Blood biomarkers for evaluation of perinatal encephalopathy: state of the art

Ernest M. Graham\textsuperscript{a,b}, Allen D. Everett\textsuperscript{b,c}, Jean-Christophe Delpech\textsuperscript{d}, and Frances J. Northington\textsuperscript{b,e}

Purpose of review
The rapid progress in biomarker science is on the threshold of significantly changing clinical care for infants in the neonatal ICU. Infants with neonatal brain injuries will likely be the first group whose management is dramatically altered with point-of-care, rapidly available brain biomarker analysis. Providing an interim update on progress in this area is the purpose of this review.

Recent findings
Highlighted findings from the past 18 months of publications on biomarkers in neonatal brain injury include; Specific nonbrain markers of cardiac health and global asphyxia continue to provide information on brain injury after hypoxic–ischemic encephalopathy (HIE). Prediction of injury in the piglet hypoxia-ischemia model is improved with the use of a combination score of plasma metabolites. In a neonatal piglet model of perinatal hypoxia–ischemia, a systemic proinflammatory surge of cytokines has been identified after rewarming from therapeutic hypothermia. New biomarkers identified recently include osteopontin, activin A, neutrophil gelatinase-associated lipocalin, secretoneurin, Tau and neurofilament light protein. Brain-based biomarkers differ in their ability to predict short-term in-hospital outcomes and long-term neurologic deficits.

Summary
Neonatal brain biomarker research is currently in its very early development with major advances still to be made.

Keywords
cytokines, digital ELISA, metabolites, neonatal brain injury, outcomes

INTRODUCTION
Hypothermia is currently the only recognized standard of care treatment for moderate-to-severe perinatal hypoxic–ischemic encephalopathy (HIE), but about 45% of neonates have abnormal outcomes despite treatment [1]. Management of neonates with HIE is hindered by the lack of quantifiable biomarkers that could measure the degree of injury, assist in triage to therapy and give prognostic information. Currently, the diagnosis and prognosis of HIE in a neonate is based on clinical manifestations, imaging and electrophysiological monitoring [2]. MRI biomarkers for HIE have generally been performed several days following birth because of the lack of early sensitivity for hypoxic injury, and MRI requires that the infant be moved from the neonatal ICU (NICU) to the imaging suite, a task that can be difficult under certain circumstances. Both of these can be overcome with the use of head ultrasound (HUS) as an imaging biomarker [3]; however, HUS is not well tested nor universally accepted for this purpose because of lower sensitivity [4]. Biochemical evaluation of the severity of birth asphyxia has traditionally been performed using umbilical arterial blood gases at birth, but this test is poorly

\textsuperscript{a}Division of Maternal-Fetal Medicine, Department of Gynecology and Obstetrics, \textsuperscript{b}The Neuroscience Intensive Care Nursery program is multidisciplinary and contains members from numerous departments, \textsuperscript{c}Division of Cardiology, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, \textsuperscript{d}Department of Newborn Medicine, Boston Children’s Hospital, Harvard Medical School, Boston, Massachusetts and \textsuperscript{e}Division of Neonatology, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Correspondence to Ernest M. Graham, MD, Division of Maternal-Fetal Medicine, Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Phipps 228 GYN/OB, 600 North Wolfe Street, Baltimore, MD 21287, USA. Tel: +1 410 955-3187; e-mail: egraham5@jhmi.edu

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KEY POINTS

- Although new single-biomarker studies continue to emerge, utilizing metabolomic profiling to more comprehensively understand the effects of injury and treatment on entire pathways following neonatal brain injury may identify new therapeutic targets in neonatal brain injury.
- A number of biomarkers used to identify other conditions are now being used to identify neonatal neurologic injury. Markers of cardiac injury are increasingly useful in this respect.
- Cytokine biomarkers in a relevant preclinical model of HIE identify a proinflammatory surge during the rewarming period following therapeutic hypothermia. If confirmed, these studies may reveal an additional therapeutic target in neonatal HIE.
- Until recently, very few biomarker studies in neonatal brain injury included posthospital outcomes. Future studies will need to include these data to provide maximal information about their utility.

predictive of injury. Taken together, these constraints make long-term neurodevelopmental outcome difficult to predict in the first few days of life. Multiorgan involvement, although common when brain injury occurs, is not part of the diagnosis of HIE. In the presence of HIE, the most frequently injured organs are the heart, liver, kidneys and hematological system which may release organ-specific biomarkers into the blood which will have a role in the assessment of injury severity and long-term outcome [5*]. Although new single-biomarker studies continue to emerge, biomarker science is also utilizing metabolomic profiling to more comprehensively understand the effects of injury and treatment on entire pathways following neonatal brain injury [6**] and may likely unveil new therapeutic targets. The measurement of quantitative biomarkers that are able to detect subclinical lesions at a stage when routine brain monitoring or imaging is still silent would be a major advance in the care of neonates with suspected brain injury [5*]. Biomarkers could allow the screening of neonates for brain injury with high sensitivity and specificity, monitor the progression of injury and response to therapy through serial measurements due to their short half-life and correlate with the extent of brain lesions later seen on ultrasound or MRI [5*].

Other detailed reviews are available for brain biomarkers, and some are specific to the neonatal brain [7*,8*]. This review is meant to update very recent data on biochemical biomarkers related to hypoxic–ischemic brain injury in the newborn.

BIOMARKERS FOR OTHER CONDITIONS NOW BEING APPLIED TO HYPOXIC–ISCHEMIC ENCEPHALOPATHY

A number of biomarkers used to identify other conditions are now being used to identify neonatal neurologic injury. Cardiac biomarkers show good correlation with echo-derived markers of myocardial function, and a significant elevation of cord blood troponin has been found to be an excellent early predictor of severity of HIE and mortality in term infants [9**]. Dickkopf-1 is a regulatory antagonist in the Wnt family of glycoproteins which are involved in the signaling of embryonic development and numerous diseases [10*]. Dickkopf-1 is one of several mediators that are released from platelets on activation and is involved in platelet-mediated endothelial activation in patients with coronary artery disease. It is elevated in patients with stable and unstable angina pectoris. A study of 20 neonates with HIE compared with 20 controls found that Dickkopf-1 was released into the circulation of neonates with HIE and correlated with HIE severity which may allow it to be useful for prognosis [10*]. It appears from this report that therapeutic hypothermia was not used in the treatment of these infants; thus, cardiac depression from cooling should not have interfered with the interpretation. Phenobarbital was used liberally; however, there is no analysis for this as a confounder.

Post-hoc analysis of glycemic control in 234 neonates with moderate-to-severe HIE from the CoolCap study showed that both hypoglycemia and hyperglycemia were independently associated with unfavorable outcome. Any glucose derangement during the early postnatal period in infants with moderate-to-severe HIE was independently associated with an unfavorable outcome at 18 months, independent of severity of HIE and cooling therapy [11]. Lactate dehydrogenase (LDH), an enzyme that exists in all cells and is released into the plasma by cellular damage, has been investigated as an inexpensive and well tolerated prognostic biomarker in neonates with HIE [12*]. A study of 92 neonates with HIE found that although LDH levels did not differ for those with brain lesions on MRI, the change in LDH was significantly higher on day 3 of life in neonates with central gray matter lesions, which led these investigators to claim that changes in serum LDH may be a useful biomarker for predicting future neurodevelopmental prognosis in infants with HIE [12*].

METABOLIC PROFILING OF NEONATAL HYPOXIC–ISCHEMIC ENCEPHALOPATHY

When mass spectrometry was applied to provide metabolic profiles of brain tissue and plasma...
following an excitotoxic lesion to the neonatal mouse brain, it was found that a short list of amino acids and glycerophospholipids explained the effects of the excitotoxic lesion [13][21]. These investigators concluded that these targets may be useful starting points for studies on acute and tertiary phase biomarkers specific to the excitotoxic processes [13][21]. In a newborn piglet model of HIE, time-dependent metabolic biomarker profiles of choline, betaine, cytidine and uridine were observed to have patterns similar to lactate levels, which are currently considered the gold standard for assessing hypoxia, and they concluded that the prediction of injury could be improved with the use of a combination of plasma metabolites [14][22]. A metabolite score calculated based on the relative intensities of three metabolites (choline, 6,8-dihydroxypurine and hypoxanthine) was compared with lactate as a biomarker for the intensity and duration of perinatal hypoxia in a neonatal piglet model [15][23]. For plasma samples drawn before and directly after a hypoxic insult, the metabolite score performed similar to lactate, but it provided better predictive capacity at 2 h after resuscitation. Serial determinations of such a score in a minimally invasive bi-fluid may improve the early assessment of the severity of the hypoxic insult and assist in the diagnosis and triage to treatment [15][23]. In a nonhuman primate model using in-utero umbilical cord occlusion to induce HIE, 63 metabolites were examined as potential biomarkers of brain injury. Of this group, eight (arachidonic acid, botanic acid, citric acid, fumaric acid, lactate, maleate propanoic acid and succinic acid) were identified that correlated with early and/or long-term neurodevelopmental outcomes [16]. When hypoxia and hypotension were induced in newborn piglets, the plasma metabolome showed an increased plasma concentration of analytes reflecting a metabolic adaptation to prolonged anaerobiosis; however, after resuscitation, metabolite levels returned to the starting values [17][24]. Choline was the most significantly increased analyte during the hypoxic insult, and they concluded that these metabolites could have applicability in predicting the severity of hypoxia in the clinical setting [17][24].

**NEW BIOMARKERS FOR HYPOXIC–ISCHEMIC ENCEPHALOPATHY**

Recent studies have identified a number of biomarkers that may improve identification of neonatal neurologic injury in the period shortly after birth. Studies in 10-day-old mice reveal that hypoxia-ischemia significantly increases osteopontin, a finding not observed after lipopolysaccharide administration [21][25]. Osteopontin mRNA was induced in the brain but not in the blood, and immunostaining revealed osteopontin expression by microglia/macrophages in the hypoxia-ischemia-injured brain. They concluded that osteopontin may be a prognostic blood biomarker in HIE related to brain microglial activation, and that an increase of plasma osteopontin may indicate a perinatal event at least 24-h old. Therefore, it might be useful as a marker of prior intrauterine hypoxia-ischemia stress and a poorer response to hypothermia treatment [21][25]. Analysis of umbilical cord blood from 24 term neonates with perinatal hypoxia revealed a significant increase in activin A and neutrophil gelatinase-associated lipocalin (NGAL) compared with 34 healthy controls, with NGAL being a better marker of perinatal hypoxia [22][26]. In adults suffering from brain injury, secretoneurin is a promising early biomarker of poor outcome. Elevated levels of secretoneurin were similarly noted in cord blood from neonates with HIE following perinatal asphyxia [23][27]. Tau and neurofilament light protein levels are significantly elevated in cord blood after moderate–severe HIE and correlate...
with the severity of the insult [24]. Ubiquitin C-terminal hydrolase-L1, measured in the serum, is another potential prognostic biomarker for various forms of central nervous system injury, including neonatal HIE [25,26].

Oxidative stress is a crucial step in the development of HIE, and biomarkers such as lipid peroxidation products have been found to be increased in cord blood at delivery with severe metabolic acidosis [27]. Selenium is a constituent of the antioxidant enzyme glutathione peroxidase and is vital to antioxidant defense. Neonates with HIE have been found to have lower serum selenium levels than normal healthy neonates, independent of maternal levels, and selenium levels were negatively correlated with the severity of HIE [28].

NEW TECHNOLOGIES IN BIOMARKER DETECTION

The development of novel methodologies to detect brain injury proteins with improved sensitivity and speed may provide results that are clinically actionable. Breakthroughs in digital ELISA assays such as the Simoa platform (Quanterix, Lexington, Massachusetts, USA) allow femtogram/microliter detection of brain proteins such as Tau [29]. Advances are also being made in developing rapid detection biosensor platforms that may enable bedside detection of brain injury proteins. Organic field effect transistors have already demonstrated less than 3-min detection of glial fibrillary acidic protein (GFAP) [30,31]. Alternatively, silicon nanowires coated with detection antibodies also have the potential for protein detection within 2 min [32]. These platforms have the potential to revolutionize how we care for neonatal brain injury.

NEURODEVELOPMENTAL FOLLOW-UP FOR PREVIOUSLY STUDIED BRAIN INJURY BIOMARKERS

Only recently have studies evaluated neurodevelopmental follow-up associated with biomarkers found to be associated with neonatal brain injury during the perinatal period. In neonates born at more than 35 weeks with HIE, serum lactate levels measured after 72 h of whole body hypothermia correlated with neurodevelopmental outcome at 24-month corrected age [33]. Although IL-6 and IL-16 measured in umbilical cord blood at birth were correlated with HIE severity, IL-6 did not show any association with outcome at the age of 3 years [34]. IL-16 levels at birth were predictive of severe deficits, especially when used in combination with electroencephalogram findings [34].

Prior studies have shown GFAP measured in neonatal blood at 1–2 and 4–7 days of life (but not in cord blood) were associated with abnormal brain MRI at 7–10 days of life [35]. Furthermore, GFAP levels at time of NICU admission were significantly higher in those infants with feeding abnormalities at time of NICU discharge [35]. Chalak et al. [36] also demonstrated that GFAP in neonates with moderate-to-severe HIE was elevated at birth and associated with abnormal neurologic outcomes. Looney et al. [37] utilizing a different laboratory assay, noted that GFAP measured in cord blood at birth was not increased in those with brain injury compared with healthy controls, and no correlation was found between cord blood levels of GFAP and outcome at 36 months. These data point out the potential exquisite time dependence of the biomarker in relation to time of injury.

CONCLUSION

Given the variation of causes and symptoms at presentation of neonatal HIE, it is unlikely that a single early biomarker will be able to predict clinical outcomes in the perinatal period [8,15]. A panel of multiple inflammatory and neuronal biomarkers measured via a point of care bedside tool at various time points is likely to be the most accurate way to identify and assess the severity, timing and pattern of injury. Furthermore, full pathway analysis via various-omic strategies may identify new therapeutic targets for neonatal hypoxia-ischemia brain injury. Neonatal brain biomarker research is currently in its very early development with major advances still to be made.

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Conflicts of interest

A.D.E. is a consultant to Immumarray, Inc.

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

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sonography resistive indices as a prognostic tool in neonatal hypoxic–
ischemic encephalopathy before and after treatment with therapeutic hy-

Precooling and postcooling transfontanelle duplex brain sonographic resistive index values may be a useful prognostic tool, in conjunction with other clinical information, for the identification of hypoxic–ischemic encephalopathy (HIE).

4. Bano S, Chaudhary V, Garg UC. Neonatal hypoxic–ischemic encephalo-

The article reviews the etiopathophysiology and clinical manifestations of HIE, role of imaging in the management of the condition, patterns of brain injury in term and preterm neonates, the treatment and prognosis.

5. Saito A, Puchinotta F, Gazzolo F, et al. The potentials and limitations of

The review reports the potentials and limitations of biomarkers in predicting outcomes in hypoxic–ischemic encephalopathy complicated by perinatal asphyxia.

and pathway discovery in perinatal asphyxia and hypoxic–ischemic ence-

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33:580–600.

The review covers inflammatory serum biomarkers in the setting of birth asphyxia that can help assess the degree or severity of encephalopathy at birth and neurodevelopmental outcomes.


Significant elevation of troponin is an excellent early predictor of severity of HIE and mortality in term infants.

10. Albanna EA, Ahmed HS. Circulating Dickkopf-1 in hypoxic ischemic ence-

There is an increasing level of Dickkopf-1 released into the circulation of neonates with HIE with higher levels in severe injury.

associated with unfavourable outcome in infants with hypoxic ischaemic encepha-

The studies demonstrate metabolic profiling is a useful approach to identify acute and chronic treatments in an excised brain tissue. It provides a short list of biomarker targets with future potential in the hunt for identification of injury, stratification and possibly therapy.

12. Yum SK, Moon CJ, Youn YA, Sung IK. Changes in lactate dehydrogenase are
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The study demonstrates that metabolic profiling can be a useful approach to identify acute and chronic treatments in an excised brain tissue. It provides a short list of biomarker targets with future potential in the hunt for identification of injury, stratification and possibly therapy.


The study demonstrates the usefulness of choline and related metabolites for improving the early assessment of the severity of the hypoxic insult.


The study shows the usefulness of the metabolite score for improving the early assessment of the severity of the hypoxic insult based on serial determinations in a minimally invasive bioloid.


Severe hypoxia induces early, significant and transient changes of specific metabolites in the plasma metabolome, which represent a snapshot of the biochemical adaptation of mammals to intense hypoxia.

lome of newborn piglets subjected to postnatal hypoxia and resuscitation with

Severe hypoxia induces early, significant and transient changes of specific metabolites in the plasma metabolome, which represent a snapshot of the biochemical adaptation of mammals to intense hypoxia.

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Following hypoxia, there was a systemic proinflammatory surge after reawakening in the hypothermia group, which is counterevulsive to the putative neuroprotective effects of hypothermia.