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Clinical Observations

Neuropathology Associated With Diffuse Excessive High Signal Intensity Abnormalities on Magnetic Resonance Imaging in Very Preterm Infants

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ABSTRACT

BACKGROUND: Diffuse excessive high signal intensity abnormality is the most common finding on term-equivalent age magnetic resonance imaging in extremely preterm infants. Yet its clinical significance remains a matter of debate, in part because of a lack of prior imaging–pathology correlational studies. **PATIENT PRESENTATIONS:** We present two 24-week-gestation infants with complicated clinical courses who died at 33 and 46 weeks post-menstrual age with magnetic resonance imaging evidence of diffuse excessive high signal intensity. Two patients with periventricular leukomalacia and two without injury were examined for comparison. Immunohistochemistry characterized the presence of reactive astrocytes, microglia, myelin, and axons. Infants with periventricular leukomalacia demonstrated the typical microscopic necrosis with spheroids, gliosis/microgliosis with reduction in stainable myelin and axons. Infants with diffuse excessive high signal intensity showed vacuolated regions with increased reactive astrocytes and microglia and fewer oligodendroglial cell bodies/processes and dramatic reduction in axon number. **CONCLUSION:** These two individuals with diffuse excessive high signal intensity exhibited pathologic characteristics that were overlapping but distinct from those of periventricular leukomalacia.

Keywords: premature infant, magnetic resonance imaging, white matter abnormality, neuropathology, oligodendrocytes

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Introduction

A large majority of infants born extremely premature (less than 30 weeks gestational age) now survive. Of these, up to 40% develop cognitive and behavioral deficits. The structural antecedents of these functional deficits remain elusive. Although the incidence of cystic periventricular leukomalacia (PVL) has decreased dramatically in the past 15 years, diffuse excessive high signal intensity (DEHSI) abnormality on T2-weighted (w) magnetic resonance imaging (MRI) has been observed in up to 75% of preterm infants. Yet its clinical significance remains a matter of debate—does it represent a maturational delay of the developing white matter, white matter injury (WMI), or a combination of both?[1-5]

Several studies have failed to note an association with neurodevelopmental impairments, leading some to suggest that DEHSI represents maturational delay.[1-4] However, multiple studies have reported microstructural abnormalities in preterm infants with DEHSI, and four single-center studies that quantified DEHSI severity objectively or reported neurodevelopmental outcomes beyond two years of age observed a correlation with cognitive and language impairments.[5-8] If confirmed, DEHSI could represent a promising biomarker that could facilitate early diagnosis of high-risk infants. However, there are no reported concurrent neuroimaging and neuropathology studies in preterm

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infants with documented DEHSI on MRI. Khwaja and Volpe[9] speculated that, in addition to cystic and noncystic PVL where necrosis is predominant, a third form of white matter abnormality consisting of diffuse astrogliosis *without* focal necrosis seems likely but lacked conclusive evidence. They further speculated that this diffuse white matter gliosis is the mildest form of injury in a spectrum that includes cystic PVL as the most severe form. Here we describe two extremely preterm infants studied with postmortem MRI and autopsy with histologic evidence of pathology sampled from regions of MRI-defined DEHSI.

Methods

DEHSI patient 1

Patient 1 was a 24-week-gestation preterm male infant (birth weight, 588 g), born to a 37-year-old mother after a pregnancy complicated by urinary tract infections, preterm labor, and placental abruption. This infant experienced multiple morbidities including, respiratory distress syndrome; cystic bronchopulmonary dysplasia (BPD) requiring several weeks of high-frequency oscillatory ventilation, inhaled nitric oxide, and two courses of dexamethasone; Gram-negative sepsis; *Pseudomonas* and *Klebsiella* tracheitis/pneumonitis; and hypotension requiring volume resuscitation, dopamine, and hydrocortisone therapy. There was no evidence of intraventricular hemorrhage or PVL on early or late cranial ultrasounds. At 33 weeks postmenstrual age, high-frequency ventilation was withdrawn to allow natural death after an acute deterioration secondary to presumed sepsis that resulted in respiratory failure and hypoxemia. A postmortem MRI of his formalin fixed brain was performed.

DEHSI patient 2

Patient 2 was a 24-week-gestation extremely male infant (525 g birth weight) who was born to an 18-year-old mother whose pregnancy was complicated by pre-eclampsia, HELLP syndrome, and preterm prolonged rupture of membranes. The hospital course was complicated with multiple morbidities including respiratory distress syndrome, severe BPD requiring intermittent high frequency ventilation, culture-positive sepsis, Pseudomonas tracheitis/pneumonitis, hypotension, and severe retinopathy of prematurity. Severe anasarca (head and neck greater than body) developed late in the hospital course without evidence of superior vena cava syndrome. No lymphatic abnormalities were noted at the time of autopsy. There was no evidence of intraventricular hemorrhage or PVL on multiple cranial ultrasound examinations. There was concern for ventriculomegaly however, that was first noted at one month of age. At 46 weeks postmenstrual age, he developed an exacerbation of his BPD with persistent hypoxemia that was unresponsive to high-frequency ventilation and dexamethasone therapy. Life support was withdrawn the next day. A postmortem MRI was done within 12 hours of death.

Immunoreactivities of white matter sampled from two best-available age-matched infants with PVL and two with no pathologic evidence of WMI were carefully selected from the archives of Nationwide Children's Department of Pathology and analyzed for comparison purposes. The first PVL patient was a 24-week-gestation twin male infant who survived to 31 weeks postmenstrual age and died of sepsis. The second PVL patient was a male infant born at 39 weeks gestation and died seven weeks later because of congenital heart disease. The first non-WMI patient was a 24-week-gestation female infant who survived five weeks and died after development of necrotizing enterocolitis. The second non-WMI patient was a term female infant who succumbed to congenital heart disease five months after birth.

Neuropathology

As per current standard, the brain and spinal cord were removed and placed in 20% formalin for seven days. A neuropathologist (C.R.P.) examined and dissected all brains. The study neuroradiologist (J.A.R.) used postmortem MR images to identify DEHSI, and this guided sampling for histopathology. The PVL and non-WMI comparison patients did not undergo postmortem imaging. Ten to 15 hematoxylin-eosin–stained sections were studied from all DEHSI and comparison patients to characterize the overall neuropathology and to select suitable areas for immunohistochemical study. Immunohistochemistry was performed as previously described.[10] The specific antibodies and epitopes they label and the dilution and antigen retrieval parameters are described in the Table. Six antibodies were used: CNPase for oligodendrocytes and myelin, neurofilament for axons, glial fibrillary acidic protein (GFAP) for astrocytes, ionized calcium-binding adaptor molecule 1 (Iba1) for microglia, synaptophysin for neuronal synapses, and nestin for radial glial fibers.

Postmortem MRI

Imaging was performed on a General Electric (Milwaukee, Wisconsin) Signa HDxt 3.0 Tesla magnet running 16.0 software. High-resolution fast spin-echo T2w sequences in the axial, coronal, and sagittal planes were obtained as the primary diagnostic sequences using the following parameters: repetition time, 4000 ms; echo time, 102 ms; echo train length, 12; matrix size, 512 × 256; excitations, 4; slice thickness, 2 mm with no gap; bandwidth, 32 kHz; and field of view, 12 to 20 cm.

Patient 1 was imaged in a water bath using a wrist coil after the brain was excised and fixed in formalin. Patient 2 was imaged immediately after demise before brain excision using an eight-channel head coil. T1w and susceptibility-weighted sequences were also performed but proved to be of no diagnostic benefit for Patient 1 secondary to poor contrast and susceptibility artifacts. A premortem cranial MRI was also performed for Patient 2 at 37.5 weeks postmenstrual age.

Results

Both Patients 1 and 2 showed asymmetric abnormal increased T2w signal within the periatrial white matter on the left side for Patient 1 and right side for Patient 2, consistent with DEHSI (Fig 1). The signal change was

TABLE.
Antibodies Used for Immunohistochemistry

Antigen, Cells Labelled	Vendor	Catalog Number	Dilution	Antigen Retrieval
2′,3′-Cyclic nucleotide 3′-phosphodiesterase (CNPase), oligodendrocytes and myelin	Dako	Ab3619 Mouse monoclonal, clone: 11-58	1:800	Heat, 20 minutes, citrate buffer
Neurofilament, axons	Leica	PA0371 Mouse monoclonal, clone: 2F11	Predilute	Heat, 20 minutes, citrate buffer
Glial fibrillary acidic protein, astrocytes	Thermo Lab Vision	RB-087-R7 Rabbit polyclonal	Predilute	None
Ionized calcium-binding adaptor molecule 1, microglia	Biocare Medical	Iba1; 290AA Rabbit polyclonal	1:1000	Heat, 20 minutes, citrate buffer
Synaptophysin, neuronal synapses	Leica	PA0299 (clone 27612)	Predilute	Heat, 20 minutes, EDTA buffer
Nestin, radial glial fibers	Abcam	Ab105389	1:200	Heat, 20 minutes, EDTA buffer