OBSTETRICS

Glial fibrillary acidic protein as a biomarker for periventricular white matter injury

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OBJECTIVE: Periventricular white matter injury (PWMI), a precursor of cerebral palsy, traditionally is not diagnosed until 6 weeks of life by head ultrasound scanning. We sought to determine whether early neonatal glial fibrillary acidic protein (GFAP) levels could identify PWMI in low birthweight (<2500 g) infants.

STUDY DESIGN: Each case with PWMI on head ultrasound scanning at 6 weeks of life from April 2009 to April 2011 was matched by gestational age and mode of delivery to 2 subsequent neonates with a normal head ultrasound scan. GFAP was measured in cord blood at birth, at neonatal intensive care unit admission, and on days 1-4 of life.

RESULTS: During this 2-year period, 21 cases with PWMI with gestational age 27.4 ± 3.3 weeks were compared with 42 control infants. The incidence of cesarean delivery was 61.9% in both groups. GFAP was not significantly different in cord blood or at neonatal intensive care unit admission but was significantly elevated on day 1 (median, 5-95%; 0, 0-0.98 ng/mL cases; 0, 0-0.06 ng/mL control infants; $P = .03$), day 2 (0, 0-1.21 ng/mL; 0, 0-0.05 ng/mL, respectively; $P = .02$), day 3 (0.05, 0-0.33 ng/mL; 0, 0-0.04 ng/mL, respectively; $P = .004$), and day 4 (0.02, 0-1.03 ng/mL; 0, 0-0.05 ng/mL, respectively; $P < .001$). The odds of the development of PWMI significantly increased with increasing levels of GFAP from day 1-4 of life when adjustment was made for preeclampsia, antenatal steroid administration, and neonatal chronic lung disease.

CONCLUSION: The ability to predict PWMI with a blood test for GFAP shortly after birth opens the possibility for rapid identification of infants for early intervention and provides a benchmark for the qualification of new therapies to improve neurodevelopmental outcomes.

Key words: cerebral palsy, glial fibrillary acidic protein, periventricular white matter injury

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As a consequence of preterm birth, low birthweight (LBW; <2500 g) infants are at increased risk for a spectrum of cerebral white matter abnormalities termed *periventricular white matter injury* (PWMI). Head ultrasound imaging routinely is performed in premature neonates in the first week of life to rule out intraventricular hemorrhage (IVH) and at 6 weeks for identification of PWMI. The incidence of PWMI is higher in infants who sustain IVH possibly because the hemorrhage provides a rich source of iron for the generation of reactive oxygen species that leads to oligodendrocyte death.[1]

★ EDITORS' CHOICE ★

When PWMI is present, it is associated with cerebral palsy in 60-100% of survivors.[2] Cerebral palsy affects 6-9% of infants who are born at <32 weeks' gestation and as many as 28% of infants who are born at <26 weeks' gestation.[3]

The fetal transition at birth is known to be a particularly vulnerable time for the preterm infant brain; however, we lack the means to identify infants objectively who are at risk for long-term neurologic injury in the early neonatal period. Currently available tools to identify the neonate who is at risk for encephalopathy shortly after birth include fetal heart rate tracings, Apgar scores, umbilical cord gases, and physical examination of the newborn infant, all of which lack precision.

In adult brain injury, such as traumatic brain injury and stroke, the measurement of circulating brain proteins have demonstrated significant diagnostic and prognostic potential.[4-7] A number of proteins, which includes

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the highly brain-specific glial fibrillary acidic protein (GFAP), have been used in adults and children to identify patients with stroke or traumatic brain injury in an effort to provide prognostic data on survival or density of residual deficits.[5] GFAP is a brain-specific cytoskeletal intermediate filament protein that is found in the astroglia of the central nervous system and is a specific marker of differentiated astrocytes. Serum GFAP is derived entirely from brain and not secreted routinely in blood but is released only after cell injury or death. In adult patients who had sustained mild traumatic brain injury, a relation was found between GFAP measured directly after admission, imaging studies, and outcome determined by return to work.[8]

Our group has found that elevated serum GFAP levels in neonates who underwent extracorporeal membrane oxygenation were associated with acute brain injury and death.[9] We have also found that, in term and near-term neonates with hypoxic-ischemic encephalopathy who were treated with whole body cooling, elevated GFAP levels on admission to the neonatal intensive care unit (NICU) and through day 4 of life were predictive of brain magnetic resonance imaging (MRI) abnormalities at 7 days of life and abnormal neurodevelopmental outcomes.[10] Our aim in this study was to determine whether GFAP levels that were measured within the first 4 days of life could be used to identify LBW neonates who are at risk for the development of PWMI.

Materials and Methods

This was an institutional review board–approved case control study of all liveborn, nonanomalous LBW (<2500 g) neonates who were born at a single university hospital and who were admitted to the NICU. Neonates with major congenital malformations, chromosomal abnormalities, or genetic syndromes were excluded. For GFAP studies, cord blood and the residual unused portion of serum from daily routine clinical laboratory tests were used. We collected cord blood at delivery and the remaining portion of serum from daily neonatal blood draws during the first 4 days of life for all neonates who were admitted to our NICU weighing <1500 g and for those with birthweights of 1500-2500 g with suspected neurologic morbidity at birth, which included prolonged hypotonia or seizures. Most of these samples were obtained by heel stick, but some samples were obtained from umbilical arterial lines. Residual serum was aliquoted and stored at −80°C until assayed. After neonatal discharge, we reviewed the results of head ultrasound scans that were performed at 6 weeks of life to identify infants with PWMI whose data were then compared with the subsequent 2 infants with normal head ultrasound scans at the same gestational age within 1 week and delivered by the same mode, matching in a 2:1 fashion. In addition, from this group, we compared those infants who experience the development of both IVH and PWMI with those infants with IVH only.

Our GFAP assay has been described previously.[9,10] Case and control samples that were blinded to the neonatal head ultrasound imaging results were assayed at the same time. Samples and standards were assayed in duplicate. Assays were analyzed on a Sector Imager 2400 (Meso Scale Discovery, Rockville, MD) according to the manufacturer's protocol. Values were reported as the mean concentration of a single sample that was assayed in duplicate. GFAP concentrations are a continual range from the lower to the upper limit of quantification for the assay (0.04−40 ng/mL).[9] GFAP values <0.04 ng/mL, which is the lower limit of quantitation, were reported as 0. The GFAP assay has an interassay coefficient of variability of <2.5%. The intraassay coefficient of variability ranged from 0.18−3.07%. GFAP values that were obtained from plasma and serum were equivalent.

All neonates had a head ultrasound scan within 1 week and at 2 weeks to rule out IVH and a third ultrasound scan at 6 weeks to rule out PWMI, as per our standard clinical practice. Head ultrasound scans were all performed in the NICU. Transfontanellar head ultrasonography was performed with state-of-the-art ultrasound equipment (Zonare Medical Systems, Mountain View, CA). Standardized optimized sets of coronal and sagittal images were obtained through the anterior fontanel with the use of curved and linear array transducers (8-17 MHz). Head ultrasound scans were evaluated by 2 pediatric neuroradiologists (A.T., T.A.G.M.H.) for overall white matter echointensity, gray-white matter differentiation, echointensity of the central gray matter, ventricular size (based on the largest ventricle size), germinal matrix hemorrhage (based on Papille's classification from I-III),[11] periventricular hemorrhagic infarction (previously known as grade IV hemorrhage), and cystic periventricular leukomalacia. *Diffuse white matter injury* was defined as generalized white matter volume loss, with or without echo intensity alterations. *Focal white matter* injury was defined as focal areas of decreased echo intensity that represent remote ischemia or hemorrhage.

Neonates with PWMI were scheduled for a comprehensive neonatal neurodevelopmental examination after discharge from the NICU, as per our standard of care. This examination draws from the work of many researchers in the area and was reported previously.[12] This examination is corrected for gestational age at birth and assessed the emergence of extremity flexor tone, axial (neck, trunk, shoulder, and hip) tone, deep tendon reflexes, pathologic reflexes, primitive reflexes, postural head control, and sensory responses. These examinations were conducted by a single examiner (M.C.A.) who is certified in pediatrics, neonatology, and neurodevelopmental disabilities. The diagnosis of cerebral palsy was based on a persistently abnormal neurologic examination (eg, spasticity and/or variable tone and/or persistent primitive and pathologic reflexes) and functional impairment (including abnormal quality of movement).[13] In the case group with abnormal head ultrasound scans, GFAP levels were compared between infants who had cerebral palsy and those who did not.

Infant and maternal medical records were reviewed to identify relevant clinical