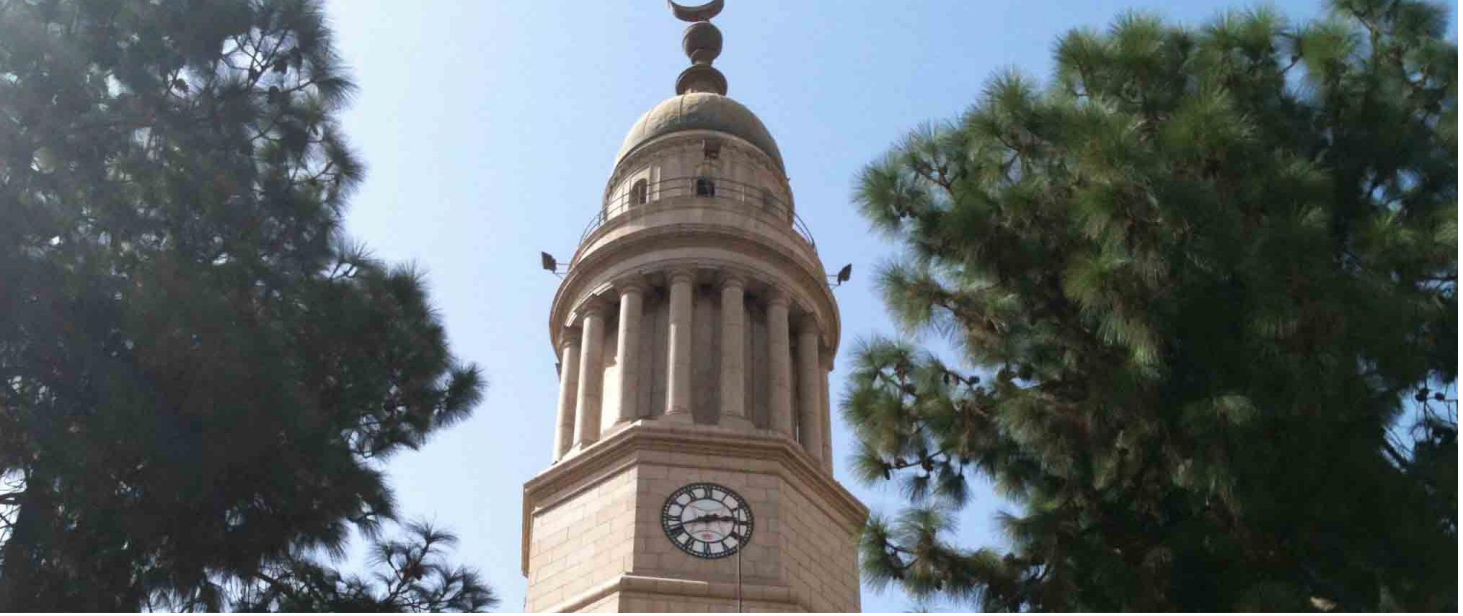


Novel Biomarkers in Multiple Sclerosis

Amr Hassan
Associate Professor of Neurology
Cairo University

Agenda

A Biomarkers for Multiple Sclerosis

- **WHY?**
- WHAT?
- WHERE?
- HOW?
- WHICH?

A Biomarkers for Multiple Sclerosis:WHY ?

- 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 Biomarkers for Multiple Sclerosis:WHY ?

- 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

Shirani A, Zhao Y, Karim ME, Evans C, Kingwell E, van der Kop ML, et al. Association between use of interferon beta and progression of disability in patients with relapsing–remitting multiple sclerosis. JAMA. 2012;308(3):247–56.

A Biomarkers for Multiple Sclerosis:WHY ?

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.

Lucchinetti CF, Bruck W, Rodriguez M, Lassmann H. Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. Brain Pathol. 1996;6(3):259–74.

A Biomarkers for Multiple Sclerosis:WHY ?

- 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.

Sadiq S. Multiple sclerosis. In: Rowland L, editor. Merritt's neurology. Philadelphia: Lippincott Williams and Wilkins; 2005. p. 941–63.
Barkhof F. The clinico-radiological paradox in multiple scle- rosis revisited. Curr Opin Neurol. 2002;15(3):239–45.

A Biomarkers for Multiple Sclerosis:WHY ?

- 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.

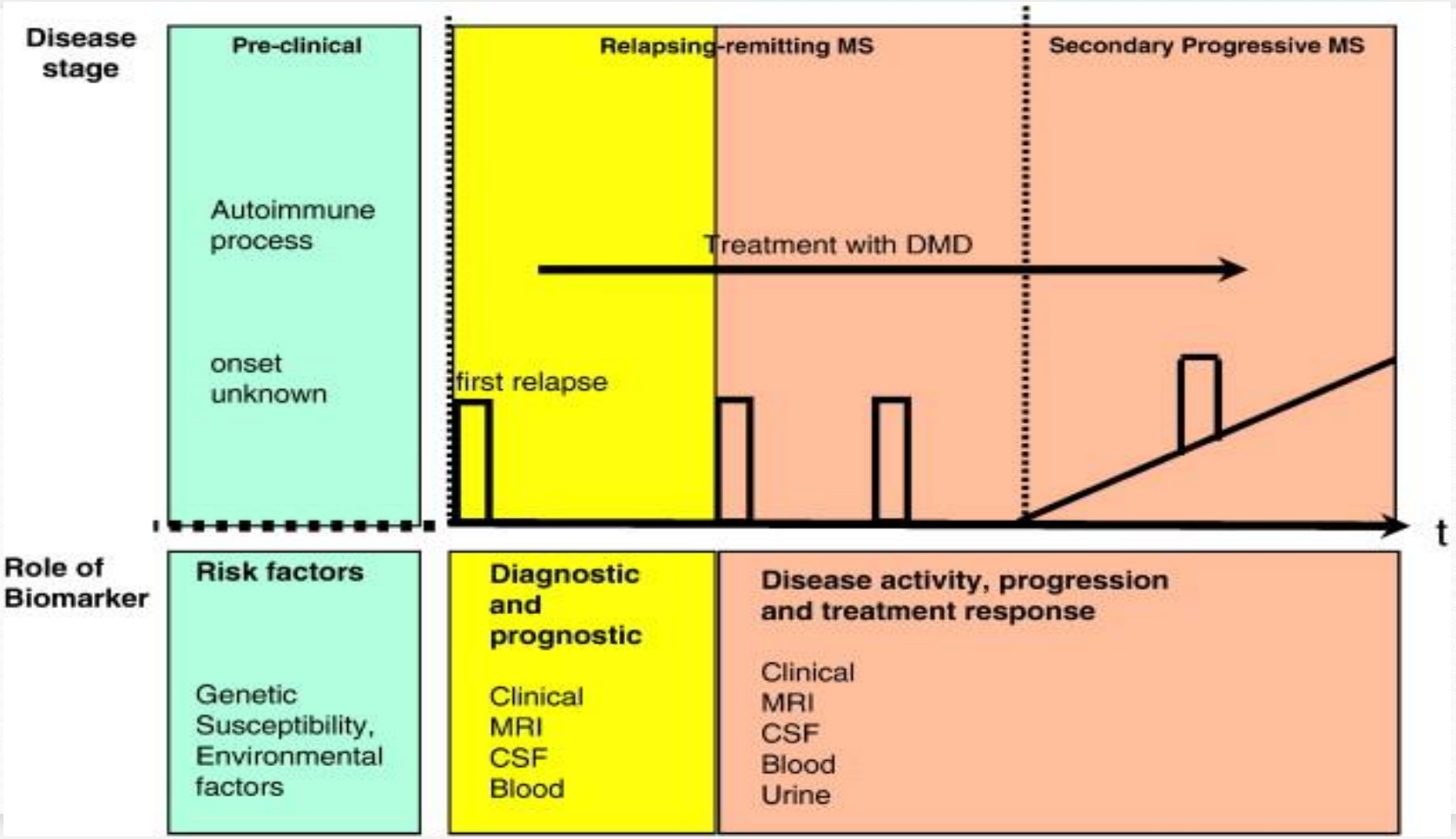
- Identification and validation of predictive biomarkers of therapeutic response are urgently needed to help guide optimal treatment management strategies in patients with MS.

Harris VK, Sadiq SA. Disease biomarkers in multiple sclerosis: potential for use in therapeutic decision making. Mol Diagn Ther. 2009;13(4):225–44.

A Biomarkers for Multiple Sclerosis:WHY ?

- 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 ?



Agenda

A Biomarkers for Multiple Sclerosis

- WHY?
- **WHAT?**
- WHERE?
- HOW?
- WHICH?

A Biomarkers for Multiple Sclerosis:WHAT ?

A Biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutical intervention

Agenda

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. Diurinal variation

2- CSF

Advantages:

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

1. The levels of a CSF biomarker cannot be influenced by liver or kidney function

Gafson AR, Giovannoni G. Towards the incorporation of lumbar puncture into clinical trials for multiple sclerosis. Mult Scler. 2012;18(10):1509–11.

2- CSF

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.

G. Calais, G. Forzy, C. Crinquette et al., “Tear analysis in clinically isolated syndrome as new multiple sclerosis criterion,” Multiple Sclerosis, vol. 16, no. 1, pp. 87–92, 2010.

5- Saliva

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

A. Minagar, I. Adamashvili, R. E. Kelley, E. Gonzalez-Toledo, J. McLarty, and S. J. Smith, “Saliva soluble HLA as a potential marker of response to interferon-βIa in multiple sclerosis: a preliminary study,” Journal of Neuroinflammation, vol. 4, article 16, 2007

6- MRI

- There are though considerable difficulties in correlating MRI findings with disability progression. Certain novel ambitious techniques promise to overcome all these problems.

Agenda

A Biomarkers for Multiple Sclerosis

- WHY?
- WHAT?
- WHERE?
- **HOW?**
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A Biomarkers for Multiple Sclerosis:HOW ?

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. “-Omics” Technologies

Agenda

A Biomarkers for Multiple Sclerosis

- WHY?
- WHAT?
- WHERE?
- HOW?
- **WHICH?**

A Biomarkers for Multiple Sclerosis: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.

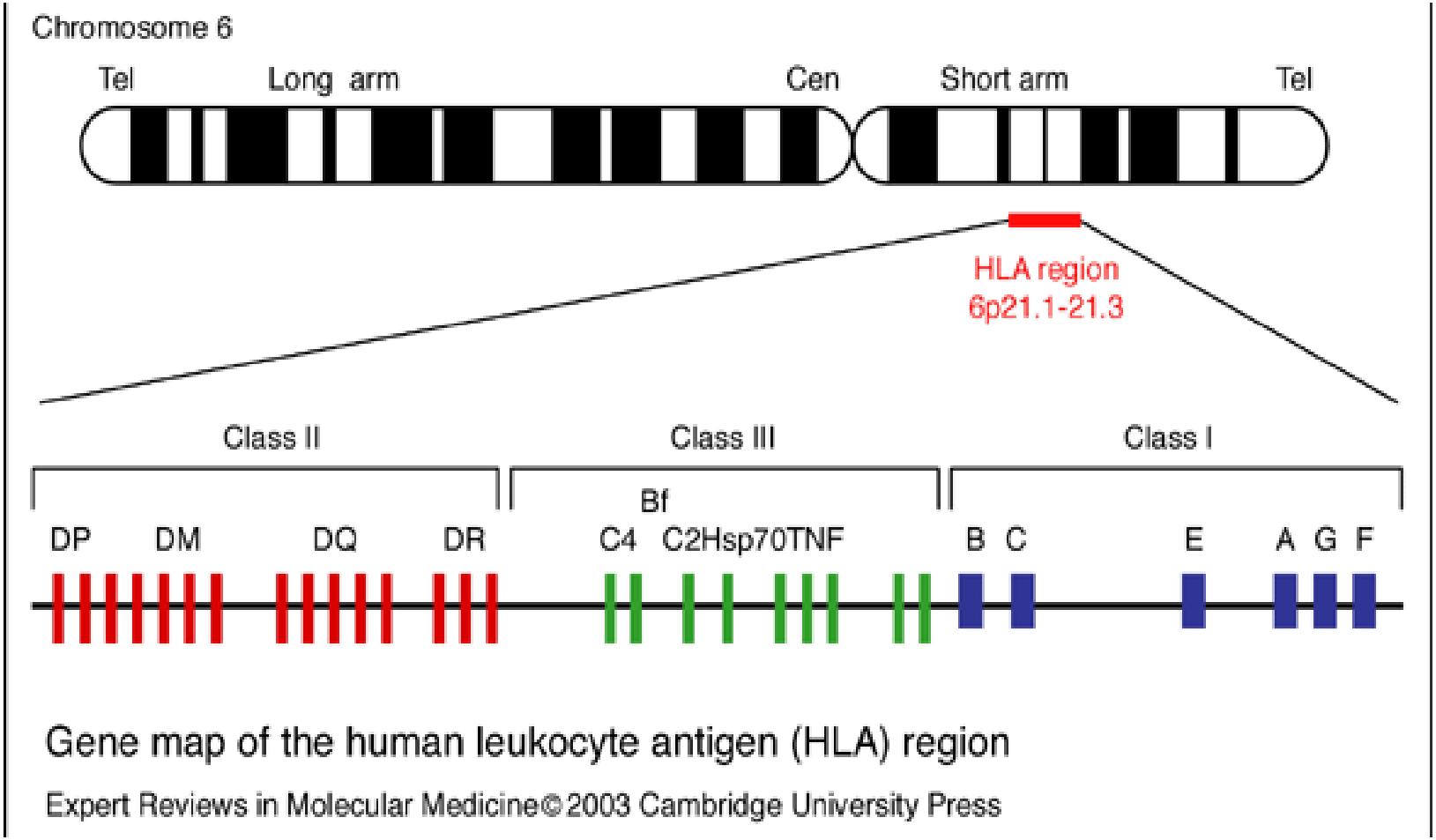
A. GENETIC AND IMMUNOGENETIC BIOMARKERS

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.

D. Buck, S. Cepok, S. Hoffmann et al., "Influence of the HLA-DRB1 genotype on antibody development to interferon beta in multiple sclerosis," Archives of Neurology, vol. 68, no. 4, pp. 480–487, 2011.

HLA gene map



Gene map of the human leukocyte antigen (HLA) region

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.

International Multiple Sclerosis Genetics Consortium, “Risk alleles for multiple sclerosis identified by a genomewide study,” The New England Journal of Medicine, vol. 357, no. 9, pp. 851–862, 2007.

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).

J. C. Corvol, D. Pelletier, R. G. Henry et al., “Abrogation of T cell quiescence characterizes patients at high risk for multiple sclerosis after the initial neurological event,” Proceedings of the National Academy of Sciences of the United States of America, vol. 105, no. 33, pp. 11839–11844, 2008.

4. Apolipoprotein E (ApoE)

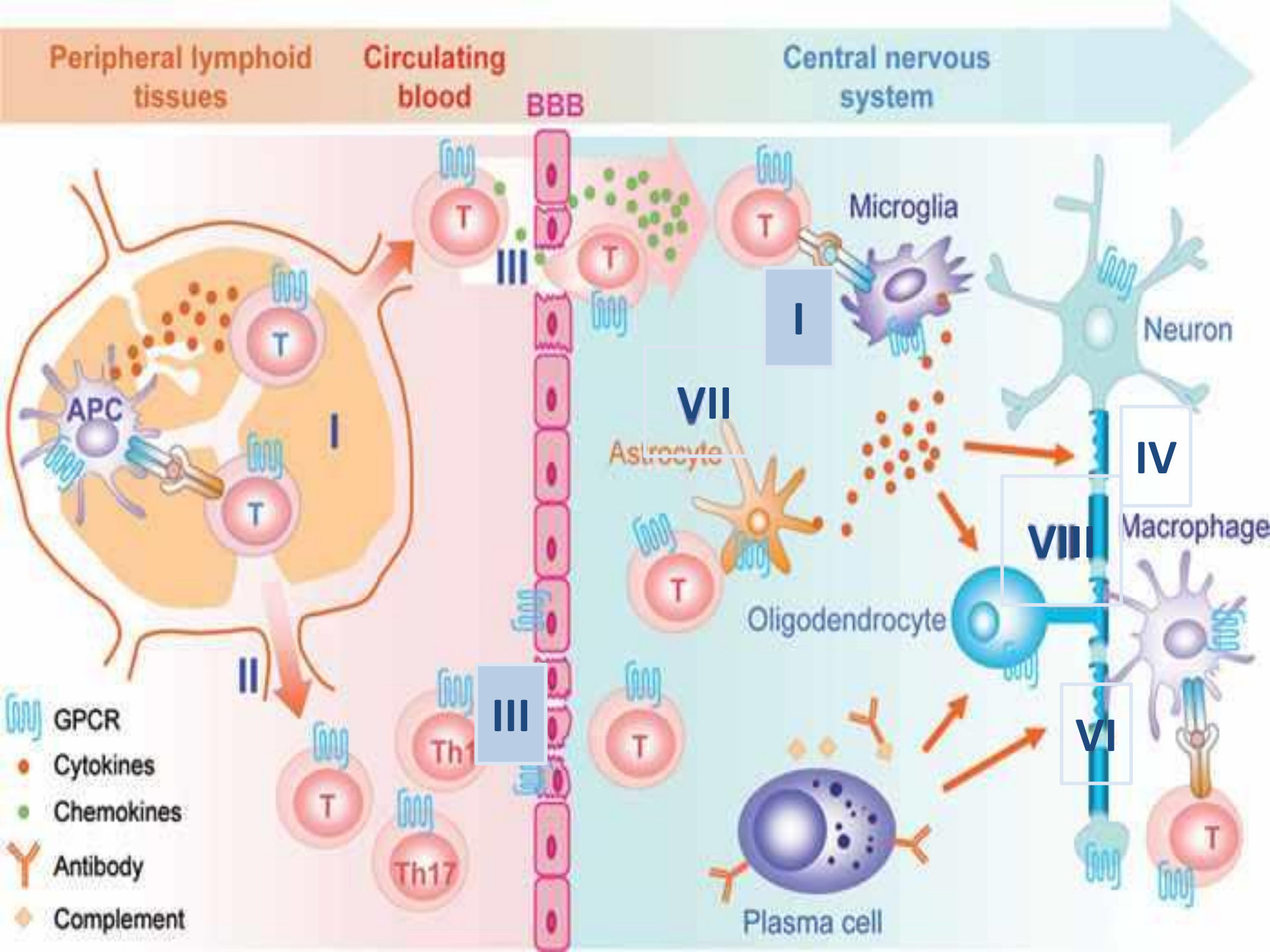
- ApoE is a protein regulating lipid homeostasis, located mostly in astrocytes.
- Carrying allele of ApoE seems to attribute greater risk of developing mental disorders in MS patients.

H. L. Zhang, J. Wu, and J. Zhu, "The immune-modulatory role of apolipoprotein E with emphasis on multiple sclerosis and experimental autoimmune encephalomyelitis," Clinical and Developmental Immunology, vol. 2010, Article ID 186813, 10 pages, 2010.

B. LABORATORY BIOMARKERS

B. Laboratory Biomarkers

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. Emering biomarkers


Peripheral lymphoid tissues
Circulating blood
BBB
Central nervous system
APC
T
I
II
III
Th1
Th17
Microglia
Neuron
VII
Astrocyte
IV
VIII
Macrophage
Oligodendrocyte
VI
Plasma cell
GPCR
Cytokines
Chemokines
Antibody
Complement

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.

M. Tintoré, A. Rovira, J. Río et al., "Do oligoclonal bands add information to MRI in first attacks of multiple sclerosis?" Neurology, vol. 70, no. 13, pp. 1079–1083, 2008.
P. Nilsson, E. M. Larsson, P. Maly-Sundgren, R. Perfekt, and M. Sandberg-Wollheim, "Predicting the outcome of optic neuritis: evaluation of risk factors after 30 years of follow-up," Journal of Neurology, vol. 252, no. 4, pp. 396–402, 2005.

I. Biomarkers of Immunological Activation

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.

J. Mandrioli, P. Sola, R. Bedin, M. Gambini, and E. Merelli, "A multifactorial prognostic index in multiple sclerosis: cerebrospinal fluid IgM oligoclonal bands and clinical features to predict the evolution of the disease," Journal of Neurology, vol. 255, no. 7, pp. 1023–1031, 2008.
L. Villar, N. Garcia-Barragan, M. Espino, et al., "Influence of oligoclonal IgM specificity in multiple sclerosis disease course," Multiple Sclerosis, vol. 14, pp. 183–187, 2008.

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.

S. Presslauer, D. Milosavljevic, T. Brücke, P. Bayer, and W. Hübl, "Elevated levels of kappa free light chains in CSF support the diagnosis of multiple sclerosis," Journal of Neurology, vol. 255, no. 10, pp. 1508–1514, 2008.

L. M. Villar, M. Espino, L. Costa-Frossard, A. Muriel, J. Jimenez, and J. C. Alvarez-Cermeno, "High levels of cerebrospinal fluid free kappa chains predict conversion to multiple sclerosis," Clinica Chimica Acta, vol. 413, no. 23-24, pp. 1813–1816, 2012.

B. Arneth and F. Birklein, "High sensitivity of free lambda and free kappa light chains for detection of intrathecal immunoglobulin synthesis in cerebrospinal fluid," Acta Neurologica Scandinavica, vol. 119, no. 1, pp. 39–44, 2009.

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].

J. Brettschneider, H. Tumani, U. Kiechle et al., "IgG antibodies against measles, rubella, and varicella zoster virus predict conversion to multiple sclerosis in clinically isolated syndrome," PLoS One, vol. 4, no. 11, article e7638, 2009.
E. Meinl, M. Krumbholz, and R. Hohlfeld, "B lineage cells in the inflammatory central nervous system environment: migration, maintenance, local antibody production, and therapeutic modulation," Annals of Neurology, vol. 59, no. 6, pp. 880–892, 2006.

I. Biomarkers of Immunological Activation

5. Epstein-Barr Virus (EBV) Reaction

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

S. Cepok, D. Zhou, R. Srivastava et al., "Identification of Epstein-Barr virus proteins as putative targets of the immune response in multiple sclerosis," Journal of Clinical Investigation, vol. 115, no. 5, pp. 1352–1360, 2005.
D. Buljevac, G. Van Doornum, H. Flach, et al., "Epstein-Barr virus and disease activity in multiple sclerosis," Journal of Neurology, Neurosurgery & Psychiatry, vol. 76, pp. 1377–1381, 2005.

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.

M. C. Kowarik, S. Cepok, J. Sellner, et al., "CXCL13 is the major determinant for B cell recruitment to the CSF during neuroinflammation," Journal of Neuroinflammation, vol. 9, article 93, 2012.
F. Sellebjerg, L. Börnsen, M. Khademi et al., "Increased cerebrospinal fluid concentrations of the chemokine CXCL13 in active MS," Neurology, vol. 73, no. 23, pp. 2003–2010, 2009.

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.

Y. C. Chen, X. Yang, L. Miao, et al., "Serum level of interleukin-6 in Chinese patients with multiple sclerosis," Journal of Neuroinflammation, vol. 249, no. 1-2, pp. 109–111, 2012.

I. Biomarkers of Immunological Activation

7. Cytokines

- 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.

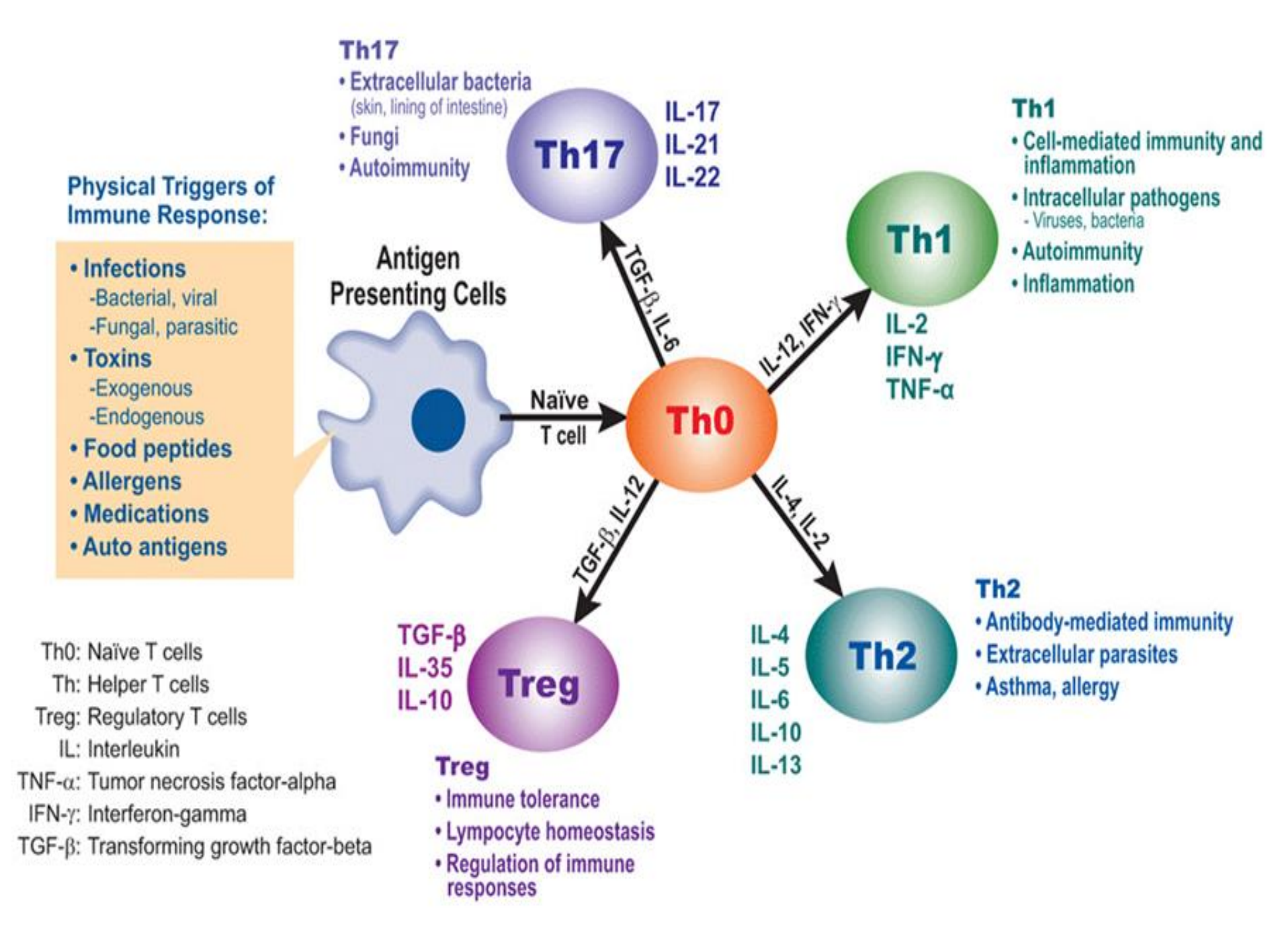
B. S. Kim, Y. H. Jin, L. Meng, et al., "IL-1 signal affects both protection and pathogenesis of virus-induced chronic CNS demyelinating disease," Journal of Neuroinflammation, vol. 9, no. 1, article 217, 2012.
M. N. Karimabad, M. K. Arababadi, E. Hakimizadeh, et al., "Is the IL-10 promoter polymorphism at position -592 associated with immune system-related diseases?" Inflammation. In press.

I. Biomarkers of Immunological Activation

7. Cytokines

- 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.

R. Schneider, A. N. Mohebiany, I. Ifergan, et al., "B cell-derived IL-15 enhances CD8 T cell cytotoxicity and is increased in multiple sclerosis patients," The Journal of Immunology, vol. 187, no. 8, pp. 4119–4128, 2011.
M. Rentzos, C. Cambouri, A. Rombos et al., "IL-15 is elevated in serum and cerebrospinal fluid of patients with multiple sclerosis," Journal of the Neurological Sciences, vol. 241, no. 1-2, pp. 25–29, 2006.


Th17
• Extracellular bacteria (skin, lining of intestine)
• Fungi
• Autoimmunity
Th17
IL-17
IL-21
IL-22
Th1
• Cell-mediated immunity and inflammation
• Intracellular pathogens - Viruses, bacteria
• Autoimmunity
• Inflammation
Th1
IL-2
IFN-γ
TNF-α
Physical Triggers of Immune Response:
• Infections
-Bacterial, viral
-Fungal, parasitic
• Toxins
-Exogenous
-Endogenous
• Food peptides
• Allergens
• Medications
• Auto antigens
Antigen Presenting Cells
TGF-β, IL-6
IL-12, IFN-γ
Naïve T cell
Th0
TGF-β, IL-12
IL-4, IL-2
TGF-β
IL-35
IL-10
Treg
IL-4
IL-5
IL-6
IL-10
IL-13
Th2
Th2
• Antibody-mediated immunity
• Extracellular parasites
• Asthma, allergy
Treg
• Immune tolerance
• Lympocyte homeostasis
• Regulation of immune responses
Th0: Naïve T cells
Th: Helper T cells
Treg: Regulatory T cells
IL: Interleukin
TNF-α: Tumor necrosis factor-alpha
IFN-γ: Interferon-gamma
TGF-β: Transforming growth factor-beta

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.

G. Acar, F. Idiman, G. Kirkali et al., "Intrathecal sICAM-1 production in multiple sclerosis correlation with triple dose Gd-DTPA MRI enhancement and IgG index," Journal of Neurology, vol. 252, no. 2, pp. 146–150, 2005.

A. Uzawa, M. Mori, S. Masuda, and S. Kuwabara, "Markedly elevated soluble intercellular adhesion molecule 1, soluble vascular cell adhesion molecule 1 levels, and blood-brain barrier breakdown in neuromyelitis optica," Archives of Neurology, vol. 68, no. 7, pp. 913–917, 2011.

I. Biomarkers of Immunological Activation

8. Adhesion Molecules

- Finally, laminin 411, which is situated within the vascular endothelium, interacts with adhesion molecule CD146, allowing Th17 cells to overcome the BBB.

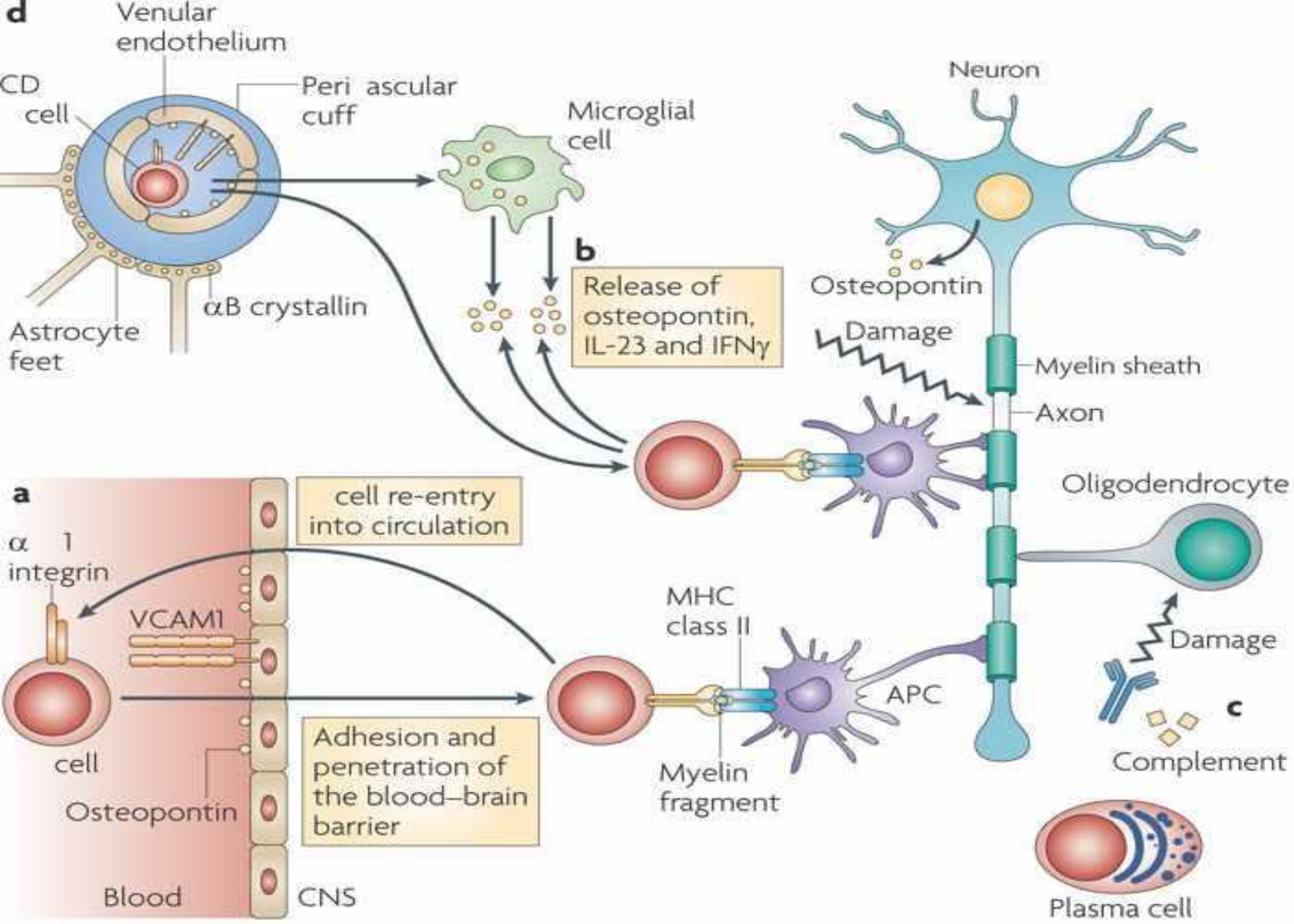
K. Flanagan, K. Fitzgerald, J. Baker, et al., "Laminin-411 is a vascular ligand for MCAM and facilitates TH17 cell entry into the CNS," PLoS One, vol. 7, no. 7, article e40443, 2012.

I. Biomarkers of Immunological Activation

9. Osteopontin

- 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.

M. Braitch, R. Nunan, G. Niepel, L. J. Edwards, and C. S. Constantinescu, "Increased osteopontin levels in the cerebrospinal fluid of patients with multiple sclerosis," Archives of Neurology, vol. 65, no. 5, pp. 633–635, 2008.


d
Venular endothelium
CD cell
Peri ascular cuff
Microglial cell
Neuron
b
Release of osteopontin, IL-23 and IFNγ
Osteopontin
αB crystallin
Astrocyte feet
Damage
Myelin sheath
Axon
Oligodendrocyte
a
cell re-entry into circulation
α 1 integrin
VCAM1
MHC class II
APC
Damage
c
Complement
cell
Osteopontin
Adhesion and penetration of the blood–brain barrier
Myelin fragment
Blood
CNS
Plasma cell

I. 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.

Q. Yan, R. Donelan, J. Dinzey, M. Rammal, and S. Sadiq, "Fetuin-A is a biomarker for disease activity and treatment efficacy in multiple sclerosis," in Proceedings of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS '08), p. 897, Montreal, Canada, 2008.

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.

E. Iacobaeus, P. Amoudruz, M. Ström et al., "The expression of VEGF-A is down regulated in peripheral blood mononuclear cells of patients with secondary progressive multiple sclerosis," PLoS One, vol. 6, no. 5, article e19138, 2011.

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.

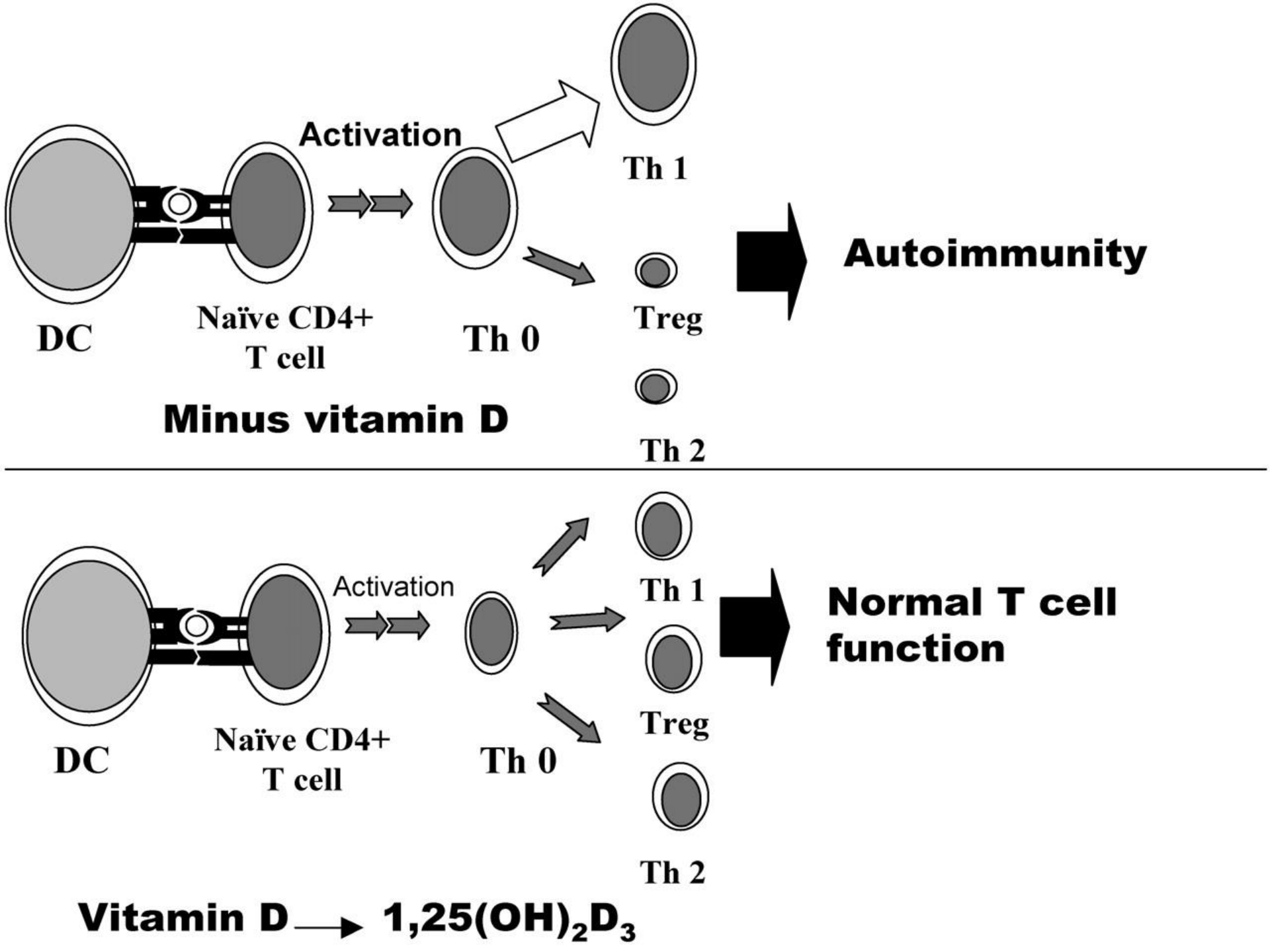
K. I. Løken-Amsrud, T. Holmøy, S. J. Bakke, et al., "Vitamin D and disease activity in multiple sclerosis before and during interferon-*β* treatment," *Neurology*, vol. 79, no. 3, pp. 267–273, 2012.

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.

S. V. Ramagopalan, N. J. Maugeri, L. Handunnetthi et al., "Expression of the multiple sclerosis-associated MHC class II allele HLA-DRB1*1501 is regulated by vitamin D," PLoS Genetics, vol. 5, no. 2, article e1000369, 2009.
N. Stewart, S. Simpson Jr., I. van der Mei, et al., "Interferon-β and serum 25-hydroxyvitamin D interact to modulate relapse risk in MS," Neurology, vol. 79, no. 3, pp. 254–260, 2012.


Activation
Th 1
Autoimmunity
DC
Naïve CD4+
T cell
Th 0
Treg
Th 2
Minus vitamin D
Activation
Th 1
Normal T cell
function
DC
Naïve CD4+
T cell
Th 0
Treg
Th 2
Vitamin D → 1,25(OH)2D3

III. Biomarkers of BBB disruption

1. Matrix Mettalloproteinase 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.

C. Avolio, M. Ruggieri, F. Giuliani et al., "Serum MMP-2 and MMP-9 are elevated in different multiple sclerosis subtypes," Journal of Neuroimmunology, vol. 136, no. 1-2, pp. 46–53, 2003.
W. Sato, A. Tomita, D. Ichikawa, et al., "CCR2+CCR5+ T cells produce matrix metalloproteinase-9 and osteopontin in the pathogenesis of multiple sclerosis," The Journal of Immunology, vol. 189, no. 10, pp. 5057–5065, 2012.

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.

I. Ifergan, H. Kebir, S. Terouz, et al., "Role of Ninjurin-1 in the migration of myeloid cells to central nervous system inflammatory lesions," Annals of Neurology, vol. 70, no. 5, pp. 751–763, 2011.

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.

A. Uzawa, M. Mori, S. Masuda, and S. Kuwabara, "Markedly elevated soluble intercellular adhesion molecule 1, soluble vascular cell adhesion molecule 1 levels, and blood-brain barrier breakdown in neuromyelitis optica," Archives of Neurology, vol. 68, no. 7, pp. 913–917, 2011.

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.

A. Reijerkerk, K. A. Lakeman, J. A. Drexhage, et al., "Brain endothelial barrier passage by monocytes is controlled by the endothelin system," Journal of Neurochemistry, vol. 121, no. 5, pp. 730–737, 2012.

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%).

F. Sellebjerg, M. Christiansen, and P. Garred, “MBP, anti-MBP and anti-PLP antibodies, and intrathecal complement activation in multiple sclerosis,” Multiple Sclerosis, vol. 4, no. 3, pp. 127–131, 1998.

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.

J. M. van Noort, M. Bsibsi, W. H. Gerritsen et al., "αB-crystallin is a target for adaptive immune responses and a trigger of innate responses in preactive multiple sclerosis lesions," Journal of Neuropathology and Experimental Neurology, vol. 69, no. 7, pp. 694–703, 2010.

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.

Lassmann H, van Horssen J, Mahad D. Progressive multiple sclerosis: pathology and pathogenesis. Nat Rev Neurol. 2012;8(11):647–56.

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.

K. Rejdak, M. J. Eikelenboom, A. Petzold et al., “CSF nitric oxide metabolites are associated with activity and progression of multiple sclerosis,” Neurology, vol. 63, no. 8, pp. 1439–1445, 2004.

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.

A. Diestel, O. Aktas, D. Hackel et al., "Activation of microglial poly(ADP-ribose)-polymerase-1 by cholesterol breakdown products during neuroinflammation: a link between demyelination and neuronal damage," Journal of Experimental Medicine, vol. 198, no. 11, pp. 1729–1740, 2003.

V. Biomarkers of Oxidative Stress

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.

Miller E, Mrowicka M, Saluk-Juszczak J, Ireneusz M. The level of isoprostanes as a non-invasive marker for in vivo lipid per- oxidation in secondary progressive multiple sclerosis. Neuro- chem Res. 2011;36(6):1012–6.

Teunissen CE, Sombekke M, van Winsen L, Killestein J, Barkhof F, Polman CH, et al. Increased plasma 8,12-iso-iPF2alpha-VI levels in relapsing multiple sclerosis patients are not predictive of disease progression. Mult Scler. 2012;18(8):1092–8.

Mir F, Lee D, Ray H, Sadiq SA. Cerebrospinal fluid isoprostane levels are a biomarker of oxidative stress in multiple sclerosis. Neurol Neuroimmunol Neuroinflammation. 2014 (in press).

V. Biomarkers of Oxidative Stress

4. Lactate

- Mitochondrial dysfunction is an important feature in MS and of particular relevance to the neurodegenerative phase of the disease.
- Measurement of ***serum lactate*** in MS might be a relative inexpensive test for longitudinal monitoring of "virtual hypoxia" in MS and also a secondary outcome for treatment trials aimed to improve mitochondrial function in patients with MS.

Amorini AM, Nociti V, Petzold A, Gasperini C, Quartuccio E, Lazzarino G. et al. Serum lactate as a novel potential biomarker in multiple sclerosis. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2014 1842(7):1137-1143.

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.

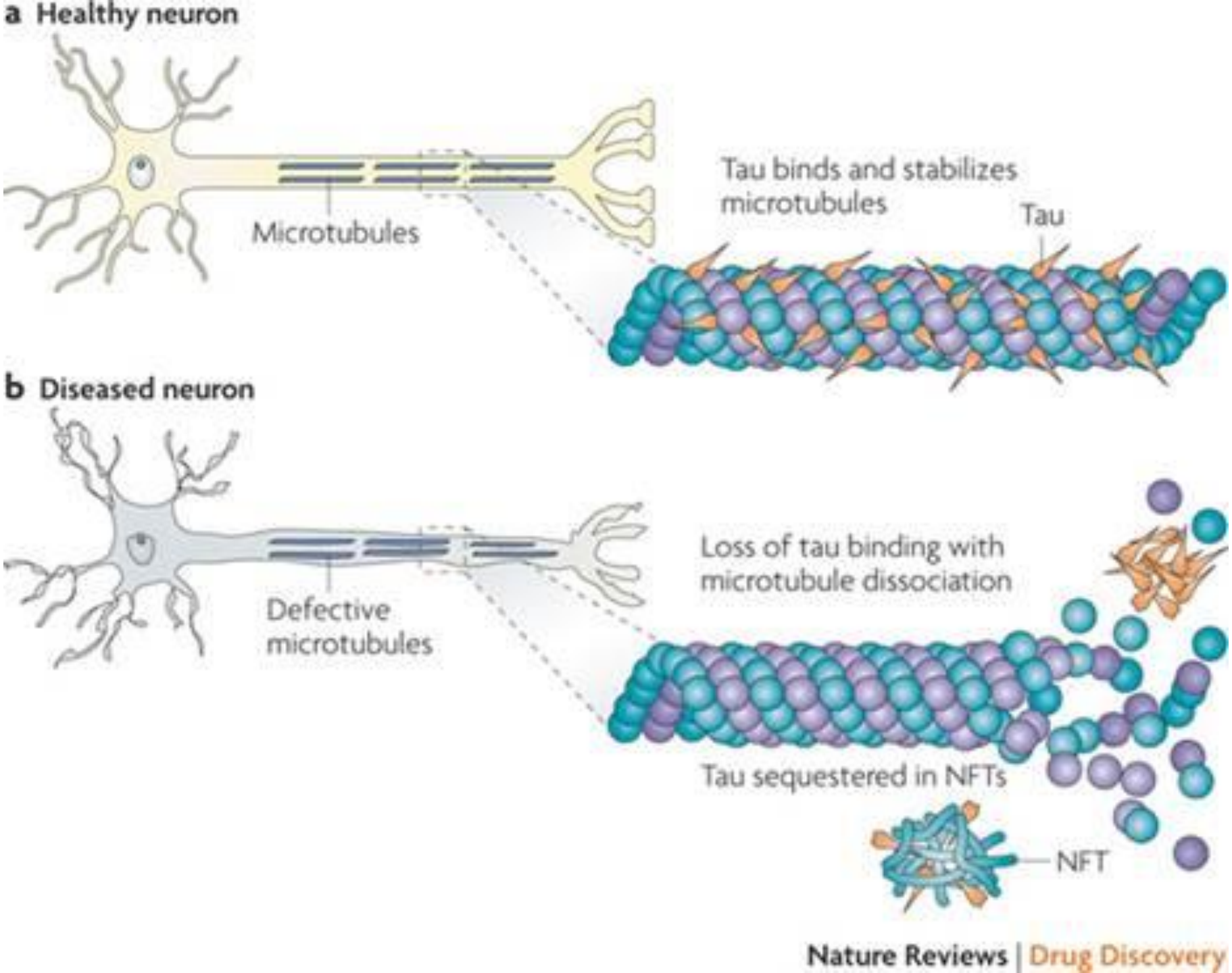
M. Gunnarsson, C. Malmeström, M. Axelsson et al., "Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab," Annals of Neurology, vol. 69, no. 1, pp. 83–89, 2011.
A. Petzold, M. J. Eikelenboom, G. Keir et al., "Axonal damage accumulates in the progressive phase of multiple sclerosis: three year follow up study," Journal of Neurology, Neurosurgery and Psychiatry, vol. 76, no. 2, pp. 206–211, 2005.

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.

J. Brettschneider, A. Petzold, A. Junker, and H. Tumani, "Axonal damage markers in the cerebrospinal fluid of patients with clinically isolated syndrome improve predicting conversion to definite multiple sclerosis," Multiple Sclerosis, vol. 12, no. 2, pp. 143–148, 2006.


a Healthy neuron
Microtubules
Tau binds and stabilizes microtubules
Tau
b Diseased neuron
Defective microtubules
Loss of tau binding with microtubule dissociation
Tau sequestered in NFTs
NFT

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.

Kanter JL, Narayana S, Ho PP, Catz I, Warren KG, Sobel RA, et al. Lipid microarrays identify key mediators of autoimmune brain inflammation. Nat Med. 2006;12(1):138–43.

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.

Wang D, Bhat R, Sobel RA, Huang W, Wang LX, Olsson T, et al. Uncovering Cryptic Glycan Markers in Multiple Sclerosis (MS) and Experimental Autoimmune Encephalomyelitis (EAE). Drug Dev Res. 2014;75(3):172–88.

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.

Kanter JL, Narayana S, Ho PP, Catz I, Warren KG, Sobel RA, et al. Lipid microarrays identify key mediators of autoimmune brain inflammation. Nat Med. 2006;12(1):138–43.

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

C. E. Teunissen, P. C. Dijkstra, and C. Polman, "Biological markers in CSF and blood for axonal degeneration in multiple sclerosis," Lancet Neurology, vol. 4, no. 1, pp. 32–41, 2005.
C. Tortorella, M. Ruggieri, E. Di Monte, E. Ceci, P. Iaffaldano, and V. Direnzo, "Serum and CSF N-acetyl aspartate levels differ in multiple sclerosis and neuromyelitis optica," Neurology, vol. 72, pp. 1322–1329, 2009.

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.

A. Petzold, M. J. Eikelenboom, D. Gveric et al., "Markers for different glial cell responses in multiple sclerosis: clinical and pathological correlations," Brain, vol. 125, no. 7, pp. 1462–1473, 2002.
C. Malmeström, S. Haghighi, L. Rosengren, O. Andersen, and J. Lycke, "Neurofilament light protein and glial fibrillary acidic protein as biological markers in MS," Neurology, vol. 61, no. 12, pp. 1720–1725, 2003.

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.

A. R. Massaro, "Are there indicators of remyelination in blood or CSF of multiple sclerosis patients?" Multiple Sclerosis, vol. 4, no. 3, pp. 228–231, 1998.
T. Ziemssen, T. Kümpfel, H. Schneider, W. E. F. Klinkert, O. Neuhaus, and R. Hohlfeld, "Secretion of brain-derived neurotrophic factor by glatiramer acetate-reactive T-helper cell lines: implications for multiple sclerosis therapy," Journal of the Neurological Sciences, vol. 233, no. 1-2, pp. 109–112, 2005.

VIII. Biomarkers of Remyelination Repair

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.

A. Jurewicz, M. Matysiak, C. S. Raine, and K. Selmaj, "Soluble Nogo-A, an inhibitor of axonal regeneration, as a biomarker for multiple sclerosis," Neurology, vol. 68, no. 4, pp. 283–287, 2007.

IX. Biomarkers of Therapeutic Response

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.

Sorensen PS, Koch-Henriksen N, Ross C, Clemmesen KM, Bendtzen K. Appearance and disappearance of neutralizing antibodies during interferon-beta therapy. Neurology. 2005; 65(1):33–9.
Bertolotto A, Deisenhammer F, Gallo P, Solberg Sorensen P. Immunogenicity of interferon beta: differences among products. J Neurol. 2004;251 Suppl 2:II15–24.

IX. Biomarkers of Therapeutic Response

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

Calabresi PA, Giovannoni G, Confavreux C, Galetta SL, Havrdova E, Hutchinson M, et al. The incidence and signifi- cance of anti-natalizumab antibodies: results from AFFIRM and SENTINEL. Neurology. 2007;69(14):1391–403.

IX. Biomarkers of Therapeutic Response

2. CSF fetuin-A

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

Harris VK, Donelan N, Yan QJ, Clark K, Touray A, Rammal M, et al. Cerebrospinal fluid fetuin-A is a biomarker of active multiple sclerosis. Mult Scler. 2013;19(11):1462–72.

IX. Biomarkers of Therapeutic Response

3. Circulating CD49d expression

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

Defer G, Mariotte D, Derache N, Toutirais O, Legros H, Cau- quelin B, et al. CD49d expression as a promising biomarker to monitor natalizumab efficacy. J Neurol Sci. 2012;314(1–2):138–42.

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.

K. Mitosek-Szewczyk, Z. Stelmasiak, H. Bartosik-Psujek, and E. Belniak, “Impact of cladribine on soluble adhesion molecules in multiple sclerosis,” Acta Neurologica Scandinavica, vol. 122, no. 6, pp. 409–413, 2010.

X. Prognostic Biomarkers

Chitinase 3-like 1 (CHI3L1)

- It 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.

Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, Kang MJ, et al. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Annu Rev Physiol. 2011;73:479–501.
Comabella M, Fernandez M, Martin R, Rivera-Vallve S, Borras E, Chiva C, et al. Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. Brain. 2010;133(Pt 4):1082–93.

Stoop MP, Singh V, Stingl C, Martin R, Khademi M, Olsson T, et al. Effects of natalizumab treatment on the cerebrospinal fluid proteome of multiple sclerosis patients. J Proteome Res. 2013;12(3):1101–7.
Thouvenot E, Hinsinger G, Galeotti N, Nabholz N, Urbach S, Rigau V, et al., editors. Chitinase 3-like 1 and chitinase 3-like 2 as diagnostic and prognostic biomarkers of multiple sclerosis. Philadelphia: American Academy of Neurology Annual Meet- ing; 2014.

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.

Comabella M, Fernandez M, Martin R, Rivera-Vallve S, Borras E, Chiva C, et al. Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. Brain. 2010;133(Pt 4):1082–93.

XI. Emerging Biomarker Categories

A. Transcriptomic Signatures

A. Circulating MicroRNAs

A. Exosomes/Microvesicles

B. Antigen Arrays

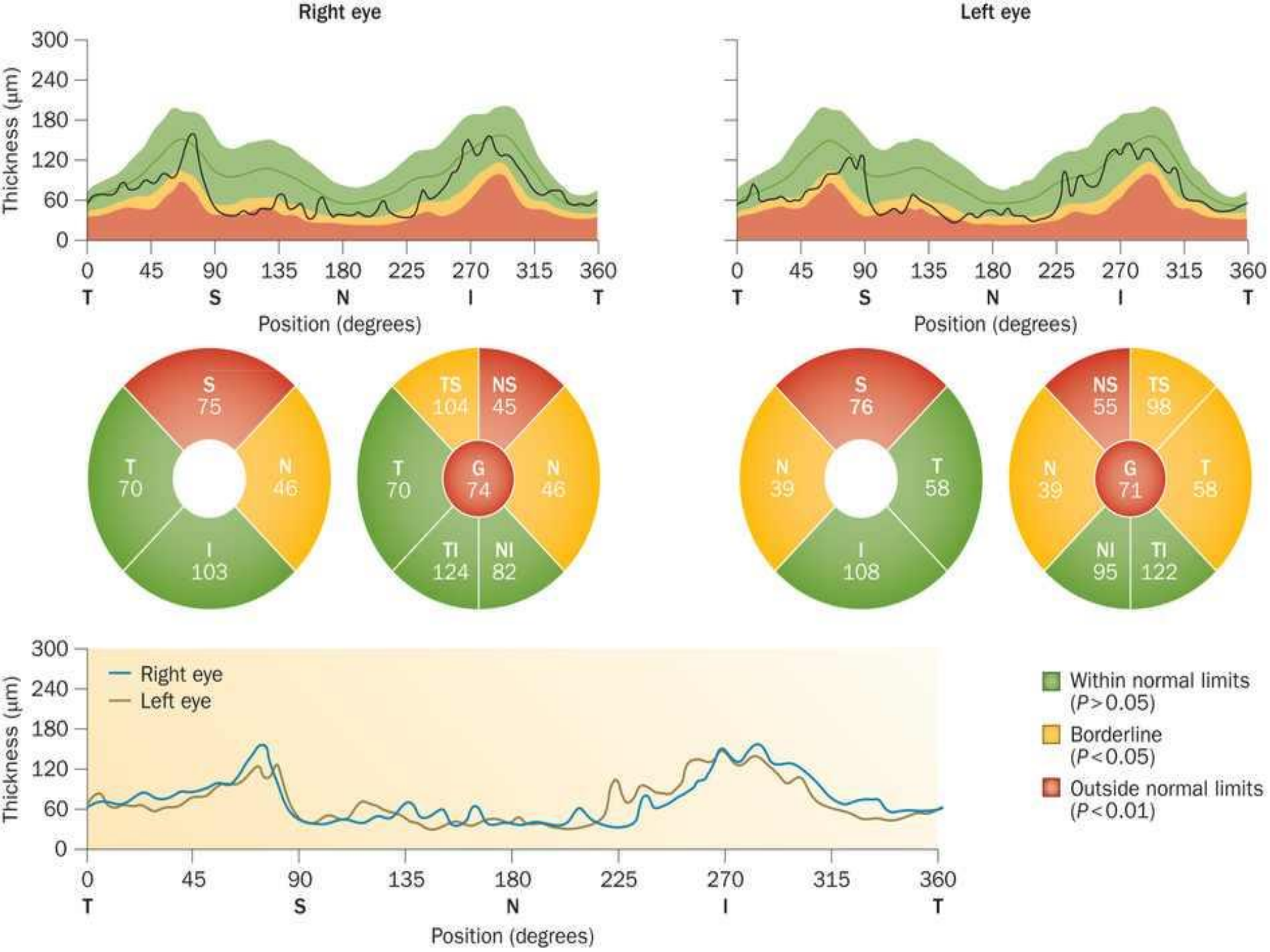
Comabella M, Fernandez M, Martin R, Rivera-Vallve S, Borras E, Chiva C, et al. Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. Brain. 2010;133(Pt 4):1082–93.

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.

E. Grazioli, R. Zivadinov, B. Weinstock-Guttman et al., "Retinal nerve fiber layer thickness is associated with brain MRI outcomes in multiple sclerosis," Journal of the Neurological Sciences, vol. 268, no. 1-2, pp. 12–17, 2008.
R. Herrero, E. Garcia-Martin, C. Almarcegui, et al., "Progressive degeneration of the retinal nerve fiber layer in patients with multiple sclerosis," Investigative Ophthalmology & Visual Science, vol. 53, no. 13, pp. 8344–8349, 2012.


Right eye
Left eye
Thickness (µm)
300
240
180
120
60
0
0 45 90 135 180 225 270 315 360
T S N I T
Position (degrees)
S 75
T 70
N 46
I 103
TS 104
NS 45
T 70
G 74
N 46
TI 124
NI 82
S 76
N 39
T 58
I 108
NS 55
TS 98
N 39
G 71
T 58
NI 95
TI 122
Right eye
Left eye
Within normal limits (P>0.05)
Borderline (P<0.05)
Outside normal limits (P<0.01)

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.

R. T. Shinohara, J. Goldsmith, F. J. Mateen, C. Crainiceanu, and D. S. Reich, "Predicting breakdown of the blood-brain barrier in multiple sclerosis without contrast agents," American Journal of Neuroradiology, vol. 33, no. 8, pp. 1586–1590, 2012.
P. A. Brex, O. Ciccarelli, J. I. O'Riordan, M. Sailer, A. J. Thompson, and D. H. Miller, "A longitudinal study of abnormalities on MRI and disability from multiple sclerosis," The New England Journal of Medicine, vol. 346, no. 3, pp. 158–164, 2002.

2. Magnetic Resonance Imaging (MRI)

iii. **Hypointense T1-weighted lesions (black holes):** considered as satisfactory biomarkers of axonal damage. Their correlation with disability remains debatable.

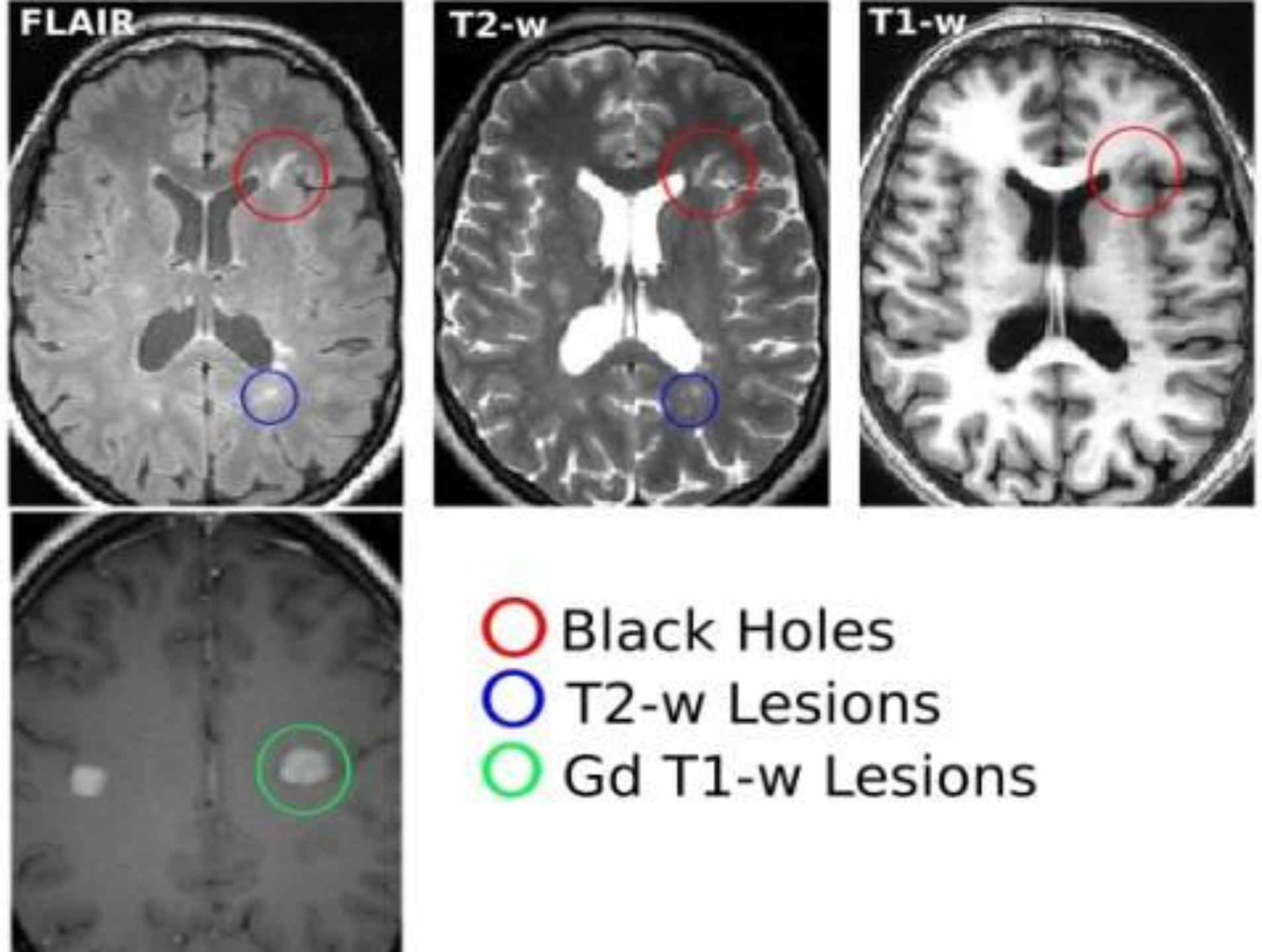
iv. **Whole brain atrophy biomarkers:** the most widely used measure is the brain parenchymal fraction. Brain atrophy worsening rates are higher in untreated MS patients (0.5%–1% annualized decrease) in comparison with healthy controls (0.1%–0.3%) . Brain atrophy worsening rate at initial diagnosis has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

P. A. Brex, G. J. M. Parker, S. M. Leary et al., "Lesion heterogeneity in multiple sclerosis: a study of the relations between appearances on T1 weighted images, T1 relaxation times, and metabolite concentrations," Journal of Neurology Neurosurgery and Psychiatry, vol. 68, no. 5, pp. 627–632, 2000.
M. A. Sahraian, E. W. Radue, S. Haller, and L. Kappos, "Black holes in multiple sclerosis: definition, evolution, and clinical correlations," Acta Neurologica Scandinavica, vol. 122, no. 1, pp. 1–8, 2010.
N. De Stefano, A. Giorgio, M. Battaglini et al., "Assessing brain atrophy rates in a large population of untreated multiple sclerosis subtypes," Neurology, vol. 74, no. 23, pp. 1868–1876, 2010.
. Fisher, R. A. Rudick, J. H. Simon et al., "Eight-year follow-up study of brain atrophy in patients with MS," Neurology, vol. 59, no. 9, pp. 1412–1420, 2002.

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.

E. Fisher, J. C. Lee, K. Nakamura, and R. A. Rudick, "Gray matter atrophy in multiple sclerosis: a longitudinal study," Annals of Neurology, vol. 64, no. 3, pp. 255–265, 2008.
C. M. Dalton, D. T. Chard, G. R. Davies et al., "Early development of multiple sclerosis is associated with progressive grey matter atrophy in patients presenting with clinically isolated syndromes," Brain, vol. 127, no. 5, pp. 1101–1107, 2004.


FLAIR
T2-w
T1-w
Black Holes
T2-w Lesions
Gd T1-w Lesions

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.

W. Rashid, G. R. Davies, D. T. Chard et al., "Increasing cord atrophy in early relapsing-remitting multiple sclerosis: a 3 year study," Journal of Neurology, Neurosurgery and Psychiatry, vol. 77, no. 1, pp. 51–55, 2006.

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 and with reduction of amplitude in visual evoked potentials, suggesting that MTR is primarily an axonal damage biomarker.

I. Van Den Elskamp, D. L. Knol, H. Vrenken et al., "Lesional magnetization transfer ratio: a feasible outcome for remyelinating treatment trials in multiple sclerosis," Multiple Sclerosis, vol. 16, no. 6, pp. 660–669, 2010
A. Klistorner, J. Chaganti, R. Garrick, K. Moffat, and C. Yiannikas, "Magnetisation transfer ratio in optic neuritis is associated with axonal loss, but not with demyelination," NeuroImage, vol. 56, no. 1, pp. 21–26, 2011.
R. A. Brown, S. Narayanan, and D. L. Arnold, "Segmentation of magnetization transfer ratio lesions for longitudinal analysis of demyelination and remyelination in multiple sclerosis," NeuroImage, vol. 66, pp. 103–109, 2012. .

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.

M. Bozzali, M. Cercignani, M. P. Sormani, G. Comi, and M. Filippi, "Quantification of brain gray matter damage in different MS phenotypes by use of diffusion tensor MR imaging," American Journal of Neuroradiology, vol. 23, no. 6, pp. 985–988, 2002.

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

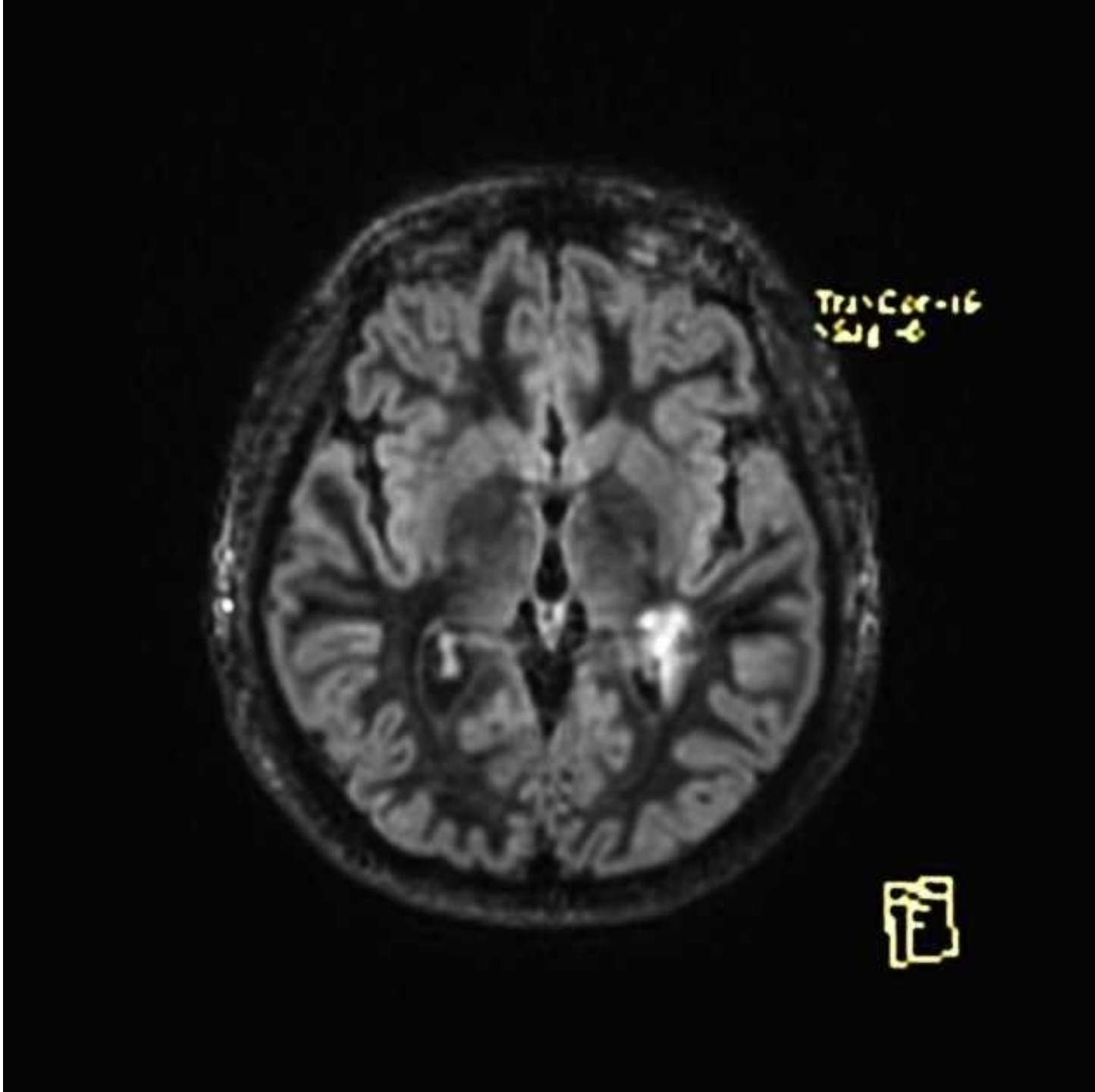
- Corpus callosum DTI abnormalities are present in early MS stages, even when lesions in conventional MRI are still absent .
- MD alterations precede visible in conventional MRI BBB injury by at least 5 months, being thus a reliable predictive biomarker for MS relapse.
- Corpus callosum DTI abnormalities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

M. Wahl, A. Hübers, B. Lauterbach-Soon et al., "Motor callosal disconnection in early relapsing-remitting multiple sclerosis," Human Brain Mapping, vol. 32, no. 6, pp. 846–855, 2011.
Y. Liu, P. J. Mitchell, T. J. Kilpatrick, et al., "Diffusion tensor imaging of acute inflammatory lesion evolution in multiple sclerosis," Journal of Clinical Neuroscience, vol. 19, no. 12, pp. 1689–1694, 2012.
W. Tian, T. Zhu, J. Zhong et al., "Progressive decline in fractional anisotropy on serial DTI examinations of the corpus callosum: a putative marker of disease activity and progression in SPMS," Neuroradiology, vol. 54, no. 4, pp. 287–297, 2012.

5. Double inversion recovery (DIR)

A sequence that suppresses both CSF and white matter signal for better delineation of the plaques.

DIR



6. 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
iv. Glutamate: biomarker of acute inflammation.

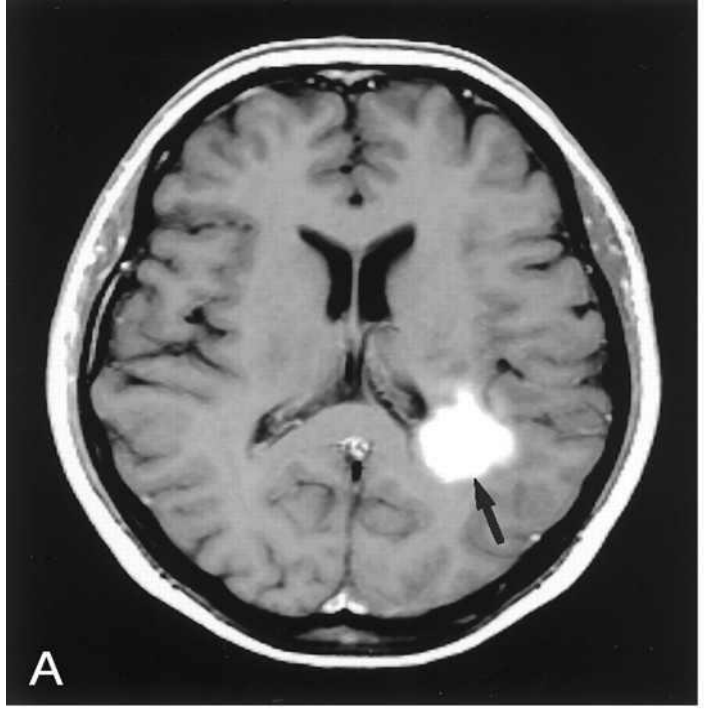
D. J. Rigotti, M. Inglese, I. I. Kirov, et al., "Two-year serial whole-brain N-acetyl-L-aspartate in patients with relapsing-remitting multiple sclerosis," Neurology, vol. 78, no. 18, pp. 1383–1389, 2012.

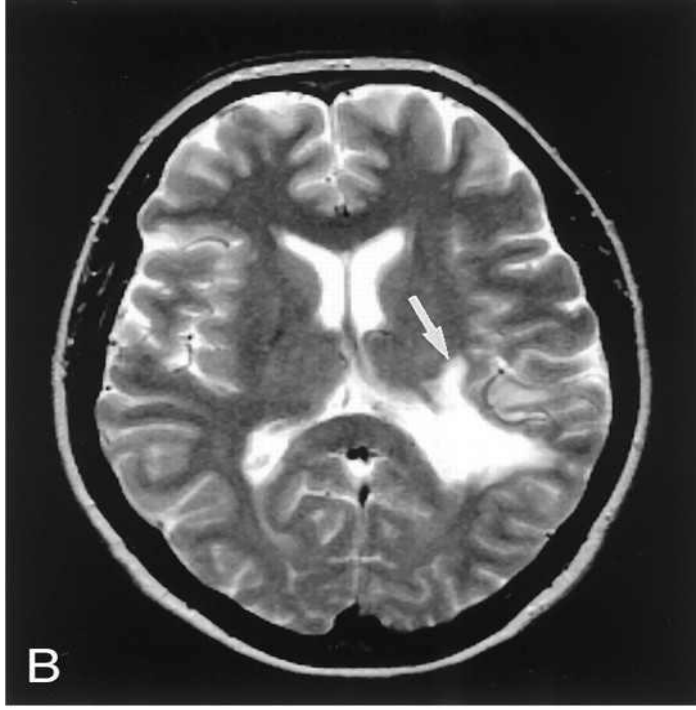
6. Magnetic Resonance Spectroscopy (MRS)

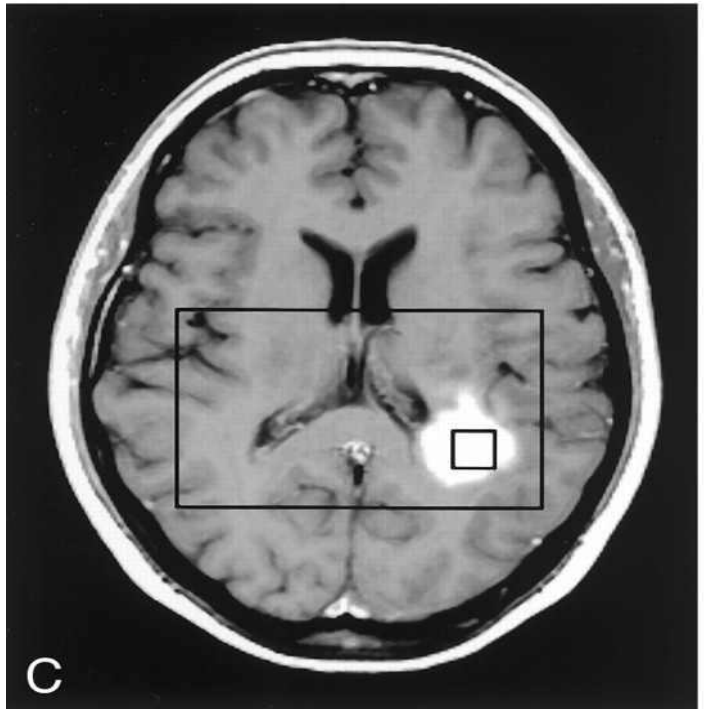
- Early spectroscopic changes represent a bad prognostic factor of future disability. Spectroscopic findings suggest that white matter abnormalities in RRMS are more prominent than grey matter abnormalities where the injury is less diffuse.

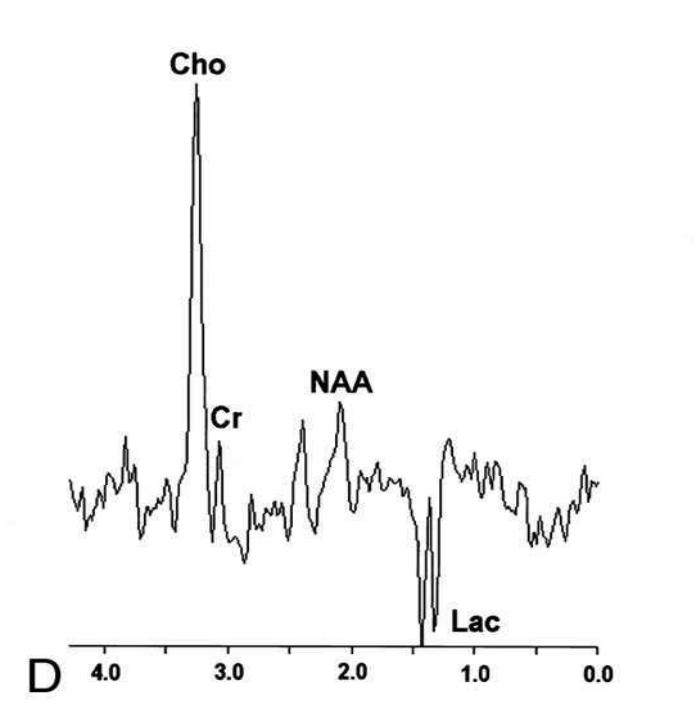
E. Hattingen, J. Magerkurth, U. Pilatus, A. Hubers, M. Wahl, and U. Ziemman, "Combined 1H and 31P spectroscopy provides new insights into the pathobiochemistry of brain damage in multiple sclerosis," NMR in Biomedicine, vol. 24, no. 5, pp. 536–546, 2011.
I. I. Kirov, A. Tal, J. S. Babb, J. Herbert, and O. Gonen, "Serial proton MR spectroscopy of gray and white matter in relapsing-remitting MS," Neurology, vol. 80, no. 1, pp. 39–46, 2012.

MRS









7. Positron Emission Tomography (PET)

- 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.

U. Oh, M. Fujita, V. N. Ikonomidou et al., "Translocator protein PET imaging for glial activation in multiple sclerosis," Journal of Neuroimmune Pharmacology, vol. 6, no. 3, pp. 354–361, 2011.

8. Diffusion tensor spectroscopy (DTS)

- Diffusion tensor spectroscopy (DTS), a technique combining properties of DTI and MRS, seems promising in better distinguishing axonopathy, demyelination, inflammation, edema, and gliosis.

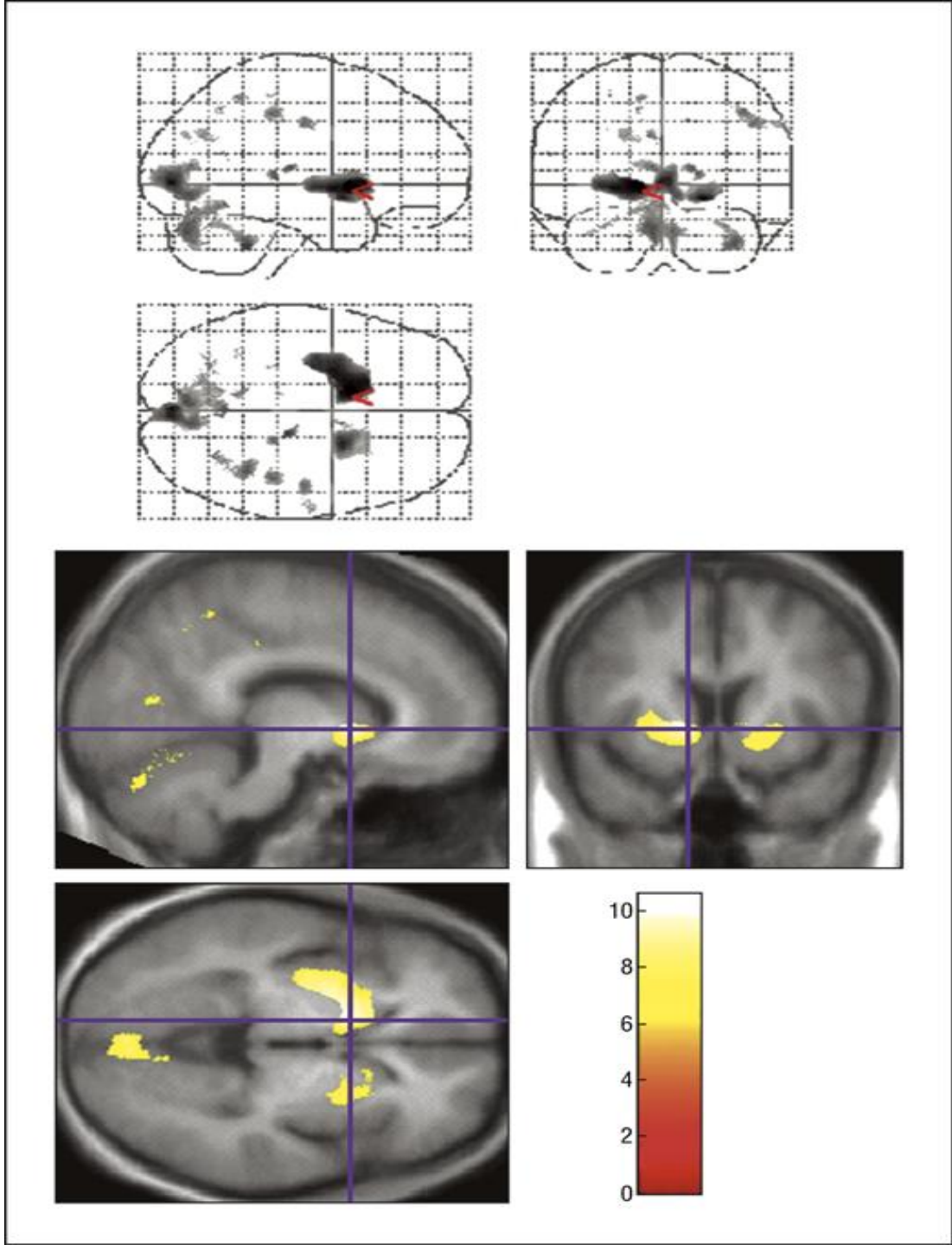
E. T. Wood, I. Ronen, A. Techawiboonwong, et al., "Investigating axonal damage in multiple sclerosis by diffusion tensor spectroscopy," Journal of Neuroscience, vol. 32, no. 19, pp. 6665–6669, 2012.

9. Voxel-based morphometry (VBM)

- Voxel-based morphometric (VBM) analysis is an accurate method that includes segmentation of brain volumes into GM, WM, and CSF, normalization to a standard space, and quantification of ***GM atrophy*** on a voxel-by-voxel basis.

Grossman MMcMillan CMoore P et al. What's in a name: voxel-based morphometric analyses of MRI and naming difficulty in Alzheimer's disease, frontotemporal dementia and corticobasal degeneration. *Brain* 2004;127628- 649

VBM

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THANK YOU