

# Perinatal asphyxia in the term newborn

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## Proceedings

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*The role of the clinical pathological dialogue in problem solving*

Guest Editors: Gavino Faa, Vassilios Fanos, Peter Van Eyken

## Abstract

Despite the important advances in perinatal care in the past decades, asphyxia remains a severe condition leading to significant mortality and morbidity. Perinatal asphyxia has an incidence of 1 to 6 per 1,000 live full-term births, and represents the third most common cause of neonatal death (23%) after preterm birth (28%) and severe infections (26%). Many preconceptional, antepartum and intrapartum risk factors have been shown to be associated with perinatal asphyxia.

The standard for defining an intrapartum hypoxic-ischemic event as sufficient to produce moderate to severe neonatal encephalopathy which subsequently leads to cerebral palsy has been established in 3 Consensus statements. The cornerstone of all three statements is the presence of severe metabolic acidosis (pH < 7 and base deficit ≥ 12 mmol/L) at birth in a newborn exhibiting early signs of moderate or severe encephalopathy.

Perinatal asphyxia may affect virtually any organ, but hypoxic-ischemic encephalopathy (HIE) is the most studied clinical condition and that is burdened with the most severe sequelae.

The feasibility of providing neuroprotection after HIE has been proven by hypothermia therapy, which is able to reduce the risk of death or major neurodevelopmental disability. Many promising neuroprotective agents might contribute to reduce hypoxic-ischemic brain injury through different mechanisms of action, but further studies are required to confirm their efficacy.

The prognosis is dependent on the severity of the perinatal asphyxia. Only a minority of infants with severe HIE survive without handicap.

## Keywords

Perinatal asphyxia, hypoxic-ischemic encephalopathy, Apgar score, newborn.

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## Background

Despite the important advances in perinatal care in the past decades, asphyxia remains a severe condition leading to significant mortality and morbidity. The term “asphyxia” is derived from the Greek and means “stopping of the pulse”. Perinatal asphyxia is a condition characterized by an impairment of exchange of the respiratory gases (oxygen and carbon dioxide) resulting in hypoxemia and hypercapnia, accompanied by metabolic acidosis [1].

According to Volpe [2], hypoxemia may be defined as the “diminished amount of oxygen in the blood supply”, while cerebral ischemia is defined as the “diminished amount of blood perfusing the brain”. Cerebral ischemia is the more important of the two forms of oxygen deprivation because it also leads to glucose deprivation. The terms hypoxia-ischemia and asphyxia are often used interchangeably, but they are not equivalent from a pathophysiological viewpoint. Hypoxia-ischemia or pure ischemia are rarely observed in the newborn, while some combination of hypoxia, ischemia and hypercapnia are more common.

Estimates of the incidence of perinatal asphyxia are quite variable from one study to another. De Haan et al. [3] reported an incidence of perinatal asphyxia of 1 to 6 per 1,000 live full-term births. Moreover, asphyxia has been shown to be the third most common cause of neonatal death (23%) after preterm birth (28%) and severe infections (26%) [4].

Asphyxial injury may involve virtually every organ system of the body, but hypoxic-ischemic encephalopathy (HIE) is the most studied clinical

condition and that exhibiting the most serious sequelae.

## Etiology

In term newborns, asphyxia can occur in utero and during labor and delivery as a result of impaired placental gas exchange. Preconceptional risk factors for asphyxia are maternal age  $\geq 35$  years, social factors, family history of seizures or neurologic disease, infertility treatment, previous neonatal death etc. Antepartum risk factors include maternal prothrombotic disorders and proinflammatory states, maternal thyroid disease, severe preeclampsia, multiple gestation, chromosomal/genetic abnormalities, congenital malformations, intrauterine growth restriction, trauma, breech presentation and antepartum hemorrhage. Numerous intrapartum risk factors for asphyxia are recognized, including abnormal fetal heart rate during labor, chorioamnionitis/maternal fever, thick meconium, operative vaginal delivery, general anesthesia, emergency cesarean delivery, placental abruption, umbilical cord prolapse, uterine rupture, maternal cardiac arrest, and fetal exsanguination. Asphyxia can also occur in the immediate postnatal period, usually secondary to pulmonary, neurological or cardiovascular abnormalities. It should be noted that, in many cases, the timing of asphyxia cannot be established with certainty.

## Criteria for the diagnosis of intrapartum asphyxia

The standard for defining an intrapartum hypoxic-ischemic event as sufficient to produce moderate to severe neonatal encephalopathy which subsequently leads to cerebral palsy has been established in three Consensus statements. In 1996, the American Academy of Pediatrics and American College of Obstetrics and Gynecology published the first statement that included the following criteria: (a) profound metabolic acidosis (pH  $< 7.0$ ) in umbilical artery blood; (b) Apgar score  $\leq 3$  for longer than 5 minutes; (c) neonatal encephalopathy; (d) multi-organ system dysfunction [5]. The second Consensus statement was approved by the International Cerebral Palsy Task Force in 1999, and included 3 essential criteria and 5 additional criteria. The essential criteria were the following: (a) metabolic acidosis in early neonatal blood sample (pH  $< 7.0$  and base deficit  $\geq 12$  mmol/L); (b) moderate or severe encephalopathy; (c) cerebral

palsy of spastic quadriplegia, dyskinetic or mixed type. The 5 additional criteria were: (a) sentinel event; (b) severe changes in fetal heart rate; (c) Apgar score < 6 beyond 5 min; (d) multi-system involvement; (e) early imaging evidence [6]. The third consensus statement was developed by the American College of Obstetrics and Gynecology in 2002, including 4 essential criteria and 5 additional criteria. The essential criteria were the following: (a) metabolic acidosis (pH < 7.0 and base deficit  $\geq 12$  mmol/L) in umbilical artery sample; (b) moderate or severe encephalopathy; (c) cerebral palsy of spastic quadriplegia or dyskinetic type; (d) exclusion of other etiologies. The 5 additional criteria were: (a) sentinel event; (b) abrupt changes in fetal heart rate; (c) Apgar score  $\leq 3$  beyond 5 min; (d) multi-system failure within 72 h of life; (e) early imaging evidence [7]. The cornerstone of all three statements is the presence of severe metabolic acidosis (pH < 7.0 and base deficit  $\geq 12$  mmol/L) at birth in a newborn exhibiting early signs of moderate or severe encephalopathy. Both arterial and venous cord blood should be obtained because the former reflects fetal status more directly while the latter reflects the uteroplacental oxygen exchange [8]. In the two most recent statements, difficulties result from the use of a long term outcome, namely cerebral palsy, as an essential diagnostic criterion for intrapartum asphyxia. Moreover, these two statements have included cerebral imaging findings as supportive evidence of intrapartum asphyxia; this may be a problematic criterion, especially in countries where the availability of cerebral imaging technology is restricted.

### Clinical manifestations

#### *Apgar score*

The Apgar score is a clinical indicator commonly used to describe the newborn's physical condition at birth. A hypoxic-ischemic insult, but also many other non-asphyxial factors such as maternal analgesia, prematurity and infection, may cause depression of the Apgar score. A prolonged depression of the Apgar score has been shown to be related with death or severe neurodevelopmental outcome [9].

#### *Neonatal encephalopathy*

Neonatal encephalopathy (NE) has been defined as “a clinically defined syndrome of disturbed

neurological function in the earliest days of life in the term infant, manifested by difficulty with initiating and maintaining respiration, depression of tone and reflexes, subnormal level of consciousness, and often by seizures” [10].

In the past, it was assumed that the primary etiology of NE was hypoxia-ischemia. Indeed, the term NE is simply a clinical description of disturbed neurological function, irrespective of etiology or pathogenesis. Currently, NE is considered a nonspecific response of the brain to injury that may occur through multiple causal pathways [11]. Hypoxia-ischemia represents one of these pathways, and therefore the term hypoxic-ischemic encephalopathy (HIE) should be reserved for the sub-set of cases of NE with a good evidence of a recent hypoxic-ischemic cause. Robertson et al. [12] define HIE as “an acute non-static encephalopathy caused by intrapartum or late antepartum brain hypoxia and ischemia”. This clinical condition evolves during the first days of life after significant hypoxic-ischemic insult, and is a leading predictor of neurodevelopmental disability [13].

Sarnat and Sarnat [14] classified HIE into 3 clinical stages: mild (stage 1), moderate (stage 2) and severe (stage 3) encephalopathy. Infants who develop HIE show alterations in the level of consciousness and in the behavior ranging from hyper-alertness/irritability through lethargy/obtundation to stupor/coma. Disorders of tone ranging from an increase to a marked decrease, and a spectrum of abnormal movements from tremors and jitteriness to frank seizures may be observed. Other clinical manifestations of HIE include apnea with bradycardia and oxygen desaturation, feeding difficulty, shrill cry, exaggeration of the Moro reflex, increased deep tendon reflexes, and decorticate or decerebrate posturing. The severity of HIE symptoms reflects the timing and duration of the insult [15].

In contrast to other etiologies of NE (genetic disorders, brain malformations, metabolic defects etc.), HIE is a potentially modifiable condition, and therefore it is of crucial importance to ascertain the presence or absence of hypoxia-ischemia.

The estimation of incidence of NE and HIE and the identification of their risk factors are problematic due to the lack of universal agreed definitions. The incidence of NE has been estimated to be 2.5 to 3.5 per 1,000 live births (combined point estimate of 3.0 per 1,000 live births); on the other hand, the incidence of HIE has been estimated to be 1.3 to 1.7 per 1,000 live births (combined point estimate

of 1.5 per 1,000 live births). It has been estimated that 30% of cases of NE in developed countries and 60% of cases in developing countries have evidence of intrapartum hypoxia-ischemia [16].

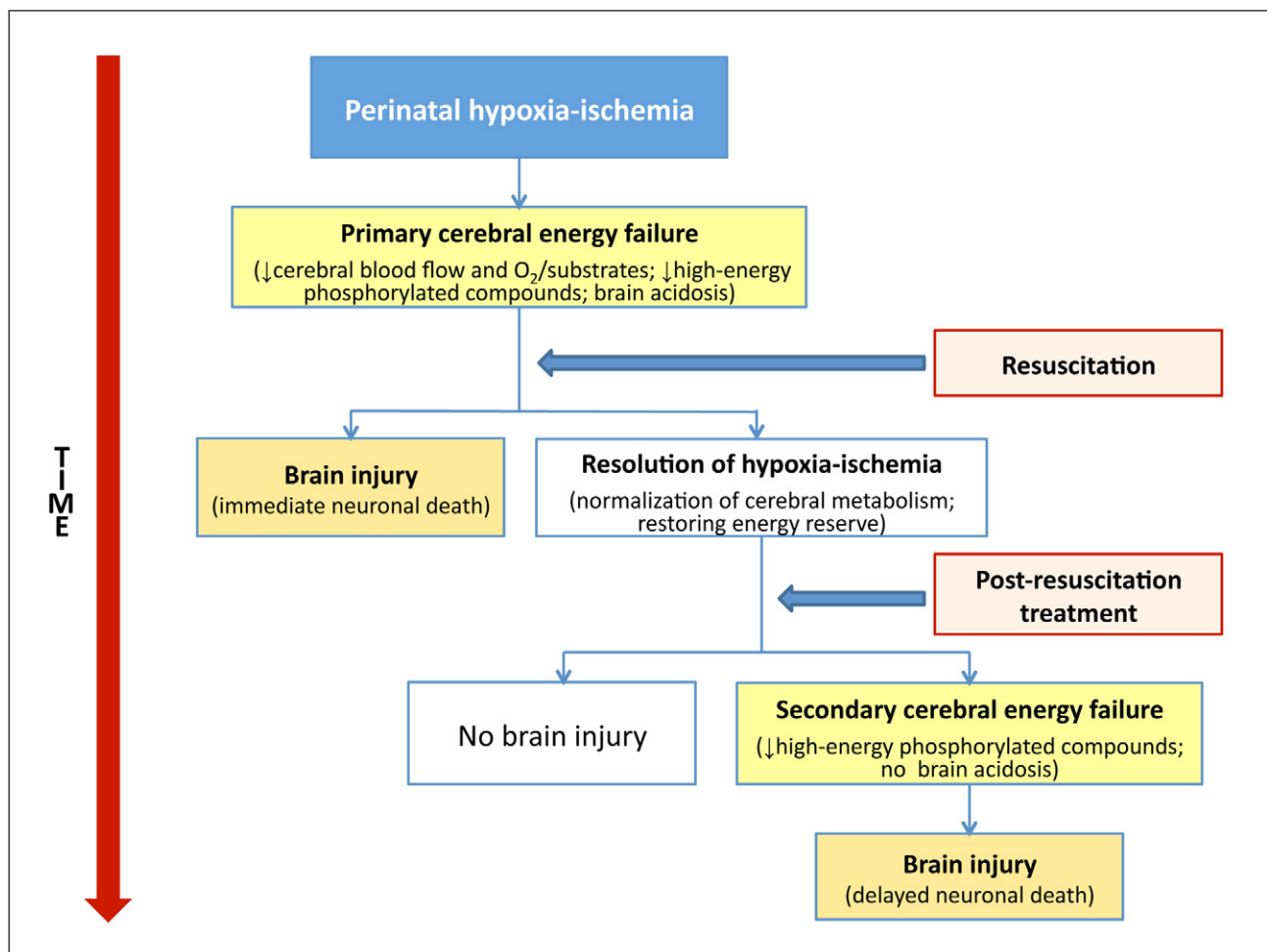
#### Pathogenesis of hypoxic-ischemic encephalopathy

After perinatal hypoxia-ischemia, different sequences of pathologic events may occur, culminating in brain injury (**Fig. 1**). In newborn animals, the phases of primary and secondary energy failure have been recognized, based on characteristics of the cerebral energy state [17]. In the phase of primary energy failure, reductions in cerebral blood flow, in  $O_2$ /substrates and in high-energy phosphorylated compounds (ATP and phosphocreatine) have been observed; furthermore, tissue acidosis is prominent. This phase represents an essential prerequisite for all subsequent pathologic events. Primary energy failure is associated with a complex series of acute intracellular derangements,

including loss of membrane ionic homeostasis, defective osmoregulation, release/blocked reuptake of excitatory amino acids, and inhibition of the synthesis of proteins [18]. Overstimulation of neurotransmitter receptors, associated with loss of ionic homeostasis, mediate an elevation in intracellular calcium and osmotic dysregulation. The increase in intracellular calcium triggers several destructive pathways by activating proteases, lipases and endonucleases [19].

The fall in high-energy phosphorylated compounds and intracellular pH subsequently is reversed, and recycling of neurotransmitters is promoted, if the resolution of hypoxia-ischemia occurs within a specific interval of time (**Fig. 1**); the duration of this interval is affected by various factors including maturation, substrate availability, body temperature and simultaneous pathological conditions.

If the injury is sufficiently severe, a secondary energy failure occurs within hours to days after



**Figure 1.** Schematic representation of primary and secondary energy failure in the brain following perinatal asphyxia.

the primary insult. Secondary energy failure is characterized by declines in phosphocreatine and ATP without brain acidosis, differently from primary energy failure [17]. In this process, secondary neurotoxic mechanisms are activated, including the ones reported below.

- Extracellular accumulation of excitatory amino acids (mainly glutamate) due to increased release as well as impaired uptake; this causes the overactivation of neuronal glutamate receptors, mainly the N-methyl-D-aspartate (NMDA) receptor, which results in an excessive intracellular influx of calcium.
- The resulting intracellular calcium accumulation has the following effects: (a) activation of cell-degrading enzymes (lipases, phospholipases, proteases and endonucleases); (b) production of oxygen free radicals through activation of Xanthine oxidase, increased prostaglandin synthesis, and activation of Nitric Oxide (NO) synthase.
- Peroxidation of membrane lipids and direct damage of protein and DNA as a result of the increase in free radical formation and the subsequent depletion of normal antioxidant defenses.
- Impaired mitochondrial function as a combined result of intracellular calcium accumulation and excessive amounts of free radicals. Mitochondrial dysfunction leads to (a) additional release of oxygen free radicals; (b) release of cytochrome C that causes neuronal apoptosis through the activation of a proteolytic cascade, including caspases; (c) release of proteins able to induce apoptosis through a caspase-independent mechanism. Extrinsic pathway not involving mitochondria can also induce apoptosis after hypoxic-ischemic injury.
- In the neonatal hypoxic-ischemic brain, an inflammatory reaction develops: it is characterized by early expression of multiple inflammatory genes followed by chemokine and cytokine production. Inflammatory cells, in particular macrophages and microglia, accumulate at the injury site and may contribute to damage by the production of excitatory amino acids, oxygen free radicals, NO, and proinflammatory cytokines. During early reperfusion, neutrophils contribute to injury by their accumulation in the vascular bed, thus plugging microvessels and impairing blood circulation.

In asphyxiated newborns, there is a correlation between the severity of secondary energy failure

and the adverse neurodevelopmental outcome. The presence of a latent phase between primary and secondary energy failure suggests that specific therapeutic interventions are possible. Strategies to prevent brain injury through inhibition of secondary neurotoxic mechanisms have been and are under investigation.

#### Neuronal death

Neuronal death after hypoxia-ischemia can occur by different mechanisms, i.e. necrosis or apoptosis. Necrosis is caused by a severe injury and occurs within minutes: depletion of cellular energy and loss of membrane integrity result in leakage of cytoplasmic contents and a subsequent inflammatory reaction. Conversely, apoptosis is a highly controlled, energy-requiring process that takes more time to develop, and leads to cellular “suicide.” Apoptosis is the dominant form of cell death after less severe brain injury and in the later phases of the injury process. However, it is noteworthy that both forms of cell death coexist.

#### Clinical conditions aggravating hypoxic-ischemic brain injury

There is evidence that a number of common clinical events including hypoglycemia, hyperthermia and seizures may aggravate the hypoxic-ischemic brain injury; therefore, particular attention should be paid to the prevention and treatment of them to avoid additional injury. The administration of oxygen during resuscitation after asphyxia may also contribute to hypoxic-ischemic brain injury. In fact, hyperoxia resulting from the use of high concentrations of oxygen can lead to an excessive release of free oxygen radicals, thus aggravating brain injury. Accordingly, oxygen delivery during neonatal resuscitation should be carefully controlled, and oxygen saturation monitored, in order to avoid hyperoxia.

#### *Multisystem organ involvement*

The consequences of hypoxic-ischemic insult usually extend to other organ systems in addition to the brain. In a minority of cases (< 15%), the brain is the only organ that exhibits dysfunction after asphyxia. In most cases, systemic hypoxia-ischemia results in multiorgan dysfunction.

The lungs of asphyxiated newborns can be injured by hypoxia, as a result of inhaled meconium,

secondary to cardiac dysfunction, or compromised due to pulmonary hypertension [20]. Accordingly, gas exchange is impaired and assisted ventilation may be needed.

Hypoxia-ischemia causes a direct damage to the myocardium which, together with the negative consequences of compensatory mechanisms to maintain cerebral perfusion, leads to a recognizable clinical and laboratory picture [21, 22]. Myocardial ischemia compromises cardiac conduction and contractile efficiency, often requiring an inotropic support to maintain adequate circulation. Functional and conduction abnormalities may be detected by echocardiography and electrocardiogram, while heart muscle damage is reflected by the increase of cardiac enzymes.

The other multisystem effects regard kidneys, liver, and bone marrow. Kidney injury represents the best systemic marker of brain injury. Oligo-anuria following hypoxic-ischemic injury is common, frequently associated with hematuria, and results from renal tubular damage. Serum creatinine and blood urea concentrations increase progressively, reaching the peak in the days following the injury. Fluid retention and hyponatremia may occur due to inappropriate secretion of antidiuretic hormone.

The effects on the bone marrow include an increased release of nucleated red blood cells (NRBC) and thrombocytopenia. The NRBC count reaches a peak at 6-8 hours following brain injury and returns to normal by 36-72 hours. On the other hand, the platelet count falls sometimes by 12 hours, and reaches the nadir at 2-3 days. Thrombocytopenia can be severe enough to determine or aggravate bleeding (risk of intracerebral bleeding).

Liver dysfunction may be manifested by increased hepatocellular enzymes, even though more extensive damage may develop.

Fluctuations of blood glucose concentration may be observed, with hypoglycemia being most common. Hypoglycemia may result in neurological sequelae, particularly when it causes or accompanies seizures. On the other hand, hyperglycemia may also lead to, or aggravate, brain damage through a mechanism involving a hyperosmolar state [23].

## Diagnosis

A clinical evaluation and laboratory and instrumental examinations are required in order to assess and manage the asphyxiated newborn.

The differential diagnosis for a term newborn with suspected perinatal asphyxia includes acute

hemorrhage, depression from maternal anesthesia or analgesia, infection, cardiac or pulmonary disorders, trauma, neurological disorders and metabolic diseases.

## Blood gas analysis

The blood gas criteria that define perinatal asphyxia causing brain injury are uncertain. Nevertheless, the pH and base deficit on the umbilical cord or first blood gas is useful for determining which newborns have asphyxia requiring further evaluation for the development of HIE. The best indicator for intrapartum asphyxia is severe metabolic acidosis (pH < 7.0 and base deficit  $\geq 12$  mmol/L) in umbilical cord arterial blood at delivery.

## Brain imaging

Cranial and Doppler ultrasonography, computerized tomography (CT) and magnetic resonance imaging (MRI) are the most commonly used brain imaging techniques in the newborn with NE.

## Cranial ultrasonography

Cranial ultrasonography (CS) has been used widely in neonatal practice, as it is a convenient, non-invasive, safe and quick imaging technique to visualize the neonatal brain parenchyma and ventricular system serially without disturbing or moving the patient. CS is helpful to exclude structural abnormalities and to detect calcifications and cysts, atrophy or cerebral hemorrhage. Sequential cranial ultrasound examinations following a recent hypoxic-ischemic insult are helpful for assessing the evolution of injury, and particularly for defining the pattern of lesions and the timing of their onset. In neonates affected by severe forms of encephalopathy, this neuroimaging method is able to detect cortex and basal ganglia lesions. Nevertheless, CS does not allow early identification of asphyxial brain injury that becomes evident between 24 and 72 hours after birth [24]. The strong correlation between the brain lesions found on CS (focal echodense areas) and those detected on CT (decreased density areas) is well recognized [25]. Additionally, in term infants with hypoxic-ischemic injury, a strong correlation between sonographic findings and MRI findings has been found when the two techniques were used at the same time [26].

### Doppler ultrasonography

It is a non-invasive diagnostic technique that changes sound waves into an image which can be displayed on a monitor. Doppler ultrasonography (DS) completes the CS exam, providing important information about two parameters, the resistive index (RI) and the end-diastolic flow velocity (EDFV). Reduced RI and increased EDFV values have been revealed in the anterior cerebral artery in case of neonatal asphyxia; they seem to indicate an alteration in the cerebral blood flow due to the vasodilation resulting from hypercapnia or metabolites accumulation [26, 27]. At present, no sufficient data are available on diagnostic and prognostic utility of DS, so it is not widely used in the clinical assessment of neonatal asphyxia.

### Computerized tomography

This imaging technique has been used in the past in term infants with HIE. MRI has been shown to be superior to CT in all aspects; even the detection of calcifications, previously only possible with CS or CT scans, can be obtained with appropriate MRI sequences. The study by Chau et al. [28], compared the patterns of brain injury detected by conventional MRI, diffusion-weighted MRI and CT on the third day of life in a cohort of term infants with NE. Diffusion-weighted MRI was found to be the most sensitive technique for assessing brain injury on day 3 in the study population; the agreement for the predominant injury pattern was good for CT and diffusion-weighted MRI (67% agreement). In a very recent study, the diagnostic performance of CT has been shown to be inferior to MRI in identifying cerebral injury in newborns with NE. The results of this study suggest that the evaluation of newborns with NE should be based on CS as a screening test, followed by MRI rather than CT [29]. Finally, the patient exposure to ionizing radiation from CT must be kept in mind, and thus MRI should be used if possible.

### Magnetic resonance imaging

MRI is the optimum imaging modality for the early assessment of brain injury in asphyxiated term neonates. Compared with CS and CT, MRI can visualize cerebral hypoxic-ischemic lesions with higher resolution, sensitivity and specificity. It is able to detect 75-100% of cerebral lesions resulting from asphyxia, particularly those affecting white-

matter, basal ganglia and thalamus [30]. MRI has the advantage of not exposing the newborn to ionizing radiation. On the other hand, its scanning time is quite long, and therefore sedation is frequently required in newborns undergoing MRI scan. Brain lesions may be detected with MRI by the third day of life and become more evident and defined in the following two weeks. It was found that MRI performed in the second week after birth can predict the outcome in neonates affected by HIE [31-34]. A recent cohort study has found a strong correlation between early (4<sup>th</sup> day of life) and late (second week of life) sequential MRI studies performed in newborns with HIE undergoing therapeutic hypothermia. In particular, the localization, extension and severity of hypoxic-ischemic brain injury in the two scans have been shown to be strongly correlated. This study's results suggest that MRI can be a useful prognostic tool in the first days of life [35].

Advanced MRI techniques, including MR spectroscopic imaging (MRSI) and diffusion tensor imaging (DTI), can more accurately identify brain injury in an earlier stage. Recently, a cohort of term newborns with HIE has been prospectively investigated with MRI, with DTI and MRSI, on days 1 and 3 of life. Quantitative MR values obtained by DTI and MRSI on day 1 have been found to be highly correlated with those observed on day 3 after birth, suggesting that quantitative MRI techniques can provide an objective measure of brain injury before qualitative images [36].

### Near-infrared spectroscopy

Near-infrared spectroscopy (NIRS) is a non-invasive tool for assessing cerebral hemodynamics and oxygenation. It has been documented that newborns with HIE may exhibit brain hyperperfusion early after birth, probably as a consequence of reperfusion injury [37]. Therefore, the measurement of brain perfusion may be useful to assess and treat this category of newborns. MRI can offer this type of information but MRI scans are not easily obtained in critically-ill patients. NIRS represents a good alternative and complementary tool, since it permits cerebral hemodynamic monitoring at patient bedside; additionally, it is cheaper, can be easily used, and is able to perform serial measurements of brain perfusion. A recent study has assessed the correlation between measurements of cerebral perfusion by NIRS and by MRI in full-term infants with HIE treated with hypothermia. A high correlation has been found between the two

tools regarding brain perfusion measurements in term infants with severe encephalopathy. Therefore, MRI and NIRS could be used together in this category of patients to obtain important information for tailoring neuroprotective therapies [38].

### *Electroencephalography and cerebral function monitoring*

Different tools are available to study electric function of the neonatal brain, in particular standard electroencephalography (EEG) and cerebral function monitoring (CFM). A recent study has evaluated the relation between EEG patterns and neurological outcomes in term newborns with HIE, showing that a normal EEG is correlated with a normal outcome, whereas the presence of “burst suppression” on EEG is predictive of death or pathological outcome [39]. Sequential EEG, in newborns with seizures, has been found to have more predictive value to estimate the neurological outcome and postnatal death, as compared to a single EEG recording [40].

The CFM is a real-time monitoring device that uses a method known as amplitude-integrated EEG (aEEG). This method consists of recording a single-channel EEG from biparietal or central electrodes [41]. CFM is commonly used for bedside monitoring of background neurological activity in term and near-term infants with encephalopathy. CFM patterns are well correlated with those obtained with regular EEG, even though short or low amplitude seizures cannot be detected with CFM. Therefore, CFM should never replace regular EEG, and a standard EEG is always recommended in newborns with HIE. CFM has been shown to be useful to assess asphyxiated newborns in combination with neurologic examination, and to select and manage those infants requiring particular treatments such as hypothermia. CFM can reveal different patterns: abnormal ones in the first 6 hours of life identify newborns with a worse outcome (death or disability); conversely, normal voltage patterns are associated with normal development [42]. A recent study has shown that CFM is a helpful tool for evaluating neonatal encephalopathy when used by adequately trained staff, according to a protocol based on the local resources [43].

### *Laboratory evaluation*

Neonatal asphyxia is often followed by a multiorgan failure involving mainly the kidney, brain, and heart. Multiorgan failure is associated with poor prognosis and high mortality. Currently,

several biomarkers are available and may help clinicians to globally assess newborns with hypoxic-ischemic injury. However, some of these markers have not been routinely used in neonatal practice until now [44].

### **Markers of kidney damage**

Kidney is one of the most important organs commonly involved in the multiple organ dysfunction caused by perinatal asphyxia. The severity of renal damage ranges from mild disorders to acute kidney failure.

Evaluations of blood urea and serum creatinine levels are the tests most frequently used to assess renal injury caused by perinatal asphyxia. Currently, these indicators are not considered helpful to the early identification of renal damage. In fact, they reflect the glomerular damage, and the consequent reduction of glomerular filtration rate (GFR), that occurs at least 24 hours after the hypoxic insult and when about 50% of nephrons are compromised. Furthermore, serum creatinine level at birth reflects the maternal level. At present, more useful markers of kidney injury are available.

Asphyxial insult causes an earlier and subtler damage of tubular cells, determining their necrosis. Accordingly, markers of tubular dysfunction such as urinary  $\beta_2$  microglobulin have been found to be better indicators of early renal injury. The study by Banerjee et al. [45] revealed that a raised urinary level of  $\beta_2$  microglobulin was related with HIE term newborns, irrespective of clinical staging; conversely, serum creatinine and blood urea were shown to be increased only in newborns with severe HIE (Sarnat stage III). Furthermore, a recent study highlighted that the increase of urinary  $\beta_2$  microglobulin is directly related both to asphyxia grading (Apgar score) and to Sarnat and Sarnat staging of HIE [46].

Cystatin C is another marker of renal damage. This protein, produced by all nucleated cells, is filtered by the glomeruli and degraded by proximal tubular cells. It does not cross the placental barrier and is not influenced by any newborn conditions. Recently, cystatin C level has been shown to be a good indicator of GFR; it is able to identify even mild forms of glomerular dysfunction, and decreases in newborns with perinatal asphyxia [47].

### **Markers of central nervous system damage**

Intrapartum asphyxia is one of the most frequent causes of neonatal encephalopathy. The availability



of markers of neurological damage may be very important to target therapy, evaluate response to treatment, and predict neurodevelopmental outcomes. Therefore, many biomarkers of brain injury have been investigated in the past years. Hypoxia, both acute and chronic, is a well-known cause of an increased count of NRBC. A recent study has been conducted to evaluate the power of this parameter in predicting neurological outcomes in asphyxiated newborns. Both NRBC count and NRBC count per 100 white blood cells (NRBC/100WBCs) have been found to be higher in those patients that exhibited a convulsion in the first 12 hours after birth, and in those patients that subsequently developed HIE stage III. The newborns that died or those with sequelae had a significantly higher NRBC count [48].

At present, no specific and reliable markers of brain injury have been identified. Nevertheless, some biomarkers have shown to be involved in the hypoxic mechanism that leads to CNS damage.

Glial fibrillary acid protein (GFAP) and ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), normally expressed either in neurons and in astrocytes, are easily measurable markers of neuronal apoptosis as they are released into blood circulation after a blood-brain barrier damage caused by hypoxia. A pilot study found that UCH-L1 and GFAP levels were associated with the worst outcomes in newborns with HIE, being increased in those patients that died or in those with severe brain injury at MRI [49]. Chalak et al. [50] have recently conducted a study to measure in umbilical cord serum the above mentioned biomarkers and others related to neurological injury to classify the severity of antenatal damage in the newborn with encephalopathy, to determine if they arise during the rewarming phase of hypothermia and to assess their correlation with neurological outcomes. The Authors have found a correlation between serum GFAP level at birth and encephalopathy severity. The investigated biomarkers have not been shown to be increased after the rewarming phase and some of them have been found to be related with neurodevelopmental outcomes at 15-18 months of age.

Another biomarker of CNS damage is S-100 $\beta$  protein. It is mainly synthesized by astroglial cells and increases in the blood after their death and when the blood-brain barrier is damaged. This marker was documented to be increased in the first urine of newborns with HIE [51], and to be a good predictor of neonatal death when its value is higher than 1 mcg/l [52]. S-100 $\beta$  level has been studied also in blood samples, documenting that it correlates with

the development of severe or moderate forms of HIE [53, 54]. Finally, serum S-100 $\beta$  protein was found to increase very early after asphyxial insult, from about 2 hours after birth [53].

Other markers seem to be related to neuronal damage associated with neonatal asphyxia. The neuron-specific enolase (NSE), normally produced by central and peripheral neurons, was found to be increased in serum immediately after birth in newborns with moderate or severe forms of HIE [55], but this finding was not confirmed in another study [53]. Brain-derived neurotrophic factor, a neurotrophin that normally fosters brain cell growth and differentiation, has been shown to have higher levels in newborns with HIE compared with normal newborns [56]. The interleukin-6, a biomarker non specific for brain damage, was found to be related with both severity of HIE and neurological outcomes at 2 years after birth [57]. Finally, creatine kinase BB (CK-BB), normally contained in astrocytes and neurons, can be found in serum after neonatal asphyxia. Nagdyman et al. [53] observed that serum concentration of CK-BB was an early predictor of HIE as its rise occurred from 2 hours after birth. Furthermore, the Authors documented that the level of CK-BB was very higher in infants with moderate or severe forms of asphyxia if compared to those infants with no or mild forms of asphyxia. Literature data suggest that no single biomarker is able to assess neurological damage after perinatal asphyxia; a panel of multiple biomarkers should probably be considered in evaluating the clinical consequences of asphyxia, and in initiating, continuing or stopping neuroprotective therapy.

#### Markers of cardiac damage

A temporary increase in myocardial work is observed soon after a neonatal asphyxial insult in order to increase blood flow and protect organs against hypoxia. Myocardial injury normally develops when this compensation mechanism fails. ECG, echocardiography and measurement of cardiac enzymes are used to assess myocardial dysfunction but only few studies have investigated their diagnostic value in newborns, with non-definitive results [58, 59]. Nevertheless, some studies have found it useful to assess myocardial function in asphyxiated newborns by measuring cardiac enzymes. Cardiac troponin I (cTnI) and Cardiac troponin T (cTnT) are regulatory proteins that control the calcium-mediated interaction between actin and myosin. They are markers of

myocardial damage, and their levels increase in newborns with evidence of asphyxia. Serum level of cTnI at 72 hours after birth appears to be a significant predictor of mortality in term newborns with HIE [60]. Furthermore, Agrawal et al. [61] have shown that mean creatine kinase total levels, creatine kinase-myocardial band (CK-MB) and cTnI values rise proportionally with the severity of HIE. In asphyxiated newborns, ECG monitoring and enzyme measurement appear to be useful to evaluate cardiac function and establish an adequate treatment.

## Treatment

A detailed description of the therapy of perinatal asphyxia is beyond the scope of this article. Therefore, only a few relevant aspects of treatment will be briefly discussed.

The treatment of asphyxia starts with a correct perinatal management of high-risk pregnancies. The management of the hypoxic-ischemic newborns in the delivery room is the second fundamental step of the treatment. Low Apgar scores and need for cardiopulmonary resuscitation at birth are common but nonspecific findings. Most newborns respond rapidly to resuscitation and make a full recovery. The outcomes for newborns who do not respond to resuscitation by 10 minutes of age are very poor, with a very low probability of surviving without severe disability. Resuscitation in room air is advised for term newborns, since the use of 100% oxygen is associated with worse outcomes compared to the use of room air.

The initial management of asphyxiated newborns following admission to the neonatal intensive care unit (NICU) does not target the pathophysiologic sequence resulting in hypoxic-ischemic brain injury, but rather is aimed at avoiding injury resulting from secondary events related to hypoxia-ischemia. An early identification and treatment of the most common events that could aggravate brain damage and a good and early supportive intensive care have been shown to be essential to avoid or to reduce the ongoing brain injury in asphyxiated newborns. Temperature control, respiratory and cardiac support, seizures treatment, maintaining normal blood glucose, hematocrit, and electrolytes values, correcting blood gases and acid-base status alterations are a must in the management of this category of newborns [62].

In neonatal hypoxic-ischemic brain, the reoxygenation and reperfusion that follow re-

suscitation trigger a neurotoxic cascade that usually leads to neuronal death. Between the initial insult and the brain damage there is a time window that has been shown to be useful to start neuroprotective treatments [63]. In the past 20 years, many efforts have been made to identify specific neuroprotective therapies able to block or reduce the negative effects of hypoxia and ischemia [41]. At the moment, hypothermia represents the neuroprotective treatment of choice for term neonates with NE following perinatal asphyxial insult, as only this therapy has been shown to have neuroprotective effects in larger clinical studies. The most studied neuroprotective pharmacological agents are allopurinol, deferoxamine, topiramate, xenon [63], melatonin [64], erythropoietin [65], and magnesium [66]; they might contribute to reduce brain injury through different mechanisms of action [63]. These agents appear to be beneficial when administered alone or in combination with hypothermia, but further studies are required to confirm their neuroprotective efficacy.

Therapeutic hypothermia has multiple neuroprotective effects, including reduction of cerebral metabolism, prevention of seizures, stabilization of the blood-brain barrier, inhibition of glutamate and NO release, selective reduction of apoptosis, and suppression of microglia activation. Hypothermia is usually applied in a selected category of newborns meeting specific inclusion criteria, and is performed in NICU settings due to its complexity. There are two different typologies of active hypothermia therapy: cool-cap and total body hypothermia. The first modality consists of external cooling of the head, while the second one uses a little mattress full of a coolant liquid that envelopes the newborn body [67]. Two types of control systems to manage therapeutic hypothermia are available: manual and servo-controlled system. The first one has been shown to be associated with a greater variability in rectal temperature control that might expose the newborn to adverse cerebral consequences. On the other hand, the servo-controlled system has been demonstrated to be more reliable as it is able to maintain a steady rectal temperature [68].

A recent Cochrane review of 11 randomized controlled trials has revealed that therapeutic hypothermia is beneficial in term and late preterm infants with HIE. Cooling has been found to reduce mortality without causing an increase in major disability in survivors. According to the results of this study, hypothermia should be employed in term

and late preterm newborns with moderate-to-severe HIE if identified before 6 hours of life [69]. A recent study has documented that moderate hypothermia in newborns with perinatal asphyxia results in better neurocognitive outcomes at 6-7 years of age [70]. Conversely, two follow-up studies have found that the incidence of disability at school age in cooled survivors is similar to that of children not treated with hypothermia (control group) [71, 72]. Taken together, literature data indicate that hypothermia treatment should be started in the first 6 hours after the occurrence of hypoxic-ischemic insult and generally has to be prolonged for 72 hours. Moreover, Thoresen et al. [73] found a better motor outcome at 18-20 months of age in those asphyxiated newborns that had been cooled in the first 3 hours of life. These results suggest that hypothermia should be started as soon as possible after birth in those patients fulfilling inclusion criteria. Hypothermia treatment may result in a number of short-term adverse effects. Bradycardia, thrombocytopenia, hypotension, seizures, skin lesions, and pulmonary hemorrhage are the side effects most frequently observed [74]. As regards bradycardia and thrombocytopenia, they have been considered as not so meaningful if compared with benefits of hypothermia [69]. A recent study has shown that a passive modality of hypothermia without any additional cooling treatment could be feasible and safe for inducing and maintaining hypothermia in asphyxiated term newborns, but further studies are required to confirm these data [75].

Recent advances in regenerative medicine suggest that cell therapies could improve repair of the damaged brain. Neural Stem/Progenitor Cells (NSPC), Mesenchymal Stem/progenitor Cells (MSC) and Human Umbilical Cord Blood Cells (HUCBC) might reduce brain injury through a combination of different mechanisms such as immunomodulation, neuroprotection and, in the case of NSPC, cell replacement.

## Outcome

Perinatal asphyxia is burdened with high morbidity and mortality. Reported mortality rate is about 20% in full-term asphyxiated infants, while the incidence of neurological impairments in survivors is estimated to be of about 25% [76, 77]. Nevertheless, there is a large variety of literature data regarding mortality and neurological outcome rates.

A recent literature review has reported the rate of individual long-term neurodevelopmental

outcomes after HIE: 45% of sequelae were represented by cognition and developmental delay or learning difficulties, 29% by cerebral palsy, 26% by blindness or vision defects, 17% by gross motor and coordination problems, 12% by epilepsy, 9% by hearing loss or deafness, and 1% by behavioral problems [78]. A recent review of the literature by Ellenberg et al. [79] has revealed that cerebral palsy rate ranges from less than 3% to more than 50% in different studies. The study by Graham et al. [80] has shown that HIE results in cerebral palsy in at least 14% of cases. Furthermore, van Handel et al. [81] investigated the behavior at school age in children who had been affected by neonatal encephalopathy, demonstrating that they had a problematic behavior, with social and attention problems.

Multiple factors have been shown to influence the prognosis of HIE. The rate of adverse developmental outcomes, including cognitive impairment, sensory-motor impairments or death, varies with the severity of HIE. In children under 3 years of age, this rate is nil for mild, 32% for moderate and almost 100% for severe forms of HIE [82]. In fact, mild forms of HIE are associated with better intellectual, educational and neuropsychological outcomes when compared with severe forms. However, more subtle cognitive deficits and behavioral alterations have been revealed through long-term evaluations, even in mild forms of HIE [83]. On the other hand, moderate forms of HIE show a wide range of outcomes that are not easily predictable in neonatal period [84, 85]. A retrospective study documented that both cerebral palsy and epilepsy at one year of age were more frequently observed in those term infants that exhibited the onset of epilepsy within 6 months of age [86].

Blood glucose values have been shown to influence the outcome in newborns with asphyxia. Recently, Spies et al. [87] have found that hyperglycemia (blood glucose > 150 mg/dL) in the first 12 hours of life is associated with poor gross motor outcome in asphyxiated term infants.

Recently, the prognostic value of different diagnostic tests routinely used in newborns with HIE has been investigated. T1/T2-weighted MRI, aEEG, and EEG have been proven to be very good prognostic tests, with a high sensitivity. Two other tests, sensory and visual evoked potentials, are promising but their reliability in predicting outcome has to be tested in further studies. The combined use of more than one test could be the most useful tool in predicting prognosis after perinatal asphyxia [33].

## Declaration of interest

The Authors declare that they have nothing to disclose related to this article.

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