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Neonatal encephalopathy: Focus on epidemiology and underexplored aspects of etiology

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A B S T R A C T

Neonatal Encephalopathy (NE) is a neurologic syndrome in term and near-term infants who have depressed consciousness, difficulty initiating and maintaining respiration, and often abnormal tone, reflexes and neonatal seizures in varying combinations. Moderate/severe NE affects 0.5–3/1000 live births in high-income countries, more in low- and middle-income countries, and carries high risk of mortality or disability, including cerebral palsy. Reduced blood flow and/or oxygenation around the time of birth, as with ruptured uterus, placental abruption or umbilical cord prolapse can cause NE. This subset of NE, with accompanying low Apgar scores and acidemia, is termed Hypoxic-Ischemic Encephalopathy. Other causes of NE that can present similarly, include infections, inflammation, toxins, metabolic disease, stroke, placental disease, and genetic disorders. Aberrant fetal growth and congenital anomalies are strongly associated with NE, suggesting a major role for maldevelopment. As new tools for differential diagnosis emerge, their application for prevention, individualized treatment and prognostication will require further systematic studies of etiology of NE.

Neonatal Encephalopathy (NE) is identified by the World Health Organization as one of the 10 leading causes of lost years of life [1] and is extremely expensive in lifetime costs and in emotional distress for individuals and families. Although NE is traditionally attributed to birth asphyxia, population-based studies have consistently observed that most NE and some of the subset of NE considered to be hypoxic-ischemic in origin occurs in infants who have not experienced acute peripartum or intrapartum events. Therapeutic hypothermia (TH), undertaken soon after birth, is an important advance in treatment, but does not benefit all affected infants [2] The clinical management of infants with NE is complex, demanding, and resource intensive, but etiology is also important. The underlying etiology of NE may influence the infant's response to treatment and understanding of etiology can help caregivers identify preventive strategies and individualized approaches to treatment as well as provide a more accurate prognosis. Thoughtful differential diagnosis is a key step for optimal clinical management.

"NE" describes disturbed neurological function in a neonate with abnormal level of consciousness and any combination of seizures, difficulty initiating and maintaining respiration, abnormal tone or reflexes, or disturbances of suck or swallowing. Initially described in neonates ≥37 weeks at birth, recent trends have included late preterm births 35–36 weeks. In most neonates with NE, onset is in the first 24 h of life, commonly in the delivery room, but onset may be any time in the first week of life. Although presenting in the neonatal period, NE often has its origins in prenatal life. The etiology of NE is diverse and no single test defines it.

One of the many etiological pathways to NE includes a period of peripartum or intrapartum hypoxia-ischemia. In 2014, the American College of Obstetricians and Gynaecologists (ACOG) and the American Academy of Pediatrics (AAP) Task Force on NE defined a set of markers concerning neonatal status, contributing events and developmental outcome that should be used to determine if NE is due to peripartum or intrapartum hypoxia-ischemia[3] (See Table 1).

The more criteria that are fulfilled, the more likely it is that hypoxia-

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Table 1
ACOG/AAP criteria to determine whether NE is due to peripartum or intrapartum hypoxia ischemia[a].

1. Does the baby meet the definition for NE?
35+ weeks gestation, abnormal consciousness±difficulty initiating and maintaining respiration, seizures, abnormal tone/reflexes
2. What is the likelihood that an acute peripartum or intrapartum event was the major contributor?
Neonatal signs
a. Apgar score of less than 5 at 10 min of life
b. Fetal umbilical artery acidemia: pH < 7 or base deficit ≥12
c. MRI obtained between 24 and 96 h and up to day 10 showing distinct basal-ganglia-thalamus, watershed or near-total cortical injury pattern
d. Presence of multisystem organ failure – can include cardiac, renal, hepatic, metabolic, hematologic and gastrointestinal dysfunction
Type and timing of contributing factors consistent with an acute or peripartum event
a. Sentinel hypoxic or ischemic event immediately before or during labor/delivery: e.g., ruptured uterus, umbilical cord prolapse
b. Fetal heart rate pattern that deteriorates to absent variability with: recurrent late or variable decelerations, or with bradycardia, or a sinusoidal pattern for ≥20 min
c. MRI obtained between 24 and 96 h and up to day 10 showing distinct basal-ganglia-thalamus or watershed pattern
d. No evidence of other proximal or distal factors that could contribute substantially or indicate other underlying pathobiology e.g. abnormal fetal growth, congenital microcephaly, maternal infection, neonatal sepsis
Developmental outcome is spastic quadriplegic or dyskinetic cerebral palsy (CP)
a. Other CP subtypes are less likely to be associated with an acute peripartum or intrapartum event and, spastic quadriplegia and dyskinesia can also have other causes.
b. Other developmental disabilities may occur, but are not specific to acute peripartum or intrapartum events and may arise from a variety of causes

[a] Adapted from Reference 3

ischemia has played a major role in the etiological pathway to NE. Hypoxic-ischemic encephalopathy (HIE) is the subset of NE, in which potentially asphyxiating birth events ("sentinel events") such as major abruption or cord prolapse are thought to have occurred. Other determinative factors should also be thoroughly investigated. Even when efforts are made to gather complete data, caution is appropriate when concluding that intrapartum events are causal in a neonate with NE because the antecedents of the intrapartum risk factors may themselves influence outcome. For example, premature rupture of membranes, thick meconium, prolonged second stage of labor, and abnormal cardiotocography (CTG) are often listed as intrapartum factors [3] because they are *observed* in the intrapartum period, but these can obviously be the results of pathologic mechanisms that operated well before labor began. The same is true of low Apgar scores and acidosis. These signs may indicate the severity, but not necessarily the nature of the insult. Except in the case of evident sentinel events such as uterine rupture, the critical questions often remain: At what point was oxygen and/or blood supply decreased? At what point would an intervention directed to oxygen and/or blood supply have prevented neurologic abnormality? And this is commonly not clearly identifiable.

Population-based studies have been consistent in finding that most neonates with NE, including those who later develop cerebral palsy (CP), did not experience sentinel events, yet most experimental models of NE and of CP are based upon assumptions about the primacy of hypoxia-ischemia in these disorders. Such models may not be relevant to most NE or CP in human infants. Assumptions about hypoxic-ischemic etiology in NE may blind us to other etiologic possibilities.

The nature of study samples are important in the interpretation of study results. Studies from hospitals that are referral centers, including randomized trials of TH, provide much of our knowledge about clinical features of NE. They can include detailed observations about differences *among* neonates being cooled, correlates of those differences, and initial observations on effects of treatment. The samples for such studies may not however, be representative of all NE in the community. Population-based studies, on the other hand, are the appropriate approach for robust information about incidence of NE and about the frequency and impact of risk factors. These two study types hospital-based and population-based are complementary and both are needed. It is necessary, however, to recognize the strengths and limitations of each.

1. Epidemiology of NE and HIE

We sought to update the landmark paper "Epidemiology of neonatal encephalopathy and hypoxic-ischemic encephalopathy"[4] published in 2010. The estimated incidence of NE was 3/1000 and HIE was 1.5/1000 live births[4]. These estimations were derived from two population-based studies (last birth year 1995) for the NE estimate and three population-based studies (last birth year 1996) for the HIE estimate. There were several important differences in the studies included in the review. First and foremost was the case definition – no two studies used the same definition/inclusion criteria. The differences in reported incidence between population-based and hospital-based studies were large, with hospital-based studies reporting higher incidence estimates, due to the nature of referral centers for both neonates and high-risk pregnancies. If all hospital-based studies had been included, the range for NE would have been 2–6/1000 and for HIE 1–8/1000 live births. There was a combination of retrospective and prospective designs, high- and low-income countries, and differences in the birth years covered (ranging between 1970's to late 1990s). Similar difficulties were identified during this update.

In Fig. 1 [4–15], Kurinczuk et al.[4] and Lee et al.[5] are review

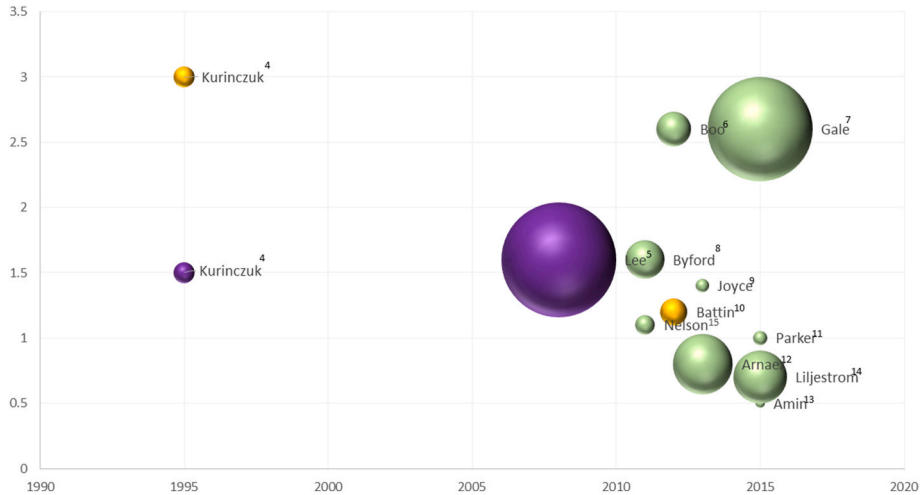

Fig. 1. NE and HIE incidenc

papers and all others represent single studies, ranging from whole countries to single hospitals[6–14]. The larger the circle, the larger the denominator as seen in Table 2 (Supplementary Data) [6–18]. The only new study of NE incidence [10], reported that, across New Zealand, incidence was 1.2/1000 live births, a much lower estimate than (3/1000 liv births) in the 2010 review.

A recent global summary modelled that, in one year, 1.15 million neonates (8.5 cases per 1000 live births) were estimated to have developed NE associated with intrapartum events (HIE), with 96% born in Low- and Middle-Income Countries (LMICs) [19]. The median incidence in high-income countries was 1.6/1000 live births (Fig. 1). The remaining studies reported on HIE incidence, and there was large variation between studies, from 0.5/1000[13] to 2.6/1000 live births[7]. These studies were from the United Kingdom but the designs were very different, one relying on careful review of notes for eligibility to a strict definition and the other, much larger, relying on diagnostic codes. Both study designs are useful for their individual aims, but they are surely measuring different things. Although eligibility and exclusion criteria differed (Table 2 Supplementary Data), there was less variation in incidence than in the previous review. In high-income countries, most studies reported an incidence of HIE between 0.5 and 1.5/1000 live births, suggesting a reduction from the HIE in the mid 2000s.

The concerns reported by Kurinczuk et al. [4] about definitions can be highlighted by two recent studies that used the same cohort of neonates, but different definitions. In a South African cohort, the incidence of moderate to severe HIE varied, depending upon the definition used, between 1.5 and 3.7 per 1000 live births[20]. In the UK, Yates et al.[21] reported a range between 1.3 and 2.5/1000 live births dependent on definition.

In line with our findings, a number of European studies have reported recent reductions each using the *same* definitions over time, attributed to the many improvements in prenatal and perinatal care. Amin et al. (2019) [13] in Wales reported a steady decline from 1.0/1000 live births in 2007 to <0.5/1000 live births in 2015. A marked reduction in HIE (between 4 and 5/1000 live births in the early 2000s to 0.4–1.9/1000 in 2010–14) has also been reported in Portugal, accompanied by an overall 4% decrease in caesarean deliveries [22]. In the Netherlands, the incidence of moderate and severe NE was 1.56 per 1000 births in the years 2000–2007, falling to 0.86 in 2008–2013[23].

In LMICs, both the incidence and severity of NE were higher than high-income countries and the case-mortality rate was also higher[24]. Lee et al. (2013) estimated a median rate of 12.1/1000 in low-income countries [5]. Three studies from LMICs [16–18] were identified for this paper, all with extremely high rates of HIE; however, their definitions were quite different to those used in high-income countries, making comparisons difficult (Table 2 Supplementary Data). It is likely that the causal pathways to NE are different in LMICs with an over-representation of sentinel events, obstructed labor, hypoxia-ischemia and infectious etiologies [24,25]. The impact of unrecognized pathology such as pre-eclampsia, gestational diabetes, fetal growth restriction, neonatal jaundice and hypoglycemia is compounded by the lack of trained birth attendants [26]. While these problems currently magnify risk, they identify opportunities for interventions to improve outcomes in LMIC settings.

As neonatal deaths and stillbirths decrease with progress towards the Global Millennium Goals, it is likely that survival among children with NE will increase creating a spike in long-term disability in these countries[27]. This is particularly so in countries early in their experience with neonatal intensive care. Partnerships with neonatal intensive care units (NICUs) in high-income countries may help avoid pitfalls and optimize outcomes worldwide.

2. The neonatal neurological examination and description of seizures

A thorough neonatal neurological examination is among the first steps in the evaluation of potential etiologies of NE. The Sarnat stages of NE observed in 1976 are a tool for grading encephalopathy severity [28]. The evolution of physical examination findings and neurological state during the first 72 h of age differs based on cause of the encephalopathy, severity, and injury timing and should be carefully documented. Medications administered to mother or child, such as sedatives and antiseizure medications, and level of NICU support should be taken into consideration when interpreting neurological examination findings. The modified Sarnat grade and the Amiel-Tison Neurological Assessment at Term (ATNAT) at three days of age correlate strongly with outcome at 24 months[29]. However, the best correlation of neurological exam with outcome is the examination performed at discharge from the NICU, when confounders such as sedatives, mechanical ventilation, and other medications are often no longer present [29]. Normalization of the neurological exam during the first three days of age predicted a normal outcome; in contrast, a static course on serial examinations is worrisome and may suggest the possibility of a severe degree of abnormality and perhaps chronic prenatal etiology.[29]. Similarly, persistent encephalopathy as observed by abnormal amplitude-integrated electroencephalogram at 48 h of age is associated with abnormal neurodevelopmental outcomes [30] By 3 months of age, abnormal general movements are highly predictive of CP [31].

Seizures occur in many infants with NE and are often the indicator leading to neurodiagnostic evaluation and diagnosis. Timing of the onset of seizures and electroencephalographic findings vary by etiology of NE. In a multicenter European study, seizure onset was earlier in infants with NE due to HIE than for infants with metabolic/genetic causes (median of 16.5 h for moderate HIE vs. 56.6 h for metabolic/genetic disorder, $P < .001$)[32]. In addition, seizures decreased in frequency earlier in infants with moderate HIE (median 34.4 h of age) than in infants with seizures due to stroke or metabolic/genetic conditions[32]. Thus the timing and evolution of seizures over the first few days of age can help direct the work-up for etiologies of NE presenting with seizures.

3. Risk factors and etiologies of NE, underestimation of aberrant brain development and under exploration of its mechanisms

Risk factors for NE span the pre-conceptional to perinatal period and include etiologies originating from maternal, placental, and fetal sources that can impact fetal status and transition at delivery[33]. Without careful consideration of the multiple potential etiologies of NE and gathering of all available information, the role of contributing or causal factors and their mechanism in NE may be missed. Information concerning factors operating in fetal development requires transfer of maternal prenatal records to clinicians involved in care of the infant.

Low socioeconomic status (SES) is more common in infants with NE than in term healthy controls; this was first noted in Western Australia [34], and has now been confirmed in other regions of the world. In a population-based study in the United States, SES was measured separately by three factors: low median income neighborhoods at birth, level of maternal education, and private insurance. Each indicated lower SES in NE compared to term healthy controls[35]. In New Zealand, the rate of NE was higher in the presence of standardized measures of deprivation[10]. SES is also a powerful predictor of cognitive performance. In one landmark study, as measured by individual Stanford Binet R testing in about 40,000 4-year-old children, IQ varied by about 20 IQ points – more than a standard deviation– according to SES. [36]. Any credible study of cognitive function of survivors of NE or other perinatal factor must take SES into account. The links between SES and risk of NE await further investigation, including whether disagreements about the association of placental inflammation with NE might relate to differing SES in the groups studied [37,38].

Maternal psychiatric disorders and drug exposures also can have important consequences in the developing brain[39]. Even exposure to maternal stress, which is common, can affect development of the fetal autonomic nervous system and impact fetal-neonatal transition [33].

Maternal antidepressant use during pregnancy has been associated with a three-fold increase in risk for NE[40]. Untreated depression during pregnancy, however, can also lead to deleterious effects on pregnancy outcome, including low-birth weight, preeclampsia and eclampsia and on maternal-infant bonding[39].

Maternal substance use, highly correlated to psychiatric disorders, can also contribute to impaired fetal brain development, intrauterine growth restriction, preterm birth, abnormal fetal-neonatal transition, and neonatal withdrawal syndromes. Neonatal withdrawal syndromes have been recognized following birth to a mother on antidepressant medication, especially venlafaxine. A venlafaxine neonatal withdrawal syndrome has been described, which presents as NE typically within the first four days of age[41]. Infants can be jittery and hyperreflexic with poor feeding, tremors, irritability, and myoclonic jerks. Most infants have a normal EEG and there is no epileptic correlate to the movements [41]. The NE associated with withdrawal subsides over days to weeks.

Congenital infections are an important cause of NE and may go unrecognized without focused testing. Congenital infectious agents can contribute to destructive lesions (i.e. intracranial hemorrhage, cystic lesions) that can present with NE. Specific congenital infections vary based on maternal exposures and risks, but include *toxoplasmosis gondii*, *cytomegalovirus* (CMV), *parvovirus B19*, *human immunodeficiency virus* and *group beta streptococcus*[42]. We recommend documentation of testing performed during pregnancy.

CMV is the most common congenital infection in developed countries, can present as NE[43] and may be on the pathway to CP for up to 10%. [44]. Since postnatal antiviral treatment is available, this is an important infection to recognize. About ten percent of neonates with symptomatic congenital CMV have seizures as a presenting sign[45]. Other clinical findings such as hepatosplenomegaly and thrombocytopenia should raise suspicion of this viral infection, which is often asymptomatic in the mother. Diagnostic evaluation should begin early, with urine or saliva for CMV DNA by polymerase chain reaction within the first three weeks of birth, neuroimaging, ophthalmology evaluation, and auditory brainstem response testing. Neuroimaging findings include lenticulostriate vasculopathy, subependymal cysts, and/or periventricular calcifications best seen on cranial ultrasound, as well as neuronal migration disorders and cerebellar hypoplasia best seen on brain MRI[45]. Postnatal treatment with oral valganciclovir for six months has been shown to decrease sensorineural hearing loss and may lead to modest improvement in neurodevelopmental outcome at 24 months of age[46].

Population-based studies indicate that neonates who are growth-restricted and/or have congenital anomalies are at high risk for NE [34]. One in four or five surviving infants with NE develops CP, and these same factors – fetal growth restriction and birth defects – are important contributors to CP in term and near-term infants, greatly outweighing birth asphyxia and infection/inflammation in the attributable fraction of CP they account for. [42]. They deserve much greater focus than they have received as predictors of neurologic disability and as identifiers of underlying aberrant developmental processes[47].

Ideally, the history for an infant presenting with NE should include information about tests for malformations, congenital infections, genetics, fetal growth trajectory, maternal autoimmune conditions, substance exposures, thyroid disease and other medical conditions that can influence the fetal and neonatal condition[48]. For most NE births this information must be assembled after delivery.

4. Placental role in the pathogenesis of NE

A noted perinatal pathologist observed “In-labor catastrophes are rare and, when present, are rarely either diagnosable (in advance) or intervenable. Most cases of unexpected bad outcomes have chronic insults in the placenta.” [49]. Effects of hypoxia and ischemia on the placenta include impaired villous development, reduced trophoblast invasion, and altered placental blood flow. There is an hypothesis that these changes are secondary to oxidative stress as a result of the abnormal remodeling of the uterine spiral arteries [50].

Abnormalities observable on gross inspection were more common in the placentas of children with a later diagnosis of cerebral palsy, especially in those who had NE, than in controls [51].

Abnormal microscopic findings were also common in the placenta of neonates with NE [52] and included vasculopathy and/or placental inflammation that can be caused by infection or impaired immune tolerance [50,53,54]. Vasculopathy is associated with worse neurodevelopmental outcomes and stillbirth, while thrombosis and decreased fetoplacental flow are significant independent factors for development of NE. Fetal vascular malperfusion (FVM) is a recent clinical term indicating a group of placental lesions causing reduced or absent perfusion of the villous parenchyma by the fetus. It is an important placental finding in NE[38], observed in 20% of neonates with NE and 7% of controls (p=<.001) [38]. FVM is manifested as extensive avascular villi, necrotic fragments in the villous stroma, vascular thrombi, and abnormalities of the umbilical cord. Based on histological characteristics, FVM is thought to develop not later than 48 h before labor[38]. Abnormalities on electronic fetal monitoring were considerably more common in infants with NE whose placentas revealed FVM[38]. Infection with an inflammatory fetal response (funisitis) is a strong predictor of severe NE in term neonates, while inflammation limited to the maternal side of the placenta (chorioamnionitis without funisitis) is less clearly related to neurological outcomes in neonates with NE[38,53,54]. High-grade villitis (patchy/diffuse villitis) has been associated with NE, neonatal seizures, intrauterine growth restriction, and placental hypoplasia [53,54]. All samples with high-grade villitis had focal avascular villi and small infarcts. There is a higher risk of unfavorable neurodevelopmental outcomes in neonates who experienced meconium aspiration accompanied by a high-grade villitis. In a retrospective cohort study, Mir et al. identified high-grade placental inflammatory villitis, a fetal lesion – but not chorioamnionitis, a maternal lesion– as an adverse factor in neurologic development[37]. In contrast, inflammatory placental lesions and maternal vascular malperfusion were not linked significantly with NE in the Vik et al. study, who have primarily associated vascular malperfusion of subacute or chronic origin on the fetal side of the placenta (i.e. FVM) with increased risk of NE [38]. What might underlie conflicting results about placental inflammation and NE in different studies has not been explored, but may include differences in the populations studied, the methodology employed or the interpretation by the pathologist.

5. Genetics, inflammation, epigenetics, transcriptomics, and metabolomics of NE

5.1. Genetic markers

Evidence is increasing that genetic factors are important in NE. A recent review makes it evident that the genetics and genomics influencing brain development may have a large role in NE and in cerebral palsy[42]. For example, an association has been reported of a single nucleotide polymorphism (SNP) rs1835740 with clinical complications at birth: neonates homozygous for the minor allele required resuscitation more frequently than the wild type neonates, and the proportion with a low Apgar score was higher in those with the minor allele[55]. Perhaps relevant to mechanism is that the SNP rs1835740 is involved in glutamate homeostasis and signaling and sensitizes to hypoxia to produce glutamate concentration in the brain high enough to cause respiratory depression at birth[55]. In term neonates with a clinical diagnosis of NE, there was a correlation of abnormal findings on electroencephalography, transfontanellar ultrasound, and a mutation in the IL-6 coding gene[56]. Carriers of the *IL6* 174G > C mutation (CC genotype) were also more likely to have neurological symptoms compared to those with the *IL-6* 174 GC genotype [56]. Mutations in the *MECP2* gene associated with X-linked Rett syndrome, can cause severe NE in boys[57,

58]. Affected infants present with microcephaly, hypotonia, motor disorders, and seizures, and suffer early mortality.

5.2. Inflammation biomarkers

Biomarkers play a potential role in the early diagnosis, assessment of response to treatment, and prognosis in NE[59]. Despite differing etiologies leading to NE, infants have a systemic inflammatory response, which appears proportional to the severity of NE. In addition, cytokine responses are predictive of MRI anomalies, seizures and neurodevelopmental outcome in infants with NE[60–63]. Early neutrophilia has been associated with increased infarct size on MRI and, in a neonatal animal model of inflammation-sensitized hypoxic-ischemic brain injury, early neutrophil infiltration is critical in the injury process[64,65]. In human neonates, neutrophilia is associated with poor neurological outcome following NE[66]. However, inflammation has long been recognized as vital for its dual role in repair, as well as a trigger for neuronal damage in perinatal brain injury[67,68]. Perinatal inflammation demonstrated by chorioamnionitis or elevated cord blood cytokines has been associated with NE, as well as longer term neuropsychiatric disorders[54]. Although cerebrospinal fluid biomarkers have previously been shown to be associated with adverse outcomes in the pre TH era, this has not been found post-TH[63], perhaps related the later timing of lumbar puncture after day 3 when TH is completed.

Persistent systemic inflammation has been demonstrated in animal models of neonatal brain injury with elevated pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) at day 7. TH delays the peak in proinflammatory cytokines in infants with NE randomized to TH versus normothermia[61], and sustained inflammation during the first week of life correlates with severity of NE[69]. The combination of infection and hypoxia/ischemia synergizes to increase the degree of brain injury[70, 71]. Infection and inflammation have been implicated in both the etiology and the response to injury.

Cytokine dysregulation has been found in school-aged children with CP who had brain injury in the neonatal period[72], with significantly higher monocyte TNF-α and toll like receptor (TLR)-4 expression. Numis et al.[73] demonstrated early changes in proinflammatory cytokines, such as IL-6 and TNF-α in the IL-1 β pathway, were associated with remote epilepsy in infants following NE. Perinatal inflammation is associated with many neuropsychiatric and neuro-psychological disorders. Fleiss et al. suggest that the injury processes can persist for months and years and propose a tertiary mechanism of damage, which includes inflammation and epigenetic changes[74].

IL-1β production is increased following hypoxic-ischemic injury and this injury is reduced by IL-receptor antagonist (IL-1RA) or by caspase 1 [75], but was less effective in conjunction with hypothermia [76]. This pathway has also been implicated in numerous diseases like gout, Alzheimer's disease, obesity, and diabetes[77]. The inflammasomes are complex proteins in macrophages and neutrophils which play a fundamental role in the production of inflammation in innate immunity and are formed by activation of the nucleotide-binding oligomerization domain like receptor family proteins (NLRP). NLRP3 is the most widely studied inflammasome and can be activated by microbes, LPS and fungi, and induce IL-1β secretion in the presence of adenosine triphosphate (ATP). Regulation of the NLRP3 inflammasome complex is described in 3 possible ways: expression of the inflammasome complex, the amount of NLRP3 activators present and post-transcriptional modulation via microRNAs.

MicroRNAs (miRNAs) are small non-coding RNA of 19–24 nucleotides, which have key gene regulatory activity in cell maintenance and homeostasis and rapid physiological and behavioral responses. MicroRNAs have been studied in NE in cord blood and correlated with neonatal outcomes[78]. miR-223 downregulates NLRP3 to inhibit inflammation through caspase-1 and IL-1β, reduce brain edema and improve neurological functions[79]. The inflammasome pathway and miRNAs have been implicated in neonatal complications, such as BPD and cerebral injury, and therefore are potential targets for drug development. In a piglet model, mRNA and miRNA could differentiate between a hypoxic or inflammation-sensitized hypoxic etiology. IL-10 mRNA in particular was highest in inflammation and hypoxia and lowest in hypoxia alone. Several miRNAs were significantly increased within 6 h in hypoxia [80]. Maternal blood hypoxia-regulated miRs can highlight at-risk pregnancies associated with fetal hypoxia, which may allow earlier intervention [81].

Further research should address the degree to which systemic inflammatory responses can serve as biomarkers to predict outcome. In a preclinical model of HIE, there was evidence or persistent immune dysregulation and hyperactivity. Persistent cognitive defects were seen in a preclinical model. A more in-depth knowledge of dysregulated immune function will be required for the development of specific treatments.

5.3. Epigenetics markers

Epigenetic changes are heritable, functionally relevant changes to the genome that do not involve alteration of the DNA sequence. Epigenetic modifications, notably DNA methylation and demethylation, histone modifications, and miRNA, form stable gene expression patterns that, among others, enable cellular differentiation[57]. Epigenetics are susceptible to environmental influences: adverse stimuli, such as hypoxia, infection, and hemorrhage, may lead to epigenetic modifications during fetal brain development and increase the risk of perinatal neurological complications[82].

A complex epigenetic system regulates cellular response to hypoxia, with HIF-1α as a major controlling factor. HIF-1α signaling modifies the expression of a set of miRNAs, whereas HIF-1α itself is controlled by various miRNAs. Furthermore, different miRNAs have been found to regulate numerous other processes present in hypoxia ischemia, ranging from inflammation to apoptosis to circadian rhythm disturbances following HIE[83].Transcriptomics is an analysis of the transcriptome or the complete set of RNA transcripts, both coding and non-coding. It provides an insight into the biological responses elicited by internal or external stimuli. Growing evidence suggests notable differences in transcriptomic signatures in neonates with NE, compared to healthy controls [84,85].

5.4. Transcriptomics

Next-generation sequencing performed on whole blood RNA from 12 infants with NE and healthy controls identified 950 genes that were expressed significantly differentially between the two groups. Pronounced differential expression was identified in several key regulators of the hypoxic response[85].The majority (71%) of these genes were under-expressed in NE, while 29% were over-expressed. None of the 950 genes are known to be cold-inducible, making it unlikely that therapeutic hypothermia caused these modifications. Analysis of transcriptomic profiles in 45 infants after NE identified 855 genes that were expressed significantly differently between favorable and poor clinical outcome groups. The genes with the most prominent expression differences were Regulator of G-protein signaling 1 (*RGS1)*, involved in immunomodulatory response to inflammation in the brain, and Structural Maintenance Of Chromosomes 4 (*SMC4)*, known to regulate chromosome organization[84].

5.5. Metabolomics

Metabolic changes are the earliest response to hypoxic injury in NE. A disruption of glucose and oxygen supply launches a series of detrimental biochemical events. Oxygen deprivation disturbs the normal functioning of the electron transport chain and tricarboxylic acid cycle, thus forcing a switch to anaerobic metabolism. ATP stores get depleted

quickly, causing the ion pumps to fail. Blockage of ionic transport leads to depolarization of a cellular membrane and accumulation of Ca^{2+}, extracellular glutamate, and free radicals in the cell. All these events can culminate in cell death [86]. A recent study used untargeted metabolomics to examine early metabolomic alterations in infants with perinatal asphyxia who were resuscitated at birth and recovered quickly (PA n = 41), those who developed HIE (n = 30) and healthy controls (n = 71). [87]. Metabolomic pathway analysis of cord blood from revealed 29 putatively annotated metabolic features that were significantly different in the perinatal asphyxia group, with eight of these also significant in the HIE group, compared to healthy controls. Pathway analysis in HIE group revealed alterations in 50% of the detected tryptophan pathway metabolites and 75% of the detected pyrimidine pathway metabolites versus healthy controls[87].

6. Research

Most studies of risk factors for NE have focused chiefly or exclusively on factors present or absent during delivery or the immediate newborn period. Most such studies originate in specialty hospital units rather than in representative populations. Most do not indicate how much effort was made to identify characteristics such as family history of neurologic disease, maternal health history, pregnancy course or socioeconomic factors, all of which have been associated with NE risk in the only wide-ranging population-based study available to date, that from Western Australia, completed a quarter-century ago. [34]. There is serious need for such research, updated to include genetic investigation.

Precision medicine and individualized therapies based on etiology, placental abnormalities, and genetics will be crucial to optimize adjunctive therapies. Future studies, including randomized trials of hypothermia plus adjunct therapies, need to collect common data elements including placental descriptors. Longitudinal studies are greatly needed, incorporating genetic and genomic investigations. Large population-based registries should be utilized and – because several phenotypically differing forms of developmental disabilities are often present in the same sibship – investigations should combine registries for birth defects, CP, intellectual deficit, autism, psychiatric disorders, with one another and with genetic analysis.

New research is also required to focus on biomechanisms and genes unique to the placenta, as recent studies indicate potentially powerful interactions between placenta and fetal/neonatal (and maternal) brain.

7. Concluding remarks

NE is an important clinical entity for which optimal diagnostic and therapeutic methods are still actively evolving. There are new diagnostic capabilities, some of which are not yet widely employed in perinatal medicine. Identification of useful biomarkers may be enhanced by advanced methodology, as rapid multiplexed micro-assays become feasible that require only tiny amounts of blood or other biologic materials. CP is the outcome of one in four infants who survive NE, and strong genetic components are now recognized in the etiology. Genetic and genomic studies, in cooperation with teratology and developmental neurobiology, are likely to solve many mysteries in NE, and cast new light on recurring medicolegal issues. Studies employing appropriate research methodology indicate that in relatively high resource settings, the contribution of birth asphyxia to NE has been over-estimated, aberrant development manifested by birth defects including congenital microcephaly, abnormal fetal growth and/or genetic abnormalities is under-estimated and its mechanisms under-explored, information from the placenta markedly under-utilized, and genetic analysis of endophenotypes scarcely begun. We still have a lot to learn about what causes NE and what can be done about it. Is it time for an international and multidisciplinary Baby Brain Initiative?

Practice points

- Review family history of neurological disorders and consanguinity, previous babies with perinatal/neonatal death, neonatal encephalopathy and neurodevelopmental conditions including cerebral palsy and autism
- For term and near-term neonates requiring NICU admission, submit placentas for examination. Follow up results, request expert consultation if in question.
- Review maternal antenatal and perinatal records including maternal medical conditions such as thyroid disease, prenatal genetic testing, fetal biometry, delivery records and placental pathology which can provide insight into potential etiologies of NE
- Consider evaluation for congenital infections based on the presentation of NE, imaging findings, laboratory assessment, and prior maternal testing.
- Consider genome studies for etiology in babies with NE, if considered informative test parents as well

Research directions

- Seek a consensus on recommended workup for etiology of NE with focus on root causes, proposing both a minimal workup and a preferred more extensive workup.
- In representative populations, studies of NE that are prospective or in registries, examining etiologic information and clinical features of NE, co-morbidities in the newborn period (especially birth defects and aberrant fetal growth) and at outcome, placental features, and genetics/genomics.

Declaration of competing interest

There are no conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.siny.2021.101265.

References

[1] Naghavi M, Abajobir AA, Abbafati C, et al. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017;390(10100):1151–210.
[2] Tagin MA, Woolcott CG, Vincer MJ, et al. Hypothermia for neonatal hypoxic ischemic encephalopathy: an updated systematic review and meta-analysis. Arch Pediatr Adolesc Med 2012;166(6):558–66. https://doi.org/10.1001/archpediatrics.2011.1772 [published Online First: 2012/02/09].
[3] AAo Pediatrics. Neonatal encephalopathy and neurologic outcome, second Edition Report of the American College of Obstetricians and gynecologists' Task Force on neonatal encephalopathy. Pediatrics 2014;133(5):e1482–8.
[4] Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic–ischaemic encephalopathy. Early Hum Dev 2010;86(6):329–38.
[5] Lee AC, Kozuki N, Blencowe H, et al. Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. Pediatr Res 2013;74(S1):50–72.
[6] Boo N-Y, Cheah IG-S. The burden of hypoxic-ischaemic encephalopathy in Malaysian neonatal intensive care units. Singap Med J 2016;57(8):456.
[7] Gale C, Statnikov Y, Jawad S, et al. Neonatal brain injuries in England: population-based incidence derived from routinely recorded clinical data held in the National Neonatal Research Database. Arch Dis Child Fetal Neonatal Ed 2018;103(4):F301–6.
[8] Byford S, Weaver E, Anstey C. Has the incidence of hypoxic ischaemic encephalopathy in Queensland been reduced with improved education in fetal surveillance monitoring? Aust N Z J Obstet Gynaecol 2014;54(4):348–53.
[9] Joyce NM, Tully E, Kirkham C, et al. Perinatal mortality or severe neonatal encephalopathy among normally formed singleton pregnancies according to obstetric risk status:" is low risk the new high risk?" A population-based cohort study. Eur J Obstet Gynecol Reprod Biol 2018;228:71–5.
[10] Battin M, Sadler L, Masson V, et al. Neonatal encephalopathy in New Zealand: demographics and clinical outcome. J Paediatr Child Health 2016;52(6):632–6.

[11] Parker S-J, Kuzniewicz M, Niki H, et al. Antenatal and intrapartum risk factors for hypoxic-ischemic encephalopathy in a US birth cohort. J Pediatr 2018;203:163–9.
[12] Arnaez J, García-Alix A, Arca G, et al. Population-based study of the national implementation of therapeutic hypothermia in infants with hypoxic-ischemic encephalopathy. Ther Hypothermia Temp Manag 2018;8(1):24–9.
[13] Amin P, Zaher S, Penketh R, et al. Falling caesarean section rate and improving intra-partum outcomes: a prospective cohort study. J Matern Fetal Neonatal Med 2019;32(15):2475–80.
[14] Liljestrom L, Wikstrom AK, Agren J, et al. Antepartum risk factors for moderate to severe neonatal hypoxic ischemic encephalopathy: a Swedish national cohort study. Acta Obstet Gynecol Scand 2018;97(5):615–23.
[15] Nelson DB, Lucke AM, McIntire DD, et al. Obstetric antecedents to body-cooling treatment of the newborn infant. Am J Obstet Gynecol 2014;211(2):155. e1–55. e6.
[16] Simiyu IN, Mchaile DN, Katsongeri K, et al. Prevalence, severity and early outcomes of hypoxic ischemic encephalopathy among newborns at a tertiary hospital, in northern Tanzania. BMC Pediatr 2017;17(1):131.
[17] Manandhar SR, Basnet R. Prevalence of perinatal asphyxia in neonates at a tertiary care hospital: a descriptive cross-sectional study. J Nepal Med Assoc JNMA 2019; 57(219).
[18] Biselele T, Naulaers G, Bunga Muntu P, et al. A descriptive study of perinatal asphyxia at the university hospital of Kinshasa (democratic republic of Congo). J Trop Pediatr 2013;59(4):274–9.
[19] Lee J, Croen LA, Backstrand KH, et al. Maternal and infant characteristics associated with perinatal arterial stroke in the infant. Jama 2005;293(6):723–9.
[20] Horn AR, Swingler GH, Myer L, et al. Defining hypoxic ischemic encephalopathy in newborn infants: benchmarking in a South African population. J Perinat Med 2013; 41(2):211–7.
[21] Yates H, McCullough S, Harrison C, et al. Hypoxic ischaemic encephalopathy: accuracy of the reported incidence. Arch Dis Child Fetal Neonatal Ed 2012;97(1): F77–8.
[22] Lopes-Pereira J, Costa A, Ayres-De-Campos D, et al. Computerized analysis of cardiotocograms and ST signals is associated with significant reductions in hypoxic-ischemic encephalopathy and cesarean delivery: an observational study in 38,466 deliveries. Am J Obstet Gynecol 2019;220(3):269. e1–69. e8.
[23] Landman AJ, Immink-Duijker ST, Mulder EJ, et al. Significant reduction in umbilical artery metabolic acidosis after implementation of intrapartum ST waveform analysis of the fetal electrocardiogram. Am J Obstet Gynecol 2019;221 (1):63. e1–63. e13.
[24] Ellis M, Manandhar N, Manandhar DS, et al. Risk factors for neonatal encephalopathy in Kathmandu, Nepal, a developing country: unmatched case-control study. Bmj 2000;320(7244):1229–36.
[25] Tann CJ, Nakakeeto M, Willey BA, et al. Perinatal risk factors for neonatal encephalopathy: an unmatched case-control study. Arch Dis Child Fetal Neonatal Ed 2018;103(3):F250–6.
[26] Higashi H, Barendregt J, Kassebaum N, et al. Surgically avertable burden of obstetric conditions in low-and middle-income regions: a modelled analysis. BJOG An Int J Obstet Gynaecol 2015;122(2):228–36.
[27] Wang H, Coates MM, Coggeshall M, et al. Global, regional, national, and selected subnational levels of stillbirths, neonatal, infant, and under-5 mortality, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet (British edition) 2016;388(10053):1725–74. https://doi.org/10.1016/S0140-6736(16)31575-6.
[28] Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol 1976;33(10):696–705. https://doi.org/10.1001/archneur.1976.00500100030012.
[29] Murray DM, Bala P, O'Connor CM, et al. The predictive value of early neurological examination in neonatal hypoxic-ischaemic encephalopathy and neurodevelopmental outcome at 24 months. Dev Med Child Neurol 2010;52(2): e55–9. https://doi.org/10.1111/j.1469-8749.2009.03550.x.
[30] Chandrasekaran M, Chaban B, Montaldo P, et al. Predictive value of amplitude-integrated EEG (aEEG) after rescue hypothermic neuroprotection for hypoxic ischemic encephalopathy: a meta-analysis. J Perinatol 2017;37(6):684–9.
[31] Einspieler C, Bos AF, Krieber-Tomantschger M, et al. Cerebral palsy: early markers of clinical phenotype and functional outcome. J Clin Med 2019;8(10):1616.
[32] Rennie JM, de Vries LS, Blennow M, et al. Characterisation of neonatal seizures and their treatment using continuous EEG monitoring: a multicentre experience. Arch Dis Child Fetal Neonatal Ed 2019;104(5):F493–501.
[33] Mulkey SB, Plessis AD. The critical role of the central autonomic nervous system in fetal-neonatal transition. Semin Pediatr Neurol 2018;28:29–37. https://doi.org/10.1016/j.spen.2018.05.004.
[34] Badawi N, Kurinczuk JJ, Keogh JM, et al. Antepartum risk factors for newborn encephalopathy: the Western Australian case-control study. BMJ 1998;317(7172): 1549–53. https://doi.org/10.1136/bmj.317.7172.1549.
[35] Blume HK, Loch CM, Li CI. Neonatal encephalopathy and socioeconomic status: population-based case-control study. Arch Pediatr Adolesc Med 2007;161(7): 663–8.
[36] Broman SH, Nichols PL, Kennedy WA. Preschool IQ: prenatal and early developmental correlates. Oxford, England: Lawrence Erlbaum; 1975.
[37] Mir IN, Johnson-Welch SF, Nelson DB, et al. Placental pathology is associated with severity of neonatal encephalopathy and adverse developmental outcomes following hypothermia. Am J Obstet Gynecol 2015;213(6):849. e1-49. e7.
[38] Vik T, Redline R, Nelson KB, et al. The placenta in neonatal encephalopathy: a case-control study. J Pediatr 2018;202:77–85 e3. https://doi.org/10.1016/j.jpeds.2018.06.005.
[39] Dubovicky M, Belovicova K, Csatlosova K, et al. Risks of using SSRI/SNRI antidepressants during pregnancy and lactation. Interdiscipl Toxicol 2017;10(1): 30–4. https://doi.org/10.1515/intox-2017-0004.
[40] Peebles PJ, Duello TM, Eickhoff JC, et al. Antenatal and intrapartum risk factors for neonatal hypoxic ischemic encephalopathy. J Perinatol 2020;40(1):63–9. https://doi.org/10.1038/s41372-019-0531-6.
[41] Holland J, Brown R. Neonatal venlafaxine discontinuation syndrome: a mini-review. Eur J Paediatr Neurol 2017;21(2):264–8. https://doi.org/10.1016/j.ejpn.2016.11.003.
[42] Ford-Jones EL. An approach to the diagnosis of congenital infections. Paediatr Child Health 1999;4(2):109–12. https://doi.org/10.1093/pch/4.2.109.
[43] Muller WJ. Treatment of perinatal viral infections to improve neurologic outcomes. Pediatr Res 2017;81(1–2):162–9. https://doi.org/10.1038/pr.2016.191.
[44] Smithers-Sheedy H, Raynes-Greenow C, Badawi N, et al. Congenital cytomegalovirus among children with cerebral palsy. J Pediatr 2017;181:267–271. e1.
[45] de Vries LS. Viral infections and the neonatal brain. Semin Pediatr Neurol 2019;32: 100769. https://doi.org/10.1016/j.spen.2019.08.005.
[46] Kimberlin DW, Jester PM, Sanchez PJ, et al. Valganciclovir for symptomatic congenital cytomegalovirus disease. N Engl J Med 2015;372(10):933–43. https://doi.org/10.1056/NEJMoa1404599.
[47] McIntyre S, Blair E, Badawi N, et al. Antecedents of cerebral palsy and perinatal death in term and late preterm singletons. Obstet Gynecol 2013;122(4):869–77.
[48] Scher MS. Fetal neurology: principles and practice with a life-course perspective. Handbook of clinical neurology: Elsevier; 2019. p. 1–29.
[49] Roberts DJ. Placental pathology, a survival guide. Arch Pathol Lab Med 2008;132 (4):641–51. https://doi.org/10.1043/1543-2165(2008)132[641. Ppasg]2.0.Co;2 [published Online First: 2008/04/04].
[50] Burton GJ, Jauniaux E. Development of the human placenta and fetal heart: synergic or independent? Front Physiol 2018;9:373.
[51] Blair E, de Groot J, Nelson KB. Placental infarction identified by macroscopic examination and risk of cerebral palsy in infants at 35 weeks of gestational age and over. Am J Obstet Gynecol 2011;205(2):124. e1–24. e7.
[52] Wintermark P, Boyd T, Gregas MC, et al. Placental pathology in asphyxiated newborns meeting the criteria for therapeutic hypothermia. Am J Obstet Gynecol 2010;203(6):579. e1–79. e9.
[53] Burton GJ, Fowden AL, Thornburg KL. Placental origins of chronic disease. Physiol Rev 2016;96(4):1509–65.
[54] Aslam S, Strickland T, Molloy EJ. Neonatal encephalopathy: need for recognition of multiple etiologies for optimal management. Front pediatr 2019;7:142.
[55] Odd D, Váradi A, Rajatileka S, et al. Association between neonatal resuscitation and a single nucleotide polymorphism rs1835740. Acta Paediatr 2016;105(7): e307–12.
[56] Calkavur S, Akisu M, Olukman O, et al. Genetic factors that influence short-term neurodevelopmental outcome in term hypoxic-ischaemic encephalopathic neonates. J Int Med Res 2011;39(5):1744–56.
[57] Kaur Simranpreet, Adam John ChristodoulouMP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G, Amemiya A, editors. In: GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 2001 Oct 3. 1993–2021. [updated 2019 Sep 19].
[58] Jin SC, Lewis SA, Bakhtiari S, et al. Mutations disrupting neuritogenesis genes confer risk for cerebral palsy. Nat Genet 2020:1–11.
[59] Ramaswamy V, Horton J, Vandermeer B, et al. Systematic review of biomarkers of brain injury in term neonatal encephalopathy. Pediatr Neurol 2009;40(3):215–26.
[60] O'Hare FM, Watson RWG, O'Neill A, et al. Serial cytokine alterations and abnormal neuroimaging in newborn infants with encephalopathy. Acta Paediatr 2017;106 (4):561–7.
[61] Jenkins DD, Rollins LG, Perkel JK, et al. Serum cytokines in a clinical trial of hypothermia for neonatal hypoxic-ischemic encephalopathy. J Cerebr Blood Flow Metabol 2012;32(10):1888–96.
[62] Chalak LF, Sánchez PJ, Adams-Huet B, et al. Biomarkers for severity of neonatal hypoxic-ischemic encephalopathy and outcomes in newborns receiving hypothermia therapy. J Pediatr 2014;164(3):468–74.
[63] Dietrick B, Molloy E, Massaro AN, et al. Plasma and cerebrospinal fluid candidate biomarkers of neonatal encephalopathy severity and neurodevelopmental outcomes. J Pediatr 2020;226:71–9.
[64] Buck BH, Liebeskind DS, Saver JL, et al. Early neutrophilia is associated with volume of ischemic tissue in acute stroke. Stroke 2008;39(2):355–60.
[65] Yao H-W, Kuan C-Y. Early neutrophil infiltration is critical for inflammation-sensitized hypoxic-ischemic brain injury in newborns. J Cerebr Blood Flow Metabol 2020;40(11):2188–200.
[66] Morkos A, Hopper A, Deming D, et al. Elevated total peripheral leukocyte count may identify risk for neurological disability in asphyxiated term neonates. J Perinatol 2007;27(6):365–70.
[67] Nelson KB, Willoughby RE. Infection, inflammation and the risk of cerebral palsy. Curr Opin Neurol 2000;13(2):133–9.
[68] Hagberg H, Mallard C, Ferriero DM, et al. The role of inflammation in perinatal brain injury. Nat Rev Neurol 2015;11(4):192.
[69] O'Hare FM, Watson R, O'Neill A, et al. Persistent systemic monocyte and neutrophil activation in neonatal encephalopathy. J Matern Fetal Neonatal Med 2016;29(2):309–16.
[70] Mallard C, Wang X. Infection-induced vulnerability of perinatal brain injury. Neurol Res Int 2012;2012.
[71] Martinello KA, Meehan C, Avdic-Belltheus A, et al. Acute LPS sensitization and continuous infusion exacerbates hypoxic brain injury in a piglet model of neonatal encephalopathy. Sci Rep 2019;9(1):1–17.

[72] Lin CY, Chang YC, Wang ST, et al. Altered inflammatory responses in preterm children with cerebral palsy. Ann Neurol 2010;68(2):204–12.
[73] Numis AL, Foster-Barber A, Deng X, et al. Early changes in pro-inflammatory cytokine levels in neonates with encephalopathy are associated with remote epilepsy. Pediatr Res 2019;86(5):616–21.
[74] Fleiss B, Gressens P. Tertiary mechanisms of brain damage: a new hope for treatment of cerebral palsy? Lancet Neurol 2012;11(6):556–66.
[75] Hedtjärn M, Mallard C, Iwakura Y, et al. Combined deficiency of IL-1β18, but not IL-1αβ, reduces susceptibility to hypoxia-ischemia in the immature brain. Dev Neurosci 2005;27(2–4):143–8.
[76] Chevin M, Guiraut C, Sébire G. Effect of hypothermia on interleukin-1 receptor antagonist pharmacodynamics in inflammatory-sensitized hypoxic-ischemic encephalopathy of term newborns. J Neuroinflammation 2018;15(1):214.
[77] Ozaki E, Campbell M, Kiang A-S, et al. Inflammation in age-related macular degeneration. Retinal Degener Diseases 2014;801:229–35. https://doi.org/10.1007/978-1-4614-3209-8_30.
[78] Looney A-M, Walsh BH, Moloney G, et al. Downregulation of umbilical cord blood levels of miR-374a in neonatal hypoxic ischemic encephalopathy. J Pediatr 2015;167(2):269–73. https://doi.org/10.1016/j.jpeds.2015.04.060.
[79] Yang Z, Zhong L, Xian R, et al. MicroRNA-223 regulates inflammation and brain injury via feedback to NLRP3 inflammasome after intracerebral hemorrhage. Mol Immunol 2015;65(2):267–76.
[80] Lingam I, Avdic-Belltheus A, Meehan C, et al. Serial blood cytokine and chemokine mRNA and microRNA over 48 h are insult specific in a piglet model of inflammation-sensitized hypoxia–ischaemia. Pediatr Res 2020. https://doi.org/10.1038/s41390-020-0986-3.
[81] Whitehead CL, Teh WT, Walker SP, et al. Circulating MicroRNAs in maternal blood as potential biomarkers for fetal hypoxia in-utero. PloS One 2013;8(11):e78487.
[82] Ma Q, Zhang L. Epigenetic programming of hypoxic–ischemic encephalopathy in response to fetal hypoxia. Prog Neurobiol 2015;124:28–48.
[83] Bustelo M, Barkhuizen M, van den Hove DL, et al. Clinical implications of epigenetic dysregulation in perinatal hypoxic-ischemic brain damage. Front Neurol 2020:11.
[84] Montaldo P, Cunnington A, Oliveira V, et al. Transcriptomic profile of adverse neurodevelopmental outcomes after neonatal encephalopathy. Sci Rep 2020;10(1):1–7.
[85] Montaldo P, Kaforou M, Pollara G, et al. Whole blood gene expression reveals specific transcriptome changes in neonatal encephalopathy. Neonatology 2019;115(1):68–76.
[86] du Plessis AJ, Volpe JJ. Perinatal brain injury in the preterm and term newborn. Curr Opin Neurol 2002;15(2):151–7.
[87] Denihan NM, Kirwan JA, Walsh BH, et al. Untargeted metabolomic analysis and pathway discovery in perinatal asphyxia and hypoxic-ischaemic encephalopathy. J Cerebr Blood Flow Metabol 2019;39(1):147–62.

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