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Serum biomarkers to evaluate the integrity of the neurovascular unit

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A B S T R A C T

Biomarkers have the potential to enable the clinicians to screen infants for brain injury, monitor progression of disease, identify injured brain regions, assess efficacy of neuroprotective therapies, and offer hope to identify the timing of the injury, thus shedding light on the potential pathophysiology and the most effective therapy. Currently, clinicians do not routinely use biomarkers to care for neonates with Neonatal Encephalopathy (NE) and brain injury due to prenatal hypoxia–asphyxia. This review will cover potential biomarkers of the neurovascular unit in the setting of NE that (i) can help assess the degree or severity of encephalopathy at birth; (ii) can help monitor progression of disease process and efficacy of neuroprotective therapy; (iii) can help assess neurodevelopmental outcome. These biomarkers will be summarized in two categories: 1) Specific biomarkers targeting the neurovascular unit such as glial fibrillary acidic protein (GFAP), ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), S100B, and neuron specific enolase (NSE) and 2) general inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1b (IL-1b), and pNF-H, among others.

1. Introduction

Neonatal encephalopathy, resulting from hypoxic–ischemic encephalopathy (HIE), is a fetal disorder due to prenatal hypoxia–asphyxia. The incidence is variable, occurring in 1.5/1000 live term births (95% confidence interval [CI], 1.3–1.7) with substantial associated morbidity and mortality [1]. Those with severe encephalopathy have a higher risk of death (up to 85%), and survivors have a >50% risk of cerebral palsy (CP) and/or mental retardation [2]. Recent evidence suggests there is a progression in the severity of the insult in neonates with HIE; thus, neonates with mild to moderate encephalopathy are less likely to die, but are at risk of significant motor deficits, fine motor disability, memory impairment, visual or visual–motor dysfunction, increased hyperactivity, and delayed school readiness [2–5]. Since the advent of hypothermia, the outcomes have improved in neonates with moderate to severe encephalopathy, but the long term morbidity remains elevated [2–5].

Although the time of onset, duration and severity of injury is nearly impossible to establish, it clearly occurs before and/or during delivery. To predict the severity and outcome of neonates with NE, clinicians use the grading system based upon clinical criteria [6]. This scoring system divides neonates into mild, moderate, or severe categories, and measures the progression of the neurologic insult to predict a neonate's prognosis [7]. Nevertheless, the Sarnat score system is subjective and changes over time. Amplitude integrated electroencephalogram (aEEG) may help stage the severity of injury and predict prognosis [8]. Unfortunately, the predictive value of both Sarnat score and the aEEG has been reported to be decreased in neonates undergoing hypothermia [9].

2. Markers of injury in the setting of the neurovascular unit

The neurovascular unit or blood–brain barrier (BBB) represents the key interface of molecular and cellular exchange between blood and neural tissues. The concept of the neurovascular unit emphasizes cell–cell signaling among the various neuronal, glial, and vascular compartments as a key factor to normal brain function. The cerebrovascular endothelial cells separate blood and brain interstitial fluid. Cell–cell interactions between astrocytes and endothelial cells sustain the functionality of the blood–brain barrier (BBB) [10]. The BBB is dynamic and can be modified by circulating factors secreted by inside cells or transferred from distant organs following injury to the BBB [10,11]. Commonly known factors up-regulated after hypoxia have also been reported to impair the adult BBB permeability, such as bradykinin, histamine, serotonin, glutamate, ATP, adenosine, platelet-activating factor, interleukins (IL-1α, IL-1β, IL-6), TNFα, free radicals, and nitric oxide (NO) to list a few [11]. The microglia and astrocytes are key components of the neurovascular unit and have been also reported to produce cytokines, chemokines, and other factors [12,13].

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The review will cover potential biomarkers of injury to the neurovascular unit, divided into two categories: 1) Brain specific biomarkers targeting the neurovascular unit, such as glial fibrillary acidic protein (GFAP), ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1), S100B, and neuron specific enolase (NSE), and 2) general inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1b (IL-1b), and pNF-H, among others.

3. Brain specific biomarker

3.1. Glial fibrillary acidic protein (GFAP)

This cytoskeleton intermediate filament protein of the astrocytes is released into the blood upon astrocyte death and has been correlated with poor outcomes in adult patients after stroke, cardiac arrest, or traumatic brain injury [14]. GFAP has also been used as a predictor of mortality or poor neurological outcomes in children requiring ECMO [15–18]. Serum GFAP has been recently reported in newborns with NE and undergoing hypothermia therapy. A recent small study by Ennen et al. reported GFAP levels in infants with NE to be significantly elevated when compared with controls. In addition, a GFAP threshold of 0.08 ng/ml at admission distinguished neonates with moderate/severe NE who underwent SHT from controls without NE. GFAP levels equal to or greater than 0.15 ng/mL upon NICU admission were predictive of an abnormal brain MRI despite hypothermia therapy [19]. One recent prospective pilot cohort study we conducted at parkland hospital has also identified umbilical arterial serum GFAP levels as the sole marker correlating with severity of NE, with higher levels in moderate to severe NE, as compared to those with mild NE [20].

Neuronal glial fibrillary acidic protein (GFAP), along with ubiquitin carboxyl-terminal hydrolase L1, and cytokines were measured in serum from umbilical artery at 6–24, 48, 72, and 78 h of age. Neurodevelopmental outcomes (Bayley Scales of Infant and Toddler Development-III scales) were performed at 18–24 months. Twenty neonates received hypothermia; while 7 had mild HIE and were not cooled. At birth, serum GFAP increased with the severity of HIE Fig. 1 ($p < .001$), and serial GFAP remained elevated in neonates with moderate to severe NE who were undergoing hypothermia compared to mild NE, but did not increase further with rewarming. Elevated GFAP, along with other biomarkers such as IL-1, IL-6, IL-8, tumor necrosis factor, interferon at 6–24 h were associated with abnormal neurological outcomes [20]. GFAP levels of >0.05 ng/ml beyond 72 h. of age have been associated with abnormal BSID-III outcome at 24 months of age in the latter study as well as others examining outcomes [21].

3.2. Ubiquitin carboxyl-terminal esterase L1 (UCH-L1)

UCH-L1 is a neuron-specific cytoplasmic enzyme, a marker of neuronal apoptosis, that is concentrated in dendrites [22]. Serum UCH-L1 reflects the extent of neuronal injury, because it is expressed in neurons, then released into the circulation after breakdown of the blood–brain barrier, and is recently easily measured [23]. UCH-L1 has been reported elevated in the cerebrospinal fluid of patients after traumatic brain injury and surgically induced circulatory arrest [24,25]. UCH-L1 values in circulating blood were>100 ng/mL in a small study in neonates with NE who subsequently died [26]. One recent study showed that UCH-L1 was elevated in the umbilical arterial cord plasma and after 72 h of SHT in infants with NE, who developed adverse outcomes compared with those with favorable outcomes [21]. In a pilot study we have reported UCH-L1 levels to be significantly elevated at birth (umbilical arterial blood) and to decrease after the first 6–24 h of SHT [20]. Both latter studies [20,21] speak to the importance of determining exact times of biomarker measurement, showing that timing is critical for interpretation. In addition, another study also reported that serum levels of UCH-L1 were elevated in neonates with HIE in the first 24 h of life compared with a control population of neonates in the newborn nursery who had no other medical issues [26].

3.3. Neuron specific enolase (NSE)

NSE belongs to the family of enolases, enzymes present in all tissues and organisms capable of glycolysis, but is an intracytoplasmic glycolytic enzyme derived from neuronal cytoplasm and neuroendocrine cells [27,28]. High levels of NSE in CSF and serum have been correlated with poor outcome in patients with cardiac arrest [29,30], in patients with cerebrovascular accident [31] and in pediatric patients with traumatic brain injury [32]. Elevated serum NSE concentrations in neonates undergoing cardiac surgery were also correlated with poor prognosis [33]. Additionally, serum NSE concentrations have also been associated in a small neonatal study with severity of NE and a cut-off point of 45.4 mcg/L could distinguish infants with poor outcomes from infants with normal outcomes [34].

3.4. S-100

S-100 is a calcium binding protein and is a major component of the cytosol in various cell types. It is present in high concentrations in glial

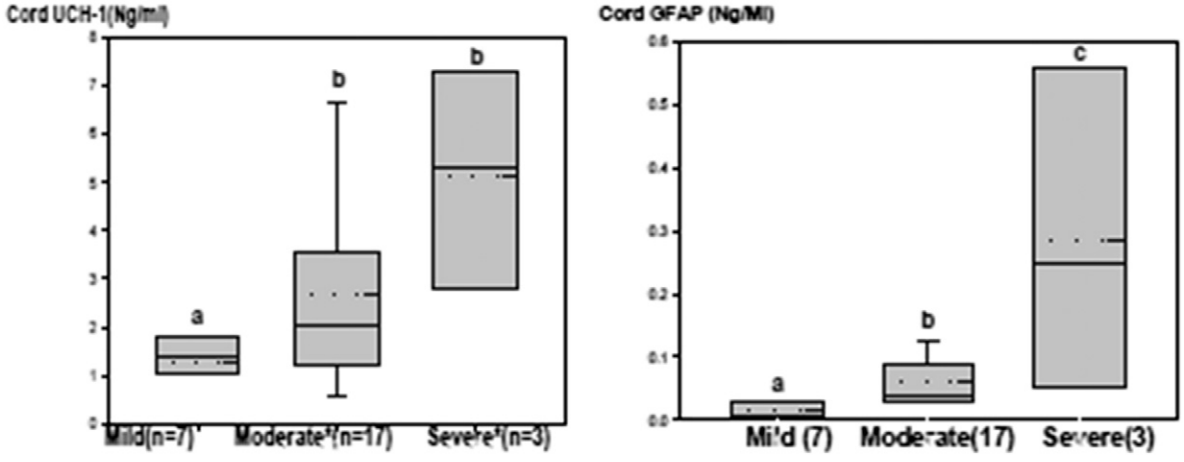

Fig. 1. Umbilical cord GFAP and UCH-1 and severity of encephalopathy at birth. Biomarkers are reported in ng/ml on the Y axis, while level of encephalopathy in the first 6 h is shown on the X axis. A box and whisker plot is shown with median as solid line, mean as dotted line, 25th and 75th quartiles as lower and upper borders respectively. Analyses include neonates with mild (n = 7), moderate (n = 17) and severe (n = 3) HIE. $p = 0.001$ (GFAP) and $p = 0.03$ (UCH-1) by Jonckheere–Terpstra. Annotations denote significant differences between groups. With permission from Elsevier, JPEDS, 164:468-74.