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Original Article

Antecedents of Objectively Diagnosed Diffuse White Matter Abnormality in Very Preterm Infants

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ABSTRACT

Background: Diffuse white matter abnormality (diffuse excessive high signal intensity) is the most common finding on structural brain magnetic resonance imaging (MRI) at term-equivalent age in very preterm infants. Yet, there remains a large gap in our understanding of the etiology of diffuse white matter abnormality. Our objective was to evaluate perinatal and neonatal inflammation-associated antecedents of diffuse white matter abnormality on MRI.

Methods: We prospectively enrolled 110 very preterm infants born at ≤31 weeks gestational age and collected data on multiple perinatal/neonatal exposures, especially inflammation initiating-illnesses. We performed structural MRI at term-equivalent age and quantified the volume of diffuse white matter abnormality objectively. Multivariable regression was used to identify clinical antecedents of diffuse white matter abnormality.

Results: The mean (S.D.) birth gestational age of the final study sample of 98 very preterm infants was 28.3 (2.5) weeks. Multiple inflammation initiating-illnesses were associated with diffuse white matter abnormality in univariate analyses. In multivariable linear regression analyses controlling for gestational age, severe retinopathy of prematurity ($P < 0.001$) and bronchopulmonary dysplasia ($P = 0.006$) were independent risk factors, whereas maternal treatment with 17-hydroxyprogesterone ($P < 0.001$) was protective of later development of objectively quantified diffuse white matter abnormality.

Conclusions: We identified several perinatal and neonatal antecedent clinical factors associated with diffuse white matter abnormality. Although we found some support for inflammation as a common underlying mechanism, larger studies are needed to validate inflammation as a potential common pathway to the development of diffuse white matter abnormality in very preterm infants.

Introduction

Very preterm infants are at an increased risk of infection, inflammation, and inflammation-initiating illnesses that have been associated with long-term neurodevelopmental impairments.[1-4] Yet, no clear neuroimaging intermediate biomarker for such inflammation-initiating illnesses has been identified. Diffuse excessive high signal intensity (DEHSI) on T2-weighted magnetic resonance imaging (MRI) at term-equivalent age could represent one such biomarker. Since its initial description two decades ago,[5] where DEHSI was reported in 75% infants born before 30 weeks' gestational age, multiple cohort studies have confirmed a similarly

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high prevalence, between 50% and 80% in very preterm infants.[6-9] Despite DEHSI's common occurrence, our understanding about its etiology, pathophysiology, or long-term consequences remain limited.[10]

The only published postmortem analysis of infants with DEHSI[11] identified pathology that resembled periventricular leukomalacia (e.g., increased reactive astrocytes and microglia) and diffuse vacuolated regions, a finding that was distinct from periventricular leukomalacia. Other investigators have speculated, based on postmortem studies without associated neuroimaging, that high-signal-intensity abnormalities on MRI without cystic changes may be a milder form of periventricular leukomalacia presenting as diffuse injury without necrosis on postmortem histopathology.[12,13] Considering this neuropathology as well as microstructural[9,14] and metabolic imaging abnormalities[15] within white matter regions of DEHSI reported in other studies, *diffuse white matter abnormality* (DWMA) represents a more appropriate name for this imaging abnormality. Most investigators have not reported any antecedent risk factors for DWMA[6-8] other than patent ductus arteriosus (PDA) ligation surgery.[9] However, these studies diagnosed DWMA visually/qualitatively, which has been shown to be unreliable,[16-19] likely due to measurement error significantly biasing the results. Many of these studies may also have been underpowered to identify antecedents owing to their relatively small size and/or because they defined DWMA categorically. When defined quantitatively as volume of DWMA, we previously identified severe retinopathy of prematurity (ROP) and surgery for necrotizing enterocolitis (NEC) or spontaneous intestinal perforation (SIP) as independent risk factors of DWMA volume.[20] Another quantitative study identified prolonged mechanical ventilation as a risk factor for abnormal microstructure in the centrum semiovale of extremely preterm infants with a high prevalence of DWMA.[21] Inflammation appears to be a common theme among these identified risk factors, but these provocative findings have not been validated. In this study we define DWMA using our recently developed, intensity-based algorithm to semiautomatically quantify DWMA.[22,23] Our objective was to examine antecedent perinatal and neonatal clinical factors of objectively diagnosed DWMA in a prospective, population-based cohort of very preterm infants. We hypothesized that perinatal and neonatal inflammation-initiating illnesses will be independently associated with objectively quantified volume of DWMA.

Methods

Population

We prospectively enrolled 110 very preterm infants admitted to four Columbus, Ohio, regional level III neonatal intensive care units (NICUs) from November 2014 to March 2016. These four NICUs cared for approximately 80% of all very preterm infants in the Columbus, Ohio, region. Data collection occurred between January 2015 and April 2016. The four NICUs included (with total subjects enrolled) two academic NICUs, Nationwide Children's Hospital (NCH) (N = 15) and Ohio State University Medical Center (N = 41), and two nonacademic NICUs, Riverside Hospital (N = 30) and Mount Carmel St. Ann's Hospital (N = 24). Any very preterm infant born at 31 weeks' gestational age or younger and cared for in one of these four NICUs was eligible for inclusion. We excluded any infants who had congenital or chromosomal anomalies that affected the central nervous system resulting in a known poor outcome or infants who remained hospitalized at 44 weeks postmenstrual age (PMA) (unless they were at the NCH, the only site of imaging). For the latter, there were only three such exclusions. The NCH Institutional Review Board approved the study. Written informed consent was obtained from a parent or a guardian of all very preterm infants.

Magnetic resonance imaging acquisition

We imaged all study infants except from the NCH after NICU discharge between 39 and 44 weeks PMA on a 3T Siemens Skyra MRI scanner at the NCH. All study infants from the NCH NICU were also imaged during the same interval; however, a majority of these infants were still hospitalized and were transported to the same MRI scanner by a skilled neonatal nurse and neonatologist. Heart rate and oxygen saturation of all infants was monitored during the scan. We performed all imaging without sedation by following these procedures: infant was fed 30 minutes before the scan, silicone earplugs were placed (Instaputty, E.A.R. Inc, Boulder, CO, USA), and a blanket and vacuum immobilization device (MedVac, CFI Medical Solutions, Fenton, MI, USA) were applied to promote natural sleep. The following axial T2-weighted MRI sequence was used: echo time 147, repetition time 9500 ms, refocusing flip angle 150°, voxel dimensions $0.93 \times 0.93 \times 1.0$ mm^3, scan time 4:09 min; 3-dimensional magnetization-prepared rapid gradient echo: echo time 2.9, repetition time 2270 ms, echo spacing time 8.5 ms, flip angle 13°, voxel dimensions $1.0 \times 1.0 \times 1.0$ mm^3, 3:32 min; and axial SWI: echo time 20, repetition time 27 ms, flip angle 15°, voxel dimensions $0.7 \times 0.7 \times 1.6$ mm^3, 3:11 min.

Image postprocessing

We objectively segmented DWMA regions in the centrum semiovale (two slices immediately above the lateral ventricles on axial T2) using our published algorithm (Fig).[22] Briefly, voxels with signal intensity values greater than 1.8 S.D. above the mean of cerebral tissue (white and gray matter) were labeled as DWMA. Cerebral tissue segmentation was achieved by unified segmentation with spatial priors obtained from a neonatal probabilistic brain atlas. To enhance detection accuracy, we manually removed erroneous, randomly isolated voxels. DWMA volume was calculated as the product of voxel volume and total number of voxels in the detected DWMA region. To account for individual differences in total brain white matter volume, DWMA volume was divided by that infant's total white matter volume and labeled hereafter as normalized volume of DWMA.

Data collection and definitions

A trained neonatal research nurse collected a detailed predefined list of maternal characteristics, pregnancy/delivery data, and infant data from birth to study MRI examination. Chorioamnionitis was defined as clinically present if documented by the obstetrician in the maternal records. Any maternal receipt of 17-hydroxyprogesterone (17P) or similar progesterone therapies during current pregnancy for prior history of preterm birth was coded as "Yes." Severe bronchopulmonary dysplasia (BPD) was defined using the NIH definition—need for more than 30% effective fractional inspiratory oxygen concentration via nasal cannula or need for any positive pressure support at 36 weeks PMA. Proven NEC Bell's stage IIIB or SIP that required surgery was documented as surgery for NEC/SIP. Using the International Classification of ROP, we defined severe ROP as any of the following: stage 3 ROP, any stage ROP with plus disease, or any prethreshold ROP requiring treatment with laser ablation or Avastin intraocular injections. General anesthesia/major surgery was defined using a predefined list of operations that required general anesthesia during the NICU hospitalization. In addition to these inflammation-initiating illnesses/therapies, we also examined maternal infection within

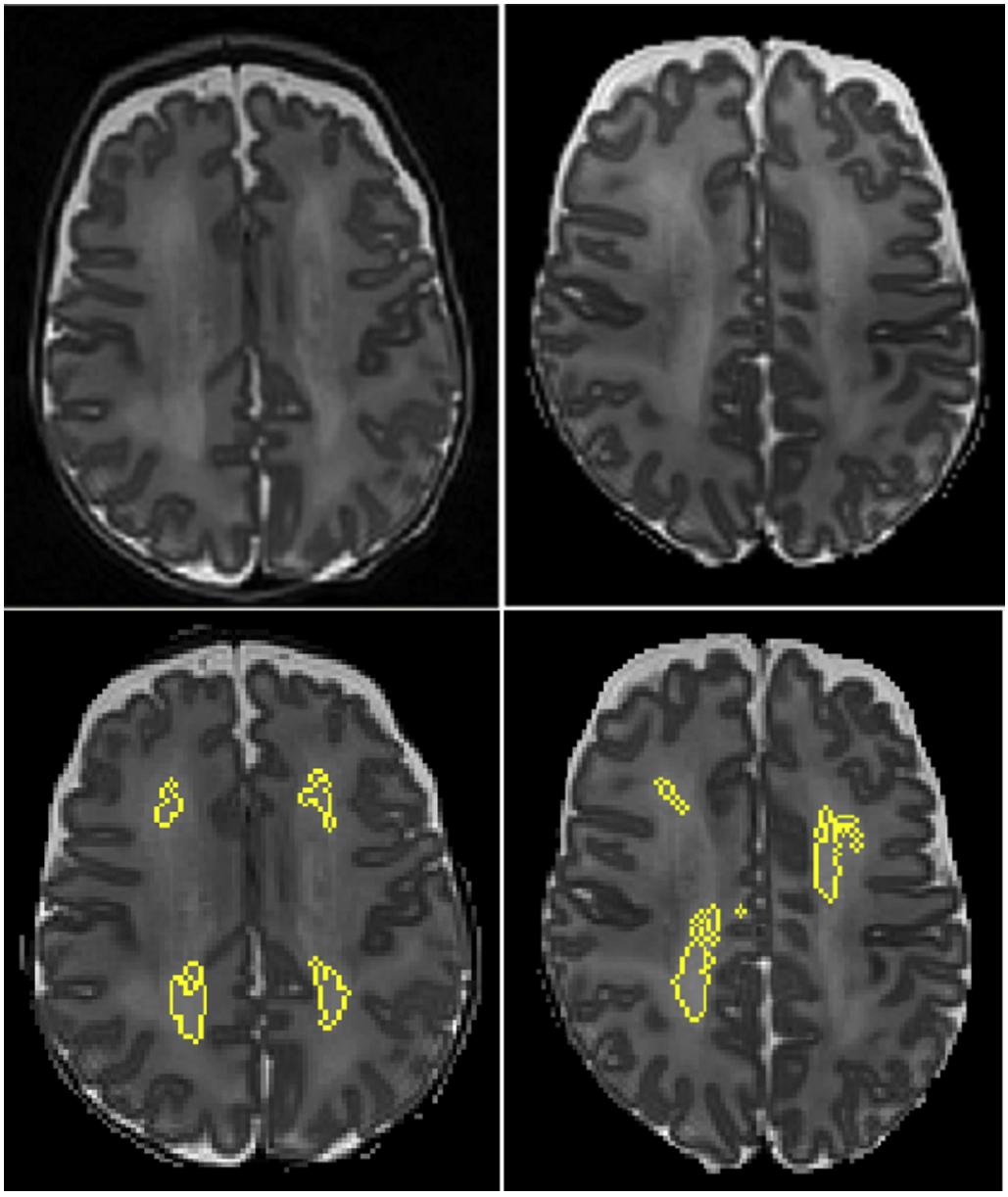

FIGURE. Semiautomated segmentation of diffuse white matter abnormality (DWMA) in the centrum semiovale. The two top panels display raw axial T2-weighed magnetic resonance images through the centrum semiovale (immediately above the lateral ventricles) from 27-week (left) and 31-week (right) gestational age preterm infants, scanned at term-equivalent age. Higher signal intensity can be appreciated in the centrum semiovale white matter from the surrounding subcortical white matter. The bottom panels display the corresponding slices with automatically segmented DWMA in yellow. Both infants were diagnosed with severe DWMA. The color version of this figure is available in the online edition.

seven days of delivery and culture-positive late-onset sepsis. Gestational age was defined using the best available obstetric estimate. Transitional hypotension was defined as low systemic blood pressure within 48 hours of birth that warranted clinical treatment with volume and/or cardiac pressors. We also examined the lowest blood pressure as preferentially defined by central arterial monitoring if available or non-invasive blood pressure cuff measurements within the first 24 hours after birth. To assess perinatal acidosis, we recorded the base deficit from cord blood gas or neonatal blood gas within the first hour after birth. Nutritional factors such as total days of total parenteral nutrition and days of maternal breast milk in the first month of life were also evaluated. Structural brain MRI was read by one of three pediatric neuroradiologists using an established neonatal MRI monitoring system[24] to derive a global abnormality score.

Statistical analyses

Normalized volume of DWMA exhibited a non-Gaussian distribution that was skewed to the right and was therefore log transformed for all analyses. Base deficit within the first hour after birth was missing for 24 subjects. Of these, 22 did not require intubation or any delivery room resuscitation (e.g., positive pressure ventilation, chest compressions, epinephrine). Also, their mean (S.D.) 5-minute Apgar score was 8.1 (0.8) and gestational age was 29.4 (1.7) weeks. For these 22 infants we reasoned that clinicians did not

obtain a blood gas due to presumed clinical stability. Therefore, we felt it was appropriate to impute a "normal" base deficit of -2 for each of these 22 infants. We did not impute data for the other two infants with missing data as they both had lower 5-minute Apgar scores and required intubation and positive pressure support in the delivery room. We tested over 50 clinical variables[20,21] present before or during pregnancy (antenatal), present at birth (intrapartum), and factors occurring between the first postnatal day and study MRI examination (NICU) with normalized volume of DWMA in univariate linear regression analyses. Clinical variable selection was based on supporting literature and biological plausibility. Each of the chosen variables had previously been suspected antecedents with previously reported association with perinatal brain injury and/or neurodevelopmental impairments. As postnatal clinical covariates can overshadow antepartum or intrapartum variables that may be causative, we created multivariable regression models in which we ordered clinical factors temporally so that the earliest occurring factors were entered first and would not be displaced by later occurring covariates.[20,25] Variables with P value <0.10 were entered into a multivariable linear regression model in a backward stepwise fashion to evaluate their independent association ($P < 0.05$) with normalized volume of DWMA. Because younger infants are at risk for brain abnormalities, we decided to include gestational age in the final model, irrespective of its significance. To control for differences in clinical care practices between the four NICUs, we included NICU/Center as a covariate in the final model. Two-sided P values <0.05 were considered to indicate statistical significance. We performed all analyses using STATA 15.1 (Stata Corp., College Station, TX, USA).

Results

Of the original cohort of 110 very preterm infants, we excluded 12 infants, one that exhibited significant motion artifact on the study MRI and all 11 infants with severe brain injury where such injury, typically in the central white matter, interfered with our ability to accurately quantify DWMA. The mean (S.D.) birth gestational age of the final study sample of 98 infants was 28.3 (2.5) weeks and birth weight was 1132 (396) g (Table 1). One infant was diagnosed with severe injury (1.0%), six with moderate injury (6.1%), 21 with mild injury (21.4%), and 70 with no injury (70.4%) on their structural MRI at term-equivalent age.

Table 1 summarizes important antenatal, intrapartum, and postnatal maternal and infant clinical factors for the full cohort before term-equivalent age MRI. The mean (S.D.) age at MRI scan was 40.4 (0.6) weeks. Age at MRI was not significantly associated with DWMA volume. In univariate analyses, several antecedents were adversely associated (P<0.10) with normalized volume of DWMA, including 5-minute Apgar score less than 5, hypothermia at birth, late-onset culture-positive sepsis, PDA, surgery for NEC/SIP, duration of oxygen supplementation, duration of continuous positive airway pressure therapy, severe BPD, severe ROP, and undergoing general anesthesia/major surgery. One factor was associated with a reduced risk of DWMA—maternal treatment with 17P therapy (Table 2).

TABLE 1.
Distribution of Important Antenatal, Intrapartum, and Postnatal Clinical Factors Before MRI at Term-Equivalent Age by Center in a Cohort of Very Preterm Infants

Clinical variables*	All Infants (N = 98)	Nationwide (N = 14)	Ohio State (N = 34)	Riverside (N = 26)	St. Ann's (N = 24)	*P*
Maternal hypertension	32 (32.7%)	4 (28.6%)	13 (38.2%)	7 (16.9%)	8 (33.3%)	0.808
Antepartum hemorrhage	21 (21.4%)	7 (50.0%)	7 (20.6%)	3 (11.5%)	4 (16.7%)	*0.036*
Maternal infection within 7 days of delivery	18 (18.4%)	2 (14.3%)	8 (23.5%)	4 (15.4%)	4 (16.7%)	0.813
Chorioamnionitis (clinical)	15 (15.3%)	0 (0%)	9 (26.5%)	3 (11.5%)	3 (12.5%)	0.104
17-OH progesterone therapy	24 (24.5%)	4 (28.6%)	6 (17.7%)	7 (26.9%)	7 (29.2%)	0.720
Antenatal steroids (any)	85 (86.7%)	7 (50.0%)	32 (94.1%)	24 (92.3%)	22 (91.7%)	*<0.001*
Antenatal magnesium therapy	64 (66.0%)	6 (42.9%)	25 (73.5%)	17 (65.4%)	16 (66.7%)	0.374
Gestational age at birth (weeks), mean (S.D.)	28.3 (2.5)	27.7 (3.0)	27.3 (2.4)	28.2 (2.3)	29.6 (2.4)	*0.005*
Birth weight (g), mean (S.D.)	1132 (396)	1198 (538)	976 (314)	1109 (354)	1330 (376)	*0.003*
Male	53 (54.1%)	8 (57.1%)	16 (47.1%)	16 (61.5%)	13 (54.2%)	0.431
Intubation at birth	29 (29.6%)	8 (57.1%)	11 (32.4%)	7 (26.9%)	3 (12.5%)	0.035
Apgar score <5 at 5 minutes†	19 (19.6%)	6 (46.2%)†	4 (11.8%)	5 (19.2%)	4 (16.7%)	0.066
Base deficit within first hour after birth, median (range)‡	−4.0 (−12.9, 2.0)	−4.0 (−8.5, −1.0)‡	−2.0 (−12.9, −0.1)	−4.0 (−12.0, 0)	−4.5 (−10.0, 2.0)	0.495
Hypothermia, delivery room	28 (28.6%)	3 (21.4%)	14 (41.2%)	9 (34.6%)	2 (8.3%)	*0.042*
Pneumothorax or PIE	11 (11.2%)	0 (0%)	4 (11.8%)	5 (19.2%)	2 (8.3%)	0.306
Lowest mean blood pressure in first 24 hours after birth (mm Hg), median (range)	28 (18-51)	29 (23-31)	28 (19-36)	27 (18-38)	29 (20-51)	0.450
Transitional hypotension requiring therapy	8 (8.2%)	2 (14.3%)	4 (11.8%)	0 (0%)	2 (8.3%)	0.316
Culture-positive late-onset sepsis	13 (13.3%)	2 (14.3%)	3 (8.2%)	4 (15.4%)	4 (16.7%)	0.818
Patent ductus arteriosus	21 (21.4%)	3 (21.4%)	8 (23.5%)	8 (30.8%)	2 (8.3%)	0.279
BPD (oxygen supplementation at 36 weeks PMA)	48 (49.0%)	10 (71.4%)	20 (58.8%)	11 (42.3%)	7 (29.2%)	*0.040*
Severe BPD (NIH definition)	29 (29.6%)	7 (50%)	8 (23.5%)	7 (26.9%)	7 (29.2%)	0.327
Surgery for NEC or SIP	5 (5.1%)	0 (0%)	3 (8.8%)	1 (3.9%)	1 (4.2%)	0.608
Severe ROP	9 (9.2%)	0 (0%)	4 (11.8%)	3 (11.5%)	2 (8.3%)	0.602
General anesthesia/major surgery	12 (12.2%)	2 (14.3%)	4 (11.8%)	2 (7.7%)	4 (16.7%)	0.804
PMA at MRI scan (weeks), mean (S.D.)	40.4 (0.6)	40.3 (0.4)	40.4 (0.6)	40.3 (0.6)	40.7 (0.8)	0.561

Abbreviations:
BPD = Bronchopulmonary dysplasia
MRI = Magnetic resonance imaging
NEC = Necrotizing enterocolitis
NICUs = Neonatal intensive care units
PIE = Pulmonary interstitial emphysema
PMA = Postmenstrual age
ROP = Retinopathy of prematurity
SIP = Spontaneous intestinal perforation
Italics highlight significant group differences for that clinical factor between the four NICUs.
* All values are N (%) unless otherwise noted.
† Apgar score at 5 minutes was missing for one infant (home birth).
‡ Base deficit was missing for two subjects.

TABLE 2.
Univariate Analyses Displaying the Coefficients of Several Clinical Antecedents That Were Associated (at $P<0.10$) With Normalized Volume of DWMA, Objectively Defined on Structural Brain MRI at Term-Equivalent Age

Clinical Antecedent	Coefficient (95% CI)*	*P* Value
Maternal 17-hydroxyprogesterone therapy	0.27 (0.13, 0.56)	0.001
Apgar score <5 at 5 minutes	2.46 (1.06, 5.70)	0.035
Hypothermia, delivery room	0.70 (0.50, 0.98)	0.035
Base deficit within the first hour after birth	0.89 (0.80, 1.00)	0.044
Days on oxygen supplementation	1.01 (1.00, 1.02)	0.066
Days on CPAP therapy	1.01 (1.00, 1.03)	0.050
Severe BPD	2.98 (1.46, 6.05)	0.003
Patent ductus arteriosus	3.64 (1.66, 7.97)	0.002
Culture-positive late-onset sepsis	2.48 (0.93, 6.62)	0.070
Severe ROP	8.12 (2.71, 24.26)	<0.001
General anesthesia/major surgery	3.14 (1.14, 8.60)	0.027

Abbreviations:
BPD = Bronchopulmonary dysplasia
CPAP = Continuous positive airway pressure
DWMA = Diffuse white matter abnormality
MRI = Magnetic resonance imaging
ROP = Retinopathy of prematurity
* The coefficients for the categorical variables, represent a ratio of the normalized volume of diffuse white matter abnormality for infants with a given antecedent divided by the volume for infants without that antecedent. For antecedent continuous variables, the coefficient is the ratio of the normalized volume of diffuse white matter abnormality for a one-unit increase in the clinical variable.

In multivariable linear regression analyses, maternal 17P therapy was the only significant variable from the antepartum and intrapartum periods that was retained in the postpartum model. In the final model, maternal 17P therapy was independently protective, whereas severe ROP and severe BPD were associated with increased normalized volume of DWMA (Table 3). Gestational age was inversely associated with normalized volume of DWMA but was not significant in the final model. We also evaluated sex but did not retain it in the final model as it was not significant ($P = 0.75$) and did not significantly impact the other variables.

We did not find a significant relationship between normalized volume of DWMA and any of the following clinical variables: maternal antenatal steroid or magnesium therapy, maternal hypertension (chronic or pregnancy induced), maternal infection within seven days of delivery, antepartum hemorrhage, clinical chorioamnionitis, sex, the lowest systemic blood pressure in the first 48 hours, therapy for transitional hypotension, any air leak (pneumothorax or pulmonary interstitial emphysema), need for chest compressions, air leak syndrome, severe anemia prompting blood transfusion therapy, duration of maternal milk within the first 28 days after birth, time to full enteral feedings, or duration of total parenteral nutrition. We also examined the association with several types of injury on structural MRI, but none of them were associated with normalized volume of DWMA, including gray or white matter signal abnormality, cystic changes, cerebral or intraventricular hemorrhage, cerebellar hemorrhage/injury/atrophy, ventriculomegaly, enlarged extra-axial space, brain atrophy, or the global abnormality score.

TABLE 3.
Final Multivariable Linear Regression Model Displaying the Coefficients of Several Clinical Antecedents That Were Associated With the Development of DWMA, Objectively Defined on Structural Brain MRI at Term-Equivalent Age

Clinical Antecedent	Coefficient (95% CI)*	*P* Value
Maternal 17-hydroxyprogesterone therapy	0.27 (0.14, 0.53)	<0.001
Severe retinopathy of prematurity	7.03 (2.39, 20.77)	<0.001
Severe bronchopulmonary dysplasia	2.73 (1.31, 5.68)	0.008
Gestational age at birth	1.11 (0.97, 1.27)	0.135

Abbreviations:
CI = Confidence interval
DWMA = Diffuse white matter abnormality
MRI = Magnetic resonance imaging
* The coefficients for the top three variables, which are categorical, represent a ratio of the normalized volume of diffuse white matter abnormality for infants with a given antecedent divided by the volume for infants without that antecedent. For the gestational age continuous variable, the coefficient is the ratio of the normalized volume of diffuse white matter abnormality for a one-week increase in gestational age. The final model also included NICU/Center where the infant was born and/or primarily cared for.

Discussion

We identified several unique antecedent factors for the development of DWMA in a geographically defined, prospective cohort of very preterm infants. Notably, both severe ROP and severe BPD share systemic inflammation as a common mechanism. In addition, the one protective factor we found, maternal 17P therapy, is known to have anti-inflammatory properties.[26] Of the other antecedents that were also significant in univariate analyses, a large majority have also been associated with inflammation, including culture-positive late-onset sepsis, surgical NEC/SIP, PDA,[27] and general anesthesia/major surgery.[28] However, inflammation may only be one of several risk factors for some of these antecedents, and hypoxia, ischemia, and hyperoxia may co-occur in the development of some of these conditions. Conversely, we did not find any significant associations between DWMA and clinical factors mostly associated with hypoxia, ischemia, or nutritional deficits.

Previously, we identified severe ROP and surgery for NEC/SIP as independent risk factors in a different cohort of extremely preterm infants.[20] However, we had defined DWMA unconventionally by relabeling regions of white matter as DWMA that were erroneously identified by our brain tissue segmentation software as cerebrospinal fluid (due to its high signal intensity). By using an updated algorithm that was specially designed to quantify DWMA in this study, we were able to identify severe ROP and surgery for NEC/SIP again in univariate analyses ($P = 0.10$). However, surgical NEC/SIP was no longer significant in multivariable analyses, likely because we lacked sufficient power (N = 5). Similar to surgical NEC/SIP, severe ROP has been reported to be an independent risk factor for neurodevelopmental impairments in several cohort studies,[2,4] yet few studies have identified a link between ROP and brain structural abnormalities.[29] Severe BPD was also associated with higher volume of DWMA. Pogribna et al.[21] reported duration of mechanical ventilation as a risk factor for abnormal microstructure in the centrum semiovale, supporting our findings. Although BPD is a well-established, independent risk factor for neurodevelopmental impairments, structural abnormalities other than ventriculomegaly have not been consistently reported in infants with BPD to explain the poor developmental outcomes in these infants.[2] Interestingly, ventriculomegaly often accompanies DWMA diagnosis and its development has perhaps been most closely associated with perinatal inflammation.[5,30] The independent association of severe BPD and ROP with DWMA that we identified suggests that DWMA may be the structural antecedent of the neurodevelopmental impairments commonly observed in infants with severe BPD and ROP. Unlike Skiold et al.,[9] we did not find an association between PDA ligation surgery and DWMA. However, our study was underpowered to examine this association because only three infants in our cohort underwent this procedure.

Severe ROP was highly significant in univariate and multivariable analyses with exposure associated with a seven-fold increase in normalized volume of DWMA when compared with infants without severe ROP, after controlling for other antecedents. However, only nine infants developed severe ROP, which explains the wide confidence intervals (2.4- to 20.8-fold increase) and need for independent replication. The pathogenesis of ROP has primarily

been attributed to an initial phase of hyperoxia after birth followed by hypoxemia and poor control of oxygenation during the NICU course.[31,32] More recent evidence from both experimental models and large epidemiologic studies also implicates antenatal inflammation and postnatal infection/inflammation as strong risk factors in the development of ROP.[33-37]

Similar to ROP, hyperoxia and hypoxia are well-established risk factors for BPD in animal models.[38,39] However, the most robust data come from randomized trials in preterm infants that implicate inflammation as a predominant causal factor because treatment with inhaled and systemic corticosteroids results in reduced rates of BPD,[40-42] whereas antioxidant therapies or tighter control of oxygen saturations does not reduce BPD.[43,44] These lines of evidence from human preterm infants suggests that inflammation plays a greater role than hypoxia, hyperoxia, or ischemia in the development of BPD. We also could not find an association with antecedent factors known to be associated with hypoxia and ischemia either directly (e.g., antepartum hemorrhage, maternal hypertension, delivery room resuscitation, lowest mean blood pressure in the first 24 hours, transitional hypotension prompting therapy, chest compressions during NICU stay) or indirectly (e.g., low Apgar score at 5 minutes, air leaks, perinatal acidosis, severe anemia prompting blood transfusion therapy) in our multivariable model. Therefore, inflammation may play a greater role than hypoxia or ischemia in the development of DWMA. However, we did not collect serial blood gas, oxygen saturation, or proteomic data to definitively rule out a role for hypoxia, ischemia, or hyperoxia in the development of DWMA. Moreover, inflammation may sensitize the developing white matter to secondary injury from these other factors, which are themselves potent inducers of inflammation.[45,46] A common outcome of these exposures is arrested oligodendrocyte maturation, resulting in persistent myelination abnormalities in animal models of perinatal white matter damage.[12,46,47] Larger studies that are better able to quantify the contribution of each of these mechanisms will be needed to definitively determine a common pathway, if present, to the development of DWMA.

The independent association with 17P that we identified represents a promising lead to explore for neuroprotection. Both 17P and its naturally occurring analog, progesterone, a steroid hormone, have been tested in randomized trials for mothers with a prior history of preterm birth, and 17P treatment was approved by the US Food and Drug Administration in 2011 for this indication.[48] However, progesterone's mechanism of action for reduced risk of preterm births remains unclear. In experimental models of adult brain injury, progesterone therapy exhibits a wide range of mechanisms and benefits, including inhibiting the inflammatory response, reducing oxidative stress, promoting recovery of mitochondrial dysfunction, and mitigating the severity of brain damage.[49-53]

We previously reported a significant inverse association between DWMA volume measured in the centrum semiovale and standardized cognitive and language scores on the Bayley Scales of Infant and Toddler Development, Third Edition, at age two years in two independent cohorts.[22,54] Two other studies that queried the centrum semiovale region using different objective, quantitative measures of injury, also reported an adverse association with cognitive and language development.[55,56] However, when investigators have diagnosed DWMA visually/qualitatively, most have not found an association with neurodevelopmental deficits.[7,8,16,57] A visual diagnosis of DWMA has been shown to be subjective and prone to measurement error, thus potentially biasing study results.[16,18,19] The balance of the more robust evidence appears to be suggestive of a significant association between DWMA and adverse developmental outcomes, especially cognitive impairments. This finding is significant because cognitive impairments are the most common adverse neurodevelopmental outcome in very preterm infants, yet there are no other validated objective imaging biomarkers of this prevalent outcome. Moreover, pro-inflammatory biomarkers and inflammation-initiating illnesses have been linked to adverse cognitive outcomes.[3] Could DWMA represent an intermediate prognostic biomarker between perinatal inflammation initiating-illnesses and long-term cognitive impairments? If so, it could be developed as a surrogate end point for clinical trials of perinatal neuroprotective therapies.

Our study has several limitations. Although we found a significant association between maternal 17P therapy during pregnancy and DWMA, we did not collect information about drug timing, dosing, or duration to better understand how these factors influenced its relationship with DWMA. Therefore, these findings should be viewed as hypothesis generating. Also, it is often difficult to disentangle any association with a therapy with its underlying indication. However, it is unlikely that the underlying indication for 17P therapy, prior preterm birth, is driving the association between 17P and reduced DWMA volume. We did not analyze cerebrospinal fluid or serum cytokine levels to confirm our suspicion of the link with inflammation. We also did not collect placental pathology about inflammatory and vascular disease. These limitations are being addressed in our larger follow-up study. The significant strengths of our study include the use of a population-based, prospective cohort examination of a large number of *a priori*-defined perinatal/neonatal variables and use of our robust algorithm to derive a more objective and quantitative definition of DWMA. Our work, if validated, will facilitate evaluation of novel neuroprotective agents within weeks of the intervention, when compared with the current two to five years it takes to accurately diagnose neurodevelopmental impairments.

In conclusion, we identified severe BPD and ROP as potential risk factors for DWMA. Although inflammation plays a prominent role in the development of both conditions, other mechanisms also contribute to its development. Therefore, we need larger studies with more in-depth data collection as we are currently doing to conclusively confirm inflammation as a common pathway to the development of DWMA in very preterm infants.

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