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# Acute symptomatic neonatal seizures, brain injury and long-term outcome: the role of neuroprotective strategies.

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## Abstract

**Introduction:** Neonatal seizures are frequent but underdiagnosed manifestations of acute brain dysfunction and an important contributor to unfavorable outcomes. Etiology and severity of brain injury are the single strongest outcome determinants.

**Areas covered:** The authors will discuss the prognostic role of acute symptomatic seizures versus brain injury and the main neuroprotective and neurorestorative strategies for full-term and preterm infants.

**Expert opinion:** Prolonged acute symptomatic seizures likely contribute to long-term outcomes by independently adding further brain injury to initial insults. Correct timing and dosing of therapeutic interventions, depending on etiology and gestational ages, need careful evaluation. Although promising strategies are under study, the only standard of care is whole-body therapeutic hypothermia in full-term newborns with hypoxic-ischemic encephalopathy.

**Keywords:** acute symptomatic; antiseizure medications; brain injury; erythropoietin; experimental models; hypothermia; neonatal seizures; neuroprotection; outcome; status epilepticus

**Article highlights**

- Acute symptomatic neonatal seizures are associated with worsening long-term outcome.
- The main prognostic factors are etiology and severity of underlying brain injury.
- Experimental and clinical data suggest and independent role of seizures in brain injury, especially if prolonged.
- Whole body therapeutic hypothermia in full term neonates with hypoxic-ischemic encephalopathy results in improved long-term outcome and reduced seizure burden.

- Additional neuroprotective and neurorestorative strategies have been proposed to prevent or reduce detrimental secondary changes, with mixed results.

## 1. Introduction

Epidemiology:

Seizures are the most frequent manifestation of acute brain injury in newborns. Recent population-based studies documented an incidence of 2.29/1000 live births [1], and the following risk factors for seizures: at least one complication of pregnancy, at least one neonatal complication, low Apgar score, need for resuscitation at birth, intraventricular hemorrhage (IVH) grades II–IV for preterm, and acute hypoxic–ischemic encephalopathy (HIE) for full-term infants [2].

Etiologies:

Acute symptomatic seizures are the most frequent cause of neonatal seizures, full-term and near-term neonates being mainly affected by hypoxic-ischemic encephalopathy (HIE) or stroke, and very or extremely preterm newborns by intraventricular haemorrhage (IVH) and its complications, or by white matter injury of prematurity. Additional etiologies include acute metabolic derangements and central nervous system infections [3].

Inborn errors of metabolism and genetically-determined neonatal-onset epilepsies require a different management and will not be reviewed here.

Definition of Neonatal Seizures:

The current definition of neonatal seizures is EEG-based and requires the presence of at least 10 second-long paroxysmal activity with definite beginning and end [4]. From a clinical point of view, various classifications have been provided in the literature [5-8] and there has been a recent proposal for a new classification by the International League Against Epilepsy (ILAE), which has not been published yet. However, diagnosis requires conventional EEG or amplitude-integrated EEG recordings [8-11]. Based on the association of EEG discharges with a clinical component, they are divided into electroclinical or eletrographic-only, while the category of clinical seizures has been discouraged, as clinical recognition of seizures in newborns is prone to a high risk of misdiagnosis [12,13] and clear-cut clinical evidence has demonstrated the need to treat electrographic seizures just as aggressively as electroclinical ones [14,15].

Treatment:

Available guidance on therapy is mainly based on low-levels of evidence and traditionally relies on phenobarbital, phenytoin, benzodiazepines or lidocaine (depending on regional-specific practice), but the use of levetiracetam is emerging, based on low drug-to-drug interactions, linear kinetics, availability of an intravenous formulation, and evidence for a lower risk of neurotoxicity [16], although a randomized controlled study comparing levetiracetam with phenobarbital documented higher effectiveness of the latter [17]. Overall, response rates approximately range between 40 and 60% [18-21] but considering the self-limiting nature of acute symptomatic seizures and the diagnostic pitfalls, this is obviously affected by study population and study design (ascertainment tools, timing of administration and of evaluation of effectiveness).

Outcomes:

Although innovations in critical care, such as improvements in parenteral nutrition and non-invasive respiratory support [22], have reduced mortality, the rate of normal outcomes has decreased in some countries, mainly due to the higher percentage of preterm babies. Importantly, legislative differences concerning end of care also need consideration, and can sometimes account for some inconsistencies, as high rates of transition to palliative care can reduce percentages of patients with unfavorable neurological outcomes. For example, in a population-based study, the incidence of epilepsy after neonatal seizures was 15.2% (16.3% in patients born at term, and 14.3% in preterm-born subjects) [23] in keeping with historical data from a review from the same group [24], documenting stable rates in the last 60 years.

Prognostic factors:

The main prognostic factors in acute symptomatic neonatal seizures include: antenatal factors (placental factors including chorioamnionitis), perinatal factors (gestational age, aetiology, severity and patterns of brain injury as expressed by cranial ultrasound and brain magnetic resonance imaging (MRI), Apgar scores, birth weight, neurological examination, abnormal background EEG) [25-27]. Seizure aetiology is among the key players for outcome [26]. Background EEG and electrographic-only seizures have been found as independent predictors of lack of response to phenobarbital in one study [19]; moderately and severely abnormal EEG was confirmed to be associated with poor response in an additional study, which also identified higher mean seizure score and higher degrees of brain MRI injury (white matter, cortex, and watershed regions) to be associated with poor response to phenobarbital [28]. Based on these readily available variables, prediction models have been constructed, which can be performed at the bed-side by combining clinical, EEG and imaging data [27,29,30].

## 2. Body

A huge body of literature has tried to separate the detrimental effects of seizures per se from those of their underlying etiology.

2.1 Brain injury and pathophysiology of acute seizures

### 2.1.1 Neonatal HIE in full-term newborns

The pathophysiology of brain injury in HIE has been extensively reviewed [31]. In neonatal HIE, progression of clinical signs occurs after a latent period and then resolves within several weeks [32].

This time-line of clinical evolution is determined by the occurrence of secondary energy failure, clinically announced by seizures and other signs of encephalopathy, the third step after the initial asphyxia and the subsequent latent period of 8–24 h after resuscitation [33,34]. This delayed energy failure is determined by the activation of an excito-oxidative cascade. The earliest event is the activation of excitatory glutamate receptors, especially N-methyl-dextro-aspartate (NMDA) receptors (NMDAR), which determine massive membrane depolarization and entry of calcium in neurons, followed by oxidative stress, worsening mitochondrial dysfunction with lactic acidosis and mitochondrial failure, which can end up in either apoptosis or necrosis, depending on the energy supplies to the cell [35-37] and intrinsic factors to the developing brain [38]. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors, also activated, probably contribute to seizure occurrence at this stage [39]. Inflammation (through glial activation) and delayed cell death also additionally worsen brain damage [31].

### 2.1.2 Preterm injury to the white matter

The main predisposing factors to IVH are the immaturity of the vasculature of the germinal matrix and its passivity to systemic blood pressure changes, which are

typical of preterm newborns. White matter injury can be favored by cerebral oxygenation changes, infection and inflammation, but also by intrinsic vulnerability to oxidative stress under hypoxic-ischemic conditions [40,41], which are in turn favored by immature vascular supply and autoregulation mechanisms [40,42]. In the acute phase, injury to the subplate neurons can transiently increase cortical excitability [43], resulting in acute seizures. The ensuing disruption of projection and association fibers and the secondary effects on the cortical plate and its connections to the thalamus are thought to be key determinants for long-term outcomes, especially relating to epilepsy and cognition [44].

### 2.2 Interaction between seizures, brain damage and additional detrimental pre/perinatal factors

Of note, a body of evidence exists, favoring the view that one sub-injurious event (such as gestational or neonatal systemic inflammation, infection, gestational chronic mild maternal stress, and gestational hypoxia) can sensitize the developing brain to second injurious factors (neonatal insults), causing an exacerbated cascade of deleterious effects on the developing brain, including activation of glutamate receptors, cytokines, toll-like receptor pathways, and apoptosis [45].

#### 2.2.1 Seizure-related variables and seizure burden

Semiology per se should not be considered as a reliable prognostic indicator, for two main reasons: the frequent co-occurrence of different seizures types in single newborns [46], and its dependence on etiology [47]. However, there is an important point to make: newborns with exclusively subclinical seizures have higher mortality rates [48], probably because of their poorer neurological and general state [49]. Literature data on humans suggest that outcome is a matter of seizure duration, rather than an all-or-nothing effect. Mortality is significantly associated with seizure

burden, especially status epilepticus [25,48]. A synergistic effect of status epilepticus with the underlying brain disorder in determining epilepsy has been suggested [50] However, status epilepticus independently predicts outcome, even in comparison with recurrent seizures [27] and is an independent risk factor for epilepsy [46]. Refractory seizures are associated with twice the risk of death than those controlled with the initial antiseizure drug loading dose [48]. However, drug-resistance occurs in the most severely affected infants, and status epilepticus is more frequent in critically ill, encephalopathic newborns [27,49]. However, this is unlikely to be the whole story, as standardized treatment protocols have been shown to result in better short-term outcomes, with lower maximum phenobarbital concentrations, lower progression to status epilepticus and shorter in-hospital stay [51]. It would be noteworthy to know what the long-term outcomes of these babies will be, because if improved prognosis is confirmed, this would mean that by applying more rigorous protocols we will be able to address the modifiable amount of brain injury possibly directly linked to the effect of ongoing seizures. An independent role for seizures on outcome has been suggested by clinical research. The relative risk for adverse outcome in neonates with perinatal asphyxia was 8.41 (4.07–17.39) and for neonates with stroke was 4.95 (1.07–23.0) when suffering neonatal seizures compared to newborns with the same types of brain injury but no seizures [52].

Ictal fraction (calculated as the total duration of seizures/duration of the EEG recording × hour) is also predictive of outcome, when it exceeds 10 minutes [26]. The number of electrographic seizures (none, 1-75, > 75 seizures) correlates with subsequent mortality and morbidity in a cohort of at-risk newborns and newborns with HIE [14].

Furthermore, the odds for abnormal outcome increase over nine-fold if seizure burden exceeds 40 minutes and eight-fold if the hourly seizure burden is over 13 minutes/hour, and this is independent from having electrographic seizures, HIE grade or therapeutic hypothermia. Noteworthy, the mere presence or absence of seizures is not associated with outcome [53]. Longer seizure duration is associated with higher 1-year risk of epilepsy in children with arterial ischemic stroke (including newborns), and each 10-minutes increase in seizure burden is associated with a five-fold risk increase, while having more than 10 seizures increases the risk of epilepsy by 30-fold compared to no seizures [54]. In newborns with HIE, seizure burden also correlates with severity of brain injury [55]. Infants at risk for hypoxic-ischemic brain injury, after controlling for injury severity on brain MRI, have a worse motor and cognitive outcome if they have seizures, compared to patients with no seizures [56].

Importantly, it must be emphasized that the critical seizure burden for aggravation of brain damage is still unknown [57], and as a result, the definition of status epilepticus in neonates is still controversial and has not changed [58], in spite of these clinical research data.

### 2.3 Mechanisms for seizure-induced detrimental effects

#### 2.3.1 Clinical data

How do seizures result in additional brain damage to that provoked by the initial insult to the developing central nervous system? Some lines of research have been delineated, documenting various involved pathogenic pathways.

##### 2.3.1.1 Cerebral blood flow (CBF)

Seizures can induce detrimental cerebrovascular effects [60], especially in preterm babies, lacking cerebral pressure autoregulation [61]. This means that ictal cerebral

haemodynamic changes passively reflect systemic haemodynamic events, and notably increases in arterial pressure, which can damage the germinal matrix by increasing intracranial pressure [60, 61]. A secondary effect from muscle contraction is to be excluded, as these changes are documented irrespective of the presence of a clinical correlate to seizures and in mechanically ventilated neonates [60, 62]. Even more importantly, ictal changes in CBF can also occur without systemic pressure fluctuations. [63]

Brain-derived circulating endothelial cells, which were used in animal models as an indicator of cerebral vascular endothelial damage and seizure-induced cerebral blood flow dysregulation, were looked for in a pilot study on infants with either HIE or IVH with documented clinical seizures and were found to be significantly higher than in infants with cerebrovascular insults and subclinical or no seizures. Furthermore, their levels decreased with seizure control [64].

**2.3.1.2 Lactic acidosis**

Hyperlactacidemia is a measure of hypoxia, and in full-term newborns with HIE persistent lactic acidosis correlates with severity of EEG abnormalities and seizure burden (mean time to normal lactate: 10.0 hours in infants without seizures versus 27.3 hours in infants with seizures) [65].

Seizures can contribute to lactic acidosis in infants with HIE progressing to severe neurological disability or death [66]. Abnormal lactate levels on spectroscopy in newborns with HIE correlate with neurodevelopmental outcome [67], and in infants with abnormal outcome recovery takes much longer, as the increase is still detectable at 1 month of age [68].

Reliance on anaerobic metabolism has been confirmed by near-infrared spectroscopy (NIRS), which detected a decline in cerebral oxygenation during

repetitive seizures [62], suggesting an uncoupling between increased metabolic requirements and energy supply [59], resulting in relative cerebral hypoxia [62]. The magnitude of this effect seems to increase in a dose-dependent manner, as each increase in seizure score is independently associated with increased lactate/choline ratio on spectroscopy, even after controlling for confounders [69], demonstrating an independent association of seizure severity with brain injury and wider extent of the hypoxic areas compared to MRI-detectable structural damage [69].

**2.3.2 Animal data**

Animal data offer a heterogeneous landscape of weaknesses and strengths which are unique to the immature brain.

In the secondary phase of hypoxic-ischemic damage, severe seizures can contribute to the spreading of injury from the core to mildly affected regions [70]. Additionally, in animal models of induced cord occlusion, prolonged lactic acidosis is found in those sustaining the most severe cerebral injury on histology [71]. Similarly, in PN1 rats with hypoxic brain injury and prolonged seizures, micro-dialysis performed during seizures detects decreased glucose and adenosine triphosphate, with raised lactate and b-hydroxybutyrate [72], and a marked exacerbation of the extent of cerebral tissue damage. These data are in complete agreement with findings from clinical research in human newborns.

Changes in glutamate and γ-amino-butyric acid (GABA) receptors have also been reported after status epilepticus in young animals [73, 74] and recurrent seizures can adversely affect neurogenesis [75], and induce synaptic reorganization [76-78]. However, compared to older ages, the immature brain shows peculiar characteristics, making it less vulnerable to the excito-toxic effects of glutamate [79, 80], probably due to lower calcium entry into the cell, and this resistance may be due

to smaller density of active synapses, lower energy consumption, and a relative immaturity of cell death cascades.

Interestingly, seizures in the newborn brain are also associated with less oxidative stress than in adults [81]. High concentrations of the mitochondrial uncoupling protein (UCP2), due to the high fat content of maternal milk, also seem to reduce reactive oxygen species (ROS) production in the neonatal brain [82].

However, oxidative stress caused by seizures still represents an important contributor to cerebral vascular injury and cerebral blood flow dysregulation, as documented in a piglet model, in which seizures have been associated with increased ROS in cerebral vessels and cortical astrocytes, contributing to the long-term adverse cerebrovascular effects of seizures [83], although these also show adaptive effects by activating endogenous antioxidant neuroprotective pathways [83]. We discussed some of the main pathways activated by insults to the developing brain and we reviewed the main detrimental effects promoted by seizing activity.

What are the main strategies counteracting these cascades? Essentially from a temporal point of view, we can distinguish between neuroprotective and neurorestorative interventions [84] (Figures 1 and 2), which are based on the greater neuroplasticity of the neonatal brain compared to older ages [85].

## 3. Neuroprotective strategies

### 3.1 Hypothermia

Therapeutic hypothermia is the current standard of care for term infants with moderate-to-severe HIE in high-income countries [86]. Newborns receiving standard care plus hypothermia have higher survival without neurological abnormalities, significant reduction in their risk of cerebral palsy or moderate/severe disability [87]. The functional outcome at 7–8 years correlates with the 18-month

neurodevelopmental assessment, supporting the long-term predictive value of the favorable midterm outcomes reported in the original trials [88]. Based on current evidence, more benefit can be expected in newborns with moderate than severe encephalopathy [89]. Additionally, an even lower incidence of epilepsy at 2 years of age has been reported in comparison with the original randomized controlled trials [90].

If this is exclusively linked to mitigation of brain injury or also positively affected by reduction of seizure-induced damage, it is hard to know. However, a huge body of evidence from animal studies indicates that seizures and epileptiform activity in HIE can be reduced by therapeutic hypothermia [91]. Data on humans have been more conflicting, as reduced incidence and severity of seizures was documented in observational studies but not in meta-analyses of randomized controlled trials [92, 93]. Two observational studies showed lower electrographic seizures burden on continuous EEG in neonates with moderate encephalopathy [94], and after accounting for severity of brain injury on MRI [95], while the third evaluated newborns with arterial ischemic stroke and found lower likelihood of seizures in treated patients compared to neonates with stroke and no hypothermia [96].

The risk of having seizures is lower in cooled than in non-cooled newborns if they have moderate encephalopathy, while no difference was documented between the two groups if the degree of HIE is severe [97]. It is possible that the beneficial effect of hypothermia is insufficient in reversing the excito-toxic changes increasing seizure probability in severe cases, as the degree of injury is exceedingly high and cannot be efficiently counteracted, or that the secondary detrimental effects continue to develop over a longer time frame than in moderate cases, or both. In fact, more severe HIE is associated with greater primary cerebral damage, earlier onset of secondary

deterioration, and greater neuronal loss, but also (as we discussed above) with more prolonged metabolic derangement.

Many controversies also exist with respect to clinical scenarios falling outside currently approved guidelines, such as mild HIE, delayed cooling, post-natal collapse, late preterm infants, and infants with IVH or other types of brain injury. In animal models, late cooling (>6 hours) in the setting of severe hypoxic–ischemic injury, longer (120 hours vs. 72 hours) and deeper cooling (32°C vs. 33.5°C) are not beneficial and may prove detrimental [98, 99]. We already discussed above the critical role of time in the sequential changes during hypoxic-ischemic events. This results in the same interventions to possibly determine different effects when administered in different phases. The short latent phase before secondary cellular deterioration is the period during which therapeutic interventions are most likely to improve outcome. This is true for blood flow restoration, as well as for glucose levels [100]. It is also likely that maturational changes in the developing brain account for higher vulnerability of preterm infants compared to fullterm infants. Moreover, the higher incidence of adverse effects can also negatively affect outcome. Clinical studies evaluating infants not satisfying current cooling criteria document potential areas of intervention, but also a need for caution in specific sub-populations. In fact, in a retrospective observational study, no significant differences in 2-year neurodevelopmental outcomes were found in infants cooled according to or outside the standard entry criteria [101], except for newborns with severe degrees of intracranial hemorrhage, experiencing high rates of coagulopathy, death or disability. A retrospective cohort of preterm infants <36 weeks' gestation with HIE receiving whole body hypothermia documented death or moderate to severe neurodevelopmental impairment at 18-24 months in 50% infants with known

outcomes [102], and some studies documented increased incidence of hyperglycemia and leukopenia, more frequent and severe brain injury patterns, and death [103].

### 3.2 Haemodynamic management

Hemodynamic management aiming at optimizing autoregulation can be considered as an adjunctive therapeutic strategy to hypothermia in HIE. In a study on newborns, the mean arterial blood pressure “with optimized autoregulatory function” was measured in order to evaluate autoregulation. An association between greater duration and deviation of blood pressure below the optimal values and greater injury in the paracentral gyri and white matter was documented, while optimal mean values were related to lesser injury in the white matter, putamen, globus pallidus, and brainstem. Finally, higher blood pressure levels were associated with reduced injury in the paracentral gyri [104].

### 3.3. Pharmacological agents

#### 3.3.1 Allopurinol

Allopurinol has anti-oxidant properties through xanthine oxidase inhibition, chelation of unbound iron and scavenging of free hydroxyl radicals. It also preserves NMDAR integrity [105]. While in preclinical studies it was shown to decrease brain injury in rodent models in the acute phase of hypoxic-ischemic brain injury [106], data in human neonates have been more conflicting.

When administered intravenously at 40 mg/kg within 4 hours of birth in severe HIE, it decreased serum-free radical levels, and improved cerebral blood flow, with no toxicity [107]. A small randomized control trial tested the same dose within 2 hours of birth and then repeated daily in the first 3 days of life for term newborns with mild, moderate, or severe encephalopathy [108]. Better developmental outcome at 12

months was documented compared with placebo. In a follow-up study of neonates enrolled in these two trials, neurodevelopmental and cognitive outcomes at 4 to 8 years showed no difference in adverse developmental outcomes between treatment and control groups, although a significant decrease in the risk of severe adverse outcome (death or severe disability) was found in a subgroup analysis in moderate HIE [109]. The need to exclude the most severe cases in order to find impact on outcomes raises the question of not gaining any advantage if treatment commences when brain damage is too severe. Therefore, a randomized multi-center placebo-controlled trial was performed on women in labour during suspected fetal hypoxia, but failed to improve long-term developmental and behavioral outcome at 5 years of age [110].

**3.4 Xenon**

Xenon is a monoatomic gas with very high tissue solubility. It is a non-competitive inhibitor of NMDA glutamate receptor, with proven antiapoptotic and neuroprotective effects following hypoxic-ischaemic injury in animals. Out of fourteen human newborns treated with 30% inhaled xenon for 24 h combined with 72 h of moderate systemic hypothermia, five had seizures, for which they received phenytoin and/or phenobarbital and which stopped during xenon therapy, but recurred within a few minutes after withdrawal. Xenon displayed an anticonvulsant and EEG depressant effect at substantially lower doses than anaesthetic ones [111].

3.3.2 Magnesium

Magnesium links to NMDA channels in a voltage-dependent manner and was proposed for clinical use to contrast glutamate excito-toxicity in order to protect the developing brain from NMDAR-mediated injury [112, 113]. Its effects in clinical cohorts of newborns with HIE were evaluated by a meta-analysis with the primary

aim to assess the composite outcome of death or moderate-to-severe neurodevelopmental disability at 18 months. Magnesium was administered within the first 24 hours after birth with one of the following schemes: three doses of 250 mg/kg per dose 24-h apart [114,115] plus dopamine 5 mg/kg/min in [116] or 250 mg/kg within 30 minutes of birth followed by 125 mg/kg at 24 and 48 h [117, 118]. The authors found no difference in the composite primary outcome of death or moderate-to-severe neurodevelopmental disability at age 18 months, while the short-term composite outcome of survival with abnormalities in neurodevelopmental exam, neuroimaging or neurophysiologic investigations improved [119].

A retrospective observational study of singleton pregnancies complicated by prolonged premature rupture of membranes at 23+°-31+6 weeks receiving magnesium for tocolysis or not, documented that magnesium can prolong the latent period, and showed neuroprotective effects for neonatal IVH, periventricular leukomalacia and developmental delay during infancy. These benefits were limited to the subgroup between 23 and 27+6 weeks' gestation. Furthermore, prolonged in utero exposure to magnesium sulphate was associated with bone mineralization [120].

3.3.3 Melatonin

Melatonin has antioxidant, anti-inflammatory and anti-apoptotic properties [121]. It is a scavenger for reactive oxygen and nitrogen species [122], it decreases lipid peroxidation and serum nitrite/nitrate concentrations [123], and stimulates both antioxidant enzyme activity and their mRNA levels [122]. It also reduces proinflammatory cytokines by preventing translocation of the nuclear factor-kappa B of activated B cells, reduces phospholipase A2, lipoxygenase and cyclooxygenases activation [124] and leukocytes recruitment to inflammatory sites [125] and vascular

endothelial growth factor levels [126]. Finally, together with anti-apoptotic activity mainly targeting the mitochondria, it also enhances cell rescue pathways [121].

In a randomized controlled pilot study, newborns with HIE were allocated either to whole-body hypothermia alone or plus melatonin (five daily 10 mg/kg enteral doses). Compared with healthy newborns, patients with HIE had higher levels of serum melatonin and nitric oxide, and plasma superoxide dismutase at birth, while the two HIE groups did not differ for clinical, EEG or other laboratory characteristics. At 5 days, greater increase in melatonin and decline in nitric oxide, but less decline in superoxide dismutase were found in patients treated with hypothermia plus melatonin, and this group had fewer seizures, less brain white matter abnormalities and improved survival without neurological or developmental abnormalities (evaluated by neurological examination and Denver Developmental Screening Test II) than controls, suggesting correlation with favorable mid-term outcome. A small trial in infants with HIE randomized to receive melatonin plus hypothermia or hypothermia alone reported improved survival at 6 months of age without neurological or developmental abnormalities [127].

One of the main limitations is the unavailability of a safe intravenous solution (most contain alcohol), and risk for limited enteral absorption, especially in multi-organ failure [105].

3.3.6 Erythropoietin

Erythropoietin (Epo) is a cytokine originally known for its role in erythropoiesis, but also providing important functions in the central nervous system as a neuroprotective agent and a growth factor [128]. Clinical and pre-clinical studies suggest that chronic exposure to hyperoxia results in subtle, diffuse injury to the immature brain [129-131], microglial activation [130, 132] induced by oxidative stress, inflammation and

cellular degeneration, which can lead to hypomyelination and long-term cognitive deficits [129, 130]. Importantly, such hyperoxic conditions might be unavoidable during neonatal intensive care stay.

Experimental models of preterm brain injury seem to suggest a positive effect of high dose Epo in long-term neurocognitive function in rats. In one study, 20,000 IU/kg Epo by single intraperitoneal injection at the onset of hyperoxia in P6 rats improved hyperoxia-induced oligodendrocyte degeneration with preservation of white matter structures an improved cognitive outcome by reducing hyperoxia-induced deleterious effects on neuronal plasticity [133].

Clinical studies were encouraged by positive results from experimental research. Pharmacokinetic and safety studies show that 500-3000 U/kg Epo is safe [134, 135]. In one study, 24 newborns with HIE received a maximum of six Epo doses in addition to hypothermia. Eight (36%) had moderate-to-severe brain injury on neonatal MRI, but just one (4.5%) had moderate-to-severe disability at latest follow-up (mean age in the whole cohort: 22 months) [136]. In a retrospective cohort of extremely low birth weight infants, Epo was associated with higher cognitive and motor scores at BSID-II or BSID-III and no long-term detrimental effects (last examined at 36 months) [137]. Preterm infants born between 26 and 31weeks + 6 days of gestational age took part into a randomized, double-blind, placebo-controlled trial in which they received either recombinant human Epo (3000 IU/kg) or placebo given intravenously < 3 hours, at 12-18 hours, and at 36-42 hours after birth. White matter injury was assessed at term-equivalent age by conventional brain MRI. Fewer infants treated with recombinant human erythropoietin had abnormal scores for white matter injury, white matter signal intensity, periventricular white matter loss, and gray matter injury compared to untreated infants [138]. A positive role in developmental

processes in the preterm brain was suggested by tractography, demonstrating a diffuse (although weak) increase in the strength of local connections and a possible trophic effect, facilitating the development of peripheral and frontal, temporal, subcortical and limbic core connections [139]. However, a randomized trial on preterm infants born between 24 weeks and 27+6 weeks of gestation receiving Epo or placebo in the first 24 hours after birth failed to document significant differences in death or severe neurodevelopmental impairment at 2 years [140]. Finally, Epo also works as a trophic factor, stimulating neurogenesis [141, 142]. Experimental studies with neonatal stroke models showed that Epo reduces infarct volume [143, 144] and improves motor and cognitive function [145, 146], up to 1 week after the onset of neonatal stroke [147]. Although experimental models and human brain MRI data seem promising, prospective, randomized case-control clinical studies with long-term follow-up are needed, using standardized neurodevelopmental scales to assess the overall, neurological and neurocognitive outcome of patients treated with Epo.

3.3.4 Vitamin E

Vitamin E is a chain-breaking antioxidant and the main lipid peroxyl radical scavenger. A meta-analysis documented reduced risk of intracranial hemorrhage but increased risk of sepsis in preterm infants [148].

3.3.5 Caffeine

Caffeine reduces the incidence of bronchopulmonary dysplasia and improves survival without neurodevelopmental disability when used for apnoea of prematurity [149].

However, caution is required in its use outside this indication. First of all, prenatal exposure predisposes to intrauterine growth restriction and small growth for gestational age at birth. Even more importantly, in animal models, exposure during

pregnancy and breast-feeding seems to adversely affect neuronal development. The potential for multiple impacts on developing brain derives, first of all, from its role as a non-selective antagonist on adenosine receptors [150], which are highly expressed in the immature brain with a modulating role [151], thus their inactivation can result in precocious oligodendrocytes maturation, possibly leading to reduced oligodendrocytes pool in later life. Additionally, aside from acting as a free radicals scavenger, caffeine also inhibits phosphodiesterases, promoting intracellular calcium release and interfering with GABA-A receptors [152]. In fact, the development of GABAergic neuronal networks in the primary visual cortex was shown to be affected by caffeine in rats, with increased synaptic activity in vitro and elevated network activity in vivo. Similarly, in vivo abnormalities in hippocampal network activity were found in the neonatal period, persisting until adulthood. Most importantly, animal data suggest increased seizure susceptibility in a hyperthermia-induced seizure model [153], and both a trend towards increased seizure incidence and a three-fold increase in seizure burden [154] in a secondary post-hoc analysis of a trial of early high-dose caffeine citrate administration to preterm infants, aiming to improve white matter microstructural development [155].

Caffeine also increases oxygen extraction, suggesting transient stimulating effect on brain metabolism. However, in a cohort of preterm babies < 32 weeks of gestational age, no significant changes occurred in brain perfusion or in electrical brain activity when exposed to 10 mg/kg caffeine [155], even if a different study, using Doppler ultrasound, showed decreased CBF velocity [156].

Finally, phenobarbital-mediated impaired neurogenesis was largely restored by 10 mg/kg caffeine preconditioning [157], and this finding might pave the way to new lines of research.

### 3.5 Mesenchymal stem cells and neurotrophic factors

Neurotrophic factors and mesenchymal stem cells (MSCs) have been studied in experimental models of stroke and intracranial hemorrhage. The state of the art on their use in perinatal arterial ischemic stroke (PAIS) has been recently reviewed [158].

MSC treatment reduces infarct volume and blood–brain barrier disruption and increases angiogenesis, leading to neurovascular repair and improved cerebral blood flow. They also stimulate neurogenesis [159]. These effects are thought to be reached by a paracrine effect [158], as the majority of these cells do not survive more than 72 hours [160].

One of the main issues in applying neuroprotection to PAIS is that its neurological signs become apparent after the therapeutic time window [158]. A correlation between neurotrophic factors and progenitor cells in serum and the severity and outcome of ischemic stroke has been documented in adults [161], while abnormal levels of neurotrophic factors were detected in newborns with various perinatal brain insults, including HIE, hydrocephalus, and IVH, although not PAIS, in some cases correlating with the severity of brain injury [162-164]. Autologous umbilical cord blood cells have been safely administered to neonates with HIE [165]. Reduced levels of VEGF receptor-2 after neonatal stroke worsen injury, increase cell death, and reduce endothelial cell proliferation in rats, indicating a role for vascular endothelial growth factor (VEGF) signaling in repair after ischemic injury and in neuroprotection against apoptosis [166]. Importantly, motor and cognitive functions are also improved and brain infarct volume is attenuated [167]. Epidermal growth factor (EGF) and insulin-like growth factor (IGF-1) showed beneficial effects in experimental models of

preterm white matter injury. Selective overexpression of human EGF receptor in oligodendrocyte (OL) lineage cells and intranasal administration of EGF immediately after injury decreased OL death and induced generation of new OLs from progenitor cells [168]. IGF-1 also showed protective effects in newborn animals and in cultured pre-OLs [169], with positive functional effects in immature rats [170]. Intranasally-administered stem cells in rat models of preterm injury mitigate myelin injury and improve behavioral outcome [160, 171]. In a different experimental model of preterm white matter injury, transplantation of OL progenitor cells produced from embryonic stem cells resulted in their successful differentiation, myelin sheath formation, and proliferation of endogenous neural stem cells. Functional benefits were also documented at 6 weeks [172].

**3.6 Antiseizure medications**

Based on the contribution of acute seizures in increasing the detrimental effects of the initial brain insult, a timely and effective treatment is likely to represent a critical step in protecting the newborn brain. Consequently, the first neuroprotective strategy when managing acute symptomatic seizures is effectively stopping them. As response rate is around 50% for medications used as first-line [18], and acute symptomatic seizures are self-limiting phenomena, one major issue is to find an effective and safe drug to administer. The most widely used first-line antiseizure medications include phenobarbital and phenytoin, due to the longest “real world” use and evidence from randomized controlled trials [18]. However, due to concerns relating to their safety, other medications are widely used, usually as second or third-line, including benzodiazepines (midazolam, lorazepam, diazepam), or lidocaine, or have been proposed even as first-line due to safety and kinetics, such as levetiracetam, which nonetheless was documented to be less efficacious than

phenobarbital [17], resulting in better reserving it to second or third-line treatment. Alternative drugs, such as topiramate, have more limited use due to the unavailability of an intravenous formulation (Table 1).

Additionally, there is a huge body of evidence for a potential detrimental effect of antiseizure medications. Descriptions of deleterious effects on long-term outcome, especially cognition, have been numerous, especially in animal models [173]. Results from experimental studies on immature rodent models suggest that phenobarbital can induce apoptosis, involving the cortex, hypothalamus, thalamus, basal ganglia and the white matter [174-176]. However, the average phenobarbital dose in these studies was much higher than typical doses administered to human neonates (75 mg/kg) [177]. These same loading doses were associated with detrimental long-term effects, including schizophrenia-like behavior and impaired learning, memory, and social interactions [178]. Both phenobarbital and phenytoin interfere with synaptic maturation of neonatal rat brain and impair behavior [177]. Large doses of phenytoin (50 mg/kg) cause similar effects to phenobarbital, with apoptosis and synaptic disruption in the developing brain [176, 177]. On the contrary, levetiracetam showed mixed results. A loading dose of 80 mg/kg and a 40 mg/kg maintenance daily dose in the hypoxic-ischemic rat resulted in decreased apoptosis, suggesting a neuroprotective role [179], possibly linked to increased expression of superoxide dismutase and glutathione peroxidase [180]. Interestingly, in a different model, a dose of 50 m/kg every12 h for 3 days increased brain injury under normothermic conditions, but not under hypothermic conditions [181], highlighting the need to use models recapitulating clinical scenarios and always evaluating hypothermic conditions in models of full term perinatal brain injury, as this is the current standard of care, and might impact on results in a clinically relevant way. A

recent study on levetiracetam use during therapeutic hypothermia in rats demonstrated dose-dependent occurrence of apoptosis, not documented at low doses [182]. A clinical study comparing levetiracetam (20 mg/kg increased by 10 mg/kg up to 40-60 mg/kg) and phenobarbital (20 mg/kg increased by 10 mg/kg up to 40 mg/kg) monotherapy for clinical neonatal seizures, for a mean duration of 8 ± 6 months documented no significant differences in neurocognitive outcome [183].

In the lack of high quality evidence, four main strategies exist in order to protect the developing brain: choosing the right time for their administration and withdrawal, avoiding deleterious drugs, favoring drugs without pro-apoptotic or with neuroprotective effects, and adding neuroprotective agents to anti-seizure medications in order to counteract their potentially deleterious effect.

Although no guidelines currently define the correct timing for treatment, the goal of a prompt treatment seems to be often missed [184]. As a high seizure burden and especially status epilepticus are associated with worse brain injury and worse long-term outcomes, correct timing in drug administration is likely to be crucial in lowering seizure burden [51], and providing the best balance between effectiveness and side effects. In fact, after prolonged seizures, activity-dependent NKCC (sodium-potassium-chloride channel) changes lead to a shift back towards an excitatory effect of GABA due to chloride accumulation mediated by NKCC1 receptors with downregulation and internalization of chloride-potassium cotransporter 2 (KCC2) receptors, thus lowering drug efficacy. [185] Even more concernedly, detrimental effects were demonstrated in case of late phenobarbital administration in an experimental model: while early phenobarbital was associated with reduced ictal-like events and prevention of a mirror focus, the epileptiform activity was even

aggravated by late phenobarbital administration (after repeated ictal-like events) [186].

If antiseizure medications have a potential for long-term detrimental effects, time is also a critical player with respect to their withdrawal. Even if no guidance and no clear-cut evidence exist, some papers documented that early discontinuation at discharge in newborns with HIE [187] or different aetiologies [188] is not associated with an increased risk of seizure recurrence [187] nor with any differences in neurological development [188]. However, although a shift towards less treatment and shorter treatment duration after seizures resolution has been documented over time, significant inconsistencies seem to persist in clinical practice [189]. The presence of brain injury at MRI was associated with a trend towards higher risk of seizures during follow-up period. Therefore, in the context of HIE, patients experiencing few seizures with response to medications, mildly abnormal EEG and absence of severe brain MRI injury are considered as candidates for early drug withdrawal [187].

Among antiseizure medications with evidence of a neuroprotective effect, topiramate is probably the most widely studied in newborns. It acts by inhibiting AMPA and kainate receptors and potentiating GABA signaling, resulting in dose-dependent brain injury reduction, and neurobehavioural improvement in animal models [190]. Preclinical studies suggest a synergistic effect with hypothermia [191-193]. Safety pilot studies documented no statistically or clinically significant differences for safety, death or severe neurologic disability, but a lower prevalence of epilepsy in newborns treated with topiramate (10 mg/kg/day) and hypothermia compared to controls [194]. In a randomized, controlled, multicentre, double-blind study, the use of topiramate (5 mg/kg loading dose, followed by 3 mg/kg maintenance dose) in newborns with HIE

correlated with lower seizure burden in the first 24 hours of hypothermia, less need for additional antiseizure medications and lower mortality, although not statistically significant. However, of the utmost importance, only 37.5% of the patients achieved therapeutic levels within the first 24 hours, and 75.5% at 48 hours [195]. There is a critical issue regarding pharmacokinetics under hypothermic conditions, which might undermine topiramate efficacy during the latent period, as an intravenous formulation is unavailable, and underdosing might have impacted on study results.

Clinical trials administering drugs with a mechanism of action directed to the immature brain have failed to provide additional benefit, as it is the case for bumetanide, a loop diuretic acting as a NKCC1 (Na-K-Cl) transporter, which was proven to increase phenobarbital efficacy in animal models [196-197], but caused hearing loss and failed to demonstrate efficacy (defined as 80% seizure reduction) in an open-label feasibility study on human newborns, determining its early termination [198].

However, an interesting experimental study investigated a lipophilic bumetanide derivative (bumepamine), which exhibits higher brain penetration than bumetanide, and found it more potent than bumetanide in potentiating phenobarbital anticonvulsant effect in two rodent models of epilepsy, despite lower inhibition of the NKCC1 channel, thus suggesting the existence of alternative mechanisms of action [199], which should be further investigated and also applied to acute symptomatic seizure models, before definite conclusions can be drawn in the context of perinatal brain injury and symptomatic neonatal seizures.

A promising additional strategy to stop or prevent acute symptomatic neonatal seizures is represented by Kv7 (KCNQ) channel openers, such as flupirtine. This drug has been proven to act more efficaciously than diazepam and phenobarbital in

rat models of kainic acid and fluorothyl induced neonatal seizures, by completely preventing or stopping seizures after the injection of the chemoconvulsants [200]. In a further rat model of global hypoxia, flupirtine was shown to dose-dependently stop behavioural seizures during hypoxia and to inhibit electroclinical seizures and electrographic seizures. However, in this study, flupirtine was administered before the insult took place, and therefore further studies should explore the time window for its use, with special emphasis to the reperfusion phase of hypoxic-ischemic events [201]. However, a rationale for a favourable effect of flupirtine and its analogues on acute symptomatic seizures and neuroprotection in the context of therapeutic hypothermia is also suggested by their dose-dependent hypomotor, sedative and hypothermic effect in mice [202].

**4. Neurorestorative interventions**

In a recent and exhaustive review on white matter injury in preterm infants, it was acknowledged that, aside from the acute detrimental effects resulting in pre-OL death, secondary long-term changes take place, resulting in dysmaturation. This late, developmentally-regulated phase involves activated microglia and reactive astrocytes, and offers a longer time window for therapeutic interventions, including pharmacological approaches, stem cell therapy (outlined above), but also nutritional (breastfeeding, polyunsaturated fatty acids, iron, zinc) and experiential factors (optimal auditory and visual exposure, minimization of pain and stress, environmental enrichment) [84]. “Extra-uterine” preterm newborns have reduced cerebral and cerebellar volumes, cortical gyrification, delayed maturation [40] and impaired functional connectivity compared to their counterparts [203].

Aside from a negative role of specific therapies, including dexamethasone, and the negative role of hyperoxia, hypocapnia, blood pressure changes,

hypo/hyperglycemia [22], the effects of the neonatal intensive care unit (NICU) environment itself have been studied, with respect to noise, lights and early life stimulation, with conflicting results [204, 205]. Pain and stress in the neonatal period have also been suggested to result in negative neurobehavioural and cognitive outcomes and to correlate with cerebral functional connectivity [206]. Nutritional factors have a crucial role in immature brain development. During the last trimester of pregnancy, transplacental transfer of polyunsaturated fatty acids (PUFA) contributes to neural membranes formation, and their reduced levels in preterm-born infants have been shown to correlate with micro and macrostructural white and grey matter characteristics at term-equivalent age [207, 208].

**5. Expert opinion**

For the time being, only therapeutic hypothermia has been demonstrated to result both in clinically significant outcome improvement and in reduction of seizure burden and later epilepsy. Apart from associating hypothermia with prophylactic antiseizure medications, the effects of neuroprotective interventions in reducing acute seizure burden and the possible long-term beneficial effects have not been directly investigated. This field would give unique insights into the independent role of seizures in worsening outcome.

As seizures likely contribute to brain injury exacerbation by further activating the same pathways as the initial deleterious event causing them, strategies addressing key involved players (oxidative stress, inflammation, hemodynamic changes) might result in a positive effect on both brain injury and seizures, and consequently on clinically meaningful outcomes. However, the occurrence of seizures, in many conditions, announces the end of the latent period and the activation of multiple cascades of detrimental events, which should be best prevented rather than treated,

although so far such (including prenatal) strategies have not been successful, except hypothermia. The issue is, this lack of significant results might have multiple explanations, and therefore future studies should aim to control the number of confounding variables. Additional factors, from gestational age, to choice of correct timing and dosing for each drug, possibly lead to inconsistencies in study results. Further complicating this subject, the window of opportunity for neuroprotective actions is limited and might change depending on gestational age, severity of brain injury, etiology, and other –possibly still unknown- factors.

A huge body of data has been collected in animal models and further effort should be made in constructing specific models addressing the unique characteristics of different gestational ages, etiologies, degrees of brain injury, timing of insult and of administration and dosing of neuroprotective agents. When studying post-mortem specimen (possibly after behavioural testing), it would be important to analyze specific, more susceptible brain areas according to gestational ages and their functionally-linked networks, depending on the specifically analyzed outcome (i.e. epilepsy, cognition, motor function). In fact, for some brain structures, such as the cerebellum, the white matter or the frontal lobes, maturational processes continue postnatally and might be the target of specific neurorestorative interventions. Furthermore, better understanding and comparability of studied outcomes is warranted. Systemic, neurological and cognitive outcomes need to be prospectively collected with standardized scales. It is also important to identify reliable and clinically relevant biomarkers since the earliest stages of the pre/perinatal period. To this end, research on new neuroimaging tools might become increasingly useful (tractography, spectroscopy, perfusion analysis, NIRS, functional connectivity, microstructural changes), and increasingly available in the future, and may possibly

provide deeper insight into the relationships between seizures, brain injury and outcome.

In the meantime, providing evidence-based supportive care during the NICU stay is useful in improving outcome by controlling systemic factors involved in worsening of brain injury, especially in complex patients.

In five years' time, results from trials on therapeutic hypothermia in preterm infants will be available, as well as more clinical trials on allopurinol, erythropoietin, xenon and melatonin in hypoxic-ischemic encephalopathy and neuroprotective strategies for preterm infants, including administration of melatonin, erythropoietin, magnesium with respect to white matter injury. Trials are also recruiting patients in order to collect brain MRI data following neuroprotective interventions and to address the effects of NICU environment on newborns. A placebo-controlled study on enteral topiramate for neonates under hypothermia for HIE is also expected to be completed in 2022 and will report on the rate of neonatal seizures in the two groups. Additional options for acute seizure management might also become available once studies currently under way for brivaracetam and lacosamide are completed (www.clinicaltrials.gov, last visited on 21st October 2020). Hopefully, based on results of these ongoing studies, better care will be provided.



**Figure and table legends**

Figure 1. Neuroprotective interventions for fullterm newborns with HIE according to the phase and timing of their action. (HIE: hypoxic-ischemic encephalopathy)

Figure 2. Neuroprotective and neurorestorative interventions for preterm newborns.

Table 1. Antiseizure medications for acute symptomatic neonatal seizures: dosing, order and route of administration, with reference to the pertinent literature.

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| DRUG | DOSING | CHOICE/LINE | REFERENCES |
|---|---|---|---|
| Lorazepam | 0.05-0.1 mg/kg every 8-12 hours | First/iv | 209, 210 |
| Diazepam | 0.2-0.5 mg/kg every 4-6 hours | First/iv | 209, 210 |
| Phenobarbital | 15-20 mg/kg every 15-20 minutes (maximum total dosing: 40 mg/kg) | First/iv | 18 |
| Phenyotin | 18-20 mg/kg | First/iv | 18 |
| Midazolam | Bolus: 0.1-0.2 mg/kg; maintenance: 0.05 mg/kg/h by continuous infusion, (maximum speed: 1 mg/kg/h) | Second-(third)/iv | 211, 212 |
| Levetiracetam | Bolus: 10-60 mg/kg (30-60) | Second-(third)/iv | 213 |
| Lidocaine | 2 mg/kg | Second-third/iv | 214 |
| Topiramate | Maintenance: 3.5-10 mg/kg | (Second)-third/os | 215, 216 |

Table 1. Antiseizure medications for acute symptomatic neonatal seizures: dosing, order and route of administration, with reference to the pertinent literature.

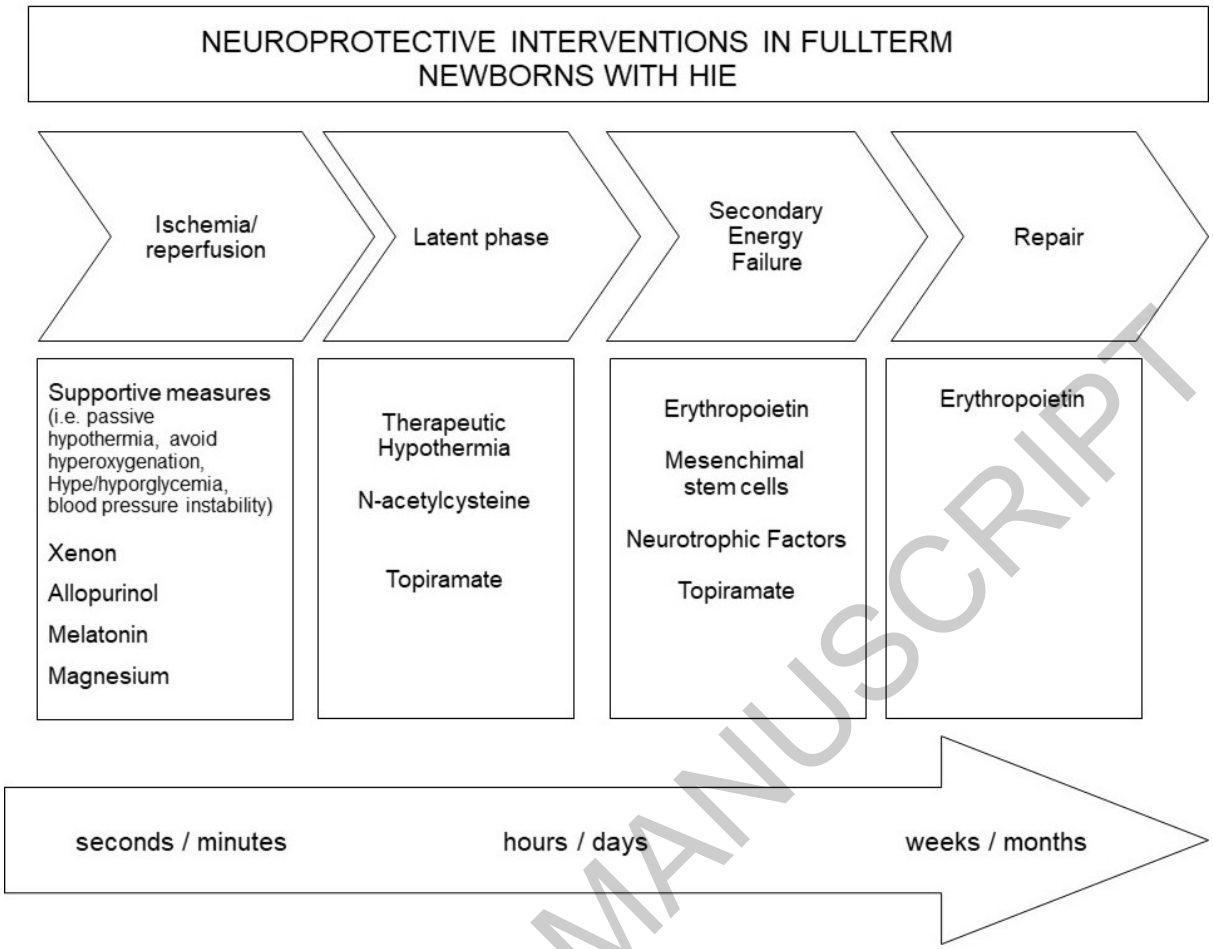


Figure 1

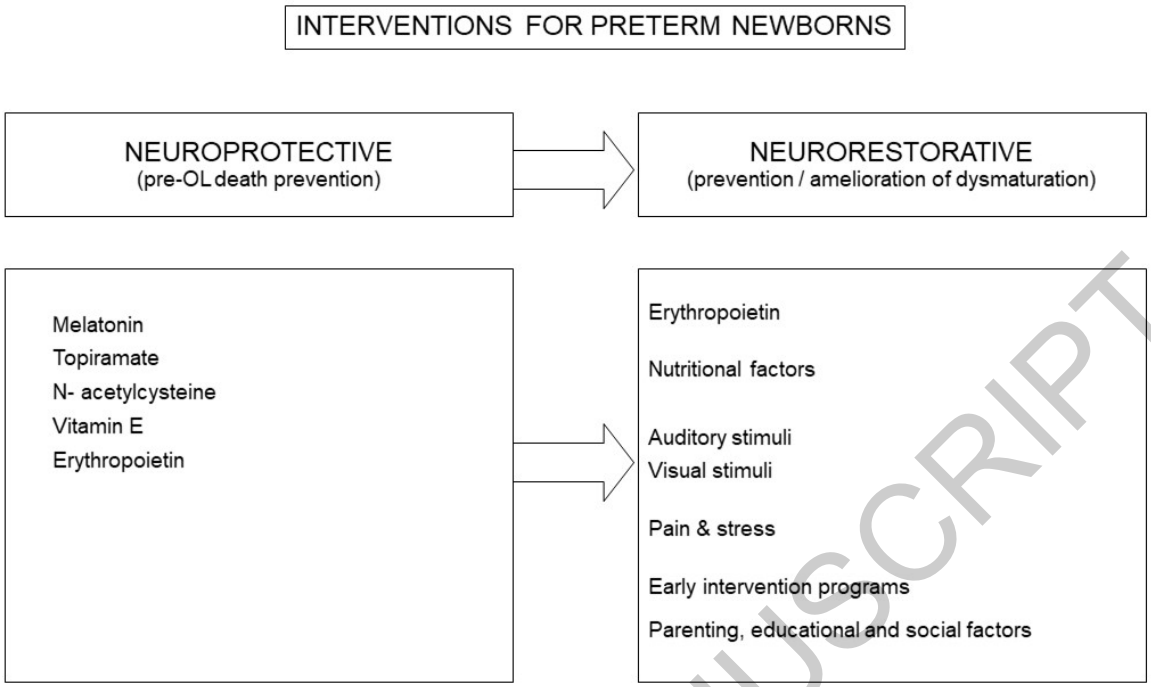


Figure 2