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# The potentials and limitations of neuro-biomarkers as predictors of outcome in neonates with birth asphyxia☆

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ABSTRACT

Perinatal asphyxia and its complication, hypoxic-ischemic encephalopathy, are still among the major causes of perinatal mortality and morbidity. Despite accurate standard postnatal monitoring procedures, the post-insult period is crucial because at a time when radiologic pictures are still silent, brain damage may already be at a subclinical stage. Against this background, the measurement of quantitative parameters, such as constituents of nervous tissue, that are able to detect subclinical lesions at a stage when routine brain monitoring procedures are still silent, could be particularly useful.
Therefore, in the present review we report the potentials and limitations of biomarkers in predicting outcome in neonates complicated by perinatal asphyxia.



## 1. Introduction

About 0.1–0.4% of full-term newborns (FTN) can be affected by perinatal asphyxia (PA) and its dramatic complication, hypoxic-ischemic encephalopathy (HIE) [1]. PA-HIE accounts for 15–20% of perinatal mortality and an additional 25% develop childhood disabilities. While mild HIE is known to be at relatively low risk for motor or cognitive deficits, in severe HIE the common clinical repertoires are cerebral palsy (CP) and intellectual disabilities. Moreover, in moderate HIE motor deficits, memory impairment and visual motor or visual perceptive dysfunction, increased hyperactivity and delayed school readiness can occur [2,3].

HIE pathogenesis is fairly complex and still not fully understood. The developing fetal brain is highly reliant on a constant sustained blood flow and, during hypoxic-ischemic insult (H-I), different intracellular mechanisms are activated, which in turn lead to cell damage [4].

The intensity, severity and timing of asphyxia, as well as peculiar ischemic susceptibility and the degree of maturity of the brain, define the dimension and grade of severity of the consequent damage [4]. There are several circumstances in which the developing brain is especially vulnerable to ischemia [5], and late preterm infants born between 34 and 36 weeks of gestation are known to be at highest risk of permanent neurological sequelae [6]. The reason for this is that at this stage central nervous system (CNS) growth is at its peak in terms of brain weight, volume, structure and function [6].

The pathophysiological steps leading to CNS damage need to be updated: there is evidence that, in addition to the bi-phasic post-H-I insult, the so-called third phase, which may last for weeks, months and even years, has to be included [7,8]. The mechanisms of this persisting damage, which are still a matter of debate, involve gliosis, persistent inflammatory receptor activation and epigenetic changes.

The combined effects of the above pathophysiological phases act by disrupting essential components of the cell, leading ultimately to death or apoptosis [7,8] and to a significant increase in peculiar CNS neurobiomarkers (BM) in biological fluids such as brain constituents (activin A; glial fibrillary acidic protein, GFAP; neuron specific enolase, NSE; S100B) and vasoactive agents (adrenomedullin, AM).

In the present review we report the potentials and limitations of biomarkers in predicting outcome in neonates complicated by PA.

## 2. Biomarkers: where are we?

In recent years there has been increased interest in the usefulness of BMs for the early diagnosis of CNS damage, as well as for the monitoring

*Abbreviations:* FTN, full-term newborns; PA, perinatal asphyxia; HIE, hypoxic-ischemic encephalopathy; CP, cerebral palsy; H-I, hypoxic-ischemic insult; CNS, central nervous system; BM, biomarkers; GFAP, glial fibrillary acidic protein; NSE, neuron specific enolase; AM, adrenomedullin; FDA, food and drug administration; EMA, european medicine agency; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; ICH, cerebral hemorrhage; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; BBB, brain blood barrier; HT, hypothermia.

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and evaluation of the extent of the lesion. The Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have been shown to support research on BMs [9,10]. The main points of interest regard the specificity of BMs for determining the extent of CNS damage and the timing of the insult. Briefly, a BM may enable clinicians to: (i) screen infants for brain injury with high sensitivity and specificity, (ii) monitor the progression of disease through longitudinal monitoring, thanks to their short half-life, (iii) correlate the findings of BMs with those of validated standard procedures able to diagnose brain injury and the extent of lesions, such as ultrasound and magnetic resonance imaging (MRI). In addition to these criteria, the new generation of BMs in perinatal medicine have the following properties: (i) they have been well studied in the pediatric/neonatal population; (ii) they can be measured using kits that are commercially available worldwide, are simple to use and provide measurements with good reproducibility; (iii) reference ranges are available for pediatric/neonatal populations; (iv) they can be assessed in different biological fluids such as urine, blood, cerebrospinal fluid (CSF), amniotic fluid, saliva and milk, possibly reducing perinatal stress related to testing.

## 3. Biomarkers

### 3.1. Activin A

Activin A is a member of the transforming growth factor-β superfamily, which in the CNS regulates neurons differentiation and proliferation [10,11]. The neuroprotective action of activin A is exerted via the activation of different pathways: in animal models and cell cultures activin A has a beneficial role in neuronal recovery, supporting the survival of neurogenic cell lines and retinal neurons and offering protection against neurotoxic damage [11]. In rats, activin A enhances neuron survival, rescues against neurotoxic damage and decreases H-I injury [11].

#### 3.1.1. Potentials

Activin A has been measured in milk and in CSF, cord and peripheral blood and urine of PA-HIE FTN [10]. In CSF, activin A concentrations were higher in PA FTN who developed severe HIE than in those who did not or in controls. An activin A cut-off of 1.3 pg/L as an early diagnostic test for HIE [12] had a sensitivity and specificity of 100% with a positive predictive value (PPV) of 100% and a negative predictive value (NPV) of 0%. Notwithstanding these promising results, the assessment of activin A in CSF has been gradually abandoned due to the invasive sample collection procedure and stress for infants. In blood, elevated longitudinal activin A levels in PA-FTN correlated with impaired fetal circulation and hypoxia [13]. As an HIE diagnostic test an activin A cut-off of 0.66 ng/L achieved a sensitivity of 93.33%, a specificity of 96.63%, with positive and negative likelihood ratios (PLR, NLR) of 27.69 and 0.069, respectively. In both blood and CSF, activin A increased in PA-HIE before the appearance of related clinical and laboratory signs [14]. More recently, activin A levels measured in urine were significantly higher in PA newborns with moderate or severe HIE than in those with absent or mild HIE. An activin A cut-off >0.08 ng/L at first void had a sensitivity of 83.3% and a specificity of 100% for predicting the development of moderate or severe HIE [15].

These observations suggest that activin A could provide additional information to physicians on the occurrence of perinatal brain injuries at a stage when diagnostic procedures have a limited benefit.

#### 3.1.2. Limitations

To date, few data are available regarding the correlation of activin A levels with the extent of brain lesions, assessed using standard techniques such as ultrasound and MRI, and with short-term outcomes. Reference curves in the neonatal/pediatric period are requested due to the trophic role of activin in CNS development [9–11,16].

### 3.2. GFAP

A monomeric protein of the astroglial skeleton with a molecular mass of 40–53 kDa located in the white and gray matter [9,10] where it can be released rapidly out of damaged areas and up-regulated through astrogliosis. Changes in blood concentrations of GFAP have also been shown to reflect damage to the blood-brain barrier (BBB).

#### 3.2.1. Potentials

GFAP has been investigated mainly in conventional biological fluids (i.e. CSF, blood) of PA infants. In CSF, GFAP was assayed between 12 and 48 h after birth of PA-HIE FTN. GFAP levels increased up to 5-fold in accordance with HIE severity [17]. Indeed, GFAP measured in the first 4 days of life was significantly increased and correlated with other indicators of long-term prognosis and with neurological impairment at 1 year of age, or death before that time [17,18]. The PPV of a GFAP measurement higher than the 98th percentile of normal infants was 69%, while a GFAP level below this limit invariably predicted a good outcome [18].

In blood, increased GFAP levels are closely correlated with the severity of HIE. At a GFAP cut-off of 0.08 pg/mL, the PPV value was 100% and NPV 0% for predicting infants with abnormal outcomes at 15–18 months of age [19]. Reports also showed that GFAP at a cut-off value of 0.07 ng/mL, had a sensitivity and specificity for HIE of 77% and 78%, respectively, and correlated with MRI patterns [20].

#### 3.2.2. Limitations

Few data are currently available regarding reference curves for GFAP in different biological fluids. This is of relevance, bearing in mind that GFAP is reasonably involved in CNS development and is therefore gestational age-dependent. Notably, data on GFAP levels in unconventional biological fluids are still lacking, and no conclusive results have been reported regarding correlations between GFAP and cerebral ultrasound and MRI patterns.

### 3.3. NSE

An enzyme that catalyzes the conversion of 2-phospho-D-glycerate to phosphoenolpyruvate in a glycolytic pathway. It has a molecular weight of 78 kDa and a half-life of 24 h. In the CNS it is characterized by its stable presence in mature sensory and endocrine neurons, suggesting that a unique system of intracellular energy metabolism may be shared by neurons and paraneurons [9,10]. Immunohistochemistry shows that NSE can be traced in the cerebral circulation after experimentally induced cerebral lesions, and plasma concentrations correlated with events of neuronal NSE loss in focally ischemic neurons [9,10].

NSE is also present in erythrocytes, platelets, plasmatic cells, lymphocytes, capillary walls, and myoepithelial cells, which explains its physiologically low concentrations in blood. NSE is secreted after cell injury into the extracellular fluids, CSF and blood, where it represents an attempt to maintain homeostasis [21].

#### 3.3.1. Potentials

NSE has been investigated mainly in conventional biological fluids (i.e. CSF, blood) of PA infants and is thought to be released into the blood as a consequence of damage to BBB, or brain injury due to tissue ischemia or edema [22]. In blood, higher NSE levels were observed in PA-HIE FTN than in controls. At a cut-off value of >40.0 mg/L NSE achieved a sensitivity and specificity as a predictor of moderate/severe HIE of 79% and 70% respectively, and a PPV and NPV of 51% and 89%, respectively. Moreover, NSE as a predictor of poor outcome at a cut-off value >45.4 mg/L had a sensitivity and specificity of 84% and 70%, and a PPV and NPV of 39% and 95%, respectively [23]. Conversely, Nagdyman et al. found no differences in NSE concentrations in FTN with no or mild HIE and those with moderate or severe HIE [24]. Roka et al. [25] measured NSE blood concentrations in PA infants subjected to whole-body hypothermia (HT) and found that NSE levels were highly elevated

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