Infection and Neonatal Brain Injury

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Infection and Brain Damage

Chorioamnionitis

- Microbiologic
  - Identification microorganisms AF
- Clinical
  - Fever (T > 38° C)
  - ↑ WBC, fetal or maternal tachycardia, uterine tenderness, foul-smelling AF
  - 3% to 59% (very preterm)
- Histologic
  - 10% term births
  - 40%-70% PTD not preceded by labor
Complications IAI

- Maternal sepsis
- Neonatal sepsis
- Neonatal pneumonia
- Neonatal meningitis
- Neonatal death

Expanded neonatal complications

- Periventricular leukomalacia (PVL)
- Cerebral palsy
- RDS
- Broncho pulmonary dysplasia
- Neocrotizing enterocolitis

Cerebral Palsy

- Chronic disability characterized by aberrant control of movement or posture appearing early in life and not result of recognized progressive disease
- 2 (1.5-2.5) per 1,000 LBs
  - Little variation past 40 years

Cerebral Palsy

- Past – CP due to poor obstetric care/management
- Epi studies – usually antenatal timing
  - 70%-80%
- Risk Factors
  - Multiple gestation
  - Prematurity
  - Low birth weight
  - IUGR
  - Intrauterine infection
  - Maternal pyrexia
  - Thrombophilia
  - Antepartum hemorrhage
  - Congenital abnormalities
Cerebral Palsy

- Old Paradigm
  - Ischemia predominant cause CP

- New Paradigm
  - Infection plays important role in development CP

Hypothesis: Infection/Inflammation of Brain Leads to Cerebral Palsy

- Sigmund Freud – Infantile Cerebral Paralysis
  - “Diffuse cerebral sclerosis represents chronic interstitial inflammation that sometimes involves vascular wall, at other times interstitial connective tissue, and results in scar formation” (Schmars)
  - Marie 1st suggested infection remote from brain might cause CP via “products of inflammation that reached specific arterial region, radiating a chronic, damaging effect”

Association Chorioamnionitis and CP

- Eastman & DeLeon (AJOG 1955)
- Nelson & Ellenberg (Adv Neurol 1978)
  - Collaborative Perinatal Project
  - Chorio ↑ risk CP from 3 to 8/1000 LBs

Chorioamnionitis and CP: Preterm Infants

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Outcome</th>
<th>No. studies</th>
<th>Summary RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Chorio</td>
<td>CP</td>
<td>12</td>
<td>1.9 (1.5-2.5)</td>
</tr>
<tr>
<td>Clinical Chorio</td>
<td>Cystic PVL</td>
<td>7</td>
<td>2.6 (1.7-3.9)</td>
</tr>
<tr>
<td>Histologic Chorio</td>
<td>CP</td>
<td>8</td>
<td>1.5 (0.9-2.5)</td>
</tr>
<tr>
<td>Histologic Chorio</td>
<td>Cystic PVL</td>
<td>3</td>
<td>1.6 (1.0-2.5)</td>
</tr>
</tbody>
</table>

Chorioamnionitis and CP: 
Term/Near Term Infants

- Nelson & Ellenberg (Adv Neurol 1978) – Collob. Perinatal Project
  - Chorioamnionitis ↑ risk CP from 3 to 8/1000 LBs
- Grether & Nelson (JAMA 1997) – Pop. based/case-control
  - ≥ 1 indicator maternal infection
    - 3% control
    - 22% CP – OR 9.3 (3.7-23)
    - 37% spastic quadriplegia – OR 19 (6.5-56)
- Wu & Colford (JAMA 2000) – Metaanalysis
  - RR 4.7 (1.3-16.2) Clinical chorio and risk CP

Extrauterine Maternal Infection 
and Neonatal Brain Injury

- Dammon & Leviton (Semin Pediatri Neurol 1998)
  - Extrauterine infections able to initiate cascade events linking infection, labor, and neonatal brain injury
- Leviton (Dev Med Child Neurol 1993)
  - WMD associated maternal UTI
- Mays et al (Obstet Gynecol 1995)
  - Acute appendicitis assoc IVH and PVL (gest. age controlled)

Mechanisms Chorioamnionitis Cause 
Hypoxic-Ischemic Brain Injury

- ↓ O₂ transfer across inflammed placenta/membranes
- ↓ fetal/neonatal cardiac output or cerebral blood flow
- Maternal fever increases fetal temperature and O₂ needs
- Inflammation, ↑ inflammatory cytokines
- Activation coagulation factors
Evidence for Role Maternal and Intrauterine Infection in CP

- Histologic (and clinical) chorioamnionitis more common in newborns with PVL and CP
- ↑ risk PVL and CP
  - ↑ AF levels cytokines
  - ↑ umbilical cord levels IL-6
- In vitro animal models
  - Induction white matter damage (PVL) with ascending IUI (rabbits)
  - Injection LPS → WMD (kittens)
- Overexpression TNF-α and IL-6 histologic sections neonatal brains with PVL
- Funisitis strong, independent risk factor CP

Evidence for Role Maternal and Intrauterine Infection in CP

- Inflammation activates microglial cells developing brain
  - Secretion proinflammatory cytokines (IL-1β and TNF-α)
  - Secretion toxic molecules (NO, reactive O₂ species, quinolonic acid)
  - Damage adjacent neurons and glia
  - Induce apoptosis
- FIRS plays critical role pathogenesis neonatal brain damage
- Genetic susceptibility and gene polymorphisms → excessive production cytokines → ↑ risk PVL and CP

Hypothesis: Mechanism of Inflammation-Induced Cerebral Palsy

Infection Decidua
Maternal Inflammatory Response
Passage bacteria and/or maternal inflammatory Mediators across fetal membranes, AF and to fetus
FIR

Hypothesis: Mechanism of Inflammation-Induced Cerebral Palsy

FIR
mid 2nd trimester early 3rd trimester
Apoptosis immature precursors of oligodendocytes
prior onset myelinization
Periventricular Leukomalacia
Periventricular Leukomalacia

- Development lesion cerebral white matter
  - Characterized by foci necrosis white matter adjacent to lateral ventricles
- Antecedant to CP
  - Especially spastic diplegia
- Peak incidence premature infants
- Etiologic mechanisms
  - Ischemia-reperfusion
  - Release cytotoxic cytokines during infection, ischemia
  - Interaction vascular factors and coagulation factors

- PVL develops secondary loss oligodendrocytes (precursors)
  - Disrupted myelinization
- Experimentally endotoxin induces WMD
- Suggests role for cell mediators
  - Presence ↑ microglia and reactive astrocytes at sites WMD
  - Identification cytokines with toxic or trophic effects on OL survival

Infection and Development PVL

<table>
<thead>
<tr>
<th>Study</th>
<th>Maternal Infection</th>
<th>Neonatal Population</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varma et al</td>
<td>Clinical chorio</td>
<td>500-1750 g</td>
<td>↑ risk abnormal cranial US (PVL/IVH) OR 2.03 (1.4-3.3)</td>
</tr>
<tr>
<td>Zupan et al</td>
<td>Chorio &amp; PROM</td>
<td>Preterm</td>
<td>PVL 22%</td>
</tr>
<tr>
<td>Perlman et al</td>
<td>Prolonged PROM &amp; chorio</td>
<td>Preterm</td>
<td>Significant predictors PVL prolonged PROM – OR 6.6 Chorio – OR 6.8</td>
</tr>
<tr>
<td>Alexander et al</td>
<td>Clinical chorio</td>
<td>500-1500 g</td>
<td>Independent risk factors PVL OR 3.4 (1.6-7.3)</td>
</tr>
<tr>
<td>Wu &amp; Colford</td>
<td>Clinical chorio</td>
<td>Preterm</td>
<td>↑ risk cystic PVL RR 3.0 (2.2-4.0)</td>
</tr>
<tr>
<td></td>
<td>Histologic chorio</td>
<td>Preterm</td>
<td>↑ risk cystic PVL RR 2.1 (1.5-2.9)</td>
</tr>
</tbody>
</table>

PVL and Infection

- > 30 years ago – neonates with sepsis ↑ risk PVL
- Animal models
  - Endotoxin/intrauterine infection → WMD
- Chorioamnionitis in > 50% preterm infants develop PVL within 3 days of birth
  - 9.4 fold ↑ risk PVL with amnionitis (75% vs 8%)
- IL-6 (cord blood) significantly ↑ newborns with WMD
  - PVL initiated prior to birth
Pathogenesis of intrauterine infection causing cerebral palsy

- Preterm Birth
- IUI → IVH → White Matter Disease
- Cerebral Palsy

Cytokines and PVL

- Major pathologic feature white matter lesions is myelin damage
- Human fetal myelogenesis occurs predominantly 28-32 weeks
- Oligodendrocytes - source of myelin
- TNF-α and IL-1β induce death of oligodendrocytes (necrosis, ?apoptosis)
- TNF-α and IL-1β stimulate astrocytes

Pathogenesis White Matter Damage in Response to IUI

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Boundary</th>
<th>Cytokine Cascade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine cavity</td>
<td>PLACENTA</td>
<td>LPS → Cytokines (AF)</td>
</tr>
<tr>
<td>Fetal Systemic Circulation</td>
<td>BLOOD BRAIN BARRIER</td>
<td>Cytokines (intraplacenta) → Cytokines (cord, serum) → Damage → Cytokines</td>
</tr>
</tbody>
</table>

Pathogenesis White Matter Damage in Response to IUI

- Cytokines affect Endothelial Platelets
- Astrocytes Oligodendroglia
- IVH → White Matter Damage

**Inflammatory Cytokine Response Linked to Development PVL and CP**

- Dammon & Leviton – Infection/Neurologic Damage Model
  - Maternal Infection – IUI
  - Fetal Cytokinemias/Proinflammatory Response
    - (IL-1, IL-6, TNF-α)
    - Disruption oligodendrocyte development
  - Neonatal WMD, PVL, and CP
- FIRS mediated by cytokines
  - Participates pathogenesis PVL and CP

**Neither Intrauterine Infection nor Inflammation Sufficient to Cause Brain Injury and CP**

- CP complex multifactorial syndrome determined by interaction environmental and genetic factors
- CP not develop in
  - 82% fetuses with documented microbial invasion AF
  - 76% those with intrauterine inflammation (↑ IL-6) (Yoon et al AJOG 2003;182:675)

**Neither Intrauterine Infection nor Inflammation Sufficient to Cause Brain Injury and CP**

- Factors predispose brain injury in setting intrauterine infection
  - Gestational age
  - Integrity BBB
  - Vulnerability CNS
  - Fetal infection rate
  - Nature and intensity of inflammatory response
  - Coagulation

**Systemic Activation of Inflammation and Coagulation in Newborn Brain Damage**

- Study term infants developed CP
- Post natal day 2 - ↑ levels factor V Leiden mutation product (impaired anti-coag)
- Prominent ↑ proteins with anti-coagulant properties – protein C, protein S and antithrombin III
- ↑ coagulation factors accompanied by ↑ blood levels pro-inflammatory cytokines

Nelson K et al Ann Neurol 1998;44:665
Chorioamnionitis and Brain Injury

- Marked inflammation fetal side of placenta associated adverse neurologic outcome
- Co-existence infection and thrombotic lesions associated ↑ risk of CP or neurologic impairment
  - Preterm
  - Term

Redline & O’Riordan Arch Pathol Lab Med 2000; 124:1785
Redline et al Arch Pathol Lab Med 1998;122:1091

Coagulation, Inflammation and Risk of Neonatal White Matter Damage

- Coexistence inflammatory and thrombotic lesions in preterm placenta
  - Associated ↑ risk neurologic impairment (Redline et al 2000)
- Co-occurrence markers inflammation and coagulation in term newborns develop CP (Nelson et al 1998)
- ↑ circulating levels activated coagulation factors enhance influence of inflammation factors (Leviton & Dammann 2004)

Coagulation, Inflammation and Risk of Neonatal White Matter Damage

- Activated coagulation factors contribute to occurrence cerebral white matter damage
  - Exacerbating inflammatory phenomena
  - Not by occluding cerebral vessels
- Indicators coagulation activation
  - ↑ blood newborns (adults) with SIR
- Coagulation factors exacerbate inflammation
  - In turn promotes coagulation


Coagulation, Inflammation and Risk of Neonatal White Matter Damage

- Activated protein C
  - Only therapy → ↓ mortality SIRS/MOD
  - Both anti-coagulation and anti-inflammation effects

Intrauterine Infection and IVH

- Groome et al (AJOG 1992) – March of Dimes multicenter study
  - Clinical chorioamnionitis associated 2-3 fold ↑ risk IVH
  - IUI associated 2 fold ↑ risk IVH, PVL and ventriculomegalgy
  - Early-onset and late-onset sepsis VLBW newborns associated ↑ IVH

Combined Effect Infection and Hypoxia-Ischemia

- Epidemiologic studies
  - Markedly ↑ risk CP following combined exposure of infection and birth asphyxia (Nelson & Grether 1998)
    - Spastic CP – OR 78
    - Spastic quadriplegic CP – OR 367
- Wu et al (JAMA 2003) – term/near term
  - 4-fold ↑ risk CP with chorio
  - OR 17.5 with chorio and HI injury
**Combined Effect Infection and Hypoxia-Ischemia**

- Lab studies suggest activation innate immune system exert toxic effects on brain
  - LPS activates innate immune system via interaction with toll-like receptors
  - TLR stimulation mediates synthesis pro-inflammatory cytokines (IL-1\(\beta\) and TNF-\(\alpha\))
  - ↑ levels IL-1\(\beta\) and TNF-\(\alpha\) expressed immature CNS in response HI

- Investigated combined effects of LPS and HI on vulnerability of immature rat brain
  - Neuropathology
  - Cerebral blood flow
  - Expression TLR-4 & CD14 mRNA
- LPS dramatically ↑ vulnerability of immature brain to HI
  - Comb 20 mins HI and LPS induced as extensive brain injury as 40-50 mins HI
  - Not explained by alterations cerebral blood flow or hyperthermia
  - Altered expression TLR-4 in RNA and CD14 mRNA


**LPS Administration Enhances Hypoxic-Ischemic Brain Damage**

- Dose-response effect LPS on HI insult in neonatal rat pups
- LPS added to HI
  - Neuronal loss cerebral cortex and hippocampus significantly ↑ in dose-response manner
- Intrauterine infection may induce fetal and neonatal brain damage when combined with a mild degree of hypoxia-ischemia


**Association Acidosis, Maternal Fever, and Risk of Encephalopathy**

<table>
<thead>
<tr>
<th></th>
<th>Infants with encephalopathy</th>
<th>Infants without encephalopathy</th>
<th>Adjusted(^a) OR</th>
<th>Adjusted(^b) OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal acidosis</td>
<td>40%</td>
<td>4.2%</td>
<td>11.5</td>
<td>11.8</td>
</tr>
<tr>
<td>Maternal fever</td>
<td>44%</td>
<td>6.8%</td>
<td>8.1</td>
<td>6.3</td>
</tr>
<tr>
<td>Maternal fever and neonatal acidosis</td>
<td>20%</td>
<td>0.4%</td>
<td>93.9</td>
<td>76.2</td>
</tr>
</tbody>
</table>

\(^a\)– acidosis or fever, nulliparity, gender
\(^b\)– acidosis or fever, nulliparity, gender, induction, epidural

Impey et al AJOG 2008;198:49e1-49eb
Amniotic Fluid Infection (AFI) Primarily Fetal

- PMNs in AF fetal in origin
- AF cortisol (fetal adrenal) ↑ AFI
- Markers of infection (IL-6)
  - ↑ AF
  - ↑ umbilical cord blood
- Macrophages in chorioamnion produce IL-6
  - Fetal origin

Fetal Inflammatory Response to Intra-Amniotic Infection

- Fetus contributes to cellular inflammatory response evident in AF
- Funisitis better than membrane inflammation in predicting PTD, perinatal death and echolucency cerebral white matter
- IAI accompanied fetal cytokinemia > maternal cytokines
- More severe histologic inflammatory response
  - Higher level of cytokines in AF

Fetal Inflammatory Response to Intraamniotic Infection

- ↑ levels pro-inflammatory cytokines in AF, cord blood and early neonatal specimens among best predictors PTD, cerebral white matter damage and CP
- Levels of cytokines elevated in brains of infants die with histologic evidence of white matter damage
  
  Leviton A et al Internat Ped Research Foundation 1999;45:566

Fetal Inflammatory Response to Intraamniotic Infection

- Concentration pro-inflammatory cytokines AF ↑s with ↑ing grade histologic chorioamnionitis (Negishi et al J Perinat Med 1996)
- Fetal (not maternal) cytokine levels correlate with severity funisitis (Salafia et al Am J Perinatal 1997)
Fetal Inflammatory Response Syndrome (FIRS)

- Fetal immune system activation
- Elaboration (variable intensity) numerous proinflammatory cytokines
- Associated PTL (especially pPROM)
- Associated perinatal morbidity/mortality

Fetal Inflammatory Response Syndrome (FIRS)

- Analogous adult SIRS
- Fetal vasculitis
  - Umbilical cord (funisitis)
  - Chorionic plate
- Humoral: cytokinemia (AF)
  - IL-1α, IL-6, IL-8
  - TNF-α

Fetal Inflammatory Response System

- Multisystem disorder
- May result in
  - Preterm delivery
  - Adverse neonatal outcomes

Fetal Inflammatory Response System

- Lung
- FIR
- Bowel
- Brain
- Sepsis
- Eye
**Why This Baby?**

- VLBW infants
  - Prevalence infection (AF micro, placenta histology) 40%-80%
  - 5%-13% develop CP
- 85% infants in infected and inflamed fetoplacental unit, sufficiently affected to cause PTB, not sustain neurologic damage

**FIRS**

**Short Term**
- Multi-organ dysfunction
- Sepsis
- Fetal death

**Long Term**
- BPD
- NEC
- CP

**Inflammation Induced Brain Injury**

- Activation of Microglia → cytokines, NO, excitatory amino acids
- Astrocytes → fibrosis

- ↑Susceptibility to ischemic injury

- Direct and Indirect Mechanisms
  - Endothelial target BBB disruption
  - Neuronal Damage
  - Oligodendroglial Damage

**Why This Baby?**

- Host factors contribute to pathogenesis CP
  - Genetic predisposition inflammation (cytokine gene polymorphisms)
  - Genetic predisposition thrombosis
Cerebral Palsy

• Complex multifactorial syndrome
  • Determined by interaction of environmental and genetic factors
• Environmental – exposure infection
• Genotype – determines intensity inflammatory response
  • Polymorphisms genes coding for proinflammatory and anti-inflammatory cytokines – regulate host response to infection/tissue injury
  • Fetal genotype may determine intensity FIR to infection and predispose to CP

Polymorphism TNF-Locus and Increased TNF-α Production

• Genetic variability TNF locus contributes to determination of susceptibility to or outcomes
  • Infectious diseases
  • Autoimmune diseases
  • Neoplastic diseases
• ↑ risk morbidity and death
  • ↑ levels
  • ↓ levels

Modification Infection Associated Risks PTD and White Matter Damage
Polymorphisms TNF-α

• TNF-α critical mediator of inflammation and immunity
• Produced in excess TNF-α link between
  • Maternal infection
  • PTB
  • Neonatal white matter damage
    • Most important predictor CP among preterm

Maternal and Fetal Genetic Factors

Regulation TNF-α Generation

Woman’s Infection-Associated PTB
Infant’s Risk White Matter Damage
**Association Inherited Cytokine Polymorphisms and Cerebral Palsy**

- Case-control study with DNA from newborn screening cards
  - 443 CP infants, 883 controls
- Carriage of polymorphisms in TNF alpha and mannose-binding lectin genes
  - Associated ↑ risk CP

Gibson CS et al AJOG 2006;194:674-80
South Australian Cerebral Palsy Research Group

**Duration Labor and Risk Cerebral White-Matter Damage Very Preterm Infants with IUI**

- Cohort of 296 cases PTL or PPROM at < 32 weeks’ gestation
  - 126 (43%) complicated by IUI
    - 49 (39%) clinical chorioamnionitis
    - 107 (84%) histologic chorioamnionitis
      - 15 (12%) funisitis
    - 18 (14%) early neonatal sepsis

Locatelli et al AJOG 2005;193:928
Duration Labor and Risk
Cerebral White-Matter Damage
Very Preterm Infants with IUI

- White-matter damage 13 (10%)
  - IVH stage 3+
  - PVL
  - Ventriculomegaly without hydrocephalus

Duration Labor and Risk
Cerebral White-Matter Damage
Very Preterm Infants with IUI

- No difference G.A. at delivery and rates of labor or cesarean delivery
- Duration active labor similar
  - 66 ± 45 mins vs. 88 ± 75 mins. (P=.49)
- Duration clinical chorioamnionitis similar
  - 310 ± 186 mins vs. 529 ± 544 mins. (P=.44)

"No evidence that duration labor related to increased risk of white-matter brain lesions in preterm neonates born with clinical, histologic, or laboratory evidence of intrauterine infection."