| MR spectroscopy   | Neurodegeneration, brain atrophy |
| OCT               | Retinal markers of gray matter atrophy |

Abbreviations: CH3L1, chitinase 3-like protein 1; DTI, diffusion-tensor imaging; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; OCT, optical coherence tomography.
Summary

Biomarker discovery is an area of unmet need and MRI-guided therapy has become the standard of care. Increased utility of this modality can be expected in the future with improved technology and decreasing costs. Markers in body fluid will complement imaging, especially serum markers because frequent sampling of CSF, although desirable is simply not practical. In this regard, examination of NFl shows great promise since serum levels of NFl correlated well with CSF levels. Improved understanding of the pathogenesis of MS affords not only opportunities for early intervention with treatment modalities but also the opportunities to develop better and meaningful markers of inflammation, injury, and most of all, repair.