Molecular biomarkers in the neurological ICU: is there a role?

Ramon Diaz-Arrastia[a], *Pashtun Shahim*[b], *and Danielle K. Sandsmark*[a]

Purpose of review
The aim of the article is to summarize recent advances in the field of molecular biomarkers in neurocritical care.

Recent findings
Advances in ultrasensitive immunoassay technology have made it possible to measure brain-derived proteins that are present at subfemtomolar concentrations in blood. These assays have made it possible to measure neurofilament light chain (NfL) in serum or plasma, and early studies indicate that NfL is a promising prognostic and pharmacodynamic biomarker across a broad range of neurologic disorders, including cardiac arrest and traumatic brain injury. However, as acquired brain injury is a complex and heterogeneous disorder, it is likely that assays of panels of biomarkers will ultimately be needed to maximally impact practice. Micro-RNAs are a novel but exciting class of molecules that also show potential to provide clinically actionable information.

Summary
Although not yet ready for adoption into routine clinical practice, several molecular biomarkers are on the cusp of clinical validation. The availability of such tests likely will revolutionize the practice of neurocritical care.

Keywords
glial fibrillary acidic protein, micro-RNA, neurofilament light, neuron-specific enolase, ubiquitin carboxyl-terminal hydrolase L1

INTRODUCTION

Biomarkers are molecules that can be measured in accessible biological fluids that reflect physiological, pharmacological, or disease processes and can suggest the cause of, susceptibility to, activity levels of, or progress of a disease. According to the Food and Drug Administration, biomarkers fall into several categories (which are not mutually exclusive) [1,2]. In the management of neurologically critically ill patients, *prognostic*, *predictive*, and *pharmacodynamic* biomarkers are particularly relevant. *Prognostic* biomarkers are baseline measurements that categorize patients by degree of risk for disease progression and informs about the natural history of the disorder. *Predictive* biomarkers are baseline characteristics that categorize patients by their likelihood of response to a particular treatment. Finally, *pharmacodynamic* biomarkers are dynamic measurements that show that a biologic response has occurred in a patient after a therapeutic intervention. Biomarkers have historically been critical to progress in a broad range of clinical conditions. Therapeutic advances in fields as disparate as cardiology and oncology have relied on the ability to measure biomarkers that are reliable indicators of the underlying disease. The absence of validated biomarkers in the neurocritical care field is a major factor limiting our understanding the natural history and the long-term effects of acute brain injury, as well as a barrier to drug development in this area.

Blood and cerebrospinal fluid (CSF) levels of structural protein components of brain cells that are released in the aftermath of brain injury have been widely studied for the past two decades and are a promising adjunct to detect and monitor secondary brain injury in the neurological ICU setting [3–10].

[a]Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania and [b]Clinical Center, National Institutes of Health, Bethesda, Maryland, USA

Correspondence to Ramon Diaz-Arrastia, MD, Ph.D., Penn Presbyterian Medical Center, Andrew Mutch Building, Room 409, 51 North 39th Street, Philadelphia, PA 19104, USA. Tel.: +1 215 662 8732;.
e-mail: Ramon.Diaz-Arrastia@pennmedicine.upenn.edu

Curr Opin Crit Care 2020, 26:000–000
DOI:10.1097/MCC.0000000000000703

1070-5295 Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

Copyright © 2020 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.

KEY POINTS

- Molecular biomarkers that provide prognostic, predictive, and pharmacodynamics information are particularly relevant to neurocritical care.
- Biomarkers such as GFAP and UCH-L1 may be used as aid in the evaluation of mild TBI.
- NfL appears to be a useful biomarker across a range of neurological disorders that involve axonal degeneration.
- A panel of brain injury biomarkers will likely be required to provide actionable information rather than use a single biomarker.
- mi-RNAs well suited to assess complex and heterogeneous clinical situations and have shown promise as prognostic biomarkers for OHCA.

More recently, studies on small noncoding RNA molecules, particularly micro-RNAs (mi-RNAs), show promise to provide complementary prognostic information and to identify molecular pathways involved in the neurodegenerative process. This review will focus on publications over the past 2–3 years that represent important advances, and on the biomarkers that are on the cusp of adoption in routine critical care.

CONTEXT OF USE OF MOLECULAR BIOMARKERS IN NEUROCRITICAL CARE

Patients who have suffered severe acquired brain injuries, for example, from hypoxic–ischemic insults, traumatic brain injury (TBI), hemorrhagic stroke, or ischemic infarction, are at high risk for secondary brain injury from brain edema, tissue ischemia, hematoma expansion, and metabolic derangements, among other insults. Biomarkers may be used to identify tissue at risk, ideally while the injury is reversible, and so allow for the institution of therapeutic interventions to potentially limit secondary injury. In addition, biomarkers may also be used to provide prognostic information to inform decisions about the potential benefits of referral to neurorehabilitation units, or about decisions about withholding care. Finally, predictive and pharmacodynamic biomarkers could help select patients for clinical trials of targeted neuroprotective and neurorestorative therapies. In this review, the term 'context of use' is conceptualized more broadly to encompass different settings where biomarkers could be useful in clinical research, and eventually in clinical practice. Consideration of the context of use is important, as the particular purpose for which the biomarker is used greatly impacts issues related to the required specificity, sensitivity, and analytical details.

NEUROFILAMENT LIGHT CHAIN

The past two years have witnessed an explosion of interest in neurofilament light chain (NfL) as a biomarker in a wide range of neurological disorders. NfL is an intermediate filament protein abundantly expressed in the long myelinated subcortical axons [11▪]. It is the smallest and most abundant of the three major neurofilament subunits (Nf-light chain, Nf-medium chain, and Nf-heavy chain) and consequently the most likely to be found in circulation after neurologic insults [12]. Most pathological processes that cause axonal damage release neurofilaments into extracellular fluid, CSF, and blood [11▪]. High levels of neurofilaments are general indicators of axonal damage in many neurologic conditions, including multiple sclerosis [13,14▪,15], HIV-associated encephalopathy [16,17], neurodegenerative disorders [18–20], aging [21], stroke [22,23], and TBI [24–27]. Although not diagnostically specific, blood levels of neurofilaments are potentially useful to monitor and predict disease progression in a spectrum of acute and chronic neurological diseases, and to assess treatment efficacy.

NfL has been widely studied over the past three decades as a biomarker of axonal injury, and there is an extensive literature supporting the value of measuring NfL in CSF in human disease [28–30]. However, only the advent of fourth-generation Single Molecule Array (SiMoA) technology [31–33] has made possible reliable quantification of NfL in blood, across the range of concentrations observed in disease and physiological conditions [13,16,34]. The analytical sensitivity of SiMoA is 100–1000-fold higher than that obtained using the same antibodies in an enzyme-linked immunoabsorbent assay format. Further, reliable measurement of the low NfL concentrations present in blood samples from young, healthy individuals allows even minor changes in levels of this protein that occur with neural injury to be monitored. There is a strong correlation between CSF and serum or plasma levels of NfL, which has been demonstrated in numerous studies and various neurological diseases [13,14▪,18,20,26]. These findings provide confidence that NfL measurements in peripheral blood closely monitor neurodegenerative processes in the central nervous system.

The evolving literature of serum NfL in multiple sclerosis is particularly relevant, as it points to the usefulness of this molecule as a pharmacodynamic biomarker of therapeutic efficacy. Multiple sclerosis is a chronic disease of presumed autoimmune origin, which is characterized, at least initially, by

Copyright © 2020 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.

episodes of inflammation in the brain and spinal cord that predominantly affect the white matter [35]. The formation of new lesions can be visualized with MRI, the only established biomarker of disease activity in routine clinical practice [36]. However, MRI does not allow selective detection of axonal degeneration, which seems to be the most important determinant of long-term disability [37]. CSF NfL has been widely studied as an multiple sclerosis biomarker [38], but the requirement for invasive CSF sampling limited its widespread adoption in multiple sclerosis research and clinical practice. Recent studies, using the highly sensitive SiMoA assay, demonstrate that not only can serum NfL distinguish between patients with multiple sclerosis and healthy controls [13], but can distinguish between patients with enhancing MRI lesions from patients without such lesions [13]. Serum NfL levels in multiple sclerosis patients have been independently associated with disability and relapse status [13,39▪], and the risk of future relapses is higher among patients with higher serum neurofilament light chain (NF-L) levels [13,39▪]. In a longitudinal observational study, patients with higher serum NfL levels at baseline, independent of other clinical and MRI variables, experienced significantly more brain and spinal cord volume loss over two years and five years of follow-up [39▪]. Finally, serum NfL shows promise as a pharmacodynamic biomarker in multiple sclerosis, as starting disease-modifying treatments leads to reductions in serum NfL [14▪].

The management of out of hospital cardiac arrest (OHCA) represents an area where highly sensitive and specific prognostic biomarkers can make an impact in clinical practice. Neural proteins such as S100β and neuron-specific enolase (NSE) have been studied for many years in this setting. However, because of the high false-positive rates, neither is recommended to be used alone in the 2015 American Heart Association Guidelines [40]. A recently published study from the Target Temperature Management After Cardiac Arrest (TTM) trial [41▪▪] indicates that serum NfL substantially outperforms S100β, NSE, and Tau for predicting poor outcome in OHCA. This study included 717 participants in the TTM trial, of whom 360 (50%) had poor neurologic recovery six months after OHCA, based on the Cerebral Performance Category Scale. NfL was measured in serum using the Simoa HD-1 analyzer at 24, 48, and 72 h after resuscitation. NfL levels increased between 24 and 48 h, but remained stable at 72 h. NfL levels were higher in patients with poor outcome than in those with good outcome at all three time points. Only 29 patients had poor neurologic outcomes despite low or only moderately elevated NfL levels (<100 pg/ml, corresponding to the upper inter-quartile range for patients with good outcome). The area under the curve (AUC) of a Receiver Operator Characteristic curve for predicting poor outcome was 0.94 for NfL, compared with AUCs in the range of 0.75–0.81 for NSE, S100β, and tau. At an NfL cutoff of 478 pg/ml, corresponding to 98% specificity, the sensitivity was 69%, and the false-positive rate was under 1%. At a specificity of 98%, NfL had greater sensitivity for poor outcome compared with electroencephalography, somatosensory evoked potentials, head computed tomography, and both pupillary and corneal reflexes [41▪▪].

NfL also shows substantial promise as a prognostic biomarker in TBI. Axonal injury is a prominent finding in TBI, and it has been known for decades that NfL is elevated in CSF after severe TBI [42] and even after mild brain injuries sustained during participation in contact sports, such as boxing [43]. The advent of highly sensitive SiMoA assays has made possible detailed studies settings where CSF sampling is not routinely feasible [24,25,44,45]. There was a high correlation between CSF and serum NF-L levels ($r = 0.88$), providing confidence that serum NfL levels mirror NfL release in brain. Unlike most other structural protein components, which are found at highest concentrations in biological fluids soon after the injury and are promptly cleared [46▪▪], CSF, and serum NfL, although elevated within a few hours of injury, continues to rise over the first two weeks [24,46▪▪]. One year after injury serum levels had substantially decreased but remained elevated over uninjured controls. The delayed release of NfL into the circulation suggests that it may be a marker of secondary neural injury, rather than primarily a marker of the initial insult, potentially making it useful as a pharmacodynamic biomarker for neuroprotective therapies. High NfL levels within the first 24 h are weakly associated with poor neurologic outcome 12 months later [24], although including NfL in a predictive model only marginally improves the predictive ability compared with a model based on clinical variables alone (AUC increases only from 0.65 to 0.7). Hence, NfL may not be useful as a standalone biomarker in TBI in routine clinical settings.

PROTEIN BIOMARKER PANELS

As the neural proteins studied as potential biomarkers in brain injury are produced by different cell types and may reflect different features of the injury [47], it is likely that a panel that measures several biomarkers may outperform a single biomarker for prognostic, predictive, or pharmacodynamic purposes. This hypothesis was recently directly tested. Thelin *et al.* [46▪▪] measured S100β, NSE, glial fibrillary acidic protein (GFAP), ubiquitin carboxyl-

1070-5295 Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

Copyright © 2020 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.

terminal hydrolase L1 (UCH-L1), tau, and NfL in serial samples obtained over the first two weeks in patients with severe TBI admitted to a single neurological ICU. All biomarkers were associated with injury severity as classified on cranial computed tomography at admission. In univariate analysis, all biomarkers outperformed other known outcome predictors for predicting unfavorable outcome, with UCH-L1 displaying the best discriminative ability (pseudo R^2 0.260). A correlation matrix showed substantial covariance between all biomarkers, with the strongest correlation between UCH-L1, GFAP, and tau ($r = 0.83–0.88$). NfL was weakly correlated with the other biomarkers ($r = 0.27–0.44$), and a principal component analysis showed that NfL was orthogonal to the other biomarkers, suggesting that it provides unique information about the injury process. After adjusting for known TBI outcome predictors, such as those used in the IMPACT model [48], GFAP and NfL added the most independent information to predict favorable versus unfavorable outcome, improving the multivariate model from 0.38 to 0.51 pseudo-R^2. This innovative study provides convincing proof of principle that a panel of brain injury biomarkers will likely be required to provide actionable information that will impact clinical practice in a complex and heterogeneous condition such as TBI.

MICRO-RNAS

miRNAs are synthesized from longer primary RNA precursors and, after processing, are incorporated into an RNA-induced silencing complex that directs the miRNA to target miRNAs. miRNAs also are secreted from cells, and play a signaling role in nearby cells and remote tissues [49]. In the circulation, miRNAs are found in association with exosomes [50–52], lipoproteins [50,53], and other macromolecular complexes. A single miRNA can target and block numerous mRNAs, many of which may encode multiple components of complex-signaling cascades and intracellular networks. In this way, miRNAs can act to fine-tune gene expression, alter cellular function at a network level, and operate as potent governors of cell biology. As such, miRNAs are also attractive disease biomarkers, as they are found in accessible biological fluids, are resistant to RNAase activity, are stable in human plasma, and are readily measurable using robust and highly sensitive analytical techniques [49]. The ability to measure all miRNAs (the miRNA-ome) in plasma, through unbiased techniques such as RNA-sequencing methods or with highly multiplexed arrays such as Nanostring [54] makes miRNAs well suited to assess complex and heterogeneous clinical situations.

Several recent studies from the TTM trial collaborators have explored the value of candidate miRNAs as prognostic biomarkers for OHCA. So far, these studies have focused on candidate miRNAs, chosen based on prior associations with cardiovascular metabolism and ischemic heart disease. Further, these miRNAs were analyzed using relatively simple statistical models, such as multivariate logistic regression and Cox proportional hazard models, and thus only scratch the surface of the potential value of this technology. Deveaux *et al.* [55] measured circulating levels of miR-122-5p and miR-124-3p in blood samples obtained 48 h after resuscitation in 590 patients enrolled in the TTM trial. Levels of miR-122-5p were lower in patients with poor outcome, independent of the assigned target temperature, and were weakly predictive of poor recovery (AUC 0.62). miR-124-3p levels were weakly correlated with levels of miR-122-5p ($r = 0.35$), and a model including both miRNAs showed modest improvement in the predictive accuracy, compared with a model based on clinical variables along. The same group of investigators [56▪] recently published analysis on miR-574-5p in the same cohort ($n = 590$). This miRNA was elevated in patients with poor outcome, and independently predicted neurologic outcome in women but not men. These studies, while early, point to the promise that miRNAs have for providing clinically actionable information in complex disorders.

CONCLUSION

Identification and validation of biomarkers that can guide the management of critically brain-injured patients has been a holy grail in the field for over 30 years. Although much progress remains to be made, the last several years have witnessed meaningful advances, and it is likely that at least some of the biomarkers under current intense investigation will become adopted into routine clinical practice over the next several years. In particular, GFAP and UCH-L1, which were recently cleared by the Food and Drug Administration as an aid in the evaluation of mild TBI (https://www.fda.gov/news-events/press-announcements/fda-authorizes-marketing-first-blood-test-aid-evaluation-concussion-adults) also show promise as prognostic biomarkers in severe TBI and other acute neurologic conditions. In addition, NfL appears to be a useful biomarker across a range of neurological disorders that involve axonal degeneration and has potential both as a prognostic and a pharmacodynamic biomarker. Further over the horizon, the use of biomarker panels promises to provide additional information beyond what is available from measurements of single molecules. Finally, studies aiming to unlock the

Copyright © 2020 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.

potential utility of miRNAs as biomarkers in neurocritical illness have successfully established proof-of-principle. Further progress will rely on availability of large sample collections from well-characterized patients with neurocritical illness.

Acknowledgements

Support in the authors' laboratories is supported by NINDS (U01 NS099046, U24 NS107199, U01 NS086090 (to RD-A) and K23 NS104239 (to DKS)), the Department of Defense (DM180187, W81XWH1920002, BA 170613, MTEC18–03-DTTBI (to R.D.A.)), and the Pennsylvania Department of Health.

Financial support and sponsorship

None.

Conflicts of interest

R.D.A. is a consultant to Quanterix, BRAINBox Solutions, and MesoScale Discoveries.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Food and Drug Administration CfDEaRC. Guidance for industry and FDA staff: qualification process for drug development tools. Food and Drug Administration, 2014, 1–32. Available from: http://www.fda.gov/cder/guidance/index.htm.
2. Food and Drug Administration CfDEaR. E16 biomarkers related to drug or biotechnology product development: context, structure, and format of qualification submissions. Food and Drug Administration, 2011 August. Available from: www.fda.gov/downloads/Drugs/GuidanceComplicanceRegulatoryInformation/Guidelines/UCM 267449.pdf.
3. Thelin EP, Zeiler FA, Ercole A, *et al.* Serial sampling of serum protein biomarkers for monitoring human traumatic brain injury dynamics: a systematic review. Front Neurol 2017; 8:300.
4. Al NF, Thelin E, Nystrom H, *et al.* Comparative assessment of the prognostic value of biomarkers in traumatic brain injury reveals an independent role for serum levels of neurofilament light. PLoS One 2015; 10:e0132177.
5. Thelin EP, Johannesson L, Nelson D, Bellander BM. S100B is an important outcome predictor in traumatic brain injury. J Neurotrauma 2013; 30:519–528.
6. Thelin EP, Nelson DW, Bellander BM. Secondary peaks of S100B in serum relate to subsequent radiological pathology in traumatic brain injury. Neurocrit Care 2014; 20:217–229.
7. Ercole A, Thelin EP, Holst A, *et al.* Kinetic modelling of serum S100b after traumatic brain injury. BMC Neurol 2016; 16:93.
8. Thelin EP, Jeppsson E, Frostell A, *et al.* Utility of neuron-specific enolase in traumatic brain injury; relations to S100B levels, outcome, and extracranial injury severity. Crit Care 2016; 20:285.
9. Thelin EP, Nelson DW, Bellander BM. A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. Acta Neurochir (Wien) 2017; 159:209–225.
10. Bellander BM, Olafsson IH, Ghatan PH, *et al.* Secondary insults following traumatic brain injury enhance complement activation in the human brain and release of the tissue damage marker S100B. Acta Neurochir (Wien) 2011; 153:90–100.
11. ■ Khalil M, Teunissen CE, Otto M, *et al.* Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol 2018; 14:577–589.

This is an excellent review of the recent advances on neurofilament light chain as a biomarker in a broad range of neurologic disorders, based on breakthroughs in ultrasensitive immunoassays that allow assessment of this protein in blood.

12. Lee MK, Xu Z, Wong PC, Cleveland DW. Neurofilaments are obligate heteropolymers in vivo. J Cell Biol 1993; 122:1337–1350.
13. Disanto G, Barro C, Benkert P, *et al.* Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann Neurol 2017; 81:857–870.
14. ■ Piehl F, Kockum I, Khademi M, *et al.* Plasma neurofilament light chain levels in patients with MS switching from injectable therapies to fingolimod. Mult Scler 2018; 24:1046–1054.

This manuscript documents an example of neurofilament light as a pharmacodynamic biomarker.

15. Novakova L, Zetterberg H, Sundstrom P, *et al.* Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology 2017; 89:2230–2237.
16. Gisslen M, Price RW, Andreasson U, *et al.* Plasma concentration of the neurofilament light protein (NFL) is a biomarker of CNS injury in HIV infection: a cross-sectional study. EBioMedicine 2016; 3:135–140.
17. Anderson AM, Easley KA, Kasher N, *et al.* Neurofilament light chain in blood is negatively associated with neuropsychological performance in HIV-infected adults and declines with initiation of antiretroviral therapy. J Neurovirol 2018; 24:695–701.
18. Gaiottino J, Norgren N, Dobson R, *et al.* Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. PLoS One 2013; 8:e75091.
19. Wilke C, Preische O, Deuschle C, *et al.* Neurofilament light chain in FTD is elevated not only in cerebrospinal fluid, but also in serum. J Neurol Neurosurg Psychiatry 2016; 87:1270–1272.
20. Preische O, Schultz SA, Apel A, *et al.* Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. Nat Med 2019; 25:277–283.
21. Mattsson N, Andreasson U, Zetterberg H, Blennow K. Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. JAMA Neurol 2017; 74:557–566.
22. Gattringer T, Pinter D, Enzinger C, *et al.* Serum neurofilament light is sensitive to active cerebral small vessel disease. Neurology 2017; 89:2108–2114.
23. Traenka C, Disanto G, Seiffge DJ, *et al.* Serum neurofilament light chain levels are associated with clinical characteristics and outcome in patients with cervical artery dissection. Cerebrovasc Dis 2015; 40:222–227.
24. Shahim P, Gren M, Liman V, *et al.* Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. Sci Rep 2016; 6:36791.
25. Shahim P, Tegner Y, Marklund N, *et al.* Neurofilament light and tau as blood biomarkers for sports-related concussion. Neurology 2018; 90:e1780–e1788.
26. Shahim P, Tegner Y, Wilson DH, *et al.* Blood biomarkers for brain injury in concussed professional ice hockey players. JAMA Neurol 2014; 71:684–692.
27. Ljungqvist J, Zetterberg H, Mitsis M, *et al.* Serum neurofilament light protein as a marker for diffuse axonal injury: results from a case series study. J Neurotrauma 2017; 34:1124–1127.
28. Rosengren LE, Karlsson JE, Sjogren M, *et al.* Neurofilament protein levels in CSF are increased in dementia. Neurology 1999; 52:1090–1093.
29. Norgren N, Karlsson JE, Rosengren L, Stigbrand T. Monoclonal antibodies selective for low molecular weight neurofilaments. Hybrid Hybridomics 2002; 21:53–59.
30. Norgren N, Rosengren L, Stigbrand T. Elevated neurofilament levels in neurological diseases. Brain Res 2003; 987:25–31.
31. Rissin DM, Kan CW, Campbell TG, *et al.* Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol 2010; 28:595–599.
32. Rissin DM, Fournier DR, Piech T, *et al.* Simultaneous detection of single molecules and singulated ensembles of molecules enables immunoassays with broad dynamic range. Anal Chem 2011; 83:2279–2285.
33. Wilson DH, Rissin DM, Kan CW, *et al.* The Simoa HD-1 analyzer: a novel fully automated digital immunoassay analyzer with single-molecule sensitivity and multiplexing. J Lab Autom 2016; 21:533–547.
34. Kuhle J, Barro C, Andreasson U, *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; 54:1655–1661.
35. Brownlee WJ, Hardy TA, Fazekas F, Miller DH. Diagnosis of multiple sclerosis: progress and challenges. Lancet 2017; 389:1336–1346.
36. Lucchinetti CF, Popescu BF, Bunyan RF, *et al.* Inflammatory cortical demyelination in early multiple sclerosis. N Engl J Med 2011; 365:2188–2197.
37. Trapp BD, Peterson J, Ransohoff RM, *et al.* Axonal transection in the lesions of multiple sclerosis. N Engl J Med 1998; 338:278–285.
38. 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; 64:402–404.
39. ■ Barro C, Benkert P, Disanto G, *et al.* Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain 2018; 141:2382–2391.

This manuscript discusses the utility of neurofilament light chain as a prognostic biomarker in multiple sclerosis.

40. Callaway CW, Donnino MW, Fink EL, *et al.* Part 8: post-cardiac arrest care: 2015 American Heart Association Guidelines update for cardiopulmonary resuscitation and emergency cardiovascular care. Circulation 2015; 132: S465–S482.

Copyright © 2020 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.

41. Moseby-Knappe M, Mattsson N, Nielsen N, *et al.* Serum neurofilament light ■■ chain for prognosis of outcome after cardiac arrest. JAMA Neurol 2019; 76:64–71.

This manuscript reports the findings on serum neurofilament light chain as a prognostic biomarker of neurologic outcome in out of hospital cardiac arrest, showing that it outperforms other molecular biomarkers as well as clinical and physiologic measures.

42. Bagnato S, Grimaldi LM, Di RG, *et al.* Prolonged cerebrospinal fluid neurofilament light chain increase in patients with post-traumatic disorders of consciousness. J Neurotrauma 2017; 34:2475–2479.
43. Zetterberg H, Hietala MA, Jonsson M, *et al.* Neurochemical aftermath of amateur boxing. Arch Neurol 2006; 63:1277–1280.
44. Shahim P, Zetterberg H, Tegner Y, Blennow K. Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports. Neurology 2017; 88:1788–1794.
45. Korley FK, Yue JK, Wilson DH, *et al.* Performance evaluation of a multiplex assay for simultaneous detection of four clinically relevant traumatic brain injury biomarkers. J Neurotrauma 2019; 36:182–187.
46. Thelin E, Al NF, Frostell A, *et al.* A serum protein biomarker panel improves ■■ outcome prediction in human traumatic brain injury. J Neurotrauma 2019; 36:2850–2862.

This manuscript demonstrates the value of panels using multiple biomarkers which provide orthogonal information to provide better prognostic information that what is possible through analysis of single biomarkers.

47. Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. Nat Rev Neurol 2013; 9:201–210.
48. Maas AI, Murray GD, Roozenbeek B, *et al.* Advancing care for traumatic brain injury: findings from the IMPACT studies and perspectives on future research. Lancet Neurol 2013; 12:1200–1210.
49. Wang J, Chen J, Sen S. MicroRNA as biomarkers and diagnostics. J Cell Physiol 2016; 231:25–30.
50. Vickers KC, Remaley AT. Lipid-based carriers of microRNAs and intercellular communication. Curr Opin Lipidol 2012; 23:91–97.
51. Li Y, Shen Z, Yu XY. Transport of microRNAs via exosomes. Nat Rev Cardiol 2015; 12:198.
52. Valadi H, Ekstrom K, Bossios A, *et al.* Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9:654–659.
53. Vickers KC, Palmisano BT, Shoucri BM, *et al.* MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol 2011; 13:423–433.
54. Stylli SS, Adamides AA, Koldej RM, *et al.* miRNA expression profiling of cerebrospinal fluid in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg 2017; 126:1131–1139.
55. Devaux Y, Salgado-Somoza A, Dankiewicz J, *et al.* Incremental value of circulating MiR-122-5p to predict outcome after out of hospital cardiac arrest. Theranostics 2017; 7:2555–2564.
56. Boileau A, Somoza AS, Dankiewicz J, *et al.* Circulating levels of miR-574-5p ■ are associated with neurological outcome after cardiac arrest in women: a target temperature management (TTM) trial substudy. Dis Markers 2019; 2019:1802879.

This manuscript represents an early finding in the field of using micro-RNAs as biomarkers in neurocritical care settings.

Copyright © 2020 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.