Model animal de pneumònia associada al ventilador: descripció del model i utilitat

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Presenter Disclosure Information

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Compensated service for a relevant commercial entity

1. Covidien Honoraria for lectures

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SUMMARY

- Ventilator-Associated Pneumonia
- Model of severe *P. aeruginosa* pneumonia
- Effects of Glucocorticoids in severe *PA* pneumonia
- Model of severe MRSA pneumonia
- Model of ventilator-associated pneumonia and future studies
VENTILATOR ASSOCIATED PNEUMONIA

HAP
- Pneumonia occurring ≥ 48 hours after hospital admission
- Risk factors for MDR bacteria causing HAP
  - Antibiotic therapy within 90 days of infection
  - Current hospitalization of ≥ 5 days
  - High frequency of antibiotic resistance in community or specific hospital unit
  - Immunosuppressive disease or therapy
  - Presence of HCAP risk factors for MDR

VAP
- Pneumonia occurring > 48 hours after endotracheal intubation
- Risk factors for MDR bacteria causing VAP
  - Presence of HCAP or HAP risk factors for MDR

HCAP
- Pneumonia occurring ≤ 48 hours of hospital admission in patients with ≥ 1 of the following risk factors for MDR bacteria as cause of infection:
  - Hospitalization for ≥ 2 days in an acute-care facility within 90 days of infection
  - Residence in a nursing home or long-term care facility
  - Antibiotic therapy, chemotherapy, or wound care within 30 days of current infection
  - Hemodialysis treatment at a hospital or clinic
  - Home infusion therapy or home wound care
  - Family member with infection due to MDR bacteria
# ICU Infections 2006-2007 (NHSN)

<table>
<thead>
<tr>
<th>Type of ICU</th>
<th>Type of Infection</th>
<th>Mean Infection Incidence Density*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical</td>
<td>Ventilator-associated pneumonia</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Catheter-associated UTI</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Catheter-associated BSI</td>
<td>2.4</td>
</tr>
<tr>
<td>Surgical</td>
<td>Ventilator-associated pneumonia</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>Catheter-associated UTI</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>Catheter-associated BSI</td>
<td>2.3</td>
</tr>
</tbody>
</table>

*Number of infections/device days x 1000

Am J Infect Control 2008 36:609-26
## Mortality and Costs of VAP

<table>
<thead>
<tr>
<th>Variable</th>
<th>No VAP (n=692)</th>
<th>VAP (n=127)</th>
<th>$p$ value $\leq$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis n(%)</td>
<td>75 (11)</td>
<td>43 (44)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ICU LOS</td>
<td>4 days</td>
<td>26 days</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hospital LOS</td>
<td>13 days</td>
<td>38 days</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Death n (%)</td>
<td>237 (34)</td>
<td>64 (50)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Total cost</td>
<td>$21,620</td>
<td>$70,568</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Warren et al *Crit Care Med* 2003 31:1312-17
VAP: ETIOLOGY

- Pseudomonas aeruginosa: 24.7%
- Staphylococcus aureus: 20.4%
- Enterobacteriaceae: 14.1%
- Haemophilus spp.: 9.8%
- Streptococcus pneumoniae: 9.5%
- Acinetobacter spp.: 7.9%
- Streptococcus spp.: 6.4%
- Neisseria spp.: 2.6%
- S. maltophilia: 1.7%
- Fungi: 1.9%
- Anaerobes: 1.9%
- Others: 3.8%

Chastre et al AJRCCM 2002 Apr 1;165(7):867-903
ANIMAL MODELS OF VAP

• Animal models are an essential step between in vitro testing and clinical studies, and are necessary for the understanding of pathophysiology, pharmacology and efficacy of therapy.

• An animal model provides a unique opportunity to study some of the incompletely understood mechanisms involved in VAP, such as the role of inflammation, the dynamics of bacterial colonization and/or infection, and the response to antimicrobial therapy.
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SUMMARY

- Ventilator-Associated Pneumonia
- Model of severe *P. aeruginosa* pneumonia
- Effects of Glucocorticoids in severe *PA* pneumonia
- Model of severe MRSA pneumonia
- Model of ventilator-associated pneumonia and future studies
Experimental *Pseudomonas aeruginosa* pneumonia: evaluation of the associated inflammatory response

O. Sibila*, C. Agustí*, A. Torres*, S. Baquero*, S. Gando*, J.R. Patrón†, J.G. Morato†, D.H. Guifredo‡, N. Bassi§ and C.M. Luna§

*Eur Respir J* 2007; 30: 1167–1172
METHODS

• Animal model of severe pneumonia in piglets, mechanically ventilated up to 96 hours, through bronchial inoculation of a 75 ml solution of *Pseudomonas aeruginosa* (10^6 cfu/ml)

The following variables were assessed:

• Clinical, hemodynamic and biochemical data every 12 hours.
• Inflammatory response (IL-6, IL-1beta, IL-8, TNF-alfa and PCR) in serum and BAL every 24 hours.
• Microbiological studies (cultures from blood, BAL and lung tissue).
• Histopathology studies.
Large White-Landrace pig (30 kg) Following surgical preparation, bronchoscopic inoculation of *P. aeruginosa*

Ventilated pig (96 hours) Macroscopic pneumonia
RESULTS

Clinical Data

PaO2/FiO2

Temperature

Mean Arterial Pressure

Heart Rate
RESULTS

Inflammatory Response (Serum)

serum IL-1 beta

- Time (hours): 0, 2, 4, 8, 72, 96
- pg/dl: 0, 25, 50, 75
- p-value: 0.97

serum IL-6

- Time (hours): 0, 2, 4, 8, 72, 96
- pg/dl: 0, 10, 20, 30, 40, 50
- p-value: 0.04

serum IL-8

- Time (hours): 0, 2, 4, 8, 72, 96
- pg/dl: 0, 25, 50, 75
- p-value: 0.67

serum TNF-alfa

- Time (hours): 0, 2, 4, 8, 72, 96
- pg/dl: 0, 25, 50, 75, 100, 125, 150
- p-value: 0.30
RESULTS

Inflammatory Response (BAL)

**BAL IL-1 beta**
- Time (hours): 0, 6, 96
- pg/ml: 0, 200, 400, 600, 800, 1000
- p = 0.06

**BAL IL-6**
- Time (hours): 0, 6, 96
- pg/ml: 0, 50, 100, 150, 200
- p = 0.04

**BAL IL-8**
- Time (hours): 0, 6, 96
- pg/ml: 0, 50, 100, 150, 200
- p = 0.14

**BAL TNF-α**
- Time (hours): 0, 6, 96
- pg/ml: 0, 50, 100, 150
- p = 0.46
RESULTS

Histology Studies

P. Aeruginosa concentration
Lung Tissue

Histological severity

Pneumonia Confluent P Abscessed P

<10^4 cfu/ml 10^4 cfu/gr 10^5 cfu/gr
SUMMARY

- Ventilator-Associated Pneumonia
- Model of severe *P. aeruginosa* pneumonia
- Effects of Glucocorticoids in severe *PA* pneumonia
- Model of severe MRSA pneumonia
- Model of ventilator-associated pneumonia and future studies
Effects of glucocorticoids in ventilated piglets with severe pneumonia

O. Sibila*,†, C.M. Luna†, C. Agustí*,‡, S. Baquero†, S. Gando†, J.R. Patrón†, J.G. Morato†, R. Absi‡, N. Bassi† and A. Torres*,‡

Eur Respir J 2008; 32: 1037–1046
STUDY DESIGN

Data Collection

Randomization:
- Control group (n=5)
- Ciprofloxacin group (n=5) (ciprofloxacin, 200 mg, every 12 h)
- Ciprofloxacin + Glucocorticoids group (n=5)
  (Ciprofloxacin, 200 mg every 12 h plus GCs, i.v. methylprednisolone 0.5 mg/kg-1 every 12 h)

- Animal preparation
- Bronchoscopic inoculation of pathogens
- Baseline data

Autopsy
RESULTS

Clinical Data (12h-96h)

### Changes in PaO$_2$/FiO$_2$

![Graph showing changes in PaO$_2$/FiO$_2$ with p=0.30](image)

### Changes in temperature

![Graph showing changes in temperature with p=0.76](image)

### Changes in Cst

![Graph showing changes in Cst with p=0.01](image)
RESULTS

Inflammatory Response (BAL)

CRP BAL

IL-6 BAL

IL-1 BAL

IL-8 BAL

TNF-alfa BAL

CRP BAL

IL-6 BAL

IL-1 BAL

IL-8 BAL

TNF-alfa BAL

Control
CIP
CIP+GC

p=0.41

p=0.03

p=0.40

p=0.19

p=0.30
RESULTS

Histology Studies

LUNG TISSUE

control CIP CIP+GC

Log cfu/gr

control CIP CIP+GC

p=0.01
SUMMARY

- Ventilator-Associated Pneumonia
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- Model of severe MRSA pneumonia
- Model of ventilator-associated pneumonia and future studies
Animal model of severe pneumonia in piglets mechanically ventilated up to 24 hours through bronchial inoculation of a solution containing 75 ml of MRSA (106 ufc/ml)

The following variables were assessed:

1.- Clinical, hemodynamic and biochemical data.
2.- Inflammatory response (CRP, IL-1 beta, IL-6, IL-8 and TNF-alfa) in serum and BAL
3.- Microbiological studies (cultures from blood, BAL and lung tissue)
4.- Histopathology studies.
RESULTS

Inflammatory Response (Serum)

- **serum IL-6**
  - 0 hours: 0 pg/ml
  - 24 hours: 60 pg/ml

- **serum PCR**
  - 0 hours: 10 pg/ml
  - 24 hours: 6 pg/ml

- **serum IL-1 beta**
  - 0 hours: 40 pg/ml
  - 24 hours: 80 pg/ml

- **serum IL-8**
  - 0 hours: 20 pg/ml
  - 24 hours: 40 pg/ml

* p< 0.05
RESULTS

Inflammatory Response (BAL)

* p < 0.05
**ONGOING STUDIES**

Laboratory study to assess effects of Vancomycin and Linezolid in a pig model of VAP due to MRSA

**Data Collection**

- Animal preparation
- Bronchoscopic inoculation of MRSA
- Baseline data

**Randomization:**
- Control group (n=10)
- Vancomycin Continuous Infusion (n=10) (In order to reach trough levels 15-20 µg/ml)
- Vancomycin Bolus (n=10) (10 mg/kg/12h, which corresponds to 15-20 mg/kg in humans)
- Linezolid (n=10) (600 mg/12h)

**Autopsy**
SUMMARY

• Ventilator-Associated Pneumonia

• Model of severe *P. aeruginosa* pneumonia

• Effects of Glucocorticoids in severe *PA* pneumonia

• Model of severe MRSA pneumonia

• Model of ventilator-associated pneumonia and future studies
Pathogenesis of VAP

- It is universally believed that the most common sequence leading to VAP is through the aspiration of colonized oropharyngeal secretions.
STUDY DESIGN

Oropharyngeal bacterial challenge

0 4 8 24 48 72

• Animal Preparation
• Baseline Data Collection
BACTERIAL CHALLENGE

After 4 and 8 hours of mechanical ventilation oropharyngeal instillation of 5 mL of $10^7$ cfu/mL culture of genetically modified green fluorescent *P. aeruginosa* Ceftriaxone resistant
PIG POSITION
AUTOPSY and MICROBIOLOGY STUDIES

Bacterial Count

Cfu/g

Trachea Carina RUL RML RLL LUL LLL

Sample site
HYSTOPATHOLOGY

Right Medium Lobe
HYSTOPATHOLOGY

Right Upper Lobe
Conclusions

• Animal models are an essential step between in vitro testing and clinical studies, and are necessary for the understanding of pathophysiology, pharmacology and efficacy of therapy.

• We developed several animal models to provide a unique opportunity to study some of the incompletely understood mechanisms involved in HAP, such as the role of inflammation, the dynamics of bacterial colonization and/or infection, and the response to antimicrobial therapy.

• In current studies we have developed the first accurate model of VAP to fully understand its pathogenesis and devise new strategies to reduce associated morbidity and mortality.
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Physiotherapist: Dani Marti, RRP
Research Nurses: Isabel Martin Lopez, RRN
Alicia SanJose, RRN

Thank You
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