Effect of chorioamnionitis on brain development and injury in premature newborns

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Vann Chau MD[1], Kenneth J. Poskitt MDCM[2], Deborah E. McFadden MD[3], Tim Bowen-Roberts BA[1], Anne Synnes MDCM[1], Rollin Brant PhD[4], Michael A. Sargent MD[2], Wendy Soulikias RN[1], Steven P. Miller MAS MDCM[1]

From the Departments of [1]Pediatrics, [2]Radiology, [3]Pathology, and [4]Statistics, University of British Columbia, Vancouver, Canada

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Corresponding Author:

Steven P. Miller, MAS MDCM FRCPC

British Columbia Children's Hospital

Department of Pediatrics/Division of Neurology – University of British Columbia

K3-180, 4480 Oak Street

Vancouver, British Columbia, V6H 3V4

CANADA

Telephone: 604-875-2345 ext. 5904/ Facsimile: 604-875-2285

E-mail address: smiller6@cw.bc.ca

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ABSTRACT

Objective

The association of chorioamnionitis and non-cystic white matter injury, a common brain injury in premature newborns, remains controversial. Our objectives were to determine the association of chorioamnionitis and postnatal risk factors with white matter injury, and the effects of chorioamnionitis on early brain development, using advanced MR imaging.

Methods

Ninety-two preterm newborns (24-32 weeks gestation) were studied at a median age of 31.9 weeks and again at 40.3 weeks gestation. Histopathological chorioamnionitis and white matter injury were scored using validated systems. Measures of brain metabolism (N-acetylaspartate/choline and lactate/choline) on magnetic resonance spectroscopy, and microstructure (average diffusivity and fractional anisotropy) on diffusion tensor imaging were calculated from predefined brain regions.

Results

Thirty-one newborns (34%) were exposed to histopathological chorioamnionitis, and 26 (28%) had white matter injury. Histopathological chorioamnionitis was not associated with an increased risk of white matter injury (relative risk: 1.2; P=0.6). Newborns with postnatal infections and hypotension requiring therapy were at higher risk of white matter injury (P<0.03). Adjusting for age at scan and regions of interest, histopathological chorioamnionitis did not significantly affect brain metabolic and microstructural development (P>0.1). In contrast, white matter injury was associated with

lower N-acetylaspartate/choline (–8.9%; P=0.009) and lower white matter fractional

anisotropy (–11.9%; P=0.01).

Interpretation

Histopathological chorioamnionitis does not appear to be associated with an

increased risk of white matter injury on magnetic resonance imaging or with

abnormalities of brain development. In contrast, postnatal infections and hypotension are

associated with an increased risk of white matter injury in the premature newborn.

INTRODUCTION

Cystic periventricular leukomalacia (PVL) is an important pattern of brain injury in the premature newborn. A meta-analysis found that both clinical and histopathological chorioamnionitis were significantly associated with cystic PVL.[1] However, the findings were inconsistent among the studies summarized [1], and the most recent prospective cohort study failed to show an association between histopathological chorioamnionitis and PVL, or brain volumes at term in newborns delivered prematurely.[2]

Over the last decade, the incidence of cystic PVL in premature newborns has decreased dramatically. In contrast, with the increasing use of magnetic resonance (MR) imaging, focal or multifocal non-cystic white matter injury (WMI) is increasingly recognized as the most prevalent pattern of brain injury in premature newborns.[3] The severity of WMI, best visualized with MRI, is associated with adverse neurodevelopmental outcome at 12-18 months of age.[4, 5] The MR signal changes that characterize this form of WMI (i.e. multifocal WMI) are most easily recognized in the first weeks of life, becoming harder to detect near term-equivalent age. Unfortunately, the risk factors of this spectrum of WMI are poorly understood. Experimental evidence suggests that specific developmentally regulated cell populations prevalent in the white matter of premature newborns, such as late oligodendrocyte progenitor cells and subplate neurons, are vulnerable to oxidative stress and inflammation.[6] To date, studies have not addressed the association of histopathological chorioamnionitis and WMI on early-life MRI, the time at which WMI is most readily apparent. In order to develop strategies for preventing WMI, it is critical to determine whether prenatal factors, such as

chorioamnionitis, or postnatal factors, such as hypotension, play significant roles in the pathogenesis of WMI.

The use of advanced MR techniques such as MR spectroscopic imaging (MRSI) and diffusion tensor imaging (DTI) in the first weeks of life now allows the examination of brain development and injury,[7] at a time closer to *in utero* exposures, and prior to the development of later neonatal co-morbidities such as chronic lung disease. MRSI measures the concentration of biochemical compounds such as N-acetylaspartate (NAA), choline, and lactate, reflectors of regional brain metabolism.[8] DTI characterizes the three-dimensional spatial distribution of water diffusion in each voxel of the MR image, providing an indirect measure of microstructural development.[9-11] With maturation, average diffusivity (D_{AV}) decreases presumably due to developing neuronal and glial cell membranes restricting water diffusion.[9-11] However, fractional anisotropy (FA), the directionality of diffusion, increases with white matter maturation, reflecting the maturation of the oligodendrocyte lineage and early events of myelination.[10, 12]

The objectives of this prospective cohort study of premature newborns uniformly studied with placental pathology and advanced MR techniques were to determine: (i) the association of chorioamnionitis and early postnatal risk factors with WMI, (ii) the effects of chorioamnionitis on early brain development as measured by MRSI and DTI. We hypothesized that both chorioamnionitis and postnatal hypotension are important risk factors for WMI, and that exposure to chorioamnionitis would be associated with abnormalities in early brain metabolic and microstructural development.

MATERIAL AND METHODS

Study Population

This study was approved by the *University of British Columbia Clinical Research Ethics Board* and informed consent was obtained from the parent or legal caregiver of each newborn. The study population consisted of a prospective cohort of preterm newborns born at *Children's & Women's Health Centre of British Columbia*, the provincial tertiary-level neonatal referral center, from April 2006 to June 2008. The newborns were eligible if they were delivered between 24–32 weeks gestation. Exclusion criteria comprised: (1) clinical evidence of a congenital malformation or syndrome, (2) antenatal infections, or (3) ultrasound evidence of a large parenchymal hemorrhagic infarction (>2 cm).[13] Of the eligible newborns, 96 (58%) consented to participate in this study: enrolled newborns were slightly younger (median: 28 versus 29 weeks; P<0.001) and had lower birthweight (median: 1045 versus 1268 g; P<0.001).

Placental Histopathology

Every placenta was sent fresh to the Pathology Department for macroscopic and microscopic analysis. In addition to gross examination, a minimum of 4 sections were submitted for histopathological examination: reflected membranes, distal and proximal umbilical cord, fetal and maternal surfaces of placenta, and full thickness section. Any grossly identified pathology was also sampled. In the case of multiple gestations, the routine sections were submitted for each infant, in addition to sections of the separating membranes. All tissue sections were fixed in formalin and processed with tissue sections of 3−5 microns thickness cut and stained with hematoxylin and eosin. An experienced placental pathologist (DM) assessed the degree of placental inflammation and scored for

stage (extent) and grade (severity) of both the maternal and fetal inflammatory responses, using the scale proposed by Redline et al.[14] For the maternal and fetal inflammatory responses, the stage ranges from early to advanced (score 1 to 3) and the grade from mild/moderate (1) to severe (2), according to specific histopathological features.[14] Additional findings (e.g. infarcts, vascular malformations) were noted.

Magnetic resonance imaging studies

All newborns were scanned without pharmacological sedation using an MR-compatible isolette (Lammers Medical Technology) and specialized neonatal head coil (Advanced Imaging Research) as soon as they were clinically stable; this provides the shortest interval between *in utero* exposure and occurrence of focal non-cystic WMI which is most readily apparent on early scans.[4] The newborns were scanned again at term-equivalent age. The majority of MR scans were of diagnostic quality: T2-weighted images from one term-equivalent scan were excluded for motion. One newborn had its scan repeated as the first attempt was of inadequate quality.

MRI studies were carried out on a Siemens 1.5 Tesla *Avanto* using VB 13A software and included the following sequences: 3D coronal volumetric T_1-weighted images (TR 36/ TE 9.2/ FOV 200 mm/ Slice thickness 1 mm/ No gap), and axial fast spin echo T_2-weighted images (TR 4610/ TE 107/ FOV 160/ Slice thickness 4 mm/ Gap 0.2 mm).

An experienced neuroradiologist (KP) reviewed the images blinded to the newborn's medical history and placental pathology. WMI was defined as foci of abnormal white matter T_1 hyperintensity in the absence of marked T_2 hypointensity, or by low-intensity T_1 foci (cysts).[4] The severity of WMI was scored as minimal, moderate, or

severe, using a system found to be predictive of adverse neurodevelopmental outcome 12-18 months of age.[4] Intraventricular hemorrhage, ventriculomegaly, and cerebellar hemorrhage were also noted.[4, 13] Twenty random scans were re-scored: intra-observer reliability was comparable to that previously reported for these scores (Kappa>0.9).[4]

Proton Magnetic Resonance Spectroscopic Imaging

Magnetic resonance spectroscopic imaging was acquired using multivoxel chemical shift imaging (CSI) (TR 1500/ TE 144/ Averaging 4). The volume of interest [50x50x10mm(thick)] was placed at 2 levels of the brain: high centrum semi-ovale just above the body of the lateral ventricles (to exclude cerebrospinal fluid) and basal ganglia at the level of the foramen of Monro (Figure 1). All spectra were analyzed off-line by a single observer with voxels (6x6x10mm) centered bilaterally on eight predefined anatomical regions (Figure 1). As reported previously, only voxels with adequate signal to noise and fully included in the volume of interest were considered for statistical analyses (>90%). [7] The mean NAA/choline and lactate/choline ratios were calculated bilaterally for each region of interest. Using Bland Altman analyses,[15] intra-rater reliability assessed in 15 scans was high: NAA/choline mean difference <0.001 (limits of agreement: −0.03 to 0.03) and lactate/choline mean difference −0.006 (limits of agreement: −0.06 to 0.05).

Diffusion tensor imaging

Diffusion tensor imaging was acquired with a multirepetition, single-shot echo planar sequence with 12 gradient directions (TR 4900/ TE 104/ FOV 160 mm/ Slice thickness 3 mm/ No gap), and three averages of two diffusion weightings of 600 and 700 seconds per square millimeter (b value) and an image without diffusion weighting,

resulting in an in-plane resolution of 1.3 mm.[16] The diffusion tensor describes an

ellipsoid in space, with size, shape, and orientation given by the "maximum",

"intermediate" and "minimum" eigenvalues and their corresponding eigenvectors. The

average diffusivity (D_{AV}) reflects the mean of these eigenvalues, expressed as 10^{-3}

mm^2/sec, whereas the fractional anisotropy (FA) reflects their variance. Parametric maps

for D_{AV} and FA were generated with values of these DTI parameters calculated by a

single observer bilaterally from each of the seven white matter regions as reported

previously (Figure 2).[16] Using Bland Altman analyses,[15] intra-rater reliability assessed in

15 scans was high: D_{AV} mean difference 0.003 (limits of agreement: –0.05 to 0.05) and

FA mean difference –0.001 (limits of agreement: –0.02 to 0.02).

Clinical data collection

Demographic data and clinical variables were collected systematically.[4, 17]

Prolonged premature rupture of membrane (ROM) was defined as ROM >18 hours.

Clinical chorioamnionitis was defined as a combination of two of: (1) uterine tenderness,

(2) maternal and fetal tachycardia, (3) foul-smelling amniotic fluid, (4) maternal fever

and leucocytosis in the absence of identified infectious source. Definite episodes of

necrotizing enterocolitis (NEC) prior to the first scan were recorded using Bell's

criteria.[18] Postnatal infections prior to the first scan were considered present when

cultures were positive in the blood, urine, or cerebrospinal fluid, or if ≥4 white blood

cells were found in the tracheal aspirates associated with clinical pneumonia. In the

absence of a universally accepted definition,[19] newborns were considered to have

hypotension prior to the first scan if they were treated with saline boluses or vasopressors

for low blood pressure. Clinical cranial ultrasound was interpreted using standard criteria

for cystic PVL.[20] The clinical condition of the newborns at term equivalent age was described using a neuromotor score as well as respiratory support and feeding route. The neuromotor score, previously found to predict adverse neurodevelopmental outcomes when used at this age, summarizes tone, power, and cranial nerve function, ranging from normal (0) to quadriparesis (5).[4]

Data Analysis

Statistical analysis was performed using Stata 9.2 software (Stata Corporation, College Station, Texas) and R.[21] Clinical characteristics of the newborns were compared using Fisher's exact test and Mann-Whitney U test for categorical and continuous data respectively. Relative risks with 95% confidence intervals were calculated to measure the univariate association between histopathological chorioamnionitis and WMI. Logistic regression for repeated measures (generalized estimating equation) was used to account for twin pairing and evaluate the association of histopathological chorioamnionitis and clinical variables with WMI. A linear mixed-effects model, accounting for twin gestation, multiple regions of interest in each infant, and adjusting for postmenstrual age at MRI scan, was used to compare mean values of ratios of NAA and lactate to choline, and D_{AV} and FA. The correlation between regions within newborns was described using an unstructured covariance matrix. A random effect for twin pairs was assigned to account for intra-class correlation. A log-transformed outcome variable was used in all regressions to determine the percent differences of the MR measures in newborns with and without chorioamnionitis or WMI.

RESULTS

Clinical characteristics of the newborns

Of the 96 preterm newborns studied, 92 had placental pathology and are described here. Forty-four were born from twin gestations. Newborns were delivered at a median GA of 27.8 weeks (interquartile range: 26.3–29.7 weeks) and scanned at a median postmenstrual age of 31.9 weeks (IQR: 30.1–33.4 weeks). Seventy-seven newborns were scanned again at "term-equivalent" age [median of 40.3 weeks (IQR: 38.7–42.6 weeks)].

Chorioamnionitis

Chorioamnionitis was diagnosed histopathologically in 31 (33.7%) newborns: 13 mild and 18 moderate/severe. The grade and stage of the maternal and fetal inflammatory responses were closely related. When discordant (N=12), the maternal inflammatory scores were always higher. Funisitis was seen in 16 newborns (17%). All 6 newborns with clinical chorioamnionitis had histopathological chorioamnionitis: 3 mild and 3 moderate/severe.

Compared to newborns without histopathological chorioamnionitis, those with chorioamnionitis were more likely to be female, have received antenatal antibiotics, and less likely to have pregnancy-induced hypertension (Table 1). While newborns with chorioamnionitis were scanned at a slightly earlier postmenstrual age, the number of days between birth and MRI was not significantly different (P=0.1).

White matter injury and chorioamnionitis

WMI was found in 26 (28.3%) newborns: 7 mild, 12 moderate, and 7 severe. Cystic PVL was seen on clinical head ultrasound in 3 newborns.

Exposure to chorioamnionitis was not associated with an increased risk of WMI [Relative Risk: 1.2 (95% Confidence Interval: 0.6 to 2.4); P=0.6], with an absolute risk difference of 6%. When comparing newborns with moderate to severe WMI to the others in the cohort, histopathological chorioamnionitis was not a significant risk factor [RR: 1.1 (95% CI: 0.5 to 2.6); P=0.8] and the absolute risk difference dropped to 3%. Of the three children with cystic PVL, only one had histopathological chorioamnionitis (severe).

The stage and grade of both the maternal and fetal inflammatory responses were similar in the newborns with and without WMI of any severity (P>0.1) and in newborns with and without moderate to severe WMI (P>0.5). Funisitis was not a significant risk factor for WMI of any severity or of moderate/severe WMI (P>0.1). Of the 6 newborns with clinical chorioamnionitis, only one had WMI.

White matter injury on the first MRI and early postnatal risk factors

Compared to newborns without WMI, those with WMI were less likely to have pregnancy-induced hypertension and C-section delivery, and more likely to have postnatal hypotension requiring intervention (Table 2). Among the 19 newborns exposed to pregnancy-induced hypertension, only one had WMI; 11 of 19 were treated with magnesium sulfate before delivery (P<0.001). Newborns with and without WMI did not differ significantly with respect to hyaline membrane disease (88%/79%), patent ductus arteriosus (58%/41%), and definite necrotizing enterocolitis (11%/3%) prior to the first scan (all P>0.1). Culture-positive postnatal infections prior to the first MRI were more common in newborns with WMI (Table 2). Most of these infections were from staphylococcus species (21); only 3 newborns were infected with Candida, 3 with Escherichia coli, or 2 with others organisms. The occurrence of chronic lung disease was

not associated with WMI on the first scan (44%/41%; P=0.8). Two newborns with WMI

(8%) were conceived by *in vitro* fertilization, compared to 10 (15%) without WMI

(P=0.5). Of the 22 twin pairs in this cohort, 12 (27%) newborns had WMI with only one

twin pair in which both newborns were affected.

In a multivariable model accounting for twin pairs and adjusting for GA at birth,

postnatal hypotension requiring intervention was significantly associated with an

increased odds of WMI [Odds Ratio: 4.7 (95% CI: 1.6 to 13.6); P=0.004] while

histopathological chorioamnionitis was not [OR: 1.6 (95% CI: 0.4 to 4.0); P=0.3]. The

effects of hypotension or chorioamnionitis were not meaningfully affected by adding

either C-section delivery or pregnancy-induced hypertension to the model. Again

accounting for twin pairs and adjusting for GA at birth, postnatal infection prior to the

first scan was associated with an increased odds of WMI [OR: 4.8 (95% CI: 1.6 to 14.1);

P=0.005]. The effect of postnatal infection is attenuated [OR: 3.9 (95% CI: 1.2 to 12.1);

P=0.02] when hypotension is added to this model [OR: 4.0 (95%CI: 1.4 to 11.5);

P=0.01].

Early brain development: chorioamnionitis and white matter injury

Accounting for twin pairs and adjusting for age at scan and regions of interest,

histopathological chorioamnionitis was not significantly associated with NAA/choline or

lactate/choline (Table 3a). When restricting the analysis to only the three white matter

MRSI regions of interest, the effect of histopathological chorioamnionitis on the

metabolite ratios was similar (P>0.2). In a similar model, histopathological

chorioamnionitis was not significantly associated with white matter FA and D_{AV} values.

The effect of chorioamnionitis on the MRSI and DTI measures did not differ significantly

across the regions of interest; no significant interaction is observed between

chorioamnionitis and regions of interest for these measures (F-test P=0.08–0.7 for

interaction terms).

Accounting for twin pairs and adjusting for age at scan, regions of interest, and

histopathological chorioamnionitis, WMI was associated with lower NAA/choline but not

lactate/choline (Table 3b). In a similar model, WMI was significantly associated with

lower FA but not D_{AV} (Table 3b).

Term-equivalent brain development and injury: effect of chorioamnionitis

Histopathological chorioamnionitis was not significantly associated with white

matter injury on the term-equivalent scan (RR=0.7; 95% CI=0.2 to 1.9; P=0.6).

Accounting for twin pairs and adjusting for age at scan and regions of interest,

histopathological chorioamnionitis was not significantly associated with NAA/choline or

lactate/choline ratios (both P=0.6), or with white matter FA (P=0.4) and D_{AV} (P>0.9) on

the term-equivalent scan.

Term-equivalent brain injury: effect of postnatal infection

On the term-equivalent scan, exposure to culture-positive postnatal infections

increased the risk of white matter injury, although this effect was not statistically

significant [RR=2.2; 95% CI=0.9 to 5.2; P=0.08).

Outcomes

All newborns in this cohort survived to term-equivalent age with clinical outcomes available in 83 (90%). Neonatal outcomes were similar in newborns with and without histopathological chorioamnionitis (Table 1). In contrast, the newborns with WMI had higher neuromotor scores (i.e. were neurologically more impaired) than the newborns without WMI (Table 2).

DISCUSSION

Chorioamnionitis and white matter injury

In a prospective cohort of premature newborns uniformly assessed with placental pathology and early-life MRI, we find that histopathological chorioamnionitis does not significantly increase the risk of non-cystic white matter injury or abnormalities of brain development in early life or at term-equivalent age. The severity of the maternal and fetal inflammatory responses did not distinguish newborns with and without WMI. To our knowledge, our study is the first to address the association of histopathological chorioamnionitis with brain abnormalities on MRI, including advanced MR techniques, at the time focal non-cystic WMI is most readily apparent.[4, 17, 22]

Many studies have examined the association of chorioamnionitis with cystic PVL in premature newborns, the most severe pattern of white matter injury.[1, 2] While most individual reports failed to detect an association between chorioamnionitis and cystic PVL, the most recent meta-analysis found both clinical and histopathological chorioamnionitis to be independent risk factors (relative risk of 3.0 and 2.1 respectively).[1] These authors highlight that the findings from individual studies were often conflicting because of heterogeneous methodologies used to detect chorioamnionitis and brain injury, and suggest the need to adjust for pregnancy-induced hypertension when examining this association. In our cohort, chorioamnionitis was not associated with WMI even after adjusting for pregnancy-induced hypertension. In most studies exposure to chorioamnionitis was diagnosed clinically rather than with placental pathology.[2, 23-26] Our study highlights the difficulty of clinical diagnosis as only 6 of 31 newborns with histopathological chorioamnionitis met clinical chorioamnionitis criteria. Furthermore,

most previous studies defined cystic PVL using cranial ultrasonography.[1] [2] Given the dramatic decline in PVL, and the increasing recognition of WMI on MRI, our failure to detect an association of histopathological chorioamnionitis with the broader spectrum of WMI is relevant to contemporary cohorts. This is important as WMI on MRI is now recognized as an important predictor of early motor and cognitive deficits.[4, 5] Our findings are also consistent with the most recent cohort study where histopathological chorioamnionitis was neither a significant risk for cystic PVL on ultrasound nor smaller brain volumes on MRI at term-equivalent age.

Early brain development: chorioamnionitis and white matter injury

In our study, histopathological chorioamnionitis was not associated with abnormalities of early brain metabolic or microstructural development. These findings persist to at least term-equivalent age and build on the recent observation that chorioamnionitis does not impact brain volumes.[2] These data suggest that exposure to chorioamnionitis does not make the premature brain more vulnerable to WMI. In contrast, WMI is associated with early and widespread abnormalities of brain development. This is the first demonstration of the *early* widespread abnormalities associated with focal WMI lesions on MRI. This finding extends previous observations that newborns with WMI are at higher risk for abnormalities of microstructural[10, 27] and metabolic[28] brain development, and have smaller brain volumes at term-equivalent age.[29-31] These findings stress the need to prevent or treat WMI in its earliest phase.

Early postnatal factors for white matter injury

In our cohort, and consistent with earlier reports, hypotension requiring intervention and neonatal infections increased the risk of WMI.[32-35] The increased risk of

early WMI with postnatal infection is consistent with the observation that recurrent

postnatal sepsis is a risk for progressive WMI.[17] The increased risk of early WMI with

postnatal infection is attenuated by adjusting for hypotension, suggesting that

hypotension may be a mechanism by which neonatal infection impacts the brain. It is

important to note that our definition of hypotension cannot distinguish the risk of

hypotension *per se* from that of its therapy with saline boluses or vasopressors.[36, 37]

Recently, chorioamnionitis has been shown to increase the risk of hypotension in very

low birthweight infants on the first postnatal day of life.[38] Systemic inflammation impairs

cerebrovascular autoregulation[39] and potentiates hypoxic-ischemic insults.[40, 41] While,

preterm newborns with WMI have markers of intrauterine T-cell activation and increased

pro-inflammatory cytokines [42, 43], early serum measures of inflammation were not

associated with adverse neurodevelopmental outcome in this population.[44] Given the

limitations of current technologies to measure the brain inflammatory response *in vivo*,

cytokine measures were not obtained in our cohort. Our results suggest that

chorioamnionitis alone is not associated with WMI. Future studies are warranted to

clarify how systemic inflammation, *in utero* and postnatally, may cause or interact with

hypoxia-ischemia in the genesis of WMI.

Limitations

While the sample size of our cohort may preclude the detection of small

differences in the risk of WMI between the newborns with and without chorioamnionitis,

we found no difference in MR measures of early brain development. Although the rate of

WMI in our study is similar to that reported by others, [4, 45, 46] the early MR scans in this

cohort may have been obtained prior to the full extent of brain abnormalities related to

chorioamnionitis being evident. However, no significant association of chorioamnionitis

on our measures of brain injury and development were seen at term-equivalent age.

Technical limitations of MRSI and DTI may have also precluded detecting a small effect

of chorioamnionitis on these brain development measures. The slice thickness of the

MRSI may have resulted in some partial volume averaging of unintended structures in

the regions of interest. However, these regions' size compare favorably with other

neonatal MRSI studies[47] and are considerably smaller than prior single-voxel MRS

studies.[8, 28] A region-of-interest-based approach was used for DTI given the profound

changes in brain size and shape over the time period studied, and to our knowledge, tract-

based spatial statistic approaches have not yet been validated across this age range. The

DTI reliability measures also compare favorably with previous tract-based DTI

measures.[48] The effect of WMI on NAA/choline and FA also suggests any unmeasured

effect of chorioamnionitis related to these technical issues would be of lesser magnitude.

The association between histopathological chorioamnionitis and neurodevelopmental

outcome will be examined as this cohort is followed through childhood.

Conclusions

Our data suggest that neonatal infection and hypotension requiring intervention are more significant risk factors for white matter injury than *in utero* exposure to chorioamnionitis. Importantly, the risk of white matter injury is not directly related to the severity of the fetal and maternal inflammatory responses. Furthermore, while chorioamnionitis is not associated with early abnormalities in brain development, white matter injury is. Clinical trials are needed to determine whether the prevention or treatment of postnatal hypotension reduces the burden of white matter injury in this vulnerable population.

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Figure 1 Proton magnetic resonance imaging and regions of interest

Figure 1 shows the magnetic resonance (MR) spectroscopic imaging and the 8 regions of interest that were analyzed in a premature newborn with a normal MR imaging and born at $26^{+6/7}$ weeks gestation and scanned at $29^{+3/7}$ weeks postmenstrual age, at the level of (A) the high centrum semi-ovale and (B) the basal ganglia. The values of each region were averaged bilaterally: high white matter [(1) anterior, (2) central, and (3) posterior], (4) caudate, (5) lentiform nuclei, (6) thalamus, (7) optic radiations, (8) calcarine region. The spectrum of the right thalamus is shown in C.

Legend: Cho=Choline; Cr=Creatine; NAA=N-acetylaspartate; Lac=Lactate.

Figure 2 Diffusion tensor imaging and regions of interest

Figure 2 shows the axial diffusion tensor imaging encoded anisotropy color map and the 7 regions of interest that were analyzed in a premature newborn with a normal MR imaging and born at $26^{+6/7}$ weeks gestation and scanned at $29^{+3/7}$ weeks postmenstrual age, at (A) the high centrum semi-ovale and (B) the basal ganglia. The values of each region were averaged bilaterally: high white matter [(1) anterior, (2) central, (3) posterior], (4) genu of the corpus callosum, (5) posterior limb of the internal capsule, (6) splenium of the corpus callosum, and (7) optic radiations. The color convention used to display the predominant diffusion direction has red representing right–left, green representing anterior-posterior, and blue representing superior-inferior anatomical directions.[16, 49]

Table 1 Demographic and clinical characteristics of the newborns with and without

histopathological chorioamnionitis (univariate analysis)

Number (%) or Median (interquartile range)	Histopathological chorioamnionitis		
	Yes	**No**	**P**
Number	**31 (34%)**	**61 (66%)**	
Male sex	9 (29%)	35 (57%)	0.02
GA at birth (weeks)	27.3 (26.3-28.1)	28.6 (26.0-30.0)	0.1
PMA at first scan (weeks)	30.3 (29.4-31.4)	32.4 (31.3-34.0)	<0.001
Birth weight (grams)	1035 (810-1235)	1095 (835-1285)	0.9
Head circumference (cm)	25.3 (23.5-26.0)	26.1 (24.1-28.0)	0.2
PIH	0	19 (31%)	0.001
Antenatal antibiotics	27 (87%)	32 (53%)	0.001
Antenatal steroids	23 (74%)	37 (62%)	0.3
C-section delivery	15 (48%)	38 (62%)	0.3
SNAP-PE	24 (9-40)	18 (9-36)	0.6
Definite NEC	1 (3%)	4 (7%)	0.7
Culture positive postnatal infection	8 (26%)	21 (34%)	0.5
Neonatal hypotension	9 (29%)	20 (33%)	0.8
Brain abnormalities			
WMI	10 (32%)	16 (26%)	0.6
IVH	11 (35%)	29 (48%)	0.4
Ventriculomegaly	10 (32%)	13 (21%)	0.5

Cerebellar hemorrhage	6 (19%)	4 (7%)	0.08
Outcome* at term equivalent-age			
GA at assessment (weeks)	40.3 (38.0-41.3)	40.3 (39.1-42.6)	0.5
Neuromotor score	1 (0-2)	1 (0-2)	0.5
NG feeds	9	18	0.4
CPAP	1	4	>0.9

GA=Gestational age

PMA=Postmenstrual age

PIH=Pregnancy-induced hypertension

ROM=Rupture of membranes

SNAP-PE=Score of neonatal acute physiology- perinatal extension

NEC=Necrotizing enterocolitis

WMI=White matter injury

IVH=Intraventricular hemorrhage

NG=Nasogastric

CPAP=Continuous positive airway pressure

*Neonatal outcomes available for 90% of the cohort

Table 2 Demographic and clinical characteristics of the newborns with and without white matter injury on the first scan (univariate analysis)

Number (%) or Median (interquartile range)	White matter injury		
	Yes	No	P
Number	**26 (28%)**	**66 (72%)**	
Male sex	11 (42%)	33 (50%)	0.6
GA at birth (weeks)	27.9 (26.4-29.7)	27.6 (26.2-29.7)	0.9
PMA at first scan (weeks)	31.6 (30.1-32.4)	32.0 (30.1-33.6)	0.6
Birth weight (grams)	1150 (910-1330)	1045 (813-1230)	0.2
Head circumference (cm)	25.5 (25.0-28.0)	25.3 (23.5-27.0)	0.2
PIH	1 (4%)	18 (27%)	0.03
Antenatal antibiotics	16 (62%)	43 (65%)	>0.9
Antenatal steroids	16 (62%)	44 (67%)	0.8
C-section delivery	10 (38%)	43 (65%)	0.03
SNAP-PE	26 (15-57)	18 (9-32)	0.1
Definite NEC	3 (11%)	2 (3%)	0.1
Culture positive postnatal infection	13 (50%)	16 (24%)	0.03
Neonatal hypotension	14 (54%)	15 (23%)	0.006
Histopathological chorioamnionitis	10 (38%)	21 (32%)	0.6
Other brain abnormalities			
IVH	14 (54%)	26 (39%)	0.2

Ventriculomegaly	11 (42%)	12 (18%)	0.03
Cerebellar hemorrhage	4 (15%)	6 (9%)	0.5
Outcome* at term-equivalent age			
GA at assessment	40.4 (38.6-43.0)	40.3 (38.7-42.3)	>0.9
Neuromotor score	2 (2-3)	1 (0-1)	<0.001
NG feeds	5	18	0.1
CPAP	1	4	0.3

GA=Gestational age

PMA=Postmenstrual age

PIH=Pregnancy-induced hypertension

ROM=Rupture of membranes

SNAP-PE=Score of neonatal acute physiology- perinatal extension

NEC=Necrotizing enterocolitis

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*Neonatal outcomes available for 90% of the cohort

Table 3 Comparison of metabolic and microstructural brain development on the early

MR scan

A. Newborns without and with histopathological chorioamnionitis

Mean	Without histopathological chorioamnionitis *	With histopathological chorioamnionitis *	Adjusted difference ** % (95% CI)	P
MRSI				
NAA/choline	0.450	0.435	–3.8% (–10.9 to +3.9)	0.3
Lactate/choline	0.158	0.148	–12.6% (–26.2 to +3.5)	0.1
DTI				
D_{AV}	1.431	1.446	+1.1% (–5.3 to +7.9)	0.7
FA	0.270	0.284	+5.4% (–4.9 to 16.8)	0.3

B. Newborns without and with white matter injury

Mean	Without white matter injury *	With white matter injury *	Adjusted difference ** % (95% CI)	P
MRSI				
NAA/choline	0.448	0.408	–8.9% (–14.9 to –2.6)	0.009
Lactate/choline	0.159	0.150	–9.9% (–21.9 to +8.7)	0.3
DTI				
D_{AV}	1.418	1.488	+2.8% (–1.0 to +6.8)	0.1
FA	0.282	0.251	–11.9% (–20.0 to –2.9)	0.01

* Mean values on the early scan across all brain regions, adjusted for gestational age at

scan

**Analyses were adjusted for gestational age at the time of early MRI, and accounted for

multiple brain regions in an individual newborn and twin pairs

95% CI=95% confidence interval

MRSI=Magnetic resonance spectroscopic imaging

DTI=Diffusion tensor imaging

NAA=N-acetylaspartate
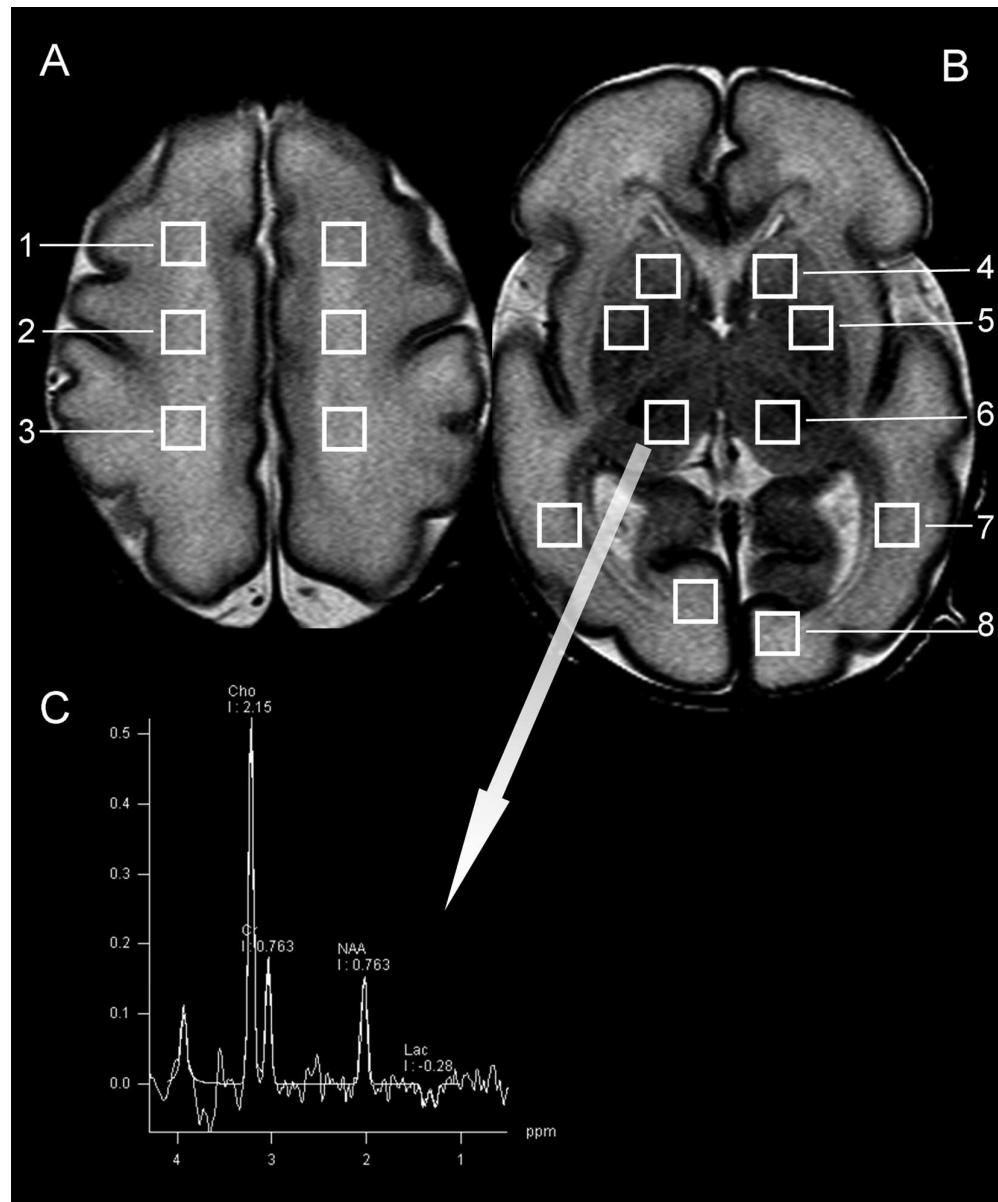
D_{AV}=Average diffusivity

FA=Fractional anisotropy

Figure 1 Proton magnetic resonance imaging and regions of interest
Figure 1 shows the magnetic resonance (MR) spectroscopic imaging and the 8 regions of interest that were analyzed in a premature newborn with a normal MR imaging and born at 26+6/7 weeks gestation and scanned at 29+3/7 weeks postmenstrual age, at the level of (A) the high centrum semi-ovale and (B) the basal ganglia. The values of each region were averaged bilaterally: high white matter [(1) anterior, (2) central, and (3) posterior], (4) caudate, (5) lentiform nuclei, (6) thalamus, (7) optic radiations, (8) calcarine region. The spectrum of the right thalamus is shown in C.
Legend: Cho=Choline; Cr=Creatine; NAA=N-acetylaspartate; Lac=Lactate.
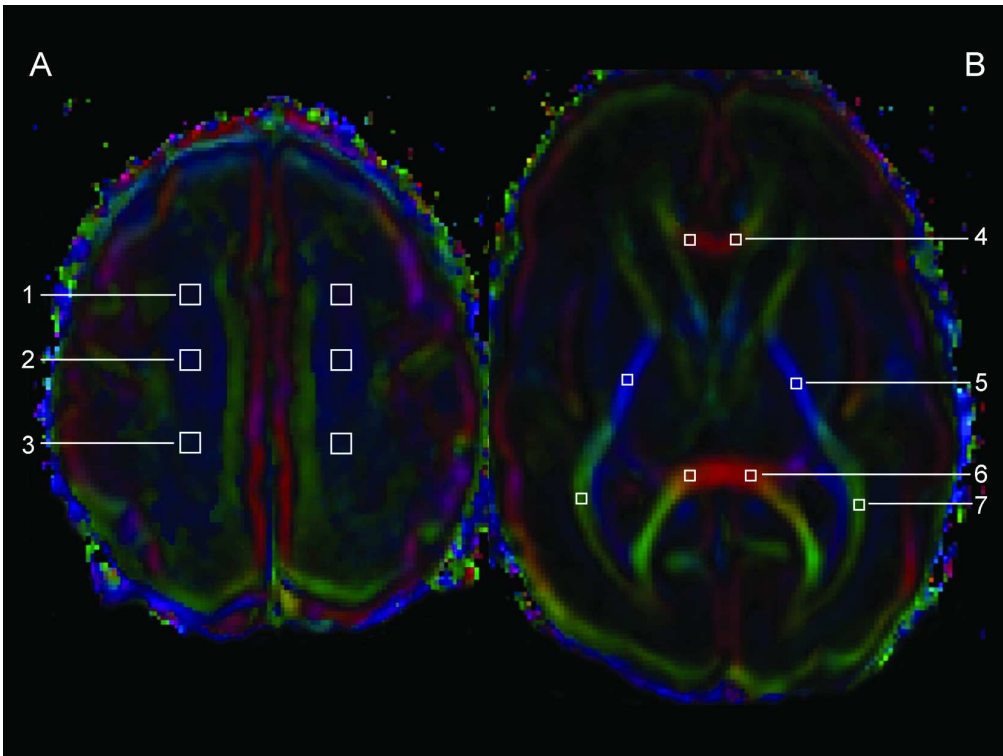70x84mm (600 x 600 DPI)

Figure 2 Diffusion tensor imaging and regions of interest
Figure 2 shows the axial diffusion tensor imaging encoded anisotropy color map and the 7 regions of interest that were analyzed in a premature newborn with a normal MR imaging and born at 26+6/7 weeks gestation and scanned at 29+3/7 weeks postmenstrual age, at (A) the high centrum semi-ovale and (B) the basal ganglia. The values of each region were averaged bilaterally: high white matter [(1) anterior, (2) central, (3) posterior], (4) genu of the corpus callosum, (5) posterior limb of the internal capsule, (6) splenium of the corpus callosum, and (7) optic radiations. The color convention used to display the predominant diffusion direction has red representing right–left, green representing anterior-posterior, and blue representing superior-inferior anatomical directions.
70x52mm (600 x 600 DPI)