PERIVENTRICULAR WHITE MATTER INJURY IN THE PREMATURE INFANT IS FOLLOWED BY REDUCED CEREBRAL CORTICAL GRAY MATTER VOLUME AT TERM


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Running Title: White Matter Injury and Cerebral Cortical Development

ABSTRACT

Periventricular white matter injury, i.e., periventricular leukomalacia (PVL), the dominant form of brain injury in the premature infant, is the major neuropathological substrate associated with the motor and cognitive deficits observed later in such infants. The nature of the relationship of this lesion to the subsequent cognitive deficits is unclear, but such deficits raise the possibility of cerebral cortical neuronal dysfunction. Although cortical neuronal necrosis is not a prominent feature of brain injury in premature infants, the possibility of a deleterious effect of PVL on subsequent cerebral cortical development has not been investigated. An advanced quantitative volumetric 3D-MRI technique was used to measure brain tissue volumes at term in premature infants with earlier ultrasonographic and MRI evidence of periventricular leukomalacia (PVL) (mean gestational age (GA) at birth 28.7± 2.0 weeks, n=10), in premature infants with normal imaging studies (mean GA at birth 29.0± 2.1 weeks, n=10), and in control term infants (n=14). Premature infants with PVL had a marked reduction in cerebral cortical gray matter at term when compared with either premature infants without PVL or normal term infants (mean ± sd: PVL, 157.5 ± 41.5 cc; no PVL, 211.7 ± 25.4 cc; normal term, 218.8 ± 21.3 cc, p<0.001). As expected, a reduction in the volume of total brain myelinated white matter was also noted (mean ± sd: PVL, 14.5 ± 4.6 cc; no PVL, 23.1 ± 6.9 cc; normal term, 27.6 ± 10.3 cc, p=0.002). An apparent compensatory increase in total CSF volume also was found (mean ± sd: PVL, 64.5 ± 15.2 cc; no PVL, 52.0 ± 24.1 cc; normal term, 32.9 ± 13.5 cc, p<0.001). PVL in the premature infant is shown for the first time to be followed by impaired cerebral cortical development. These
findings may provide insight into the anatomical correlate for the intellectual deficits associated with PVL in the premature infant.

Key words: prematurity, periventricular leukomalacia, cerebral cortical development, volumetric MRI

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Periventricular leukomalacia (PVL) has become the dominant form of brain injury in the premature infant, and, indeed, is the major neuropathological substrate associated with the motor and cognitive deficits observed later in such infants. PVL consists of two components, i.e., focal necrosis with loss of all cellular elements deep in the periventricular white matter, and less severe but more diffuse white matter involvement characterized principally by injury to glial cells, presumed to be oligodendroglial precursors. The pathogenesis of this lesion is considered to be related to ischemia, maternal/fetal infection or a combination of such factors. An understanding of the effects of PVL on subsequent cerebral development has been limited, but qualitative evidence for a subsequent impairment in myelination has been obtained by conventional imaging studies. Many premature infants with PVL who survive the neonatal period later exhibit a variety of cognitive deficits. Such deficits suggest the possibility of cerebral cortical impairment, although neuropathological studies have not shown evidence of prominent cortical neuronal injury. However, the possibility that the PVL could lead to subsequent impairment of cerebral cortical neuronal development, suggested by a recent neuroanatomical study, has not been investigated in the living infant. The principal reason for this lack of investigation has been the difficulties inherent in the use of currently available qualitative imaging modalities.

The objective of this study was to use advanced quantitative volumetric 3D-MRI techniques to assess the impact of PVL on subsequent cerebral development in three groups of infants at term: premature infants with and without MR evidence of PVL (n=10 in each group) and a group of healthy term infants (n=14). Because of the basic nature of the lesion, we hypothesized that PVL in the premature infant would result in a reduction in cerebral myelin volume at term. However, we aimed particularly to
determine whether PVL would result in an alteration of cerebral **cortical** volume at term.

**SUBJECTS AND METHODS**

*Subjects*

Premature infants from the neonatal intensive care unit at the Brigham and Women’s Hospital were selected consecutively for the study if gestational age were less than 32 weeks, the infants were able to undergo initial MR imaging and volumetric study within the first 14 days of life by no longer requiring ventilatory support, and after parental consent was obtained. The initial MR study was undertaken in all premature infants within the first 16 days of life. All infants also received cranial ultrasound scans during the first 72 hours of life, and at 7, 28 and 42 days of age. A full repeat MR scan protocol was undertaken between 39 and 41 weeks gestational age. Healthy control term infants (n=14) born between 39 - 41 weeks gestational age were selected from the postnatal wards to undergo MR scanning, after parental consent. These infants had their MRI scans in the first 2 days of life. Thus, quantitative volumetric analysis was carried out at term in the 20 premature and the 14 control term infants.

Ten premature infants on their initial MR scan had periventricular white matter abnormalities consistent with PVL; these abnormalities were characterized by diffuse or nodular periventricular hyperintensities on T2-weighted images (n=9), and/or cystic or cavitary lesions (n=5). Seven of these 10 infants also had abnormalities in the periventricular white matter on cranial ultrasound scans at some stage in their neonatal course (5 infants with multiple small echolucencies, 2 infants with transient echogenicities in the periventricular white matter). Three infants with diffuse periventricular abnormalities on MRI displayed only mild ventriculomegaly on cranial ultrasound scans undertaken between the third and tenth weeks of life.

Ten premature infants had no abnormality in the periventricular white matter (no PVL) on early or term MR imaging or on any ultrasound scans. Three of these 10 infants did have evidence of small germinal matrix hemorrhage on the initial ultrasound scans. There was no ventriculomegaly on cranial ultrasound scans at any time in this group of 10 infants.

The mean gestational age and birthweights of the two groups of premature infants were not significantly different (PVL: 28.7 ± 2.0 weeks, 1297 ± 298 grams; no PVL: 29.0 ± 2.1 weeks, 1315 ± 375 grams, p>0.5). The mean gestational age of the term infants was 40.2 ± 0.6 weeks. Infants with evidence of PVL had lower Apgar scores (PVL: 7/10 with 1 min score <5 and 4/10 with 5 min score <6; no PVL: 9/10 with 1 and 5 min scores >7, p<0.05), and greater use of inotropes (PVL: 6/10 had dopamine >48 hours; no PVL: 2/10 had dopamine >48 hours, p=0.01).

The study was approved by the institutional Human Subjects Research Committee. Informed consent was obtained from the infants’ parents. No sedation was necessary for the MR studies. The infants were positioned in the MR magnet in a vacuum fixation-pillow and monitored by electrocardiography and pulse oximetry (MR-Equipment Corp, Bay Shore, NY). A neonatologist remained with the infant in the scanner room for the entire MR study.

**MR Image Acquisition**

MRI scanning was performed with a 1.5 Tesla General Electric Signa System (GE-Medical Systems, Milwaukee, WI). For the acquisition of the primary MR data two different imaging modes were applied:
a three-dimensional (3D) Fourier transform spoiled gradient recalled (SPGR) sequence (1.5-mm coronal slices; flip angle, 45E; repetition time, 35msec; echo time, 5msec; field of view, 18cm; matrix, 256 x 256) and a double-echo (proton density [PD] and T2-weighted) spin-echo sequence (DE) (3-mm axial slices; repetition time, 3,000 msec; echo times, 36 and 162 msec; field of view 18cm; matrix 256 x 256, interleaved acquisition). The voxel (volume of the pixel) dimensions for the SPGR acquisition were 0.7 x 0.7 x 1.5 mm, and for the spin-echo acquisition 0.7 x 0.7 x 3.0 mm.

**MR Image Processing**

Postacquistion processing was carried out on workstations (Sun Microsystems, Mountain View, CA) with newly developed software. A sequence of image processing algorithms was used to segment each of the MRI slices into separate tissue classes: cortical gray matter, basal ganglia/thalamus, unmyelinated white matter, myelinated white matter, and cerebrospinal fluid (CSF). These algorithms were designed to reduce imaging system noise, identify a linear transformation to align the DE spin-echo images with the SPGR images to form a three-channel data set, resample the DE spin-echo images according to this transform, classify tissue types on the basis of the MR intensity in the three channels, using a template moderated classification which adds to the statistical classification algorithm (knn classification) an anatomical template, registered to the initial classification. This approach allows classification of tissue not only according to signal behavior but also according to anatomical localization. With this technique anatomically different structures with similar image acquisition characteristics (pixel intensity) can be classified correctly (i.e., subcortical gray matter vs cortical gray matter).

**Statistical analysis**

Statistical analyses were performed to compare the volume of cerebral tissues between the three groups by using analysis of variance (ANOVA, one-way) with pairwise multiple comparison procedures. To isolate the group or groups that differ from the others a multiple comparison procedures using the Bonferroni t-test or Dunn’s method for non-parametric data were performed. Analyses between cystic and diffuse PVL (see later) and the analyses of perinatal variables were carried out using t-test or Kruskall-Wallis.

**RESULTS**

Quantitative volumetric analysis was carried out at term in 10 premature infants with PVL, 10 premature infants without PVL and 14 healthy term infants. Determinations of individual volumes of gray matter (cortical, subcortical), white matter (unmyelinated and myelinated), and CSF were carried out, as detailed next.

**Gray Matter Volumes: Total, Cortical, and Subcortical**

Premature infants who had evidence of PVL had a marked reduction in cerebral cortical gray matter volume at term when compared with both premature infants without PVL and control term infants (Table 1, Figure 1, Bonferroni F=14.25, p<0.001; PVL vs no PVL, mean vol=54 cc, t=4.15, p<0.05; PVL vs Term, mean vol=61 cc, t=5.1, p<0.05; no PVL vs Term, mean vol=7 cc, t=0.59, NS). Cerebral cortical gray matter volume at term was reduced 28% in the premature infants with PVL relative to the volume in normal term infants. Of the 10 infants with MRI findings of PVL, the five with findings consistent with cystic PVL had significantly lower cortical gray matter volumes when compared with the five infants with MRI findings consistent with diffuse PVL only (mean ± sd: cystic with or without
diffuse white matter lesions [n=5], 125.9 ± 28.5 cc; diffuse white matter lesions only [n=5]: 189.2 ±22.6; t=3.89, p=0.005). Four of the five infants with cystic white matter lesions also had MR evidence of diffuse white matter injury. Although the premature infants with evidence of cystic white matter lesions had the lowest cortical gray matter volumes, the premature infants with evidence of only diffuse white matter lesions still had significantly lower cortical gray matter volumes at term compared to control term infants (Mann-Whitney T=27.00, p=0.03).

Subcortical gray matter volumes, i.e., basal ganglia and thalami, were not significantly different between the three groups (Table 1). Because nearly 90% of total gray matter volume at this age is composed of the cerebral cortical gray matter volume, there was a reduction in total gray matter volume in the premature infants with PVL of a similar magnitude to the reduction in cerebral cortical volume (Table 1, Dunn’s method H=13.17, p=0.001; PVL vs no PVL, median vol=63 cc, Q=3.0, p<0.05; PVL vs Term, median vol=66 cc, Q=3.33, p<0.05; no PVL vs Term, median vol=3 cc, Q=0.3, NS).

**White Matter Volumes: Unmyelinated and Myelinated**

There was a significant reduction in the volume of total brain myelin in the premature infants with evidence of PVL at term when compared to the volumes in the premature infants with no PVL and in control term infants (Table 1, Dunn’s method H=12.55, p=0.002; PVL vs no PVL, median vol=6.34 cc, Q=2.56, p<0.05; PVL vs Term, median vol=11.2 cc, Q=3.44, p<0.05; no PVL vs Term, median vol=4.9 cc, Q=0.7, NS). Myelin volume was reduced 47% in the premature infants with PVL relative to the myelin volume in control term infants. There was no difference in myelin volumes between infants with cystic or diffuse PVL (cystic PVL [n=5] 14.2±4.9 cc; diffuse PVL [n=5] 14.7±4.8 cc, t=0.16, p=0.9).

By contrast, the volumes of unmyelinated white matter did not differ between the three groups (Table 1). Unmyelinated white matter accounted for 41% of intracranial volume in the PVL group, 40% of total intracranial volume in the no PVL group and 40% of intracranial volume in the term group. Because approximately 90% of total white matter volume is composed of unmyelinated white matter volume, there were no significant differences between total white matter volumes between the three groups.

**Cerebrospinal Fluid Volumes**

Premature infants who had evidence of PVL had a marked increase in CSF volume at term when compared with healthy term infants (Table 1, Bonferroni F=9.69, p<0.001; PVL vs Term, mean vol=31.6 cc, t=4.3, p<0.05). Premature infants with no PVL also had a significantly greater CSF volume at term than did control term infants (no PVL vs Term, mean vol=19.1 cc, t=2.6, NS). The higher CSF volume in the premature infants with PVL versus those without PVL did not reach statistical significance (PVL vs no PVL, mean vol=12.5 cc, t=1.6, NS).

**Qualitative Analysis**

A qualitative impression of the quantitative data defined above is gained by depiction of representative coronal SPGR (Figure 2) and axial T2-weighted (Figure 3) MR images in a premature infant without PVL at 31 weeks, a healthy term infant, a premature infant with PVL at term, and a premature infant without PVL at term. The increase in myelination between 31 weeks (Figure 2A) and term (Figure 2B) is clearly apparent. However, one can also appreciate qualitatively the reduction in myelination at term in the premature infant with PVL (Figure 2C) in comparison to the premature infant without PVL (Figure 2D) and the healthy term infant (Figure 2B). A striking increase in cerebral gyral development is
apparent between the healthy premature infant at 31 weeks (Figure 3A) and the healthy term infant (Figure 3B). With an awareness of the quantitative data just described, the subtle difference in cerebral gyral development between the premature infant with PVL (Figure 3C) and both the healthy term infant (Figure 3B) and the premature infant without PVL at term can be seen (Figure 3D).

**DISCUSSION**

This study documents for the first time in-vivo evidence for an impairment of cerebral cortical gray matter development, at term, in premature infants with evidence of PVL, by the use of the technique of 3D quantitative volumetric MRI with tissue segmentation. The findings have implications concerning the impact of PVL in the premature infant on cerebral cortical gray matter development and on the promise of this technique in the study in vivo of normal and abnormal brain development. Concerning the value of these advanced MRI techniques, we have quantitated previously the sequence of normal volumetric maturation of the major regions of human brain between 29 and 41 weeks gestation.\(^{14}\) Postacquisition MR processing techniques, using tissue segmentation methods with 3D renderings, earlier had been applied in adults and older infants to provide quantification of volumetric changes.\(^{18-26}\) Limited prior studies of a small number of newborns had not provided specific quantitation of cortical gray matter or myelinated versus unmyelinated white matter.\(^{24}\) Brain volumes recorded in our current study in the control term infants lie within the normal ranges reported in our previous study,\(^{14}\) confirming the utility of this technique as a non-observer biased method of quantitation of brain regions in-vivo in a manner not previously possible with conventional imaging techniques.

This study documents for the first time a reduction in cerebral cortical gray matter volume at term in premature infants with PVL. This reduction occurred in the absence of any MRI evidence for neuronal necrosis, and this negative finding is consistent with the lack of neuropathological evidence for major cortical neuronal necrosis in PVL.\(^{3, 4, 11-13}\) The subtle nature of the impairment in gyral development in the premature infants with PVL observable by conventional MRI emphasizes the value of the 3D-MRI technique in detection and quantitation of these differences. The reduction in cortical gray matter volume raises the possibility that the PVL was causally related to a disturbance in subsequent cortical neuronal development. Our previous study\(^{14}\) showed that there is a four-fold increase in cortical gray matter volume from 30 to 40 weeks gestational age. This increase is linked closely with the marked increase in cerebral gyral development during this period (see Figure 3). At least two possibilities can be raised concerning the question of how PVL might cause a developmental disturbance of cerebral cortex. Firstly, Marin-Padilla utilized specialized histopathological methods to detect distinct alterations in the morphology and organization of neurons in the cerebral cortex overlying PVL.\(^{13}\) These neuronal alterations were postulated to result from destruction of the corticopetal, corticofugal, and association fibers, thereby resulting in input deprivation and output isolation of the overlying gray matter and, as a consequence, impaired neuronal differentiation.\(^{13}\) Secondly, theoretical modeling and experimental observations suggest that fiber development in cerebral white matter is crucial to gyral development.\(^{27}\) With PVL such "tension-based morphogenesis" might be impaired and thereby alter cerebral cortical development. These two mechanisms are not mutually exclusive. However regardless of the mechanism(s), the alteration in cerebral cortical gray matter volume documented in this study may provide insight into the established association of PVL with deficits in cognitive functions.

This study also documents a reduction in the volume of brain myelin in the premature infants with PVL. Impairment of cerebral myelination subsequent to the occurrence of PVL and presumably secondary to\(^{3, 4, 28, 29}\)
both axonal and oligodendroglial injury has been suggested by neuropathological studies\textsuperscript{3, 4, 28, 29} and conventional brain imaging.\textsuperscript{6, 7, 30-38} However, because cerebral myelination is principally a post term event, subsequent studies of myelin development will be required to delineate the full impact of PVL on this crucial process.

In conclusion, the findings in this study demonstrate a major reduction in cerebral cortical gray matter volume at term in premature infants with PVL. These findings may provide insight into the anatomical correlate for the intellectual deficits associated with PVL in the premature infant.

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Table 1. Quantitative 3D-MRI Volumes of Cerebral Tissues at Term in Premature Infants with Evidence of PVL, Premature Infants with no Evidence of PVL and Healthy Term Infants.

<table>
<thead>
<tr>
<th></th>
<th>PREMATURE WITH PVL, AT TERM (n=10)</th>
<th>PREMATURE WITH PVL, AT TERM (n=10)</th>
<th>NORMAL TERM (n=14)</th>
<th>p-VALUE ANOVA</th>
<th>PAIRWISE MULTIPLE COMPARISONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortical gray matter</td>
<td>157.5±41.5</td>
<td>211.7±25.4</td>
<td>218.8±21.3</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>Basal ganglia/thalamus</td>
<td>22.5±7.6</td>
<td>29.7±14.2</td>
<td>24.7±9.1</td>
<td>Not significant</td>
<td></td>
</tr>
<tr>
<td>Total gray matter</td>
<td>180.1±49.5</td>
<td>241±39.6</td>
<td>243±30.4</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Myelinated white matter</td>
<td>14.5±4.6</td>
<td>23.1±6.9</td>
<td>27.6±10.3</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Unmyelinated white matter</td>
<td>178.3±33.9</td>
<td>212.8±28.4</td>
<td>200.3±32.9</td>
<td>Not significant</td>
<td></td>
</tr>
<tr>
<td>Total white matter</td>
<td>192.8±38.9</td>
<td>235.9±35.3</td>
<td>227.9±43.7</td>
<td>Not significant</td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>64.5±15.2</td>
<td>52.0±24.1</td>
<td>32.9±13.5</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Values are volumes in cc and are means ± SD.

3D-MRI= three-dimentional magnetic resonance imaging
PVL= periventricular leukomalacia
ANOVA= analysis of variance
CSF= cerebrospinal fluid

FIGURE LEGENDS
Figure 1. Cerebral cortical gray matter volumes at term in premature infants with (n=10) and without (n=10) evidence of PVL and in normal term infants (n=14) [expressed as medians, with 25th/75th centile box, 10th/90th centile error bars, and outliers]

Figure 2. Coronal SPGR MR images from (A) a premature infant at 31 weeks postconceptional age, (B) a healthy term infant, (C) a premature infant with PVL at term, and (D) a premature infant without PVL at term.

Figure 3. Axial T2 weighted MR images from (A) a premature infant at 31 weeks postconceptional age, (B) a healthy term infant, (C) a premature infant with PVL at term, and (D) a premature infant without PVL at term.
PVL at term.

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