Perinatal Infection and Brain Injury

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Colonization of second trimester placentas

Biopsy of the chorion from 1,083 placentas (preterm labor) before the 28th week.

<table>
<thead>
<tr>
<th>Initiator of Delivery</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm Labor (cesarean)</td>
<td>56%</td>
<td>62%</td>
<td>42%</td>
<td>44%</td>
<td>34%</td>
</tr>
</tbody>
</table>

Onderdonk and the ELGAN study group Am J Obstet Gynecol 2008

Objective

- To present a balanced viewpoint of the evidence both supporting a role for infection/inflammation in causation of brain injury and against that relationship.
Case History

Baby Patrick is a 1390g male infant born following a 29 week gestation. The pregnancy was complicated by bacterial vaginosis. There was PPROM at a gestational age of 27 weeks. The mother received a full course of dexamethasone. Approximately 12 hours before delivery, the mother developed fever and uterine tenderness. Antibiotics were started.

The infant delivered vaginally in the cephalic position. The Apgar scores were 7 at one minute and 8 at five minutes. The infant exhibited mild respiratory distress and was placed on CPAP and supplemental oxygen for 2 days. In addition, the received a sepsis workup and was treated with antibiotics for 7 days. The blood culture was negative and the chest x-ray was normal.

The only pertinent clinical finding was recurrent apnea and bradycardia requiring theophylline. The infant was never hypotensive. Placental pathology was consistent with acute and chronic chorioamnionitis. An ultrasound scan was obtained on days 3, 14 & 28.
Why did this Infant Develop Brain Injury?

**Prevalence of Cerebral Palsy**

<table>
<thead>
<tr>
<th>Birth Weight (g)</th>
<th>Gestational Age (wks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>Term</td>
</tr>
<tr>
<td>240</td>
<td></td>
</tr>
<tr>
<td>230</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

OR for gestational age <32 weeks: 70.62 (CI 34.38-145.04)

CP Mean BW 2.538 Kg, mean GA 35.3 weeks

**Neonatal Morbidity/Mortality**

UAB (2000-2001)

- RDS
- IVH
- NEC
- Sepsis
- Survival

% survival vs Gestational Age (Weeks):

- 23
- 25
- 27
- 29
- 31
- 33
- 35
- >37
**Etiology of Cerebral Palsy**

- Hypoxia-Ischemia (3-20%)
- *Intrauterine infection* (28%)
- Congenital malformations (30-50%)
- Postnatal events

**Pathogenesis of PVL**


Progressive neuronal injury
Low dose lipopolysaccharide selectively sensitizes hypoxia-ischemia induced white matter injury in the immature brain

Oligodendrocyte Precursors

Day 2 rat pups injected with low dose endotoxin (0.05 mg/kg) or saline and 3 hours later subjected to hypoxia-ischemia


Lipopolysaccharide Preconditioning Reduces Neuroinflammation

LPS greatly reduced macrophage activation, TNF-α expression and reactive oxygen species production

Rat pups injected with low dose endotoxin (0.05 mg/kg) or saline 24 hours before hypoxia/ischemia


Endotoxin (+ Ischemia) Disturbs Fetal Hemodynamics

Arterial Blood Pressure

Asphyxia

LPS resulted in sustained hypotension

**Endotoxin Disturbs Fetal Oxygenation**

![Graph showing oxygen saturation in control and LPS treated fetuses.](image)


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**Endotoxin Disturbs Fetal Hemodynamics**

![Graph showing combined ventricular output in control and LPS treated placentae.](image)


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**Endotoxin Exacerbates Hypoxia Ischemia**

![Graph showing oxygen delivery in cerebrum with and without LPS.](image)

Neuropathological Correlates of Cerebral Palsy

- Focal & multifocal ischemic brain injury
- Selective neuronal necrosis
- Parasagittal cerebral injury
- Periventricular leukomalacia

Periventricular Leukomalacia

- **Focal** necrosis of deep white matter (destroying all cellular elements)
- **Diffuse** injury of central white matter (destroying preoligodendrocytes with astrogliosis and microglial infiltration (microgliosis))

PVL is often accompanied by secondary injury to cerebral cortex, thalamus, cerebellum, basal ganglia and brainstem.

Encephalopathy of Prematurity
Pathogenesis of Diffuse White Matter Injury

Differentiation of O4+ cells to O1+ cells occurs between 28 & 40 weeks

Inhibition of Pre-oligodendrocyte Maturation

Injury to Pre-oligodendrocytes
**Intrapartum & Postnatal Risk Factors for Periventricular Leukomalacia**

- Perinatal Hypoxia/Ischemia
- Systemic hypotension
- Sepsis
- PDA (retrograde flow in diastole)
- Hypocarbia
- Apnea & bradycardia

Active development of the periventricular vasculature occurs during the last 16 weeks of gestation when baseline blood flow to that region is low and autoregulation of blood flow is diminished.

**Prenatal Risk Factors for Periventricular Leukomalacia**

- Chorioamnionitis/PROM/
  - Placental Inflammation

**Pathogenesis of Periventricular Leukomalacia**

- Systemic infection/inflammation
- Prematurity
- Cytokines
- PAMPs
- Microglia
-Activated
- Glutamate
- Reperfusion
- ROS/RNS
- Pre-OL Injury
What is the Evidence that Intrauterine Infection Causes Brain Injury?

Cytokine levels are increased in cord blood in infants born to women with chorioamnionitis.

Shalak et al Pediatrics 110, 673, 2002

Cytokine concentrations in umbilical cord blood are elevated in infants who develop PVL.

Pathophysiology of Fetal Brain Injury

How do cytokines signal across the blood-brain barrier?

Cytokines May be Actively Transported Across the Blood-brain Barrier

- Cytokines are actively transported across the murine blood-brain barrier by a saturable transport mechanism

Threlkeld et al. Neuroimmunomodulation 17: 405-410 2010
Cytokines leak across the blood-brain barrier

Cytokines leak across the BBB at CVOs (Circumventricular Organs): Structures located around the ventricles that lack a complete blood-brain barrier (covered by fenestrated capillaries).

Allows them to monitor substances in the blood that normally would be excluded by the blood brain barrier (e.g., monitoring osmolality)

Intrapulmonary lipopolysaccharide exposure increases cytokine expression in the neonatal brainstem

Balan et al Acta Paediatrica pages 466-471, 11 JAN 2012

Intrapulmonary lipopolysaccharide exposure up-regulates IL-1β expression in the neonatal brainstem

Balan et al Acta Paediatrica pages 466-471, 11 JAN 2012
Antenatal Inflammation alters Postnatal Hemodynamics


Study population: 22 infants chorioamnionitis and 33 controls without chorioamnionitis.

Cytokines Alter Hemodynamics


Antenatal Inflammation & Postnatal Hemodynamics

In a prospective study by Been et al*, chorioamnionitis was associated with a lower blood pressure (most notably in infants with fetal involvement).

Does Intrauterine exposure to infection and inflammation (i.e., chorioamnionitis) predispose the fetus to increased risk of morbidity over and above that due simply to prematurity alone?

Perinatal Inflammation and Cerebral Palsy in Preterms

Shatrov et al (2010) reviewed 388 studies; 15 were abstracted:
The associations with cerebral palsy were significant.

HCA - OR 2.42. CCA - OR 1.43

Controversial

<table>
<thead>
<tr>
<th>Study identification</th>
<th>Effect size (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RK et al (2000)</td>
<td>2.96 (1.19-7.38)</td>
</tr>
<tr>
<td>Gay et al (2001)</td>
<td>1.76 (0.88-3.58)</td>
</tr>
<tr>
<td>Jacobsson et al (2002)</td>
<td>1.36 (0.69-2.68)</td>
</tr>
<tr>
<td>Wu et al (2005)</td>
<td>0.49 (0.23-1.02)</td>
</tr>
<tr>
<td>Nield et al (2005)</td>
<td>0.48 (0.27-0.85)</td>
</tr>
<tr>
<td>Chatelain et al (2007)</td>
<td>0.70 (0.29-1.66)</td>
</tr>
<tr>
<td>Skinhøj et al (2008)</td>
<td>0.66 (0.35-1.26)</td>
</tr>
<tr>
<td>Sklair et al (2009)</td>
<td>0.88 (1.83-18.48)</td>
</tr>
<tr>
<td>Overall (I-squared=70.5%; P &lt;.001)</td>
<td>2.41 (1.52-3.84)</td>
</tr>
</tbody>
</table>

Meta-analysis of the association of clinical chorioamnionitis and cerebral palsy.
Histological chorioamnionitis decreased risk of cerebral palsy
Histological chorioamnionitis increased risk of cerebral palsy

Why do some studies fail to demonstrate a relationship between inflammation & adverse outcomes?

- Increased plasma cytokine levels may be unrelated to development of cerebral palsy in preterm infants.
- Cytokine levels may have been drawn at the wrong postnatal time.
- Cytokines may only be toxic only at a critical stage of brain development.
- Plasma cytokine levels may not correlate with CNS cytokine levels
- Fetal infection/inflammation was not rigidly defined, nor differentiated from maternal infection.

Cytokine levels were drawn at the wrong postnatal time.
Inflammation at birth is associated with subnormal development

Why do some studies fail to demonstrate a relationship between inflammation & adverse outcomes?

- Cytokines may only be toxic at a critical stage of brain development.

Cytokines May Enter the Brain during a Critical Time of Altered BBB permeability

- There is a restricted period in brain development when the blood-brain barrier to proteins is susceptible to systemic inflammation.
Increased Permeability of the Blood Brain Barrier May be a Critical Variable (but not the only variable)

<table>
<thead>
<tr>
<th>Age</th>
<th>Increased permeability</th>
<th>White Matter Volume</th>
<th>Microglia No. increased</th>
</tr>
</thead>
<tbody>
<tr>
<td>P23</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>P44*</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>P51</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

- In human fetuses the density of microglia in white matter peaks during the 3rd trimester & declines after 37 weeks gestation
- * Corresponds to 20-32 weeks gestation in the human fetus

Plasma cytokine levels may not correlate with CNS cytokine levels

Why do some studies fail to demonstrate a relationship between inflammation & adverse outcomes?

- Plasma cytokine levels may not correlate with CNS cytokine levels

CSF and Plasma Cytokine levels in Infants with and without White Matter Injury

<table>
<thead>
<tr>
<th>Abnormal CUS ≤ 28 weeks</th>
<th>Adj OR (CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-6 ≥ 17 pg/ml</td>
<td>1.55 (0.7-3.4)</td>
<td>0.27</td>
</tr>
<tr>
<td>Serum IL-1β ≥ 5.4 pg/ml</td>
<td>1.65 (0.7-3.7)</td>
<td>0.216</td>
</tr>
<tr>
<td>Serum TNF-α ≥ 3 pg/ml</td>
<td>1.15 (0.5-2.8)</td>
<td>0.761</td>
</tr>
<tr>
<td>CSF IL-6 ≥ 6.5 pg/ml</td>
<td>3.0 (1.3-6.7)</td>
<td>0.007</td>
</tr>
<tr>
<td>CSF IL-1β ≥ 78 pg/ml</td>
<td>1.0 (0.43-2.2)</td>
<td>0.96</td>
</tr>
<tr>
<td>CSF TNF-α ≥ 3 pg/ml</td>
<td>3.5 (1.1-11.3)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Fetal infection/inflammation was not rigidly defined, nor differentiated from maternal infection.

Why do some studies fail to demonstrate a relationship between inflammation & adverse outcomes?

- Histological
- Biochemical
- Microbiological
- Clinical

Fetal Inflammation or Neonatal sepsis

Positive culture
Positive PCR

Polymorphonuclear infiltration of placenta, membranes and umbilical cord

Elevated cytokines or amniotic fluid

Maternal fever, tachycardia, leukocytosis, CRP, vaginal discharge, uterine tenderness, fetal tachycardia.

Fetal vasculitis (funisitis)

Perinatal Inflammation and Adverse Outcomes in Preterms

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polam-1999 (HCA)</td>
<td>177 VLBW</td>
<td>not predictive CP</td>
<td>19 months F/U</td>
</tr>
<tr>
<td>Scott-1999 (CCA)</td>
<td>71 VLBW</td>
<td>not diff. PD/MD</td>
<td>7 months F/U (CCA)</td>
</tr>
<tr>
<td>Scott-2003 (HCA, CCA)</td>
<td>154 VLBW</td>
<td>not diff. PD/MD</td>
<td>7 months F/U</td>
</tr>
<tr>
<td>Jenster et al 2003</td>
<td>170 VLBW</td>
<td>not diff. CP</td>
<td>7 months F/U</td>
</tr>
<tr>
<td>Redline–2007 (HCA)</td>
<td>129 VLBW</td>
<td>not predictive CP</td>
<td>8 year F/U</td>
</tr>
<tr>
<td>Andrews 2008 (HCA)</td>
<td>261 VLBW</td>
<td>not predictive CP</td>
<td>5-8 year F/U</td>
</tr>
<tr>
<td>Nasel 2012 (HCA &amp; CCA)</td>
<td>230 VLBW</td>
<td>not predictive of CP</td>
<td>18 months F/U</td>
</tr>
<tr>
<td>Jansel 2014 (HCA &amp; CCA)</td>
<td>258 Term</td>
<td>Decreased brain injury &amp; adverse cognitive outcomes</td>
<td>30 months F/U</td>
</tr>
</tbody>
</table>
Amniotic fluid infection, inflammation and colonization in preterm labor with intact membranes

Amniotic fluid IL-6 as a marker of inflammation was measured prospectively in 305 women with preterm labor and intact membranes. MIAC was defined by amniotic fluid culture and/or detection of 16S ribosomal DNA. Cases were categorized into 5 groups: Infection (MIAC and IL-6 ≥ 11.3 ng/ml), severe inflammation (IL-6 ≥ 11.3 ng/ml) but no MIAC, mild inflammation IL-6 2.6-11.2 ng/ml (no MIAC), colonization (MIAC and IL-6 < 2.6 ng/ml) and negative (no MIAC and IL-6 < 2.6 ng/ml).

MIAC was confirmed in 10.1%, but there was discordance between culture and PCR.

Most intra-amniotic infections are occult. Both the “infection” and “severe inflammation” had similar composite perinatal morbidity (81% & 72%) and mortality rates (19% & 19%). Colonization without inflammation was associated with rates of morbidity (21%) and mortality (0%) much lower than the “infection” and “severe inflammation” groups and not significantly from negative controls. When controlled for gestational age at delivery, all significant associations disappeared. This suggests that the major effect of inflammation is on preterm delivery.

Histological Characteristics of the Fetal Inflammatory Response Associated with Neurodevelopment Impairment

347 infants (23-28 weeks gestation) with or without funisitis had a neurodevelopmental assessment at 18-22 months (mean BW 830 grams and mean GA 26 weeks)

Placental inflammation was detected in 43% of cases.

32% of the placentas/umbilical cords had demonstrated evidence of a fetal inflammatory response.

In 26% of cases there was chorioamnionitis without funisitis

11% of placentas with intact membranes and absence of clinical chorioamnionitis had evidence of FIR

Salas et al J Pediatrics. 2013

Severe FIR (subacute necrotizing funisitis or grade 2 chorionic vasculitis) was associated with higher rates of neurodevelopmental impairment aOR 2.52 (1.11-5.72) and death aOR 2.01 (1.12-3.59)

In infants with stage 1 or 2 funisitis, the presence of chorionic vasculitis was associated with a greater risk of neurodevelopmental impairment.

Milder stages of funisitis without chorionic vasculitis was not associated with NDI

Salas et al J Pediatrics. 2013
Every placenta was biopsied and cultured (under sterile conditions).
899 ELBW infants had a neurological examination at ~24 months.
32% of the inflamed placentas did not harbor a microorganism.
39% of the non-inflamed placentas harbored a microorganism.

Recovery of 2 or more species increased the OR for ventriculomegaly, echolucent lesions, quadraparesis & diaparesis.
The ELGAN Study: Odds Ratios for Colonization ± Placental Lesions

<table>
<thead>
<tr>
<th>Organism</th>
<th>Placental Lesion</th>
<th>Ventriculomegaly</th>
<th>Echolucency</th>
<th>Q Paresis</th>
<th>Diplegia</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>2.5</td>
<td>1.7</td>
<td>1.5</td>
<td>0.8</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>0.6</td>
<td>0.7</td>
<td>0.9</td>
<td>2.8</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Only those odds ratios with statistical significance are shown in red.

Cytokines and Central Nervous System Injury

Pentoxifylline Attenuates Hypoxic-Ischemic Brain Injury

Cytokines and the Central Nervous System

- Cytokines play a key role in normal brain development (regulation of lineage commitment and cellular differentiation).
- The pattern of cytokines produced in response to injury is complex.

Inflammatory Gene Expression after Hypoxia and LPS

- Hypoxia: 148 inflammatory genes were differentially expressed
- Endotoxin (72h): 656 upregulated & 890 down regulated

The local milieu of mediators at a given time point after brain injury appears to determine whether the outcome is neuroprotective or neurotoxic.
Pathogenesis of CNS Injury with Intrauterine Inflammation

Fetal Cytokemia

Chorioamnionitis

Exposure to inflammatory mediators and/or pathogens

Preterm Birth

Arrested development or injury to pre-oligodendrocytes

Leakage of Cytokines & plasma proteins into CNS

Activation/Priming of CNS Microglia

Local production of CNS cytokines

CNS Injury

Vagus Nerve

* Restricted time in development
Current data strongly suggest (but does not prove) that inflammation/infection plays a causative role in the development of PVL in preterm infants.

The cytokine responses in the fetus and neonate are complex and interventions aimed at inhibiting a single (or a few) cytokine(s) are likely to be unsuccessful.

Identification (and treatment) of infected women at an early time point may offer the opportunity to decrease neonatal morbidity.