Developmental Neuroscience

Review Article

Dev Neurosci
DOI: 10.1159/000522617

Received: October 30, 2021
Accepted: February 9, 2022
Published online: February 15, 2022

Prognostic Neurobiomarkers in Neonatal Encephalopathy

Guido Wassink Steven Harrison Simerdeep Dhillon Laura Bennet
Alistair Jan Gunn

The Department of Physiology, University of Auckland, Auckland, New Zealand

Keywords
Biomarkers · Neuromonitoring · Hypoxia-ischemia · Encephalopathy · Hypothermia

Abstract
Therapeutic hypothermia (TH) is now a standard treatment for infants with moderate-to-severe neonatal encephalopathy (NE), and improves brain damage on neuroimaging and neurodevelopmental outcomes. Critically, for effective neuroprotection, hypothermia should be started within 6 h from birth. There is compelling evidence to suggest that a proportion of infants with mild NE have material risk of developing brain damage and poor outcomes. This cohort is increasingly being offered TH, despite lack of trial evidence for its benefit. In current practice, infants need to be diagnosed within 6 h of birth for therapeutic treatment, compared to retrospective NE grading in the pre-hypothermia era. This presents challenges as NE is a dynamic brain disorder that can worsen or resolve over time. Neurological symptoms of NE can be difficult to discern in the first few hours after birth, and confounded by analgesics and anesthetic treatment. Using current enrolment criteria, a significant number of infants with NE that would benefit from hypothermia are not treated, and vice versa, some infants receive hypothermia when its benefit will be limited. Better biomarkers are needed to further improve management and treatment of these neonates. In the present review, we examine the latest research, and highlight a central limitation of most current biomarkers: that their predictive value is consistently greatest after most neuroprotective therapies are no longer effective.

Introduction

Perinatal brain injury from oxygen deprivation (hypoxia-ischemia, HI) at birth occurs in 1–4 live-born babies per 1,000 deliveries at term, and is a leading cause of neonatal encephalopathy (NE), and subsequent death or disability such as vision and hearing loss, and neurocognitive and behavioral impairments including cerebral palsy (CP) [1]. Further, infants with NE who do not develop CP still have lower IQ, perceptual reasoning, and verbal comprehension [2]. Therapeutic hypothermia (TH) is now routine care for infants with moderate-severe NE, to reduce death or disability [3], but needs to be started within 6 h from birth for effective neuroprotec-

Karger@karger.com
www.karger.com/dne

Correspondence to:
Guido Wassink, g.wassink@auckland.ac.nz

Dev Neurosci
DOI: 10.1159/000522617

Table 1. Studies of potential biochemical and neurophysiological markers of outcome

Markers	Design	Subjects	Finding	Correlated outcomes
Apgar score	Ancillary analysis of NICHD-NRN RCT on TH for NE [61]	174 TH and non-TH infants with SS grade II–III NE	Low 10-min (0–3) Apgar scores in 64/85 infants	Death or disability at 6–7 years of age on WPPSI-III [12], with OR of 0.68 (95% CI: 0.57–0.82) for each point increase in 10-min Apgar
Sarnat stage	Retrospective cohort, 2011–2013, National Asphyxia and Cooling Register, Switzerland	164 TH infants with SS grade II–III NE	102 (62%) infants had short-term improvement on SS, from admission to 4 days of life	OR of 0.118 (95% CI: 0.05–0.27) for disability at 18–24 months of age on BSID-II/III and GMDS [3]
Thompson score	Retrospective cohort, 2007–2011, University Ulm, Germany	33 TH infants with SS grade II–III NE	Increase in peak TS	OR of 1.5 (95% CI: 1.1–2.0) for death or cognitive impairment on WPPSI-III. Better prediction from 3 days of life [17]
Acid-base	Multicenter prospective cohort	230 infants with SS grade I-II-III NE	Increased BD in neonatal blood at 30–45 min after birth	Threshold of 14 mmol/L; predictive of SS grade II–III NE; sens 73.2% and spec 82% [24]
	*Prospective cohort	174 non-TH infants with NE	BD of 12–16 mmol/L and >16 mmol/L on cord blood at birth	Increased incidence of SS grade II–III NE and neonatal complications, respectively [23]
S100B	*Retrospective cohort, 1999–2001, Skåne University, Sweden	13 infants with SS grade II–III NE, 21 healthy infants	Increased S100B levels in cord blood at birth	SS grade of NE, aEEG abnormalities, acidosis, death or disability at 6 years of age [27]
	Prospective cohort	83 TH infants with SS grade II–III NE	Increased neonatal or cord blood S100B at 0–72 h of life	Brain injury on MRI, death or BSID-II MDI (OR 2.5 [95% CI: 1.3–4.8]) or PDI (OR 2.6 [95% CI: 1.2–5.6]) scores <70 at 15 months [108, 29]
	Prospective cohort	176 infants with SS grade I–II–III NE	Increased S100B in neonatal serum <6 h of birth	SS grade of NE (AUC = 0.73 for SS I/II, and 0.85 for SS II/III), NBNA scores <35 at day 7 (r −0.585, $p < 0.001$) [65]
NSE	Prospective cohort	43 TH infants with SS grade I–II–III NE	Increased NSE concentrations in CSF at 12–72 h of life	Seizures, abnormal aEEG and/or MRI, adverse outcomes at 2 years (death, CP, and/or BSID-III <70) (PPV 1.00 [95% CI 0.49, 1.00], NPV 0.96 [95% CI 0.69, -]) [28]
	Prospective cohort	83 TH infants with SS grade II–III NE	Increased NSE in neonatal or cord blood at 0–72 h of life	Brain injury on MRI, death or BSID-II MDI (OR 2.1 [95% CI: 1.2–3.6]) or PDI (OR 2.1 [95% CI: 1.2–3.6]) scores <70 at 15 months [108, 29]
	Prospective cohort	176 TH infants with SS grade I–II–III NE, 80 healthy infants	Increased NSE in neonatal serum <6 h of birth	SS grade of NE (AUC = 0.81 for SS I/II, and 0.89 for SS II/III), NBNA scores <35 at day 7 of life (r −0.61, $p < 0.001$) [65]
NfL	Brain injury biomarkers in newborns RCT [32]	37 TH infants with SS grade I–II–III NE	Increased NfL in neonatal plasma during and after TH	SS grade of NE, MRI brain injury, abnormal BSID-III at 2.7 years (after TH, median 98 h, NfL cut-off level >436 pg/mL, PPV 75%, NPV 77%) [31, 32]
Tau	Prospective cohort	103 TH infants with SS grade II–III NE	Increased tau protein in neonatal plasma at 72–96 h of life	MRI brain injury, death or development delay at 1 year of age on BSID-III (accuracy 81%, OR 2.6 [95% CI: 1.36–4.95]) [41]
	Prospective cohort	41 infants with NE, 35 healthy infants	Increased tau protein in neonatal serum <24 h of life	Abnormal GDS at 9 months of life (cut-off level 933.04 pg/mL, AUC 0.86 [95% CI: 0.74–0.98], sens 100%, spec 70.8%), SS grade of NE [38]
	Ancillary analysis of RCT of rEpo for neuroprotection in NE [109]	50 TH infants with SS grade II–III NE	Increased tau in neonatal serum <24 h and day 5 of life	Abnormal WIDEA at 1 year of age (r −0.24, $p = 0.05$), MRI global injury scores [39]
MCP-1	Ancillary analysis of NICHD-NRN RCT of TH for NE [61]	109 infants with SS grade II–III NE	Increased MCP-1 in neonatal plasma at day 7 of life	Death or disability on BSID-III at 6–7 years of age (OR 3.7, 95% CI 1.42–9.61) [62]

Dev Neurosci
DOI: 10.1159/000522617

Table 1 (continued)

Markers	Design	Subjects	Finding	Correlated outcomes
AvA	Prospective cohort	24 infants with PA, 34 healthy infants	Increased AvA concentrations on cord blood at birth	PA, with cut-off 0.208 ng/mL, sens 93.1%, spec 26.7% [110]
	Ancillary analysis of BiHiVE 2 RCT [66]	28 infants with PA, 23 infants with NE, 50 healthy infants	No differences in AvA and Acvr2b mRNA on cord blood at birth between groups	Not predictive of SS grade of NE or disability up to 36 months of age on BSID-III [36]
UCH-L1	Prospective cohort	41 infants with NE, 50 infants with PA, 40 healthy infants	Increased mUCH-L1 in cord blood at birth; no difference between NE and PA groups	Not predictive of NE [44]
	Prospective cohort	20 TH infants with SS grade II–III NE	Increased UCH-L1 in neonatal blood at start of TH and 72 h	Death or brain injury on MRI. Sens 75%, spec 100% at start of TH [29]
	Retrospective cohort, 1990–2001, Skåne University, Sweden	15 infants with SS grade II–III NE, 31 healthy infants	UCH-L1 in cord blood at birth; no difference between groups	Not predictive of SS grade of NE, abnormal EEG, death or deficits up to 6 years of age [42]
	*Prospective cohort	11 non-TH infants with SS grade II NE, 15 healthy infants	UCH-L1 in neonatal serum at 24–48 h of life; no difference between groups	No outcomes reported [43]
MDA	*Prospective cohort	40 infants with SS grade I–II–III NE, 40 healthy infants	Increased MDA in cord and neonatal blood at birth and at 48 h of life	Seizure burden, mortality [46]
	*Prospective cohort	20 infants with PA, 20 healthy infants	Increased MDA in cord blood at birth	SS grade of NE, mortality [47]
	Prospective cohort	31 infants with NE, 30 healthy infants	Increased MDA in neonatal blood at day 5 of life	SS grade of NE (r 0.466, $p = 0.008$) [49]
ISOPs	*Ancillary analysis of HYPOTOP RCT of topiramate plus TH for NE [111]	51 TH infants with SS grade II–III NE	Increased total isoprostanoids in neonatal urine <6 h, and at 12, 24, 48, 72, and 96 h of life	Brain injury on MRI [48]
SOD	Prospective cohort	31 infants with NE, 30 healthy infants	Increased SOD in neonatal blood at day 5 of life	SS grade of NE (r 0.506, $p = 0.004$) [49]
GFAP	Prospective cohort	23 infants with NE, 23 healthy infants	Increased GFAP in neonatal serum <6 h and first week of life	SS grade of NE, abnormal brain MRI (GFAP at admission; sens 50%, spec 84.6%, PPV 60%, NPV 78.6%) [54]
	Prospective cohort	103 TH infants with SS grade II–III NE	Increased GFAP in neonatal serum at 96 h of life (sens 31%, spec 96% for outcome)	Death or neurodevelopmental delay up to 2.5 years of age on BSID-III assessment [41]
	Prospective cohort	11 non-TH infants with SS grade II NE, 15 healthy infants	GFAP in neonatal serum at 24–48 h of life; no difference between groups	No outcomes reported [43]
	Prospective cohort	20 TH infants with SS grade II–III NE	Increased GFAP in neonatal blood at 24 and 72 h of life	Death or MRI brain injury (GFAP at 72 h; cut-off level of 0.2 ng/mL, sens 87.5%, spec 82%) [29]
	Ancillary analysis of BiHive RCT [66]	86 TH infants with SS grade I–II–III NE, 83 healthy infants	GFAP in cord blood at birth; no difference between groups	Not predictive of PA versus NE, SS grade of NE, eligibility for TH, or abnormal BSID-III outcome at 36 months of life [56]
	Retrospective cohort, 1990–2001, Skåne University, Sweden	15 infants with SS grade II–III NE, 31 healthy infants	GFAP in cord blood at birth; no difference between groups	Not predictive of NE, SS grade of NE, acidemia, abnormal EEG, or death or neurological sequelae up to 6 years of age [42]
Interleukins	Ancillary analysis of RCT of rEpo for neuroprotection in NE [109]	50 infants with SS grade II–III NE	Increased IL-1ß, IL-6, IL-8, IL-10, and IL-13 in neonatal serum at day 1 of life	Global brain injury on MRI (Spearman; IL-1ß = 0.1, IL-6 = 0.13, IL-8 = 0.25, IL-10 = 0.24, IL-13 = 0.1 [39]
	*Prospective cohort	82 infants with SS grade I–II–III NE, 12 healthy controls	Increased IL-8 in neonatal serum at days 1 and 2 of life	Death and SS grade II/III NE, respectively [60]
	Prospective cohort	103 TH infants with SS grade II–III NE	Increased IL-6, IL-8, IL-10 in neonatal plasma <24 h of life	Death or severe MRI brain injury (accuracy 78.7%, AUC 0.83) [41]

Dev Neurosci
DOI: 10.1159/000522617

Table 1 (continued)

Markers	Design	Subjects	Finding	Correlated outcomes
TNF-alpha	Ancillary analysis of NICHD-NRN RCT on TH for NE [61]	109 infants with SS grade II–III NE	Increased TNF-α in neonatal plasma at day 0–1 of life	No association with death or CP [62]
	Ancillary analysis of RCT of rEpo for neuroprotection in NE [109]	50 infants with SS grade II–III NE	Increased TNF-α in neonatal plasma on day 1 of life	Global brain injury on MRI (Spearman; 0.08) [39]
Interferon-γ	Ancillary analysis of RCT of rEpo for neuroprotection in NE [109]	50 infants with SS grade II–III NE	Increased IFNγ in neonatal plasma on day 1 of life	Global brain injury on MRI (Spearman; −0.10) [39]
GM-CSF	*Prospective cohort	82 infants with NE, 12 healthy infants	Increased GM-CSF in neonatal serum at day 1 and days 6–7 of life	Death and composite language BSID-III scores at 18–24 months of age, respectively [60]
RANTES	Ancillary analysis of NICHD-NRN RCT of TH for NE [61]	109 TH and non-TH infants with SS grade II–III NE	Lower RANTES in neonatal blood at days 0–7 of life	Death or disability at 6–7 years of life on BSID-III (OR 0.31 [95% CI: 0.13–0.74]), hearing loss, epilepsy [62]
miRNAs	Prospective cohort	167 TH infants with SS grade I–II–III NE, 82 healthy infants	Reduced miR-210 and miR-374a levels in cord blood at birth	SS grade of NE, NBNA scores at day 7 of life (miR-210, $r = 0.573$; miR-374a, $r = 0.651$) and GDS scores at 48 and 52 weeks of life [64]
	Prospective cohort	176 TH infants with SS grade I–II–III NE, 80 healthy infants	Reduced miR-199a levels in cord blood at birth	SS grade of NE, NBNA scores at day 7 ($r = 0.644$) and GDS scores at 48 ($r = 0.528$) and 52 weeks ($r = 0.509$) of life [65]
	*BiHiVe 2 RCT [66]	25 infants with SS grade I–II–III NE, 26 infants with PA, 22 healthy infants	Reduced miR-374a-5p, miR-376c-3p, and miR-181b-5p levels in cord blood at birth	Presence NE and/or PA, SS grade of NE, and eligibility for TH, respectively [44, 66]
	BiHiVe 1/2 RCT [66]	44 infants with SS grade I–II–III NE, 43 infants with PA, 37 healthy infants	Increased Fzd4 and Nfat5 mRNA levels in cord blood at birth	SS grade of NE and eligibility for TH (Fzd4 mRNA only), and adverse BSID-III outcomes (Nfat5; sens 71%, spec 76%, PPV 29%, NPV 95%, Fzd4; sens 71%, spec 90%, PPV 50%, NPV 96%) [67]
CK	*Prospective cohort	43 TH infants with SS grade I–II–III NE	Increased CK in neonatal serum at admission	SS grade of NE [73]
LDH	Prospective cohort	43 TH infants with SS grade I–II–III NE	Increased LDH in neonatal serum at day 3 of life	SS grade of NE [73]
ALT	Retrospective cohort, 2012–2014, Southmead Hospital, North Bristol NHS Trust, UK	79 infants with PA	Increased ALT in cord blood <6 h of birth	SS grade II/III NE (plus TH); AUC 0.78 (0.60–0.96) [71]
	*Retrospective cohort, 2006–2011, 4 NICUs in the UK	361 TH infants with SS grade I–II–III NE	Increased ALT in neonatal blood in first 7 days of life	SS grade of NE [70]
CRP	*Prospective cohort	225 infants with SS grade I–II–III NE	Increased CRP in neonatal blood in first 120 h of life	SS grade of NE [72]
	*Retrospective cohort, 2006–2011, 4 NICUs in the UK	361 TH infants with SS grade I–II–III NE	Increased CRP in neonatal blood in first 7 days of life, peaking at day 4	SS grade of NE [70]
Troponin	Retrospective cohort, 2012–2014, Southmead Hospital, North Bristol NHS Trust, UK	79 infants with PA	Increased Troponin T in cord blood <6 h of birth	SS grade II/III NE (with TH); AUC 0.81 (0.64–0.98) [71]
	Retrospective cohort, 2015–2020, Chung Shan University Hospital, Taiwan	73 TH infants with SS grade I–II–III NE	Increased Troponin I (level ≥180 pg/mL) in neonatal serum <6 h of birth	SS grade of NE, and abnormal BSID-III or death at 1 year of age (sens 34.8%, spec 89.5%, PPV 66.7%, NPV69.4%) [74]

Table 1 (continued)

Markers	Design	Subjects	Finding	Correlated outcomes
(a) EEG	Meta-analysis of 26 eligible studies (2010–2019) from Embase, Cochrane and Pubmed	111 (5 studies) TH infants with PA/NE	Abnormal EEG tracing, recording times not reported	Adverse neurological outcomes up to 18 months of age; EEG pooled sens 0.63 (95% CI: 0.49–0.76), spec 0.82 (95% CI: 0.70–0.91) [76]
	Meta-analysis of 26 eligible studies (2010–2019) from Embase, Cochrane and Pubmed	741 (9 studies) TH infants with PA/NE	Abnormal aEEG tracing, recording times not reported	Adverse neurological outcomes up to 18 months of age; aEEG background pattern pooled sens 0.90 (95% CI: 0.86–0.94), spec 0.46 (95% CI: 0.42–0.51) [76]
	Prospective cohort	74 TH and non-TH infants with SS grade II–III NE	aEEG pattern abnormalities at 3–6 h of age; PPV 84% for normothermia, 59% for TH. Abnormal voltage not predictive of outcome	Disability at 18 months of age on BSID-II, early aEEG pattern was predictive for infants with normal care but not TH [77]
	Meta-analysis of 9 eligible studies (2000–2014) from Medline, Cochrane, and CINAHL	520 TH infants with SS grade II–III NE	Abnormal aEEG pattern, pooled sens 96% (95% CI: 91–98), spec 39% (95% CI: 32–46) at 6 h. OR highest at 48 h; 66.9 (95% CI: 19.7–227.2)	Death or disability on BSID-II and GMFCS at 1 year of age, hearing and / or blindness. aEEG at 48 h predicted outcome; PPV of aEEG at 6 h was poor [112]
	*Multicenter prospective cohort (PRIME study)	63 non-TH infants with SS grade I NE	24/50 infants with normal early aEEG (at 5.5 h median age)	Abnormal MRI/neurological exam at discharge [9, 19]
MRS	Prospective cohort	223 TH infants with SS grade I–II–III NE	Reduced thalamic NAA at 7 days, sens 100% [95% CI 74–100], spec 97% [95% CI 90–100]	Adverse neurodevelopment at a median age of 23 months on BSID-III and GMFCS, hearing loss, blindness [91]
	*Prospective cohort	14 TH infants with SS grade II–III NE	Reduced basal ganglia NAA, NAAG, and GPC-PCh, and white matter GPC-PCh	SS grade of NE [93]
MRI	Prospective cohort	223 TH infants with SS grade I–II–III NE	Lower fractional anisotropy in the PLIC ($n = 65$) by DWI-MRI; AUC 0.92 (95% CI: 0.76–1.00)	Hearing loss, blindness, poor neurodevelopment at a median age of 23 months on BSID-III and GMFCS [91]
	Prospective cohort	54 TH infants with SS grade II–III NE	Lower ADC values in thalami (PPV 84.21, NPV 80) and the PLIC (PPV 91.18, NPV 75) at 4–6 days	Death or abnormal neurodevelopment on BSID-III and GMFCS at 2 years of age [97]
	Retrospective cohort, 2013–2017	107 TH infants with SS grade II–III NE	NICHD scoring; lesion size (AUC 0.718) and count (AUC 0.705) in the PLIC, basal ganglia, thalamus on DWI-MRI	Abnormal neurodevelopment at 18–24 months on BSID-III [99]
	Meta-analysis of 17 eligible studies (1990–2016) from Medline, Embase, PsycINFO, and Google Scholar	799 TH and non-TH infants with NE	Abnormal MRI at <4 weeks of life	OR 18.2 (95% CI: 9.4–34.9) for adverse neurodevelopment up to 12 months of age [98]

ADC, apparent diffusion coefficient; aEEG, amplitude-integrated EEG; ALT, alanine aminotransferase; AvA, activin A; AUC, area under curve; BD, base deficit; BiHiVE, Biomarkers in Hypoxic-ischemic Encephalopathy; BSID, Bayley Scales of Infant and Toddler Development; CI, confidence interval; CINAHL, Cumulative Index to Nursing and Allied Health Literature; CK, Creatine kinase; CRP, c-reactive protein; CSF, cerebrospinal fluid; DWI, diffusion-weighted imaging; EEG, electroencephalogram; GDS, Gesell Developmental Schedules; GFAP, Glial fibrillary acidic protein; GM-CSF, granulocyte-macrophage-colony-stimulating-factor; GMDS, Griffiths Mental Developmental Scales; GMFCS, Gross Motor Function Classification System; GPC + PCh, glycerophosphorylcholine + phosphatidylcholine; IL, interleukin; LDH, lactate dehydrogenase; IFNγ, interferon-gamma; ISOPs, isoprostanoids; MCP-1, monocyte chemotactic protein-1; MDA, malondialdehyde; MiRNA, microRNA; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAA, N-acetylaspartate; NAAG, NAA + N-acetylaspartylglutamate; NBNA, Neonatal Behavioral Neurological Assessment; NE, neonatal encephalopathy; NICHD-NRN, National Institute of Child Health and Human Development Neonatal Research Network; NfL, neurofilament light chain protein; NSE, neuron-specific enolase; OR, odds ratio; PA, perinatal asphyxia; PLIC, posterior limb of the internal capsule; PPV, positive predictive value; r = correlation; RANTES, regulated-upon-activation-normal-T-cell-expressed-and-secreted; RCT, randomized controlled trial; S100B, S100 calcium-binding protein-beta; sens, sensitivity; spec, specificity; SOD, superoxide-dismutase; SS, Sarnat stage; TH, therapeutic hypothermia; TNF-alpha, tumor necrosis factor-α; TOBY, Total Body Hypothermia for Neonatal Encephalopathy; TS, Thompson score; UCH-L1, ubiquitin C-terminal hydrolase L1; WIDEA, Warner Initial Developmental Evaluation of Adaptive and Functional Skills; WPPSI, Wechsler Preschool and Primary Scale of Intelligence. Studies in near-term or term infants; * studies without predictivity values.

tion. This corresponds with a latent phase after reperfusion from HI that lasts ~1–6 h, when brain metabolism transiently recovers but pathological intracellular mechanisms can be triggered, leading to mitochondrial dysfunction and delayed bulk cell death in the brain from ~12 to 72 h [4]. Interestingly, the rates of death or disability after TH were just 29% in a recent large controlled trial, compared with 44% in the first NICHD-NRN cooling trial [5]. This likely reflects multiple factors, including better monitoring, greater care to avoid pyrexia, a higher proportion of neonates with moderate NE being cooled now, greater experience with TH for these sick infants and related clinical care, and earlier initiation of TH.

Critically, the need to start TH as soon as possible from birth means that rapid identification of infants with NE has become a pressing clinical issue. Before TH, the grading of NE was made in retrospect with serial neurological assessments in the first week of life, typically using Sarnat staging, adjunct electroencephalograph (EEG) monitoring, and magnetic resonance imaging (MRI) [6]. Instead, clinical assessment of possible NE is now conducted within 6 h from birth, in order to start cooling infants as early as possible within the therapeutic window for neuroprotection. This is challenging, as NE is a dynamic brain disorder that evolves over time [7]. Indeed, a small retrospective study showed that 7 of 11 of infants diagnosed with mild NE within 6 h of birth progressed to moderate NE later [8]. However, there is compelling evidence that a proportion of infants with mild NE have high risk of poor neurodevelopmental outcomes [9]. In response, there has been therapeutic drift such that ~38% of these neonates are now offered TH [10], despite lack of evidence from clinical trials. An ideal biomarker would establish the severity of NE, and determine long-term prognosis in the first hours after birth. In addition, it will inform on response to treatment for clinical decision-making. In this review, we synthesize the latest literature in the field, and discuss the merits and limitations of prominent tools and potential biomarkers for predicting outcome. All studies that were sourced for this review are detailed in Table 1, including estimates of predictive value.

Apgar Score

The Apgar clinical assessment of infants' condition at birth is routinely performed in neonatal care, and most studies have used it as a pre-qualification criterion for TH. This score mainly reflects the extent and duration of resuscitation. The score is associated with outcome, but at 5 min has low sensitivity and specificity [11]. Historically, need for continuing resuscitation at 10 min was associated with worse outcomes. In the NICHD-NRN controlled cooling trial, low 10-min Apgar scores (0–3) were associated with death or disability in 64/85 (75%) infants. Nevertheless, ~21% of infants with 10-min Apgar scores of 0 survived without disability to school age [12]. Critically, the risk of death or disability was significantly lower in cooled infants with Apgar scores of 0–3, showing that prognosis of infants with even very prolonged resuscitation is improving. By comparison, profoundly depressed 10-min Apgar scores are uncommon in infants with mild NE at birth [13].

Neurological Exam

Modified Sarnat and Thompson scoring (SS and TS) of infant's neurological state are very helpful to categorize severity of neurological dysfunction into 3 stages of NE; mild (I), moderate (II), and severe (III) NE. SS is the predominant tool for infant qualification for TH [14], whereas the TS is popular in resource-limited settings, and used as adjunct to SS to confirm NE grading or as a qualifying factor for TH [15, 16]. Short-term improvement in SS or TS are predictive of long-term outcomes [17, 18], and efforts are being made to further refine SS criteria [6].

Nevertheless, the existing methods can be pragmatically useful for identifying risk of disability. In the PRIME study, a recent multicenter, prospective study of untreated mild NE defined as one or more abnormalities on the modified Sarnat examination within 6 h of birth but not enough to meet criteria for cooling, 7/43 (16%) infants had disability at 18–22 months [19]. Only one infant had CP and 2 had autism. Notably, 17/43 had Bayley scores <85 in one or more of the cognition, motor, or language domains. The median total Sarnat score (the sum of the subcategories of the modified Sarnat examination) was greater in infants who went on to develop disability than those who did not (median 7 [IQR, 5–7] vs. median 4 [IQR, 2–5]) [20]. Although not perfect, a total Sarnat score of ≥5 predicted risk of disability with an area under the curve of 0.83 ($p = 0.004$).

Acid-Base Balance

The most common biomarkers for infants with hypoxic-ischemic NE are pH and base deficit (BD) obtained from cord or neonatal blood. Clinical guidelines for TH

generally recommend prequalification cut-off values of a pH <7.0 or a base deficit of ≥16 mM. Population-based studies indicate that raising the threshold pH <7.1 is less specific [21, 22], but would capture more infants with moderate to severe NE, and presumptively more mild NE [7]. Although the extremes of BD measurement (e.g., BD >18 mM or conversely BD <10–12 mM) correlate with high and low risk of NE, intermediate BD values have variable outcomes [23, 24].

Neurobiochemical Markers

Neurobiochemical markers have been of particular interest for prediction in infants with NE [25]. Most of these biomarkers are structural proteins that provide neuronal and axonal support, and traumatic events that compromise neuronal integrity can trigger their release into interstitial cell fluid, cerebrospinal fluid (csf) and the circulation [25, 26]. Multiple markers have been examined in animal and clinical studies. The focus of this review is on the latest clinical research of bedside biomarkers of outcome.

S100B, NSE, and NfL Neuroproteins

S100 calcium-binding protein-beta (S100B) is expressed in astroglial and certain neuronal populations, and neurofilament light chain protein (NfL) and neuron-specific enolase (NSE) are a protein filament and glycolytic enzyme found in the neuronal cytoplasm. Small cohort studies in normothermia and hypothermia-treated infants show these protein concentrations become elevated in NE and correlate with outcomes [25]. For example, higher S100B levels in cord blood at birth from infants (n = 13) with moderate-severe NE correlated with acidosis, amplitude-integrated EEG (aEEG) pattern and NE severity, and death or disability at 6 years of follow-up, compared to healthy (n = 21) infants [27]. Elevated CSF-NSE levels <72 h of birth were predictive of seizure burden and abnormal findings in aEEG, MRI, and neurodevelopment in infants with mild-severe NE, with the most accurate CSF-NSE cut-off level for poor outcome in survivors being 108 ng/mL compared with 50 ng/mL in normal infants [28]. In a clinical trial of moderate-severe NE term infants, serum S100B and NSE concentrations during whole-body cooling (at 0, 12, 24, and 72 h) also correlated with basal ganglia, global and cortical damage on MRI (at 7–10 days), and short-term neurological outcomes [29]. Overall, the findings are consistent between studies [25, 30].

Plasma NfL levels after TH had started, and before and after rewarming, were higher in cooled infants with moderate-severe NE and unfavorable outcome on neuroimaging than in cases with mild NE and cooled infants that had a favorable outcome, with a cut-off level >29 pg/mL at 24 h being predictive of unfavorable outcome (sensitivity 77%, specificity 69%) [31, 32]. Further, cord blood NfL and Tau protein levels were elevated in term infants with birth asphyxia, although not just in infants that developed NE [33]. Other potential biomarkers include monocyte chemotactic protein-1, osteopontin, secretoneurin, Activin A (AvA), and neuronal exosomes [34, 35]. AvA is a member of the transforming growth-factor B super-family (TGF-B) with increased csf, serum, and urine levels in infants with moderate-to-severe NE compared with mild NE and healthy infants [30]. However, recent findings suggest that cord blood AvA protein and Acvr2b mRNA are not reliable biomarkers [36].

Tau Protein, Ubiquitin C-terminal Hydrolase L1, and Oxidative Stress Markers

Tau protein is expressed in neurons, astrocytes, and oligodendrocytes, and is a known neurobiomarker for neurodegenerative diseases. It has a critical role in microtubule stabilization, and so contributes to cell signaling, and synaptic and genomic regulation [26]. Common tauopathies such as Alzheimer's and Pick's disease are characterized by pathological deposition of tau protein in the brain [37]. Recent studies suggest that tau protein also has predictive value after perinatal HI. Plasma and serum tau protein levels within 24 h of birth were higher in infants with severe NE than infants with moderate NE, and in cases with poor neuroimaging findings and neurodevelopmental delay [38, 39]. Interestingly, lower serum tau correlated with improved short-term behavioral scores in NE infants treated with rEpo (200 IU/kg, daily from 2 to 10 days of birth) plus therapeutic cooling, compared with infants treated with hypothermia alone [40]. Finally, in cooled infants, tau protein was a late predictive marker (72–96 h) for brain damage on MRI and adverse neurological outcome [41]. Ubiquitin C-terminal hydrolase L1 is a neuron-specific, deubiquitinating enzyme that facilitates removal of pathological proteins, which helps maintain neuronal and axonal health. Like tau protein, its expression is increased after brain trauma in adult and pediatric patients. However, overall, recent neonatal evidence on outcome prediction has been disappointing [42–44].

Reactive oxygen species increase during and after HI, and can overwhelm neonatal antioxidant mechanisms

and lead to oxidative stress and brain damage [4]. Thus, oxidative stress markers may be good biomarkers for prediction of outcome [45]. In infants with perinatal asphyxia, malondialdehyde (MDA) and protein carbonyl concentrations at birth and at 48 h of life correlated with seizure burden, mortality, and degree of NE (SS grade I vs. II vs. III), but not developmental delay [46, 47]. Changes before, during, and after TH in lipid peroxidation markers such as total isoprostanoids and 15(RS)-15-F_{2t}-IsoP also correlated with lesions on MRI (at 4–8 days) in infants with NE [48]. Further, superoxide-dismutase and MDA levels were elevated at 6–14 h in infants with stage II–III NE, whereas vitamin D3 was lower compared with healthy control infants [49].

Consistent with this, serum selenium, a glutathione peroxidase constituent that is vital to anti-oxidant function, was reduced within 48 h in newborns with NE, and lowest in patients with severe NE [50]. Of note, elevated total hydroperoxides were reduced in hypothermia-treated infants with NE compared with infants that received normothermia [51]. Superoxide-dismutase and MDA levels also recovered during head cooling for 72 h, and were associated with greater scores on the Neonatal Behavioral Neurological Assessment (NBNA) at 3, 7, and 10 days of life, and Bayley-III scores at 8 months of age [52]. Of course, this functional improvement likely reflects the broad protective effects of hypothermia, rather than being mediated by anti-oxidant effects. A large prospective clinical trial in infants with grade II–III NE is recruiting (clinical trials identifier: NCT03162653) that will assess the effects of the oxygen free radical scavenger, allopurinol, in addition to TH on death or disability at 2 years of age, aEEG, and brain injury on MRI and ultrasound [53]. Peroxidation products in blood will also be measured as a marker of brain damage.

GFAP and Inflammation Markers

Glial fibrillary acidic protein (GFAP) is the main intermediate filament protein that supports the astroglial cytoskeleton. It is specific to the central nervous system and is released after HI. Evidence that it can predict neurological outcome in neonates is mixed, as reviewed [26, 30]. For example, serum GFAP concentrations were elevated within 6 h of birth and in the first week of life in infants with grade II–III NE and abnormal MRI findings [54]. Supporting this, increased serum GFAP levels were found at 24 and 72 h of life in infants who either died or had unfavorable outcome on neuroimaging (at 5–14 days), with a sensitivity of 87.5% and specificity of 82% at rewarming from TH [55]. By contrast, there was no difference in cord blood GFAP levels within 3 h of birth between infants born with perinatal asphyxia and NE (n = 86) and infants who had uncomplicated births (n = 83) [42, 56]. Serum GFAP concentration also was not predictive in infants with NE before (<6 h of life) or during TH, or in NE infants who did not qualify for TH [41, 43, 57]. Overall, these findings suggest that in isolation serum GFAP is not a reliable marker for guiding treatment or prognosis after NE.

There is consistent evidence that perinatal HI increases cytokine expression in the developing brain and circulation. Elevated levels of tumor necrosis factor-alpha, interferon-γ, and various interleukins (IL) such as IL-1ß, IL-6, IL-8, IL-10, and IL-13 have been reported in infants with NE, and shown to correlate with outcome measures [39, 58]. For example, increased plasma IL-6, 8, and 10 concentrations within 24 h of life predicted death or severe MRI-defined brain damage (at 4–7 days) in infants with NE who underwent TH. The combination of these biomarkers were more predictive than each of these cytokines alone, with an accuracy of ~78% [41]. In support of this, Pang et al. [59] also reported increased IL-6 and 10 levels in infants with NE compared with non-NE controls (159 vs. 157 infants), with IL-10 elevation within 12 h of birth being predictive of NE severity, mortality, and early childhood outcomes (OR 2.28, 95% CI: 1.35–3.86) [59]. In term infants with NE, serum IL-8 levels on days 1 and 2 of life also predicted infant death and NE grade II–III, whereas changes at 2 and 6–7 days of life in granulocyte-macrophage-colony-stimulating-factor were associated with abnormal MRI findings and Bayley-III scores at 2 years [60].

Finally, in a follow-up study of the NICHD randomized controlled trial of TH for NE [61], regulated-upon-activation-normal-T-cell-expressed-and-secreted and monocyte chemotactic protein-1 levels were associated with death or impairment (OR 0.31, 95% CI: 0.13–0.74, and OR 3.70, 95% CI: 1.42–9.61). Increased tumor necrosis factor-alpha levels in the first day of life were found in infants that died or developed CP [62]. Overall, these findings indicate that certain cytokines can be useful biomarkers for prognosis, but that most cytokine changes occur too late for potential neuroprotection therapies to be viable.

microRNAs and Transcriptional Markers

Single-stranded microRNAs (miRNA) are non-coding ribonucleic acid fragments that are ~22 nucleotides in length, and silence gene expression to inhibit protein synthesis. Recent research has implicated miRNAs in

pathological processes after perinatal HI [63]. For example, changes in hsa-miR-374a, miR-210, and miR-199 correlated with NE severity and neurological outcomes [64, 65]. Supporting this, in the Biomarker in Hypoxic-Ischemic Encephalopathy clinical trial, miR-374a-5p, miR-376c-3p, and miR-181b-5p expressions were associated with perinatal asphyxia and TH eligibility [66]. Finally, cord blood Fzd4 and Nfat5 mRNA were predictive of NE severity (SS III vs. I and II) and long-term functional outcomes [67]. Interestingly, miR-210 antagonist treatment reduced microglial inflammation and brain infarction in post-hypoxic neonatal rats [68], suggesting that miRNAs after perinatal HI are promising biomarkers and potential therapeutic targets.

Other Metabolic Markers

Multiple organ dysfunction is almost universal in infants born with NE, with high correlation between severity of NE and organ impairment during the first 3 days of life [69]. Liver enzymes, myocardial proteins, and other markers that assess end-organ damage are frequently measured in neonatal blood and may support outcome prediction. Changes in creatine and creatine kinase, lactate dehydrogenase, alanine aminotransferase, and c-reactive protein have all shown predictive value in the first 3 days of life [70–73], whereas Troponin-I levels within 6 h of birth distinguished mild NE from moderate-to-severe NE [74].

Electrophysiological Monitoring

aEEG and conventional video-EEG have a fundamental role in the monitoring and management of babies with NE. Although video-EEG is the gold standard for seizure detection, aEEG has become the method of choice for most neonatal centers as it is simpler to implement [75]. In a recent meta-analysis of 26 studies, including 1,458 near-term or term infants, multichannel EEG demonstrated sensitivity of 0.63 (95% CI: 0.49–0.76), specificity of 0.82 (95% CI: 0.70–0.91), and area under the curve (AUC) of 0.88, and for aEEG background pattern pooled sensitivity, specificity and AUC were 0.90 (95% CI: 0.86–0.94), 0.46 (95% CI: 0.42–0.51), and 0.78, respectively, for predicting an unfavorable neurological outcome in infants with NE treated with TH [76]. The times of monitoring in these studies were not reported; however, previous evidence shows that the positive predictive value of an abnormal aEEG is much less at 3–6 h after birth in TH-treated infants than normothermic infants [77], presumptively due to neuroprotection after hypothermia [78]. Moreover, aEEG monitoring has limited predictivity <6 h for mild NE [9]. Continuous and Discontinuous Normal Voltage were the predominant background patterns [73, 79], although excessive discontinuity and disrupted sleep-wake cycling are also common in these infants [79, 80]. By contrast, conventional EEG has higher accuracy, but requires resources and expertise that may not always be available in NICUs [81].

These limitations have renewed interest in novel EEG-derived biomarkers. Evoked potentials have been noted for their usefulness in improving prognosis in small cohorts of infants that received TH [82], and can be measured with standard aEEG setups [83]. Other EEG features of recent interest for prediction are cortical power bursts [84], spectral power and micro-scale transients [85–87], and neurovascular coupling [88] in moderate-to-severe NE, and sharp and diffuse delta waves, mild asymmetry, and spectral edge frequency in mild NE [7, 80].

Modern Neuroimaging

Phosphorus-31 (^{31}P) and proton (^{1}H) magnetic resonance spectroscopic (MRS) measurements in NE neonates are highly predictive of outcome [89–92]. In a prospective trial in term infants ($n = 223$) treated with TH for NE, thalamic N-acetylaspartate (NAA) levels obtained with ^{1}H-MRS at a median age of 7 days (IQR; 5–10) had higher accuracy (AUC 0.99, 95% CI: 0.94–1.00) than other clinical measures in predicting neurological outcome at 18–24 months of age [91]. In ^{1}H-MRS conducted 18–24 h after initiating TH, infants with severe NE also had lower basal ganglia NAA (0.62 ± 0.08 vs. 0.72 ± 0.05), NAA + N-acetylaspartylglutamate (NAAG; 0.66 ± 0.11 vs. 0.77 ± 0.06), and glycerophosphorylcholine plus phosphatidylcholine (GPC + PCh; 0.28 ± 0.05 vs. 0.38 ± 0.06) than infants with moderate NE [93].

MRI including diffusion-weighted MRI in the first week of life is the preferred method in most centers to assess HI brain injury [94]. Signal abnormalities on conventional T1/T2 weighted images are common in parasagittal regions and white matter, and basal ganglia and thalami in infants with moderate-to-severe NE. In a retrospective cohort analysis of 89 infants who had received TH, there was no difference in the overall rate of MRI injury by grade of NE (Barkovich classification; mild NE 54%, moderate NE 54%, severe NE 50%; $p = 0.89$), although basal ganglia/thalamic lesions were more com-

mon in severe NE ($p = 0.03$) [95]. However, infants with mild NE and subtle MRI abnormalities may benefit from more detailed scoring systems [96]. Signal hyperintensity in the posterior limb of the internal capsule (PLIC), thalami, and basal ganglia in particular have high predictive value for adverse outcomes [97–99], even after TH [100].

The timing of imaging is important for prognostication. Late MRI scans at a median age of 8 days in TH-treated infants had 95% sensitivity, 94% specificity, and 91% positive predictive value for death or disability at 2 years of age [101]. By contrast, cell death is still developing in the first days of life and so early brain imaging can underestimate the severity of injury [102]. In 12 asphyxiated term infants, MRI during TH at 2–3 days of life was predictive of brain lesions at 8–13 days [103]. However, visual and quantitative evaluation were required to determine basal ganglia abnormalities [104]. Diffusion-weighted and conventional MRI also have high correlation at day 4 and 2 weeks after birth [105]. Other techniques such as diffusion tensor imaging and tractography are more advanced and may identify brain lesions earlier [94]. Cranial ultrasound and Doppler ultrasonography are adjunct technologies for diagnostic imaging, and have limited prognostic value [58]. Overall, MRI imaging is a fundamental tool for prognosis and informing clinicians on treatment response; however, it is not yet useful for guiding early neurotherapy.

Some Reflections on Biomarkers

Biomarkers can be helpful in a variety of settings, including initial recruitment for treatment within 6 h of birth, determining whether infants are responding during treatment, and finally, assessment of prognosis after the end of treatment for parental counseling and enrolment with developmental support services. Each goal has different constraints. Modern imaging and EEG have strong, established, albeit not perfect, prognostic value after the end of treatment, with a relatively wide window in which testing is reliable.

By contrast, determining prognosis shortly after perinatal HI is critical to guide clinicians in whether or not the infant should be offered potentially neuroprotective therapies such as TH. There is only a brief window for clinical decision-making and thus an optimal biomarker must be accessible and rapidly and accurately measurable. CSF biomarkers are invasive, and so not practical since most infants would not otherwise require a lumbar puncture. Similarly, urine markers will be unreliable for targeting TH in infants who commonly have oliguria due to acute kidney injury after global HI, although they may be useful for assessing treatment response and outcome prediction. Moreover, it is well known that NE evolves over time, with corresponding changes in the infant's Sarnat stage. The pathological brain processes that occur after perinatal HI are associated with changes in biomarker response. Thus, the timing of biomarker acquisition is critical, and both their robustness and value likely depend on this. Finally, it is important to appreciate that most biomarkers have been tested within well-defined cohorts. It is likely that their predictive value would be attenuated in more diverse cohorts. Given these issues, it is not surprising that only clinical examination and EEG monitoring are widely used at present.

Conclusion

This short review of the literature shows that at present none of the proposed biomarkers has been established to be better than clinical assessment of NE for identifying infants who are likely to benefit from neuroprotective treatments. There is still only emerging evidence for biomarkers to identify mild NE, and so profiling biomarkers relative to the deleterious processes that underlie mild NE is an important area of future research. Critically, even the most promising markers show predictive values at time-points that are too late (>6 h from birth) for optimal therapeutic interventions. Speculatively, a combination of multiple modalities may offer better prognostication. Such neonatal scoring systems have shown promise in early studies, and their predictive value can likely be further improved through machine learning and deep learning algorithms to better support clinical decision-making [106, 107].

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

The authors' work reported in this review was supported by grants from the Health Research Council (HRC) of New Zealand (17/601), the Auckland Medical Research Foundation, Cure Kids, the Neurological Foundation of New Zealand, and Lottery Health Research.

Author Contributions

G.W. wrote the first draft of the MS. All authors contributed to identification of studies and writing and revising the MS.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Campbell H, Eddama O, Azzopardi D, Edwards AD, Strohm B, Rivero-Arias O. Hypothermia for perinatal asphyxia: trial-based quality of life at 6–7 years. Arch Dis Child. 2018;103(7):654–9.
2 Chakkarapani E. Cognitive and behavioural outcomes: are they impaired in children without cerebral palsy following neonatal hypoxic-ischaemic encephalopathy? Acta Paediatr. 2020;109(1):11–3.
3 Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. Cochrane Database Syst Rev. 2013;1:CD003311.
4 Wassink G, Davidson JO, Dhillon SK, Zhou K, Bennet L, Thoresen M, et al. Therapeutic hypothermia in neonatal hypoxic-ischemic encephalopathy. Curr Neurol Neurosci Rep. 2019;19(2):2.
5 Shankaran S, Laptook AR, Pappas A, McDonald SA, Das A, Tyson JE, et al. Effect of depth and duration of cooling on death or disability at age 18 months among neonates with hypoxic-ischemic encephalopathy: a randomized clinical trial. JAMA. 2017;318(1):57–67.
6 Sarnat HB, Flores-Sarnat L, Fajardo C, Leijser LM, Wusthoff C, Mohammad K. Sarnat grading scale for neonatal encephalopathy after 45 years: an update proposal. Pediatr Neurol. 2020;113:75–9.
7 McDouall A, Wassink G, Bennet L, Gunn AJ, Davidson JO. Challenges in developing therapeutic strategies for mild neonatal encephalopathy. Neural Regen Res. 2022;17(2):277–82.
8 Perretta L, Reed R, Ross G, Perlman J. Is there a role for therapeutic hypothermia administration in term infants with mild neonatal encephalopathy? J Perinatol. 2020;40(3):522–9.
9 Prempunpong C, Chalak LF, Garfinkle J, Shah B, Kalra V, Rollins N, et al. Prospective research on infants with mild encephalopathy: the PRIME study. J Perinatol. 2018 Jan;38(1):80–5.
10 Shipley L, Gale C, Sharkey D. Trends in the incidence and management of hypoxic-ischaemic encephalopathy in the therapeutic hypothermia era: a national population study. Arch Dis Child Fetal Neonatal Ed. 2021;106(5):529–34.
11 Ahearne CE, Boylan GB, Murray DM. Short and long term prognosis in perinatal asphyxia: an update. World J Clin Pediatr. 2016;5(1):67–74.
12 Natarajan G, Shankaran S, Laptook AR, Pappas A, Bann CM, McDonald SA, et al. Apgar scores at 10 min and outcomes at 6–7 years following hypoxic-ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed. 2013;98(6):F473–9.
13 Goswami IR, Whyte H, Wintermark P, Mohammad K, Shivananda S, Louis D, et al. Characteristics and short-term outcomes of neonates with mild hypoxic-ischemic encephalopathy treated with hypothermia. J Perinatol. 2020;40(2):275–83.
14 Chansarn P, Torgalkar R, Wilson D, Fan CS, Widjaja E, Whyte H, et al. Correlation of Thompson and modified Sarnat scores in neonatal hypoxic ischemic encephalopathy. J Perinatol. 2021;41(6):1522–3.
15 Weeke LC, Vilan A, Toet MC, van Haastert IC, de Vries LS, Groenendaal F. A comparison of the Thompson encephalopathy score and amplitude-integrated electroencephalography in infants with perinatal asphyxia and therapeutic hypothermia. Neonatology. 2017;112(1):24–9.
16 Mrelashvili A, Russ JB, Ferriero DM, Wusthoff CJ. The Sarnat score for neonatal encephalopathy: looking back and moving forward. Pediatr Res. 2020;88(6):824–5.
17 Mendler MR, Mendler I, Hassan MA, Mayer B, Bode H, Hummler HD. Predictive value of Thompson-score for long-term neurological and cognitive outcome in term newborns with perinatal asphyxia and hypoxic-ischemic encephalopathy undergoing controlled hypothermia treatment. Neonatology. 2018;114(4):341–7.
18 Grass B, Scheidegger S, Latal B, Hagmann C, Held U, Brotschi B, et al. Short-term neurological improvement in neonates with hypoxic-ischemic encephalopathy predicts neurodevelopmental outcome at 18–24 months. J Perinat Med. 2020;48(3):296–303.
19 Chalak LF, Nguyen KA, Prempunpong C, Heyne R, Thayyil S, Shankaran S, et al. Prospective research in infants with mild encephalopathy identified in the first six hours of life: neurodevelopmental outcomes at 18–22 months. Pediatr Res. 2019;85(4):442–8.
20 Chalak LF, Adams-Huet B, Sant'Anna G. A total Sarnat score in mild hypoxic-ischemic encephalopathy can detect infants at higher risk of disability. J Pediatr. 2019;214:217–e1.
21 Yeh P, Emary K, Impey L. The relationship between umbilical cord arterial pH and serious adverse neonatal outcome: analysis of 51,519 consecutive validated samples. BJOG. 2012;119(7):824–31.
22 Vesoulis ZA, Liao SM, Rao R, Trivedi SB, Cahill AG, Mathur AM. Re-examining the arterial cord blood gas pH screening criteria in neonatal encephalopathy. Arch Dis Child Fetal Neonatal Ed. 2018;103(4):F377–F82.
23 Low JA, Lindsay BG, Derrick EJ. Threshold of metabolic acidosis associated with newborn complications. Am J Obstet Gynecol. 1997;177(6):1391–4.
24 Wayenberg JL. Threshold of metabolic acidosis associated with neonatal encephalopathy in the term newborn. J Matern Fetal Neonatal Med. 2005;18(6):381–5.
25 Chalak L, Hellstrom-Westas L, Bonifacio S, Tsuchida T, Chock V, El-Dib M, et al. Bedside and laboratory neuromonitoring in neonatal encephalopathy. Semin Fetal Neonatal Med. 2021;26(5):101273.
26 Murray DM. Biomarkers in neonatal hypoxic-ischemic encephalopathy-review of the literature to date and future directions for research. Handb Clin Neurol. 2019;162:281–93.
27 Zaigham M, Lundberg F, Olofsson P. Protein S100B in umbilical cord blood as a potential biomarker of hypoxic-ischemic encephalopathy in asphyxiated newborns. Early Hum Dev. 2017;112:48–53.
28 Leon-Lozano MZ, Arnaez J, Valls A, Arca G, Agut T, Alarcon A, et al. Cerebrospinal fluid levels of neuron-specific enolase predict the severity of brain damage in newborns with neonatal hypoxic-ischemic encephalopathy treated with hypothermia. PLoS One. 2020;15(6):e0234082.
29 Massaro AN, Chang T, Baumgart S, McCarter R, Nelson KB, Glass P. Biomarkers S100B and neuron-specific enolase predict outcome in hypothermia-treated encephalopathic newborns*. Pediatr Crit Care Med. 2014;15(7):615–22.
30 Bersani I, Pluchinotta F, Dotta A, Savarese I, Campi F, Auriti C, et al. Early predictors of perinatal brain damage: the role of neurobiomarkers. Clin Chem Lab Med. 2020;58(4):471–86.
31 Shah DK, Ponnusamy V, Evanson J, Kapellou O, Ekitzidou G, Gupta N, et al. Raised plasma neurofilament light protein levels are associated with abnormal MRI outcomes in newborns undergoing therapeutic hypothermia. Front Neurol. 2018;9:86.
32 Shah DK, Yip PK, Barlas A, Tharmapoopathy P, Ponnusamy V, Michael-Titus AT, et al. Raised plasma neurofilament light protein levels after rewarming are associated with adverse neurodevelopmental outcomes in newborns after therapeutic hypothermia. Front Neurol. 2020;11:562510.
33 Toorell H, Zetterberg H, Blennow K, Sävman K, Hagberg H. Increase of neuronal injury markers Tau and neurofilament light proteins in umbilical blood after intrapartum asphyxia. J Matern Fetal Neonatal Med. 2018;31(18):2468–72.
34 Graham EM, Everett AD, Delpech JC, Northington FJ. Blood biomarkers for evaluation of perinatal encephalopathy: state of the art. Curr Opin Pediatr. 2018;30(2):199–203.

35 Pineles B, Mani A, Sura L, Rossignol C, Albayram M, Weiss MD, et al. Neuronal exosome proteins: novel biomarkers for predicting neonatal response to therapeutic hypothermia. Arch Dis Child Fetal Neonatal Ed. 2022 Jan;107(1):60–4.
36 O'Sullivan MP, Denihan N, Sikora K, Finder M, Ahearne C, Clarke G, et al. Activin A and Acvr2b mRNA from umbilical cord blood are not reliable markers of mild or moderate neonatal hypoxic-ischemic encephalopathy. Neuropediatrics. 2021;52(4):261–7.
37 Guo T, Noble W, Hanger DP. Roles of tau protein in health and disease. Acta Neuropathol. 2017;133(5):665–704.
38 Lv HY, Wu SJ, Gu XL, Wang QL, Ren PS, Ma Y, et al. Predictive value of neurodevelopmental outcome and serum tau protein level in neonates with hypoxic ischemic encephalopathy. Clin Lab. 2017;63(7):1153–62.
39 Massaro AN, Wu YW, Bammler TK, Comstock B, Mathur A, McKinstry RC, et al. Plasma biomarkers of brain injury in neonatal hypoxic-ischemic encephalopathy. J Pediatr. 2018 Mar;194:67–75.e1.
40 Lv HY, Wu SJ, Wang QL, Yang LH, Ren PS, Qiao BJ, et al. Effect of erythropoietin combined with hypothermia on serum tau protein levels and neurodevelopmental outcome in neonates with hypoxic-ischemic encephalopathy. Neural Regen Res. 2017;12(10):1655–63.
41 McGowan MM, O'Kane AC, Vezina G, Chang T, Bendush N, Glass P, et al. Serial plasma biomarkers of brain injury in infants with neonatal encephalopathy treated with therapeutic hypothermia. Pediatr Res. 2021 Dec;90(6):1228–34.
42 Zaigham M, Lundberg F, Hayes R, Undén J, Olofsson P. Umbilical cord blood concentrations of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) and glial fibrillary acidic protein (GFAP) in neonates developing hypoxic-ischemic encephalopathy. J Matern Fetal Neonatal Med. 2016;29(11):1822–8.
43 Patil UP, Mally PV, Wachtel EV. Serum biomarkers of neuronal injury in newborns evaluated for selective head cooling: a comparative pilot study. J Perinat Med. 2018;46(8):942–7.
44 Looney AM, O'Sullivan MP, Ahearne CE, Finder M, Felderhoff-Mueser U, Boylan GB, et al. Altered expression of umbilical cord blood levels of miR-181b and its downstream target mUCH-L1 in infants with moderate and severe neonatal hypoxic-ischaemic encephalopathy. Mol Neurobiol. 2019;56(5):3657–63.
45 Shi Z, Luo K, Deol S, Tan S. A systematic review of noninflammatory cerebrospinal fluid biomarkers for clinical outcome in neonates with perinatal hypoxic brain injury that could be biologically significant. J Neurosci Res. 2021.
46 Mondal N, Bhat BV, Banupriya C, Koner BC. Oxidative stress in perinatal asphyxia in relation to outcome. Indian J Pediatr. 2010;77(5):515–7.
47 El Bana SM, Maher SE, Gaber AF, Aly SS. Serum and urinary malondialdehyde (MDA), uric acid, and protein as markers of perinatal asphyxia. Electron Physician. 2016;8(7):2614–9.
48 Cascant-Vilaplana MM, Sanchez-Illana A, Pineiro-Ramos JD, Llorens-Salvador R, Quintas G, Oger C, et al. Do levels of lipid peroxidation biomarkers reflect the degree of brain injury in newborns? Antioxid Redox Signal. 2021 Dec 10;35(17):1467–75.
49 Mutlu M, Sariaydin M, Aslan Y, Kader S, Dereci S, Kart C, et al. Status of vitamin D, antioxidant enzymes, and antioxidant substances in neonates with neonatal hypoxic-ischemic encephalopathy. J Matern Fetal Neonatal Med. 2016;29(14):2259–63.
50 El-Mazary AA, Abdel-Aziz RA, Mahmoud RA, El-Said MA, Mohammed NR. Correlations between maternal and neonatal serum selenium levels in full term neonates with hypoxic ischemic encephalopathy. Ital J Pediatr. 2015;41:83.
51 Perrone S, Szabo M, Bellieni CV, Longini M, Bango M, Kelen D, et al. Whole body hypothermia and oxidative stress in babies with hypoxic-ischemic brain injury. Pediatr Neurol. 2010;43(4):236–40.
52 Yang T, Li S. Efficacy of different treatment times of mild cerebral hypothermia on oxidative factors and neuroprotective effects in neonatal patients with moderate/severe hypoxic-ischemic encephalopathy. J Int Med Res. 2020;48(9):300060520943770.
53 Maiwald CA, Annink KV, Rudiger M, Benders M, van Bel F, Allegaert K, et al. Effect of allopurinol in addition to hypothermia treatment in neonates for hypoxic-ischemic brain injury on neurocognitive outcome (ALBINO): study protocol of a blinded randomized placebo-controlled parallel group multicenter trial for superiority (phase III). BMC Pediatr. 2019;19(1):210.
54 Ennen CS, Huisman TA, Savage WJ, Northington FJ, Jennings JM, Everett AD, et al. Glial fibrillary acidic protein as a biomarker for neonatal hypoxic-ischemic encephalopathy treated with whole-body cooling. Am J Obstet Gynecol. 2011;205(3):251 e1–7.
55 Massaro AN, Jeromin A, Kadom N, Vezina G, Hayes RL, Wang KK, et al. Serum biomarkers of MRI brain injury in neonatal hypoxic ischemic encephalopathy treated with whole-body hypothermia: a pilot study. Pediatr Crit Care Med. 2013;14(3):310–7.
56 Looney AM, Ahearne C, Boylan GB, Murray DM. Glial fibrillary acidic protein is not an early marker of injury in perinatal asphyxia and hypoxic-ischemic encephalopathy. Front Neurol. 2015;6:264.
57 Maggiotto LV, Sondhi M, Shin BC, Garg M, Devaskar SU. Circulating blood cellular glucose transporters – surrogate biomarkers for neonatal hypoxic-ischemic encephalopathy assessed by novel scoring systems. Mol Genet Metab. 2019;127(2):166–73.
58 Walas W, Wilińska M, Bekiesińska-Figatowska M, Halaba Z, Śmigiel R. Methods for assessing the severity of perinatal asphyxia and early prognostic tools in neonates with hypoxic-ischemic encephalopathy treated with therapeutic hypothermia. Adv Clin Exp Med. 2020;29(8):1011–6.
59 Pang R, Mujuni BM, Martinello KA, Webb EL, Nalwoga A, Ssekyewa J, et al. Elevated serum IL-10 is associated with severity of neonatal encephalopathy and adverse early childhood outcomes. Pediatr Res. 2021.
60 Sweetman DU, Strickland T, Melo AM, Kelly LA, Onwuneme C, Watson WR, et al. Neonatal encephalopathy is associated with altered IL-8 and GM-CSF which correlates with outcomes. Front Pediatr. 2020;8:556216.
61 Shankaran S, Pappas A, Laptook AR, McDonald SA, Ehrenkranz RA, Tyson JE, et al. Outcomes of safety and effectiveness in a multicenter randomized, controlled trial of whole-body hypothermia for neonatal hypoxic-ischemic encephalopathy. Pediatrics. 2008;122(4):e791–8.
62 Pappas A, Shankaran S, McDonald SA, Carlo WA, Laptook AR, Tyson JE, et al. Blood biomarkers and 6- to 7-year childhood outcomes following neonatal encephalopathy. Am J Perinatol. 2020.
63 Mehta SL, Chokkalla AK, Vemuganti R. Non-coding RNA crosstalk in brain health and diseases. Neurochem Int. 2021;149:105139.
64 Wang Z, Liu Y, Shao M, Wang D, Zhang Y. Combined prediction of miR-210 and miR-374a for severity and prognosis of hypoxic-ischemic encephalopathy. Brain Behav. 2018;8(1):e00835.
65 Li M, Ye M, Zhang G. Aberrant expression of miR-199a in newborns with hypoxic-ischemic encephalopathy and its diagnostic and prognostic significance when combined with S100B and NSE. Acta Neurol Belg. 2021;121(3):707–14.
66 O'Sullivan MP, Looney AM, Moloney GM, Finder M, Hallberg B, Clarke G, et al. Validation of altered umbilical cord blood microRNA expression in neonatal hypoxic-ischemic encephalopathy. JAMA Neurol. 2019;76(3):333–41.
67 O'Sullivan MP, Casey S, Finder M, Ahearne C, Clarke G, Hallberg B, et al. Up-regulation of Nfat5 mRNA and Fzd4 mRNA as a marker of poor outcome in neonatal hypoxic-ischemic encephalopathy. J Pediatr. 2021;228:74–81 e2.
68 Li B, Dasgupta C, Huang L, Meng X, Zhang L. MiRNA-210 induces microglial activation and regulates microglia-mediated neuroinflammation in neonatal hypoxic-ischemic encephalopathy. Cell Mol Immunol. 2020;17(9):976–91.
69 Alsina M, Martín-Ancel A, Alarcon-Allen A, Arca G, Gayá F, García-Alix A. The severity of hypoxic-ischemic encephalopathy correlates with multiple organ dysfunction in the hypothermia era. Pediatr Crit Care Med. 2017;18(3):234–40.
70 Muniraman H, Gardner D, Skinner J, Paweletz A, Vayalakkad A, Chee YH, et al. Biomarkers of hepatic injury and function in neonatal hypoxic ischemic encephalopathy and with therapeutic hypothermia. Eur J Pediatr. 2017;176(10):1295–303.
71 Jones R, Heep A, Odd D. Biochemical and clinical predictors of hypoxic-ischemic encephalopathy after perinatal asphyxia. J Matern Fetal Neonatal Med. 2018;31(6):791–6.
72 Cilla A, Arnaez J, Benavente-Fernandez I, Ochoa C, Vega C, Lubian-Lopez S, et al. Effect of hypothermia and severity of hypoxic-ischemic encephalopathy in the levels of c-reactive protein during the first 120 hours of life. Am J Perinatol. 2020;37(7):722–30.

73 Doandes FM, Manea AM, Lungu N, Cioboata D, Brandibur T, Costescu O, et al. Clinical, biological and electroencephalographic monitoring of newborns with neurological risk in the Neonatal Intensive Care Unit. Exp Ther Med. 2021;22(1):760.
74 Lee IC, Yu CS, Wong SH, Lue KH. Troponin I levels in neonatal hypoxic-ischemic encephalopathy are related to cardiopulmonary comorbidity and neurodevelopmental outcomes. J Clin Med. 2021;10(17):4010.
75 Azzopardi D. Clinical applications of cerebral function monitoring in neonates. Semin Fetal Neonatal Med. 2015;20(3):154–63.
76 Liu W, Yang Q, Wei H, Dong W, Fan Y, Hua Z. Prognostic value of clinical tests in neonates with hypoxic-ischemic encephalopathy treated with therapeutic hypothermia: a systematic review and meta-analysis. Front Neurol. 2020;11:133.
77 Thoresen M, Hellström-Westas L, Liu X, de Vries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. Pediatrics. 2010;126(1):e131–9.
78 Ouwehand S, Smidt LCA, Dudink J, Benders M, de Vries LS, Groenendaal F, et al. Predictors of outcomes in hypoxic-ischemic encephalopathy following hypothermia: a meta-analysis. Neonatology. 2020;117(4):411–27.
79 Pu Y, Zhu Z, Yang Q, Zhang Y, Zhao J, Liu M, et al. Significance of amplitude integrated electroencephalography in early stage of neonatal hypoxic-ischemic encephalopathy and cerebral function monitoring in Neonatal Intensive Care Units. Am J Transl Res. 2021;13(8):9437–43.
80 Garvey AA, Pavel AM, O'Toole JM, Walsh BH, Korotchikova I, Livingstone V, et al. Multichannel EEG abnormalities during the first 6 hours in infants with mild hypoxic-ischaemic encephalopathy. Pediatr Res. 2021;90(1):117–24.
81 Martinello K, Hart AR, Yap S, Mitra S, Robertson NJ. Management and investigation of neonatal encephalopathy: 2017 update. Arch Dis Child Fetal Neonatal Ed. 2017;102(4):F346–F58.
82 Cainelli E, Trevisanuto D, Cavallin F, Manara R, Suppiej A. Evoked potentials predict psychomotor development in neonates with normal MRI after hypothermia for hypoxic-ischemic encephalopathy. Clin Neurophysiol. 2018;129(6):1300–6.
83 Nevalainen P, Metsäranta M, Marchi V, Toiviainen-Salo S, Vanhatalo S, Lauronen L. Towards multimodal brain monitoring in asphyxiated newborns with amplitude-integrated EEG and simultaneous somatosensory evoked potentials. Early Hum Dev. 2021;153:105287.
84 Koskela T, Kendall GS, Memon S, Sokolska M, Mabuza T, Huertas-Ceballos A, et al. Prognostic value of neonatal EEG following therapeutic hypothermia in survivors of hypoxic-ischemic encephalopathy. Clin Neurophysiol. 2021;132(9):2091–100.
85 Abbasi H, Gunn AJ, Bennet L, Unsworth CP. Deep convolutional neural network and reverse biorthogonal wavelet scalograms for automatic identification of high frequency micro-scale spike transients in the post-hypoxic-ischemic EEG. Annu Int Conf IEEE Eng Med Biol Soc. 2020;2020:1015–8.
86 Kota S, Massaro AN, Chang T, Al-Shargabi T, Cristante C, Vezina G, et al. Prognostic value of continuous electroencephalogram delta power in neonates with hypoxic-ischemic encephalopathy. J Child Neurol. 2020;35(8):517–25.
87 Zhang Q, Hu Y, Dong X, Feng X. Clinical significance of electroencephalography power spectrum density and functional connection analysis in neonates with hypoxic-ischemic encephalopathy. Int J Dev Neurosci. 2021;81(2):142–50.
88 Das Y, Wang X, Kota S, Zhang R, Liu H, Chalak LF. Neurovascular coupling (NVC) in newborns using processed EEG versus amplitude-EEG. Sci Rep. 2021;11(1):9426.
89 Hope PL, Costello AM, Cady EB, Delpy DT, Tofts PS, Chu A, et al. Cerebral energy metabolism studied with phosphorus NMR spectroscopy in normal and birth-asphyxiated infants. Lancet. 1984;2(8399):366–70.
90 Roth SC, Baudin J, Cady E, Johal K, Townsend JP, Wyatt JS, et al. Relation of deranged neonatal cerebral oxidative metabolism with neurodevelopmental outcome and head circumference at 4 years. Dev Med Child Neurol. 1997;39(11):718–25.
91 Lally PJ, Montaldo P, Oliveira V, Soe A, Swamy R, Bassett P, et al. Magnetic resonance spectroscopy assessment of brain injury after moderate hypothermia in neonatal encephalopathy: a prospective multicentre cohort study. Lancet Neurol. 2019;18(1):35–45.
92 Mitra S, Kendall GS, Bainbridge A, Sokolska M, Dinan M, Uria-Avellanal C, et al. Proton magnetic resonance spectroscopy lactate/N-acetylaspartate within 2 weeks of birth accurately predicts 2-year motor, cognitive and language outcomes in neonatal encephalopathy after therapeutic hypothermia. Arch Dis Child Fetal Neonatal Ed. 2019;104(4):F424–F32.
93 Lucke AM, Shetty AN, Hagan JL, Walton A, Stafford TD, Chu ZD, et al. Early proton magnetic resonance spectroscopy during and after therapeutic hypothermia in perinatal hypoxic-ischemic encephalopathy. Pediatr Radiol. 2019;49(7):941–50.
94 O'Dea M, Sweetman D, Bonifacio SL, El-Dib M, Austin T, Molloy EJ. Management of multi organ dysfunction in neonatal encephalopathy. Front Pediatr. 2020;8:239.
95 Walsh BH, Neil J, Morey J, Yang E, Silvera MV, Inder TE, et al. The frequency and severity of magnetic resonance imaging abnormalities in infants with mild neonatal encephalopathy. J Pediatr. 2017;187:26–33.e1.
96 Machie M, Weeke L, de Vries LS, Rollins N, Brown L, Chalak L. MRI score ability to detect abnormalities in mild hypoxic-ischemic encephalopathy. Pediatr Neurol. 2021;116:32–8.
97 Heursen EM, Zuazo Ojeda A, Benavente Fernandez I, Jimenez Gomez G, Campuzano Fernandez-Colima R, Paz-Exposito J, et al. Prognostic value of the apparent diffusion coefficient in newborns with hypoxic-ischaemic encephalopathy treated with therapeutic hypothermia. Neonatology. 2017;112(1):67–72.
98 Sanchez Fernandez I, Morales-Quezada JL, Law S, Kim P. Prognostic value of brain magnetic resonance imaging in neonatal hypoxic-ischemic encephalopathy: a meta-analysis. J Child Neurol. 2017;32(13):1065–73.
99 Chang PD, Chow DS, Alber A, Lin YK, Youn YA. Predictive values of location and volumetric MRI injury patterns for neurodevelopmental outcomes in hypoxic-ischemic encephalopathy neonates. Brain Sci. 2020;10(12):991.
100 Bonifacio SL, deVries LS, Groenendaal F. Impact of hypothermia on predictors of poor outcome: how do we decide to redirect care? Semin Fetal Neonatal Med. 2015;20(2):122–7.
101 Tharmapoopathy P, Chisholm P, Barlas A, Varsami M, Gupta N, Ekitzidou G, et al. In clinical practice, cerebral MRI in newborns is highly predictive of neurodevelopmental outcome after therapeutic hypothermia. Eur J Paediatr Neurol. 2020;25:127–33.
102 Chakkarapani E, Poskitt KJ, Miller SP, Zwicker JG, Xu Q, Wong DS, et al. Reliability of early magnetic resonance imaging (MRI) and necessity of repeating MRI in noncooled and cooled infants with neonatal encephalopathy. J Child Neurol. 2016;31(5):553–9.
103 Wintermark P, Hansen A, Soul J, Labrecque M, Robertson RL, Warfield SK. Early versus late MRI in asphyxiated newborns treated with hypothermia. Arch Dis Child Fetal Neonatal Ed. 2011;96(1):F36–44.
104 Imai K, de Vries LS, Alderliesten T, Wagenaar N, van der Aa NE, Lequin MH, et al. MRI changes in the thalamus and basal ganglia of full-term neonates with perinatal asphyxia. Neonatology. 2018;114(3):253–60.
105 Agut T, León M, Rebollo M, Muchart J, Arca G, Garcia-Alix A. Early identification of brain injury in infants with hypoxic ischemic encephalopathy at high risk for severe impairments: accuracy of MRI performed in the first days of life. BMC Pediatr. 2014;14:177.
106 Ambalavanan N, Shankaran S, Laptook AR, Carper BA, Das A, Carlo WA, et al. Early determination of prognosis in neonatal moderate or severe hypoxic-ischemic encephalopathy. Pediatrics. 2021;147(6):e2020048678.
107 Mooney C, O'Boyle D, Finder M, Hallberg B, Walsh BH, Henshall DC, et al. Predictive modelling of hypoxic ischaemic encephalopathy risk following perinatal asphyxia. Heliyon. 2021;7(7):e07411.