Brain injury in the preterm infant: Unexpected mechanisms of dysmyelination and cortical dysmaturation

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Is Perinatal White Matter Injury (WMI) a Static Lesion?

- ~62,000 Premature infants born in the U.S. each year < 1500g
- ~15% Develop Cerebral Palsy
  
  Hack et al., JAMA 294:318-325, 2005
- >40% of children with CP have WMI
  
  Bax et al., JAMA 296: 1602-8, 2006
- 25-50% with Cognitive Disabilities
  
  Litt et al., J Learn Disabil 8:130-141, 2005
- Intrinsic glial regeneration and repair mechanisms
Macroscopic PVL (Cystic Necrotic)
Changing Spectrum of MRI-defined WMI

- Cystic PVL < 5%
  
  Counsell et al., Pediatrics 112:176-180, 2003
  Hamrick et al., J Pediatr 145:593-599, 2004
  Inder et al., AJNR 24:805-809, 2003
  Miller et al., AJNR 24:1661-1669, 2003

- Noncystic focal or diffuse WMI predominates.

- MRI does not detect microcysts—Burden of microscopic necrosis is unclear

- Decreased cortical and subcortical gray matter volume in preterm survivors at term

- Co-morbid with WMI related to H-I or IVH
Spectrum of Chronic WMI without Cystic PVL?

Focal Cystic Necrosis

- Pan-cellular degeneration
- Lipid-Laden Macrophages

Diffuse White Matter Gliosis

- Reactive Astrocytes
- Reactive Microglia
- PreOL death
- ?Microscopic Necrosis
- ?Axonal Degeneration
- ?Myelination Failure
# Chronic Diffuse WMI Case Selection

## Retrospective Archival Cases
(formalin/paraffin, 1983-2000)
- 27 potential WMI cases (UBC)

## Pathological Diagnosis by H&E
- 17 cases with confirmed WMI
- 11 cases excluded
  - 6 cystic-necrotic PVL
  - 2 stillbirth
  - 1 IVH with extension into WM
  - 1 microhemorrhage with WM thrombi

## Diagnosis confirmed by independent quantification of WM gliosis (GFAP) in 27 cases
- cohort (17 WMI, 10 con; Table S1)

## 10 age and region matched controls identified (OHSU)

## Prospectively Collected Cases
(PFA/Frozen, 2003-2010)
- 25 potential WMI cases
  - 15 NYMC, 10 OHSU

## Pathological Diagnosis by H&E
- 12 cases with confirmed WMI (6 NYMC, 6 OHSU)
- 6 cases excluded (5 NYMC, 1 OHSU)
  - 3 age not meeting criteria (23, 28, 58 wks)
  - 2 insufficient tissue quality
  - 1 hemorrhagic necrosis in WM

## Diagnosis confirmed by independent quantification of WM gliosis (GFAP) in 27 cases
- cohort (12 WMI, 7 con; Table S14)

## 7 age and region matched controls with no WMI identified from cohort (4 NYMC, 3 OHSU)

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*Buser et al., Ann Neurol 71:93-109, 2012*
Quantification of Astrogliosis

Spectrum of Necrosis in Chronic PVL

**Macroscopic PVL (~8%)**
- Focal cystic necrosis (> 2-5 mm; Detected by MRI)

**Microscopic Necrosis (~35%)**
- Microcysts (<1mm)— overall burden is unclear

**Diffuse White Matter Gliosis (~40%)**
- Reactive astrogliosis and microgliosis
- Occurs in isolation or overlaps with necrosis

- Diffuse Gliosis +/- microscopic necrosis occurs in > 80% of WMI.

Haynes et al., *Pediatr Res* 63, 656-661, 2008
Burden of Necrosis is Declining in Human WMI

- Compared retrospective archival cases (1983-2000) vs. prospective contemporary cases (2003-2010)

- Necrosis defined by H & E staining was confirmed by quantifying reactive astrocytes and microglia.

  - Overall burden of necrosis 10-fold lower in contemporary cases (p<0.007)

  - Incidence of necrosis significantly lower in contemporary cases (p=0.02)

Buser et al., Ann Neurol 71:93-109, 2012
Microscopic Necrosis is Declining

- 59% of retrospective cohort vs. 36% of contemporary cases vs. 34% in PVL study (Pierson, 2007)
  - Mean area was low in retrospective (0.4 ± 0.6 mm²) and contemporary cohorts (0.2 ± 0.5 mm²)
  - 2.5 ± 2.2 % of the total area of white matter injury
- Three axonopathy markers were restricted to microcysts.
What is the mechanism of myelination failure?

Does myelination failure in chronic WMI arise from selective loss preOLs that are required to generate mature OLs and myelin?

Back and Volpe, MRDD Research Reviews, 1998
PreOL Death In Early WMI

- PreOLs are selectively susceptible to oxidative damage and are markedly depleted in acute human WMI.

*Annals of Neurology* 58:108-120, 2005
The Paradox of Myelination Failure

Since hypoxia-ischemia results in selective but partial loss of preOLs in acute WMI,

Why don’t surviving preOLs differentiate to generate myelin in chronic lesions?
Quantification of Human Oligodendrocyte Lineage Cells in Chronic WMI Lesions

- Olig 2 is a nuclear transcription factor that identifies cells from all stages of the oligodendrocyte lineage.

- Double-labeling for GFAP and Olig2 allows confirmation that analysis was done within lesions with gliosis.
Chronic WMI triggers proliferation of Human OL lineage cells beyond the boundaries of gliosis

Human pre-OLs are elevated in chronic lesions with diffuse white matter gliosis.
Human pre-OLs are elevated in chronic lesions with diffuse white matter gliosis

- PreOLs significantly increased in human chronic WMI lesions
- Percentage of oligodendrocytes reduced in lesions
- PreOL maturation arrest significantly associated with the magnitude of astrogliosis (p<0.005)
Mechanism of Myelination Failure for Diffuse White Matter Injury without Cystic PVL

Focal Cystic Necrosis
- Pan-cellular degeneration
- Lipid-Laden Macrophages

Diffuse White Matter Injury
- Reactive Astrocytes
- Reactive Microglia
- PreOL Maturation Arrest
- +/- Microscopic Necrosis
- +/- Axonal Degeneration

Diffuse white matter injury with preOL maturation arrest was the major form of WMI identified.
Role of Diffuse Gliosis in Chronic WMI

- Chronic WMI leads to formation of a glial scar.
- Glial scar accumulates Hyaluronic Acid (HA) and its receptor CD44.
- CD44 was significantly associated with GFAP as an independent marker of astrogliosis.

Myelination Failure in the Glial Scar

- Glial scar expresses high CD44
- CD44 high lesions have reduced myelination.
- Glial scar is high in Hyaluronic Acid (HA)
Hyaluronic Acid Blocks PreOL Differentiation to Mature OLs

Back et al., Nature Medicine 11:966-972, 2005
Role of the Glial Scar: Hyaluronic Acid Blocks Remyelination

- HA
+HA

Back et al., Nature Medicine 11:966-972, 2005
Working Model: Pathogenesis of Myelination Failure in Chronic WMI
Is there a role for high field MRI for more sensitive early WMI Detection?

- Focal or diffuse non-cystic MR signal abnormalities
- MRI shows irregular walls of ventricles and T2 signal abnormalities.
- DWI signal abnormalities seen in ~70% of preterm survivors at term
  Pediatrics 112:176-180, 2003
- DTI detects advanced lesions missed by conventional MRI
Immature (0.65 gestation) Fetal Sheep Global Ischemia Model

- Widely used preclinical model (Hypothermia!)
- Brain development similar to ~24-28 week human premature infant
- Limited cerebral autoregulation
- Reversible bilateral carotid occlusion
- Monitor fetal heart rate, blood pressure, blood gases

*Riddle et al., Journal of Neuroscience, 26:3045-3055, 2006*
Timing of Human and Fetal Sheep White Matter Maturation and Myelination

Chronic WMI retards WM Growth

A Segmentation

B

Volume (mm$^3$)

Control

Ischemia

31.1 ± 1.8 g

25.3 ± 2.8 g

p < 0.01
High Field (12 T) ex vivo MRI-Defined Chronic WMI ($T_2$)

A  Control  1 wk

B  Ischemia  D-hypo
     D-hypo

C  Ischemia  F-hyper
     D-hypo

D  Control  2 wk

E  Ischemia  D-hyper
     F-hypo

F  Ischemia  F-hyper
     D-hyper
     D-hypo
F-Hypo Lesions are Microcysts
Histopathology of MRI-defined Lesions

Microscopic Necrosis (Microcysts)
- Hypo-intense on T2W at 2 weeks
- 50% of animals but 1.5% of total lesion volume
- Microglia markedly increased by 2 weeks
- Depleted of astrocytes, OLs, axons at 2 weeks
Focal Necrotic Gyral White Matter Injury (PVL)

- Hyper-intense on T$_2$W at 1 and 2 weeks; FA$^{\text{low}}$; ADC$^{\text{hi}}$
- 11% of total lesion volume at 1 week; 16% at 2 weeks
  - Astrocytes modestly increased at 1 week
- Marked microglial enrichment; Astrocyte/Axon loss at 2 weeks
Spectrum of Chronic WMI: Focal Necrosis and Microcysts with Axonopathy
Astrolgliosis in D-Hypo Lesions

A

NeuN

4mm

B

T2w

4mm

C

MRI-based lesion ROIs

4mm

D

GFAP

4mm

E

GFAP

400μm

F

GFAP

100μm
Histopathology of MRI-defined Lesions

**Diffuse White Matter Gliosis**

- Hypo-intense on T\(_2\)W at 1 week
- 89% of total lesion volume at 1 week; 82% at 2 weeks
  - Astrocyte-enriched at 1 and 2 weeks
  - Microglia modestly increased by 2 weeks
Does Axonal Injury Occur in Diffuse WMI Defined by High-Field MRI in Fetal Sheep?

- EM study of 16 animals with 1 or 2 week survival after H-I.
- WMI defined by 12T MRI and analyzed by EM found normal:
  - Axon Density
  - Axon Calibers
  - Axonal Degeneration

Riddle et al., *Stroke* 43: 178-184, 2012
MRI (T₂W) is highly sensitive to clinically-significant astrogliotic lesions

<table>
<thead>
<tr>
<th>Lesion Size</th>
<th>1 week</th>
<th>2 week</th>
<th>p-value 1 vs 2 week</th>
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<tbody>
<tr>
<td>Large Lesions &gt; 2.5mm³</td>
<td>100%</td>
<td>75%</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.007</td>
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<td>Small Lesions &lt; 2.5mm³</td>
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- Large Lesions > 97% of Total at 1 and 2 weeks
• Novel registration algorithms identified three major forms of WMI by MRI with distinct histopathological signatures.

• $T_2W$ identified early diffuse astrogliosis with preOL arrest.

• $T_2W$ identified focal microscopic necrosis at sub-millimeter resolution

• $T_2W$ and ADC identified focal macroscopic necrosis

• Up to 100% sensitive and 92% specific for astrogliotic lesions $>2.5 \text{ mm}^3$
High Field (12T) Ex Vivo Imaging: Role of Fixation

- $T_2$ hypointense MRI artifacts have been identified in tissue preserved for years in formalin
  
  *van Duijn et al., Magn Reson Med, 2011*

- Tissue in our study fixed for 24 hours in buffered paraformaldehyde (PFA) followed by long-term storage in PBS.

- Fixation in PFA optimized detection of oligodendrocyte progenitors populations and other neural precursors
High Field (12T) Ex Vivo Imaging of T2W-Contrast

T2 values decrease ex vivo, but retain in vivo contrast patterns between gray and white matter and lesion vs. control

- T2w diffuse hypo-intense lesions that correspond to astrogliosis were not well-defined at 3 T

- Imaging modalities that maximize sensitivity to paramagnetic susceptibility, such as T2*-weighting, may enhance the contrast of lesions at lower field strengths.

- Recent study of MS lesions demonstrated T2 hypo-intensities related to T2* contrast at 7 T.

  *Pitt et al., Arch Neurol. 2010; 67(7): 812-818.*
**MRI (T₂W) is highly sensitive to clinically-significant astrogliotic lesions**

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**p < 0.001**

**p < 0.007**

- Large Lesions > 97% of Total at 1 and 2 weeks
Does PreOL Maturation Arrest Expand the Window of Developmental Vulnerability for WMI?

- Recurrent hypoxia-ischemia (rH-I) is common in critically-ill preterm infants.
- Oligodendrocyte maturation is accompanied by increased resistance to H-I.
- As the preterm brain matures, do preOL-rich chronic white matter lesions retain persistent susceptibility to rH-I?
Recurrent H-I (rH-I) Rat Model
Recurrent Hypoxia-Ischemia Triggers Massive Caspase-Dependent PreOL Death in Chronic Lesions
PreOL Maturation Arrest Confers Enhanced Susceptibility to Recurrent Hypoxia-Ischemia

Total Cells

Degenerating Cells

Caspase+ Cells

[Graphs showing data for Total Cells, Degenerating Cells, and Caspase+ Cells across different time points (P0, P3, P7) for HI and Control groups.]

* p < 0.05
** p < 0.01
*** p < 0.001
Registration of MRI and Histopathology

Registration Requirements

Serial sections through entire tissue block

Multiple antibody-labeling

- remove confounders due to alignment to a marker of interest

High-resolution montage of all histopathological specimens

- allow accurate quantification of cellular and molecular targets
OL lineage expands and undergoes maturation arrest in diffuse white matter gliosis

- PreOLs increase ~2-fold rather than undergo their normal decline
- Oligodendrocyte differentiation fails to progress: OLs are ~2-fold higher in controls
PreOL Maturation Arrest is Associated with the Magnitude of Astrogliosis

Riddle et al., Ann Neurol 70: 493-507 (2011)