Glial fibrillary acidic protein as a biomarker for neonatal hypoxic-ischemic encephalopathy treated with whole-body cooling

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OBJECTIVE: Glial fibrillary acidic protein (GFAP) is specific to astrocytes in the central nervous system. We hypothesized that serum GFAP would be increased in neonates with hypoxic-ischemic encephalopathy (HIE) treated with whole-body cooling.

STUDY DESIGN: We measured GFAP at birth and daily for up to 7 days for neonates in the intensive care unit. We compared neonates with HIE treated with whole-body cooling to gestational age–matched controls without neurological injury and neonates with HIE by brain abnormalities on magnetic resonance imaging (MRI).

RESULTS: Neonates with HIE had increased GFAP levels compared with controls. Neonates with HIE and abnormal brain imaging had elevated GFAP levels compared with neonates with HIE and normal imaging.

CONCLUSION: Serum GFAP levels during the first week of life were increased in neonates with HIE and were predictive of brain injury on MRI. Biomarkers such as GFAP could help triage neonates with HIE to treatment, measure treatment efficacy, and provide prognostic information.

Key words: fetal acidosis, glial fibrillary acidic protein, hypoxic-ischemic encephalopathy, neonatal seizures, whole-body cooling

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Obstetricians and neonatologists routinely face the challenging task of identifying the hypoxic-ischemic fetus because early intervention with head or whole-body cooling[1] or experimental therapies such as erythropoietin,[2] xenon,[3] newer anticonvulsants,[4] and umbilical cord stem cells[5] may ameliorate devastating injuries. Current markers for identification of brain injury because of intrapartum hypoxia-ischemia, such as nonreassuring fetal heart rate tracings, meconium, metabolic acidosis, and Apgar scores are imprecise.[6] Newer technologies for intrapartum monitoring such as fetal pulse oximetry and fetal electrocardiogram also lack precision.[7]

Although metaanalyses have shown the benefit of hypothermia in reducing death and disability,[8,9] there are risks, so correct identification of at-risk neonates is critical. In addition, although hypothermia is effective in improving neurodevelopmental outcomes in hypoxic-ischemic encephalopathy (HIE), we have no means to monitor this therapy or direct it at the neonate with the most severe injury. For example, hypotonia is commonly used to identify clinical HIE; however, most neonates that are hypotonic at the time of birth were not suffering from intrapartum hypoxia-ischemia.[10] Additional means are needed to identify the neonate with true intrapartum hypoxia-ischemia so that therapies with risk are avoided.

A number of circulating proteins, including glial fibrillary acidic protein (GFAP), have been measured in adult patients after stroke, cardiac arrest, or traumatic brain injury in an effort to provide prognostic data on survival or residual deficits.[11-15] GFAP is a cytoskeletal intermediate filament protein found in the astroglia of the central nervous system and is a specific marker of differentiated astrocytes. Serum GFAP is specific to brain tissue and is not routinely secreted in blood but is released only after astrocyte death. This makes it an ideal blood marker for brain injury in neonates. We have found GFAP to be a significant predictor of

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survival and neurological outcome in neonates and children requiring extracorporeal membrane oxygenation.[16]

The aims of this study were to determine whether circulating GFAP levels measured at the time of birth and during the first week of life may identify neonates with moderate/severe HIE and among neonates with moderate/severe HIE that qualified for hypothermia to determine whether circulating GFAP levels predicted an abnormal brain magnetic resonance imaging (MRI) and were associated with functional outcomes.

Materials and Methods

Subjects

This is an institutional review board–approved prospective cohort study that examined neonates admitted to the neonatal intensive care unit (NICU) at a single tertiary university hospital. Subjects were live-born, nonanomalous, nonsyndromic infants born at 36-41 weeks' gestation. Our study included neonates born at our institution as well as those born within our state and transported to our NICU within 6 hours of birth.

Records were reviewed to abstract clinical information available at the time of maternal and neonatal discharge. Preeclampsia was defined as proteinuria and new-onset hypertension. Administration of intravenous magnesium sulfate to the mother prior to delivery was recorded because this therapy has been linked with a reduced risk of neonatal brain injury.[17] Intrauterine growth restriction was defined as an estimated fetal weight less than the 10th percentile for gestational age.[18] Nonreassuring fetal heart rate tracings were those significant enough to prompt operative vaginal or cesarean delivery. Sepsis was considered present only for neonates with positive blood and/or cerebrospinal fluid cultures.

Neonates with moderate to severe encephalopathy, defined as, per Sarnat and Sarnat,[19] that met criteria for whole-body cooling[20] were compared with neonates without neurologic injury admitted to the NICU matched by gestational age within 1 week in a 1:1 fashion. Neonates of 36 weeks' gestation or longer that qualify are cooled using a conductive water-based hypothermia system and hypothermia blankets within 6 hours of birth and are kept at a rectal temperature of 33.5°C for 72 hours.

Routinely all neonates with HIE treated with whole-body cooling have a standard neonatal imaging brain MRI prior to discharge from the NICU. For this study, these images were reviewed by an experienced pediatric neuroradiologist (T.A.G.M.H.) blinded to the GFAP results.

Neonates with an abnormal brain MRI were compared with those whose brain MRI was normal. The images were reviewed for focal or diffuse lesions related to hypoxic ischemic injury. MRI brain abnormalities were defined as brain swelling; cortical highlighting; focal or global loss of gray-white matter differentiation; abnormal signal intensity in the basal ganglia and thalami; loss of normal signal intensity in the posterior limb of the internal capsule; acute and subacute parenchymal, intraventricular, or extracerebral hemorrhage; and acutely evolving focal infarction in an arterial territory or in a parasagittal or watershed distribution.[21]

Specimens

Specimens collected for measurement of GFAP included umbilical cord blood and neonatal serum. For the umbilical cord blood, a small aliquot was taken from the umbilical cord venous blood sample routinely collected at delivery. For neonatal samples, the remaining fraction of serum from daily laboratory tests was collected after clinically indicated testing was completed. Neonatal serum specimens were collected at the time of admission to the NICU (within 6 hours of birth) and then daily for the first 4 days of life for the non–neurologically injured controls and daily for 7 days for neonates with HIE that underwent whole-body cooling.

GFAP assay

Using the Mesoscale platform (MesoScale Discovery, Gaithersburg, MD), we developed an electrochemiluminescent sandwich immunoassay for GFAP.[15] This was developed after the method of Petzold et al[22] using a trio of mouse monoclonal antibodies for capture and a rabbit polyclonal for detection.[16] Serum samples were assayed in duplicate, and the mean concentration was used for analysis. The lower limit of quantitation was 0.04 ng/mL; values below this were reported as zero.

Statistical analysis

Comparisons were made using a χ^2 or Fisher's exact test for categorical variables and unpaired Student t test for continuous variables. GFAP levels were compared using the Wilcoxon rank-sum test for nonparametric data. Significance was set at $P < .05$. Linear regression was used to determine the effect of gestational age on GFAP level in the non–neurologically injured control population.

Receiver-operator characteristic (ROC) curves were constructed to determine the optimal cutoff (as determined by maximal area under the curve) of serum GFAP level at NICU admission to identify HIE qualifying for whole-body cooling and to identify neonates with brain abnormalities on MRI. The area under the ROC curve was used to compare the ability of admission GFAP to identify neonates with abnormalities on brain MRI scans compared with other currently used tests to identify these infants: nonreassuring fetal heart rate tracing, meconium, 5 minute Apgar less than 7, and umbilical arterial pH less than 7.0 or base deficit greater than 12 mM. Statistical analyses were performed with Stata 10 (StataCorp, College Station, TX).

Results

Study population and clinical characteristics

During the period from April 28, 2009, to July 11, 2010, there were 652 admissions to our NICU of which 23 consecutive neonates were diagnosed with clinical moderate/severe HIE that qualified for whole-body cooling. These 23 neonates were matched 1:1 by gestational age at birth within 1 week to neonates admitted to the NICU for nonneurological indications. The mean (SD) gestational age for cooled neonates was 38.7 (1.5) weeks and 39 (1.4) weeks for the controls.

Maternal and neonatal characteristics are summarized in Table 1. Maternal de-