Assessing the Impact of anti-RSV Interventions: Clinical Endpoints and Biomarkers

Asunción Mejías, MD, PhD
Center for Vaccines and Immunity
Division of Pediatric Infectious Diseases

ReSViNET
Disclosures

• Advisory Boards
  Janssen, Alios

• Lecture honoraria
  Abbvie, Novartis

• Grant Support
  Gilead, Janssen
Outcomes & Endpoints in RSV clinical trials

Goal of interventions against RSV

Prevent/reduce severe disease

but.. the lack of precise clinical and laboratory markers of disease severity during natural RSV infection has made evaluating the impact of interventions challenging.....
Outline

Composite endpoints for clinical interventions

• Clinical endpoints to evaluate anti-RSV intervention

• Viral factors

• Host immune profiles & antibody responses

• Genomics for the diagnosis, pathogenesis and assessment of RSV disease severity

• Role of bacterial colonization
RSV Infections Variability

- <0.1% Mortality
- 1% Hospitalization
- 10% LRTI
- 100% Infection
Clinical Endpoints Used

• **Outpatients**
  – Fever, otitis media, URI that interferes with PO intake/sleep\(^1\)
  – Need for hospitalization

• **Inpatients**
  – Length of stay (LOS), use of chest-x ray/antibiotics, tachypnea\(^2\)
  – Respiratory distress, retractions, prostration, coma, hypoxemia <90%\(^3\)
  – Need and duration of O\(_2\), PICU, LOS\(^4\)
  – LOS, PICU, mechanical ventilation\(^5\)

### RSV Disease Severity in Hospitalized Patients (PICNIC*): Pre-prophylaxis

<table>
<thead>
<tr>
<th></th>
<th>Cardiac Disease</th>
<th>Chronic Lung Disease</th>
<th>Gestation &lt; 37 wk</th>
<th>No Known Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of patients</strong></td>
<td>57</td>
<td>79</td>
<td>148</td>
<td>372</td>
</tr>
<tr>
<td><strong>Hospital days</strong></td>
<td>11</td>
<td>10</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td><strong>ICU %</strong></td>
<td>32</td>
<td>37</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td><strong>ICU days</strong></td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td><strong>Ventilation %</strong></td>
<td>19</td>
<td>25</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td><strong>Ventilation days</strong></td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td><strong>Fatality ratio,%</strong></td>
<td>5.2</td>
<td>5.1</td>
<td>3.4</td>
<td>0</td>
</tr>
</tbody>
</table>

*Pediatric Investigators Collaborative Network on Infections in Canada

Why there is so much clinical variability during RSV Infection?
1. Viral factors and disease severity

RSV loads
RSV genotypes

Do they play a role in disease severity?
<table>
<thead>
<tr>
<th>Study Year</th>
<th>Study Population</th>
<th>N</th>
<th>Assay</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Sheeran 1999</td>
<td>Intubated vs Non-intubated</td>
<td>28</td>
<td>Plaque assay</td>
<td>No differences</td>
</tr>
<tr>
<td>(2) Wright 2002</td>
<td>Low &amp; high risk</td>
<td>77</td>
<td>Plaque assay</td>
<td>No correlation</td>
</tr>
<tr>
<td>(3) DeVincenzo 2005</td>
<td>Healthy &amp; Preterm</td>
<td>141</td>
<td>Plaque assay</td>
<td>(+) correlation length hospit, respir. failure</td>
</tr>
<tr>
<td>(4) Fodha 2007</td>
<td>Healthy &amp; Preterm</td>
<td>81</td>
<td>RT-PCR</td>
<td>Associated* with severity (Resp rate, hosp, ICU)</td>
</tr>
<tr>
<td>(5) Martin 2008</td>
<td>Low &amp; high-risk</td>
<td>418</td>
<td>RT-PCR</td>
<td>(-) correlation hosp, antib use, Resp Rate</td>
</tr>
<tr>
<td>(6) Houben 2010</td>
<td>Healthy</td>
<td>30</td>
<td>RT-PCR</td>
<td>(+) correlation with disease severity score</td>
</tr>
<tr>
<td>(7) El Saleeby 2011</td>
<td>Healthy</td>
<td>62</td>
<td>Plaque assay</td>
<td>(+) correlation with duration of hospitaliz.</td>
</tr>
</tbody>
</table>


* Sequencing of the RSV A and B G glycoprotein
  (C-terminal region 212-297 aa)

RSV Genetic Variability and Viral Loads Do not Predict Disease Severity

CDSS: clinical disease severity score* (mild vs severe)

# Clinical Course RSV Bronchiolitis by Viral Loads

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Low (≥ 24.3)</th>
<th>Mid (20.8-24.2)</th>
<th>High (&lt;20.8)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=587</td>
<td>n=598</td>
<td>n=579</td>
<td></td>
</tr>
<tr>
<td>RSV subtype A</td>
<td>315 (54%)</td>
<td>317 (62%)</td>
<td>377 (65%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RSV subtype B</td>
<td>278 (47%)</td>
<td>232 (39%)</td>
<td>206 (36%)</td>
<td></td>
</tr>
<tr>
<td>LOS ≥ 3 days</td>
<td>2 (1-4)</td>
<td>3 (1-4)</td>
<td>3 (1-4)</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>244 (42%)</td>
<td>302 (51%)</td>
<td>300 (52%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Intensive care</td>
<td>87 (15%)</td>
<td>93 (16%)</td>
<td>115 (20%)</td>
<td>0.03</td>
</tr>
<tr>
<td>- Intubation or CPAP</td>
<td>35 (6%)</td>
<td>29 (5%)</td>
<td>55 (10%)</td>
<td>0.003</td>
</tr>
<tr>
<td>- ICU admission</td>
<td>83 (14%)</td>
<td>90 (15%)</td>
<td>110 (19%)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Hasegawa et al; Journal Infect Dis 2015; (10):1550-9
2. Host immune response and disease severity
Immune Response to RSV
Unfavorable Balance

Adaptive Immunity
- CD4 T cell
- CD8 T cell
- B cell

Innate Immunity
- Monocytes
- NK cells

NKG2D Ligands
- RECOGNITION
- ELIMINATION
- MEMORY

DC
- RECOGNITION
- ELIMINATION
- MEMORY

Immune Regulation
- Neutralizing AB
- Memory Cells
- Protective Immunity

LUNG INFLAMMATION
- BRONCHIOLITIS
- RECURRENT WHEEZING

Baseline Plasma Cytokine Concentrations

**TNF-α**

- Control: 30 ± 10 pg/mL (n=12)
- Ward: 40 ± 20 pg/mL (n=46)
- PICU: 50 ± 30 pg/mL (n=20)

**IL-6**

- Control: 20 ± 5 pg/mL
- Ward: 30 ± 10 pg/mL
- PICU: 40 ± 15 pg/mL

*P<0.05

**IL-8**

- Control: 20 ± 5 pg/mL
- Ward: 40 ± 10 pg/mL
- PICU: 60 ± 20 pg/mL

*P<0.05

**IL-10**

- Control: 5 ± 1 pg/mL
- Ward: 10 ± 2 pg/mL
- PICU: 15 ± 3 pg/mL

*P<0.05

*Kruskal-Wallis and Dunn’s for multiple test corrections

LPS-induced TNF-α Production

* Mann-Whitney Rank Sum Test

Impaired TNF-α Production Predicts Longer Length of Stay

Ex-vivo TNF-α production (pg/ml)

- 1-3 days
- 4-6 days
- >7 days

n=66

*p<0.01

Kruskall-Wallis test

* Impaired TNF-α production predicts longer length of stay

Logistic regression (Odds Ratio*)

Which innate immune cells are activated or suppressed in response to RSV?
Robust monocyte responses are associated with improved clinical outcomes

Heinonen S, et al. ESPID 2015
3. Neutralizing antibodies in RSV infection

Can we separate antibodies against Pre-F, Post-F and G?

What about their neutralizing activity?
ELISAs to Quantify IgG to G, Pre-F and Post-F in Serum from RSV-Infected Infants

- Nickel-coated plates enable 6His-tagged proteins to bind in an orientation similar to that on virions (more sensitive than non-coated plates)
Competition Neutralization Assay
Which RSV Antibodies Are Responsible for Neutralization?

(1) Increasing concentrations of soluble Pre-F, Post-F or sG are separately added to the IC$_{50}$ dilution of sera to adsorb antibodies to each glycoprotein (2) before adding RSV and (3) the mix is used to inoculate HeLa cells
4. Transcriptional profiles in RSV disease

- Patient Genotype (DNA)
- Environment
- Microbiome
- Unknown factors

RSV

Expression Profiles (mRNA)

Host Factors

Clinical Disease

Ramilo and Mejias, Cell Host & Microbe 2009
Host Response to RSV Bronchiolitis in Children

Mann-Whitney <0.01, Benjamini MTC x1.25 fold change

Mejias et al, PLOS Medicine 2013:10(11):e1001549
What about different age groups?
Interferon production according to age

RSV Signature

<table>
<thead>
<tr>
<th></th>
<th>&lt; 6m</th>
<th>6-24m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modules Over expressed (%)</td>
<td>53%</td>
<td>47%</td>
</tr>
<tr>
<td>Modules Under expressed (%)</td>
<td>36%</td>
<td>64%</td>
</tr>
</tbody>
</table>

RSV <6 months

<table>
<thead>
<tr>
<th></th>
<th>&lt; 6m</th>
<th>6-24m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modules Over expressed (%)</td>
<td>50%</td>
<td>81%</td>
</tr>
<tr>
<td>Modules Under expressed (%)</td>
<td>42%</td>
<td>68%</td>
</tr>
</tbody>
</table>

RSV 6-24 months

<table>
<thead>
<tr>
<th></th>
<th>&lt; 6m</th>
<th>6-24m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modules Over expressed (%)</td>
<td>71%</td>
<td>29%</td>
</tr>
<tr>
<td>Modules Under expressed (%)</td>
<td>52%</td>
<td></td>
</tr>
</tbody>
</table>

Interferon production according to age

C

\[ r = -0.52 \]

\[ P < 0.0001 \]

D

\[ r = -0.65 \]

\[ P < 0.0001 \]
Immune response to RSV across age groups

Innate Immune Response

<table>
<thead>
<tr>
<th>IFN</th>
<th>0-4 m</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>M1.2</td>
<td>94%</td>
<td></td>
</tr>
<tr>
<td>M3.4</td>
<td>79%</td>
<td></td>
</tr>
<tr>
<td>M5.12</td>
<td>52%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neutr</th>
<th>M5.15</th>
<th>87%</th>
</tr>
</thead>
</table>

n=20

Suarez et al. PAS, May 2014
Is this clinically relevant?
RSV-induced immune dysregulation correlates with disease severity

Mejias et al, PLOS Medicine 2013
MDTH Scores Correlates with RSV Disease Severity

- **Clinical Disease Severity Score**: % Sp O₂, respiratory rate, IVF, retractions, auscultation.

- **Length of Hospitalization**: Spearman Correlation, $r = 0.6$, $p < 0.001$

- **Days of Supplemental O₂**: Spearman Correlation, $r = 0.6$, $p < 0.001$

* Disease Severity Score: % Sp O₂, respiratory rate, IVF, retractions, auscultation.
Outcomes & Endpoints in RSV clinical trials

Composite endpoints ("prototype profile")

- Clinical Outcomes = severity
- Host immune profiles (Microarray + flowcytometry)
- Antibody responses (standardize assays)
- Viral loads/variants
5. Other variables that could affect severity
   NP bacterial colonization
Effects Of Pneumococcal Vaccine on Virus-associated Pneumonia

- Randomized, placebo-controlled trial of 9-valent PncCV in Soweto, SA (94% non-HIV)
- Virus-associated pneumonia in hospitalized children

<table>
<thead>
<tr>
<th>Virus</th>
<th>PncCV (n= 18,245)</th>
<th>Control (n= 18,268)</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A</td>
<td>31</td>
<td>56</td>
<td>45%*</td>
</tr>
<tr>
<td>RSV</td>
<td>90</td>
<td>115</td>
<td>22%</td>
</tr>
<tr>
<td>PIV 1-3</td>
<td>24</td>
<td>43</td>
<td>44%*</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>14</td>
<td>15</td>
<td>7%</td>
</tr>
<tr>
<td>Any of above</td>
<td>160</td>
<td>231</td>
<td>31%*</td>
</tr>
<tr>
<td>None of above</td>
<td>419</td>
<td>486</td>
<td>14%*</td>
</tr>
</tbody>
</table>

* p<0.05

RSV Activity and Pneumococcal Disease in Infants: Time series analyses

- >700,000 RSV hospitalizations and >16,000 *S. pneumo* pneumonia
- RSV attributable percent of *S. pneumoniae* PNA: 20% [17%-25%]

RSV Bronchiolitis is associated with increased bacterial colonization in the nasopharynx

- NP+ bacteria: ↑ NP WNC and % of blood nt and ↓ O2 sats
- Gram – longer duration of O2 and increased IL-8

RSV Bronchiolitis Study at Nationwide Children’s Hospital

- Patient enrollment: December 2010 to May 2012

Screened children (n=424)

Excluded
n=130

Study participants n=294

Healthy controls (HC; n=47)

RSV LRTI (n=247)

Ward (n=182)

ICU (n=65)

- Older >1 years (n=60)
- Comorbidities (n=23)
- Non-RSV (n=41)
- Other (n=6)

Bunsow E, et al ICAAC 2014
Non typable *H. influenzae* is more frequent in RSV infected infants requiring PICU

Bunsow E, et al ICAAC 2014
Non typable *H. influenzae* loads correlated with length of stay in PICU

Bacterial loads (log$_{10}$ copies/mL)

Length of stay in PICU (days)

$r=0.36$

$p=0.03$

$n=35$

Bunsow E, et al ICAAC 2014
n=132
Systemic Bacterial Clusters Profiles

de Steenhuijsen Piters W et al (under review)
Summary

• Clinical presentation and dynamics highly variable even among previously healthy children
• Viral loads, viral genotypes (?), viral gene sequence
• Antibodies against RSV: threshold for protection?
• Transcriptome profiles to monitor disease progression and assess disease severity
• Challenge: develop composite endpoints to monitor response to clinical interventions
ACKNOWLEDGMENTS

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Ioanis Ioannidis
Mark Hall
Mike Teng
Mark Peeples

Cristina Capella
Carla Garcia
Damien Chaussabel
Derek Blankenship
Julie Stephens
Gail Arthur
Michael Lawson
Paulla Davies
Grace Wentzel
Wouter Piters
Debby Bogaert

RGK Foundation; Dana Foundation; NIAID
Prefusion (Pre-F)
- Metastable
- The active form on the surface of the virion

Postfusion (Post-F)
- Refolded into a stable, 6-helix bundle

McLellan et al., 2011, 2013

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Demographics</th>
<th>RSV (n=2,961)</th>
<th>Non-RSV (n=1,628)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender (ratio)</strong></td>
<td></td>
<td>M/F 1.3:1</td>
<td>M/F 1.4:1</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Age (months)</strong></td>
<td></td>
<td>4.1 (0.3-24)</td>
<td>6.7 (0.5-24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Prematurity</strong></td>
<td></td>
<td>627 (21%)</td>
<td>477 (29.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Cong. Heart Dis</strong></td>
<td></td>
<td>97 (3.3%)</td>
<td>102 (6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Chron. Lung Dis</strong></td>
<td></td>
<td>66 (2.2%)</td>
<td>90 (5.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Down Syndr.</strong></td>
<td></td>
<td>38 (1.3%)</td>
<td>42 (2.5%)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Kruskal-Wallis  ** Fisher’s Exact Test