Mechanical Ventilation: It Doesn’t Do a Body Good!

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“So, I’m the only one who sees a conflict of interest here?”
Objectives

• Recognize association between use of invasive MV & and 2nd organ injury

• Understand “multiple-hit” contribution to lung & to 2nd organ injury

• Describe possible mechanisms that may lead 2nd organ injury associated w/ MV
Apoptosis

- Greek for natural process of leaves falling from trees
- A distinct form of programmed cell death
- Characterized by loss of cell function and rapid cellular morphologic changes
- Results in *cell death without inflammation*
Growth, Development & Response to Injury Require Balancing Apoptosis & Proliferation

Schulze-Bergkamen, Gut 2009
Example of Maturational Influence on Proliferation and Apoptosis in Brain

P:A ratio varies by EGA @ 90 d 125 d 140 d 160 d 185 d

Rees SM J Neuropath Exp Neurol 2009
What evidence is there that mechanical ventilation adversely effects organs other than the lung?
ARDS Network Study

- 6 ml/kg v 12 ml/kg
- Mortality significantly less (40% v 31%)
- Significantly less MOF & ↓ serum IL-6
In-Hospital Mortality by VT Support Mode and 1° Condition/Risk Factor

Only trauma did not have protective effect w/ low VT

Eisner MD, AJRCCM 2001
Effect of MV on Plasma Cytokines, Non-Pulmonary Function & Apoptosis

• Imai Y et al, JAMA 2003
• Ventilated adult rabbits
• Lo-VT (~ 6 ml/kg) v Hi-VT (~ 16 ml/kg)
• Also analyzed serum of adults from RCT of Lo v Hi VT
Both forms of MV increased plasma cytokines; but Hi-VT approach had greater effect.
MV associated w/ Increased Serum Enzyme Levels w/ Hi-VT Effect > Lo-VT

Imai Y JAMA 2003
Injurious MV Increases Apoptosis in Non-Pulmonary Organs

![Fluorescent TUNEL Stain](image)

- *P < 0.01

**Graph:**

<table>
<thead>
<tr>
<th></th>
<th>Lo-VT</th>
<th>Hi-VT</th>
</tr>
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<tbody>
<tr>
<td>Lung</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Kidney</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Villi</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Crypt</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

Imai Y JAMA 2003
Circulating Mediators are Associated with Apoptotic Response

- Apoptosis significantly increased in plated renal tubular cells exposed to serum from ventilated rabbits
- Ab to FAS-ligand, markedly attenuated this effect

Imai Y JAMA 2003
Interim Summary # 1

- Convincing evidence from adult studies in humans and animals show that mechanical ventilation initiates a systemic inflammatory response & this response is often accompanied by other end organ injury
What about in neonates/preterm infants

DESPAIR

It’s Always Darkest Just Before it Goes Pitch Black
Preterm brain is extremely fragile & undergoes tremendous growth & development over last trimester.
Risk Factors for White Matter Disease

• Italian study w/ 1209 infants < 32 wks
• 5-fold increase risk for WMD w/ BPD
• Compared to NO ventilation
  Ventilation x 24 hrs ↑ by 3-fold
  Ventilation 7 days ↑ by 8-fold
  Ventilation >7 days ↑ by 11-fold

Gagliardi L et al, Paediatric Perinatal Epidemiology 2009
Impact of MV and Other Risk Factors on Neurodevelopmental Outcomes

- NDI: CP &/or Bayley II MDI/PDI < 70

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>OR</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>Chorio</td>
<td>1.4</td>
<td>0.5-4.0</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>1.4</td>
<td>0.5-4.0</td>
</tr>
<tr>
<td>Poor PN Growth</td>
<td>1.9</td>
<td>1.3-2.9</td>
</tr>
<tr>
<td>Any MV</td>
<td>3.0</td>
<td>1.2-7.5</td>
</tr>
<tr>
<td>BPD</td>
<td>3.8</td>
<td>1.1-11.1</td>
</tr>
</tbody>
</table>

Schlapbach LJ, Acta Paediatr 2010
Independent of confounding factors, acute & chronic lung disease in preterm infants are associated with white matter abnormalities in the brain.
What do animal model studies add to our understanding?
Cerebral Outcomes in the Preterm Baboon

- Loeliger M et al, Pediatrics 2006
- Elective delivery after ANS at 125 d
- Low tidal volume support
- nCPAP at 24 hrs v 5 days
- Compared to fetal controls
Compared to fetal controls, preterm birth associated with marked decrease in brain (and body) growth.

Less brain injury seen in animals extubated to nCPAP by 24 hrs vs 5 days.

Loeliger M et al, Pediatrics 2006
Brain injury was correlated to degree of FiO2 & pCO2 flux as well as the length of time on ventilator support

\[ R^2 = 0.59; p < 0.01 \]

\[ R^2 = 0.45; p = 0.03 \]

Loeliger M, Pediatrics 2006
Similar decrease in regional brain volumes regardless of the ventilator approach

125-d gestation baboons ventilated x 3-4 weeks

% of fetal gestational control

- Neocortex
- White matter
- Deep grey matter

LV-PPV
HFOV

Loeliger M, Peds Res 2009
Both HFOV & LV-PPV associated with increase in regional brain astrocytosis

125-d gestation baboons ventilated x 3-4 weeks

- Deep WM
- Subcortical WM
- Neocortex
- Hippocampus

% of fetal gestational control

Loeliger M, Peds Res 2009
Cerebellar Vermis Decreased w/ DnCPAP v EnCPAP/Control

Somal IGL Neurons Decreased w/ DnCPAP v EnCPA & Control

Rees SM et al, J Neuropath Exp Neurol 2009
Cerebral Outcomes in Preterm Baboon

• Premature delivery alone is associated w/ decreased brain growth & brain injury

• Brain injury may be modified by mode & timing of respiratory therapies

• Early CPAP associated w/ less overall cerebral injury than HFV, IMV or DnCPAP

Rees SM, J Neuropath Exp Neurol 2009
Verney C, J Neuropath Exp Neurol 2010
Multi-organ Effects of MV in the Preterm Lamb Model of BPD

- Unique animal model
- Ventilator support via 3 or 21 days IMV
- Contrasted to HF-NV & fetal controls
- Histological and molecular studies
- Now extending out to 2-yrs equivalent
Intestinal Involvement

- Mechanical ventilation, but not HFNV, associated with histopathological abnormalities of the ileal mucosa
  - Reduced villous length, width & crypt depth
  - Decreased enterocyte replication
  - Increased enterocyte apoptosis
  - Increased inflammatory cellularity
MV Alters Liver Glucocorticoid & Apoptotic Pathway in Preterm Lambs

Relative mRNA expression

- GC-r
- 11-b-HSD
- p53
- Caspase 3

MV - 3 d  HFNV  Fetal Control
MV Alters Brain Expression of TLR-4 in Preterm Lambs

Brain TLR-4 mRNA expression differed w/ 3-Day MV versus early HFNV

<table>
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<tr>
<th></th>
<th>MV</th>
<th>HFNV</th>
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<tr>
<td>3 days</td>
<td>7 + 2</td>
<td>9 + 8</td>
</tr>
<tr>
<td>3-4 wks</td>
<td>115 + 13</td>
<td>54 + 15</td>
</tr>
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</table>
Management of preterm lambs with MV leads to greater apoptosis of neurons and glia in the hippocampus.
MV associated with marked increase in cortical white matter reactive astrocytes compared to HFNV
Mechanical Ventilation Affects Brain Growth & Development

• Brain derived neurotrophic factor
  – Supports neuronal/glial formation & survival
  – Decreased by MV at 3 d and 21 d
  – Associated w/ increased neuronal apoptosis
Compared to NIV modes, any form of MV is associated with increased injury in non-pulmonary organs, including brain.

Other factors may also contribute:

- Preterm birth alone
- Nutritional deficiencies
- Hypoxia-ischemia
- Other
- Infection
- Hyperoxia
- Genetic
How does the act of MV involve non-pulmonary tissues?
Injurious MV

"Gentle" MV

MSOF

??

??
NF-κB pathway

Matrix ligands, TGF-βR, Angiotensin, AT-1-r, rAGE

Cytokines, IL-1β, TNF-α, etc

AGE Advanced Glycation Products

LPS, Hyaluron, TLR-4, Oxidized Lipids, etc

Growth factors, Cell adhesion molecules, Cell surface receptors, Cytokines/Chemokines

Transcription factors, Apoptosis regulators, Immunoregulatory proteins, Enzymes

NEMO

IKKα, IKKβ

p65, p50

κβ

Proteosome Degradation
### Effects of Brief Hi-$V_T$ Support

Hillman NH et al, AJRCCM 2007

<table>
<thead>
<tr>
<th>Group</th>
<th>$V_T$ x 1$^{st}$ 15 min</th>
<th>Subsequent support</th>
</tr>
</thead>
<tbody>
<tr>
<td>FetalR + PS</td>
<td>15 ml/kg</td>
<td>Surf then placental support</td>
</tr>
<tr>
<td>FetalR + Vent</td>
<td>15 ml/kg</td>
<td>Surf then MV</td>
</tr>
<tr>
<td>NeoR + Vent</td>
<td>15 ml/kg</td>
<td>Surf then MV</td>
</tr>
<tr>
<td>Control</td>
<td>No VT</td>
<td>No Rx Stayed in utero</td>
</tr>
</tbody>
</table>

Necropsy after 3 hours of Rx
Brief resuscitation w/ increased $V_T$ promotes marked increase in pro-inflammatory receptors & cytokines in the lung as well as *non-pulmonary organs*.

Hillman NH et al, AJRCCM 2007
Preterm lamb lung & VASP: Effect of MV v HFNV

A. Duration of Ventilation: MV vs. HFNV

B. Total VASP (Normalized arbitrary densitometry units)

C. VASP phosphorylation (%)

Images A, B, and C show changes in lung tissue with different ventilation methods.
Interim Summary # 3

• Mechanical stretch of lung epithelial cells evokes a complex-signaling pathway

• Activated many unique “early response” genes
  – Often regulated by NF-κB

• Mediated by “stretch” at epithelial membrane

Copland IB, J Cell Physiol 2007
Effects may be dependent on:

• Genetic predisposition
• Gestational maturation
• Duration of support
• Magnitude of MV support
• Quantity/quality of surfactant
• Pre-existing pathophysiology
Can “Low-VT” MV contribute to VILI & systemic inflammation?
Low-VT induces an increase in plasma cytokines, though not to the same degree as Hi-VT approach.
Though Hi-VT induced more PMN influx and lung injury, even Lo-VT associated w/ injury

Control mice
Low VT (~7 ml/kg)
High VT (~15 ml/kg)

Wolthius EK, Crit Care 2009
Low Volume Ventilation Effect on TLR-4

Low VT ventilation generates ↑ TLR-4 response in lung epithelial cells of healthy mice

Veneker M, Anesthesiology 2008
Lung TLR-4 response associated with marked increase in plasma cytokines

Veneker M, Anesthesiology 2008
Why Would Low-VT Approach Produce Inflammatory Response Similar to Hi-VT?
Tidal “Hyper-Inflation” during Low Tidal Volume Ventilation in ARDS

Red color indicates areas of “Tidal H-I” during low VT support

Green color indicates areas of non-aeration

Terragni PP, AJRCCM 2007
BALF Cytokines are Significantly Increased w/ T-"HI" during Low VT

- IL-6
- IL-1β
- IL-1ra
- IL-8
- TNF-α sR55
- TNF-α sR75

P < 0.01

NO Tidal NO Tidal NO Tidal
THI HyperInflation THI HyperInflation THI HyperInflation

Terragni PP, AJRCCM 2007
So What Happens in Neonates?
MV in Newborn Infants ↑ Serum Pro-Inflammatory but ↓ Anti-Inflammatory Cytokines

- Mostly late-preterm infants w/ low-V\textsubscript{T} approach
- No evidence/suspicion for sepsis/infection
- 55% had been on CPAP prior to ETT

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Pre-MV</th>
<th>2 hrs MV</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>126 (22-420)</td>
<td>122 (54-314)</td>
<td>ns</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>78 (32-143)</td>
<td>176 (44-650)</td>
<td>0.01</td>
</tr>
<tr>
<td>IL-1b (pg/ml)</td>
<td>13 (10-19)</td>
<td>97 (75-942)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TNF-a (pg/ml)</td>
<td>1 (0.8-1.5)</td>
<td>10 (6-14)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>109 (59-266)</td>
<td>10 (8-13)</td>
<td>&lt; 0.001</td>
</tr>
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</table>

Markers of Systemic Inflammation Increase with Duration of MV Support In ELGANs

In ELGANs, ↑ pro-inflammatory proteins on days 7 & 14 are associated w/ NDI at 2 years.

Elevated Concentrations of Inflammation-Related Proteins in Postnatal Blood Predict Severe Developmental Delay at 2 Years of Age in Extremely Preterm Infants

Multiple “Hit” Hypothesis

• Even “gentle” ventilation may initiate pulmonary/systemic inflammatory state

• Pre-existing or additional “insults” to lung or body may enhance the effect
Inflammatory Response to Multiple “Hits”

• Kroon AA et al, Pediatr Res 2010

• Neonatal mice exposed to:
  – Volutrauma
  – Hyperoxia
  – LPS
  – Varying combinations
Inflammatory Response to Multiple “Hits”
Kroon AA, Peds Res 2010

• **Series I: different VT**
  1) nonventilated (NV) controls
  2) low VT (VT 3.5 mL/kg, 600 bpm, PEEP 0)
  3) moderate VT (VT 12.5 mL/kg, 160 bpm, PEEP 2)
  4) high VT (VT 25 mL/kg, 20 bpm, PEEP 2)

• **Series II: pre-exposure to LPS, low-VT and oxygen**
  Injection of either 3 mg/kg LPS or same volume of 0.9% NS
  24-hrs after treatment animals randomly assigned to:
  1) NV after NS injection or 2) NV after LPS
  3) low VT w/ RA after NS or 4) same after LPS
  5) low VT w/ 50% O2 + NS or 6) same after LPS
Inflammatory Response to Multiple “Hits”
Kroon AA, Peds Res 2010

Pro-inflammatory (but not anti-inflammatory) cytokines increased in response to varied VT support

**CXCL-2**
**IL-6**
**IL-1β**
**IL-10**
Inflammatory Response to Multiple “Hits”
Kroon AA, Peds Res 2010

Additional “hit” w/ either endotoxin or 50% FiO2 changes response
What About CPAP??
Is CPAP Protective Versus Lo-VT MV?

- Polgalse GR et al, Pediatr Res 2009
- 3 hour studies of 133 d preterm lambs
- IT-LPS via low dose Curosurf @ 15 min
- Lo-VT = 8 ml/kg + PEEP 5
- CPAP = 8 cm H2O
Both CPAP & MV increased lung & liver mRNA levels for cytokines & TLR-4

LPS augmented the effect
How Might “Low-VT” Ventilation Cause VILI & Systemic Inflammation?
710 unique genes were differentially expressed during MV as compared to spontaneous breathing.
Non-injurious MV activates a diffuse transcriptional network. This results in a broad repertoire of pro-inflammatory molecules and immune-mediated pathways.

176 different gene interactions

Gharib SA et al, *Physiol Genomics* 2009
Non-injurious MV activates a diffuse transcriptional network. This results in a broad repertoire of pro-inflammatory molecules and immune-mediated pathways. This process serves to “prime” the lung whereby sustained MV or 2nd insults can lead to pulmonary and non-pulmonary injury.

Gharib SA et al, *Physiol Genomics* 2009
Isolated/perfused mouse lungs

C – Low $V_T$ “control”

LPS – endotoxin exposed

OV – High $V_T$

<table>
<thead>
<tr>
<th>Gene Response</th>
<th>UP</th>
<th>DOWN</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS</td>
<td>396</td>
<td>596</td>
</tr>
<tr>
<td>OV</td>
<td>201</td>
<td>196</td>
</tr>
<tr>
<td>OV+LPS</td>
<td>866</td>
<td>991</td>
</tr>
</tbody>
</table>

Up-regulated genes: cytokines, transcription factors, apoptosis

Down-regulated genes: kinases, growth factors, cell cycle regulat

Dolinay T et al, Physiol Genom 2006
Factors Contributing to Neonatal Lung Injury

- Chronic Stress
- Growth Restriction
- Genetic Predisposition
- Hyperoxia O2 exposure
- Mechanical Ventilation
- Postnatal Infection
- Poor Nutrition
- Other PN Therapy
- Preterm Birth
- Delivery Room Care
- Antenatal Steroids
- Fetal lung
- Genetic Predisposition
- Surfactant
- CPAP
- Good Nutrition
- Pro-inflammatory
- Anti-inflammatory

Yoder BA, NeoRev 2008
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Yoder BA, NeoRev 2008
Factors Contributing to Neonatal Lung Injury

Chronic Stress

Growth Restriction

Fetal lung

Antenatal Steroids

Genetic Predisposition

Hyperoxia O2 exposure

Mechanical Ventilation

Postnatal Infection

Postnatal lung

Good Nutrition

CPAP

Other PN Therapy

Postnatal Care

Pro-inflammatory

Anti-inflammatory

Yoder BA, NeoRev 2008
Injurious MV

Stress Catechols/Steroids

Immune Suppression

Inflammatory Mediators

“Gentle” MV

Immature or Pre-injured Lung LPS, Oxidant Stress

MSOF

Modified from Plotz FB, Int Care Med 2004
CLUELESSNESS

There Are No Stupid Questions,
But There Are a Lot of Inquisitive Idiots