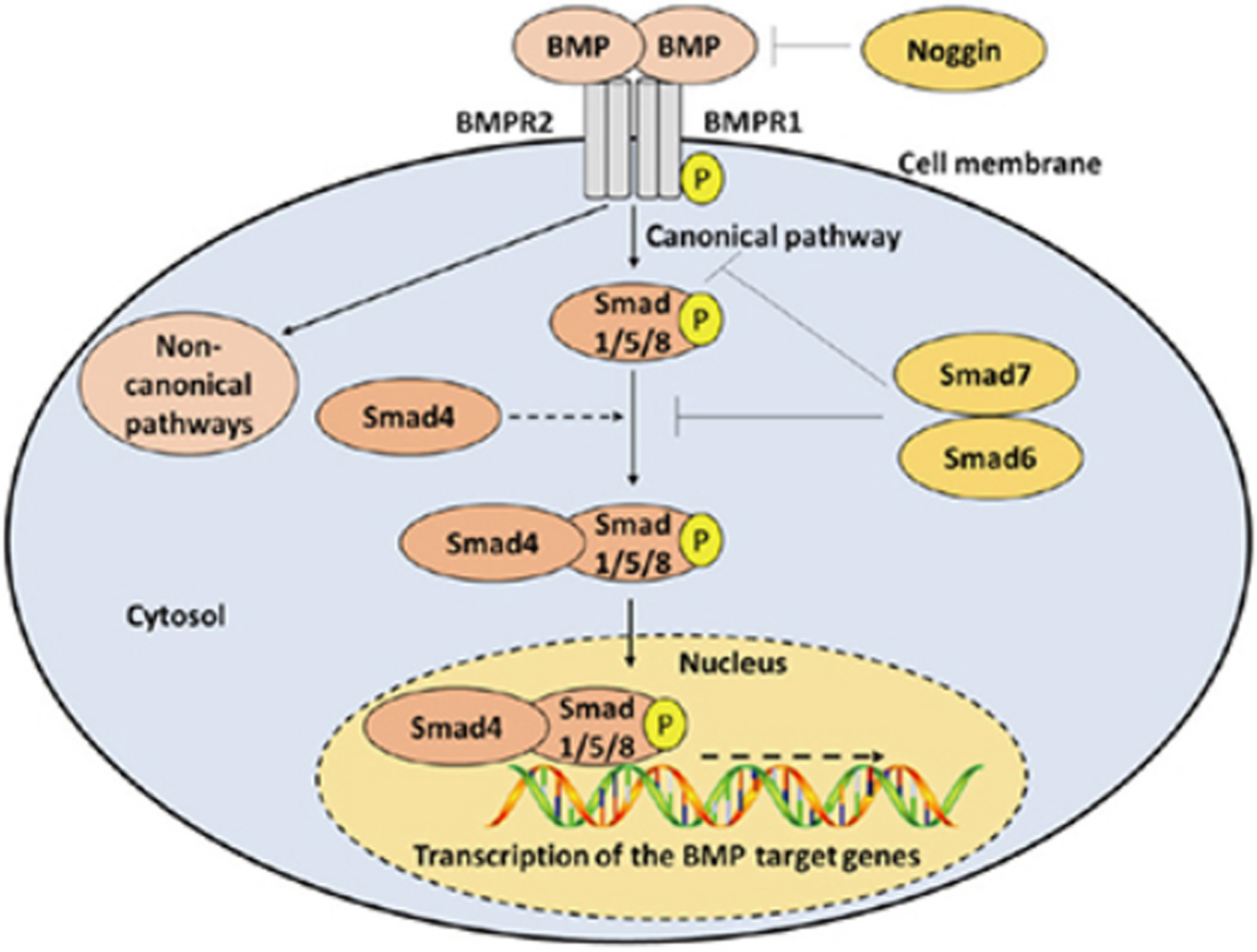
ISSN 2349-6142 (Online), ISSN 2347-8659 (Print)
Volume 8 I Issue 1 I 2021

Neuroimmunology and Neuroinflammation



OAE Publishing Inc.

EDITORIAL BOARD

Honorary Editor-in-Chief

V. Wee Yong (Canada)

Editors-in-Chief

Athanassios P. Kyritsis (Greece)
Thomas Müller (Germany)
Gang Zhao (China)

Associate Editors

Dale Ding (USA)
Dirk M. Hermann (Germany)
Michel Mittelbronn (Germany)
Domenico Praticò (USA)
Gianfranco Spalletta (USA)

Editorial Board Members

Paulo Aguiar (Brazil)
Tomohiro Aoki (Japan)
Eleonora Aronica (Netherlands)
Konstantin E. Balashov (USA)
Bi-Tao Bu (China)
Luigi Casella (Italy)
Aswin Chari (UK)
Chu Chen (USA)
Elisabeth Chroni (Greece)
Steven J. Collins (Australia)
Randall L. Davis (USA)
Vicente Felipo (Spain)
Yara Dadalti Fragoso (Brazil)
Stephen D. Ginsberg (USA)
Ye Gong (China)
Hong-Zhi Guan (China)
Ioannis Heliopoulos (Greece)
Li-Fang Hu (China)
Nunzio Iraci (UK)
Elisabeth Kapaki (Greece)
Andis Klegeris (Canada)
Spiros Konitsiotis (Greece)
Bin Li (China)
Zhu-Yi Li (China)
Sofia Markoula (Greece)
Daniele Orsucci (Italy)
Stéphane Peineau (UK)
Fabrizio Piazza (Italy)
Sara Grazia Maria Piccirillo (USA)
Christine N. Sang (USA)
Chrissa G. Sioka (Greece)
Michael J. Strong (USA)
Hua Su (USA)
Kyoungho Suk (South Korea)
Jun Tan (USA)
Rudolph E. Tanzi (USA)
Konstantinos I. Tsamis (Greece)
Georgios K. Tsivgoulis (USA)
Jorge Valero (Spain)
Kiran K. Velpula (USA)
Chao-Dong Wang (China)
Kevin K.W. Wang (USA)
Anthony R. West (USA)
Sara Xapelli (Portugal)
Jinbin Xu (USA)
Guo-Yuan Yang (China)
Yu-Feng Zang (China)
Wen-Quan Zou (USA)

Editorial Staffs

Rui-Rui Zhang (China)
Mei-Lu Jia (China)
Hai-Di Ding (China)
Huan-Liang Wu (China)
Cai-Hong Wang (China)

GENERAL INFORMATION

About the Journal

Neuroimmunology and Neuroinflammation (*NN*), ISSN 2349-6142 (Online), ISSN 2347-8659 (Print), is a peer-reviewed online journal with print on demand compilation of articles published. The journal's full text is available online at www.nnjournal.net. The journal allows free access (Open Access) to its contents and permits authors to self-archive final accepted version of the articles on any OAI-compliant institutional/subject-based repository. The journal focuses on neuroimmunology and neuroinflammation, and the coverage extends to other basic and clinical studies related to neuroscience, including molecular biology, pharmacology, endocrinology, pathology, physiology, psychology, oncology, *etc*. The journal is indexed with CAS, Chaoxing "Domain" Publishing Platform, Cite Factor, CNKI, DRJI, EBSCO, Embase, Eurasian Scientific Journal Index, Google Scholar, Hinari, JournalGuide, JournalTOCs, J-Gate, ResearchBib, Root Indexing, SHERPA/RoMEO, Wanfang Data and Worldcat.

Information for Authors

Manuscripts must be prepared in accordance with Author Instructions.
Please check www.nnjournal.net/pages/view/author_instructions for details.
All manuscripts must be submitted online at www.editorialmanager.com/neurimm.

Copyright

The entire contents of the *NN* are protected under international copyrights. The journal, however, grants to all users a free, irrevocable, worldwide, perpetual right of access to, and a license to copy, use, distribute, perform and display the work publicly and to make and distribute derivative works in any digital medium for any reasonable purpose, subject to proper attribution of authorship and ownership of the rights. The journal also grants the right to make small numbers of printed copies for their personal use under the Creative Commons Attribution 4.0 License.
Copyright is reserved by © The Author(s) 2020.

Permissions

For information on how to request permissions to reproduce articles/information from this journal, please visit www.nnjournal.net.

Disclaimer

The information and opinions presented in the journal reflect the views of the authors and not of the journal or its Editorial Board or the Publisher. Publication does not constitute endorsement by the journal. Neither the *NN* nor its publishers nor anyone else involved in creating, producing or delivering the *NN* or the materials contained therein, assumes any liability or responsibility for the accuracy, completeness, or usefulness of any information provided in the *NN*, nor shall they be liable for any direct, indirect, incidental, special, consequential or punitive damages arising out of the use of the *NN*. The *NN*, nor its publishers, nor any other party involved in the preparation of material contained in the *NN* represents or warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such material. Readers are encouraged to confirm the information contained herein with other sources.

Published by

OAE Publishing Inc.
245 E Main Street st122, Alhambra, CA 91801, USA
Website: www.oaepublish.com

Contacts

E-mail: editorialoffice@nnjournal.net
Website: www.nnjournal.net

Neuroimmunology and Neuroinflammation

CONTENTS

1 **Neurofilament light chain in demyelinating conditions of the central nervous system: a promising biomarker**
Silvia Bozzetti, Sergio Ferrari, Alberto Gajofatto, Sara Mariotto
Neuroimmunol Neuroinflammation 2021;8:1-13. http://dx.doi.org/10.20517/2347-8659.2020.26

2 **CSF biomarkers in multiple sclerosis: beyond neuroinflammation**
Simona Toscano, Francesco Patti
Neuroimmunol Neuroinflammation 2021;8:14-41. http://dx.doi.org/10.20517/2347-8659.2020.12

3 **The relation between lesions and localization of sources of slow biphasic complexes in encephalitis**
Massimo Valerio, Stefano Rivera, Luca Mesin
Neuroimmunol Neuroinflammation 2021;8:42-9. http://dx.doi.org/10.20517/2347-8659.2020.30

4 **Headache as the onset and main symptom of COVID-19 infection**
Yara D. Fragoso
Neuroimmunol Neuroinflammation 2021;8:50-2. http://dx.doi.org/10.20517/2347-8659.2020.42

5 **Bone morphogenic protein signaling in spinal cord injury**
Nadia Al-Sammarraie, Swapan K. Ray
Neuroimmunol Neuroinflammation 2021;8:53-63. http://dx.doi.org/10.20517/2347-8659.2020.34

6 **Resection of orthotopic murine brain glioma**
Bingtao Tang, Kari Foss, Terence Lichtor, Heidi Phillips, Edward Roy
Neuroimmunol Neuroinflammation 2021;8:64-9. http://dx.doi.org/10.20517/2347-8659.2020.28

Bozzetti *et al*. *Neuroimmunol Neuroinflammation* 2021;8:1-13
DOI: 10.20517/2347-8659.2020.26

Neuroimmunology and Neuroinflammation

Review

Open Access

Neurofilament light chain in demyelinating conditions of the central nervous system: a promising biomarker

Silvia Bozzetti, Sergio Ferrari, Alberto Gajofatto, Sara Mariotto

Neurology Unit, Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, Verona 37134, Italy.

Correspondence to: Dr. Sara Mariotto, Neurology Unit, Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, Policlinico GB Rossi, P.le LA Scuro 10, Verona 37134, Italy. E-mail: sara.mariotto@gmail.com

How to cite this article: Bozzetti S, Ferrari S, Gajofatto A, Mariotto S. Neurofilament light chain in demyelinating conditions of the central nervous system: a promising biomarker. *Neuroimmunol Neuroinflammation* 2021;8:1-13. http://dx.doi.org/10.20517/2347-8659.2020.26

Received: 27 Mar 2020 **First Decision:** 17 Jun 2020 **Revised:** 23 Jun 2020
Accepted: 16 Jul 2020 **Fisrt online:** 15 Aug 2020 **Published:** 21 Mar 2021

Academic Editor: Roberta Magliozzi **Copy Editor:** Cai-Hong Wang **Production Editor:** Tian Zhang

Abstract

Neurofilaments are the major structural proteins of the neuronal cytoskeleton and are classified according to molecular weight into heavy, intermediate, and light chains. They are released into the interstitial fluid and cerebrospinal fluid (CSF) as a consequence of axonal damage. In particular, the light chain (NfL) represents the most abundant and soluble subunit and has been demonstrated to be increased in the CSF of patients with inflammatory, degenerative, vascular, or traumatic injuries in correlation with clinical and radiological activity. Similar results have been obtained measuring serum NfL with high-sensitivity single-molecule array, which enables reliable and repeatable measurement of the low NfL concentrations in serum. In particular, CSF and serum NfL values are strongly correlated in patients with multiple sclerosis (MS) and have been demonstrated to be increased in patients with MS and clinically isolated syndromes (CIS) in accordance with clinical and radiological activity. NfL levels increase in patients with a recent relapse and seem to predict cognitive impairment, long-term outcome, and conversion of CIS to MS. The few available data on patients with other demyelinating diseases suggest that NfL levels are also increased in neuromyelitis optica spectrum disorders and related conditions in correlation with attack severity, suggesting that axonal damage may occur in these disorders. We herein report and discuss published data on the role of NfL as a possible predictor of disease activity, clinical outcome and treatment response in patients with demyelinating conditions of the central nervous system.

Keywords: Neurofilament light chain, multiple sclerosis, clinically isolated syndromes, radiologically isolated syndrome, neuromyelitis optica spectrum disorders, myelin oligodendrocyte glycoprotein, aquaporin-4

© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

INTRODUCTION

Inflammatory demyelinating diseases (IDD) represent a spectrum of heterogeneous disorders affecting the central nervous system (CNS). Multiple sclerosis (MS) is classified as a chronic, immune-mediated, demyelinating disorder, and it is the most well-known disease of this group[1]. Neuromyelitis optica spectrum disorders (NMOSD), which preferentially involve the spinal cord and optic nerve[2], and acute disseminated encephalomyelitis (ADEM), a typically monophasic disease of children[3], are also part of CNS IDD. Other acute inflammatory conditions including idiopathic optic neuritis[4] and acute transverse myelitis[5] also enter in the differential diagnosis. A major discovery in this field was the association between NMOSD and serum aquaporin 4 IgG (AQP4-IgG), confirming that it is a different disease from MS and needs different treatment[6]. On the other hand, the association of serum anti-myelin oligodendrocyte glycoprotein (MOG)-Abs with ADEM, NMOSD and other demyelinating events also clarify the final diagnosis in many conditions previously classified as "idiopathic"[7]. In addition to the difficulty in the diagnostic process, one of the main issues of IDD is the correct assessment of disease activity and the prediction of long-term prognosis. Different clinical scales, radiological parameters, and biological markers have been studied, with the aim of identifying reliable and easily accessible measures of disease activity, treatment response, and prognosis in these conditions. Neurofilament proteins recently emerged as a promising biomarker in this context. Neurofilaments are cylindrical proteins located in dendrites, soma and, in particular, axons of neurons, with their specific role in conferring structural stability and promoting axonal growth and intracellular transport. They are classified as intermediate filaments (i.e., 10 nm in diameter, intermediate between actin and myosin) and include neurofilament light chain (NfL), neurofilament middle chain, neurofilament heavy chain (NfH), and α-internexin, depending on the length of the carboxy-terminal region. Since NfLs are the most abundant and soluble subunit among intermediate filaments, research has mainly focused on them[8]. Low levels of NfL are constantly released from axons under normal conditions, in an age-dependent manner. However, as a consequence of axonal damage due to inflammatory, degenerative, vascular, or traumatic injury, NfL release significantly increases. After reaching the interstitial fluid, NfL are detectable in CSF and in serum at lower but comparable levels[9]. Enzyme-linked immunosorbent assay (ELISA) technology allows the measurement of the higher NfL values present in CSF; however, it is not sensitive enough to measure the significantly lower serum/plasma levels. The recent development of ultrasensitive electrochemiluminescence-based immunoassay [in particular, single-molecule immunoassay, single-molecule array (SiMoA) technology] enables reliable measurement of the low NfL concentrations in serum and the monitoring of minor changes over time[10]. This single-molecule immunoassay is based on antibody capture agents bound to the surface of paramagnetic microbeads containing approximately 250,000 attachment sites. The beads are added to the sample solution and then incubated with a second biotinylated detection antibody and beta-galactosidase-labeled streptavidin. In this manner, each bead that has captured a single protein molecule is labeled with an immunocomplex. During the detection process, a fluorescent signal is generated in sealed wells that contain beads combined with immuno-captured and enzyme-labeled protein molecules. Concentrations are determined digitally to further increase the sensitivity of the assay. With this recently developed technique, a significant increase in serum NfL levels has been demonstrated in different conditions, including Alzheimer's disease[11], Creutzfeldt-Jakob disease[12], frontotemporal dementia[13], amyotrophic lateral sclerosis[14], parkinsonian disorders[15], traumatic brain injury[16], stroke[17], peripheral neuropathies[18], autoimmune encephalitis[19], and in particular MS[20], in correlation with disease activity and *post-mortem* neurodegeneration[21]. The lower invasiveness of serum NfL measurement allows repeatable analyses over time and expands the potential utility of NfL as a biomarker of disease activity and treatment response in a wide spectrum of neurological disorders. Although the actual applicability of this assay in daily clinical practice is still limited and influenced by critical aspects that need to be considered for the correct interpretation of this measurement[22], the development of novel ultrasensitive assays and the extensive applicability of serum NfL measurement have provided major advantages in this field.

NfL IN SUBJECTS WITH CLINICALLY ISOLATED SYNDROME AND RADIOLOGICALLY ISOLATED SYNDROME

Clinically isolated syndrome (CIS) is defined as the first episode of clinical symptoms that potentially precedes MS. About 85% of patients with CIS experience a second clinical episode, thus evolving into MS within the subsequent 10 years[23]. Radiologically isolated syndrome (RIS) is defined by MRI findings suggestive of MS in the absence of clinical symptoms. Progression to MS usually occurs in approximately 66% of patients with RIS[24]. The uncertain evolution and variable long-term prognosis of patients with CIS/RIS make attractive the discovery and validation of specific biomarkers able to identify cases that will have future clinical attacks.

In this scenario, the potential utility of CSF NfL as a predictive marker of disease evolution has been recently explored. In particular, Håkansson *et al.*[25] analyzed CSF levels of NfL, NfH, and other neurodegenerative and inflammatory markers in 19 patients with CIS, 22 cases with relapsing remitting multiple sclerosis (RRMS), and 22 sex- and age-matched healthy controls in a prospective longitudinal study. Disease activity (i.e., radiological lesion load, presence of relapses, and disability worsening) was recorded at 2 years follow-up and compared with the levels of specific biomarkers measured at baseline. Interestingly, NfL values were the only prognostic marker potentially able to predict disease activity in subjects with CIS and MS. A different study compared CSF NfL levels with brain volume measured in 41 patients with CIS and 30 controls and demonstrated that NfL values were higher in subjects with CIS and were inversely associated with grey matter volume[26]. CSF NfL and progranulin levels were also evaluated in subjects with RIS and compared with those determined in subjects with CIS, MS, and healthy controls[27]. Interestingly, NfL levels were significantly lower in subjects with CIS and RIS in comparison with patients with RRMS and primary progressive multiple sclerosis (PPMS), suggesting that the detection of this biomarker could parallel clinical evolution in these disorders. In addition, CSF NfL values recently emerged as an independent risk factor for clinical conversion in subjects with RIS with higher levels associated with shorter time to progression[28].

After the discovery of highly sensitive techniques able to measure the lower values of NfL in plasma/serum, the potential utility of serum NfL in predicting CIS conversion to MS has been explored. Disanto *et al.*[29] demonstrated that serum NfL levels are higher in subjects with CIS in comparison with healthy controls and are associated with T2 hyperintense MRI lesions, gadolinium-enhancing lesions, and disability score at CIS diagnosis, but do not allow subjects with CIS who will convert to definite MS after a short interval (n = 100) to be distinguished from subjects with CIS who will not evolve (n = 100). The potential effect of riluzole treatment in subjects with CIS and early MS (n = 22 CIS/MS cases randomized to riluzole and n = 21 to placebo) in comparison with clinical parameters and serum NfL/NfH values was also analyzed. Despite the absence of treatment effect, the authors demonstrated that NfL levels correlated with Expanded Disability Status Scale (EDSS) changes and neuropsychological outcome[30]. Furthermore, higher NfL levels at baseline were associated with a more rapid decrease in brain volume and predicted higher number of enhancing lesions, confirming the role of NfL as a potential marker of neuronal and axonal damage in CIS and early MS. Finally, in a case-control study performed among US military that analyzed serum samples of 60 subjects asymptomatic at time of sampling who then developed MS (6 years later as a median), the authors observed increased serum NfL levels in cases that would develop MS in comparison to healthy controls, demonstrating a potentially useful value of serum NfL in predicting future development of/evolution to MS[31].

Taken together, available data support the role of CSF NfL levels as a predictive and prognostic marker of CIS/RIS. On the other hand, serum NfL levels are increased in patients with a recent relapse and a high number of T2/enhancing lesions on MRI. Despite the more evident association between NfL levels and

disability at baseline than at follow-up, recent studies indicate that serum NfL values could also be useful to predict MS conversion in patients with a first demyelinating event, with lower NfL levels indicating reduced risk of receiving a future MS diagnosis[32].

NfL IN RELAPSING-REMITTING MS

RRMS is the most common form of MS, involving 85% of affected patients[33]. RRMS is characterized by discrete and clearly definite attacks lasting days to weeks, followed by periods of partial/complete remission in the absence of progressive clinical deterioration[33]. Disease severity and lifelong prognosis of patients with RRMS are highly variable, so that a correct subclassification of this condition according to risk of future disease activity and final disability is of utmost importance to guide prompt therapeutic strategies[34]. The combination of clinical and radiological indicators of disease activity have long been used, despite the high costs and incomplete predictive strength. To overcome these limitations and increase the sensitivity of outcome prediction, the potential utility of different serum and CSF biomarkers have been explored over the last years using high-sensitivity technology[20,25,35,36]. In particular, NfL levels have been analyzed and compared to glial fibrillary acidic protein (GFAP)[25,36], S100B, neuron-specific enolase (NSE)[5], chitinase 3-like 1 (CHI3L1) levels[20,25], and to a panel of chemokines, matrix metalloproteinase-9, and ostepontin[25] with divergent data on the value of combining these biomarkers. The comparison between these biomarkers and previously recognized clinical/radiological parameters of disability aimed to distinguish MS patients and healthy controls, to improve the prediction of ongoing and future disease activity, to better predict long-term outcome in terms of brain and spinal cord atrophy, final disability and risk of progression, and to evaluate response to disease modifying therapy (DMT)[35].

The potential role of NfL in MS was proposed for the first time by Lycke *et al.*[37] in 1998, who detected increased levels of NfL in the CSF of RRMS patients in comparison with healthy controls. The authors also demonstrated a significant correlation between NfL values and disability as assessed by EDSS score, exacerbation rate, and time from last relapse. These findings gave important insight into MS pathogenetic mechanisms, suggesting the presence of axonal damage in subjects with a relapsing-remitting course and postulating a contribution of axonal pathology to disability[37]. Subsequently, the potential utility of measuring NfL levels in subjects with RRMS, monitoring longitudinal levels over time, offered indirect cues to the understanding of NfL kinetics in blood and CSF[20,35,38-40]. Different studies displayed substantial differences in terms of the matrix analyzed (serum, plasma, or CSF) and the performance of the assays used, giving a clear spectrum of the evolution of the detection techniques and their related sensitivity[41]. These assays ranged from a second-generation ELISA[37,39,42-44], to a third-generation electrochemiluminescence technology[9,45] and, finally, to a fourth-generation SiMoA[9,20,25,38,40,46-48] that enables a reliable and highly sensitive quantification and monitoring of serum/plasma NfL levels. In particular, the SiMoA novel ultrasensitive technology increases the sensitivity of the assay allowing comparisons between pathological and normal NfL values using small sample volume[22]. This technical improvement, together with the demonstration of a clear correlation between serum/plasma and CSF NfL levels[45,49], now enables the reliable measurement of NfL in blood samples, avoiding more costly and invasive procedures such as lumbar puncture. This concept further supports the potential use of NfL as a promising biomarker useful for longitudinal monitoring of disease activity and treatment response.

NfL values help to distinguish patients with RRMS from healthy controls

The first objective when investigating NfL levels in RRMS patients was to evaluate whether this biomarker could be useful to differentiate patients from healthy controls. Significantly higher NfL levels in both CSF[9,25,37,39,46,48] and serum[9,38,45,48] have been reported in patients *vs.* healthy controls using different techniques. However, the substantial variation in NfL values observed in different studies prevented the identification of a reproducible cut-off and suggested a great inter-individual variability, possibly influenced by differences in measurement sensitivity, but not clearly related to demographic characteristics, sample

storage, or disease duration[35]. Actually, an attempt to identify specific diagnostic cut-offs for CSF, plasma, and serum through a receiver operating characteristic analysis[46] was reported in a recent prospective phase IV study conducted with the aim of evaluating the effect of dimethyl-fumarate on NfL values. The authors identified specific NfL cut-offs to discriminate between MS patients and healthy controls with a 100% specificity, i.e., 807.5 pg/mL (80% sensitivity) for CSF, 13.0 pg/mL (47% sensitivity) for plasma, and 15.6 pg/mL (43.2% sensitivity) for serum[46].

NfL levels correlate with disease activity at sampling

NfL levels have also been demonstrated to correlate with disease activity in RRMS patients, which is commonly assessed using a combination of different surrogate biomarkers, including clinical parameters such as relapse-rate, and MRI signs (i.e., the presence of gadolinium-enhancing lesions or new or unequivocally enlarging T2 hyperintense lesions)[34]. In particular, in one of the first attempts to correlate NfL levels with disease activity, the authors demonstrated that CSF NfL values were significantly increased 2-3 months after a clinical relapse and tended to gradually decrease thereafter[37]. This pioneer finding was confirmed using more sensitive fourth-generation methods, which allowed the demonstration of increased CSF/serum NfL concentrations in patients who experienced a relapse within 3 months before sample collection, compared to those in remission[49]. In addition, a robust association between NfL values and radiological parameters of disease activity has been demonstrated. In particular, CSF/serum NfL levels are significantly higher in patients with gadolinium-enhancing lesions[9,20,49] and with new or enlarging T2 lesions[20,40,50]. Moreover, NfL concentration progressively increases in correlation with the number of contrast enhanced lesions[9,38,50] and T2 lesion load[38] detected in both brain and spinal cord[9]. As for correlations between NfL values and clinical measure of disability, a robust correlation has been reported between NfL levels and EDSS score at sampling[9,35,38,49]. In a recent cross-sectional study performed on two Swiss MS cohorts, serum NfL concentration at baseline emerged as an indicator of previous clinical disease activity, being significantly associated with a relapse within 60 days before sampling, mean annual relapse rate in the last 1 and 2 years, and with the probability of EDSS worsening during the last 6 and 12 months[9].

NfL levels have a role in the prediction of future disease activity

Attention has more recently been devoted to the possible prognostic role of NfL, to determine whether its concentration could correlate with clinical and radiological biomarkers of future disease activity[9,40,45,47-49,51,52], with treatment response[20,38,39,42,46,49,53], and with progression to a secondary progressive course[54].

In particular, serum NfL levels at baseline have displayed a significant association with the number of clinical relapses in the subsequent 18 months[40] and consequently with an increase in annual relapse rate at 1 and 2 years follow-up[9], supporting the value of this biomarker in predicting future disability. A strong and independent correlation between serum NfL levels above the 90th percentile of healthy controls values and EDSS worsening in the following 12 months was recently observed in a cohort including 189 patients with RRMS, 70 progressive cases, and 259 healthy controls[51]. Different studies have also confirmed a significant association between high serum NfL values at baseline and radiological hallmarks of disease activity/progression during the follow-up, i.e., new T2-lesions and brain volume loss during the subsequent 4 years[48] and brain/spinal cord volume loss as measured after 2 and 5 years from blood sampling[51]. In particular, Barro *et al.*[51] reported a correlation between the percentage of brain/spinal cord volume changes and serum NfL levels, that in a multivariate model remained the only predictors of brain volume loss at 2 years follow-up.

NfL levels help to predict long-term outcome

The possible role of NfL in predicting long-term clinical and radiological outcome in RRMS patients has been investigated assessing serum and/or CSF NfL values in the course of a phase 3 randomized placebo-controlled trial of intramuscular interferon-beta[47]. A robust association emerged between CSF NfL

concentration measured at year 2 from the beginning of the trial and EDSS changes, as well as brain atrophy, expressed by brain parenchymal fraction (BPF) change at 8 years follow-up. Similarly, serum NfL levels at 3 years displayed a correlation with both BPF and EDSS changes at 8 years follow-up, whereas NfL values at 4 years showed a significant association with EDSS changes over 15 years[47]. CSF NfL concentrations at year 2 and serum NfL levels at year 3 in the upper tertile predicted an increased risk of reaching an EDSS score of 6.0 or higher at 8 years follow-up[47]. During the last years, a composite clinical and paraclinical definition of "no disease activity" (NEDA) that includes the absence of relapses, disability worsening, and new or enlarging MRI lesions was proposed as the main target of MS treatment[55]. Several studies have reported that NfL levels at baseline are significantly lower in patients with no evidence of activity during the subsequent follow-up[25,48], therefore showing an accuracy of 85% in correctly classifying NEDA3 cases over the following 2 years[25]. These findings have led to the proposal of expanding the concept of NEDA, taking into consideration also the assessment of brain atrophy and the evaluation of serum and CSF biomarkers, including NfL[56]. Finally, a recent longitudinal study in a Norwegian cohort of 44 patients with newly diagnosed MS and a long-term follow-up of 10 years demonstrated that CSF NfL values were significantly higher in patients evolving from RRMS to secondary progressive multiple sclerosis (SPMS) over 5 years, suggesting a possible role of NfL in predicting the risk of a secondary progressive disease course[54].

NfL levels as a measure of treatment response

Besides the role as a diagnostic and disease activity biomarker, one of the most attractive applications of NfL is their possible use in monitoring therapeutic response. The evidence that serum NfL levels are lower in patients under DMT[9,49] and that initiation and escalation of such therapies significantly decrease NfL concentrations[49] has further confirmed this hypothesis. In particular, starting on an IFNB-1a therapy led to a sustained reduction of serum NfL levels over the following 12 and 24 months[20]. Natalizumab initiation resulted in a 3-fold reduction in CSF NfL values, which reached levels compatible with those measured in healthy controls[39]. The efficacy of fingolimod in reducing NfL concentration in serum[38] and CSF[42] has been demonstrated, also in comparison with IFN[38], in a phase 3 placebo-controlled clinical trial (FREEDOMS)[38,42] and in a phase 3 active-controlled *vs*. IFN trial (TRANSFORMS)[38]. NfL levels have been reported to be significantly reduced by 73% in CSF, 69% in serum, and 55% in plasma 1 year after dimethyl fumarate initiation in a prospective open-label phase 4 clinical trial designed to evaluate the effect of dimethyl fumarate in a cohort of newly-diagnosed RRMS patients (TREMEND). NfL values were similar to those measured in healthy controls in all serum samples, in 96% of plasma samples, and in 72% of CSF samples of treated patients 1 year after treatment initiation[46]. Finally, the therapeutic switch from IFNB or glatiramer acetate to rituximab has been demonstrated to produce a significant (i.e., 21%) reduction of CSF NfL values during the subsequent year in a cohort of 75 patients with RRMS[53]. The role of NfL as drug-response markers has recently been confirmed in a study analyzing the distribution of NfL in RRMS patients starting DMTs and the evolution of NfL values over time. The authors observed that the reduction in plasma NfL concentrations under DMT differed according to specific drugs, although levels were also influenced by baseline characteristics, clinical improvement, and possibly NfL kinetics. In particular, the largest reduction in NfL values was noted on treatment with alemtuzumab and the lowest on teriflunomide, while reduced NfL levels similar to that observed under treatment with alemtuzumab were noted under dimethyl fumarate, fingolimod, and natalizumab. However, groups were not homogeneous for characteristics influencing NfL levels, including age, disease duration, and disease severity, potentially resulting in an indication bias, which the authors tried to overcome with statistical adjustments for baseline characteristics[57].

Taken together, these data led to serum NfL being proposed as a candidate and useful biomarker for surveilling subclinical activity in clinically stable RRMS patients[49] and for measuring and predicting disease activity and treatment response, although commonly accepted cut-off values are still lacking and NfL concentrations are not comparable between different studies.

NFL IN PROGRESSIVE MS

PPMS is characterized by progressive neurological decline from disease onset, without experiencing attacks, and accounts for 15% of MS cases at presentation. SPMS in characterized by progression occurring after a RR course and involves about 50% of cases after 15 years[1]. In this context, the potential role of NfL levels in predicting and quantifying disease progression has been explored. In one of the first studies considering 95 patients with MS and a long-term follow-up (median 14 years, range 8-20), high CSF NfL levels were associated with an unfavorable prognosis and with conversion to SPMS[58]. Although divergent CSF NfL values have been observed in patients with PPMS and SPMS, the authors suggested that NfL is a useful prognostic biomarker under these conditions[59,60]. However, the absence of correlation between NfL levels and disease duration or disease severity, measured with EDSS, led to the idea that CSF NfL levels could not properly reflect disease severity in PPMS[27]. The slow axonal degeneration occurring in subjects with PPMS together with the more robust NfL increase in the course of acute axonal damage could explain these results. Different studies further support this hypothesis. Damasceno *et al.*[61] analysed CSF NfL values in consecutive patients with MS, including 32 subjects with RRMS and 15 with progressive MS, and correlated NfL values with radiological and clinical variables. Interestingly, NfL levels were significantly increased in patients with RRMS in association with cortical lesions and relapses, whereas they were not different in patients with progressive MS in comparison with healthy controls. Sellebjerg *et al.*[62] measured CSF NfL levels in 26 patients with PPMS, 26 with SPMS, and 24 healthy controls and observed higher values in cases with active progressive MS in comparison with those with inactive progressive MS, thus supporting the role of NfL in distinguishing active *vs.* inactive cases. These data further confirm the specific association between NfL concentration and active disease at sampling, which has a significant impact on axonal damage[61]. Partially divergent data emerged according to a recent meta-analysis of case-control studies, where three times higher CSF NfL levels were observed in 158 patients with progressive MS in comparison with healthy controls, although significantly lower values were detected in progressive *vs.* relapsing cases. NfL values tended to be higher, although not significantly different, in RRMS on remission (229 patients) in comparison with patients with progressive MS (158)[63]. In addition to the lower levels measured in cases with progressive *vs.* relapsing MS, a correlation between CSF levels of sCD27 (a soluble marker of T-cells) and NfL values in subjects in progression before and after treatment with natalizumab (17 patients) and methylprednisolone (23 patients) was reported, suggesting a connection between residual inflammation and axonal damage and a role of these biomarkers in monitoring treatment response[64].

The analysis of serum NfL values in patients with progressive MS further confirmed previous observations on CSF NfL measurement. In particular, higher values of serum NfL in the presence of disease activity, defined as a clinical relapse or new gadolinium-enhancing lesions on MRI, were reported in a cohort of 286 patients with MS, including both RRMS and progressive cases (19 PPMS and 63 SPMS)[49]. In a longitudinal study, Disanto *et al.*[9] examined paired serum and CSF samples of different subjects (CIS n = 48, RIS n = 13, RRMS n = 62, PPMS n = 16, and SPMS n = 3) and confirmed the strong association between CSF and serum NfL levels and the presence of 42-fold lower values in serum. A more striking association between NfL values and disability, measured with EDSS, was noted in cases with CIS/RRMS than in those with PPMS/SPMS, once again reflecting the predominant axonal damage occurring in active cases.

A study including subjects with CIS, RRMS, PPMS, and SPMS detected an association between the probability of EDSS worsening and the increase in serum NfL values, with serum NfL levels reflecting future disease progression in terms of brain and cervical spinal cord atrophy. The authors also confirmed the association between serum NfL levels and spinal cord volume loss in patients with PPMS, even in the absence of radiological signs of inflammation, thus supporting the correlation between axonal damage and spinal cord atrophy in the course of disease progression[51]. More recently, Ferraro *et al.*[65] specifically studied 27 patients with PPMS and 43 with SPMS (mean follow-up of 25 months) and demonstrated a positive correlation between plasma NfL values and disability assessed with EDSS, together with an

increase in NfL levels over time on repeated measurements. Although data on the difference of NfL levels between patients with a progressive *vs.* relapsing course are divergent, a recent systematic review confirmed that among subjects with a progressive course, higher levels are observed in those with increased clinical and radiological evidence of disease activity. The impact of disability and the possible role of NfL in predicting future disability is still debated. Finally, treatments with DMTs including natalizumab, rituximab, fingolimod, ocrelizumab, and mitoxantrone seem to affect plasma NfL levels. Unestablished treatments, first-line DMTs, or neuroprotective treatments seem less effective in influencing NfL values[66].

NFL IN NMOSD AND RELATED DISORDERS

NMOSD is an inflammatory CNS syndrome currently diagnosed on the basis of clinical, neuroimaging and laboratory features[2]. The most typical presentations of NMOSD include acute (usually bilateral) optic neuritis with severe visual acuity impairment and longitudinally extensive transverse myelitis (LETM), typically presenting with severe symptoms including paraplegia, bowel/bladder dysfunctions, and sensory loss[67,68]. However, unilateral optic neuritis, short-segment myelitis and other limited forms of neurological syndromes do not exclude NMOSD diagnosis[69]. The course is usually relapsing (90%), with increasing burden of impairment resulting from incomplete attack recovery[70], and it is influenced in particular by onset age, onset phenotype, and ethnicity[71]. In most patients with a diagnosis of NMOSD, AQP4-Abs are detectable in serum, reflecting the autoimmune pathogenesis of the disease[2]. However, seronegative cases are also part of the spectrum, and often represent a diagnostic challenge, with unpredictable disease course and final outcome. The development of cell-based assays using transfected cells and a full-length conformationally intact MOG has allowed the identification of serum[7] and, more rarely, CSF[72] antibodies to MOG in a proportion of patients with NMOSD. However, the clinical spectrum associated with MOG-Abs encompasses a broadening range of phenotypes, including NMOSD and partial forms of the disease (prevalent in adults) and ADEM (prevalent in children)[73-76].

In cases positive for MOG-Abs, isolated optic neuritis (ON) is the most common onset presentation (55%-64%), with simultaneous bilateral involvement in 34%-42% of patients[76-78], followed by acute transverse myelitis (22%-37%), which typically presents as a LETM with enhancement with blurred margins, the so called "cloud-like enhancement". Simultaneous ON and myelitis (8%)[79], an ADEM-like presentation particularly in children, and, more rarely, brainstem presentations[78] and encephalitis[77,80] are other clinical phenotypes associated with MOG-Abs positivity. Disease course can be either monophasic or relapsing (30%-70% of cases), with relapses occurring most frequently in the first year after onset and influenced by acute treatment choices[76,78]. Relapses are considered less common in this condition than in AQP4-Abs-positive NMOSD, manifest more common with ON, and have a great impact on disability[81]. Up to now, only monitoring of MOG-Abs titer has been proposed as a possible predictor of disease course. In particular, disappearance of MOG-Abs in serum is considered prognostic of cessation of relapses[77,82], although seropositivity can be maintained over years even without clinical activity[83]. On the other hand, MOG-Abs titer at onset does not predict the future disease course in terms of risk of relapses or final outcome[84]. As a consequence, antibody titers can help treatment decisions but do not seem reliable enough to be used in the clinical setting for patients' management. MOG-Abs related disorders usually have a favorable prognosis, with a full/good recovery observed in 78% of cases. However, patients can be left with significant sphincter/erectile dysfunction, cognitive impairment, and poor visual acuity, mainly driven by onset attack. Good recovery is more frequent in cases with unilateral ON or ADEM and in younger patients[78].

For the aforementioned characteristics of NMOSD and related conditions, it is evident that there is a need to improve prediction of disease course and short-/long-term prognosis.

Previous reports described astrocytic damage as a primary pathologic process in NMOSD, which is supported by the presence of AQP4-Abs in the serum of most patients (68%-91%)[85]. These antibodies

target aquaporin-4, an integral membrane protein of astrocytes and ependymal cells of CNS, and have pathogenic potential[6]. As a consequence, soluble GFAP, which reflects astrocytic damage, has been proposed as a useful disease-severity marker in subjects with AQP4-Abs related NMOSD[86]. In addition, subjects positive for both AQP4-Abs or MOG-Abs show an increase in CSF myelin basic protein in comparison with MS cases, reflecting the concomitant presence of myelin injury[87]. This concept is supported by the demonstration that the main target in MOG-Abs related conditions is located on the surface of myelin sheath and in the plasma membrane of oligodendrocytes[88].

In this scenario, the possible concomitant increase in biomarkers reflecting axonal damage (i.e., NfL) has appeared worthy of investigation in the scientific community. A possible implication of axonal damage in patients with NMOSD was first suggested by Wang *et al.*[87], who demonstrated an increase in CSF NfH and NfL in this disorder. However, this study did not explore serum NfL levels and also did not distinguish patients according to antibody status, which might influence tissue damage according to the specific target site. We recently analyzed serum NfL levels in patients with NMOSD and related disorders, and when comparing AQP4-Abs-positive, MOG-Abs-positive and seronegative patients, we observed increased serum NfL levels in patients with AQP4-Abs and MOG-Abs[89]. In particular, we detected higher NfL levels in AQP4-Abs-positive subjects, possibly reflecting the prominent axonal damage consequent to astrocytic and cellular injury, and consequently explaining the severe clinical phenotype/evolution usually described in these subjects. On the other hand, we also detected relatively increased levels of NfL in MOG-Abs-positive patients, suggesting the concomitant presence of axonal damage in this disorder and potentially explaining the long-term disability observed in some MOG-Abs-positive cases[89]. We then replicated these observations focusing on 38 MOG-Abs-positive patients and assessing serum and CSF NfL concentration according to clinical/paraclinical characteristics to investigate NfL as a biomarker of disease severity in this condition[90]. We confirmed previous observations on the increase in serum NfL levels in patients with MOG-Abs compared with healthy controls, providing more data on the concomitant presence of axonal damage in this disorder. In addition, when analyzing both serum and CSF samples, we observed a significant correlation between NfL levels in paired samples, supporting the analysis of serum as a reliable and more accessible biological fluid. Even more interestingly, we demonstrated that serum NfL values correlated with attack severity and might predict long-term outcome in patients with MOG-Abs[90]. These observations support the broader use of NfL as an accessible and repeatable biomarker of tissue damage in MOG-Abs related conditions, where it is essential to improve the prediction of short- and long-term prognosis. More recently, the analysis of NfL in a group of 33 NMOSD patients (30 seropositive for AQP4-Abs) reported increased levels in comparison with those detected in healthy controls together with a significant correlation between serum and CSF values and a significant association between NfL levels and age. In addition, serum NfL levels were increased during relapses and correlated with EDSS score but were not influenced by treatment and did not predict relapse occurrence in the subsequent year after sampling[91]. Altogether, these observations expand the utility of NfL as a possible disease activity biomarker also in NMOSD and related conditions.

CONCLUSION

NfL recently emerged as a promising biomarker in the spectrum of demyelinating CNS conditions, in particular after the development of high-sensitivity techniques, which allow us to measure and monitor serum levels over time. NfL values allow us to distinguish patients *vs.* healthy controls, as confirmed by a recent meta-analysis examining 10 studies focused on NfL in CSF and 4 studies on NfL in serum[92]. In addition, NfL levels show a correlation with clinical and radiological disease activity and help to predict MS conversion in patients with a first demyelinating event. Finally, different studies support their role in predicting future disability/long-term prognosis and in monitoring therapeutic response, further supporting their role in clinical practice. Additional evidence is needed to clarify whether CSF/blood NfL assessment is a prognostic/predictive tool in MS patients independently from currently available

biomarkers. Recent data on the presence of axonal damage also in patients with antibodies targeting astrocytes (AQP4-Abs) or oligodendrocytes (MOG-Abs) further extend the possible use of this biomarker in quantifying disease activity in these conditions, although their role in predicting disease course and long-term prognosis in these disorders has yet to be clarified.

DECLARATIONS

Authors' contributions

Analysis and interpretation of data and drafting the manuscript: Bozzetti S, Ferrari S, Gajofatto A
Design and conceptualization of the review, analysis and interpretation of data, and drafting the manuscript: Mariotto S
Read and approved the final manuscript: Bozzetti S, Ferrari S, Gajofatto A, Mariotto S

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2021.

REFERENCES

1. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 2018;17:162-73.
2. Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, et al; International Panel for NMO Diagnosis. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology 2015;85:177-89.
3. Krupp LB, Tardieu M, Amato MP, Banwell B, Chitnis T, et al; International Pediatric Multiple Sclerosis Study Group. International Pediatric Multiple Sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions. Mult Scler 2013;19:1261-7.
4. Petzold A, Plant GT. Diagnosis and classification of autoimmune optic neuropathy. Autoimmun Rev 2014;13:539-45.
5. Transverse Myelitis Consortium Working Group. Proposed diagnostic criteria and nosology of acute transverse myelitis. Neurology 2002;59:499-505.
6. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med 2005;202:473-7.
7. Reindl M, Waters P. Myelin oligodendrocyte glycoprotein antibodies in neurological disease. Nat Rev Neurol 2019;15:89-102.
8. Gaetani L, Blennow K, Calabresi P, Di Filippo M, Parnetti L, et al. Neurofilament light chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry 2019;90:870-8.
9. Disanto G, Barro C, Benkert P, Naegelin Y, Schädelin S, et al; Swiss Multiple Sclerosis Cohort Study Group. Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann Neurol 2017;81:857-70.
10. Gaiottino J, Norgren N, Dobson R, Topping J, Nissim A, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. PLoS One 2013;8:e75091.
11. Mattsson N, Cullen NC, Andreasson U, Zetterberg H, Blennow K. Association between longitudinal plasma neurofilament light and neurodegeneration in patients with alzheimer disease. JAMA Neurol 2019;76:791-9.

12. Staffaroni AM, Kramer AO, Casey M, Kang H, Rojas JC, et al. Association of blood and cerebrospinal fluid tau level and other biomarkers with survival time in sporadic Creutzfeldt-Jakob disease. JAMA Neurol 2019;76:969-77.
13. Steinacker P, Anderl-Straub S, Diehl-Schmid J, Semler E, Uttner I, et al; FTLDc Study Group. Serum neurofilament light chain in behavioral variant frontotemporal dementia. Neurology 2018;91:e1390-401.
14. Poesen K, Van Damme P. Diagnostic and prognostic performance of neurofilaments in ALS. Front Neurol 2019;9:1167.
15. Marques TM, van Rumund A, Oeckl P, Kuiperij HB, Esselink RAJ, et al. Serum NFL discriminates Parkinson disease from atypical parkinsonisms. Neurology 2019;92:e1479-86.
16. Posti JP, Takala RSK, Lagerstedt L, Dickens AM, Hossain I, et al. Correlation of blood biomarkers and biomarker panels with traumatic findings on computed tomography after traumatic brain injury. J Neurotrauma 2019;36:2178-89.
17. Korley FK, Goldstick J, Mastali M, Van Eyk JE, Barsan W, et al. Serum NfL (Neurofilament Light Chain) levels and incident stroke in adults with diabetes mellitus. Stroke 2019;50:1669-75.
18. Mariotto S, Farinazzo A, Magliozzi R, Alberti D, Monaco S, et al. Serum and cerebrospinal neurofilament light chain levels in patients with acquired peripheral neuropathies. J Peripher Nerv Syst 2018;23:174-7.
19. Mariotto S, Gajofatto A, Zuliani L, Zoccarato M, Gastaldi M, et al. Serum and CSF neurofilament light chain levels in antibody-mediated encephalitis. J Neurol 2019;266:1643-8.
20. Varhaug KN, Barro C, Bjørnevik K, Myhr KM, Torkildsen Ø, et al. Neurofilament light chain predicts disease activity in relapsing-remitting MS. Neurol Neuroimmunol Neuroinflamm 2017;5:e422.
21. Ashton NJ, Leuzy A, Lim YM, Troakes C, Hortobágyi T, et al. Increased plasma neurofilament light chain concentration correlates with severity of post-mortem neurofibrillary tangle pathology and neurodegeneration. Acta Neuropathol Commun 2019;7:5.
22. Mariotto S, Sechi E, Ferrari S. Serum neurofilament light chain studies in neurological disorders, hints for interpretation. J Neurol Sci 2020;416:116986.
23. Kinkel RP, Dontchev M, Kollman C, Skaramagas TT, O'Connor PW, et al; Controlled High-Risk Avonex Multiple Sclerosis Prevention Study in Ongoing Neurological Surveillance Investigators. Association between immediate initiation of intramuscular interferon beta-1a at the time of a clinically isolated syndrome and long-term outcomes: a 10-year follow-up of the Controlled High-Risk Avonex Multiple Sclerosis Prevention Study in Ongoing Neurological Surveillance. Arch Neurol 2012;69:183-90.
24. Granberg T, Martola J, Kristoffersen-Wiberg M, Aspelin P, Fredrikson S. Radiologically isolated syndrome--incidental magnetic resonance imaging findings suggestive of multiple sclerosis, a systematic review. Mult Scler 2013;19:271-80.
25. Håkansson I, Tisell A, Cassel P, Blennow K, Zetterberg H, et al. Neurofilament light chain in cerebrospinal fluid and prediction of disease activity in clinically isolated syndrome and relapsing-remitting multiple sclerosis. Eur J Neurol 2017;24:703-12.
26. Tortorella C, Direnzo V, Ruggieri M, Zoccolella S, Mastrapasqua M, et al. Cerebrospinal fluid neurofilament light levels mark grey matter volume in clinically isolated syndrome suggestive of multiple sclerosis. Mult Scler 2018;24:1039-45.
27. Pawlitzki M, Sweeney-Reed CM, Bittner D, Lux A, Vielhaber S, et al. CSF-Progranulin and neurofilament light chain levels in patients with radiologically isolated syndrome-sign of inflammation. Front Neurol 2018;9:1075.
28. Matute-Blanch C, Villar LM, Álvarez-Cermeño JC, Rejdak K, Evdoshenko E, et al. Neurofilament light chain and oligoclonal bands are prognostic biomarkers in radiologically isolated syndrome. Brain 2018;141:1085-93.
29. Disanto G, Adiutori R, Dobson R, Martinelli V, Dalla Costa G, et al; International Clinically Isolated Syndrome Study Group. Serum neurofilament light chain levels are increased in patients with a clinically isolated syndrome. J Neurol Neurosurg Psychiatry 2016;87:126-9.
30. Kuhle J, Nourbakhsh B, Grant D, Morant S, Barro C, et al. Serum neurofilament is associated with progression of brain atrophy and disability in early MS. Neurology 2017;88:826-31.
31. Bjornevik K, Munger KL, Cortese M, Barro C, Healy BC, et al. Serum neurofilament light chain levels in patients with presymptomatic multiple sclerosis. JAMA Neurol 2019;77:58-64.
32. Dalla Costa G, Martinelli V, Sangalli F, Moiola L, Colombo B, et al. Prognostic value of serum neurofilaments in patients with clinically isolated syndromes. Neurology 2019;92:e733-41.
33. Saleem S, Anwar A, Fayyaz M, Anwer F, Anwar F. An overview of therapeutic options in relapsing-remitting multiple sclerosis. Cureus 2019;11:e5246.
34. Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sorensen PS, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology 2014;83:278-86.
35. Domingues RB, Fernandes GBP, Leite FBVM, Senne C. Neurofilament light chain in the assessment of patients with multiple sclerosis. Arquivos de Neuro-Psiquiatria 2019;77:436-41.
36. Martínez MA, Olsson B, Bau L, Matas E, Cobo Calvo A, et al. Glial and neuronal markers in cerebrospinal fluid predict progression in multiple sclerosis. Mult Scler 2015;21:550-61.
37. Lycke JN, Karlsson JE, Andersen O, Rosengren LE. Neurofilament protein in cerebrospinal fluid: a potential marker of activity in multiple sclerosis. J Neurol Neurosurg Psychiatry 1998;64:402-4.
38. Kuhle J, Kropshofer H, Haering DA, Kundu U, Meinert R, et al. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. Neurology 2019;92:e1007-15.
39. Gunnarsson M, Malmeström C, Axelsson M, Sundström P, Dahle C, et al. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. Ann Neurol 2011;69:83-9.
40. Sormani MP, Haering DA, Kropshofer H, Leppert D, Kundu U, et al. Blood neurofilament light as a potential endpoint in Phase 2 studies in MS. Ann Clin Transl Neurol 2019;6:1081-9.

41. Khalil M, Teunissen CE, Otto M, Piehl F, Sormani MP, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol 2018;14:577-89.
42. Kuhle J, Disanto G, Lorscheider J, Stites T, Chen Y, et al. Fingolimod and CSF neurofilament light chain levels in relapsing remitting multiple sclerosis. Neurology 2015;84:1639-43.
43. Kuhle J, Plattner K, Bestwick JP, Lindberg RL, Ramagopalan SV, et al. A comparative study of CSF neurofilament light and heavy chain protein in MS. Mult Scler 2013;19:1597-603.
44. Kuhle J, Malmeström C, Axelsson M, Plattner K, Yaldizli Ö, et al. Neurofilament light and heavy subunits compared as therapeutic biomarkers in multiple sclerosis. Acta Neurol Scand 2013;128:e33-6.
45. Kuhle J, Barro C, Disanto G, Mathias A, Soneson C, et al. Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. Mult Scler 2016;22:1550-9.
46. Sejbaek T, Nielsen HH, Penner N, Plavina T, Mendoza JP, et al. Dimethyl fumarate decreases neurofilament light chain in CSF and blood of treatment naïve relapsing MS patients. J Neurol Neurosurg Psychiatry 2019;90:1324-30.
47. Kuhle J, Plavina T, Barro C, Disanto G, Sangurdekar D, et al. Neurofilament light levels are associated with long-term outcomes in multiple sclerosis. Mult Scler 2019;1352458519885613.
48. Håkansson I, Tisell A, Cassel P, Blennow K, Zetterberg H, et al. Neurofilament levels, disease activity and brain volume during follow-up in multiple sclerosis. J Neuroinflammation 2018;15:209.
49. Novakova L, Zetterberg H, Sundström P, Axelsson M, Khademi M, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology 2017;89:2230-7.
50. Burman J, Zetterberg H, Fransson M, Loskog AS, Raininko R, et al. Assessing tissue damage in multiple sclerosis: a biomarker approach. Acta Neurol Scand 2014;130:81-9.
51. Barro C, Benkert P, Disanto G, Tsagkas C, Amann M, et al. Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain 2018;141:2382-91.
52. Siller N, Kuhle J, Muthuraman M, Barro C, Uphaus T, et al. Serum neurofilament light chain is a biomarker of acute and chronic neuronal damage in early multiple sclerosis. Mult Scler 2019;25:678-86.
53. de Flon P, Gunnarsson M, Laurell K, Söderström L, Birgander R, et al. Reduced inflammation in relapsing-remitting multiple sclerosis after therapy switch to rituximab. Neurology 2016;87:141-7.
54. Bhan A, Jacobsen C, Myhr KM, Dalen I, Lode K, et al. Neurofilaments and 10-year follow-up in multiple sclerosis. Mult Scler 2018;24:1301-7.
55. Giovannoni G, Turner B, Gnanapavan S, Offiah C, Schmierer K, et al. Is it time to target no evident disease activity (NEDA) in multiple sclerosis? Mult Scler Relat Disord 2015;4:329-33.
56. Giovannoni G, Tomic D, Bright JR, Havrdová E. "No evident disease activity": The use of combined assessments in the management of patients with multiple sclerosis. Mult Scler 2017;23:1179-87.
57. Delcoigne B, Manouchehrinia A, Barro C, Benkert P, Michalak Z, et al. Blood neurofilament light levels segregate treatment effects in multiple sclerosis. Neurology 2020;94:e1201-12.
58. Salzer J, Svenningsson A, Sundström P. Neurofilament light as a prognostic marker in multiple sclerosis. Mult Scler 2010;16:287-92.
59. Norgren N, Sundström P, Svenningsson A, Rosengren L, Stigbrand T, et al. Neurofilament and glial fibrillary acidic protein in multiple sclerosis. Neurology 2004;63:1586-90.
60. Trentini A, Comabella M, Tintoré M, Koel-Simmelink MJ, Killestein J, et al. N-acetylaspartate and neurofilaments as biomarkers of axonal damage in patients with progressive forms of multiple sclerosis. J Neurol 2014;261:2338-43.
61. Damasceno A, Dias-Carneiro RPC, Moraes AS, Boldrini VO, Quintiliano RPS, et al. Clinical and MRI correlates of CSF neurofilament light chain levels in relapsing and progressive MS. Mult Scler Relat Disord 2019;30:149-53.
62. Sellebjerg F, Börnsen L, Ammitzbøll C, Nielsen JE, Vinther-Jensen T, et al. Defining active progressive multiple sclerosis. Mult Scler 2017;23:1727-35.
63. Martin SJ, McGlasson S, Hunt D, Overell J. Cerebrospinal fluid neurofilament light chain in multiple sclerosis and its subtypes: a meta-analysis of case-control studies. J Neurol Neurosurg Psychiatry 2019;90:1059-67.
64. Romme Christensen J, Komori M, von Essen MR, Ratzer R, Börnsen L, et al. CSF inflammatory biomarkers responsive to treatment in progressive multiple sclerosis capture residual inflammation associated with axonal damage. Mult Scler 2019;25:937-46.
65. Ferraro D, Guicciardi C, De Biasi S, Pinti M, Bedin R, et al. Plasma neurofilaments correlate with disability in progressive multiple sclerosis patients. Acta Neurol Scand 2020;141:16-21.
66. Williams T, Zetterberg H, Chataway J. Neurofilaments in progressive multiple sclerosis: a systematic review. J Neurol 2020; doi: 10.1007/s00415-020-09917-x.
67. Sato DK, Lana-Peixoto MA, Fujihara K, de Seze J. Clinical spectrum and treatment of neuromyelitis optica spectrum disorders: evolution and current status. Brain Pathol 2013;23:647-60.
68. Flanagan EP. Neuromyelitis optica spectrum disorder and other non-multiple sclerosis central nervous system inflammatory diseases. Continuum (Minneap Minn) 2019;25:815-44.
69. Huh SY, Kim SH, Hyun JW, Jeong IH, Park MS, et al. Short segment myelitis as a first manifestation of neuromyelitis optica spectrum disorders. Mult Scler 2017;23:413-9.
70. Wingerchuk DM, Hogancamp WF, O'Brien PC, Weinshenker BG. The clinical course of neuromyelitis optica (Devic's syndrome). Neurology 1999;53:1107-14.
71. Palace J, Lin DY, Zeng D, Majed M, Elsone L, et al. Outcome prediction models in AQP4-IgG positive neuromyelitis optica spectrum

disorders. Brain 2019;142:1310-23.
72. Mariotto S, Gajofatto A, Batzu L, Delogu R, Sechi G, et al. Relevance of antibodies to myelin oligodendrocyte glycoprotein in CSF of seronegative cases. Neurology 2019;93:e1867-72.
73. Lechner C, Baumann M, Hennes EM, Schanda K, Marquard K, et al. Antibodies to MOG and AQP4 in children with neuromyelitis optica and limited forms of the disease. J Neurol Neurosurg Psychiatry 2016;87:897-905.
74. Sato DK, Callegaro D, Lana-Peixoto MA, Waters PJ, de Haidar Jorge FM, et al. Distinction between MOG antibody-positive and AQP4 antibody-positive NMO spectrum disorders. Neurology 2014;82:474-81.
75. Cobo-Calvo Á, Ruiz A, D'Indy H, Poulat AL, Carneiro M, et al. MOG antibody-related disorders: common features and uncommon presentations. J Neurol 2017;264:1945-55.
76. Mariotto S, Ferrari S, Monaco S, Benedetti MD, Schanda K, et al. Clinical spectrum and IgG subclass analysis of anti-myelin oligodendrocyte glycoprotein antibody-associated syndromes: a multicenter study. J Neurol 2017;264:2420-30.
77. Cobo-Calvo A, Ruiz A, Maillart E, Audoin B, Zephir H, et al; OFSEP and NOMADMUS Study Group. Clinical spectrum and prognostic value of CNS MOG autoimmunity in adults: the MOGADOR study. Neurology 2018;90:e1858-69.
78. Jurynczyk M, Messina S, Woodhall MR, Raza N, Everett R, et al. Clinical presentation and prognosis in MOG-antibody disease: a UK study. Brain 2017;140:3128-38.
79. Cobo-Calvo A, Vukusic S, Marignier R. Clinical spectrum of central nervous system myelin oligodendrocyte glycoprotein autoimmunity in adults. Curr Opin Neurol 2019;32:459-66.
80. Mariotto S, Monaco S, Peschl P, Coledan I, Mazzi R, et al. MOG antibody seropositivity in a patient with encephalitis: beyond the classical syndrome. BMC Neurol 2017;17:190.
81. Juryńczyk M, Jacob A, Fujihara K, Palace J. Myelin oligodendrocyte glycoprotein (MOG) antibody-associated disease: practical considerations. Pract Neurol 2019;19:187-95.
82. López-Chiriboga AS, Majed M, Fryer J, Dubey D, McKeon A, et al. Association of MOG-IgG serostatus with relapse after acute disseminated encephalomyelitis and proposed diagnostic criteria for MOG-IgG-Associated disorders. JAMA Neurol 2018;75:1355-63.
83. de Mol CL, Wong Y, van Pelt ED, Wokke B, Siepman T, et al. The clinical spectrum and incidence of anti-MOG-associated acquired demyelinating syndromes in children and adults. Mult Scler 2019;16:1352458519845112.
84. Cobo-Calvo A, Sepúlveda M, d'Indy H, Armangué T, Ruiz A, et al; REEM Group. Usefulness of MOG-antibody titres at first episode to predict the future clinical course in adults. J Neurol 2019;266:806-15.
85. Wingerchuk DM, Weinshenker BG. Neuromyelitis optica spectrum disorder diagnostic criteria: Sensitivity and specificity are both important. Mult Scler 2017;23:182-4.
86. Takano R, Misu T, Takahashi T, Sato S, Fujihara K, et al. Astrocytic damage is far more severe than demyelination in NMO: a clinical CSF biomarker study. Neurology 2010;75:208-16.
87. Wang H, Wang C, Qiu W, Lu Z, Hu X, et al. Cerebrospinal fluid light and heavy neurofilaments in neuromyelitis optica. Neurochem Int 2013;63:805-8.
88. Brunner C, Lassmann H, Waehneldt TV, Matthieu JM, Linington C. Differential ultrastructural localization of myelin basic protein, myelin/oligodendroglial glycoprotein, and 2',3'-cyclic nucleotide 3'-phosphodiesterase in the CNS of adult rats. J Neurochem 1989;52:296-304.
89. Mariotto S, Farinazzo A, Monaco S, Gajofatto A, Zanusso G, et al. Serum neurofilament light chain in nmosd and related disorders: comparison according to aquaporin-4 and myelin oligodendrocyte glycoprotein antibodies status. Mult Scler J Exp Transl Clin 2017;3:2055217317743098.
90. Mariotto S, Ferrari S, Gastaldi M, Franciotta D, Sechi E, et al. Neurofilament light chain serum levels reflect disease severity in MOG-Ab associated disorders. J Neurol Neurosurg Psychiatry 2019;90:1293-6.
91. Watanabe M, Nakamura Y, Michalak Z, Isobe N, Barro C, et al. Serum GFAP and neurofilament light as biomarkers of disease activity and disability in NMOSD. Neurology 2019;93:e1299-311.
92. Cai L, Huang J. Neurofilament light chain as a biological marker for multiple sclerosis: a meta-analysis study. Neuropsychiatr Dis Treat 2018;14:2241-54.

Toscano *et al*. *Neuroimmunol Neuroinflammation* 2021;8:14-41
DOI: 10.20517/2347-8659.2020.12

Neuroimmunology and Neuroinflammation

Review

Open Access

CSF biomarkers in multiple sclerosis: beyond neuroinflammation

Simona Toscano, Francesco Patti

Department "GF Ingrassia", Section of Neurosciences, Multiple Sclerosis Center, University of Catania, Catania 95123, Italy.

Correspondence to: Prof. Francesco Patti, Department "GF Ingrassia", Section of Neurosciences, Multiple Sclerosis Center, University of Catania, Catania 95123, Italy. E-mail: patti@unict.it

How to cite this article: Toscano S, Patti F. CSF biomarkers in multiple sclerosis: beyond neuroinflammation. *Neuroimmunol Neuroinflammation* 2021;8:14-41. http://dx.doi.org/10.20517/2347-8659.2020.12

Received: 31 Jan 2020 **First Decision**: 20 May 2020 **Revised**: 10 Jun 2020
Accepted: 28 Jun 2020 **Fisrt online:** 21 Aug 2020 **Published:** 21 Mar 2021

Academic Editor: Roberta Magliozzi **Copy Editor**: Cai-Hong Wang **Production Editor**: Jing Yu

Abstract

For many years, quantifiable biomarkers in neurological diseases have represented a hot topic. In multiple sclerosis (MS), cerebrospinal fluid biomarkers have played a diagnostic role since the introduction of Poser's criteria in 1983, with IgG oligoclonal bands playing a supporting role in an epoch prior to magnetic resonance imaging and a complementary one after the introduction of McDonald criteria in 2001. Nowadays, that supporting role has turned into a main one in substituting for dissemination in time and defining the diagnosis of MS in patients with a first clinical event, according to the 2017 revised McDonald criteria. Possibly kappa free light chains, N-CAM, chitinase 3-like protein 1 and IgM oligoclonal bands, not yet implemented in clinical practice, could similarly gain importance in the near future. Furthermore, the increasing knowledge of molecular mechanisms leading to chronic inflammation has enhanced interest in looking for biomarkers of disease activity, better defining the MS phenotype and patients with highly active disease. Accordingly, myelin proteins, intermediate filaments, metalloproteinases and other molecules involved in the inflammatory cascade, are currently under investigation. Finally, it has long been known that axonal loss occurs from the early phases, leading to a progressive neurological deterioration. Since established criteria to assess treatment failure and transition to progressive forms are still lacking, both treatment response and prognostic biomarkers would be useful to predict MS course, and neurofilaments seem to have this potential. The purpose of this review article was to illustrate biomarkers that have been already validated or require further validation after proving to be useful in exploratory studies and potentially could prove useful in clinical practice in the coming years.

Keywords: Multiple sclerosis, biomarkers, cerebrospinal fluid, neurofilaments, oligoclonal bands, disease activity

© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

INTRODUCTION

In the framework of neurological diseases, the need for objective and measurable indicators of the underlying pathological processes is more and more pressing. Therefore, the search for biological markers, or biomarkers, is a continuously expanding field, and a large number of molecules have been explored so far; but only a few have been validated, and even fewer are currently used in clinical practice[1].

Indeed, the assessment of the clinical validity and utility of a biomarker requires a multistage process, which has been proposed as a five-phase procedure going from preclinical exploratory studies (phase 1) to clinical assay development (phase 2), retrospective studies (phase 3), prospective diagnostic accuracy studies (phase 4), and disease burden reduction studies (phase 5)[2]. Moreover, the level of evidence relies on the number of supporting studies and patients included while exploring the independence of the results in independent cohorts[3].

With effective therapeutic strategies in neurodegenerative diseases still lacking, biomarkers would allow us to define the start time of degeneration and make an early diagnosis, to monitor the disease course and predict the prognosis, but also to identify potential therapeutic targets[4].

In the context of inflammatory diseases, biomarkers would be useful to specifically define the involved actors of the immune response and potential therapeutic targets, and also to better understand the etiopathogenesis and to monitor disease activity and treatment response[5].

Multiple sclerosis (MS) is a challenging disease, since it is now clear that both inflammatory and degenerative components occur since early phases[6]. Although the introduction of the first approved medications for progressive MS (PMS) is a recent achievement[7,8], therapeutic options are actually limited in progressive phases. The majority of currently available disease-modifying drugs (DMDs) target inflammation, and therapeutic efforts are mainly focused on early phases of the disease, with the purpose of influencing long-term evolution[9]. It is for this reason that the concept of "no evidence of disease activity" (NEDA) has been introduced as the main therapeutic goal to achieve in patients with MS. Extensively used in clinical trials to define and compare the efficacy of DMDs, the concept of NEDA is still evolving, integrating an increasing number of measures able to define the absence of disease activity. Even though the achievement of this goal is the main one in a clinical real-life setting while monitoring patients under treatment as well, long-term studies are needed to provide evidence of its utility in clinical practice[10]. Currently, NEDA-3 is mainly used in clinical trials and considered the aim of therapeutic strategies in clinical practice, consisting in the absence of relapses, magnetic resonance imaging (MRI) activity and sustained disability worsening during follow-up[11]. Indeed, the increasingly prominent role of MRI and recent advances in technology have led to the inclusion of the measurement of brain volume loss in NEDA (NEDA-4), which may further evolve with the inclusion of biomarkers (NEDA-5)[12]. In this respect, the role of neurofilament light chain (NF-L) as a marker of disease activity, correlating with long-term prognosis seems to be promising[11]. The availability of biological markers reflecting such a disease heterogeneity would definitely help us to better understand its complexity and would be an instrument of unquestionable value.

According to the functional classification provided by the FDA-NIH Biomarker Working Group, such molecules can be categorized in susceptibility, diagnostic, monitoring, prognostic, safety and response biomarkers[13] [Table 1].

Susceptibility biomarkers would be useful to detect among asymptomatic individuals those at risk of developing MS, potentially including genetic investigation in first-degree relatives of MS patients[13].

Table 1. Clinically useful and validated CSF biomarker in MS

	Status	Function	Evidence
IgG OCB	Clinically useful	Diagnostic	Nearly 86% specificity and more than 95% sensitivity for the diagnosis of MS[19]. Implemented in 2017 McDonald criteria as indicator of DIT[20]
		Prognostic for conversion	Associated with higher risk of conversion in MS when detected in CIS[28,29] and RIS[30-32]
IgG index	Clinically useful	Diagnostic	Positive values found in 70-80% of MS patients[18]. Useful as a complementary tool, without replacing CSF IgG OCB[41]
		Disease-activity	Associated with MRI activity[45]
		Prognostic for conversion	Associated with higher risk of conversion in MS when detected in CIS[43]
		Prognostic for progression	Associated with disability progression[44]
KFLC	Validated	Diagnostic	Useful for the diagnosis of MS[49,51,53,54,58]. Increased levels detected in MS patients with no IgG OCB[50,55,62]
		Prognostic for conversion	Associated with higher risk of conversion in MS when detected in CIS[43,60]
		Prognostic for progression	Associated with disability progression[60,64-66]
IgM OCB	Validated	Disease-activity	Associated with aggressive disease course[248,250]
		Prognostic for conversion	Lipid-specific IgM OCB are associated with higher risk of conversion in CIS patients[252,253]
		Prognostic for progression	Associated with disability progression and conversion to SPMS[247,248,256]
		Treatment-response	Lipid-specific IgM OCB predict a decreased response to IFN-β[256]
N-CAM	Validated	Diagnostic	Lower levels detected in MS patients and in PPMS compared with RRMS ones. Considered as an indicator of poor remyelination and repair[180,181]
		Disease-activity	Increased levels detected after relapses, especially under steroid treatment, and related to clinical remission[183]
CHI3L1	Validated	Diagnostic	Increased levels in MS and NMO patients[185,188,189]
		Prognostic for conversion	Associated with higher risk of conversion to MS in CIS patients[190,192]
		Disease-activity	Increased levels associated with higher clinical and MRI disease-activity[190,193]
		Treatment-response	Increased levels in non-responder patients under IFN-β treatment compared with responders[193]
NFs	Validated	Prognostic for conversion	In RIS increased CSF NF-L are an independent risk factor for the conversion into CIS and MS, with greater values related to shorter times of conversion[32]. Associated with higher risk of conversion to MS in CIS patients[224,234]
		Disease-activity	Double NF-L levels in relapsing patients compared with remitting ones[228]. CSF NF-L levels correlate with NEDA-3, MRI activity and brain atrophy[11]. Serum NF-L in early phases contributed to predict the lesion load and brain volume loss over a period of 10 years[238]
		Prognostic for progression	High NF-L concentrations associated with progression in both clinically stable patients and relapsing ones[226,227]. In CIS patients with optic neuritis, CSF NF-L predicted long-term cognitive and physical disability over a follow-up period ranging between 9-19 years[235]. Higher NF-H levels in SPMS patients[224,225]
		Treatment-response	NF-L concentrations decreased after 12-24 months of immunosuppressive therapy in active progressive MS patients[239], after switching from first-line therapies to fingolimod[240] and after 12 months of NTZ[241,242]
MBP	Validated	Disease-activity	Higher values detected in active RRMS compared with stable patients and progressive MS. Increased levels in MS are temporally related to relapses and detectable up to 5-6 weeks after, with greater values in polysymptomatic and severe exacerbations[158,159,166-168]. Reduced levels after steroid treatment[168,169]
GFAP	Validated	Prognostic for progression	Elevated levels in MS compared with controls[265-267], with higher values in patients with EDSS greater than 6.5[266]. Associated with greater EDSS score, longer disease duration and progressive course[268]. Increased levels of GFAP in MS predictive for the disability achieved 8-10 years later[267]
		Disease-activity	Associated with MRI parameters as infratentorial chronic lesion load and the intensity of Gd+ in both CIS and RRMS patients[269]
MMP-9	Validated	Disease-activity	Elevated values during clinical relapses, related to a greater number of MRI Gd+ lesions[144]. Higher values in MS compared with controls and in RRMS compared with PPMS[148]
		Treatment-response	Decreased levels after treatment with IFN-β[152-154] and NTZ[155]
CXCL13	Validated	Diagnostic	Higher levels in MS patients compared with controls, though low specificity[126-128]
		Prognostic for conversion	Associated with higher risk of conversion to MS in CIS patients[130]
		Disease-activity	Associated with clinical and radiological activity[126,127]. Decreased levels after steroid treatment[127]
		Treatment-response	Decreased levels after treatment with NTZ[127,132], RTX[129,131]

OPN	Validated	Diagnostic	Significantly greater levels in MS patients compared with controls[102,107,108,110]
		Disease-activity	In RRMS patients, higher levels detected in active disease compared with stable disease and during relapses compared with remission phases[100-103]
NO metabolites	Validated	Disease-activity	Increased levels in body fluids of MS patients, particularly RRMS compare with SPMS. Higher values detected during relapses[78,90]
MRZ reaction	Validated	Diagnostic	A humoral response against at least 2 of 3 viruses is detected in 78% of patients with MS with high specificity[73]
		Prognostic for conversion	Associated with higher risk of conversion in MS when detected in CIS[69,70]

MS: multiple sclerosis; CIS: clinically isolated syndrome; RIS: radiologically isolated syndrome; MRI: magnetic resonance imaging; OCB: oligoclonal bands; RRMS: relapsing-remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis; NMO: neuromyelitis optica; NEDA: no evidence of disease-activity; N-CAM: neuronal cell adhesion molecule; CHI3L1: chitinase-3-like-1; MBP: myelin basic protein; GFAP: glial fibrillary acidic protein; Gd+: gadolinium-enhancing; MMP-9: matrix metalloproteinase-9; CXCL13: C-X-C motif ligand 13; NFs: neurofilaments; NF-L: light chains of neurofilaments; NF-H: heavy chains of neurofilaments; OPN: osteopontin; NO: nitric oxid; MRZ: measles-rubella-varicella; NTZ: natalizumab; RTX: rituximab

Diagnostic biomarkers should be able to confirm the diagnosis of MS, improving diagnostic accuracy when applied together with clinical and MRI criteria. Thus, they can allow clinicians to exclude other possible differential diagnoses, including different autoimmune disorders and other neurological diseases. They should also ideally detect patients with clinically isolated syndrome (CIS) and radiologically isolated syndrome (RIS) and distinguish between different subtypes of the disease[5,13].

Monitoring biomarkers play a relevant role in MS, allowing neurologists to serially assess the status of the disease[13]. Particularly, disease activity biomarkers may crucially affect therapeutic decisions by detecting high disease activity and rapid disability worsening in early phases of MS[9,14]. Correlating with clinical and radiological activity, they may aid in identifying aggressive forms of MS and also provide an indirect assessment of low therapeutic response in patients under DMDs[1].

The definition of prognostic biomarkers as a separate class is slightly more controversial, since a prognostic impact is recognized in other categories of biomarkers[1]. Indeed, those markers able to predict either the risk of relapses or progression or both would belong to this group[13]. However, this term is usually attributed to those molecules reflecting axonal damage, astrocyte activation and remyelination, prevailing in progressive phases of disease[4]. They would also be important in identifying transitional progressive forms of MS, since reliable indicators are not available[9]. Alongside these, many studies have considered those molecules that are predictive of conversion to clinically definite MS when detected in patients with CIS as being "prognostic". To distinguish these "prognostic for conversion" biomarkers from the aforementioned "prognostic for progression" ones, their role in this review will be discussed as belonging to the diagnostic category for conceptual similarity.

Finally, the monitoring of treatment response in terms of both efficacy and safety may be very important in personalizing therapies and planning switches whenever appropriate[5] and may be of benefit in the use of pharmacodynamic/response and safety biomarkers[13].

However, boundaries are blurred and some markers may exhibit more than one function. Moreover, to be validated, exploratory molecules have to be reproducible among independent studies, and only easily detectable and cost-effective ones truly impacting the diagnostic therapeutic processes would be used in clinical practice[5].

For both anatomic and physiological reasons, cerebrospinal fluid (CSF) represents the main source of potential biomarkers for MS among body fluids[4]. Indeed, its composition may reflect the impairment of brain metabolism, the breakdown of the blood-brain barrier (BBB) and many ongoing processes occurring in the central nervous system (CNS) with a consequent production of catabolites[15]. However, requiring less invasiveness and due to blood continuity with CSF, serum samples are being increasingly used and explored as a source of biomarkers[1].

Considering the extensive number of molecules that are currently under investigation and detected in body fluids, this review will focus on CSF biomarkers that are currently used in clinical practice, those that have not been clinically implemented although validated and those requiring further validation after proving to be useful in small exploratory single-site studies. Since some molecules may potentially fall in more than one category according to a functional classification, they will be classified according to their main recognized role.

DIAGNOSTIC BIOMARKERS

IgG oligoclonal bands

CSF IgG oligoclonal bands (IgG OCB) are detected in almost 90% of patients with MS and in nearly 70% of patients with CIS[16]. It does not seem that OCB-negative MS shows different characteristics, even though a different immunogenetic phenotype of HLA-DRB1 has been identified in some studies[17]. Among several techniques, isoelectric focusing followed by immunofixation in parallel CSF and serum samples is mainly used for their detection due to a high sensitivity[18,19]. Of all possible patterns, type 2 is detected when at least two bands of IgG are present in CSF but not in serum, which is suggestive of intrathecal IgG synthesis and thus of an inflammatory disease of the CNS[18].

As a qualitative assessment, CSF IgG OCB detection is actually considered a more reliable test than any quantitative assessments of intrathecal synthesis[20]. With nearly 86% specificity and more than 95% sensitivity, CSF IgG OCB do not represent a pathognomonic finding of MS, but they may strongly support the diagnosis of MS when other causes of CNS inflammation have been ruled out[19].

In 2017, the latest revised McDonald criteria gave great significance to CSF OCB as a substitute for dissemination in time[20], increasing sensitivity in the diagnosis of relapsing-remitting MS (RRMS) in patients with a first clinical event[21]. CSF IgG OCB already had a role as a diagnostic biomarker before, first in Poser's criteria (1983) to define "laboratory supported" definite and probable MS diagnosis[22]. Years later, both the McDonald *et al.*[23] and Polman *et al.*[24] criteria considered the presence of CSF IgG OCB as sufficient to provide the evidence of dissemination in space (DIS), together with the detection of at least two MRI lesions consistent with MS. Although not being included in 2010 revised criteria[25], CSF analysis with IgG OCB detection still represents a common step of the diagnostic program, particularly because it may allow us to exclude other diagnoses adding a different type of information compared with MRI, which may be unable to allow the distinction between MS and mimics at early stages[26]. Moreover, it has been argued that the presence of CSF OCB may increase the specificity of those criteria when considered together with DIS[27].

Concerning the diagnosis of primary progressive MS (PPMS), the presence of CSF OCB is one of the required criteria[20] and its role has been confirmed over time in the consecutive revisions after Poser's criteria[23-25]. In addition to a diagnostic role, CSF IgG OCB also has a prognostic role for conversion to MS, since their identification in patients with CIS increases the risk to convert into clinically definite MS with a negative predictive value of 88%[28]. In a prospective study conducted by Tintoré and coworkers in 572 patients with CIS, the detection of CSF IgG OCB almost doubled the risk of a second relapse, regardless of baseline MRI, without affecting disability outcomes during a follow-up of 50 months[29].

Despite recognizing the presence of CSF IgG OCB as one of the factors predicting an increased risk to develop MS in patients with RIS[30], specific criteria have not been established in the 2017 latest revision[20]. Results from a recent study showed that the presence of CSF OCB in children with RIS increased the risk to develop pediatric MS and improved specificity of MRI criteria in these patients[31]. In another study conducted in 75 patients with RIS, CSF OCB also proved to be an independent risk factor for the conversion to CIS and MS and were associated with shorter times of conversion[32].

It has been suggested that the presence of CSF OCB, indicative of intrathecal synthesis, may both directly and indirectly perpetuate the inflammatory damage through the chronic stimulation of microglia via immunoglobulin and immunocomplexes. Occurring even once acute perivascular inflammation has stopped, such an activation facilitates further and mutual activation of both microglia and astrocytes. As a result, antibody-mediated inflammation promotes a microenvironment of chronic inflammatory damage and neurodegeneration[33]. From this perspective, the administration of a drug able to affect local humoral production would be further useful. However, among the currently available drugs, only natalizumab (NTZ) and cladribine proved to affect intrathecal Ig synthesis, ultimately leading to CSF OCB disappearance in some cases[34-37]. Nevertheless, the presence of CSF OCB does not seem to be associated with an aggressive disease course or a faster disease progression in MS[17,38].

IgG OCB represent a validated and clinically implemented biomarker for both the diagnosis of MS and the detection of CIS converters. Its validity relies on numerous confirmatory studies conducted in more than 200 patients, thus providing a strong level of evidence[3].

IgG index

The ratio between IgG quotient and albumin quotient, known as the Link Index[39], is largely used to assess a quantitative evaluation of intrathecal synthesis, enough to be considered an alternative to the detection of CSF OCB in previous MS diagnostic criteria[22-24]. However, the latest revision of McDonald criteria clearly states that the identification of CSF IgG OCB is superior to any quantitative assessments, whose results have to be cautiously considered when isolated or conflicting with the aforementioned tool[20]. In addition, it has been clearly defined in two different consensus statements that IgG index and other quantitative assessments are just complementary tests, less sensitive than qualitative detection of CSF OCB[18,19]. A value greater than 0.70 is universally considered suggestive of pathological intrathecal synthesis for IgG index, with abnormal values detected respectively in 70%-80% of patients with clinically definite MS[18]. With a cut-off value of 0.7, a positive predictive value by 60% for the diagnosis of demyelinating CNS disease has been found[40]. Considering that a correlation exists between IgG index and positive predictive value for MS, increasing IgG index values correlate with a greater probability of MS diagnosis[40]. Nonetheless, abnormal values are rarely detected in MS patients with no CSF OCB[41].

Nephelometry is the most used technique to measure albumin in CSF and serum as well, to provide a quotient that is a reliable measure of blood-CSF barrier function, especially when age-related[18,19]. This is crucial, since the increased concentration of a substance in CSF can be the result of either intrathecal synthesis or increased permeability of the blood-CSF barrier. Moreover, interindividual variability in serum IgG concentrations is similarly reduced by using a CSF/serum IgG quotient[18].

Applying different mathematical models, several indices have been derived[42], including Tourtellotte's, Reiber's, Link's and intrathecal IgG fraction. Actually, although the IgG index is the most commonly used quantitative measure of intrathecal synthesis in clinical practice, other indices using hyperbolic mathematical functions, such as Reiber's index, are considered more accurate, resulting in few false positives[18,19]. Senel and coworkers found 43% sensitivity and 64% specificity for IgG index regarding conversion of CIS to clinically definite MS, with a positive predictive value of 53% and a negative one of 54%[43].

As a prognostic biomarker, a very high IgG index has been related to a major disability progression with greater values in secondary progressive MS (SPMS) compared with PPMS and RRMS patients in a study conducted by Izquierdo and coworkers[44].

Finally, a recent retrospective study involving 149 patients with CIS and MS investigated a possible

association between CSF parameters and MRI activity. IgG index was highly correlated with the detection of new cerebral lesions on MRI scan and proved to be an independent predictor of future MRI activity[45].

Currently, IgG index is clinically implemented as additional evidence of CNS local humoral response, with the advantage of being based on easily achievable information from a simple CSF analysis[46]. Thus, it is useful in supporting the diagnosis of MS and could represent a complementary screening test in patients suspected of MS, without replacing the diagnostic value of CSF OCB[41].

Kappa free light chains and kappa index

CSF kappa free light chains (KFLC) result from intrathecal humoral activity of plasma cells. Being normal constituents of human Ig structure together with lambda light chains, they tend to accumulate together with Ig in inflammatory disease of CNS[47] and can be detected by ELISA, Western blotting[48] or nephelometry[49-51]. Several studies reported an increased concentration of free light chains in CSF of patients with MS[49,51]. As for IgG index, the use of a ratio between KFLC CSF/serum quotient and albumin quotient has been considered by the majority as the best method to represent intrathecal FLC synthesis[47,51], with some exceptions[49,52]. Conversely, lambda FLC Index did not prove to have comparable values of sensitivity and specificity, and it is currently not considered a potential diagnostic biomarker for MS[49,53].

KFLC index has been explored as a diagnostic biomarker, despite the lack of an unequivocal cut-off value currently causes some difficulties in comparing results from several studies [Table 2]. As an indicator of intrathecal synthesis, KFLC index correlates well with IgG index[54], using a cut-off value of 5, although showing greater sensitivity (more than 96% *vs*. nearly 50%) for CSF IgG OCB identification and MS diagnosis and according to higher negative predictive values, with comparable specificity.

Although different thresholds have been used in several studies, ranging from 4.25[55] to 12.3[53], KFLC index extensively proved to have a higher sensitivity and a lower specificity with a similar diagnostic accuracy compared with IgG OCB in discriminating MS and controls[53,55-59]. In a recent study by Gaetani and coworkers, KFLC index distinguished precisely as did IgG OCB between MS and non-inflammatory diseases using a cut-off value of 7.83[56]. It has been suggested that a higher cut-off value (10.6) could be useful to differentiate MS from other inflammatory diseases by increasing specificity and to predict conversion in CIS with greater accuracy as compared with OCB[56]. Similarly, high levels of CSF KFLC have also been demonstrated in CIS patients, showing a correlation with the risk of conversion to clinically definite MS within 2 years[43,60]. Moreover, unlike KFLC index threshold, a cut-off value for intrathecal KFLC synthesis has proved to be more reproducible[58,61,62].

Noteworthy, KFLC index proved to be increased in MS patients with no evidence of IgG OCB, amounting to almost 5% of cases[50,55,62], showing a greater sensitivity but a less specificity by using a threshold of 5.9. In a recent study by Ferraro and coworkers, a KFLC index ≥ 5.8 was detected in 25% of OCB-negative MS patients and in 98% of OCB-positive ones[63].

It has been hypothesized that KFLC index may replace IgG index as a first-line test, but some disagreement remains about the need to determine both KFLC index and IgG OCB in patients with suspected MS[63] or to use them sequentially[56]. Probably, the higher sensitivity of KFLC index compared with IgG OCB would allow clinicians to screen patients in a shorter time, with lower costs and the advantage of a quantitative assessment[49,58], restricting the use of IgG OCB to patients with positive KFLC index. Such a diagnostic route would allow clinicians to reduce false positive results when faced with an inflammatory disease of the CNS. Showing a comparable or higher specificity[50,54], IgG index could still have a role as a screening test complementary to KFLC index for the detection of intrathecal Ig synthesis. However, KFLC index currently shows an intermediate level of evidence as a diagnostic biomarker, requiring other confirmatory studies in larger cohorts[3].

Table 2. Different cut-off values for kappa index and characteristics of study cohorts

	Study cohort (number of analyzed paired serum and CSF samples)	True positives	True negatives	Cut-off	Sensitivity	Specificity	McDonald's diagnostic criteria
Crespi *et al*.[54]	385	MS (127)	Other neurological diseases: IND (117) NIND (141)	≥ 5	96	78	2017
Gaetani *et al*.[56]	170	RIS, CIS, MS (64)	Other neurological diseases (106): IND (24) NIND (82)	≥ 7.83	89	81	2010
Gurtner *et al*.[57]	320	RIS, CIS, MS (67)	Other neurological diseases (258): autoimmune (53), NIND (50), IND (38), degenerative (28), peripheral neuropathy (24), infection (13), cancer (11), neuromyelitis optica (10), others (31)	≥ 10.5	87	76	2010
Leurs *et al*.[59]	745 (from 18 centers)	CIS, MS (526)	Controls (219): IND (67) NIND (76) Symptomatic controls (49) Healthy controls (27)	≥ 6.6	88 93 (MS and controls)	83 83 (MS and controls)	2010 (84%) 2005 (16%)
Pieri *et al*.[53]	176	MS (71)	Other neurological diseases: IND (33) NIND (72)	≥ 12.3	93	100	2010
Presslauer *et al*.[58]	438 (from 4 centers)	CIS/MS (70)	Other neurological diseases (368), including meningitis/encephalitis (41) Guillain-Barré (15) Neuroborreliosis (15) CIDP (7)	≥ 5.9	96	86	2010
Puthenparampil *et al*.[55]	137	MS (70)	Healthy controls (symtpomatic despite no neurological and systemic disorders) (37)	≥ 4.25	94	100	2017

MS: multiple sclerosis; CIS: clinically isolated syndrome; RIS: radiologically isolated syndrome; IND: inflammatory neurological diseases; NIND: non-inflammatory neurological diseases; CIDP: chronic inflammatory demyelinating polyneuropathy

It has also been pointed out that higher values of KFLC index are associated with greater disability[60,64-66], even though previous authors did not go in the same direction but hypothesizing a prognostic role for this marker[61,67].

Measles-rubella-varicella-zoster reaction

In the 1994 consensus report about CSF analysis in the diagnosis of MS, the detection of intrathecal Ig synthesis against neurotrophic viruses, such as measles, rubella and varicella-zoster, was considered a complementary diagnostic test for MS[18]. Such kind of local humoral response, called measles-rubella-varicella-zoster (MRZ) reaction (MRZR), has been reported in up to 94% of patients with MS if at least one intrathecal virus-specific response is detected[68], with anti-measles response as the most frequent one[69-71]. However, MRZR is usually considered positive if a humoral response against at least 2 of 3 viruses is reported, with a commonly used cut-off value of 1.5 for antibody index[72,73]. The reason for this local humoral response, which occurs without active replication of the virus[74], has not been entirely clarified[75]. An involvement of T lymphocytes promoting the differentiation of memory B cells into antibody secreting ones has been suggested[70].

High specificity of up to 97% for MRZR was also reported by Jarius and coworkers, who found a positive reaction in 78% of patients with MS compared to 3% of controls. Moreover, MRZR has proved to be able to

distinguish between MS and other diseases, such as neuromyelitis optica (NMO)[73], anti-MOG associated encephalomyelitis[73], and primary CNS lymphoma[72].

In a prospective 2-year study involving 89 patients with CIS, MRZ reaction was associated with a greater risk to convert to clinically definite MS, showing a greater positive predictive value (70%) than OCB (64%) and MRI (64%)[70]. In patients with acute optic neuritis with positive MRZR and MRI, conversion to clinically definite MS occurred in 86% of them after 4 years, with a prevalence of 73% for MRZR in those who converted[69]. Thus, MRZR can further support the diagnosis at onset and assist in discrimination between MS and other clinically similar inflammatory diseases, representing a complementary diagnostic biomarker with an intermediate level of evidence[3,74]. Nevertheless, further studies in additional cohorts are required[3].

DISEASE ACTIVITY BIOMARKERS

Nitric oxide metabolites

Due to the role of oxidative stress in MS pathogenesis, nitrate and nitrite have been investigated as disease activity biomarkers[76]. Indeed inflammatory processes produce, as a result of the activation of immune cells, reactive oxygen species, including nitrogen-based oxidants[76]. Moreover, Nitric oxide (NO) seems to have much more roles than being a blood flow controller and a synaptic transmitter, regulating the permeability of the BBB, exerting immunomodulatory properties and mediating axonal damage and demyelination[77].

Increased levels of nitrate and nitrite have been identified in body fluids of MS patients in several studies. Particularly, many studies have reported greater concentrations of these molecules in CSF[78-80], serum[81,82] and urine[83] of MS patients compared with controls. Accordingly, the inducible form of nitric oxide synthase has been detected in CSF of MS patients, while not in healthy controls[84], and its mRNA has been found in cerebral tissue of MS patients[77]. Interestingly, interferon-beta (IFN-β) has proved to exert a remarkable inhibition of inducible NO synthase expression in astrocytes[85].

Meanwhile, it is still controversial whether the concentration of NO metabolites is significantly different in RRMS compared with PMS. Indeed, some studies found higher CSF and serum levels of NO metabolites in RRMS compared with SPMS[86,87], while others did not detect any differences[80,88].

Speculating a role as a disease activity biomarker, the association between NO metabolites and the occurrence of relapses in RRMS patients has been explored, and several studies have confirmed this hypothesis[78,89-91], but longitudinal and multicenter studies are needed.

In a study by Yamashita *et al.*[78], significantly higher nitrite and nitrate levels were detected among patients in relapse compared with those in remission and patients treated with steroid in the previous 1-2 months. Acar *et al.*[90] found higher nitrate and nitrite concentrations in relapsing patients than in remitting ones, with the latter ones still showing greater values than controls. Accordingly, NO metabolites predicted disease activity with 71% specificity and 66% sensitivity. In contrast, few studies reported evidence of an association between NO metabolites and MRI findings[90,92], as well as between the development of disability and EDSS progression[92].

Osteopontin

Osteopontin (OPN) is a sialoprotein, whose role in bone remodeling has long been known[93]. Beyond this, it is closely linked to the immune system, since it mediates chemotaxis, cell adhesion and signaling, and it also promotes cytokine and interleukin (IL) function, inducing IL-12 and inhibiting IL-10 among others. In its soluble form, indeed, it is secreted by and also interacts with macrophages and activated leukocytes, reduces the inducible form of NO synthase, promoting inflammation. In its intracellular

form, it is expressed by dendritic cells and promotes Th17 and Treg differentiation[94]. Moreover, it is thought to mediate the upregulation of Th1 and Th17 cytokines, mainly IFN-γ and IL-17[95], and the inhibition of pro-apoptotic proteins, favoring T cell survival[96]. It has been suggested that a specific subset of Th1 cells, particularly arising in CSF during relapses, produces OPN, high levels of IFN-γ and matrix metalloproteinase-9 (MMP-9) after polyclonal stimulation, playing a pathogenetic role[97].

In experimental models of relapsing-remitting experimental autoimmune encephalomyelitis (EAE), OPN expression was constantly evidenced in microglia next to periventricular lesions and in neurons limited to the relapse phase, which increased in mice with greater disease severity[98]. Moreover, when recombinant OPN was given to mice, severe relapses occurred after 1-3 days. Conversely, knockout mice for OPN seemed to be protected from the development of severe EAE[96].

Accordingly, immunohistochemistry analysis of MS brain lesions in humans identified marked OPN expression immediately near the lesions, in vascular endothelial cells, microglia and astrocytes, which was greater in more active lesions[98,99].

High levels of OPN have been found in plasma of RRMS patients, with greater concentrations in patients with active disease compared with those without exacerbations[100-103] and during relapses compared with remissions[102,104,105]. Similar results were found in other studies[96,101,106], with significantly higher CSF and serum OPN levels in MS patients compared with controls[102,107-110]. A positive correlation between IL-17 and both OPN and IL-23 concentrations has also been found[106]. Moreover, CSF concentrations of OPN in MS patients, re-evaluated 5 years after sampling, proved to be not only elevated but also related to the occurence of relapses and to clinical severity[111]. It has been supposed that the increase in OPN during relapses has an inverse correlation with the concentration of serum extracellular proteasome, with marked effects on chemotaxis[112]. However, other studies did not find a clear association between OPN levels and disease activity[107,113].

According to some studies, SPMS patients exhibited elevated OPN values as well compared with controls[102,107], while a significant difference was not reported by other studies[104]. In a recent meta-analysis by Agah and coworkers, all MS patient subtypes showed higher OPN levels compared with controls, except for CIS[101]. However, greater concentrations were found in RRMS patients compared with all other groups and in those with exacerbations compared with patients with stable disease.

IFN-β proved to downregulate OPN and IL-17 in MS patients and to decrease the incidence of EAE and the amount of Th1 and Th17 cells in mice[114,115]. Indeed, RRMS patients treated with IFN-β showed OPN at similar levels compared to untreated patients in remission phase[96]. Glatiramer acetate and NTZ lead to the decrease of plasma OPN levels as well[107,116]. Several polymorphisms of the OPN gene have been investigated to find an association with disease course or activity[117-120]. A few have been correlated with the level of disability[118], with disease course and risk for conversion to SPMS[119,120], and with susceptibility to MS development and relapse rate[121].

Additional studies are needed to confirm the role of OPN as a useful disease activity biomarker.

C-X-C motif ligand 13

C-X-C motif ligand 13 (CXCL13), also known as B cell attracting chemokine (BCA-1), is a protein favoring the chemotaxis of mature B lymphocytes by interaction with its receptor CXCR5. This receptor is also expressed by CD4+ T follicular helper cells, CD4+ Th17 cells, activated Treg cells and a subgroup of CD8+ T cells[122].

Together with other lymphoid chemokines, it favors the organization of germinal centers in lymphoid follicles, including meningeal tertiary lymphoid organs in the CNS[122]. Indeed, CXCL13 has been found to be overexpressed in active MS lesions and in intrameningeal B-cell follicles of chronic white matter lesions, sustaining humoral autoimmunity and disease activity[122,123]. Not coincidentally, mice lacking CXCL13 develop milder forms of disease[124], and its expression correlates with intrathecal Ig syntesis[125].

In a recent meta-analysis conducted on 226 studies about the role of several cytokines in patients with MS, CSF CXCL13 levels proved to differentiate well between patients with MS and controls and to decrease after DMDs[126,127]. Accordingly, in a study by Khademi and coworkers, CSF CXCL13 was found to be significantly higher in infectious neurological diseases and MS. The latter group showed significantly higher values than CIS and other controls[128]. However, its lack of high specificity was confirmed by overexpression of CXCL13 in the CNS in other diseases, such as neuroborreliosis and primary CNS lymphoma[129]. Next to its diagnostic role, it also proved to be higher in CIS converting to clinically definite MS[130] and to correlate with both clinical and radiological disease activity[127,128]. Currently, its role as predictive for CIS conversion has an intermediate level of evidence, needing replication in additional cohorts[3].

Elevated levels of CSF CXCL13 also seem to decrease after B-cell depleting treatment such as rituximab[129,131], after methylprednisolone[127] and NTZ[127,132]. High CSF CXCL13 levels also correlated with low expression of immunoregulatory IL-10 and TGF-β1[127]. On the basis of this evidence, CSF CXCL13 has been mainly suggested as a disease activity and treatment response biomarker.

MMP-9

MMPs are zinc-endopeptidases, able to catalyze the cleavage of many substrates in several physiological and physiopathological processes. Indeed, MMPs play a role in tissue remodeling, angiogenesis and cell migration, but also in inflammation, wound healing and malignancies[133]. During inflammation, many molecules are able to activate MMPs, including reactive oxygen species and both TNF-α and IL-17 via NF-κB[134,135]. MMPs, in turn, are able to activate cytokines, adhesion molecules, receptors and microglia[136,137]. Moreover, MMPs may determine BBB dysfunction by proteolyzing capillary basement membrane and tight junction proteins between endothelial cells[133,138].

MMPs seem to be involved in several neurological diseases, such as MS, Alzheimer's disease, Parkinson's disease, cancer and cerebrovascular diseases[133]. In EAE, elevated levels of several MMPs have been found, considered responsible for major severity of the disease[133,139,140]. It has been supposed that MMPs may act in MS through the digestion of myelin basic protein (MBP) as well, besides favoring leukocyte leakage at post-capillary venules[138]. Among six subfamilies, gelatinases (MMP-2 and MMP-9) are constitutively expressed in brain and best explored in MS pathogenesis[133]. Particularly, there is slightly more evidence about MMP-9 as a disease activity biomarker in MS, while results on MMP-2 are more controversial[141,142].

Elevated levels of MMP-9 have been detected in serum and CSF of patients affected by MS and other neurological diseases compared with controls, showing an association with disease activity[143-149]. In a study by Lee and coworkers, higher values of MMP-9 were found during clinical relapses, also related to a greater number of MRI gadolinium-enhancing (Gd+) lesions[144]. Similarly, another study confirmed higher concentrations of CSF MMP-9 in MS patients compared with controls, more in RRMS compared with PPMS ones, but there was no unequivocal association with clinical disease activity[148].

Considering the role in MMP inhibition played by tissue inhibitors of MMPs (TIMPs), the ratio MMP-9/TIMPs has also been considered as an equally valid biomarker and has been found to be increased in the serum of MS patients compared with controls, accordingly to elevated MMP-9 levels[149].

An increased expression of MMP-9 in active MS lesions and in active borders of chronic lesions has been found in some studies employing brain tissue from MS patients[150,151], confirming previous results and corroborating MMP-9 as a potentially valid disease activity biomarker.

Some studies have explored the variations of MMPs levels in patients under DMDs. A significant decrease in serum MMP-9 mRNA in RRMS patients under IFN-β has been noted after a 12-month follow-up by Galboiz and coworkers[152] and confirmed by other studies[153]. Among these, changes in MMP-9 levels occurred under IFN-β-treatment in a study by Comabella and coworkers, with a trend of reduction during the first 3 months and then an increase until reaching baseline values. Worthy of note, a significant increase in TIMP-1 concentrations occurred in the responder group compared with non-responders[154]. A possible response to NTZ treatment has also been explored. Balasa and coworkers reported a significant decrease in serum MMP-9 after 8 months of treatment and a good correlation between the biomarker and disease activity[155], but this finding was not confirmed by other studies[156]. In NTZ-treated patients, decreased baseline levels of MMP-9 were found in patients who developed progressive multifocal leukoencephalopathy compared with those who did not[157].

However, additional studies are needed for its validation, providing evidence of its role as a potential disease activity biomarker for MS.

Myelin basic protein

It has long been known that MBP is a potential disease activity biomarker for MS[158], since it displays an acute damage to CNS myelin, despite not being specific for the disease[159]. MBP is a polypeptide that assures the preservation of myelin structure and membrane compaction[160]. Four human isoforms are known, one of them prevailing in adult CNS myelin as a polypeptide containing 170 amino acid residues[161]. MBP contains multiple epitopes, with the ones recognized by monoclonal and polyclonal antibodies mainly allocated in 80-100 residues[161,162]. MBP-specific effector T lymphocytes have proved to play an essential role in the pathogenesis of experimental EAE models[163], which is rather suppressed when T cells are inhibited by MBP-specific Tregs[164].

Several studies have found increased CSF levels of MBP in patients with MS, temporally related to relapses[158,159,165-167] and detectable up to 5-6 weeks later[168]. Accordingly, RRMS patients with disease activity show higher values than progressive MS and stable patients[165]. CSF MBP concentrations are also greater when polysymptomatic and severe relapses occur, correlating with EDSS score and MRI activity and decreasing after corticosteroid treatment[168,169]. Zhou *et al.*[170] explored the association between MBP gene variations and MS course in a 5-year prospective study involving 127 patients with CIS, identifying a risk genotype (CT+TT of rs12959006) for the risk of conversion to MS, disability progression and relapses. MBP-like material has been found in the urine of MS patients as well, although its concentration fluctuates and does not seem to be temporally related to acute myelin damage. Conversely, higher values have been found in SPMS patients and are supposed to be related to disease progression[161]. Considering the role of MBP in the pathogenesis of MS and its potential role as a therapeutic target, several clinical trials have been carried out or are currently ongoing to evaluate possible new drugs[171-174]. However, this biomarker has not been validated and the preliminary results need to be replicated in additional cohorts.

Neuronal cell adhesion molecule

Neuronal cell adhesion molecule (N-CAM) is considered a marker of repair and remyelination[175] and it is mainly expressed in the CNS, but its involvement in neoplastic diseases has also been documented[176]. During the development of the CNS, the polysialylated form of N-CAM is actively involved in myelination, axonal growth and neural cell migration[177]. It has been found to be expressed by neural precursors of oligodendrocytes, astrocytes and neurons, supporting the process of myelination in the olfactory bulb in mouse brain[177].

In animal models, increased N-CAM expression has been identified in astrocytes in acutely demyelinated areas[178] and, similarly, in areas damaged by kainic acid[179]. Soluble forms of N-CAM have also been found to be involved in peripheral nerve myelination and repair, with Schwann cells expressing specific receptors for the molecule[177]. Both soluble and membrane-bound forms of this molecule exist, with different and little known expression and specific functions, and N-CAM belongs to the immunoglobulin superfamily[180]. Normal CSF values of soluble N-CAM range between 460 and 1,060 ng/mL[177]. Among several neurological diseases, CSF levels were found to be reduced in MS patients, who showed a mean value of 250 ± 107 ng/mL, compared with healthy controls (mean value of 412 ± 109), with similar findings when comparing patients affected by Alzheimer's disease and meningitis with controls, regardless of age and gender[180]. Moreover, PPMS patients exhibited lower levels compared with RRMS ones[181]. These data confirmed the results of a previous study showing lower soluble N-CAM concentrations in non-acute phase MS patients compared with controls and acute-phase MS patients[182]. In the last group, indeed, increased CSF N-CAM levels were noted, gradually increasing in the first week after relapse and correlating well with the remission of symptoms[183]. Moreover, comparing acute-phase patients who underwent steroid treatment with those who did not, significantly greater values were recorded in the first group[183]. However, steroid treatment does not determine an increase in N-CAM levels in itself, and this finding was not reported in non-acute phase MS patients who were treated[183].

Among DMDs, NTZ and mitoxantrone proved to significantly increase N-CAM levels in MS patients, while fingolimod did not[181].

Considering the evidence of lower N-CAM levels in PPMS compared to RRMS[181], in RRMS compared to CIS, and in polyneuropathy compared to Guillain-Barré syndrome[180], this molecule is actually considered mainly as an indicator of scarce repair capability more than a marker of severe neuronal damage[180]. However, it is not currently used in clinical practice and needs further validation[1,184].

Chitinase-3-like-1

Chitinase-3-like-1 (CHI3L1) (or YKL-40) belongs to the family of chitinases, enzymes that catalyze the cleavage of chitin by hydrolysis. Its biological role in humans has not been definitely clarified, despite many proofs of its involvement in several processes exist, such as tissue remodeling, angiogenesis, tumorigenesis and inflammation[185]. Belonging to the same family, chitotriosidase is known to be associated with several diseases, including infectious and inflammatory ones[186].

Though it is not a specific marker for MS, CSF CHI3L1 levels have been found to be increased in RRMS and NMO patients compared with controls, including healthy people, patients suffering from other inflammatory diseases and SPMS patients[185,187,188]. Conversely, serum CHI3L1 levels were not significantly different between groups in the aforementioned studies[185,187]. Elevated levels of CHI3L1 were also detected in both PPMS and SPMS compared with healthy controls[189]. Patients who fulfilled diagnostic criteria for active progressive MS or showed elevated levels of MMP-9 and CXCL13 also had higher concentrations of CHI3L1[189]. However, as a diagnostic biomarker, CHI3L1 needs further replication in additional cohorts[3].

Particular attention has been given to the prognostic role of this molecule, whose CSF concentration has proved to be an independent predictor for the risk of conversion to clinically definite MS in CIS[187,190-192], but not in RIS[32]. In a study by Comabella and coworkers, CSF CHI3L1 levels additionally correlated with shorter latency time of conversion and with disability progression during follow-up and radiological disease activity[190].

In a large multicenter study involving 813 patients with CIS, the aforementioned results were confirmed. Not only CSF CHI3L1 concentration was associated with the risk of conversion to clinically definite MS,

but it also was correlated with shorter time to conversion and to disability worsening, for which it was an independent risk factor[192]. As a consequence, there is strong evidence of its role as a biomarker able to predict CIS conversion, and it should be assessed for clinical implementation[3].

As a treatment response biomarker, serum CHI3L1 levels were measured in 76 RRMS patients under IFN-β treatment and were found to be increased in the non-responder group compared with the responder one. As there was such a difference since baseline, it was suggested that non-responders had higher disease activity and accordingly greater CSF CHI3L1 levels[193].

Other biomarkers requiring further validation

Several T-cell cytokines have been explored as potential biomarkers for MS, but which are crucial in MS pathogenesis has not been entirely elucidated yet[194].

IL-12 and IL-23 respectively induce the differentiation of naive T cells in IFNγ-producing Th1 cells and IL-17-producing Th17 cells[195]. Both interleukins increase the encephalitogenic potential of T lymphocytes, but only IL-23 has been found to be a critical molecule in the development of EAE[196]. On the basis of results coming from EAE models, where animals improved after administration of neutralizing antibodies against the shared IL-12/IL-23 p40 subunit, a phase II double-blind placebo-controlled trial with the monoclonal antibody ustekinumab was conducted in 249 RRMS patients, although it did not show substantial efficacy[197].

Differently, IL-17 does not seem to be crucial to EAE development, though increasing its severity and atypical presentation, maybe through the recruiting of neutrophils and the effect of MMPs[194]. Nevertheless, increased IL-17 mRNA expression in mononuclear cells was found in MS lesions and in CSF and blood of MS patients[198,199], and Th17 cells were found to undergo a more marked increase in CSF during MS relapses than Th1 cells, which usually prevail in both blood and CSF[200]. A monoclonal antibody against IL-17A (secukinumab) has proved to reduce MRI activity in MS, but further studies are needed[201].

Tumor necrosis factor alpha (TNF-α) is a cytokine involved in the pathogenesis of several autoimmune diseases, including MS, wherein increased CSF and serum levels of this molecule have been detected[202-204]. Today, it is known that TNF-α may exert different biological effects, depending on the involved receptor, both stimulating inflammatory processes and apoptosis (via TNF receptor 1) or inducing a pro-survival pathway and reducing inflammation (via TNF receptor 2). This might explain the failure and the unexpected results of treatment approaches with unselective anti-TNF-α drugs in MS patients, which lead to an increase in disease activity in MS[205,206]. The modulation of TNF-α signaling has provided promising results in EAE, whose remission has been induced by selective inhibition of the soluble form of TNF-α, which mainly acts via TNF receptor 1[207].

B cell-activating factor (BAFF), belonging to the TNF family, is a maturation and survival factor for B lymphocytes, whose serum levels have been found to be increased in several autoimmune diseases[208]. In MS, increased BAFF concentrations in CSF and in demyelinating lesions have been detected[209]. The association with disease activity has not been elucidated, since some controversial results have been reported[1,209]. Moreover, the clinical significance of increased BAFF levels under treatment with some DMDs is not clear[209].

PROGNOSTIC BIOMARKERS

Neurofilaments

Neurofilaments (NFs) are components of the neuronal cytoskeleton, responsible for the increase in nerve conduction velocity in myelinated fibers and for their structural support[210]. Consisting of heavy (NF-H),

medium (NF-M) and light (NF-L) chains[211], their detection in CSF and blood samples has been the subject of interest for years. Several studies investigated the increase in NFs in several neurological diseases[212], such as amyotrophic lateral sclerosis[213], Alzheimer's disease[214], frontotemporal dementia[215], stroke[216], MS[211], Huntington disease[217], atypical parkinsonian syndromes and neurocognitive impairment in HIV-positive individuals[218].

In most cases, NFs have been investigated as a potential prognostic and disease activity marker related to axonal damage, speculating a relation between the quantitative amount of CSF and serum NFs and the rate of neurodegeneration[218].

In MS, NFs have also been extensively examined as a diagnostic, disease activity and drug response biomarker. Moreover, serum NF-L levels, detected through a single molecule array (Simoa), appear strictly related to CSF levels[219-222], despite being approximately 42-fold lower[223]. Cut-off values have not been unequivocally established as for CSF ones. However, serum NF-L values between 16-20 pg/mL have been identified as a normal range among a heterogeneous group of healthy controls enrolled in various studies[211], without gender difference and with a trend to increase along with age-related physiological axonal damage[223].

Particularly, while the detection of higher CSF NF-H levels in SPMS patients suggests a major correlation with chronic axonal damage and is accordingly age-related[224,225], CSF NF-L seem to be better related to acute axonal damage due to inflammation. Indeed, increased levels of NF-L were found in CSF of MS patients compared with controls, with greater concentrations during exacerbations. Moreover, such high concentrations were associated with progression in both clinically stable patients and relapsing ones[226,227]. A recent meta-analysis confirmed these results, finding higher CSF NF-L levels in RRMS patients compared with PMS and double concentrations in relapsing patients compared with remitting ones[228].

In a longitudinal study involving 22 IFNβ-1a- and riluzole-treated patients and 20 IFNβ-1a- and placebo-treated ones with early MS, serum NF-L concentrations were assessed over a 24-month period, correlating well with EDSS changes, Gd+ lesions and the development of brain atrophy. Moreover, increased serum NF-L levels were associated with worse results in neuropsychological tests assessing visuospatial functioning, recall and both verbal and non-verbal episodic learning[229].

Similar results concerning the association between serum NF-L levels and cognitive impairment in early stages of MS[230] and between serum NF-L concentrations and EDSS changes[231] were confirmed by other studies, though not all agreed[232]. Despite correlating with EDSS in PMS patients, serum NF-L levels failed to correlate with EDSS progression in the previous year and during a median follow-up of 27 months. Particularly, serum NF-L increased in all PMS patients, including those who did not exhibit changes in EDSS or an increase in disability[232].

In patients with RIS, increased CSF NF-L levels were found to be an independent risk factor for the conversion to CIS and MS. Matute-Blanch and coworkers considered a cut-off value equal to 619 ng/L, since greater values were related to shorter times of conversion[32]. CSF NF-L concentrations have been found to be increased in patients with CIS as well[233], with greater ones in those who converted to clinically definite MS[224,234]. Despite these promising results, their role as prognostic biomarker for CIS conversion is still weak, and replication in larger cohorts is needed to confirm it[3].

In 86 CIS patients with optic neuritis as the first clinical event, CSF NF-L levels also predicted long-term cognitive and physical disability over a follow-up period ranging between 9 and 19 years[235].

As a disease activity and prognostic biomarker, the amount of CSF NF-L levels showed a significant association with NEDA-3 status, MRI activity and brain atrophy and significantly correlated with serum NF-L ones[11]. In several studies, serum NF-L also correlated with MRI activity, predicted the development of brain volume loss in a period of 2 years and decreased under DMDs[236,237]. A recent study obtained similar results, with serum NF-L detected in early phases contributing to the prediction of lesion load and brain volume loss over a period of 10 years[238].

CSF NF-L concentrations proved to decrease after 12-24 months of immunosuppressive therapy in active progressive MS patients[239] and after switching from first-line therapies to fingolimod in RRMS ones[240]. Moreover, compared with NF-H, CSF NF-L has been found to be superior as a therapeutic biomarker after 12 months of NTZ-treatment in RRMS patients[241,242]. Nevertheless, the potential role of CSF NF-L as a treatment response biomarker is severely limited by the invasiveness of performing serial lumbar punctures. Conversely, serial serum NF-L assessments would represent a more easily detectable marker and a reliable indicator of CSF NF-L levels[219,221]. Results from a recent study conducted on 15 MS patients treated with alemtuzumab and monitored with serial serum NF-L measurements were significant[243]. Indeed, serum NF-L levels correlated well with clinical and radiological activity at baseline and during follow-up, decreasing within 6 months from drug administration until reaching stable values under 8 pg/ml in those patients who achieved NEDA-3. Moreover, patients who showed clinical and radiological disease activity during follow-up also exhibited increased levels of serum NF-L up to 5 months before relapses.

So far, several studies have confirmed the reliability of NF-L as a disease activity and treatment response biomarker for MS, even though it does not represent a MS-specific biomarker. However, a precise cut-off is still missing, precluding the chance to stratify the risk of clinical and radiological disease activity according to NF-L levels. The opportunity to consider only intra-individual values is still debated, without focusing on their deviation from values reported in healthy people[237].

Further replication in larger, multicenter cohorts is needed. A randomized controlled trial, prospectively recruiting 900 patients from 45 sites in the USA, will provide further information about the potential role of serum NF-L as a prognostic and treatment response biomarker for MS[244].

IgM oligoclonal bands

Unlike small and monomeric IgG, IgM are large molecules consisting of pentameter units and ten antigen-binding sites and are strong activators of complement[245]. In a similar way to CSF IgG OCB, their identification is considered a sign of intrathecal synthesis, suggesting an inflammatory disease of the CNS[246]. However, CSF IgM oligoclonal bands (IgM OCB) are mainly considered a prognostic and disease activity biomarker than a diagnostic one, though not routinely used in clinical practice[1].

In a study involving 29 MS patients who were followed-up for 5 to 16 years, the presence of CSF IgM OCB was strongly associated with conversion to SPMS and the achievement of greater EDSS scores[247]. In a similar way, IgM OCB-positivity strongly predicted a severe disease course influencing the probability of developing greater disability in a cohort of 64 MS patients[248].

In patients with CIS, the identification of CSF lipid-specific IgM OCB was associated with greater MRI lesion load and brain atrophy at the first clinical event[249] and with an aggressive disease course[250]. Periventricular lesion load during the first years of disease proved to be related as well to the entity of IgM intrathecal synthesis in CIS patients, so that an active role of IgM in the development of demyelinating lesions has been supposed[251].

Moreover, both the risk of a second clinical event and its earliness were strongly increased when both CSF lipid-specific IgM OCB and IgG OCB were detected, as in 22% of 192 patients with CIS[252]. In another

study by Ferraro and coworkers, the identification of CSF IgM OCB in CIS patients was predictive of the occurrence of another relapse within a year[253]. Results from a blinded multicenter study involving 52 neurological patients and 13 centers confirmed the reproducibility of the test[254]. However, further confirmatory studies in additional cohorts are needed, and IgM OCB detection currently has an intermediate level of evidence as a predictive biomarker for CIS conversion[3].

The presence of CSF IgM OCB has been also associated with a severe disease course in RRMS patients, while it seems to be less frequent among PPMS compared with RRMS ones[255]. Strong evidence of its value as a prognostic biomarker for RRMS exists[3,249], so its potential clinical implementation has to be evaluated.

Finally, there is little evidence for the possible interactions between DMDs and CSF IgM OCB. The response to IFN-β treatment in RRMS seems to be reduced in patients exhibiting CSF lipid-specific IgM OCB, who showed a minor reduction in relapse rate and a higher probability of achieving greater EDSS values[256].

NTZ has proved to reduce serum IgM and IgG levels after 2 years of treatment in a time-dependent manner[257]. In a study by Villar and coworkers, NTZ determined a decrease in CSF IgM OCB in patients with no active disease, with complete disappearance in 70% of them, while no effects were reported in those with active disease[258].

Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is highly expressed in the cytoskeleton of astrocytes, and it belongs to the family of intermediate filaments, which are highly cell type specific[259]. Since GFAP is upregulated in astroglial cell activation (astrogliosis), occurring in many inflammatory and non-inflammatory diseases[167,259-261], it has been explored as a biomarker for MS. Particularly, reactive astrocytes proved to be actively involved in neurodegenerative diseases, probably losing their protective role and developing neurotoxic functions[262-264]. The glial scar itself, which physically protects a damaged area, may also physically obstruct remyelination[264]. Finally, A1 astrocytes were found in MS lesions, as in EAE models where they were associated with neuronal and oligodendrocytic death[262]. On the basis of these remarks, an association between GFAP and disability in MS has been investigated. Elevated CSF GFAP levels were found in MS patients compared with controls[265-267], showing higher concentrations in patients with EDSS greater than 6.5 compared with those with minor disability[266]. In a study by Högel and coworkers, serum levels of GFAP proved to be associated with a greater EDSS score as well, but also with longer disease duration and progressive course[268]. On this issue, positive correlations have been found between CSF GFAP and disease duration, likewise between serum GFAP and disease severity, in a cohort of 93 PPMS patients[264]. In a study by Axelsson and coworkers, the increased levels of GFAP in MS patients were predictive for disability resulting 8-10 years later, confirming the association of this molecule with disability and progression in MS patients[267]. A similar result was obtained in a more recent longitudinal study involving 301 patients with CIS/MS with a mean follow-up time of 11 years, showing a correlation between GFAP levels and an early progression in the EDSS score[234]. However, further studies are needed to confirm its role as a prognostic biomarker for MS.

Evidence of an association between GFAP and high disease activity also exists, showing correlation with MRI parameters such as infratentorial chronic lesion load and the intensity of Gd+ in both CIS and RRMS patients[269]. Effectively, there is evidence that GFAP may increase in CSF and serum soon after (4-24 h) traumatic brain injuries, as a marker of acute lesion[261]. Due to its high cell type specificity and good correlation with neuronal degeneration, GFAP is currently considered a potential prognostic biomarker for progression[4].

CONCLUSION

Research on biological markers is very active and current. At present, there are few molecules available, considering the hundreds under investigation. But they are continuously increasing due to a greater knowledge of MS and its underlying physiopathology. For instance, there are no clinically useful disease activity biomarkers, despite the large number of exploratory molecules described for this functional group.

As for the group of diagnostic biomarkers, previously dominated by IgG OCB analysis, the possibility to rely on quantitative, less expensive and less time-consuming assessments as a first-line screening, is moving forward.

Though it is true that CSF is the most suitable means for getting information about CNS physiopathology[15], it is equally true that much of interest is moving towards serum biomarkers. For quite some time, their clinical use has been limited by both a greater variability and very low concentrations, a problem overcome by the introduction of increasingly sophisticated tools (e.g., the detection of serum NF-L levels through Simoa)[222]. Furthermore, treatment response biomarkers, such as anti-IFN-β and anti-NTZ antibodies, are mainly determined in serum and have not been included in this review, which is focused on CSF biomarkers. Despite requiring a more invasive approach, CSF still represents a unique source of data about the CNS, enough to have been defined as a "liquid biopsy" of CNS[4]. This is even more true since the histological analysis of brain tissue cannot be routinely performed and almost any study on new potential biomarkers has to start from CSF analysis. There is no doubt that we are now able to diagnose and treat patients in early phases and even wondering about treating asymptomatic patients with only radiological signs suggestive of the disease. Thinking of how MS diagnosis has been revolutionized by MRI in the last 20 years, it would not be impressive if new and promising biomarkers might lead to a new revolution in MS in the coming years.

HIGHLIGHTS

1. CSF is a unique source of potential biomarkers for MS, despite requiring a certain invasiveness for its collection.
2. Only CSF diagnostic biomarkers are currently used in clinical practice, though hundreds of molecules have been validated as disease activity and prognostic biomarkers.
3. IgG OCB maintain a prominent role as a validated diagnostic biomarker and are considered an alternative tool to MRI which can substitute for dissemination in time according to the 2017 revision of McDonald criteria. They also retain a prognostic role for conversion to MS when detected in patients with CIS.
4. NF-L has proved to be a useful biomarker as indicator of disease activity in MS. The possibility of measuring NF-L at different time points through serum detection makes it also suitable for the monitoring of treatment response.
5. KFLC index has proved to be a more sensitive but less specific diagnostic biomarker compared with IgG OCB, representing a potential first-line assessment in patients with suspected MS and reducing the request for IgG OCB analysis. It has a role as a prognostic for CIS conversion biomarker as well, but the lack of a universal cut-off value still represents a limit.
6. IgM OCB show good potential as a prognostic biomarker, since they are associated with an aggressive disease course, a higher risk of conversion to MS in CIS patients, disability progression and conversion to SPMS.
7. Several disease activity biomarkers seem promising, though requiring further validation. Increased levels of NO metabolites, OPN, MBP, MMP-9, N-CAM, CXCL13 and CHI3L1 have been detected in a close temporal correlation with relapses.

DECLARATIONS

Authors' contributions

The conception and design of the study, conducted the literature review, drafted the manuscript: Toscano S
The conception and design of the study, and provided critical revision and final approval of the article: Patti F

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2021.

REFERENCES

1. Comabella M, Montalban X. Body fluid biomarkers in multiple sclerosis. Lancet Neurol 2014;13:113-26.
2. Frisoni GB, Boccardi M, Barkhof F, Blennow K, Cappa S, et al. Strategic roadmap for an early diagnosis of Alzheimer's disease based on biomarkers. Lancet Neurol 2017;16:661-76.
3. Teunissen CE, Malekzadeh A, Leurs C, Bridel C, Killestein J. Body fluid biomarkers for multiple sclerosis--the long road to clinical application. Nat Rev Neurol 2015;11:585-96.
4. Giovannoni G. Multiple sclerosis cerebrospinal fluid biomarkers. Dis Markers 2006;22:187-96.
5. Ziemssen T, Akgün K, Brück W. Molecular biomarkers in multiple sclerosis. J Neuroinflammation 2019;16:272.
6. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, et al. Axonal transection in the lesions of multiple sclerosis. N Engl J Med 1998;338:278-85.
7. FDA News Release. FDA approves new oral drug to treat multiple sclerosis. Available from: http://www.fda.gov/news-events/press-announcements/fda-approves-new-oral-drug-treat-multiple-sclerosis. [Last accessed on 2 Jul 2020]
8. FDA News Release. FDA approves new drug to treat multiple sclerosis. Available from: http://www.fda.gov/news-events/press-announcements/fda-approves-new-drug-treat-multiple-sclerosis. [Last accessed on 2 Jul 2020]
9. Comi G. Induction vs. escalating therapy in multiple sclerosis: practical implications. Neurol Sci 2008;29 Suppl 2:S253-5.
10. Giovannoni G, Tomic D, Bright JR, Havrdová E. "No evident disease activity": The use of combined assessments in the management of patients with multiple sclerosis. Mult Scler 2017;23:1179-87.
11. Håkansson I, Tisell A, Cassel P, Blennow K, Zetterberg H, et al. Neurofilament levels, disease activity and brain volume during follow-up in multiple sclerosis. J Neuroinflammation 2018;15:209.
12. Pandit L. No evidence of disease activity (NEDA) in multiple sclerosis - shifting the goal posts. Ann Indian Acad Neurol 2019;22:261-3.
13. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource [Internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK338448/. [Last accessed on 2 Jul 2020]
14. Rush CA, MacLean HJ, Freedman MS. Aggressive multiple sclerosis: proposed definition and treatment algorithm. Nat Rev Neurol 2015;11:379-89.
15. Johanson CE, Duncan JA 3rd, Klinge PM, Brinker T, Stopa EG, et al. Multiplicity of cerebrospinal fluid functions: new challenges in health and disease. Cerebrospinal Fluid Res 2008;5:10.
16. Dobson R, Ramagopalan S, Davis A, Giovannoni G. Cerebrospinal fluid oligoclonal bands in multiple sclerosis and clinically isolated syndromes: a meta-analysis of prevalence, prognosis and effect of latitude. J Neurol Neurosurg Psychiatry 2013;84:909-14.
17. Imrell K, Landtblom AM, Hillert J, Masterman T. Multiple sclerosis with and without CSF bands: clinically indistinguishable but immunogenetically distinct. Neurology 2006;67:1062-4.

18. Andersson M, Alvarez-Cermeño J, Bernardi G, Cogato I, Fredman P, et al. Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. J Neurol Neurosurg Psychiatry 1994;57:897-902.
19. Freedman MS, Thompson EJ, Deisenhammer F, Giovannoni G, Grimsley G, et al. Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement. Arch Neurol 2005;62:865-70.
20. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 2018;17:162-73.
21. Schwenkenbecher P, Wurster U, Konen FF, Gingele S, Sühs KW, et al. Impact of the McDonald criteria 2017 on early diagnosis of relapsing-remitting multiple sclerosis. Front Neurol 2019;10:188.
22. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983;13:227-31.
23. McDonald WI, Compston A, Edan G, Goodkin D, Hartung P, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 2001;50:121-7.
24. Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria." Ann Neurol 2005;58:840-6.
25. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69:292-302.
26. Sandberg-Wollheim M, Olsson T. Cerebrospinal fluid oligoclonal bands are important in the diagnosis of multiple sclerosis, unreasonably downplayed by the McDonald criteria 2010: Yes. Mult Scler 2013;19:714-6.
27. Arrambide G, Tintore M, Espejo C, Auger C, Castillo M, et al. The value of oligoclonal bands in the multiple sclerosis diagnostic criteria. Brain 2018;141:1075-84.
28. Tintoré M, Rovira A, Brieva L, Grivé E, Jardí R, et al. Isolated demyelinating syndromes: comparison of CSF oligoclonal bands and different MR imaging criteria to predict conversion to CDMS. Mult Scler 2001;7:359-63.
29. Tintoré M, Rovira A, Río J, Tur C, Pelayo R, et al. Do oligoclonal bands add information to MRI in first attacks of multiple sclerosis? Neurology 2008;70:1079-83.
30. Boyko A. Radiologically isolated syndrome with oligoclonal bands in CSF (RIS + OCB) can be classified as high MS risk group. Mult Scler 2020;26:869-70.
31. Makhani N, Lebrun C, Siva A, Narula S, Wassmer E, et al; Observatoire Francophone de la Sclérose en Plaques (OFSEP), Société Francophone de la Sclérose en Plaques (SFSEP), the Radiologically Isolated Syndrome Consortium (RISC) and the Pediatric Radiologically Isolated Syndrome Consortium (PARIS). Oligoclonal bands increase the specificity of MRI criteria to predict multiple sclerosis in children with radiologically isolated syndrome. Mult Scler J Exp Transl Clin 2019;5:2055217319836664.
32. Matute-Blanch C, Villar LM, Álvarez-Cermeño JC, Rejdak K, Evdoshenko E, et al. Neurofilament light chain and oligoclonal bands are prognostic biomarkers in radiologically isolated syndrome. Brain 2018;141:1085-93.
33. Pryce G, Baker D. Oligoclonal bands in multiple sclerosis; Functional significance and therapeutic implications. Does the specificity matter? Mult Scler Relat Disord 2018;25:131-7.
34. Rejdak K, Stelmasiak Z, Grieb P. Cladribine induces long lasting oligoclonal bands disappearance in relapsing multiple sclerosis patients: 10-year observational study. Mult Scler Relat Disord 2019;27:117-20.
35. Mancuso R, Franciotta D, Rovaris M, Caputo D, Sala A, et al. Effects of natalizumab on oligoclonal bands in the cerebrospinal fluid of multiple sclerosis patients: a longitudinal study. Mult Scler 2014;20:1900-3.
36. von Glehn F, Farias AS, de Oliveira AC, Damasceno A, Longhini AL, et al. Disappearance of cerebrospinal fluid oligoclonal bands after natalizumab treatment of multiple sclerosis patients. Mult Scler 2012;18:1038-41.
37. Harrer A, Tumani H, Niendorf S, Lauda F, Geis C, et al. Cerebrospinal fluid parameters of B cell-related activity in patients with active disease during natalizumab therapy. Mult Scler 2013;19:1209-12.
38. Koch M, Heersema D, Mostert J, Teelken A, De Keyser J. Cerebrospinal fluid oligoclonal bands and progression of disability in multiple sclerosis. Eur J Neurol 2007;14:797-800.
39. Link H, Tibbling G. Principles of albumin and IgG analyses in neurological disorders. III. Evaluation of IgG synthesis within the central nervous system in multiple sclerosis. Scand J Clin Lab Invest 1977;37:397-401.
40. Mayringer I, Timeltaler B, Deisenhammer F. Correlation between the IgG index, oligoclonal bands in CSF, and the diagnosis of demyelinating diseases. Eur J Neurol 2005;12:527-30.
41. Link H, Huang YM. Oligoclonal bands in multiple sclerosis cerebrospinal fluid: an update on methodology and clinical usefulness. J Neuroimmunol 2006;180:17-28.
42. Lefvert AK, Link H. IgG production within the central nervous system: a critical review of proposed formulae. Ann Neurol 1985;17:13-20.
43. Senel M, Tumani H, Lauda F, Presslauer S, Mojib-Yezdani R, et al. Cerebrospinal fluid immunoglobulin kappa light chain in clinically isolated syndrome and multiple sclerosis. PLoS One 2014;9:e88680.
44. Izquierdo G, Angulo S, Garcia-Moreno JM, Gamero MA, Navarro G, et al. Intrathecal IgG synthesis: marker of progression in multiple sclerosis patients. Acta Neurol Scand 2002;105:158-63.
45. Klein A, Selter RC, Hapfelmeier A, Berthele A, Müller-Myhsok B, et al. CSF parameters associated with early MRI activity in patients with MS. Neurol Neuroimmunol Neuroinflamm 2019;6:e573.
46. Gastaldi M, Zardini E, Franciotta D. An update on the use of cerebrospinal fluid analysis as a diagnostic tool in multiple sclerosis. Expert Rev Mol Diagn 2017;17:31-46.
47. Hegen H, Walde J, Milosavljevic D, Aboulenein-Djamshidian F, Senel M, et al. Free light chains in the cerebrospinal fluid. Comparison

of different methods to determine intrathecal synthesis. Clin Chem Lab Med 2019;57:1574-86.
48. Kaplan B, Aizenbud BM, Golderman S, Yaskariev R, Sela BA. Free light chain monomers in the diagnosis of multiple sclerosis. J Neuroimmunol 2010;229:263-71.
49. Hassan-Smith G, Durant L, Tsentemeidou A, Assi LK, Faint JM, et al. High sensitivity and specificity of elevated cerebrospinal fluid kappa free light chains in suspected multiple sclerosis. J Neuroimmunol 2014;276:175-9.
50. Desplat-Jégo S, Feuillet L, Pelletier J, Bernard D, Chérif AA, et al. Quantification of immunoglobulin free light chains in cerebrospinal fluid by nephelometry. J Clin Immunol 2005;25:338-45.
51. Duranti F, Pieri M, Centonze D, Buttari F, Bernardini S, et al. Determination of κFLC and κ Index in cerebrospinal fluid: a valid alternative to assess intrathecal immunoglobulin synthesis. J Neuroimmunol 2013;263:116-20.
52. Zeman D, Kušnierová P, Bartoš V, Hradílek P, Kurková B, et al. Quantitation of free light chains in the cerebrospinal fluid reliably predicts their intrathecal synthesis. Ann Clin Biochem 2016;53:174-6.
53. Pieri M, Storto M, Pignalosa S, Zenobi R, Buttari F, et al. KFLC index utility in multiple sclerosis diagnosis: further confirmation. J Neuroimmunol 2017;309:31-3.
54. Crespi I, Vecchio D, Serino R, Saliva E, Virgilio E, et al. K index is a reliable marker of intrathecal synthesis, and an alternative to IgG index in multiple sclerosis diagnostic work-up. J Clin Med 2019;8:446.
55. Puthenparampil M, Altinier S, Stropparo E, Zywicki S, Poggiali D, et al. Intrathecal K free light chain synthesis in multiple sclerosis at clinical onset associates with local IgG production and improves the diagnostic value of cerebrospinal fluid examination. Mult Scler Relat Disord 2018;25:241-5.
56. Gaetani L, Di Carlo M, Brachelente G, Valletta F, Eusebi P, et al. Cerebrospinal fluid free light chains compared to oligoclonal bands as biomarkers in multiple sclerosis. J Neuroimmunol 2020;339:577108.
57. Gurtner KM, Shosha E, Bryant SC, Andreguetto BD, Murray DL, et al. CSF free light chain identification of demyelinating disease: comparison with oligoclonal banding and other CSF indexes. Clin Chem Lab Med 2018;56:1071-80.
58. Presslauer S, Milosavljevic D, Huebl W, Aboulenein-Djamshidian F, Krugluger W, et al. Validation of kappa free light chains as a diagnostic biomarker in multiple sclerosis and clinically isolated syndrome: a multicenter study. Mult Scler 2016;22:502-10.
59. Leurs CE, Twaalfhoven H, Lissenberg-Witte BI, van Pesch V,Dujmovic I, et al. Kappa free light chains is a valid tool in the diagnostics of MS: a large multicenter study. Mult Scler 2020;26:912-23.
60. Makshakov G, Nazarov V, Kochetova O, Surkova E, Lapin S, et al. Diagnostic and prognostic value of the cerebrospinal fluid concentration of immunoglobulin free light chains in clinically isolated syndrome with conversion to multiple sclerosis. PLoS One 2015;10:e0143375.
61. Presslauer S, Milosavljevic D, Huebl W, Parigger S, Schneider-Koch G, et al. Kappa free light chains: diagnostic and prognostic relevance in MS and CIS. PLoS One 2014;9:e89945.
62. Presslauer S, Milosavljevic D, Brücke T, Bayer P, Hübl W. Elevated levels of kappa free light chains in CSF support the diagnosis of multiple sclerosis. J Neurol 2008;255:1508-14.
63. Ferraro D, Trovati A, Bedin R, Natali P, Franciotta D, et al. Cerebrospinal fluid kappa and lambda free light chains in oligoclonal band-negative patients with suspected multiple sclerosis. Eur J Neurol 2020;27:461-7.
64. Vecchio D, Crespi I, Virgilio E, Naldi P, Campisi MP, et al. Kappa free light chains could predict early disease course in multiple sclerosis. Mult Scler Relat Disord 2019;30:81-4.
65. Rinker JR 2nd, Trinkaus K, Cross AH. Elevated CSF free kappa light chains correlate with disability prognosis in multiple sclerosis. Neurology 2006;67:1288-90.
66. Rudick RA, Medendorp SV, Namey M, Boyle S, Fischer J. Multiple sclerosis progression in a natural history study: predictive value of cerebrospinal fluid free kappa light chains. Mult Scler 1995;1:150-5.
67. Rathbone E, Durant L, Kinsella J, Parker AR, Hassan-Smith G, et al. Cerebrospinal fluid immunoglobulin light chain ratios predict disease progression in multiple sclerosis. J Neurol Neurosurg Psychiatry 2018;89:1044-9.
68. Felgenhauer K, Reiber H. The diagnostic significance of antibody specificity indices in multiple sclerosis and herpes virus induced diseases of the nervous system. Clin Investig 1992;70:28-37.
69. Tumani H, Tourtellotte WW, Peter JB, Felgenhauer K, The Optic Neuritis Study Group. Acute optic neuritis: combined immunological markers and magnetic resonance imaging predict subsequent development of multiple sclerosis. J Neurol Sci 1998;155:44-9.
70. Brettschneider J, Tumani H, Kiechle U, Muche R, Richards G, et al. IgG antibodies against measles, rubella, and varicella zoster virus predict conversion to multiple sclerosis in clinically isolated syndrome. PLoS One 2009;4:e7638.
71. Reiber H, Ungefehr S, Jacobi C. The intrathecal, polyspecific and oligoclonal immune response in multiple sclerosis. Mult Scler 1998;4:111-7.
72. Hottenrott T, Schorb E, Fritsch K, Dersch R, Berger B, et al. The MRZ reaction and a quantitative intrathecal IgG synthesis may be helpful to differentiate between primary central nervous system lymphoma and multiple sclerosis. J Neurol 2018;265:1106-14.
73. Jarius S, Eichhorn P, Franciotta D, Petereit HF, Akman-Demir G, et al. The MRZ reaction as a highly specific marker of multiple sclerosis: re-evaluation and structured review of the literature. J Neurol 2017;264:453-66.
74. Franciotta D, Salvetti M, Lolli F, Serafini B, Aloisi F. B cells and multiple sclerosis. Lancet Neurol 2008;7:852-8.
75. Godec MS, Asher DM, Murray RS, Shin ML, Greenham LW, et al. Absence of measles, mumps, and rubella viral genomic sequences from multiple sclerosis brain tissue by polymerase chain reaction. Ann Neurol 1992;32:401-4.
76. Ibitoye R, Kemp K, Rice C, Hares K, Scolding N, et al. Oxidative stress-related biomarkers in multiple sclerosis: a review. Biomark Med 2016;10:889-902.

77. Smith KJ, Lassmann H. The role of nitric oxide in multiple sclerosis. Lancet Neurol 2002;1:232-41.
78. Yamashita T, Ando Y, Obayashi K, Uchino M, Ando M. Changes in nitrite and nitrate (NO2-/NO3-) levels in cerebrospinal fluid of patients with multiple sclerosis. J Neurol Sci 1997;153:32-4.
79. Miljkovic Dj, Drulovic J, Trajkovic V, Mesaros S, Dujmovic I, et al. Nitric oxide metabolites and interleukin-6 in cerebrospinal fluid from multiple sclerosis patients. Eur J Neurol 2002;9:413-8.
80. Yuceyar N, Taşkiran D, Sağduyu A. Serum and cerebrospinal fluid nitrite and nitrate levels in relapsing-remitting and secondary progressive multiple sclerosis patients. Clin Neurol Neurosurg 2001;103:206-11.
81. Giovannoni G, Heales S, Silver N, O'riordan J, Miller R, et al. Raised serum nitrate and nitrite levels in patients with multiple sclerosis. J Neurol Sci 1997;145:77-81.
82. Haghikia A, Kayacelebi AA, Beckmann B, Hanff E, Gold R, et al. Serum and cerebrospinal fluid concentrations of homoarginine, arginine, asymmetric and symmetric dimethylarginine, nitrite and nitrate in patients with multiple sclerosis and neuromyelitis optica. Amino Acids 2015;47:1837-45.
83. Giovannoni G, Silver NC, O'Riordan J, Miller RF, Heales SJ, et al. Increased urinary nitric oxide metabolites in patients with multiple sclerosis correlates with early and relapsing disease. Mult Scler 1999;5:335-41.
84. Calabrese V, Scapagnini G, Ravagna A, Bella R, Foresti R, et al. Nitric oxide synthase is present in the cerebrospinal fluid of patients with active multiple sclerosis and is associated with increases in cerebrospinal fluid protein nitrotyrosine and S-nitrosothiols and with changes in glutathione levels. J Neurosci Res 2002;70:580-7.
85. Hua LL, Liu JS, Brosnan CF, Lee SC. Selective inhibition of human glial inducible nitric oxide synthase by interferon-beta: implications for multiple sclerosis. Ann Neurol 1998;43:384-7.
86. Danilov AI, Andersson M, Bavand N, Wiklund N, Olsson T, et al. Nitric oxide metabolite determinations reveal continuous inflammation in multiple sclerosis. J Neuroimmunol 2003;136:112-8.
87. Giovannoni G, Miller DH, Losseff NA, Sailer M, Lewellyn-Smith N, et al. Serum inflammatory markers and clinical/MRI markers of disease progression in multiple sclerosis. J Neurol 2001;248:487-95.
88. Tavazzi B, Batocchi AP, Amorini AM, Nociti V, D'Urso S, et al. Serum metabolic profile in multiple sclerosis patients. Mult Scler Int 2011;2011:167156.
89. Svenningsson A, Petersson AS, Andersen O, Hansson GK. Nitric oxide metabolites in CSF of patients with MS are related to clinical disease course. Neurology 1999;53:1880-2.
90. Acar G, Idiman F, Idiman E, Kirkali G, Cakmakçi H, et al. Nitric oxide as an activity marker in multiple sclerosis. J Neurol 2003;250:588-92.
91. Sellebjerg F, Giovannoni G, Hand A, Madsen H, Jensen C, et al. Cerebrospinal fluid levels of nitric oxide metabolites predict response to methylprednisolone treatment in multiple sclerosis and optic neuritis. J Neuroimmunol 2002;125:198-203.
92. Rejdak K, Eikelenboom MJ, Petzold A, Thompson EJ, Stelmasiak Z, et al. CSF nitric oxide metabolites are associated with activity and progression of multiple sclerosis. Neurology 2004;63:1439-45.
93. Merry K, Dodds R, Littlewood A, Gowen M. Expression of osteopontin mRNA by osteoclasts and osteoblasts in modelling adult human bone. J Cell Sci 1993;104:1013-20.
94. Del Prete A, Scutera S, Sozzani S, Musso T. Role of osteopontin in dendritic cell shaping of immune responses. Cytokine Growth Factor Rev 2019;50:19-28.
95. Murugaiyan G, Mittal A, Weiner HL. Increased osteopontin expression in dendritic cells amplifies IL-17 production by CD4+ T cells in experimental autoimmune encephalomyelitis and in multiple sclerosis. J Immunol 2008;181:7480-8.
96. Braitch M, Constantinescu CS. The role of osteopontin in experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS). Inflamm Allergy Drug Targets 2010;9:249-56.
97. Sato W, Tomita A, Ichikawa D, Lin Y, Kishida H, et al. CCR2(+)CCR5(+) T cells produce matrix metalloproteinase-9 and osteopontin in the pathogenesis of multiple sclerosis. J Immunol 2012;189:5057-65.
98. Chabas D, Baranzini SE, Mitchell D, Bernard CC, Rittling SR, et al. The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease. Science 2001;294:1731-5.
99. Sinclair C, Mirakhur M, Kirk J, Farrell M, McQuaid S. Up-regulation of osteopontin and alphaBeta-crystallin in the normal-appearing white matter of multiple sclerosis: an immunohistochemical study utilizing tissue microarrays. Neuropathol Appl Neurobiol 2005;31:292-303.
100. Vogt MH, Lopatinskaya L, Smits M, Polman CH, Nagelkerken L. Elevated osteopontin levels in active relapsing-remitting multiple sclerosis. Ann Neurol 2003;53:819-22.
101. Agah E, Zardoui A, Saghazadeh A, Ahmadi M, Tafakhori A, et al. Osteopontin (OPN) as a CSF and blood biomarker for multiple sclerosis: A systematic review and meta-analysis. PLoS One 2018;13:e0190252.
102. Shimizu Y, Ota K, Ikeguchi R, Kubo S, Kabasawa C, et al. Plasma osteopontin levels are associated with disease activity in the patients with multiple sclerosis and neuromyelitis optica. J Neuroimmunol 2013;263:148-51.
103. Chowdhury SA, Lin J, Sadiq SA. Specificity and correlation with disease activity of cerebrospinal fluid osteopontin levels in patients with multiple sclerosis. Arch Neurol 2008;65:232-5.
104. Comabella M, Pericot I, Goertsches R, Nos C, Castillo M, et al. Plasma osteopontin levels in multiple sclerosis. J Neuroimmunol 2005;158:231-9.
105. Vogt MH, Floris S, Killestein J, Knol DL, Smits M, et al. Osteopontin levels and increased disease activity in relapsing-remitting multiple sclerosis patients. J Neuroimmunol 2004;155:155-60.

106. Wen SR, Liu GJ, Feng RN, Gong FC, Zhong H, et al. Increased levels of IL-23 and osteopontin in serum and cerebrospinal fluid of multiple sclerosis patients. J Neuroimmunol 2012;244:94-6.
107. Kivisäkk P, Healy BC, Francois K, Gandhi R, Gholipour T, et al. Evaluation of circulating osteopontin levels in an unselected cohort of patients with multiple sclerosis: relevance for biomarker development. Mult Scler 2014;20:438-44.
108. Braitch M, Nunan R, Niepel G, Edwards LJ, Constantinescu CS. Increased osteopontin levels in the cerebrospinal fluid of patients with multiple sclerosis. Arch Neurol 2008;65:633-5.
109. Vogt MH, ten Kate J, Drent RJ, Polman CH, Hupperts R. Increased osteopontin plasma levels in multiple sclerosis patients correlate with bone-specific markers. Mult Scler 2010;16:443-9.
110. Börnsen L, Khademi M, Olsson T, Sørensen PS, Sellebjerg F. Osteopontin concentrations are increased in cerebrospinal fluid during attacks of multiple sclerosis. Mult Scler 2011;17:32-42.
111. Szalardy L, Zadori D, Simu M, Bencsik K, Vecsei L, et al. Evaluating biomarkers of neuronal degeneration and neuroinflammation in CSF of patients with multiple sclerosis-osteopontin as a potential marker of clinical severity. J Neurol Sci 2013;331:38-42.
112. Dianzani C, Vecchio D, Clemente N, Chiocchetti A, Martinelli Boneschi F, et al. Untangling extracellular proteasome-osteopontin circuit dynamics in multiple sclerosis. Cells 2019;8:262.
113. Runia TF, van Meurs M, Nasserinejad K, Hintzen RQ. No evidence for an association of osteopontin plasma levels with disease activity in multiple sclerosis. Mult Scler 2014;20:1670-1.
114. Zhao Q, Cheng W, Xi Y, Cao Z, Xu Y, et al. IFN-β regulates Th17 differentiation partly through the inhibition of osteopontin in experimental autoimmune encephalomyelitis. Mol Immunol 2018;93:20-30.
115. Hong J, Hutton GJ. Regulatory effects of interferon-β on osteopontin and interleukin-17 expression in multiple sclerosis. J Interferon Cytokine Res 2010;30:751-7.
116. Iaffaldano P, Ruggieri M, Viterbo RG, Mastrapasqua M, Trojano M. The improvement of cognitive functions is associated with a decrease of plasma Osteopontin levels in Natalizumab treated relapsing multiple sclerosis. Brain Behav Immun 2014;35:176-81.
117. Mas A, Martínez A, de las Heras V, Bartolomé M, de la Concha EG, et al. The 795CT polymorphism in osteopontin gene is not associated with multiple sclerosis in a Spanish population. Mult Scler 2007;13:250-2.
118. Biernacka-Lukanty J, Michalowska-Wender G, Michalak S, Raczak B, Kozubski W, et al. Polymorphism of the osteopontin gene and clinical course of multiple sclerosis in the Polish population. Folia Neuropathol 2015;53:343-6.
119. Caillier S, Barcellos LF, Baranzini SE, Swerdlin A, Lincoln RR, et al; Multiple Sclerosis Genetics Group. Osteopontin polymorphisms and disease course in multiple sclerosis. Genes Immun 2003;4:312-5.
120. Chiocchetti A, Comi C, Indelicato M, Castelli L, Mesturini R, et al. Osteopontin gene haplotypes correlate with multiple sclerosis development and progression. J Neuroimmunol 2005;163:172-8.
121. Comi C, Cappellano G, Chiocchetti A, Orilieri E, Buttini S, et al. The impact of osteopontin gene variations on multiple sclerosis development and progression. Clin Dev Immunol 2012;2012:212893.
122. Londoño AC, Mora CA. Role of CXCL13 in the formation of the meningeal tertiary lymphoid organ in multiple sclerosis. F1000Res 2018;7:514.
123. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol 2004;14:164-74.
124. Bagaeva LV, Rao P, Powers JM, Segal BM. CXC chemokine ligand 13 plays a role in experimental autoimmune encephalomyelitis. J Immunol 2006;176:7676-85.
125. Krumbholz M, Theil D, Cepok S, Hemmer B, Kivisäkk P, et al. Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment. Brain 2006;129:200-11.
126. Bai Z, Chen D, Wang L, Zhao Y, Liu T, et al. Cerebrospinal fluid and blood cytokines as biomarkers for multiple sclerosis: a systematic review and meta-analysis of 226 studies with 13,526 multiple sclerosis patients. Front Neurosci 2019;13:1026.
127. Sellebjerg F, Börnsen L, Khademi M, Krakauer M, Olsson T, et al. Increased cerebrospinal fluid concentrations of the chemokine CXCL13 in active MS. Neurology 2009;73:2003-10.
128. Khademi M, Kockum I, Andersson ML, Iacobaeus E, Brundin L, et al. Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course. Mult Scler 2011;17:335-43.
129. Irani DN. Regulated Production of CXCL13 within the Central Nervous System. J Clin Cell Immunol 2016;7:460.
130. Brettschneider J, Czerwoniak A, Senel M, Fang L, Kassubek J, et al. The chemokine CXCL13 is a prognostic marker in clinically isolated syndrome (CIS). PLoS One 2010;5:e11986.
131. Piccio L, Naismith RT, Trinkaus K, Klein RS, Parks BJ, et al. Changes in B- and T-lymphocyte and chemokine levels with rituximab treatment in multiple sclerosis. Arch Neurol 2010;67:707-14.
132. Romme Christensen J, Ratzer R, Börnsen L, Lyksborg M, Garde E, et al. Natalizumab in progressive MS: results of an open-label, phase 2A, proof-of-concept trial. Neurology 2014;82:1499-507.
133. Rempe RG, Hartz AMS, Bauer B. Matrix metalloproteinases in the brain and blood-brain barrier: Versatile breakers and makers. J Cereb Blood Flow Metab 2016;36:1481-507.
134. Wang Y, Wu H, Wu X, Bian Z, Gao Q. Interleukin 17A promotes gastric cancer invasiveness via NF-κB mediated matrix metalloproteinases 2 and 9 expression. PLoS One 2014;9:e96678.
135. Moon SK, Cha BY, Kim CH. ERK1/2 mediates TNF-alpha-induced matrix metalloproteinase-9 expression in human vascular smooth muscle cells via the regulation of NF-kappaB and AP-1: involvement of the ras dependent pathway. J Cell Physiol 2004;198:417-27.
136. Powell WC, Fingleton B, Wilson CL, Boothby M, Matrisian LM. The metalloproteinase matrilysin proteolytically generates active

soluble Fas ligand and potentiates epithelial cell apoptosis. Current Biology 1999;9:1441-7.
137. Woo MS, Park JS, Choi IY, Kim WK, Kim HS. Inhibition of MMP-3 or -9 suppresses lipopolysaccharide-induced expression of proinflammatory cytokines and iNOS in microglia. J Neurochem 2008;106:770-80.
138. Agrawal S, Anderson P, Durbeej M, van Rooijen N, Ivars F, et al. Dystroglycan is selectively cleaved at the parenchymal basement membrane at sites of leukocyte extravasation in experimental autoimmune encephalomyelitis. J Exp Med 2006;203:1007-19.
139. Nygårdas PT, Hinkkanen AE. Up-regulation of MMP-8 and MMP-9 activity in the BALB/c mouse spinal cord correlates with the severity of experimental autoimmune encephalomyelitis. Clin Exp Immunol 2002;128:245-54.
140. Buhler LA, Samara R, Guzman E, Wilson CL, Krizanac-Bengez L, et al. Matrix metalloproteinase-7 facilitates immune access to the CNS in experimental autoimmune encephalomyelitis. BMC Neurosci 2009;10:17.
141. Fainardi E, Castellazzi M, Tamborino C, Trentini A, Manfrinato MC, et al. Potential relevance of cerebrospinal fluid and serum levels and intrathecal synthesis of active matrix metalloproteinase-2 (MMP-2) as markers of disease remission in patients with multiple sclerosis. Mult Scler 2009;15:547-54.
142. Trentini A, Castellazzi M, Cervellati C, Manfrinato MC, Tamborino C, et al. Interplay between Matrix Metalloproteinase-9, Matrix Metalloproteinase-2, and Interleukins in Multiple Sclerosis Patients. Dis Markers 2016;2016:3672353.
143. Liuzzi GM, Trojano M, Fanelli M, Avolio C, Fasano A, et al. Intrathecal synthesis of matrix metalloproteinase-9 in patients with multiple sclerosis: implication for pathogenesis. Mult Scler 2002;8:222-8.
144. Lee MA, Palace J, Stabler G, Ford J, Gearing A, et al. Serum gelatinase B, TIMP-1 and TIMP-2 levels in multiple sclerosis. A longitudinal clinical and MRI study. Brain 1999;122:191-7.
145. Lichtinghagen R, Seifert T, Kracke A, Marckmann S, Wurster U, et al. Expression of matrix metalloproteinase-9 and its inhibitors in mononuclear blood cells of patients with multiple sclerosis. J Neuroimmunol 1999;99:19-26.
146. Waubant E, Goodkin DE, Gee L, Bacchetti P, Sloan R, et al. Serum MMP-9 and TIMP-1 levels are related to MRI activity in relapsing multiple sclerosis. Neurology 1999;53:1397-401.
147. Fainardi E, Castellazzi M, Bellini T, Manfrinato MC, Baldi E, et al. Cerebrospinal fluid and serum levels and intrathecal production of active matrix metalloproteinase-9 (MMP-9) as markers of disease activity in patients with multiple sclerosis. Mult Scler 2006;12:294-301.
148. Leppert D, Ford J, Stabler G, Grygar C, Lienert C, et al. Matrix metalloproteinase-9 (gelatinase B) is selectively elevated in CSF during relapses and stable phases of multiple sclerosis. Brain 1998;121:2327-34.
149. Benesová Y, Vasku A, Novotná H, Litzman J, Stourac P, et al. Matrix metalloproteinase-9 and matrix metalloproteinase-2 as biomarkers of various courses in multiple sclerosis. Mult Scler 2009;15:316-22.
150. Lindberg RL, De Groot CJ, Montagne L, Freitag P, van der Valk P, et al. The expression profile of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in lesions and normal appearing white matter of multiple sclerosis. Brain 2001;124:1743-53.
151. Anthony DC, Ferguson B, Matyzak MK, Miller KM, Esiri MM, et al. Differential matrix metalloproteinase expression in cases of multiple sclerosis and stroke. Neuropathol Appl Neurobiol 1997;23:406-15.
152. Galboiz Y, Shapiro S, Lahat N, Rawashdeh H, Miller A. Matrix metalloproteinases and their tissue inhibitors as markers of disease subtype and response to interferon-beta therapy in relapsing and secondary-progressive multiple sclerosis patients. Ann Neurol 2001;50:443-51.
153. Bernal F, Elias B, Hartung HP, Kieseier BC. Regulation of matrix metalloproteinases and their inhibitors by interferon-beta: a longitudinal study in multiple sclerosis patients. Mult Scler 2009;15:721-7.
154. Comabella M, Río J, Espejo C, Ruiz de Villa M, Al-Zayat H, et al. Changes in matrix metalloproteinases and their inhibitors during interferon-beta treatment in multiple sclerosis. Clin Immunol 2009;130:145-50.
155. Balasa R, Bianca C, Septimiu V, Iunius S, Adina H, et al. The matrix metalloproteinases panel in multiple sclerosis patients treated with Natalizumab: a possible answer to Natalizumab non-responders. CNS Neurol Disord Drug Targets 2018;17:464-72.
156. Castellazzi M, Bellini T, Trentini A, Delbue S, Elia F, et al. Serum gelatinases levels in multiple sclerosis patients during 21 months of Natalizumab therapy. Dis Markers 2016;2016:8434209.
157. Fissolo N, Pignolet B, Matute-Blanch C, Triviño JC, Miró B, et al; Biomarkers and Response to Natalizumab for Multiple Sclerosis Treatment (BIONAT), Best EScalation Treatment in Multiple Sclerosis (BEST-MS), and the Société Francophone de la Sclérose En Plaques (SFSEP) Network. Matrix metalloproteinase 9 is decreased in natalizumab-treated multiple sclerosis patients at risk for progressive multifocal leukoencephalopathy. Ann Neurol 2017;82:186-95.
158. Cohen SR, Brooks BR, Herndon RM, McKhann GM. A diagnostic index of active demyelination: myelin basic protein in cerebrospinal fluid. Ann Neurol 1980;8:25-31.
159. Whitaker JN, Lisak RP, Bashir RM, Fitch OH, Seyer JM, et al. Immunoreactive myelin basic protein in the cerebrospinal fluid in neurological disorders. Ann Neurol 1980;7:58-64.
160. Stadelmann C, Timmler S, Barrantes-Freer A, Simons M. Myelin in the central nervous system: structure, function, and pathology. Physiol Rev 2019;99:1381-431.
161. Whitaker JN. Myelin basic protein in cerebrospinal fluid and other body fluids. Mult Scler 1998;4:16-21.
162. Meinl E, Hohlfeld R. Immunopathogenesis of multiple sclerosis: MBP and beyond. Clin Exp Immunol 2002;128:395-7.
163. Olsson T, Sun J, Hillert J, Höjeberg B, Ekre HP, et al. Increased numbers of T cells recognizing multiple myelin basic protein epitopes in multiple sclerosis. Eur J Immunol 1992;22:1083-7.
164. Kim YC, Zhang AH, Yoon J, Culp WE, Lees JR, et al. Engineered MBP-specific human Tregs ameliorate MOG-induced EAE through IL-2-triggered inhibition of effector T cells. J Autoimmun 2018;92:77-86.
165. Whitaker JN. Myelin encephalitogenic protein fragments in cerebrospinal fluid of persons with multiple sclerosis. Neurology

1977;27:911-20.
166. Sellebjerg F, Christiansen M, Nielsen PM, Frederiksen JL. Cerebrospinal fluid measures of disease activity in patients with multiple sclerosis. Mult Scler 1998;4:475-9.
167. Noppe M, Crols R, Andries D, Lowenthal A. Determination in human cerebrospinal fluid of glial fibrillary acidic protein, S-100 and myelin basic protein as indices of non-specific or specific central nervous tissue pathology. Clinica Chimica Acta 1986;155:143-50.
168. Lamers KJ, de Reus HP, Jongen PJ. Myelin basic protein in CSF as indicator of disease activity in multiple sclerosis. Mult Scler 1998;4:124-6.
169. Barkhof F, Frequin ST, Hommes OR, Lamers K, Scheltens P, et al. A correlative triad of gadolinium-DTPA MRI, EDSS, and CSF-MBP in relapsing multiple sclerosis patients treated with high-dose intravenous methylprednisolone. Neurology 1992;42:63-7.
170. Zhou Y, Simpson S Jr, Charlesworth JC, van der Mei I, Lucas RM, et al; AUSLONG Investigators Group. Variation within MBP gene predicts disease course in multiple sclerosis. Brain Behav 2017;7:e00670.
171. Belogurov A Jr, Zakharov K, Lomakin Y, Surkov K, Avtushenko S, et al. CD206-targeted liposomal myelin basic protein peptides in patients with multiple sclerosis resistant to first-line disease-modifying therapies: a first-in-human, proof-of-concept dose-escalation study. Neurotherapeutics 2016;13:895-904.
172. Warren KG, Catz I, Ferenczi LZ, Krantz MJ. Intravenous synthetic peptide MBP8298 delayed disease progression in an HLA Class II-defined cohort of patients with progressive multiple sclerosis: results of a 24-month double-blind placebo-controlled clinical trial and 5 years of follow-up treatment. Eur J Neurol 2006;13:887-95.
173. Establish Tolerance In MS With Peptide-Coupled, Peripheral Blood Mononuclear Cells (ETIMS). Available from: https://clinicaltrials.gov/ct2/show/NCT01414634. [Last accessed on 7 Jul 2020]
174. Safety, Tolerability, and Effectiveness of CGP77116 in Patients With Multiple Sclerosis (MS). Available from: https://clinicaltrials.gov/ct2/show/NCT00001781. [Last accessed on 7 Jul 2020]
175. Tumani H, Hartung HP, Hemmer B, Teunissen C, Deisenhammer F, et al; BioMS Study Group. Cerebrospinal fluid biomarkers in multiple sclerosis. Neurobiol Dis 2009;35:117-27.
176. Zołtowska A, Stepiński J, Lewko B, Serkies K, Zamorska B, et al. Neural cell adhesion molecule in breast, colon and lung carcinomas. Arch Immunol Ther Exp (Warsz) 2001;49:171-4.
177. Massaro AR. The role of NCAM in remyelination. Neurol Sci 2002;22:429-35.
178. Massaro AR, Sbriccoli A, Tonali P. Reactive astrocytes within the acute plaques of multiple sclerosis are PSA-NCAM positive. Neurol Sci 2002;23:255-6.
179. Le Gal La Salle G, Rougon G, Valin A. The embryonic form of neural cell surface molecule (E-NCAM) in the rat hippocampus and its reexpression on glial cells following kainic acid- induced status epilepticus. J Neurosci 1992;12:872-82.
180. Gnanapavan S, Grant D, Illes-Toth E, Lakdawala N, Keir G, et al. Neural cell adhesion molecule--description of a CSF ELISA method and evidence of reduced levels in selected neurological disorders. J Neuroimmunol 2010;225:118-22.
181. Axelsson M, Dubuisson N, Novakova L, Malmeström C, Giovannoni G, et al. Cerebrospinal fluid NCAM levels are modulated by disease-modifying therapies. Acta Neurol Scand 2019;139:422-7.
182. Massaro AR, Albrechtsen M, Bock E. N-CAM in cerebrospinal fluid: a marker of synaptic remodelling after acute phases of multiple sclerosis? Ital J Neurol Sci 1987;Suppl 6:85-8.
183. Massaro AR. Are there indicators of remyelination in blood or CSF of multiple sclerosis patients? Mult Scler 1998;4:228-31.
184. Paul A, Comabella M, Gandhi R. Biomarkers in Multiple Sclerosis. Cold Spring Harb Perspect Med 2019;9:a029058.
185. Correale J, Fiol M. Chitinase effects on immune cell response in neuromyelitis optica and multiple sclerosis. Mult Scler 2011;17:521-31.
186. Kanneganti M, Kamba A, Mizoguchi E. Role of chitotriosidase (chitinase 1) under normal and disease conditions. J Epithel Biol Pharmacol 2012;5:1-9.
187. De Fino C, Lucchini M, Lucchetti D, Nociti V, Losavio FA, et al. The predictive value of CSF multiple assay in multiple sclerosis: a single center experience. Mult Scler Relat Disord 2019;35:176-81.
188. Novakova L, Axelsson M, Khademi M, Zetterberg H, Blennow K, et al. Cerebrospinal fluid biomarkers as a measure of disease activity and treatment efficacy in relapsing-remitting multiple sclerosis. J Neurochem 2017;141:296-304.
189. Sellebjerg F, Börnsen L, Ammitzbøll C, Nielsen JE, Vinther-Jensen T, et al. Defining active progressive multiple sclerosis. Mult Scler 2017;23:1727-35.
190. Comabella M, Fernández M, Martin R, Rivera-Vallvé S, Borrás E, et al. Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. Brain 2010;133:1082-93.
191. Borràs E, Cantó E, Choi M, Maria Villar L, Álvarez-Cermeño JC, et al. Protein-based classifier to predict conversion from clinically isolated syndrome to multiple sclerosis. Mol Cell Proteomics 2016;15:318-28.
192. Cantó E, Tintoré M, Villar LM, Costa C, Nurtdinov R, et al. Chitinase 3-like 1: prognostic biomarker in clinically isolated syndromes. Brain 2015;138:918-31.
193. Matute-Blanch C, Río J, Villar LM, Midaglia L, Malhotra S, et al. Chitinase 3-like 1 is associated with the response to interferon-beta treatment in multiple sclerosis. J Neuroimmunol 2017;303:62-5.
194. Lovett-Racke AE, Yang Y, Racke MK. Th1 versus Th17: are T cell cytokines relevant in multiple sclerosis? Biochim Biophys Acta 2011;1812:246-51.
195. Longbrake EE, Racke MK. Why did IL-12/IL-23 antibody therapy fail in multiple sclerosis? Expert Rev Neurother 2009;9:319-21.
196. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature 2003;421:744-8.

197. Segal BM, Constantinescu CS, Raychaudhuri A, Kim L, Fidelus-gort R, et al. Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomised, dose-ranging study. Lancet Neurol 2008;7:796-804.
198. Matusevicius D, Kivisäkk P, He B, Kostulas N, Ozenci V, et al. Interleukin-17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis. Mult Scler 1999;5:101-4.
199. Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, et al. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med 2002;8:500-8.
200. Brucklacher-Waldert V, Stuerner K, Kolster M, Wolthausen J, Tolosa E. Phenotypical and functional characterization of T helper 17 cells in multiple sclerosis. Brain 2009;132:3329-41.
201. Havrdová E, Belova A, Goloborodko A, Tisserant A, Wright A, et al. Activity of secukinumab, an anti-IL-17A antibody, on brain lesions in RRMS: results from a randomized, proof-of-concept study. J Neurol 2016;263:1287-95.
202. Ishizu T, Osoegawa M, Mei FJ, Kikuchi H, Tanaka M, et al. Intrathecal activation of the IL-17/IL-8 axis in opticospinal multiple sclerosis. Brain 2005;128:988-1002.
203. Rossi S, Motta C, Studer V, Barbieri F, Buttari F, et al. Tumor necrosis factor is elevated in progressive multiple sclerosis and causes excitotoxic neurodegeneration. Mult Scler 2014;20:304-12.
204. Trenova AG, Slavov GS, Draganova-Filipova MN, Mateva NG, Manova MG, et al. Circulating levels of interleukin-17A, tumor necrosis factor-alpha, interleukin-18, interleukin-10, and cognitive performance of patients with relapsing-remitting multiple sclerosis. Neurol Res 2018;40:153-9.
205. Pegoretti V, Baron W, Laman JD, Eisel ULM. Selective Modulation of TNF-TNFRs Signaling: insights for multiple sclerosis treatment. Front Immunol 2018;9:925.
206. Caminero A, Comabella M, Montalban X. Tumor necrosis factor alpha (TNF-α), anti-TNF-α and demyelination revisited: an ongoing story. J Neuroimmunol 2011;234:1-6.
207. Brambilla R, Ashbaugh JJ, Magliozzi R, Dellarole A, Karmally S, et al. Inhibition of soluble tumour necrosis factor is therapeutic in experimental autoimmune encephalomyelitis and promotes axon preservation and remyelination. Brain 2011;134:2736-54.
208. Ng LG, Sutherland AP, Newton R, Qian F, Cachero TG, et al. B cell-activating factor belonging to the TNF family (BAFF)-R is the principal BAFF receptor facilitating BAFF costimulation of circulating T and B cells. J Immunol 2004;173:807-17.
209. Kannel K, Alnek K, Vahter L, Gross-Paju K, Uibo R, et al. Changes in blood B cell-activating factor (BAFF) levels in multiple sclerosis: a sign of treatment outcome. PLoS One 2015;10:e0143393.
210. Friede RL, Samorajski T. Axon caliber related to neurofilaments and microtubules in sciatic nerve fibers of rats and mice. Anat Rec 1970;167:379-87.
211. Varhaug KN, Torkildsen Ø, Myhr KM, Vedeler CA. Neurofilament light chain as a biomarker in multiple sclerosis. Front Neurol 2019;10:338.
212. Rosengren LE, Karlsson JE, Karlsson JO, Persson LI, Wikkelsø C. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. J Neurochem 1996;67:2013-8.
213. De Schaepdryver M, Jeromin A, Gille B, Claeys KG, Herbst V, et al. Comparison of elevated phosphorylated neurofilament heavy chains in serum and cerebrospinal fluid of patients with amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2018;89:367-73.
214. Fyfe I. Alzheimer disease: neurofilament light in the blood marks Alzheimer degeneration. Nat Rev Neurol 2017;13:257.
215. Meeter LH, Dopper EG, Jiskoot LC, Sanchez-Valle R, Graff C, et al. Neurofilament light chain: a biomarker for genetic frontotemporal dementia. Ann Clin Transl Neurol 2016;3:623-36.
216. Mages B, Aleithe S, Altmann S, Blietz A, Nitzsche B, et al. Impaired neurofilament integrity and neuronal morphology in different models of focal cerebral ischemia and human stroke tissue. Front Cell Neurosci 2018;12:161.
217. Byrne LM, Rodrigues FB, Blennow K, Durr A, Leavitt BR, et al. Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's disease: a retrospective cohort analysis. Lancet Neurol 2017;16:601-9.
218. Bridel C, van Wieringen WN, Zetterberg H, Tijms BM, Teunissen CE, et al; and the NFL Group. Diagnostic value of cerebrospinal fluid neurofilament light protein in neurology: a systematic review and meta-analysis. JAMA Neurol 2019;76:1035-48.
219. Bergman J, Dring A, Zetterberg H, Blennow K, Norgren N, et al. Neurofilament light in CSF and serum is a sensitive marker for axonal white matter injury in MS. Neurol Neuroimmunol Neuroinflamm 2016;3:e271.
220. Novakova L, Zetterberg H, Sundström P, Axelsson M, Khademi M, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology 2017;89:2230-7.
221. Kuhle J, Barro C, Disanto G, Mathias A, Soneson C, et al. Serum neurofilament light chain in early relapsing remitting MS is increased and correlates with CSF levels and with MRI measures of disease severity. Mult Scler 2016;22:1550-9.
222. Kuhle J, Barro C, Andreasson U, Derfuss T, Lindberg R, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. Clin Chem Lab Med 2016;54:1655-61.
223. Disanto G, Barro C, Benkert P, Naegelin Y, Schädelin S, et al; Swiss Multiple Sclerosis Cohort Study Group. Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann Neurol 2017;81:857-70.
224. Teunissen CE, Iacobaeus E, Khademi M, Brundin L, Norgren N, et al. Combination of CSF N-acetylaspartate and neurofilaments in multiple sclerosis. Neurology 2009;72:1322-9.
225. Kuhle J, Leppert D, Petzold A, Regeniter A, Schindler C, et al. Neurofilament heavy chain in CSF correlates with relapses and disability in multiple sclerosis. Neurology 2011;76:1206-13.

226. Norgren N, Sundström P, Svenningsson A, Rosengren L, Stigbrand T, et al. Neurofilament and glial fibrillary acidic protein in multiple sclerosis. Neurology 2004;63:1586-90.
227. Cai L, Huang J. Neurofilament light chain as a biological marker for multiple sclerosis: a meta-analysis study. Neuropsychiatr Dis Treat 2018;14:2241-54.
228. Martin SJ, McGlasson S, Hunt D, Overell J. Cerebrospinal fluid neurofilament light chain in multiple sclerosis and its subtypes: a meta-analysis of case-control studies. J Neurol Neurosurg Psychiatry 2019;90:1059-67.
229. Kuhle J, Nourbakhsh B, Grant D, Morant S, Barro C, et al. Serum neurofilament is associated with progression of brain atrophy and disability in early MS. Neurology 2017;88:826-31.
230. Quintana E, Coll C, Salavedra-Pont J, Muñoz-San Martín M, Robles-Cedeño R, et al. Cognitive impairment in early stages of multiple sclerosis is associated with high cerebrospinal fluid levels of chitinase 3-like 1 and neurofilament light chain. Eur J Neurol 2018;25:1189-91.
231. Kuhle J, Plavina T, Barro C, Disanto G, Sangurdekar D, et al. Neurofilament light levels are associated with long-term outcomes in multiple sclerosis. Mult Scler 2019; doi: 10.1177/1352458519885613.
232. Ferraro D, Guicciardi C, De Biasi S, Pinti M, Bedin R, et al. Plasma neurofilaments correlate with disability in progressive multiple sclerosis patients. Acta Neurol Scand 2020;141:16-21.
233. Disanto G, Adiutori R, Dobson R, Martinelli V, Dalla Costa G, et al; International Clinically Isolated Syndrome Study Group. Serum neurofilament light chain levels are increased in patients with a clinically isolated syndrome. J Neurol Neurosurg Psychiatry 2016;87:126-9.
234. Martínez MA, Olsson B, Bau L, Matas E, Cobo Calvo Á, et al. Glial and neuronal markers in cerebrospinal fluid predict progression in multiple sclerosis. Mult Scler 2015;21:550-61.
235. Modvig S, Degn M, Roed H, Sørensen TL, Larsson HB, 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 2015;21:1761-70.
236. Siller N, Kuhle J, Muthuraman M, Barro C, Uphaus T, et al. Serum neurofilament light chain is a biomarker of acute and chronic neuronal damage in early multiple sclerosis. Mult Scler 2019;25:678-86.
237. Varhaug KN, Barro C, Bjørnevik K, Myhr KM, Torkildsen Ø, et al. Neurofilament light chain predicts disease activity in relapsing-remitting MS. Neurol Neuroimmunol Neuroinflamm 2018;5:e422.
238. Chitnis T, Gonzalez C, Healy BC, Saxena S, Rosso M, et al. Neurofilament light chain serum levels correlate with 10-year MRI outcomes in multiple sclerosis. Ann Clin Transl Neurol 2018;5:1478-91.
239. Axelsson M, Malmeström C, Gunnarsson M, Zetterberg H, Sundström P, et al. Immunosuppressive therapy reduces axonal damage in progressive multiple sclerosis. Mult Scler 2014;20:43-50.
240. Novakova L, Axelsson M, Khademi M, Zetterberg H, Blennow K, et al. Cerebrospinal fluid biomarkers of inflammation and degeneration as measures of fingolimod efficacy in multiple sclerosis. Mult Scler 2017;23:62-71.
241. Kuhle J, Malmeström C, Axelsson M, Plattner K, Yaldizli O, et al. Neurofilament light and heavy subunits compared as therapeutic biomarkers in multiple sclerosis. Acta Neurol Scand 2013;128:e33-6.
242. Gunnarsson M, Malmeström C, Axelsson M, Sundström P, Dahle C, et al. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. Ann Neurol 2011;69:83-9.
243. Akgün K, Kretschmann N, Haase R, Proschmann U, Kitzler HH, et al. Profiling individual clinical responses by high-frequency serum neurofilament assessment in MS. Neurol Neuroimmunol Neuroinflamm 2019;6:e555.
244. Traditional Versus Early Aggressive Therapy for Multiple Sclerosis Trial (TREAT-MS). Available from: https://clinicaltrials.gov/ct2/show/NCT03500328. [Last accessed on 9 Jul 2020]
245. Janeway CA Jr, Travers P, Walport M, Shlomchik MJ. Immunobiology: the immune system in health and disease. 5th edition. New York: Garland Science; 2001. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27162/. [Last accessed on 9 Jul 2020]
246. Sharief MK, Keir G, Thompson EJ. Intrathecal synthesis of IgM in neurological diseases: a comparison between detection of oligoclonal bands and quantitative estimation. J Neurol Sci 1990;96:131-42.
247. Villar LM, Masjuan J, González-Porqué P, Plaza J, Sádaba MC, et al. Intrathecal IgM synthesis is a prognostic factor in multiple sclerosis. Ann Neurol 2003;53:222-6.
248. Mandrioli J, Sola P, Bedin R, Gambini M, Merelli E. A multifactorial prognostic index in multiple sclerosis. Cerebrospinal fluid IgM oligoclonal bands and clinical features to predict the evolution of the disease. J Neurol 2008;255:1023-31.
249. Magraner MJ, Bosca I, Simó-Castelló M, García-Martí G, Alberich-Bayarri A, et al. Brain atrophy and lesion load are related to CSF lipid-specific IgM oligoclonal bands in clinically isolated syndromes. Neuroradiology 2012;54:5-12.
250. Villar LM, Sádaba MC, Roldán E, Masjuan J, González-Porqué P, et al. Intrathecal synthesis of oligoclonal IgM against myelin lipids predicts an aggressive disease course in MS. J Clin Invest 2005;115:187-94.
251. Durante L, Zaaraoui W, Rico A, Crespy L, Wybrecht D, et al. Intrathecal synthesis of IgM measured after a first demyelinating event suggestive of multiple sclerosis is associated with subsequent MRI brain lesion accrual. Mult Scler 2012;18:587-91.
252. Boscá I, Magraner MJ, Coret F, Alvarez-Cermeño JC, Simó-Castelló M, et al. The risk of relapse after a clinically isolated syndrome is related to the pattern of oligoclonal bands. J Neuroimmunol 2010;226:143-6.
253. Ferraro D, Simone AM, Bedin R, Galli V, Vitetta F, et al. Cerebrospinal fluid oligoclonal IgM bands predict early conversion to clinically definite multiple sclerosis in patients with clinically isolated syndrome. J Neuroimmunol 2013;257:76-81.
254. Espiño M, Abraira V, Arroyo R, Bau L, Cámara C, et al. Assessment of the reproducibility of oligoclonal IgM band detection for its application in daily clinical practice. Clin Chim Acta 2015;438:67-9.

255. Sola P, Mandrioli J, Simone AM, Ferraro D, Bedin R, et al. Primary progressive versus relapsing-onset multiple sclerosis: presence and prognostic value of cerebrospinal fluid oligoclonal IgM. Mult Scler 2011;17:303-11.
256. Bosca I, Villar LM, Coret F, Magraner MJ, Simó-Castelló M, et al. Response to interferon in multiple sclerosis is related to lipid-specific oligoclonal IgM bands. Mult Scler 2010;16:810-5.
257. Selter RC, Biberacher V, Grummel V, Buck D, Eienbröker C, et al. Natalizumab treatment decreases serum IgM and IgG levels in multiple sclerosis patients. Mult Scler 2013;19:1454-61.
258. Villar LM, García-Sánchez MI, Costa-Frossard L, Espiño M, Roldán E, et al. Immunological markers of optimal response to natalizumab in multiple sclerosis. Arch Neurol 2012;69:191-7.
259. Hol EM, Pekny M. Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. Curr Opin Cell Biol 2015;32:121-30.
260. Bélanger M, Magistretti PJ. The role of astroglia in neuroprotection. Dialogues Clin Neurosci 2009;11:281-95.
261. Yang Z, Wang KK. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci 2015;38:364-74.
262. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, et al. Neurotoxic reactive astrocytes are induced by activated microglia. Nature 2017;541:481-7.
263. Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. Lancet Neurol 2015;14:183-93.
264. Abdelhak A, Hottenrott T, Morenas-Rodríguez E, Suárez-Calvet M, Zettl UK, et al. Glial activation markers in CSF and serum from patients with primary progressive multiple sclerosis: potential of serum GFAP as disease severity marker? Front Neurol 2019;10:280.
265. Rosengren L, Lycke J, Andersen O. Glial fibrillary acidic protein in CSF of multiple sclerosis patients: relation to neurological deficit. J Neurol Sci 1995;133:61-5.
266. Petzold A, Eikelenboom MJ, Gveric D, Keir G, Chapman M, et al. Markers for different glial cell responses in multiple sclerosis: clinical and pathological correlations. Brain 2002;125:1462-73.
267. Axelsson M, Malmeström C, Nilsson S, Haghighi S, Rosengren L, et al. Glial fibrillary acidic protein: a potential biomarker for progression in multiple sclerosis. J Neurol 2011;258:882-8.
268. Högel H, Rissanen E, Barro C, Matilainen M, Nylund M, et al. Serum glial fibrillary acidic protein correlates with multiple sclerosis disease severity. Mult Scler 2020;26:210-9.
269. Kassubek R, Gorges M, Schocke M, Hagenston VAM, Huss A, et al. GFAP in early multiple sclerosis: A biomarker for inflammation. Neurosci Lett 2017;657:166-70.

Valerio *et al*. *Neuroimmunol Neuroinflammation* 2021;8:42-9
DOI: 10.20517/2347-8659.2020.30

Neuroimmunology and Neuroinflammation

Case Report

Open Access

The relation between lesions and localization of sources of slow biphasic complexes in encephalitis

Massimo Valerio, Stefano Rivera, Luca Mesin

Mathematical Biology & Physiology, Department of Electronics and Telecommunications, Politecnico di Torino, Turin 10129, Italy.

Correspondence to: Prof. Luca Mesin, Mathematical Biology & Physiology, Department of Electronics and Telecommunications, Politecnico di Torino, Corso Duca degli Abruzzi 24, Turin 10129, Italy. E-mail: luca.mesin@polito.it

How to cite this article: Valerio M, Rivera S, Mesin L.The relation between lesions and localization of sources of slow biphasic complexes in encephalitis. *Neuroimmunol Neuroinflammation* 2021;8:42-9. http://dx.doi.org/10.20517/2347-8659.2020.30

Received: 11 Apr 2020 **First Decision**: 28 May 2020 **Revised**: 12 Jun 2020
Accepted: 28 Jun 2020 **Fisrt online**: 15 Aug 2020 **Published**: 21 Mar 2021

Academic Editor: Mercè Falip **Copy Editor**: Cai-Hong Wang **Production Editor**: Jing Yu

Abstract

Slow biphasic complexes (SBC) were found in the electroencephalogram (EEG) of patients with inflammations of the brain. We have developed an automated method to identify them and proved that they represent a sensitive marker of the severity of encephalitis. Here we focus on another property of SBCs, i.e., the localization of their sources. We present two encephalitic patients, showing lesions in the magnetic resonance images, which are either spread in the brain or focused on the left hemisphere, respectively. Applying a source localization algorithm to the identified SBCs, we found either a diffused or a left-focused distribution, respectively. This result further suggests a relation between neuroinflammation and appearance of SBCs, indicating that their distribution reflects in part the localization of brain lesions. This promising result extends the information that can be extracted from EEG, promoting the reduction of expensive or invasive measurements in encephalitic patients.

Keywords: EEG, encephalitis, slow biphasic complex, source localization

INTRODUCTION

Encephalitis is an inflammatory process of the cerebral parenchyma associated with neurological dysfunctions[1], which requires prompt diagnosis and intervention[2]. It is caused mainly by infectious diseases or immune disorders, cancer, and vascular problems[3,4]. It can have different progressions (acute,

© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

subacute, or chronic) and patients frequently report neurological sequelae[3,4]. The incidence of infectious encephalitis is estimated at 1.5-7 cases per 100,000 inhabitants/year in the world[5], and 10.09 cases per 100,000 in Italian infants[4]. Encephalitis is a serious problem requiring hospitalization and giving a significant economic burden on society[6].

In more than 50% of cases the etiological cause is unknown and patients are admitted with non-specific symptoms at the time of presentation[7]. However, the main manifestations are brain suffering and/or altered state of consciousness, possibly in addition to fever, focal neurological deficits, epileptic seizures, abnormalities in the electroencephalogram (EEG) or neuroimaging, and cerebrospinal fluid (CSF) pleiocytosis. After a rapid evaluation of basic vital functions, serological and instrumental tests, empirical urgent therapy is usually adopted for symptomatic patients, in association with antiviral, antibiotic, and steroid drugs[8]. Prognosis is difficult to evaluate, mainly due to the multiple possible aetiologies (for example, in the case of herpes simplex encephalitis, which is the most common one, mortality in the range of 5%-20% was documented for patients treated with antiviral, 70% in those who did not receive treatment)[9].

The EEG has a fundamental role in the diagnostic framework. Different aetiologies of encephalitis were found to be associated with specific EEG patterns: for instance, triphasic waves are pathognomonic of hepatic encephalopathy[10] and lateralized periodic discharges or periodic lateralized epileptiform discharges are found in herpetic encephalopathy[11]. Moreover, stage II of subacute sclerosing panencephalitis is characterized by bilaterally symmetrical and synchronous generalized, stereotyped high amplitude delta waves, called Radermacker or "R" complexes[12]. Here we are interested in a specific EEG element, called slow biphasic complex (SBC), described as identical in the first part to the "R" complex even if it has different spatial and temporal properties[13]. SBC has been described as associated with the inflammatory processes of the central nervous system[13-17]. We have recently proposed an automated method to identify SBCs in an EEG trace, opening the possibility of quantifying them and investigating their origin. In particular, we have demonstrated that the number and amplitude of complexes reflect the severity of the inflammation in pediatric encephalitic patients[14]. Moreover, we have proposed to integrate information from different features of SBC to improve the diagnosis[18].

Herein, we focus on the relation between the location of SBCs and brain lesions found in magnetic resonance images (MRI). Methods for EEG source detection are applied to the identified SBCs to localize the brain areas producing them. This could be a promising tool to investigate the topography of inflammatory activity[19]. We report the application of this method in two specific cases.

CASE REPORT

We applied our processing to the EEG recorded from two patients also considered in a previous paper[14] (to which the reader can refer for details on EEG recordings), for which MRIs were also available. For each patient, we considered EEG data recorded close to the day in which the MRI was acquired. The two patients were very different, the first showing diffused lesions due to acute disseminated encephalomyelitis (ADEM) and the second with inflammatory processes, caused by infectious etiology, focused in one hemisphere. In this section, we first introduce the processing methods; then the two cases are discussed.

Methods

An algorithm we introduced before was applied to identify the SBCs[14]. Then, a method for source localization was used to identify the brain areas involved in the production of the complexes. These locations were compared to those of lesions identified in the MRIs by an expert neuro-radiologist.

Identification of slow biphasic complexes

SBCs were identified by the method described in a previous paper[14]. In brief, each EEG trace was

processed with a set of match filters, each comparing the signal with a scaled version of a prototype biphasic waveform. The identified complexes were then automatically reviewed, excluding outliers and waveforms showing repetitive firings, as some waveforms could have a shape similar to that of an SBC, but they could not satisfy some properties indicated in previous publications[14,17,18].

Source localization

Source localization in EEG refers to the detection of the sources inside the brain that generate the electrical activity acquired on the scalp. When the available electrodes are in a small number (as in our cases, in which either 12 or 18 channels were available for the two cases, respectively), the source detection may have low accuracy[20], but can provide useful information on the brain areas that are most involved in the inflammatory activity.

From the mathematical point of view, the dipoles inside the brain that produce a scalp potential that best fits the original data are sought. The problem can be written as follows:

$$M = GD + n \quad (1)$$

where each row of the matrix M contains a measured EEG, G is the Lead-Field matrix that describes the response of the activation of N different dipoles, whose level of activity (collected in D) should be estimated, and n is an additive noise, assumed spatially and temporally white. Different methods have been proposed in the literature to solve this problem[19,21]. In this study, the minimum norm estimation (MNE) was used[22]. It searches for the solution with minimum power, by minimizing the following regularized functional

$$U(D) = \| M - GD \|^2 + a\| D \|^2 \quad (2)$$

where α is a regularization parameter to constrain the power of the solution (chosen in this study to be equal to the mean of the eigenvalues of G^TG divided by 2,500; however the estimation was stable to a variation of α by an order of magnitude). It brings to the following solution to recover the sources:

$$D_{MNE} = (G^TG + aI_N)^{-1}G^TM \quad (3)$$

where I_N is the identity matrix of dimension N × N.

Localization of sources of SBCs

The waveforms of interest are concentrated in a low-frequency band. After visual inspection of the portions containing SBCs, each EEG trace was then band-pass filtered between 0.5 and 5 Hz (Chebychev filter of order 6 of type I), and the common-mode was removed. This filter provided clean EEG traces, focusing the source detection mostly on the waveforms of interest. Half second long windows centered on the identified SBCs were concatenated to generate the rows of matrix M in equation (1). Then, MNE (FieldTrip implementation[23]) provided a discrete brain model made of equivalent current dipole sources, containing the mean activation over time for each source location. The same procedure was applied to EEG data with the same duration obtained concatenating windows not including SBCs, to estimate the average background activity. The difference between the medians of dipole intensities during SBC onsets and in the background was then investigated (checking significant differences with the Wilcoxon rank-sum test for equal medians, with significance level $P = 0.001$).

First case

A 4-year-old subject was considered. At the time of the presentation, the patient presented with fever,

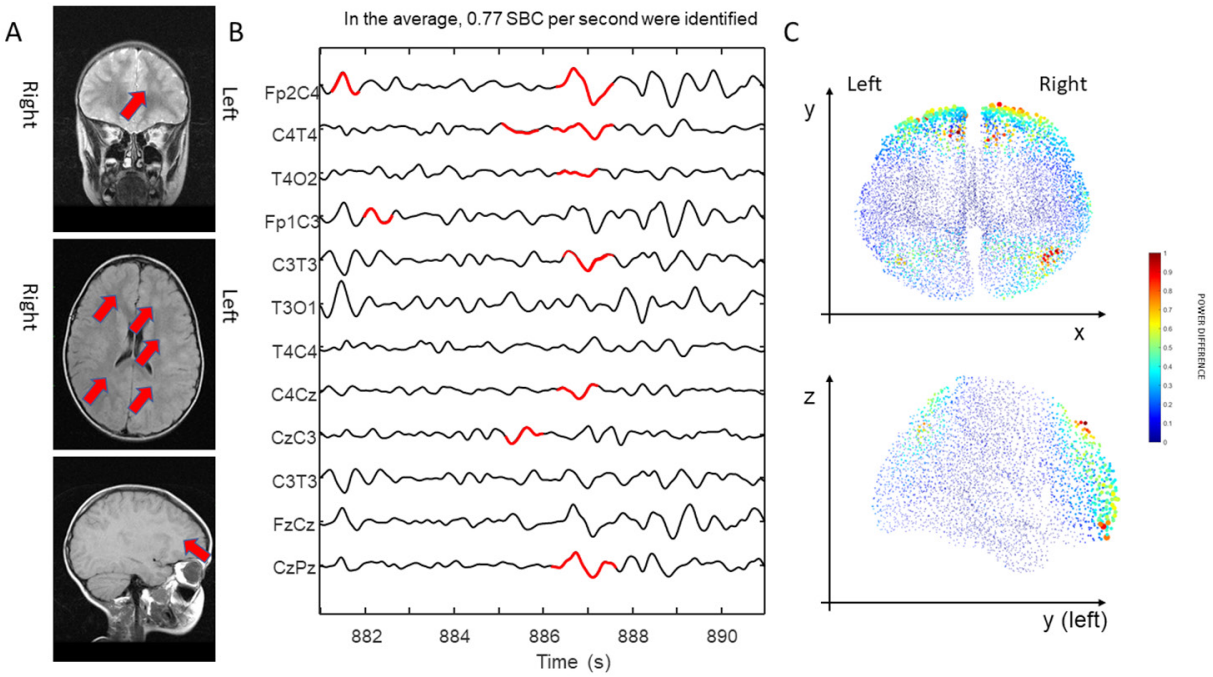


Figure 1. Coronal, axial, and sagittal magnetic resonance images sections of the brain for the first case. The lesions are highlighted with red arrows (radiological convention) (A); a portion of electroencephalogram (filtered between 0.5 and 5 Hz) with an indication of the identified slow biphasic complexes (SBC) in red color (B); the normalized mean power of the difference between the intensities of the sources of the signal during SBCs activation and background (neurological convention) (C)

headache, lower back pain, and somnolence. The symptoms, after a temporary regression with nonsteroidal anti-inflammatory drugs, deteriorated in the following days leading to confusion, plaintive, and drowsiness and patient was hospitalized. Empirical therapy was administered with antibiotics, antiviral, and steroid therapy. Lumbar puncture was performed which demonstrated an elevated CSF pressure, but laboratory tests (i.e., physical chemical test and cell counting) were negative. Blood chemistry tests showed a mild increase in white cells and high inflammation indexes.

The EEG performed at the onset showed a severe widespread brain suffering from SBCs on the frontal areas. The first brain MRI showed an ADEM-compatible result, with cortico-subcortical lesions prevalently observable in the frontal lobe (bigger on the left), medial temporal cortex, and basal ganglia.

We processed an EEG trace acquired the day before the MRI registration. The results are shown in Figure 1. The MRI is shown on the left with an indication of the main lesions. A portion of EEG is shown in the center, with the identified SBCs super-imposed in red color. The localization of SBC sources (emerging from the background) is shown on the right. The intensity of dipole sources during SBC activity resulted significantly different from the background (higher, actually) in 99% of cases. An important increase in intensity can be appreciated for the dipoles in the frontal area and the parietal-occipital lobe. The activity is quite spread across the two hemispheres.

During hospitalization, there was a slow improvement in clinical and instrumental examinations. The patient after a month was dismissed from the hospital with ADEM diagnosis and a schedule of follow-up.

Second case

A 10-year-old subject was considered. In the beginning, the child was diagnosed with a rubella type rash that after 5 days evolved presenting neurological symptoms with photophobia and drowsiness alternating

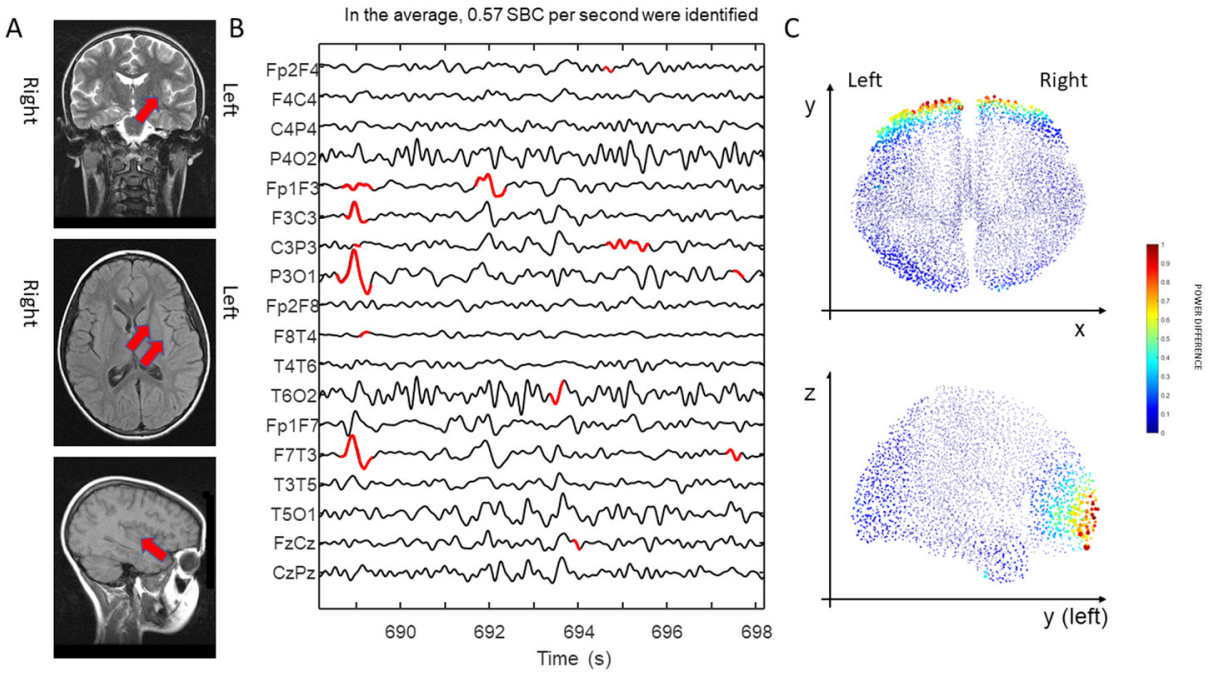


Figure 2. Coronal, axial, and sagittal magnetic resonance images sections of the brain for the second case. The lesions are highlighted with red arrows (radiological convention) (A); a portion of electroencephalogram (filtered between 0.5 and 5 Hz) with an indication of identified slow biphasic complexes (SBC) (B); the normalized mean power of the difference between the intensities of the sources of the signal during SBCs activation and background (neurological convention) (C)

with psychomotor agitation. The results of serology and bacteriological tests showed a slight increase in inflammatory parameters, in particular of lymphocytes. The examination of the CSF extracted by a lumbar puncture reported increased cellularity. Due to the worsening condition, the child was transferred to our tertiary children's center to be admitted to the intensive care unit. The adopted empirical therapy consisted of a triad of drugs formed by the antibiotic, steroid, and antiviral drugs (as in the previous case), with the addition of antifungal therapy.

The EEG traces initially showed a very slow widespread electrical activity that after a few days has been focused on the left hemisphere, in particular on the frontal areas. The MRI, acquired after the admission of the patient in the intensive care unit, showed lesions in particular in the flair sequence in the areas parasagittal frontal and mesial - insular temporal in the left hemisphere.

We processed an EEG trace acquired two days before the MRI registration. The results are shown in Figure 2. The MRI is shown on the left, a portion of EEG in the center, and the localization of SBC sources (emerging from background) on the right. Also, in this case, the intensity of dipole sources during SBC activity was significantly higher than the background in 99% of cases. An important increase in intensity can be appreciated for the dipoles in frontal area (mainly on the left) and a superficial portion of the occipital lobe. Most activity is found in the left hemisphere.

Afterward, the clinical situation improved, but the child showed a deficit in the right side of the body. She was dismissed with a diagnosis of encephalitis during rubella infection.

DISCUSSION

In the literature, several studies recognized the possibility to identify the etiological causes of different pathologies based on the EEG[24,25]. This allows us to advance diagnostic hypotheses and to outline

predictive factors, establishing different outcomes. Early EEG alterations suggest a negative prognosis, supporting the use of aggressive anti-inflammatory neuroprotection therapies[26,27].

SBCs have been observed in patients with brain inflammations[13,15,28,29]. We have developed an automated processing method to identify SBCs and we have shown in previous studies a good correlation between their onset and the severity of encephalitis[14,18]. Here we have focused on the distribution of the sources of SBCs and their relation with the lesions identified in MRIs.

Two different cases are discussed. In the first, lesions were widespread in the brain, whereas in the second they were more focused on the left hemisphere. Sources of SBCs were also found more widespread in the first case and predominantly on the left hemisphere in the second, indicating that the localization of SBC sources can provide some insights on the location of the lesions.

However, we should notice that SBC sources were identified quite superficial, mostly in frontal location and not exactly in the sites of the lesions. Notice that the low-frequency activity investigated in this study (predominantly in the delta range and increased in our patients, due to brain suffering from encephalitis) is larger in the frontal lobe, even during periods in which SBCs are not present. However, the sources of SBCs are significantly larger than those producing background activities, indicating that the predominant identification of SBC sources in the frontal lobe is not a bias.

Possibly, our results could be biased by both the source localization algorithm and the surface EEG technique in general, which emphasizes cortical contributions. Indeed, the activity of cortical neurons is recorded with a larger amplitude than that of deeper sources (possibly, even appearing under the noise level or covered by the synchronous cortical activity of areas also affected by the inflammation). Consider also that a small number of electrodes was used in our clinical recordings, hampering the identification of deep sources[20]. Moreover, lesions affect the activity of cortical neurons connected to the inflamed ones: these connected neurons could also be far apart from the lesions. Thus, it could be possible to observe altered activity not only in a contiguous area, but also distant from the lesions, i.e., produced by cortical neurons in connection with the focal area of inflammation, but located far apart (reflecting a well-known difference between anatomical and functional correlates in the brain). Notice also that, if the area of inflammation is located in the white matter, it affects only axons and action potentials propagating along them are less visible from EEG than post-synaptic potentials. However, as mentioned above, even if the exact locations of the lesions are not easy to be identified, our results suggest that the spread of the lesions and a possible asymmetry can be found (indeed, in the first case, the identified SBC sources are widespread and, in the second case, SBC sources are predominantly found in the same hemisphere in which lesions are located).

Further work is suggested to deepen the promising results found in this pilot study on a few patients. In particular, using a high-density system for EEG acquisition could allow us to better locate the sources of SBCs, to be correlated with MRI results[30]. Moreover, the use of functional MRI in synchronous with EEG registration[31] could help in investigating better SBC sources.

As EEG acquisition is cheaper and faster than MRI recording, the possible confirmation of the relation between SBC sources location and lesions could have relevance in the clinical practice. Specifically, EEG could support the follow-up of the patient, e.g., with daily monitoring of the effect of the treatment on the possible reduction of the lesions. This method, if confirmed in an extended study, could support the clinician in rapid diagnosis, allowing the fast implementation of specific therapy to improve the prognosis and simple monitoring of the progress of the patient.

DECLARATIONS

Acknowledgments

The authors are grateful to Prof. B. Vitiello and Prof. G. Capizzi who provided access to the clinical data discussed in this work.

Authors' contributions

Data selection and interpretation of results: Valerio M
Localization of SBC sources: Rivera S
Identification of SBCs and supervision of the other activities: Mesin L

Availability of data and materials

Data will not be shared.

Financial support and sponsorship

None.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

A consent for the examination and data processing was obtained from the parents of the patients.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2021.

REFERENCES

1. Tunkel AR, Glaser CA, Bloch KC, Sejvar JJ, Marra CM, et al. The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 2008;47:303-27.
2. Piquet AL, Cho TA, The Clinical Approach to Encephalitis. Curr Neurol Neurosci Rep 2016;16:45.
3. Kneen R, Michael BD, Menson E, Mehta B, Easton A, et al. Management of suspected viral encephalitis in children - Association of British Neurologists and British Paediatric Allergy, Immunology and Infection Group national guidelines. J Infect 2012;64:449-77.
4. Barbadoro P, Marigliano A, Ricciardi A, D'Errico MM, Prospero E. Trend of hospital utilization for encephalitis. Epidemiol Infect 2012;140:753-64.
5. Boucher A, Herrmann JL, Morand P, Buzelé R, Crabol Y et al. Epidemiology of infectious encephalitis causes in 2016. Med Mal Infect 2017;47:221-35.
6. Vora NM, Holman RC, Mehal JM, Steiner CA, Blanton J, et al. Burden of encephalitis-associated hospitalizations in the United States, 1998-2010. Neurology 2014;82:443-51
7. Glaser CA, Honarmand S, Anderson LJ, Schnurr DP, Forghani B, et al. Beyond viruses: clinical profiles and etiologies associated with encephalitis. Clin Infect Dis 2006;43:1565-77.
8. Thompson C, Kneen R, Riordan A, Kelly D, Pollard AJ, et al. Encephalitis in children. Arch Dis Child 2012;97:150-61.
9. Zuo XZ, Tang WJ, Chen XY, Huang W. A review with comments on herpes simplex encephalitis in adults. Neuroimmunol Neuroinflammation 2017;4:24-7.
10. Emmady PD, Murr N. EEG, Triphasic Waves. StatPearls Publishing; 2020.
11. Lin L, Drislane F. Lateralized Periodic Discharges. J Clin Neurophysiol 2018;35:189-98.
12. Andraus MEC, Andraus CF, Alves-Leon SV. Periodic EEG patterns: importance of their recognition and clinical significance. Arq Neuropsiquiatr 2012;70:145-51.
13. Beaumanoir A, Magistris M, Nahory A. Sporadic slow biphasic complex: description and clinical correlations. Electroencephalogr Clin Neurophysiol 1985;61:142.
14. Mesin L, Valerio M, Beaumanoir A, Capizzi G. Automatic identification of slow biphasic complexes in EEG: an effective tool to detect encephalitis. Biomed Phys Eng Express 2019;5:045006.

15. Beaumanoir A, Grioni D, Kullmann G, Tiberti A, Valseriati D. EEG anomalies in the prodromic phase of Rasmussen's syndrome. Report of two cases. Neurophysiol Clin 1997;27:25-32.
16. Maciel CB, Hirsch LJ. Definition and classification of periodic and rhythmic patterns. J Clin Neurophysiol 2018;35:179-88.
17. Gatti A, Guarneri M, Motto CA, Vigliano P, Beaumanoir A. Slow biphasic complex: electro-clinical considerations. Ital J Neurol Sci 1997;18:99.
18. Mesin L, Valerio M, Capizzi G. Automated diagnosis of encephalitis in pediatric patients using EEG rhythms and slow biphasic complexes. Phys Eng Sci Med 2020;[published online ahead of print, 2020 Jul 21].
19. Grech R, Cassar T, Muscat J, Camilleri K, Fabri SG, et al. Review on solving the inverse problem in EEG source analysis. J Neuroeng Rehabil 2008;5:25.
20. Vatta F, Bruno P, Inchingolo P. Accuracy of EEG source reconstruction in the presence of brain lesions: modelling errors and surface electrodes' placement. Biomed Sci Instrum 2002;38:423-8
21. Jatoi MA, Kamel N, Saeed A, Malik AS, Faye I, et al. A survey of methods used for source localization using EEG signals. Biomed Sig Proc Control 2014;11: 42-52.
22. Dale AM, Sereno MI. Improved localizadon of cortical activity by combining EEG and MEG with MRI cortical surface reconstruction: a linear approach. J Cogn Neurosci 1993;5:162-76.
23. Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput Intell Neurosci 2011;2011:156869
24. Appavu B, Riviello JJ. Electroencephalographic patterns in neurocritical care: pathologic contributors or epiphenomena? Neurocrit Care 2018;29:9-19.
25. Yildirim M, Konuskan B, Yalnizoglu D, Topaloglu H, Erol I, et al. Electroencephalographic findings in anti-N-methyl-d-aspartate receptor encephalitis in children: a series of 12 patients. Epilepsy Behav 2018;78:118-23.
26. Sutter R, Kaplan PW, Valença M, De Marchis GM. EEG for diagnosis and prognosis of acute nonhypoxic encephalopathy: history and current evidence. J Clin Neurophysiol 2015;32:456-64.
27. Mohammad SS, Soe S, Pillai SC, Nosadini M, Barnes EH, et al. Etiological associations and outcome predictors of acute electroencephalography in childhood encephalitis. Clin Neurophysiol 2016;127:3217-24.
28. Tanoue K, Oguni H, Nakayama N, Sasaki K, Ito Y, et al. Focal epileptic spasms, involving one leg, manifesting during the clinical course of west syndrome (WS). Brain Dev 2008;30:155-9.
29. Chen L, Zhu M, Zhou H, Liang J. Clinical study of West syndrome with PS and late-onset epileptic spasms. Epilepsy Res 2010;89:82-8.
30. Olivieri G, Contaldo I, Ferrantini G, Musto E, Scalise R, et al. Autoimmune encephalopathies in children: diagnostic clues and therapeutic challenges. Neuroimmunol Neuroinflammation 2016;7:147
31. Vitali P, Di Perri C, Vaudano AE, Meletti S, Villani F. Integration of multimodal neuroimaging methods: a rationale for clinical applications of simultaneous EEG-fMRI. Funct Neurol 2015;30:9-20.

Fragoso. *Neuroimmunol Neuroinflammation* 2021;8:50-2
DOI: 10.20517/2347-8659.2020.42

Neuroimmunology and Neuroinflammation

Letter to Editor

Open Access

Check for updates

Headache as the onset and main symptom of COVID-19 infection

Yara D. Fragoso

Department of Post-Graduate Studies, Universidade Metropolitana de Santos, Santos, SP 11045-002, Brazil.

Correspondence to: Prof. Yara D. Fragoso, Department of Post-Graduate Studies, Universidade Metropolitana de Santos, Avenida Conselheiro Nebias 536, Santos, SP 11045-002, Brazil. E-mail: yara@bsnet.com.br

How to cite this article: Fragoso YD. Headache as the onset and main symptom of COVID-19 infection. *Neuroimmunol Neuroinflammation* 2021;8:50-2. http://dx.doi.org/10.20517/2347-8659.2020.42

Received: 28 May 2020 **Accepted:** 29 May 2020 **Fisrt online:** 4 Sep 2020 **Published:** 21 Mar 2021

Science Editor: Athanassios P. Kyritsis **Copy Editor:** Cai-Hong Wang **Production Editor:** Tian Zhang

Abstract

This is a case report of a COVID-19 infection in a young male, previously healthy. The infection evolved with intense headache as the main symptom for 2 weeks. The headache was throbbing, severe, continuous, and worsened with efforts such as coughing. The patient presented no meningeal signs and neurological examination was normal. This is the first report of severe headache at the onset and main symptom of COVID-19 infection.

Keywords: Coronavirus, headache, pain, COVID-19

Dear Editor,

The year 2020 started with a pandemic coronavirus infection that is expected to change human behavior for years to come. The initial cases started in late 2019, and this is the second coronavirus to lead to severe acute respiratory syndrome (SARS); thus, it has been named COVID-19 or SARS-CoV-2[1]. The clinical manifestations of COVID-19 vary from none to mild, moderate, severe, and rapidly progressive and fulminant disease. Its main symptoms include fever (up to 98% of cases), dry cough (68%-76%), dyspnea (circa 55%), and myalgia or fatigue (35%-44%)[2]. Anosmia, ageusia, and diarrhea are now recognized as symptoms of the disease. Headache has been reported as affecting 8%-13% of patients[2,3]. However, headache has not been described as the most disabling symptom of COVID-19 yet. We would like to report a case recently seen in our Neurology Unit.

A healthy and athletic Brazilian male aged 29 years, who did not suffer from headaches, gave consent for his case to be reported. In mid-March 2020, the patient woke up with bitemporal throbbing headache of severe intensity. He could not tolerate light and noise and presented low fever (37.8 °C). This headache persisted

© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

for 3 days, as did the increased temperature. No analgesic or anti-inflammatory drug affected the intensity of the headache. On the fourth day, his temperature returned to normal and a persistent dry cough started. The intensity of headache during the episodes of cough increased. The patient slept for many hours a day and remained in a dark, silent bedroom. PCR was positive for COVID-19. His parents tested PCR-positive for COVID-19 a week later. His father, who is a medical doctor, had moderate symptoms of the viral infection while the mother remained asymptomatic. Two weeks after the patient's initial symptoms, he recovered fully and had no more headaches. At no time did he have abnormalities in his neurological examination, including meningeal signs. He has been followed for 6 weeks now and has returned to his usual healthy condition.

To the best of our knowledge, this is the first report of severe headache as the onset and main symptom of COVID-19 infection. It can be classified as "9.2.2.1-Acute headache attributed to systemic viral infection"[4]. Physicians at the front line of COVID-19 management should be aware of severe headache as a possible main symptom of this infection. This patient had a migraine-like headache, but he had high temperature and no previous history of migraine. Although neurological examination was normal, we must consider the possibility of meningeal involvement in the inflammatory process. The intolerance to light and sound, the worsening with coughing, and the throbbing characteristic of the headache which was irresponsive to treatment suggest meningeal vascular involvement. Although a case of meningitis and encephalitis by COVID-19 has been recently published, the clinical presentation of that patient was a lot more serious and life-threatening[5]. In our case, we emphasize that patients seen at the Emergency Department complaining of severe headache might be dismissed without a COVID-19 hypothesis.

DECLARATIONS

Authors' contributions

The author contributed solely to the article.

Availability of data and materials

Not applicable.

Financial support and sponsorship

None.

Conflicts of interest

The author declared that there are no conflicts of interest.

Ethical approval and consent to participate

This report was approved by the Ethics Committee at Universidade Metropolitana de Santos, under the number CAAE 56332016.4.0000.5509.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2021.

REFERENCES

1. Lake MA. What we know so far: COVID-19 current clinical knowledge and research. Clin Med (Lond) 2020;20:124-7.
2. Han Q, Lin Q, Jin S, You L. Coronavirus 2019-nCoV: a brief perspective from the front line. J Infect 2020;80:373-7.
3. Mao L, Jin H, Wang M, Hu Y, Chen S, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan,

China. JAMA Neurol 2020;77:1-9.
4. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition. Cephalalgia 2018;38:1-211.
5. Moriguchi T, Harii N, Goto J, Harada D, Sugawara H, et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. Int J Infect Dis 2020;94:55-8.

Al-Sammarraie *et al*. *Neuroimmunol Neuroinflammation* 2021;8:53-63
DOI: 10.20517/2347-8659.2020.34

Neuroimmunology and Neuroinflammation

Editorial

Open Access

Bone morphogenic protein signaling in spinal cord injury

Nadia Al-Sammarraie, Swapan K. Ray

Department of Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, Columbia, SC 29209, USA.

Correspondence to: Prof. Swapan K. Ray, Department of Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, 6439 Garners Ferry Road, Columbia, SC 29209, USA. E-mail: swapan.ray@uscmed.sc.edu

How to cite this article: Al-Sammarraie N, Ray SK. Bone morphogenic protein signaling in spinal cord injury. *Neuroimmunol Neuroinflammation* 2021;8:53-63. http://dx.doi.org/10.20517/2347-8659.2020.34

Received: 24 Apr 2020 **Accepted**: 30 Apr 2020 **Fisrt online:** 4 Sep 2020 **Published:** 21 Mar 2021

Science Editor: Gang Zhao **Copy Editor**: Jing-Wen Zhang **Production Editor**: Tian Zhang

Abstract

Spinal cord injury (SCI) is a debilitating injury that results from traumatic or non-traumatic insults to the spinal cord, causing significant impairment of the patient's activity and quality of life. Bone morphogenic proteins (BMPs) are a group of polyfunctional cytokines belonging to the transforming growth factor beta superfamily that regulates a wide variety of cellular functions in healthy and disease states. Recent studies suggest that dysregulation of BMP signaling is involved in neuronal demyelination and death after traumatic SCI. The focus of this article is to describe our current understanding of the role of BMP signaling in the regulation of cell fate, proliferation, apoptosis, autophagy, and inflammation in traumatic SCI. First, we will describe the expression of BMPs and pattern of BMP signaling before and after traumatic SCI in rodent models and *in vitro*. Next, we will discuss the role of BMP in the regulation of neuronal and glial cell differentiation, survival, functional recovery from traumatic SCI, and the gap in knowledge in this area that requires further investigation to improve SCI prognosis.

Keywords: Spinal cord injury, bone morphogenic protein, apoptosis, proliferation, autophagy, differentiation, inflammation

INTRODUCTION

Spinal cord injury (SCI) can be either traumatic or non-traumatic damage to the spinal cord and has a peak prevalence of approximately 906-1800 cases per million people in the United States[1-3]. SCI usually causes complete or partial motor and sensory neurological deficits with deleterious outcomes[4]. Traumatic SCI can be caused by major trauma to the spinal cord following road traffic accidents, falls in the elderly, and violent and sport-related injuries[1,3]. Non-traumatic SCI usually result from ischemic-reperfusion

© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

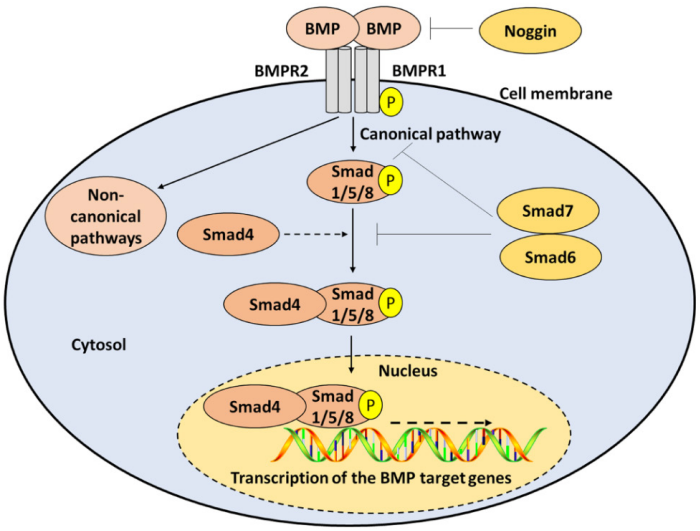


Figure 1. Molecular components and pathways of BMP signaling. The BMP signaling is initiated by the binding of BMP ligands to BMPR1 and BMPR2. In the canonical pathway, BMP receptors phosphorylate Smad1/5/8, which can bind to Co-Smad4 and are translocated to the nucleus to regulate the expression of target genes. In the non-canonical pathways, BMP receptors activate non-Smad pathways. Termination of BMP signaling is achieved by noggin, Smad6, and/or Smad7. BMP: bone morphogenic protein; BMPR: BMP receptor

injury, congenital malformations, degenerative diseases, malignancy, autoimmune diseases, or infections in the spinal cord[2,5,6]. Traumatic SCI is a devastating neurological condition characterized by both acute and chronic phases of progressive spinal cord damage that involve neuroinflammation, oligodendrocytes loss, neuronal loss, demyelination, and reactive astrogliosis with scar formation[7,8]. The acute phase is characterized by oligodendrocyte death and demyelination, reactive astrocyte proliferation, axonal swelling, and acute inflammatory cell infiltration[9,10]. The chronic phase is characterized by chronic infiltration of inflammatory cells, partial neuronal regrowth and remyelination, and glial scar establishment[10,11]. The upregulation of detrimental factors and pathways cause progressive pathogenesis leading to the activation of cysteine proteases (calpains and caspases) for neuronal and glial cell death, and declining neurological function (motor and sensory) in both acute and chronic traumatic SCI[12,13]. Current research is mostly focused on traumatic SCI for understanding of its pathogenesis and developing effective new therapeutic strategies. The main goals in developing new therapies for traumatic SCI are to minimize neural cell loss and prevent glial scar formation to promote remyelination and functional recovery[8,14].

Bone morphogenetic proteins (BMPs) are a group of approximately 15 growth regulating polyfunctional cytokines that belong to the transforming growth factor beta (TGFβ) superfamily and are widely expressed in both the intact and injured spinal cord[15,16]. Signal activation and transduction include the binding of BMP cytokines to BMP receptor 1 (BMPR1A, BMPR1B, or ActR-1A) and BMP receptor 2 complex, followed by phosphorylation and activation of Smad1/5/8 intracellular receptor regulated proteins or R-Smads[15,17,18]. Smad1/5/8 proteins then bind to the common Smad4 or the Co-Smad4 to form a complex, which is translocated to the nucleus to regulate transcription of BMP-targeted genes in a context dependent manner[15,17,18]. Inhibition of signaling is usually achieved via the activation and competitive binding of Smad6, Smad7, and noggin inhibitory proteins. Smad6 and Smad7 inhibit the interaction between BMP receptors and R-Smads and/or the interaction between the R-Smads and the common Smad4[19-21]. Noggin inhibitory proteins bind with high affinity to BMP ligand proteins and prevent their association with their receptors[22] [Figure 1].

Growing evidence from rodent models of SCI[16,23] show that BMP ligands and receptors are expressed in the intact spinal cord and are drastically upregulated post-injury. This is summarized in Table 1.

Table 1. Expression of BMP signaling components before and after SCI in rodent models

BMP signaling component	SCI model	Outcomes	Ref.
BMP ligands	Rats	BMP7 mRNA was mildly expressed in glial cells in intact spinal cord but markedly expressed in glial cells and motoneurons post-SCI	[27]
	Rats	BMP2/4 mRNA was mildly expressed in intact spinal cord but markedly expressed in oligodendrocytes, astrocytes, and microglia surrounding the damaged site post-SCI	[23]
	Mice	BMP2, 4, and 7 levels were increased in neurons, microglia, oligodendrocytes, and NSCs post-SCI, which enhanced astrocyte proliferation. BMP4 promoted differentiation of astrocytes and inhibited differentiation of neurons and oligodendrocytes	[37]
	Rats	BMP2 and 4 levels were increased post-SCI and promoted differentiation of the engrafted OPCs cells into astrocytes	[40]
	Mice	BMP7 expression was increased after SCI and further augmented after agmatine treatment, leading to reduced collagen scar formation and improved BBB score post-SCI	[30]
	Rats	BMP4 expression was increased in astrocytes cultured from injured thoracic spinal cord	[36]
	Rats	BMP2/4 expression was increased after SCI and associated with low BBB scores	[29]
	Rats	BMP7 was expressed in glial cells of the intact spinal cord and increased in glial cells and motoneurons after SCI	[27]
	Mice	BMP2 was slightly expressed in intact spinal cord and markedly increased post-SCI	[38]
	Mice	BMP4 level was increased in neurons of gray and white matter and ependyma cells near the damaged site post-SCI	[28]
	Rats	BMP4 was overexpressed after acute SCI	[57]
	Rats	BMP2, 3, 4, 5, 7, 9, 12, and 13 were expressed in intact spinal cord	[16]
BMP receptors	Rats	BMPR1A and BMPR2 expression levels were increased in neurons post-SCI	[23]
BMP antagonists	Rats	Noggin was minimally expressed in intact spinal cord	[16]
Canonical pathway	Mice	p-Smad1, 5, and 8 were activated in neurons, oligodendrocytes, OPCs, astrocytes, and NSCs post-SCI	[37]

BMP: bone morphogenic protein; SCI: spinal cord injury; OPCs: oligodendrocyte precursor cells; NSCs: neural stem cells; BBB: Basso, Beattie, and Bresnahan; BMPR: BMP receptor

Table 2. Effects of BMP treatment on neuronal and non-neuronal cells in SCI in *in vitro* models

BMP signaling component	Treatment	Outcomes	Ref.
BMP ligands	BMP7	BMP7 inhibited tumor necrosis factor α-mediated oligodendrocyte death	[56]
	BMP7	BMP7 inhibited glutamate induced neuronal cell death	[24]
	BMP4	*In vitro* culture of NSCs in the presence of BMP4 resulted in amelioration of oligodendrocyte differentiation and increase in astrocyte differentiation. Smad1 and 5 were activated in response to BMP4 treatment of NSCs	[25]
	BMP7	Noggin expressing OPCs treated with BMP7 showed less astrocytic differentiation	[16]
BMP antagonists	Noggin	Noggin treatment reduced astrocyte numbers. Inhibition of BMP4 using noggin attenuated differentiation of NSCs into astrocytes	[37]
	Noggin	Noggin treatment of OPCs partially reduced astrocytic differentiation	[40]
	Noggin	Noggin treatment reduced differentiation of OPCs into astrocytes in astrocyte conditioning media. p-Smad1, 5, and 8 levels were increased in OPCs in astrocyte conditioning media compared to control. OPCs cultured in astrocyte conditioning media predominantly differentiated into astrocytes	[36]
	Noggin and LDN193189	Treatment attenuated BMP4 induced activation of caspase-3 for cell death in neurons and oligodendrocytes post-SCI	[57]
	Noggin	Noggin treatment reduced astrocytic differentiation and increased the differentiation of NSCs into oligodendrocytes	[25]

BMP: bone morphogenic protein; SCI: spinal cord injury; OPCs: oligodendrocyte precursor cells; NSCs: neural stem cells

Furthermore, *in vitro* studies[24,25] extensively elucidated the protective and deregulatory role of BMP components in a variety of cellular events on both neuronal and non-neuronal cells, which are summarized in Table 2. Most of these studies focused mainly on the level of expression of BMP signaling proteins and the resultant cellular damage, describing only limited knowledge on molecular regulation and downstream targets. This article will focus mainly on the role of different BMP ligands and receptors on neuronal and glial cell differentiation, neuroinflammation, cell death, and autophagy in the *in vivo* and *in vitro* models

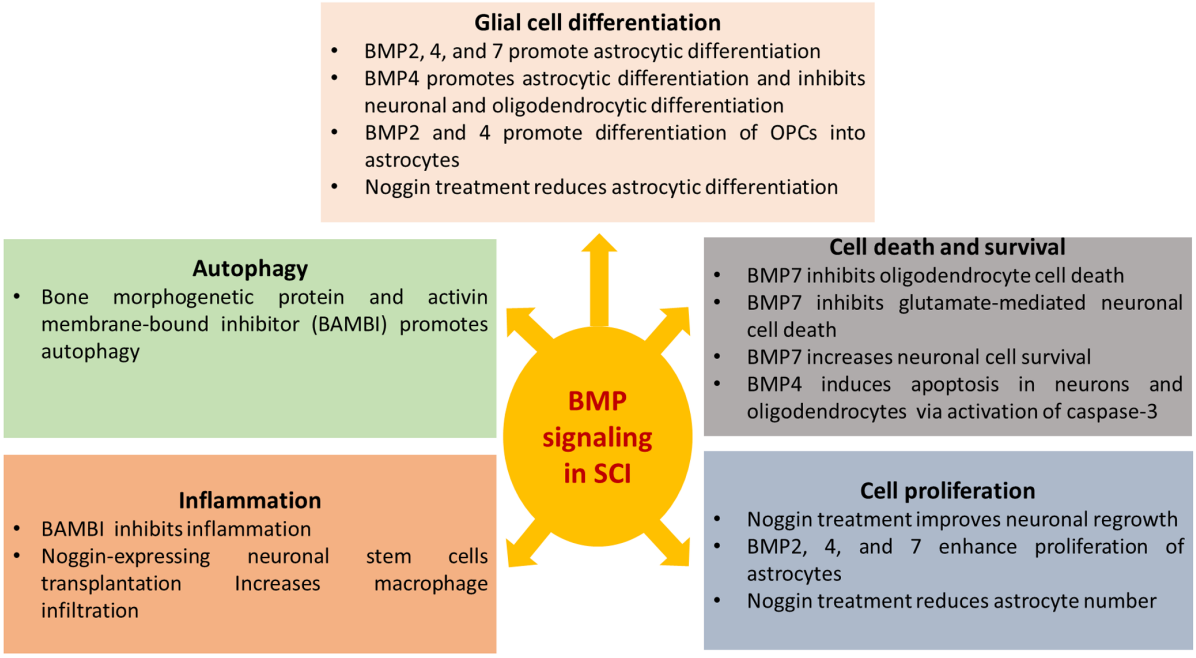


Figure 2. Cellular manifestations of BMP signaling in SCI. This diagram illustrates the *in vitro* and *in vivo* effects of activation or inhibition of BMP signaling on neuronal and/or glial cell proliferation, differentiation, survival, apoptosis, autophagy, and inflammation in SCI. BMP: bone morphogenic protein; SCI: spinal cord injury; BAMBI: BMP and activin membrane-bound inhibitor; OPCs: oligodendrocyte precursor cells

of traumatic SCI, which are summarized in Figure 2. Next, we will discuss the gap in knowledge in this area and suggest future studies for further understanding of the role of BMP signaling in pathogenesis in traumatic SCI that remains largely elusive.

EXPRESSION OF BMP LIGANDS, RECEPTORS, AND SMAD AND NON-SMAD SIGNALING IN SPINAL CORD BEFORE AND AFTER INJURY, AND THEIR ASSOCIATION WITH FUNCTIONAL RECOVERY IN SCI

Studies in rodent models of SCI have shown that BMP ligands and receptors are expressed in intact spinal cord and their expression are further increased following SCI. BMP2, 3, 4, 5, 7, 9, 12, and 13 and the BMP receptors 1A, 1B, and 2 are minimally expressed in uninjured spinal cord[16,23,26]. However, after SCI, the expression of BMP ligands and their receptors are increased considerably in neuronal and glial cells in spinal cord and exert diverse cellular effects[23,27]. The increase in BMP2, 4, and 7 expression levels are amongst the most studied BMP ligands after SCI. Expression levels of the ligands and the downstream canonical pathway and non-canonical pathways were markedly increased in response to SCI. Chen *et al.*[28] studied the expression of BMP4 and other signaling molecules critical for neuronal development in SCI in mice. This study found that BMP4 was upregulated after SCI in the neurons of the gray and white matter and ependymal cells (a type of glial cells known to produce cerebrospinal fluid and act as reservoir of neurodegeneration) surrounding the SCI lesion[28]. Setoguchi *et al.*[27] studied the expression of BMP7 before and after acute SCI in rat model[27]. This study found that BMP7 was expressed in glial cells at low levels before injury but its expression was markedly increased in glial cells and expression occurred in motoneurons after SCI[27] [Table 1].

Cui *et al.*[29] conducted a study to examine the changes in expression of BMP2 and 4 in a rat model of SCI. This study found increases in expression of both BMP2 and 4 after SCI, which correlated with low

Table 3. Therapeutic and genetic targeting of BMP signaling in SCI in *in vivo* models

Treatment	SCI model	Effects	Ref.
BMP7	Rats	BMP7 promoted neuroprotection via an increase in the number of surviving neurons, in part, via increased p38 non-canonical signaling	[24]
Agmatine	Mice	It augmented BMP7 expression, reduced collagen scar formation, and improved BBB scores	[30]
Agmatine	Mice	It reduced neuronal cell death and scar formation, leading to improved locomotive function. This effect was achieved, in part, via increased expression of BMP2/7 in neurons and oligodendrocytes, and decreased expression of BMP4 in the damaged site	[31]
Conditional deletion of astrocytic BMPR1A and 1B	Mice	Knockouts of astrocytic BMPR1A cause reduction in astrocytic hypertrophy, decrease in axonal density, and enhancement of the inflammatory response. In contrast, knockouts of astrocytic BMPR1B increase astrocytic hypertrophy and reduce lesion size and glial scar formation post-SCI	[26]
Transplantation of OPCs expressing BMPR1A, 1B, and 2	Rats	Transplantation of OPCs expressing (BMPR1A, 1B, and 2) into rat spinal cord led to their differentiation into astrocytes	[40]
Administration of AAV vector encoding BMP4	Mice	Intra-thecal administration of AAV vector encoding BMP4 led to Smad1 activation in dorsal motoneuron and increased axonal regrowth after SCI	[42]
Conditional knockout of β1-integrin in ependymal stem cells	Mice	Conditional knockout of β1-integrin in ependymal stem cells increased the movement of BMPR1B into lipid rafts while enhancing BMP signaling (canonical and non-canonical) and glial scar formation	[39]
Noggin	Rats	Administration of recombinant mouse noggin intra-thecally improved locomotive function post-SCI and enhanced axonal regrowth	[23]
Noggin	Rats	Noggin treatment reduced BMP2/4 expression and improved motor scored post-SCI	[29]
Transplantation of Smad6, Smad7, or noggin expressing NPCs	Mice	It promoted differentiation of NPCs into oligodendrocytes and neurons but inhibited their differentiation into astrocytes, leading to improvement of BBB scores in mice post-SCI	[38]
Transplantation of noggin expressing neuronal stem cells	Rats	It led to macrophage infiltration and widening of lesion size, but prevented astrocytic differentiation post-SCI	[16]
BAMBI	Rats	Overexpression of BAMBI inhibited inflammation and promoted autophagy post-SCI	[54]
BMP2	Rats	Intra-thecal administration of rhBMP2 resulted in increases in expression of p-Smad1, 5, and 8 in most spinal cord cell types	[32]

BMP: bone morphogenic protein; SCI: spinal cord injury; OPCs: oligodendrocyte precursor cells; NSCs: neural stem cells; BBB: Basso, Beattie, and Bresnahan; BMPR: BMP receptor; BAMBI: BMP and activin membrane-bound inhibitor; AAV: adeno-associated virus

Basso, Beattie, and Bresnahan (BBB) motor assessment scores when compared to controls[29]. Furthermore, they found that inhibition of BMP signaling using noggin treatment was able to improve BBB scores when compared to the untreated group after hemisection SCI[29]. Matsuura *et al.*[23] studied the changes in expression of levels of BMP2 and 4 and the BMP receptor 2 in rats after SCI, and the effect of noggin treatment on recovery from SCI. They found that BMP2 and 4 and the BMP receptor 2 were slightly expressed in intact spinal cord and expression levels were further increased after SCI. Moreover, noggin treatment was able to improve locomotive function after SCI when compared to the non-treated SCI group[23]. Besides, several treatments with endogenous BMP components or recombinant BMP protein resulted in neuroprotective effects and improved locomotive function by modulating BMP signaling[30-32]. Kim *et al.*[30] compared the effects of agmatine, an endogenous protein with neuroprotective effects, on scar formation and functional recovery after SCI in mice. This study found that agmatine reduced scar size and improved BBB scores, in part, by increasing expression of BMP7[30]. Park *et al.*[31] showed that intraperitoneal agmatine treatment in a mice model of SCI was associated with increased expression of BMP2 and 7 in neurons and oligodendrocytes while expression of BMP4 in astrocytes and oligodendrocytes surrounding the damage site was reduced. The treatment resulted in improvement of locomotive function, inhibited neuronal death, and reduced scar size[31]. Similarly, Dmitriev *et al.*[32] studied the effect of intra-thecal administration of rhBMP2 on expression of p-Smad1, 5, and 8 within the cells of the spinal cord after SCI in rats. The study found significant activation of p-Smad1, 5, and 8 in all neuronal cells, glial cells, and fibroblasts, which might affect recovery from SCI following rhBMP2 treatment [Table 3].

ROLE OF BMP SIGNALING IN DIFFERENTIATION OF GLIAL CELLS AFTER SCI

Astrocytes, oligodendrocytes, ependymal cells, and microglia are non-neuronal heterogenous cell types that maintain spinal cord integrity, homeostasis, and myelination[33]. Marked increase in astrocyte differentiation

was observed in response to SCI, which contributed to glial scar formation in SCI tissue[34]. On one hand, glial scar provides protective mechanisms to limit the lesion size after SCI; on the other hand, it leads to deleterious effects by the inhibition of axonal regeneration[34,35]. Recent studies suggest that BMP signaling promotes differentiation of neuronal stem cells (NSCs) and oligodendrocyte precursor cells (OPCs) into astrocytes predominantly[36-38]. Wang *et al.*[36] studied the effect of the microenvironment created by reactive astrocytes on the differentiation of OPCs after SCI in rats. They found that SCI increased the expression of BMP4 in astrocytes isolated from the site of injury, and it further released BMP4 in their conditioning media. They also found that *in vitro* culture of OPCs in astrocytes-derived conditioning media activated Smad1, 5, and 8, which led to differentiation of a significant number of OPCs into astrocytes, while inhibiting differentiation of oligodendrocytes[36].

In contrast, noggin treatment reduced astrocytic differentiation and increased oligodendrocytic differentiation[36]. Similarly, Xiao *et al.*[37] conducted a study to test the effect of BMP signaling on the differentiation of NSCs after SCI in mice. This study found that BMP2, 4, and 7 were expressed in intact spinal cord and their expression was further increased after SCI in the following cell types: neurons, NSCs, microglia, and oligodendrocytes, but not in astrocytes[37]. They also found that the expression of phosphorylated Smad1, 5, and 8 were increased after SCI in the above cell types, OPCs, and astrocytes[37]. Furthermore, they found that BMP4 was highly expressed in neutrospheres (free-floating clusters of neural stem cells) cultured from the spinal cord and it promoted astrocytic differentiation from NSCs, while inhibition of BMP signaling using noggin treatment reduced astrocytic differentiation[37]. Setoguchi *et al.*[38] examined the effect of BMPs on the differentiation of transplanted NPCs *in vitro* and after SCI in mice. This study found that BMP2 was expressed in the spinal cord before injury and was upregulated drastically after[38]. They also found that BMP2 and 7 promoted the differentiation of NPCs to astrocytes *in vitro*, while the inhibition of BMP signaling using Smad6, Smad7, or noggin overexpressing NPCs resulted in the differentiation of NPCs into neuronal cells and inhibited the differentiation of NPCs into astrocytes[38]. Similarly, transplanting the above-modified NPCs into a mice model of SCI resulted in improvement of the motor scores with inhibition of astrocytic differentiation and promotion of neuronal and oligodendrocytic differentiations *in vivo*[38]. Together, these studies imply that targeting BMP signaling could be beneficial for ameliorating astrocytic scar formation, and for enhancing oligodendrocytic differentiation for remyelination after SCI.

In addition, North *et al.*[39] showed that the conditional deletion of β1 integrin from ependymal stem cells resulted in an increase in their differentiation into astrocytes, which could promote glial scar formation after SCI and reduce BBB motor scores in SCI mice, which were found to be associated with increases in canonical (Smad1/5/8) and non-canonical (p38) signaling. Furthermore, Song *et al.*[25] found that BMP4 treatment of NSCs in culture promoted astrocytic differentiation via activation of Smad1/5/8, while noggin treatment resulted in reduction of astrocytic differentiation and an increase in oligodendrocytic differentiation *in vitro*. In contrast, Enzmann *et al.*[16] showed that the intra-thecal transplantation of noggin overexpressing NSCs or progenitor cells was unable to restrict astrocytic differentiation in rats after SCI, suggesting additional regulatory mechanisms were controlling astrocytic differentiation.

Studies also showed that the expression of BMP receptors was increased after SCI, particularly affecting astrocytic hypertrophy (an astrocyte grown bigger than its normal size to adapt to changes) and differentiation. Astrocytes play both physiological and pathological roles after traumatic SCI, which triggers an initial astroctytic hypertrophy and subsequently, an astrocytic hyperplasia. In astrocytic hypertrophy, astrocytes are reactive with bigger bodies, thicker processes, and overexpression of their intermediate filament proteins such as glial fibrillary acidic protein and vimentin to help repair the blood-brain barrier and reduce the spread of inflammatory cells at the site of SCI. On the other hand, in astrocytic hyperplasia, astrocytes increase their numbers around the injury site and produce much finer processes to contribute to

the development of the glial scar that becomes an impediment to axonal regeneration after SCI. Conditional deletion of astrocytic BMPR1A in mice has adverse effects on recovery after SCI via impairing astrocytic hypertrophy, reducing axonal density, and fostering inflammation[26]. In contrast, conditional deletion of astrocytic BMPR1B has more beneficial effects by increasing the number of hypertrophied astrocytes, attenuation of the glial scar, and diminishing lesion size in mice after SCI[26]. In addition, expression of BMPR1A, 1B, and 2 in OPCs that were transplanted into rat spinal cord predominantly promoted their differentiation into astrocytes[40]. Similarly, culture of OPCs in the presence or absence of BMP2/4 and noggin showed that BMP treatment increased their differentiation into astrocytes while noggin treatment enhanced it[40].

ROLE OF BMP SIGNALING IN AXONAL GROWTH AND GLIAL CELL PROLIFERATIONS AFTER SCI

Reactive astrogliosis (also known simply as astrogliosis or astrocytosis) and neuronal regrowth occur in response to the loss of glial cells and neurons after SCI to partially promote healing of tissue damage and attempt neuronal recovery[10,41]. Recent studies suggest that BMP signaling causes astrocytic proliferation and neuronal growth after SCI[23,37,42]. Parikh *et al.*[42] studied whether Smad1 activation could have a beneficial effect on axonal regeneration in mice after SCI. BMP4 overexpression in dorsal motoneurons was achieved by the intra-thecal administration of viral vectors overexpressing BMP4 in mice after SCI[42]. The results show activation of Smad1 in dorsal neurons, which is associated with improving axonal growth after SCI[42]. The administration of recombinant mouse noggin intra-thecally improved locomotive function and increased axonal regrowth after SCI[23]. Xiao *et al.*[37] found that the levels of BMP2, 4, and 7 expression were all increased in neurons, microglia, oligodendrocytes, and NSCs after SCI, which enhanced astrocytic proliferation, while noggin treatment diminished astrocyte numbers.

ROLE OF BMP SIGNALING IN AUTOPHAGY AFTER SCI

Autophagy or "self-eating" is a central molecular mechanism that regulates tissue homeostasis in health and disease[43]. Autophagy is characterized by direct or indirect lysosomal degradation of damaged mitochondria, misfolded proteins, and other cellular debris for recycling to maintain energy metabolism in response to stressful stimuli[43]. Macroautophagy is the major type of autophagy, which includes sequential events of autophagosome formation, autophagosome-lysosome fusion, and autolysosomal degradation of cargos[44]. Autophagy flux is defined as the total dynamics of autophagy and thereby it is the progression of cargo sequestration into autophagosomes, delivery to lysosomes, and degradation by lysosomal enzymes[45]. Autophagy flux is usually increased in mechanical injury such as mild traumatic SCI or metabolic stress such as starvation, but autophagy flux is decreased due to suppression of autophagy at an upstream (autophagosome formation) or downstream step (autolysosome formation)[46]. Recent studies suggest an impairment of autophagy flux after moderate to severe SCI, which leads to neuronal cell death and adversely affects oligodendrocyte-mediated neuronal myelination and functional recovery[47,48]. On the other hand, activating autophagy improves neurological recovery in rodent models of SCI due to activation of autophagosome formation and/or enhancement of autophagy flux[49-51]. Although modulation of autophagy plays a crucial role in the pathogenesis in SCI, there is limited knowledge on the role of BMP signaling in the regulation of autophagy after SCI. BMP and activin membrane-bound inhibitor (BAMBI) is a pseudo-receptor that lacks the kinase activity and inhibits the signaling of TGFβ family[52]. BAMBI has been found to be down regulated in rats after SCI, while intraspinal injection of the BAMBI expressing vector after SCI promotes autophagy and improves locomotive function in rats[52]. The BAMBI overexpression causes activation of Beclin-1 and LC3B II, two proteins critical for inducing autophagy and maintaining autophagy flux; on the other hand, it results in down regulation of autophagy inhibitor proteins such as Bim and p62[52]. The role of BMP ligands and receptors in disruption of autophagy post-SCI remains largely unknown; however, modulating BMP signaling to restore autophagy may provide a new therapeutic avenue in treating SCI.

ROLE OF BMP SIGNALING IN INFLAMMATION AFTER SCI

Inflammation is encountered in both the acute and chronic phases of SCI and results in expansion of the initial lesion, destruction of nearby tissue, neuronal loss, axonal demyelination, and fibrosis or scar formation[53]. Shortly after SCI, there is massive infiltration of neutrophils and after 24 hours, infiltration of reactive microglia/macrophages increases progressively[9]. Several weeks following SCI, there is an increase in the infiltration of CD45-positive cells and CD68-positive reactive microglia/macrophages, which mark the phase of chronic inflammation in SCI and are associated with impairment of locomotive function[11]. Recent studies suggest contradictory roles of the inhibition of BMP signaling on inflammatory responses after SCI. The transplantation of noggin-expressing NSCs in SCI rats results in a marked increase in macrophage infiltration[16]. In contrast, the overexpression of BAMBI in a rat model of SCI results in inhibition of neuroinflammation, which is characterized by reduction in the levels of expression of interleukin (IL)-1β, IL-6, IL-10, TGFβ, and mechanistic target of rapamycin[54].

ROLE OF BMP SIGNALING IN NEURONAL AND GLIAL CELL DEATH AFTER SCI

Both neurons and oligodendrocytes are highly susceptible to cellular damage and death following SCI resulting in axonal demyelination and neurological deficits[7,55]. *In vitro* and *in vivo* studies have shown that BMP7 exerts beneficial effects on neuronal and oligodendrocyte cell survival[24,56]. de Rivero Vaccari *et al.*[24] studied the protective mechanism of BMP7 on neuronal survival after SCI *in vitro* and *in vivo*. BMP7 promotes neuronal survival after SCI in rats and inhibits glutamate induced neuronal cell death *in vitro*[24]. Similarly, Wang *et al.*[56] tested the effects of BMP7 on the survival of oligodendrocytes *in vitro*. Results showed that BMP7 treatment prevented tumor necrosis factor α-induced oligodendrocyte death. These results imply that BMP7 protects neurons and oligodendrocytes from cell death in SCI models. On the other hand, a recent study conducted by Hart *et al.*[57] has shown that BMP4 induces apoptosis in both neurons and oligodendrocytes via the activation of caspase-3 (the final executioner of apoptosis) after SCI, while its inhibition using BMP signaling inhibitors attenuates the activation of caspase-3. These results suggest different roles of different BMP ligands on neuronal and glial cell survival post-SCI, which requires further investigation.

CONCLUSION AND FUTURE PROSPECTIVE

BMP ligands, receptors, and inhibitors are differentially expressed in the intact spinal cord in rodents. BMP ligands, receptors, and canonical and non-canonical pathways are upregulated after SCI. In general, augmented BMP signaling results in adverse cellular responses and impairs functional recovery in SCI animal models. On the other hand, the inhibition of BMP signaling improves neuronal cell survival, neuronal outgrowth, and functional recovery after SCI. Although BMP dysregulation is reported in SCI, the cell-type specific role of BMP signaling in SCI remains poorly understood. Several gaps in knowledge still exist regarding the molecular mechanisms underlying BMP dysregulation, the direct causal link between individual BMP ligands and receptors and progression of pathogenesis in SCI, the spatial and temporal effects of BMP signaling in the pathogenesis of acute, subacute, and chronic phases in SCI, and the mechanisms by which BMP ligands regulate autophagy, inflammation, differentiation, and apoptosis. Further *in vivo* studies using conditional knockout rodent models are needed to understand the specific requirements of different BMP ligands in SCI and neurological recovery, the ligand-receptor pairs that are involved in the regulation of SCI pathogenesis, and the downstream canonical or non-canonical pathways that impact neuronal survival after SCI.

DECLARATIONS

Authors' contributions

Conceptualized the theme and conducted the literature review process: Al-Sammarraie N

Contributed to the preparation and revision of the manuscript, interpretation of subtopics, and preparation

of figures: Al-Sammarraie N, Ray SK
Approved the final version to be published: Al-Sammarraie N, Ray SK

Availability of data and materials

Not applicable.

Financial support and sponsorship

The work was supported, in part, by an investigator-initiated research grant (SCIRF-2015-I-01) from the South Carolina Spinal Cord Injury Research Fund (Columbia, SC, USA), an award from the Soy Health Research Program (SHRP, United Soybean Board, Chesterfield, MO, USA), and earlier R01 grants (CA-091460 and NS-057811) from the National Institutes of Health (Bethesda, MD, USA).

Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2021.

REFERENCES

1. Singh A, Tetreault L, Kalsi-Ryan S, Nouri A, Fehlings MG. Global prevalence and incidence of traumatic spinal cord injury. Clin Epidemiol 2014;6:309-31.
2. Musubire AK, Meya DB, Bohjanen PR, Katabira ET, Barasukana P, et al. A systematic review of non-traumatic spinal cord injuries in sub-Saharan Africa and a proposed diagnostic algorithm for resource-limited settings. Front Neurol 2017;8:618.
3. Hagen EM, Rekand T, Gilhus NE, Grønning M. Traumatic spinal cord injuries - incidence, mechanisms and course. Tidsskr Nor Laegeforen 2012;132:831-7.
4. van Middendorp JJ, Goss B, Urquhart S, Atresh S, Williams RP, et al. Diagnosis and prognosis of traumatic spinal cord injury. Global Spine J 2011;1:1-8.
5. Simon F, Oberhuber A. Ischemia and reperfusion injury of the spinal cord: experimental strategies to examine postischemic paraplegia. Neural Regen Res 2016;11:414-5.
6. Marsala M, Sorkin LS, Yaksh TL. Transient spinal ischemia in rat: characterization of spinal cord blood flow, extracellular amino acid release, and concurrent histopathological damage. J Cereb Blood Flow Metab 1994;14:604-14.
7. Alizadeh A, Dyck SM, Karimi-Abdolrezaee S. Traumatic spinal cord injury: an overview of pathophysiology, models and acute injury mechanisms. Front Neurol 2019;10:282.
8. Hassannejad Z, Shakouri-Motlagh A, Mokhatab M, Zadegan SA, Sharif-Alhoseini M, et al. Oligodendrogliogenesis and axon remyelination after traumatic spinal cord injuries in animal studies: a systematic review. Neuroscience 2019;402:37-50.
9. Carlson SL, Parrish ME, Springer JE, Doty K, Dossett L. Acute inflammatory response in spinal cord following impact injury. Exp Neurol 1998;151:77-88.
10. Li N, Leung GK. Oligodendrocyte precursor cells in spinal cord injury: a review and update. Biomed Res Int 2015;2015:235195.
11. Arnold SA, Hagg T. Anti-inflammatory treatments during the chronic phase of spinal cord injury improve locomotor function in adult mice. J Neurotrauma 2011;28:1995-2002.
12. Ray SK, Hogan EL, Banik NL. Calpain in the pathophysiology of spinal cord injury: neuroprotection with calpain inhibitors. Brain Res Rev 2003;42:169-85.
13. Ray SK, Samantaray S, Smith JA, Matzelle DD, Das A, Banik NL. Inhibition of cysteine proteases in acute and chronic spinal cord injury. Neurotherapeutics 2011;8:180-6.
14. Plemel JR, Keough MB, Duncan GJ, Sparling JS, Yong VW, et al. Remyelination after spinal cord injury: is it a target for repair? Prog Neurobiol 2014;117:54-72.
15. Wang RN, Green J, Wang Z, Deng Y, Qiao M, et al. Bone morphogenetic protein (BMP) signaling in development and human diseases. Genes Dis 2014;1:87-105.

16. Enzmann GU, Benton RL, Woock JP, Howard RM, Tsoulfas P, et al. Consequences of noggin expression by neural stem, glial, and neuronal precursor cells engrafted into the injured spinal cord. Exp Neurol 2005;195:293-304.
17. Horbelt D, Denkis A, Knaus P. A portrait of transforming growth factor β superfamily signalling: background matters. Int J Biochem Cell Biol 2012;44:469-74.
18. Heldin CH, Moustakas A. Role of Smads in TGFβ signaling. Cell Tissue Res 2012;347:21-36.
19. Imamura T, Takase M, Nishihara A, Oeda E, Hanai J, et al. Smad6 inhibits signalling by the TGF-β superfamily. Nature 1997;389:622-6.
20. Hanyu A, Ishidou Y, Ebisawa T, Shimanuki T, Imamura T, et al. The N domain of Smad7 is essential for specific inhibition of transforming growth factor-beta signaling. J Cell Biol 2001;155:1017-27.
21. Hata A, Lagna G, Massagué J, Hemmati-Brivanlou A. Smad6 inhibits BMP/Smad1 signaling by specifically competing with the Smad4 tumor suppressor. Genes Dev 1998;12:186-97.
22. Zimmerman LB, De Jesús-Escobar JM, Harland RM. The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. Cell 1996;86:599-606.
23. Matsuura I, Taniguchi J, Hata K, Saeki N, Yamashita T. BMP inhibition enhances axonal growth and functional recovery after spinal cord injury. J Neurochem 2008;105:1471-9.
24. de Rivero Vaccari JP, Marcillo A, Nonner D, Dietrich WD, Keane RW. Neuroprotective effects of bone morphogenetic protein 7 (BMP7) treatment after spinal cord injury. Neurosci Lett 2009;465:226-9.
25. Song P, Xia X, Han T, Fang H, Wang Y, et al. BMSCs promote the differentiation of NSCs into oligodendrocytes via mediating Id2 and Olig expression through BMP/Smad signaling pathway. Biosci Rep 2018;38:BSR20180303.
26. Sahni V, Mukhopadhyay A, Tysseling V, Hebert A, Birch D, et al. BMPR1a and BMPR1b signaling exert opposing effects on gliosis after spinal cord injury. J Neurosci 2010;30:1839-55.
27. Setoguchi T, Yone K, Matsuoka E, Takenouchi H, Nakashima K, et al. Traumatic injury-induced BMP7 expression in the adult rat spinal cord. Brain Res 2001;921:219-25.
28. Chen J, Leong SY, Schachner M. Differential expression of cell fate determinants in neurons and glial cells of adult mouse spinal cord after compression injury. Eur J Neurosci 2005;22:1895-906.
29. Cui ZS, Zhao P, Jia CX, Liu HJ, Qi R, et al. Local expression and role of BMP-2/4 in injured spinal cord. Genet Mol Res 2015;14:9109-17.
30. Kim JH, Lee YW, Park YM, Park KA, Park SH, et al. Agmatine-reduced collagen scar area accompanied with surface righting reflex recovery after complete transection spinal cord injury. Spine (Phila Pa 1976) 2011;36:2130-8.
31. Park YM, Lee WT, Bokara KK, Seo SK, Park SH, et al. The multifaceted effects of agmatine on functional recovery after spinal cord injury through Modulations of BMP-2/4/7 expressions in neurons and glial cells. PLoS One 2013;8:e53911.
32. Dmitriev AE, Farhang S, Lehman RA, Ling GS, Symes AJ. Bone morphogenetic protein-2 used in spinal fusion with spinal cord injury penetrates intrathecally and elicits a functional signaling cascade. Spine J 2010;10:16-25.
33. Ahmed S, Gull A, Khuroo T, Aqil M, Sultana Y. Glial cell: a potential target for cellular and drug based therapy in various CNS diseases. Curr Pharm Des 2017;23:2389-99.
34. Okada S, Hara M, Kobayakawa K, Matsumoto Y, Nakashima Y. Astrocyte reactivity and astrogliosis after spinal cord injury. Neurosci Res 2018;126:39-43.
35. Wang H, Song G, Chuang H, Chiu C, Abdelmaksoud A, et al. Portrait of glial scar in neurological diseases. Int J Immunopathol Pharmacol 2018;31:2058738418801406.
36. Wang Y, Cheng X, He Q, Zheng Y, Kim DH, et al. Astrocytes from the contused spinal cord inhibit oligodendrocyte differentiation of adult oligodendrocyte precursor cells by increasing the expression of bone morphogenetic proteins. J Neurosci 2011;31:6053-8.
37. Xiao Q, Du Y, Wu W, Yip HK. Bone morphogenetic proteins mediate cellular response and, together with Noggin, regulate astrocyte differentiation after spinal cord injury. Exp Neurol 2010;221:353-66.
38. Setoguchi T, Nakashima K, Takizawa T, Yanagisawa M, Ochiai W, et al. Treatment of spinal cord injury by transplantation of fetal neural precursor cells engineered to express BMP inhibitor. Exp Neurol 2004;189:33-44.
39. North HA, Pan L, McGuire TL, Brooker S, Kessler JA. β1-Integrin alters ependymal stem cell BMP receptor localization and attenuates astrogliosis after spinal cord injury. J Neurosci 2015;35:3725-33.
40. Lü HZ, Wang YX, Zou J, Li Y, Fu SL, et al. Differentiation of neural precursor cell-derived oligodendrocyte progenitor cells following transplantation into normal and injured spinal cords. Differentiation 2010;80:228-40.
41. Karimi-Abdolrezaee S, Billakanti R. Reactive astrogliosis after spinal cord injury-beneficial and detrimental effects. Mol Neurobiol 2012;46:251-64.
42. Parikh P, Hao Y, Hosseinkhani M, Patil SB, Huntley GW, et al. Regeneration of axons in injured spinal cord by activation of bone morphogenetic protein/Smad1 signaling pathway in adult neurons. Proc Natl Acad Sci U S A 2011;108:E99-107.
43. Glick D, Barth S, Macleod KF. Autophagy: cellular and molecular mechanisms. J Pathol 2010;221:3-12.
44. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008;132:27-42.
45. Zhang XJ, Chen S, Huang KX, Le WD. Why should autophagic flux be assessed? Acta Pharmacol Sin 2013;34:595-9.
46. Wong YC, Holzbaur EL. Autophagosome dynamics in neurodegeneration at a glance. J Cell Sci 2015;128:1259-67.
47. Liu S, Sarkar C, Dinizo M, Faden AI, Koh EY, et al. Disrupted autophagy after spinal cord injury is associated with ER stress and neuronal cell death. Cell Death Dis 2015;6:e1582.
48. Saraswat Ohri S, Bankston AN, Mullins SA, Liu Y, Andres KR, et al. Blocking autophagy in oligodendrocytes limits functional recovery after spinal cord injury. J Neurosci 2018;38:5900-12.

49. Zhou KL, Chen DH, Jin HM, Wu K, Wang XY, et al. Effects of calcitriol on experimental spinal cord injury in rats. Spinal Cord 2016;54:510-6.
50. Zhang D, Xuan J, Zheng BB, Zhou YL, Lin Y, et al. Metformin improves functional recovery after spinal cord injury via autophagy flux stimulation. Mol Neurobiol 2017;54:3327-41.
51. Chen HC, Hsu PW, Tzaan WC, Lee AW. Effects of the combined administration of vitamins C and E on the oxidative stress status and programmed cell death pathways after experimental spinal cord injury. Spinal Cord 2014;52:24-8.
52. Tramullas M, Lantero A, Díaz A, Morchón N, Merino D, et al. BAMBI (bone morphogenetic protein and activin membrane-bound inhibitor) reveals the involvement of the transforming growth factor-beta family in pain modulation. J Neurosci 2010;30:1502-11.
53. Trivedi A, Olivas AD, Noble-Haeusslein LJ. Inflammation and spinal cord injury: infiltrating leukocytes as determinants of injury and repair processes. Clin Neurosci Res 2006;6:283-92.
54. Yang Y, Guo C, Liao B, Cao J, Liang C, He X. BAMBI inhibits inflammation through the activation of autophagy in experimental spinal cord injury. Int J Mol Med 2017;39:423-29.
55. Almad A, Sahinkaya FR, McTigue DM. Oligodendrocyte fate after spinal cord injury. Neurotherapeutics 2011;8:262-73.
56. Wang X, Xu JM, Wang YP, Yang L, Li ZJ. Protective effects of BMP-7 against tumor necrosis factor α-induced oligodendrocyte apoptosis. Int J Dev Neurosci 2016;53:10-17.
57. Hart CG, Dyck SM, Kataria H, Alizadeh A, Nagakannan P, et al. Acute upregulation of bone morphogenetic protein-4 regulates endogenous cell response and promotes cell death in spinal cord injury. Exp Neurol 2020;325:113163.

Tang *et al*. *Neuroimmunol Neuroinflammation* 2021;8:64-9
DOI: 10.20517/2347-8659.2020.28

Neuroimmunology and Neuroinflammation

Technical Note

Open Access

Resection of orthotopic murine brain glioma

Bingtao Tang[1], Kari Foss[2], Terence Lichtor[3], Heidi Phillips[2], Edward Roy[1]

[1]Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.
[2]Department of Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign, Urbana, IL 61802, USA.
[3]Carle Illinois College of Medicine, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

Correspondence to: Dr. Edward Roy, Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, 524 Burrill Hall, 407 S. Goodwin Ave, Urbana, IL 61801, USA. E-mail: eroy@illinois.edu

How to cite this article: Tang B, Foss K, Lichtor T, Phillips H, Roy E. Resection of orthotopic murine brain glioma. *Neuroimmunol Neuroinflammation* 2021;8:64-9. http://dx.doi.org/10.20517/2347-8659.2020.28

Received: 6 Apr 2020 **First Decision**: 6 Jun 2020 **Revised**: 22 Jun 2020
Accepted: 1 Jul 2020 **Fisrt online**: 1 Sep 2020 **Published**: 21 Mar 2021

Academic Editor: Terry Lichtor, Dale Ding **Copy Editor**: Cai-Hong Wang **Production Editor**: Jing Yu

Abstract

Glioma is a malignant brain tumor with a poor prognosis. Surgical resection is usually the first line of treatment. However, animal models of glioma do not include surgical resection and tumors are typically treated before they become advanced. This report demonstrates the feasibility of surgical resection of advanced gliomas in mice. The described technique establishes a murine model which could be used for the development of immunotherapy for advanced glioma after surgical resection. Use of surgical resection in murine models could increase the probability that therapies developed in mice will translate to human patients.

Keywords: Glioma, surgical resection, immunotherapy, overcoming immunosuppression, surgical stress, prolonged survival

INTRODUCTION

Glioma is a type of brain or spinal cord tumor that originates from glial cells; 80% of all malignant brain tumors are gliomas[1]. These tumors are typically asymptomatic in people until they reach an advanced stage. The standard of care for glioma includes surgery, radiotherapy and chemotherapy. Combining radiotherapy with the chemotherapy agent temozolomide may prolong survival and delay tumor progression modestly[2]. Despite the current standard of care, prognosis for glioma patients remains poor: the median survival for high-grade glioma, glioblastoma, is about 12-15 months with only 5% patients

© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

living longer than 5 years[3]. These facts indicate that there is a strong need to develop novel treatments for glioma.

Recently, our group developed a novel viro-immunotherapy for early-stage murine glioma with 83% tumor regression rate[4]. Despite the impressive response that this treatment elicits, such a response is not representative of what occurs in the clinical setting, since patients are usually diagnosed at an advanced stage of tumor progression.

Surgical resection has always been the first line of treatment for gliomas. However, surgical resection promotes an immunosuppressive tumor microenvironment[5]. It has been reported that interferon-γ secretion and cell cytotoxicity of natural killer cells are profoundly suppressed[6]; the number of cytotoxic T cells and T helper cells[7] markedly decreases, while the number of regulatory T cells (Tregs)[8,9] and low-density neutrophils[10] significantly increases in the postoperative period. All these factors could contribute to the poor prognosis and recurrence of tumor.

As a preliminary step toward testing a novel viro-immunotherapy, we asked whether mice would survive resection of an advanced murine glioma.

METHODS

Cell culture

Glioma 261 (GL261) is a murine glioma cell line. GL261 cells were purchased from the National Cancer Institute Division of Cancer Treatment and Diagnosis Repository and tested negative for mycoplasma. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; 10013CV, Corning Incorporated) containing 5 mmol/L HEPES, 1.3 mmol/L L-glutamine, 50 μmol/L 2-ME, penicillin, streptomycin and 10% fetal bovine serum (FBS) at 37 °C and 5% CO_2. GL261 neurosphere (GL261 NS) cells, which are cancer stem-like non-adherent cells, were generated by culturing in untreated cell culture flasks (08-757-501, Corning Incorporated) with DMEM/F12 + GlutaMAX (10565018, Life Technologies) culture medium containing penicillin/streptomycin (17-602E, Lonza), B27 with vitamin A (17504044, Life Technologies), 20 ng/mL recombinant human epidermal growth factor (EGF; 236-EG-200, R&D Systems), 20 ng/mL recombinant human fibroblast growth factor (FGF; 233-FB-025/CF, R&D Systems), and 5 μg/mL heparin (H3149100KU, Sigma-Aldrich) in an incubator at 37 °C with 5% CO_2[11].

Animals

C57BL/6J mice were purchased from The Jackson Laboratory and maintained as colonies in animal facilities at the University of Illinois Urbana-Champaign. Mice of both sexes were used in experiments when they were 2-3 months old. All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Illinois Urbana-Champaign.

Intracranial tumor establishment

GL261 NS cells were harvested, washed twice with Hanks' Balanced Salt Solution (HBSS; 21023CV, Corning Incorporated) and stereotactically injected into the brain of mice anesthetized with isoflurane (59399-106-01, Akorn). A total of 5×10^4 GL261 NS cells in 0.5 μL HBSS were infused into the ventral striatum (0.5 mm rostral; 2.25 mm lateral; 3.3 mm ventral). Mice were euthanized at 75% of baseline body weight or when they exhibited symptoms of neurological impairment, lethargy, or pain, in accordance with IACUC guidelines.

Intracranial tumor resection

Tumor-bearing mice were anesthetized with isoflurane (59399-106-01, Akorn) and placed in a stereotactic stage. Mice were treated with a 200-μL subcutaneous injection of carprofen (0.5 mg/mL; Division of

Figure 1. Performance of craniotomy. Four burr holes (white holes) were placed with a hand-held Jacob's chuck. A craniotomy was then performed by cutting into the skull with micro-scissors and connecting the small burr holes with the base of the flap positioned medially (dashed line)

Animal Resources, University of Illinois at Urbana-Champaign). Surgery was performed by experienced neurosurgeons (KF and TL), using a Zeiss Opmi Visu Operating Microscope with 200-mm working distance and 10-25 × magnification. Microsurgical instruments were obtained from Accurate Surgical and Scientific Instruments. A linear incision was made in a para-sagittal direction on the dorsum of the skull with a #15 scalpel blade. The skin was reflected laterally, and the peri-cranium was exposed with cotton swabs. The bregma and sagittal suture were identified, and a craniotomy procedure was performed just lateral to the sagittal suture over the location of interest (site of GL261 glioma cells). Four small burr holes (white holes shown in Figure 1) were created in the bone using a hand-held Jacob's chuck. Using micro-scissors to cut the bone, three of the four sides (white solid line shown in Figure 1) of the craniotomy were opened with the fourth side (white dashed line shown in Figure 1) remaining attached to create a bone flap. The overlying dura was opened with micro-scissors and gently peeled back from the cortical surface over the site of the tumor. Using micro-dissection under a high-power microscope, an attempt was made to remove as much of the tumor mass as possible using a combination of blunt and sharp dissection, while minimizing damage to normal neural tissue. Tumor was identified by darker coloration and gelatinous texture. No fluorescent dye was used to visualize tumor. Following tumor removal, the extirpated tumor bed was copiously irrigated, and hemostasis was ensured. The bone flap was placed gently back over the exposed brain. PBS containing penicillin (1000 U/mL) and streptomycin (1 mg/mL) (17-602E, Lonza) was used to irrigate the craniotomy. The skin was closed with cyanoacrylate glue (VetBond; 1469SB; 3M).

HE staining

After mice were euthanized, brains were snap-frozen in OCT embedding medium (23-730-571, Fisher Healthcare) for cryosectioning. Cryosections (5 μm) were fixed in cold 95% ethanol overnight and stained with hematoxylin and eosin.

Data analysis

GraphPad Prism software (La Jolla, CA) was used for all statistical analyses and graph presentation. Survival data were recorded from the time of the tumor cell implantation until euthanasia and were plotted using a Kaplan-Meier curve. Survival treatment groups were compared with a Log-rank (Mantel-Cox) test. $P < 0.05$ was considered significant.

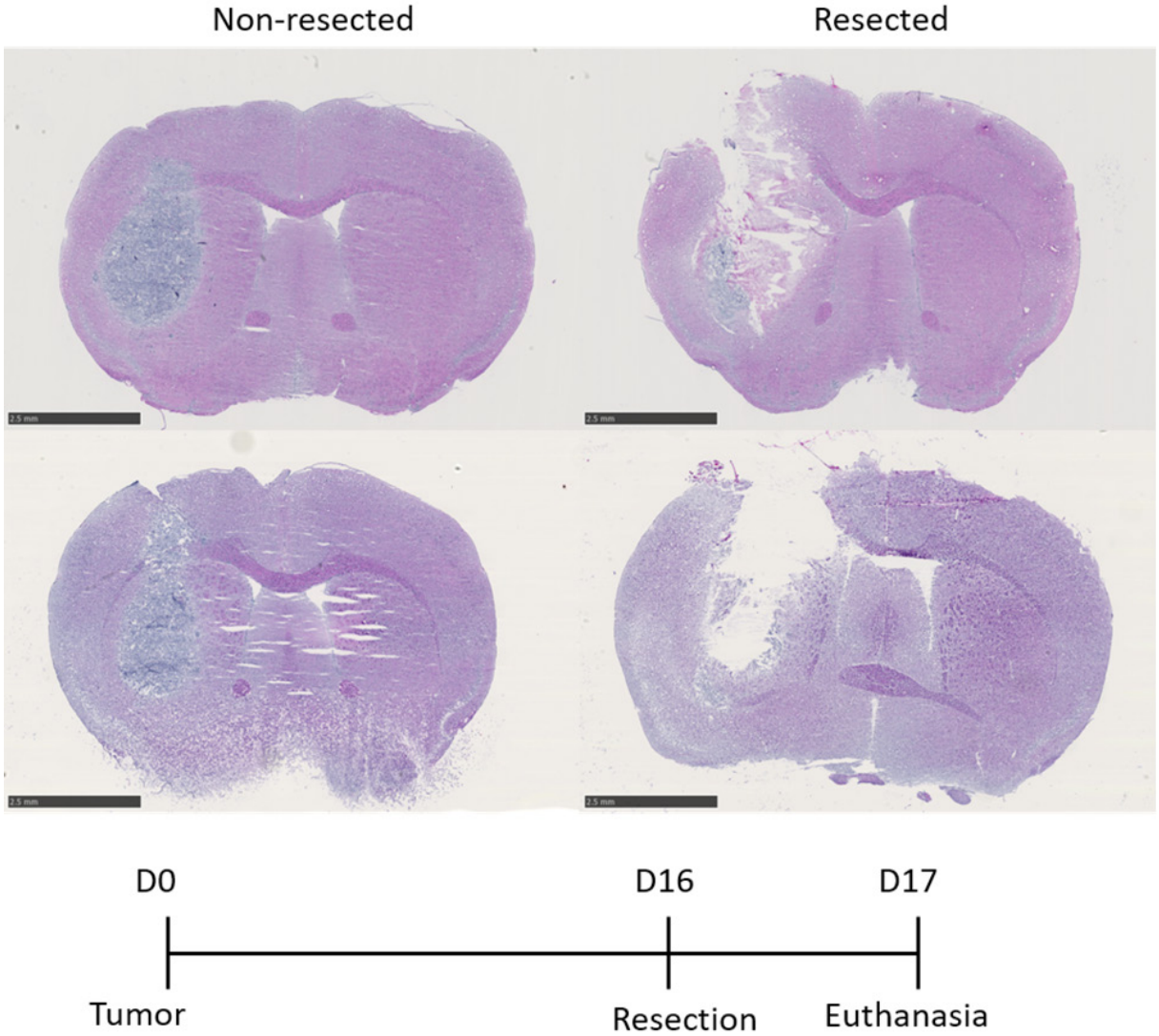


Figure 2. Efficacy of tumor resection shown by HE staining. Mice received tumor cells (i.c.) on day 0, and tumor resection occurred on day 16. Mice were euthanized on day 17, followed by overnight incubation of tissue sections in 95% ethanol and standard HE staining protocol. Representative HE stained sections are shown. Pink: normal brain tissue; dark blue: tumor. Scale bar = 2.5 mm, n = 2 for both groups

RESULTS

Resection of tumor on live mice is feasible and has the potential to prolong survival for mice bearing advanced glioma

A technique was developed to perform tumor resection on live mice bearing advanced glioma in the brain. Glioma tumors were established by direct intracranial injection of 5×10^4 GL261 NS cells/mouse into the right striatum. Tumor resection was performed 16 days after tumor implantation. Four mice were euthanized on day 17 for HE staining, and the remaining four mice were reserved for the survival experiment. Figure 2 shows that the advanced glioma occupied about 25% of area of the right hemisphere of the brain and that surgical resection removed approximately 85% of the glioma tumor. The survival curve [Figure 3] shows that the median survival for resected mice was prolonged by 5 days (although short of significant difference) compared to non-resected mice. These results indicate that it is feasible to perform tumor resection on mice bearing advanced glioma in the brain, that mice are able to survive the resection, and that surgical resection has the potential to prolong survival time.

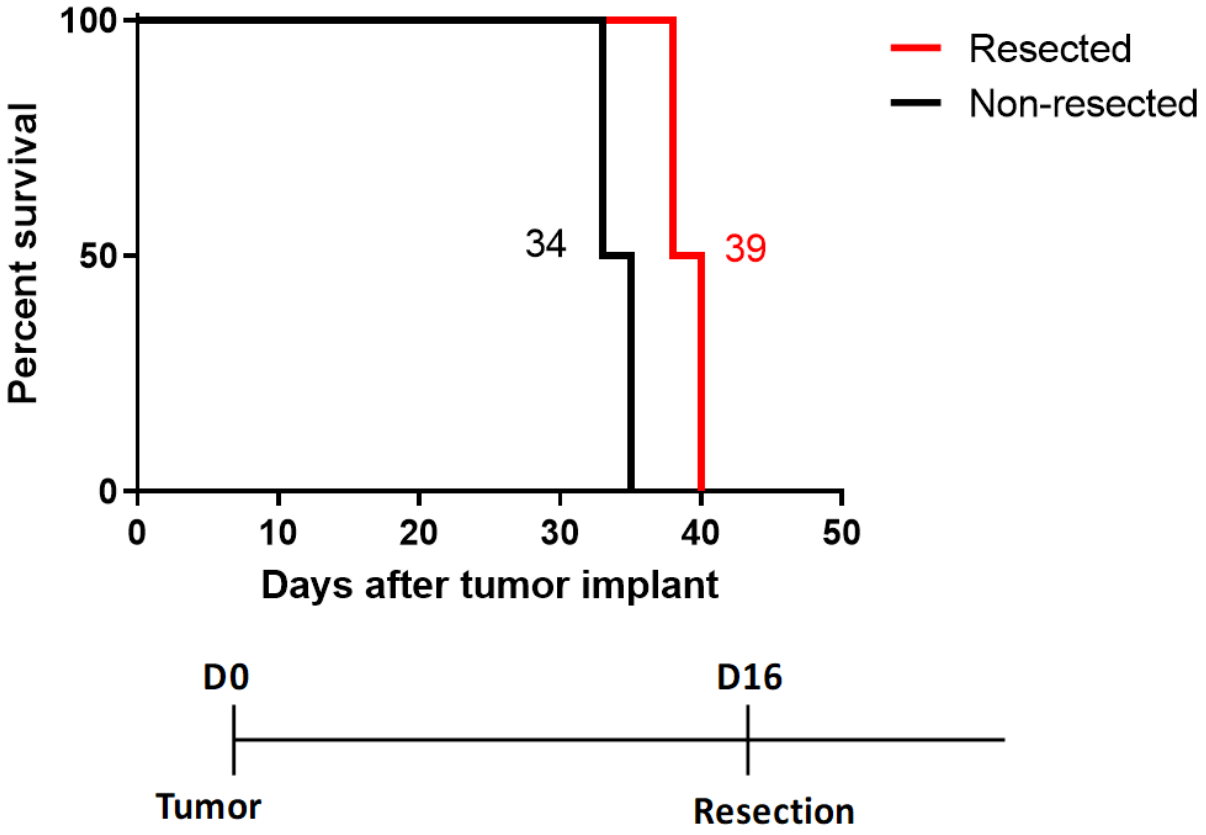


Figure 3. Prolonged survival of mice bearing advanced glioma after tumor resection. Mice received tumor (i.c.) on day 0, and tumor resection was on day 16. Mice that received the tumor resection lived longer (but short of statistical significance, $P = 0.0896$) than mice that did not receive tumor resection, $n = 2$ for both groups

DISCUSSION

Gliomas infiltrate the brain, and therefore, it is difficult to differentiate tumor tissue from normal brain. Magnetic resonance imaging (MRI) of tumors before surgical resection could help guide how aggressive removal of tissue should be. Intraoperative MRI in humans can be performed, but is not yet practical in mice. Aggressive surgical resection can lead to neurologic deficits. No neurologic deficits were observed in the animals in this study. Future work will determine whether resection as described combined with an immunotherapy strategy will lead to a prolongation of survival without adverse side effects. Since surgical stress can be immunosuppressive, including resection will provide a more rigorous test of potential immunotherapies.

DECLARATIONS

Acknowledgments

We thank the following: Dr. David Kranz for generously sharing his laboratory facilities; people of the Division of Animal Resources at University of Illinois Urbana-Champaign for cheerful and competent service; and Claire Schane and David Yan for technical assistance.

Authors' contributions

Concept and design: Tang B, Roy E
Data acquisition, analysis and interpretation: Tang B, Foss K, Lichtor T, Phillips H, Roy E
Manuscript preparation: Tang B, Roy E
Critical revision and finalizing of the manuscript: Foss K, Lichtor T, Phillips H, Roy E

Availability of data and materials

Not applicable.

Financial support and sponsorship

This research was funded by the Carle Foundation Hospital and University of Illinois Cancer Scholars for Translational and Applied Research (C*STAR) Graduate Program.

Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

All experiments were approved in advance by the Institutional Animal Care and Use Committee of the University of Illinois (protocol 19002), whose animal programs are accredited by the AAALAC International and follow the NRC Guide for the Care and Use of Laboratory Animals, Eighth Edition.

Consent for publication

Not applicable.

Copyright

© The Author(s) 2021.

REFERENCES

1. Goodenberger ML, Jenkins RB. Genetics of adult glioma. Cancer Genet 2012;205:613-21.
2. Hart MG, Garside R, Rogers G, Stein K, Grant R. Temozolomide for high grade glioma. Cochrane Database Syst Rev 2013;(4):CD007415.
3. Ostrom QT, Gittleman H, Liao P, Rouse C, Chen Y, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2007-2011. Neuro Oncol 2014;16 Suppl 4:iv1-63.
4. Tang B, Guo ZS, Bartlett DL, Yan DZ, Schane CP, et al. Synergistic combination of oncolytic virotherapy and immunotherapy for glioma. Clin Cancer Res 2020;26:2216-30.
5. Chen Z, Zhang P, Xu Y, Yan J, Liu Z, et al. Surgical stress and cancer progression: the twisted zo. Mol Cancer 2019;18:132.
6. Angka L, Martel AB, Kilgour M, Jeong A, Sadiq M, et al. Natural killer cell IFNγ secretion is profoundly suppressed following colorectal cancer surgery. Ann Surg Oncol 2018;25:3747-54.
7. Ogawa K, Hirai M, Katsube T, Murayama M, Hamaguchi K, et al. Suppression of cellular immunity by surgical stress. Surgery 2000;127:329-36.
8. Lissoni P, Brivio F, Fumagalli L, Messina G, Meregalli S, et al. Effects of the conventional antitumor therapies surgery, chemotherapy, radiotherapy and immunotherapy on regulatory T lymphocytes in cancer patients. Anticancer Res 2009;29:1847-52.
9. Saito Y, Shimada M, Utsunomiya T, Morine Y, Imura S, et al. Regulatory T cells in the blood: a new marker of surgical stress. Surg Today 2013;43:608-12.
10. Kumagai Y, Ohzawa H, Miyato H, Horie H, Hosoya Y, et al. Surgical stress increases circulating low-density neutrophils which may promote tumor recurrence. J Surg Res 2020;246:52-61.
11. Pellegatta S, Finocchiaro G. Dendritic cell vaccines for cancer stem cells. In: Yu JS, editor. Cancer Stem Cells. Totowa: Humana Press; 2009. pp. 233-47.

AUTHOR INSTRUCTIONS

1. Submission Overview

Before you decide to publish with us, please read the following items carefully and make sure that you are well aware of Editorial Policies and the following requirements.

1.1 Topic Suitability

The topic of the manuscript must fit the scope of the journal. Please refer to Aims and Scope for more information.

1.2 Open Access and Copyright

The journal adopts Gold Open Access publishing model since its establishment and has been distributing contents under Attribution 4.0 International License since October 2017, whereas Attribution-NonCommercial-ShareAlike 3.0 Unported had been adopted by then. Please make sure that you are well aware of these policies.

1.3 Publication Fees

Authors are required to pay Article Processing Charges of 360 US Dollars after the manuscript is officially accepted. For more details, please refer to Article Processing Charges.

1.4 Language Editing

All submissions are required to be presented clearly and cohesively in good English. Authors whose first language is not English are advised to have their manuscripts checked or edited by a native English speaker before submission to ensure the high quality of expression. A well-organized manuscript in good English would make the peer review even the whole editorial handling more smooth and efficient.
If needed, authors are recommended to consider the language editing services provided by Charlesworth to ensure that the manuscript is written in correct scientific English before submission. Authors who publish with OAE journals enjoy a special discount for the services of Charlesworth via the following two ways.
Submit your manuscripts directly at http://www.charlesworthauthorservices.com/~OAE;
Open the link http://www.charlesworthauthorservices.com/, and enter Promotion Code "OAE" when you submit.

1.5 Work Funded by the National Institutes of Health

If an accepted manuscript was funded by National Institutes of Health (NIH), the author may inform editors of the NIH funding number. The editors are able to deposit the paper to the NIH Manuscript Submission System on behalf of the author.

2. Submission Preparation

2.1 Cover Letter

A cover letter is required to be submitted accompanying each manuscript. It should be concise and explain why the study is significant, why it fits the scope of the journal, and why it would be attractive to readers, *etc*.
Here is a guideline of a cover letter for authors' consideration:
In the first paragraph: include the title and type (e.g., Original Article, Review, Case Report, *etc*.) of the manuscript, a brief on the background of the study, the question the author sought out to answer and why;
In the second paragraph: concisely explain what was done, the main findings and why they are significant;
In the third paragraph: indicate why the manuscript fits the Aims and Scope of the journal, and why it would be attractive to readers;
In the fourth paragraph: confirm that the manuscript has not been published elsewhere and not under consideration of any other journal. All authors have approved the manuscript and agreed on its submission to the journal. Journal's specific requirements have been met if any.
If the manuscript is contributed to a special issue, please also mention it in the cover letter.
If the manuscript was presented partly or entirely in a conference, the author should clearly state the background information of the event, including the conference name, time and place in the cover letter.

2.2 Types of Manuscripts

There is no restriction on the length of manuscripts, number of figures, tables and references, provided that the manuscript is concise and comprehensive. The journal publishes Original Article, Review, Meta-Analysis, Case Report, Commentary, *etc*. For more details about paper type, please refer to the following table.

Manuscript Type	Definition	Abstract	Keywords	Main Text Structure
Original Article	An Original Article describes detailed results from novel research. All findings are extensively discussed.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Review	A Review paper summarizes the literature on previous studies. It usually does not present any new information on a subject.	Unstructured abstract. No more than 250 words.	3-8 keywords	The main text may consist of several sections with unfixed section titles. We suggest that the author includes an "Introduction" section at the beginning, several sections with unfixed titles in the middle part, and a "Conclusion" section in the end.
Case Report	A Case Report details symptoms, signs, diagnosis, treatment, and follows up an individual patient. The goal of a Case Report is to make other researchers aware of the possibility that a specific phenomenon might occur.	Unstructured abstract. No more than 150 words.	3-8 keywords	The main text consists of three sections with fixed section titles: Introduction, Case Report, and Discussion.
Meta-Analysis	A Meta-Analysis is a statistical analysis combining the results of multiple scientific studies. It is often an overview of clinical trials.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Systematic Review	A Systematic Review collects and critically analyzes multiple research studies, using methods selected before one or more research questions are formulated, and then finding and analyzing related studies and answering those questions in a structured methodology.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Technical Note	A Technical Note is a short article giving a brief description of a specific development, technique or procedure, or it may describe a modification of an existing technique, procedure or device applied in research.	Unstructured abstract. No more than 250 words.	3-8 keywords	/
Commentary	A Commentary is to provide comments on a newly published article or an alternative viewpoint on a certain topic.	Unstructured abstract. No more than 250 words.	3-8 keywords	/
Editorial	An Editorial is a short article describing news about the journal or opinions of senior editors or the publisher.	None required	None required	/
Letter to Editor	A Letter to Editor is usually an open post-publication review of a paper from its readers, often critical of some aspect of a published paper. Controversial papers often attract numerous Letters to Editor	Unstructured abstract (optional). No more than 250 words.	3-8 keywords (optional)	/
Opinion	An Opinion usually presents personal thoughts, beliefs, or feelings on a topic.	Unstructured abstract (optional). No more than 250 words.	3-8 keywords	/
Perspective	A Perspective provides personal points of view on the state-of-the-art of a specific area of knowledge and its future prospects. Links to areas of intense current research focus can also be made. The emphasis should be on a personal assessment rather than a comprehensive, critical review. However, comments should be put into the context of existing literature. Perspectives are usually invited by the Editors.	Unstructured abstract. No more than 150 words.	3-8 keywords	/

2.3 Manuscript Structure

2.3.1 Front Matter

2.3.1.1 Title

The title of the manuscript should be concise, specific and relevant, with no more than 16 words if possible. When gene or protein names are included, the abbreviated name rather than full name should be used.

2.3.1.2 Authors and Affiliations

Authors' full names should be listed. The initials of middle names can be provided. Institutional addresses and email addresses for all authors should be listed. At least one author should be designated as corresponding author. In addition, corresponding authors are suggested to provide their Open Researcher and Contributor ID upon submission. Please note that any change to authorship is not allowed after manuscript acceptance.

2.3.1.3 Abstract

The abstract should be a single paragraph with word limitation and specific structure requirements (for more details please refer to Types of Manuscripts). It usually describes the main objective(s) of the study, explains how the study was done, including any model organisms used, without methodological detail, and summarizes the most important results and their significance. The abstract must be an objective representation of the study: it is not allowed to contain results which are not presented and substantiated in the manuscript, or exaggerate the main conclusions. Citations should not be included in the abstract.

2.3.1.4 Keywords

Three to eight keywords should be provided, which are specific to the article, yet reasonably common within the subject discipline.

2.3.2 Main Text

Manuscripts of different types are structured with different sections of content. Please refer to Types of Manuscripts to make sure which sections should be included in the manuscripts.

2.3.2.1 Introduction

The introduction should contain background that puts the manuscript into context, allow readers to understand why the study is important, include a brief review of key literature, and conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved. Relevant controversies or disagreements in the field should be introduced as well.

2.3.2.2 Methods

Methods should contain sufficient details to allow others to fully replicate the study. New methods and protocols should be described in detail while well-established methods can be briefly described or appropriately cited. Experimental participants selected, the drugs and chemicals used, the statistical methods taken, and the computer software used should be identified precisely. Statistical terms, abbreviations, and all symbols used should be defined clearly. Protocol documents for clinical trials, observational studies, and other non-laboratory investigations may be uploaded as supplementary materials.

2.3.2.3 Results

This section contains the findings of the study. Results of statistical analysis should also be included either as text or as tables or figures if appropriate. Authors should emphasize and summarize only the most important observations. Data on all primary and secondary outcomes identified in the section Methods should also be provided. Extra or supplementary materials and technical details can be placed in supplementary documents.

2.3.2.4 Discussion

This section should discuss the implications of the findings in context of existing research and highlight limitations of the study. Future research directions may also be mentioned.

2.3.2.5 Conclusion

It should state clearly the main conclusions and include the explanation of their relevance or importance to the field.

2.3.3 Back Matter

2.3.3.1 Acknowledgments

Anyone who contributed towards the article but does not meet the criteria for authorship, including those who provided professional writing services or materials, should be acknowledged. Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgments section. This section is not added if the author does not have anyone to acknowledge.

2.3.3.2 Authors' Contributions

Each author is expected to have made substantial contributions to the conception or design of the work, or the acquisition, analysis, or interpretation of data, or the creation of new software used in the work, or have drafted the work or substantively revised it.

Please use Surname and Initial of Forename to refer to an author's contribution. For example: made substantial contributions to conception and design of the study and performed data analysis and interpretation: Salas H, Castaneda WV; performed data acquisition, as well as provided administrative, technical, and material support: Castillo N, Young V.

If an article is single-authored, please include "The author contributed solely to the article." in this section.

2.3.3.3 Availability of Data and Materials

In order to maintain the integrity, transparency and reproducibility of research records, authors should include this section in their manuscripts, detailing where the data supporting their findings can be found. Data can be deposited into data repositories or published as supplementary information in the journal. Authors who cannot share their data should state that the data will not be shared and explain it. If a manuscript does not involve such issue, please state "Not applicable." in this section.

2.3.3.4 Financial Support and Sponsorship

All sources of funding for the study reported should be declared. The role of the funding body in the experiment design, collection, analysis and interpretation of data, and writing of the manuscript should be declared. Any relevant grant numbers and the link of funder's website should be provided if any. If the study is not involved with this issue, state "None." in this section.

2.3.3.5 Conflicts of Interest

Authors must declare any potential conflicts of interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. If there are no conflicts of interest, please state "All authors declared that there are no conflicts of interest." in this section. Some authors may be bound by confidentiality agreements. In such cases, in place of itemized disclosures, we will require authors to state "All authors declare that they are bound by confidentiality agreements that prevent them from disclosing their conflicts of interest in this work.". If authors are unsure whether conflicts of interest exist, please refer to the "Conflicts of Interest" of OAE Editorial Policies for a full explanation.

2.3.3.6 Ethical Approval and Consent to Participate

Research involving human subjects, human material or human data must be performed in accordance with the Declaration of Helsinki and approved by an appropriate ethics committee. An informed consent to participate in the study should also be obtained from participants, or their parents or legal guardians for children under 16. A statement detailing the name of the ethics committee (including the reference number where appropriate) and the informed consent obtained must appear in the manuscripts reporting such research.

Studies involving animals and cell lines must include a statement on ethical approval. More information is available at Editorial Policies.

If the manuscript does not involve such issue, please state "Not applicable." in this section.

2.3.3.7 Consent for Publication

Manuscripts containing individual details, images or videos, must obtain consent for publication from that person, or in the case of children, their parents or legal guardians. If the person has died, consent for publication must be obtained from the next of kin of the participant. Manuscripts must include a statement that a written informed consent for publication was obtained. Authors do not have to submit such content accompanying the manuscript. However, these documents must be available if requested. If the manuscript does not involve this issue, state "Not applicable." in this section.

2.3.3.8 Copyright

Authors retain copyright of their works through a Creative Commons Attribution 4.0 International License that clearly states how readers can copy, distribute, and use their attributed research, free of charge. A declaration "© The Author(s) 2019." will be added to each article. Authors are required to sign License to Publish before formal publication.

2.3.3.9 References

References should be numbered in order of appearance at the end of manuscripts. In the text, reference numbers should be placed in square brackets and the corresponding references are cited thereafter. Only the first five authors' names are required to be listed in the references, other authors' names should be omitted and replaced with "et al.". Abbreviations of the journals should be provided on the basis of Index Medicus. Information from manuscripts accepted but not published should be cited in the text as "Unpublished material" with written permission from the source.

References should be described as follows, depending on the types of works:

Types	Examples
Journal articles by individual authors	Weaver DL, Ashikaga T, Krag DN, Skelly JM, Anderson SJ, et al. Effect of occult metastases on survival in node-negative breast cancer. N Engl J Med 2011;364:412-21. [PMID: 21247310 DOI: 10.1056/NEJMoa1008108]
Organization as author	Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40:679-86. [PMID: 12411462]
Both personal authors and organization as author	Vallancien G, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1,274 European men suffering from lower urinary tract symptoms. J Urol 2003;169:2257-61. [PMID: 12771764 DOI: 10.1097/01.ju.0000067940.76090.73]
Journal articles not in English	Zhang X, Xiong H, Ji TY, Zhang YH, Wang Y. Case report of anti-N-methyl-D-aspartate receptor encephalitis in child. J Appl Clin Pediatr 2012;27:1903-7. (in Chinese)
Journal articles ahead of print	Odibo AO. Falling stillbirth and neonatal mortality rates in twin gestation: not a reason for complacency. BJOG 2018; Epub ahead of print [PMID: 30461178 DOI: 10.1111/1471-0528.15541]
Books	Sherlock S, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub; 1993. pp. 258-96.
Book chapters	Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. pp. 93-113.
Online resource	FDA News Release. FDA approval brings first gene therapy to the United States. Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm. [Last accessed on 30 Oct 2017]
Conference proceedings	Harnden P, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ Cell Tumour Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer; 2002.
Conference paper	Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. pp. 182-91.
Unpublished material	Tian D, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. Proc Natl Acad Sci U S A. Forthcoming 2002.

For other types of references, please refer to U.S. National Library of Medicine.
The journal also recommends that authors prepare references with a bibliography software package, such as EndNote to avoid typing mistakes and duplicated references.

2.3.3.10 Supplementary Materials

Additional data and information can be uploaded as Supplementary Material to accompany the manuscripts. The supplementary materials will also be available to the referees as part of the peer-review process. Any file format is acceptable, such as data sheet (word, excel, csv, cdx, fasta, pdf or zip files), presentation (powerpoint, pdf or zip files), image (cdx, eps, jpeg, pdf, png or tiff), table (word, excel, csv or pdf), audio (mp3, wav or wma) or video (avi, divx, flv, mov, mp4, mpeg, mpg or wmv). All information should be clearly presented. Supplementary materials should be cited in the main text in numeric order (e.g., Supplementary Figure 1, Supplementary Figure 2, Supplementary Table 1, Supplementary Table 2, *etc.*). The style of supplementary figures or tables complies with the same requirements on figures or tables in main text. Videos and audios should be prepared in English, and limited to a size of 500 MB or a duration of 3 minutes.

2.4 Manuscript Format

2.4.1 File Format

Manuscript files can be in DOC and DOCX formats and should not be locked or protected.

2.4.2 Length

There are no restrictions on paper length, number of figures, or amount of supporting documents. Authors are encouraged to present and discuss their findings concisely.

2.4.3 Language

Manuscripts must be written in English.

2.4.4 Multimedia Files

The journal supports manuscripts with multimedia files. The requirements are listed as follows:
Videos or audio files are only acceptable in English. The presentation and introduction should be easy to understand. The frames should be clear, and the speech speed should be moderate.
A brief overview of the video or audio files should be given in the manuscript text.
The video or audio files should be limited to a duration of 3 min and a size of up to 500 MB.

Please use professional software to produce high-quality video files, to facilitate acceptance and publication along with the submitted article. Upload the videos in mp4, wmv, or rm format (preferably mp4) and audio files in mp3 or wav format.

2.4.5 Figures

Figures should be cited in numeric order (e.g., Figure 1, Figure 2) and placed after the paragraph where it is first cited;
Figures can be submitted in format of tiff, psd, AI or jpeg, with resolution of 300-600 dpi;
Figure caption is placed under the Figure;
Diagrams with describing words (including, flow chart, coordinate diagram, bar chart, line chart, and scatter diagram, *etc*.) should be editable in word, excel or powerpoint format. Non-English information should be avoided;
Labels, numbers, letters, arrows, and symbols in figure should be clear, of uniform size, and contrast with the background;
Symbols, arrows, numbers, or letters used to identify parts of the illustrations must be identified and explained in the legend;
Internal scale (magnification) should be explained and the staining method in photomicrographs should be identified;
All non-standard abbreviations should be explained in the legend;
Permission for use of copyrighted materials from other sources, including re-published, adapted, modified, or partial figures and images from the internet, must be obtained. It is authors' responsibility to acquire the licenses, to follow any citation instruction requested by third-party rights holders, and cover any supplementary charges.

2.4.6 Tables

Tables should be cited in numeric order and placed after the paragraph where it is first cited;
The table caption should be placed above the table and labeled sequentially (e.g., Table 1, Table 2);
Tables should be provided in editable form like DOC or DOCX format (picture is not allowed);
Abbreviations and symbols used in table should be explained in footnote;
Explanatory matter should also be placed in footnotes;
Permission for use of copyrighted materials from other sources, including re-published, adapted, modified, or partial tables from the internet, must be obtained. It is authors' responsibility to acquire the licenses, to follow any citation instruction requested by third-party rights holders, and cover any supplementary charges.

2.4.7 Abbreviations

Abbreviations should be defined upon first appearance in the abstract, main text, and in figure or table captions and used consistently thereafter. Non-standard abbreviations are not allowed unless they appear at least three times in the text. Commonly-used abbreviations, such as DNA, RNA, ATP, *etc*., can be used directly without definition. Abbreviations in titles and keywords should be avoided, except for the ones which are widely used.

2.4.8 Italics

General italic words like *vs*., *et al., etc*., *in vivo*, *in vitro*; *t* test, *F* test, *U* test; related coefficient as *r*, sample number as *n*, and probability as *P*; names of genes; names of bacteria and biology species in Latin.

2.4.9 Units

SI Units should be used. Imperial, US customary and other units should be converted to SI units whenever possible. There is a space between the number and the unit (i.e., 23 mL). Hour, minute, second should be written as h, min, s.

2.4.10 Numbers

Numbers appearing at the beginning of sentences should be expressed in English. When there are two or more numbers in a paragraph, they should be expressed as Arabic numerals; when there is only one number in a paragraph, number < 10 should be expressed in English and number > 10 should be expressed as Arabic numerals. 12345678 should be written as 12,345,678.

2.4.11 Equations

Equations should be editable and not appear in a picture format. Authors are advised to use either the Microsoft Equation Editor or the MathType for display and inline equations.

2.5 Submission Link

Submit an article via https://oaemesas.com/nn/.

OAE Publishing Inc.
www.oaepublish.com

Neuroimmunology and Neuroinflammation
(NN)
Los Angeles Office
245 E Main Street ste122, Alhambra,
CA 91801, USA
Tel: +1 323 9987086
E-mail: editorialoffice@nnjournal.net
Website: www.nnjournal.net