DOI 10.1007/s11055-022-01310-7
Neuroscience and Behavioral Physiology, Vol. 52, No. 6, July, 2022

Prognostic Markers in Multiple Sclerosis

V. M. Alifirova, E. M. Kamenskikh, E. S. Koroleva, E. V. Kolokolova, and A. M. Petrakovich

Translated from Zhurnal Nevrologii i Psikhiatrii imeni S. S. Korsakova, Vol. 122, No. 2, pp. 22–27, February, 2022. Original article submitted February 16, 2021. Accepted March 4, 2021.

Multiple sclerosis (MS) is chronic autoimmune disease with a high level of heterogeneity in its course and prognosis. More than half of patients with MS do not discuss their long-term prognosis with the treating doctor. Most patients regard personalized information on the course of MS as extremely important in relation to taking decisions on family planning, choice of profession, and treatment. Determination of prognosis in routine clinical practice uses clinical markers, though these are nominally divided into favorable and unfavorable factors, which allows general conclusions regarding the prognosis of MS to be made. Neuroimaging and biological markers are mainly used for research purposes, though they are now actively employed in clinical studies to assess the effects of treatment on the organic causes of persistent disability. This review describes studies of the prognostic value of various clinical, neuroimaging, and biological markers.

Keywords: prognosis, prognostic marker, multiple sclerosis, biomarkers, neuroimaging.

Multiple sclerosis (MS) is an autoimmune demyelinating and simultaneously a neurodegenerative disease of the CNS, which leads to long-term loss of patients' ability to work [1]. At diagnosis, more than 80% of cases are relapsing remitting MS (RRMS), though 65% of cases of RRMS develop into secondary progressive MS (SPMS) [2].

MS is treated using MS disease-modifying drugs (DMD), which are the only evidence-based approach to influencing the rate of progression, though this treatment is not always effective because of weak responses to the tactic selected, highly active or aggressive MS, and various other reasons. The treatment of SPMS is mainly symptomatic in nature, though a number of drugs for the pathogenetic treatment of SPMS are under development and are being introduced into clinical practice [3]. An important task is that of developing effective treatments delaying and hopefully preventing the progression of RRMS to SPMS. No small role is played by the ability to prognosticate the progression of MS in each individual patient.

Depending on the method of acquiring information, several types of prognostic markers are identified: clinical markers and neuroimaging markers. Most markers are at the research stage, with assessment of specificity and sensitivity and evaluation of advantages and drawbacks. As the safest method of acquiring clinical data, neuroimaging markers characterize focal lesions but provide little detail on microstructural damage [4]. Biomarkers allow assessment of the status of processes at the microstructural level, though acquiring data on them requires invasive procedures for collection of biological fluids, which carries certain risks.

Evidence-based provision of more effective and aggressive treatment tactics before the onset of severe or irreversible clinical symptoms in patients increases the probability of preserving quality of life and slowing or stopping progression of MS. The value of prognostic markers is assessed in terms of their ability to reflect a high risk of progression in the conditions obtaining, which is reflected as an increase in the extent of disability, with the possible progression of RRMS to SPMS or clinically isolated syndrome (CIS) to RRMS. Indirect prognostic markers may be precursors of MS relapses, which may be the basis of subsequent progression. This review addresses studies of potential markers for MS progression.

Clinical Markers. At the patient consultation stage, collection of demographic and history data can identify risk factors associated with the features of the course and progression of MS. Favorable factors for MS course are young age at onset, female sex, initial symptoms in the form of sensory or visual impairments, short duration of first re-

Siberian State Medical University, Russian Ministry of Health, Tomsk, Russia; e-mail: em_kamenskih@mail.ru.

lapse, and long subsequent remission [5]. Unfavorable factors include older age at onset of MS, male sex, impairments to the pyramidal system, cerebellum, or pelvic organs, multisymptomatic onset, incomplete remission after the first relapse, short first remission, and high relapse frequency at the initial stages of MS. Data reported by Bergamaschi et al. [5] indicated that unfavorable prognoses were associated with greater age, greater number of impaired functional systems, and pelvic, motor, or sensorimotor manifestations. Results from a cohort observational study reported by Fambiatos et al. [6] (n = 15,717) indicated that the probability of transition from RRMS to SPMS increased with increases in the patient's age (odds radio (OR) = 1.02, $p < 0.001$) and duration of illness (OR = 1.01, $p = 0.038$). Higher scores on the Expanded Disability Status Scale (EDSS) (OR = 1.30, $p < 0.001$), rapid increase in disability (OR = 2.82, $p < 0.001$), and larger numbers of relapses in the preceding year (OR = 1.07, $p = 0.010$) also had statistically significant effects on the prognosis of progression. A decrease in the level of disability (OR = 0.62, $p = 0.039$) and use of DMD treatment (OR = 0.71, $p = 0.007$) were predicted to be linked with lower risks of conversion to SPMS. A number of studies [5]suggested that male sex was a more unfavorable factor for MS course, though in this case there was no statistically significant difference by gender (OR = 1.14, $p = 0.098$). A study reported by Briggs et al. [7] indicated that smoking and obesity were recognized as risk factors for a more severe course of MS.

Boiko et al. [8] assessed the prognostic significance of neuropsychological test results in patients with RRMS (n = 50). In studies over a five-year observation period, Stroop word-color interference test results indicated that difficulty in switching verbal skills to sensory perceptive functions was tightly linked with increases in disability on the EDSS in patients with RRMS.

Attempts were made to construct a mathematical model of the course and progression of MS on the basis of clinical data from large registers [9]. Kasatkin et al. [10] developed a mathematical model for assessing the personalized risk of relapse of MS over a period of six months, which is actually a system supporting doctors' decision-taking in relation to the need to change DMD. In 2020, Volkov and Popova [11] published results on the questionnaire MSProDiscuss for the early detection of RRMS progression and diagnosis of SPMS (sensitivity 0.82, specificity 0.84).

Jordy et al. [12] assessed olfactory function in patients with MS (n = 100): results from patients with EDSS ≥4 demonstrated a 5.2 times greater level of olfactory dysfunction than the control group. The authors suggested that olfactory dysfunction is a potential marker for progression of MS. In relation to the less common clinical impairments, there is interest in impairments to circadian rhythms, reflecting the functions of deep brain structures and influencing cortisol release, which may also affect the progression of MS [13].

Mirmosayyeb et al. [14] reported data from analysis of the characteristics of progression in patients with RRMS (n = 2627) with onset in adulthood (18–50 years of age; n = 2416), in youth (<18 years; n = 127), and in late life (>50 years; n = 84 years). In relation to high rates of progression, this was associated with high EDSS scores in early-onset patients, while in adult-onset patients it was also linked with younger age, shorter intervals between relapses, and spinal cord lesions, while in the late-onset group disease progression was associated with plaques in the spinal cord and accumulation of spinal contrast foci.

Neuroimaging Markers. Paraclinical methods have an important role in MS studies. Neuroimaging plays the key role in the diagnosis of MS. Prognostic neuroimaging markers are found at the investigation stage. Studies reported by Genovese et al. on patients with RRMS (n = 1612) showed that progression of disability was associated with the volume of atrophy of T2 foci (34.4 mm^3, $p < 0.001$) and the percentage change in brain volume (–0.21%, p = = 0.042) [15]. Atrophy volume in T2 foci was linked with progression from CIS to RRMS or RRMS to SPMS (+26.4 mm^3, $p = 0.002$).

Longitudinal and cross-sectional studies have shown that the extent of disability in MS correlates with atrophy of the gray matter of the brain [16–18]. More detailed study allowed this group to conclude that there is an interaction between local gray matter atrophy in the temporal lobe and deep brain structures with progressive forms of MS [19, 20]. Assessment of local gray matter atrophy in the thalamus, corpus callosum, and spinal cord was also used as a predictor for progression [21]. The thalamus underwent atrophy in MS patients earlier than other brain structures [22]. The rate of thalamic atrophy in patients correlated with the level of disability on the EDSS [23]. An eight-year longitudinal study involving patients with different MS subtypes (n = 73) showed that thalamic atrophy correlated with the level of disability [24]. It should be noted that assessment of overall and local atrophy of the brain is used mainly for scientific purposes but not in routine clinical practice [21].

Studies using the diffusion tensor imaging (DTI) MRI mode have reported more severe diffusion anomalies in the white matter in patients with SPMS than in those with RRMS [25]. Cortese et al. took the view that greater increases in diffusion in progressive forms of MS were linked with the combination of structural damage to axons and demyelination [18]. Changes noted in DTI mode pointed to a high probability of forming "black holes" and white matter damage in this area [26–28].

In progressive forms of MS, new active foci are found rarely, though existing T2 foci tend to increase gradually with loss of brain tissue without acute inflammation. These foci have cells with high iron contents – activated microglia – at the lesion edges [18, 29]. Rims of this type are rarely seen in inactive foci or those at the remyelination stage, and are not encountered in active foci [30]. Elliot et al. devel-

oped a method for automatic detection of chronic foci in standard brain MRI scans and put this forward as a marker for chronic inflammation reflecting ongoing "silent" progression [18, 31].

Proton MR spectroscopy with recording of changes in metabolite peaks yields data on biochemical changes in different parts of the brain. Decreases in the N-acetylaspartate (NAA) peak serve as a marker for neuroaxonal damage, while increases in the myoinositol peak provide evidence of increased glial cell activity. The largest changes in NAA and myoinositol peaks observed on MR spectroscopy were seen in patients in whom CIS later progressed to MS [32]. Studies reported by Golovkin et al. showed that the NAA level decreased to 30% of normal in SPMS, which is evidence of a transition from reversible inflammation to progressing degeneration [33]. MR spectroscopy in MS with assessment of γ-glutamate and γ-aminobutyric acid (GABA spectroscopy) is at the research stage [28, 33, 34].

A prospective longitudinal study reported by Kobys' [35] included patients from the moment of diagnosis of CIS to onset of RRMS (n = 180). The observation period averaged 10.3 years. The main neuroimaging predictors of reaching moderate disability in MS was the presence of nine or more T2 foci of size >3 mm with infratentorial and periventricular locations, and the risk of reaching severe disability increased when there were ≥20 T2 foci and a longer disease duration. Decreases in NAA levels in particular areas served as an indicator of neurodegenerative processes in MS. The NAA:creatinine ratio in the brain in MS decreased diffusely, reflecting ubiquitous functional impairments.

Construction of maps of dynamic interactions between the functional network structure of the brain and clinical markers of disease – MRI-based connectomics – is a neuroimaging method with potential [18, 36, 37].

Biomarkers. The search for biomarkers for the progression of MS is directly linked with studies of pathogenic processes underlying the transition from autoimmune inflammation to persistent neurodegeneration. By the time of writing this review, there was no single theory able to explain all the processes seen in progression of MS without internal contradictions [38]. The ideal biomarker, apart from sensitivity and specificity, must be obtained by a method which is safe for patients and easily detected using laboratory equipment, and must also be resistant to the systematic influences of factors such as the sampling method and biosample processing and storage [39].

Blood and cerebrospinal fluid (CSF) are suitable materials for sampling to find biomarkers in MS. Biomarkers in the blood can be measured at different time intervals and relatively large volumes can be collected. However, peripheral media, including venous blood, do not always reliably reflect the processes occurring in the CNS, show significant daily variations, and are present at low concentrations because of the excluding function of the blood:brain barrier. CSF concentrations of potential biomarkers are higher and directly reflect changes in the CNS. However, CSF sampling requires invasive lumbar puncture and only small quantities of material are obtained [4].

At present, the most actively studied prognostic biomarkers for MS are oligoclonal bands (OCB), chitinase-3-like protein-1 (CHI3L1), and neurofilaments (NF).

OCB are immunoglobulin bands found in blood or CSF, detected by a variety of electrophoretic methods. OCB were previously used only as a diagnostic marker, though some researchers now take the view that OCB can be used as a prognostic marker for MS. For example, studies reported by Tintore et al. (n = 1015) showed that detection of OCB points to an increased risk of clinically confirmed MS (OR 1.3 [1.0–1.8]) and greater stable disability (OR 2.0 [1.2–3.6]) independently of each other and other factors [40]. In addition, data reported by Kuhle et al. indicated that OCB were the most sensitive and specific prognostic factor for determining the probability that CIS would progress to MS [41].

Another actively studied marker is CHI3L1 – this is a glycosidase present as an extracellular monomeric single-chain glycoprotein secreted by monocytes, macroglia, and activated astrocytes [42]. The physiological role of CHI3L1 in the CNS has not been fully studied, though its presence in inflammatory foci of demyelination indicates that it is at least a modulator of inflammation in the CNS [43, 44]. Kusnierova et al. assessed the CSF CHI3L1 concentration in inflammatory CNS diseases, including MS and CIS [45]. Increased CSF CHI3L1 levels were seen in patients with MS, but they were much lower than in patients with other inflammatory CNS diseases. As the CSF CHI3L1 concentration correlated with the NF heavy chain concentration, this being a biomarker for structural damage to nervous tissue, but not with inflammatory biomarkers, the authors suggested that CHI3L1 reflects the extent of tissue damage rather than the extent of inflammatory activity [46, 47].

NF is a member of the intermediate filaments family of proteins and is the main component of the axon cytoskeleton [48]. NF can be divided in terms of molecular weight into neurofilament light chains (NFL) (68 kDa), neurofilament intermediate chains (160 kDa), and neurofilament heavy chains (205 kDa) [49, 50]. Current highly sensitive methods allow NFL to be detected not only in the CSF, but also in serum. A large prospective study reported by Barro et al. in MS patients showed that high NFL concentrations at the early stage of illness were associated with faster development of disabling complications and subsequently reflected increasing atrophy of the brain and spinal cord [51]. NFL are known to be associated with changes in both neuron structure and treatment sensitivity, which increases the chance that standardized, reliable, and widely available analyses with reference values for serum NFL (sNFL) will be introduced [52, 53].

Metabolic profiles of the tryptophan and kynurenine metabolic pathways have been evaluated as potential bio-

markers [54, 55]. Data on the influences of the microbiome on the course and progression of MS have been reported [56].

Both treating physicians and patients need personalized prognoses. Data reported by Dennison et al. (n = 3175) indicated that 53.1% of patients with MS never discussed the long-term prognosis with their treating physicians [57]. Some 54.2% of patients did not understand their long-term prognoses. For 76%, it was extremely important to obtain information on the long-term course of MS. More than 90% were willing to use tools which would allow personalized prognoses of the course of MS to be obtained. Patients noted that the long-term prognosis would be an important factor for taking decisions about careers, families, and everyday life, as well as selecting particular types of medical help.

Considering the limited use of prognostic markers for each separate subgroup, there is a need to continue studying this problem. Currently the most widespread prognostic strategies are built on subjective analyses of all markers available to clinicians in routine practice. The clinician's clinical experience plays no little role here. Despite the subjectivity of the results obtained, the relatively high risk of errors, and the need for a high level of professionalism on the part of the physician in making prognoses at the early stages of the course of MS, this method is successfully used.

The limited introduction of mathematical models is associated with both low market promotion of the technologies developed, but also the need to use parameters not assessed in routine clinical practice. These characteristics are generally features of neuroimaging data requiring high-power MRI or biomarkers which are not only not evaluated in the context of state-funded healthcare but are also unavailable in large clinical laboratory diagnostic centers.

Studies addressing the prognostic value of markers in MS themselves have a number of limitations. This is likely to be particularly associated with the lack of a single definition of prognostic markers, such that for each nosology the prognosis is important in relation to a particular clinical event associated with the disease of interest. Best studied is the question of prognostic markers in oncology: they are used for determining the risk of disease recurrence. For MS, there is no single strategy for assessing the prognostic strength of markers and no clinical events have been selected as the basis for analysis. For prognostic markers, it is usually difficult to assess sensitivity and specificity because of the binary nature of the results. The sensitivity and specificity of prognostic markers in reported studies determine the probability of making diagnoses of particular levels of progression, which are difficult to assess in routine practice. For this purpose, for example, use can be made of threshold values for the volume and number of cortical foci or the extent of atrophy of brain matter, whose presence correlates with sNFL levels [58]. A number of studies have assessed not the features of the course characterizing progression but rather the fact of progression of CIS to RRMS or RRMS to SPMS or the difference between patients with RRMS and progressive forms of MS course [42, 59]. Correlations with increased EDSS scores have been evaluated for many markers. On the other hand, research groups use a variety of statistical methods to assess prognostic significance: from multiparametric regression analysis to multilayered neural networks. Studies seek models with the greatest prognostic value, where the variables are the quantitative characteristics of biomarkers [60]. The introduction of prognostic markers to clinical practice unavoidably requires standardization of all stages of investigation and statistical assessment, along with direct comparative studies between markers and methods.

The shift in the MS treatment paradigm from decreasing the mean annual frequency of relapses to preventing disease progression is a trigger mechanism for new research into the long-term functional and structural CNS damage in MS. A multidisciplinary approach to solving the problem of prognosticating the course of illness and the involvement of large numbers of scientific groups in the work will soon yield new solutions and, thus, tools for personalizing the prognosis of MS.

The authors declare no conflict of interest.

REFERENCES

1. All-Russian Society of Neurologists, *Clinical Guidelines for Multiple Sclerosis* (2019), https://www.ructrims.org/files/%D0%9A%D0%BB%D0%B8%D0%BD%20%D1%80%D0%B5%D0%BA%D0%A0%D0%A1_2019_3.docx, acc. Feb. 15, 2021.
2. N. Ghasemi, S. Razavi, and E. Nikzad, "Multiple sclerosis: Pathogenesis, symptoms, diagnoses and cell-based therapy," *Cell J.*, **19**, No. 1, 1–10 (2017), https://doi.org/10.22074/cellj.2016.4867.
3. Q. Wu, E. A. Mills, Q. Wang, et al., "Siponimod enriches regulatory T and B lymphocytes in secondary progressive multiple sclerosis," *JCI Insight*, **5**, No. 3 (2020), https://doi.org/10.1172/jci.insight.134251.
4. T. Ziemssen, K. Akgün, and W. Brück, "Molecular biomarkers in multiple sclerosis," *J. Neuroinflamm.*, **16**, No. 1, 272 (2019), https://doi.org/10.1186/s12974-019-1674-2.
5. R. Bergamaschi, C. Berzuini, A. Romani, and V. Cosi, "Predicting secondary progression in relapsing-remitting multiple sclerosis: a Bayesian analysis," *J. Neurol. Sci.*, **189**, No. 1–2, 13–21 (2001), https://doi.org/10.1016/S0022-510X(01)00572-X.
6. A. Fambiatos, V. Jokubaitis, D. Horakova, et al., "Risk of secondary progressive multiple sclerosis: A longitudinal study," *Mult. Scler.*, **26**, No. 1, 79–90 (2020), https://doi.org/10.1177/1352458519868990.
7. F. B. S. Briggs, J. C. Yu, M. F. Davis, et al., "Multiple sclerosis risk factors contribute to onset heterogeneity," *Mult. Scler. Relat. Disord.*, **28**, 11–16 (2019), https://doi.org/10.1016/j.msard.2018.12.007.
8. A. N. Boiko, B. T. Mugutdinova, and T. M. Mugutdinov, "Prognostic significance of neuropsychological tests in patients with typical remitting multiple sclerosis," *Med. Alfavit*, **2**, No. 17(354) (2018), https://elibrary.ru/item.asp?id = 36574676.
9. K. Tilling, M. Lawton, N. Robertson, et al., "Modelling disease progression in relapsing-remitting onset multiple sclerosis using multilevel models applied to longitudinal data from two natural history cohorts and one treated cohort," *Health Technol. Assess.*, **20**, No. 81, 1–48 (2016), https://doi.org/10.3310/hta20810.
10. *Proceedings of the Third All-Russian Congress with International Participation, "Multiple Sclerosis and Other Demyelinating Diseases (ROKIRS/RUCTRIMS Congress)*, Ekaterinburg, Sept. 13–16, 2018,

Zh. Nevrol. Psikhiat., **118**, No. 8, Spec. Iss., 128–171 (2018), https://doi.org/10.17116/jnevro2018118082128.
11. A. I. Volkov and E. V. Popova, "New tools for early detection of the progression of multiple sclerosis," *Zh. Nevrol. Psikhiatr.*, **120**, No. 7–2 (2020), https://doi.org/10.17116/jnevro202012007243.
12. S. S. Jordy, A. Starzewski, Jr., F. A. Macedo, et al., "Olfactory alterations in patients with multiple sclerosis," *Arq. Neuropsiquiatr.*, **74**, No. 9, 697–700 (2016), https://doi.org/10.1590/0004-282X20160128.
13. L. Tonetti, F. Camilli, S. Giovagnoli, et al., "Circadian activity rhythm in early relapsing-remitting multiple sclerosis," *J. Clin. Med.*, **8**, No. 12 (2019), https://doi.org/10.3390/jcm8122216.
14. O. Mirmosayyeb, S. Brand, M. Barzegar, et al., "Clinical characteristics and disability progression of early- and late-onset multiple sclerosis compared to adult-onset multiple sclerosis," *J. Clin. Med.*, **9**, No. 5 (2020), https://doi.org/10.3390/jcm9051326.
15. A. V. Genovese, J. Hagemeier, N. Bergsland, et al., "Atrophied brain T2 lesion volume at MRI is associated with disability progression and conversion to secondary progressive multiple sclerosis," *Radiology*, **293**, No. 2, 424–433 (2019), https://doi.org/10.1148/radiol.2019190306.
16. E. Fisher, J. C. Lee, K. Nakamura, and R. A. Rudick, "Gray matter atrophy in multiple sclerosis: a longitudinal study," *Ann. Neurol.*, **64**, No. 3, 255–265 (2008), https://doi.org/10.1002/ana.21436.
17. L. K. Fisniku, P. A. Brex, D. R. Altmann, et al., "Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis," *Brain*, **131**, No. 3, 808–817 (2008), https://doi.org/10.1093/brain/awm329.
18. R. Cortese, S. Collorone, O. Ciccarelli, and A. T. Toosy, "Advances in brain imaging in multiple sclerosis," *Ther. Adv. Neurol. Disord.*, **12** (2019), https://doi.org/10.1177/1756286419859722.
19. A. Eshaghi, R. V. Marinescu, A. L. Young, et al., "Progression of regional grey matter atrophy in multiple sclerosis," *Brain*, **141**, No. 6, 1665–1677 (2018), https://doi.org/10.1093/brain/awy088.
20. A. Eshaghi, F. Prados, W. J. Brownlee, et al., and the MAGNIMS study group., "Deep gray matter volume loss drives disability worsening in multiple sclerosis," *Ann. Neurol.*, **83**, No. 2, 210–222 (2018), https://doi.org/10.1002/ana.25145.
21. M. Bross, M. Hackett, and E. Bernitsas, "Approved and emerging disease modifying therapies on neurodegeneration in multiple sclerosis," *Int. J. Mol. Sci.*, **21**, No. 12, 4312 (2020), https://doi.org/10.3390/ijms21124312.
22. A. Gajofatto, M. Calabrese, M. D. Benedetti, and S. Monaco, "Clinical, MRI, and CSF markers of disability progression in multiple sclerosis," *Dis. Markers*, **35**, 484959 (2013), https://doi.org/10.1155/2013/484959.
23. B. Audoin, G. R. Davies, L. Finisku, et al., "Localization of grey matter atrophy in early RRMS," *J. Neurol.*, **253**, No. 11, 1495–1501 (2006), https://doi.org/10.1007/s00415-006-0264-2.
24. M. A. Rocca, S. Mesaros, E. Pagani, et al., "Thalamic damage and long-term progression of disability in multiple sclerosis," *Radiology*, **257**, No. 2, 463–469 (2010), https://doi.org/10.1148/radiol.10100326.
25. P. Preziosa, M. A. Rocca, S. Mesaros, et al., "Intrinsic damage to the major white matter tracts in patients with different clinical phenotypes of multiple sclerosis: a voxelwise diffusion-tensor MR study," *Radiology*, **260**, No. 2, 541–550 (2011), https://doi.org/10.1148/radiol.11110315.
26. R. J. Fox, T. Cronin, J. Lin, et al., "Measuring myelin repair and axonal loss with diffusion tensor imaging," *AJNR Am. J. Neuroradiol.*, **32**, No. 1, 85–91 (2011), https://doi.org/10.3174/ajnr.A2238.
27. R. T. Naismith, J. Xu, N. T. Tutlam, et al., "Increased diffusivity in acute multiple sclerosis lesions predicts risk of black hole," *Neurology*, **74**, No. 21, 1694–1701 (2010), https://doi.org/10.1212/WNL.0b013e3181e042c4.
28. D. Ontaneda and R. J. Fox, "Imaging as an outcome measure in multiple sclerosis," *Neurotherapeutics*, **14**, No. 1, 24–34 (2017), https://doi.org/10.1007/s13311-016-0479-6.
29. M. Absinta, P. Sati, and D. S. Reich, "Advanced MRI and staging of multiple sclerosis lesions," *Nat. Rev. Neurol.*, **12**, No. 6, 358–368 (2016), https://doi.org/10.1038/nrneurol.2016.59.
30. A. Dal-Bianco, G. Grabner, C. Kronnerwetter, et al., "Slow expansion of multiple sclerosis iron rim lesions: pathology and 7 T magnetic resonance imaging," *Acta Neuropathol.*, **133**, No. 1, 25–42 (2017), https://doi.org/10.1007/s00401-016-1636-z.
31. C. Elliott, J. S. Wolinsky, S. L. Hauser, et al., "Slowly expanding/evolving lesions as a magnetic resonance imaging marker of chronic active multiple sclerosis lesions," *Mult. Scler.*, **25**, No. 14, 1915–1925 (2019), https://doi.org/10.1177/1352458518814117.
32. A. A. Bogdan, *Changes in the Functional State of Brain Matter in Multiple Sclerosis Based on Proton Magnetic Resonance Spectroscopy Data* (2020), publ. online, https://cutt.ly/cjFwnIN, acc. Jan. 18, 2021.
33. V. I. Golovkin, A. V. Pozdnyakov, Yu. F. Kamynin, and I. S. Martens, "New techniques for predicting therapy in multiple sclerosis," *Byull. Sib. Med.*, **9**, No. 4, 138–144 (2010), https://doi.org/10.20538/1682-0363-2010-4-138-144.
34. R. Srinivasan, N. Sailasuta, R. Hurd, et al., "Evidence of elevated glutamate in multiple sclerosis using magnetic resonance spectroscopy at 3 T," *Brain*, **128**, No. 5, 1016–1025 (2005), https://doi.org/10.1093/brain/awh467.
35. T. A. Kobys', "Neuroimaging predictors of the progression of disability in multiple sclerosis," *Vestn. Sovrem. Klin. Med.*, **9**, No. 4 (2016), https://cyberleninka.ru/article/n/neyrovizualizatsionnye-prediktory-progressirovaniya-invalidizatsii-pri-rasseyannom-skleroze, acc. Dec. 3, 2020.
36. C. Tur, A. Eshaghi, D. R. Altmann, et al., "Structural cortical network reorganization associated with early conversion to multiple sclerosis," *Sci. Rep.*, **8**, No. 1, 10715 (2018), https://doi.org/10.1038/s41598-018-29017-1.
37. T. Charalambous, C. Tur, F. Prados, et al., "Structural network disruption markers explain disability in multiple sclerosis," *J Neurol. Neurosurg. Psychiatry*, **90**, No. 2, 219–226 (2019), https://doi.org/10.1136/jnnp-2018-318440.
38. B. Nourbakhsh and E. M. Mowry, "Multiple sclerosis risk factors and pathogenesis," *Continuum (Minneap. Minn.)*, **25**, No. 3, 596–610 (2019), https://doi.org/10.1212/CON.0000000000000725.
39. C. E. Teunissen, H. Tumani, S. Engelborghs, and B. Mollenhauer, "Biobanking of CSF: international standardization to optimize biomarker development," *Clin. Biochem.*, **47**, No. 4–5, 288–292 (2014), https://doi.org/10.1016/j.clinbiochem.2013.12.024.
40. M. Tintore, À. Rovira, J. Río, et al., "Defining, high, medium and low impact prognostic factors for developing multiple sclerosis," *Brain*, **138**, No. 7, 1863–1874 (2015), https://doi.org/10.1093/brain/awv105.
41. J. Kuhle, G. Disanto, R. Dobson, et al., "Conversion from clinically isolated syndrome to multiple sclerosis: A large multicentre study," *Mult. Scler.*, **21**, No. 8, 1013–1024 (2015), https://doi.org/10.1177/1352458514568827.
42. G. Hinsinger, N. Galéotti, N. Nabholz, et al., "Chitinase 3-like proteins as diagnostic and prognostic biomarkers of multiple sclerosis," *Mult. Scler.*, **21**, No. 10, 1251–1261 (2015), https://doi.org/10.1177/1352458514561906.
43. M. S. Boesen, P. E. H. Jensen, M. Magyari, et al., "Increased cerebrospinal fluid chitinase 3-like 1 and neurofilament light chain in pediatric acquired demyelinating syndromes," *Mult. Scler. Relat. Disord.*, **24**, 175–183 (2018), https://doi.org/10.1016/j.msard.2018.05.017.
44. E. Thouvenot, G. Hinsinger, C. Demattei, et al., "Cerebrospinal fluid chitinase-3-like protein 1 level is not an independent predictive factor for the risk of clinical conversion in radiologically isolated syndrome," *Mult. Scler.*, **25**, No. 5, 669–677 (2019), https://doi.org/10.1177/1352458518767043.
45. P. Kušnierová, D. Zeman, P. Hradílek, et al., "Determination of chitinase 3-like 1 in cerebrospinal fluid in multiple sclerosis and oth-

er neurological diseases," *PLoS One*, **15**, No. 5, e0233519 (2020), https://doi.org/10.1371/journal.pone.0233519.

46. S. Modvig, M. Degn, H. Roed, et al., "Cerebrospinal fluid levels of chitinase 3-like 1 and neurofilament light chain predict multiple sclerosis development and disability after optic neuritis," *Mult. Scler.*, **21**, No. 14, 1761–1770 (2015), https://doi.org/10.1177/1352458515574148.
47. F. Baldacci, S. Lista, G. Palermo, et al., "The neuroinflammatory biomarker YKL-40 for neurodegenerative diseases: advances in development," *Expert Rev. Proteomics*, **16**, No. 7, 593–600 (2019), https://doi.org/10.1080/14789450.2019.1628643.
48. M. Khalil and J. Salzer, "CSF neurofilament light," *Neurology*, **87**, No.11, 1068 (2016), https://doi.org/10.1212/WNL.0000000000003107.
49. D. L. Dong, Z. S. Xu, M. R. Chevrier, et al., "Glycosylation of mammalian neurofilaments. Localization of multiple O-linked N-acetylglucosamine moieties on neurofilament polypeptides L and M," *J. Biol. Chem.*, **268**, No. 22, 16,679–16,687 (1993).
50. M. Khalil, C. E. Teunissen, M. Otto, et al., "Neurofilaments as biomarkers in neurological disorders," *Nat. Rev. Neurol.*, **14**, No. 10, 577–589 (2018), https://doi.org/10.1038/s41582-018-0058-z.
51. C. Barro, P. Benkert, G. Disanto, et al., "Serum neurofilament as a predictor of disease worsening and Brain and spinal cord atrophy in multiple sclerosis," *Brain*, **141**, No. 8, 2382–2391 (2018), https://doi.org/10.1093/brain/awy154.
52. O. Ciccarelli, "Multiple sclerosis in 2018: new therapies and biomarkers," *Lancet Neurol.*, **18**, No. 1, 10–12 (2019), https://doi.org/10.1016/S1474-4422(18)30455-1.
53. J. Kuhle, G. Disanto, J. Lorscheider, et al., "Fingolimod and CSF neurofilament light chain levels in relapsing-remitting multiple sclerosis," *Neurology*, **84**, No. 16, 1639–1643 (2015), https://doi.org/10.1212/WNL.0000000000001491.
54. L. Gaetani, F. Boscaro, G. Pieraccini, et al., "Host and microbial tryptophan metabolic profiling in multiple sclerosis," *Front. Immunol.*, **11** (2020), https://doi.org/10.3389/fimmu.2020.00157.
55. C. K. Lim, A. Bilgin, D. B. Lovejoy, et al., "Kynurenine pathway metabolomics predicts and provides mechanistic insight into multiple sclerosis progression," *Sci. Rep.*, **7**, 41473 (2017), https://doi.org/10.1038/srep41473.
56. P. Ak and B. Se, "The role of the gut microbiome in multiple sclerosis risk and progression: Towards characterization of the 'MS microbiome'," *Neurotherapeutics*, **15** (2018), https://doi.org/10.1007/s13311-017-0587-y.
57. L. Dennison, M. Brown, S. Kirby, and I. Galea, "Do people with multiple sclerosis want to know their prognosis? A UK nationwide study," *PLoS One*, **13**, No. 2 (2018), https://doi.org/10.1371/journal.pone.0193407.
58. R. Magliozzi, O. W. Howell, R. Nicholas, et al., "Inflammatory intrathecal profiles and cortical damage in multiple sclerosis," *Ann. Neurol.*, **83**, No. 4, 739–755 (2018), https://doi.org/10.1002/ana.25197.
59. D. A. Häring, H. Kropshofer, L. Kappos, et al., "Long-term prognostic value of longitudinal measurements of blood neurofilament levels," *Neurol. Neuroimmunol. Neuroinflamm.*, **7**, No. 5, e856 (2020), https://doi.org/10.1212/NXI.0000000000000856.
60. *Challenges in Design, Analysis and Reporting of Prognostic and Predictive Marker Research – from Single Studies to an EBM Based Assessment*, Accessed March 3, 2021, https://methods.cochrane.org/sites/methods.cochrane.org.prognosis/files/public/uploads/program+abstracts.pdf.