Biomarcadores y Sepsis

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Mutua Terrassa University Hospital
Barcelona. SPAIN
Uncontrolled infection/major trauma/circulatory shock/tissue necrosis/apoptosis/anaphylaxia

PAMPs
LPS, LTA, lipoproteins, peptidoglycans, bacterial DNA, etc.

DAMPs
HMGB-1, heat-shock protein, DNA, uric acid, etc.

Complex protein systems
- Complement system
- Coagulation system

Vascular and tissue cells
- Endothelial cells
- Epithelial cells
- Adipose tissue

Blood and lymphatic cells
- Granulocytes
- Macrophages/monocytes
- Lymphocytes (T-cells, B-cells)

Insult
Trigger

Sensors and effector cells

Mediators and biomarkers
- C5a, C3a, C5aR, C5b-9, etc.
- aPPT, PT, AT, Protein C etc.
- Endothelial stress response: ELAM-1, ICAM-1, Selectins,
- Acute phase reactants: CRP, LBP, PCT, etc.
- Cytokines/chemokines
  - Soluble receptors: IL-6, IL-8, IL-4, IL-10, MIF, HMGB1, sTNF, suPAR, STREM-1, etc.
- Cell surface markers: mHLA DR, CD64, CD48, C5aR, etc.

Impact on organ function

Brain
- Confusion

Lung
- Respiratory distress

Cardiovascular system
- Shock
- Oliguria/Anuria

Kidney
- Excretory failure

Liver
- Loss of barrier function, ileus

Gut
- Capillary leak edema, DIC

Microcirculation

Outcome

Effective source control
- Normalization of biomarker abnormalities
- Resolution of organ dysfunction; recovery

Ineffective source control
- Persistence of biomarker abnormalities
- Multiple organ failure; death

MARKER ANALYSIS

- Over 150 markers analyzed by immunoassay, including various pro-forms, variants, and fragments.

Markers of:

- **Pro-inflammation** (e.g., CRP, TNFα, IL-1β, IL-8)

- **Anti-inflammation** (e.g., IL-10, IL-6, soluble TNF receptors)

- **Coagulation and fibrinolysis** (e.g., D-dimer, tissue factor, protein C)

Markers of:

- **Apoptosis** (e.g., caspase-3)

- **Vasoregulation** (e.g., BNP, proBNP, bigET-1, calcitonin)

- **Organ and tissue dysfunction** (e.g., NGAL, myoglobin, I-FABP, pulmonary surfactant proteins)
UTILITY OF BIOMARKERS OF SEPSIS

- Screening
- Diagnosis and early recognition
- Risk Stratification
- Identify responders to therapy.
- Monitoring of the Response to Therapy.
Biomarkers of infection

• **Single determination**
  - value in the diagnosis of infection
  - Value in determining severity

• **Serial determinations**
  - as predictor of infection
  - monitoring clinical course and response to antibiotic therapy

_Póvoa ICM 2002;28:235_
Time course: Endotoxin Challenge
Which is the useful marker for the early recognition of sepsis?

- IL-6
- CRP
- PCT
- TNF-α
- WBC

ERYTHROCYTE SEDIMENTATION
The Biomarker Response in Sepsis

• Most commonly studied and correlated with outcomes
  – CRP
  – Pro-calcitonin (PCT)
  – IL-6
  – HMGB1
  – STREM1
  – Panels of Biomarkers
C-Reactive Protein (CRP)

• CRP measurement is a rapid, reproducible and inexpensive.

• Acute Phase Protein
  – The acute phase response accompanies inflammation.
  – Acute phase proteins are defined as those proteins whose plasma concentrations increase by at least 25 percent during inflammatory states
  – Changes in levels of acute phase proteins result from cytokines: IL-6, IL-8 effect on hepatocytes
CPR
Diagnostic Marker of Infection

CRP > 8.7 mg/dl

Sensitivity 93.4%
Specificity 86.1%
AUC 0.93
Positive LR 6.71
Negative LR 0.008

Povoa Clin Microbial Infect 2005;11:101-108
Sierra Intensive Care Med 2004;30:2038-2045
CRP as a marker of infection prediction
day -5 to day 0

N=63 pts (28 controls; 35 infected - documentation)

p<0.001
CRP as a marker of infection prediction day -5 to day 0

N=63 pts (28 controls; 35 infected - documentation)

Max daily Δ CPR

AUC (day -5 to day 0) 0.86 (95% CI: 0.75–0.93)

maximum daily Δ CRP > 4.1 mg/dL Æ ICU-acquired infection
sensitivity 0.92 , specificity 0.71, LR+ 3.22, LR- 0.11
Differentiation between Sepsis and SIRS

N=76 infected pts
P=0.024; r²=0.12

CRP
PCR as a Marker of Severity of Sepsis

N=313 ICU pts

Lobo Chest 2003;123:2043

N=76 infected pts

PCR = 7.00 + 1.05 * SO
R-Square = 0.12
r=0.34, r^2=0.12
p=0.004

Póvoa Clin Microbiol Infect 2005;11:101
PCR as a Marker of Severity of Sepsis

Is C-reactive protein a good prognostic marker in septic patients?

<table>
<thead>
<tr>
<th></th>
<th>Sepsis</th>
<th>Documented Sepsis</th>
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</thead>
<tbody>
<tr>
<td>CRP (mg/dL)</td>
<td>0.55 (0.45–0.65)</td>
<td>0.66 (0.53–0.79)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>0.48 (0.38–0.58)</td>
<td>0.44 (0.29–0.59)</td>
</tr>
<tr>
<td>WCC (×1,000) mL⁻¹</td>
<td>0.46 (0.35–0.56)</td>
<td>0.6 (0.46–0.73)</td>
</tr>
<tr>
<td>APACHE II</td>
<td>0.75 (0.67–0.83)</td>
<td>0.65 (0.51–0.78)</td>
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<tr>
<td>SAPS II</td>
<td>0.82 (0.75–0.89)</td>
<td>0.75 (0.63–0.86)</td>
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<tr>
<td>SOFA</td>
<td>0.80 (0.72–0.88)</td>
<td>0.77 (0.66–0.88)</td>
</tr>
</tbody>
</table>

area under the receiver operating characteristics curves

Response to initial antimicrobial therapy

The Time Course of Blood C-reactive Protein Concentrations in Relation to the Response to Initial Antimicrobial Therapy in Patients with Sepsis

X. Schmit, J.L. Vincent

Infection 2008; 36: 213–219
PCR. Conclusion

• Single determination could be useful in the diagnosis of infection.
• Daily measurement of CRP is easy and inexpensive to perform, and can aid in:
  – diagnosis of sepsis
  – assessing response to antibiotic therapy.
• Limited value in determining prognosis.
Procalcitonin (PCT)

• PCT is a 13-kd propeptide of calcitonin. In healthy individuals, levels of PCT are below 0.1 ng/mL.

• In patients with sepsis, PCT levels may increase up to 5000 to 10,000 times with calcitonin still in the normal range.

• In contrast to the short half-life of calcitonin (10 minutes), the half-life of PCT is approximately 24 hours.
Procalcitonin (PCT)

Parenchymal cell (Lung, liver etc.)

T Cell

Production of INF-γ

Virus infection

monocyte/Mø

Production of inflammatory cytokine (TNF-α, IL-1, IL-6)

Bacterial infection

Calcitonin m-RNA

Golgi apparatus

inhibition

stimulus
Procalcitonin Level in Systemic Viral Infections is Considerably Lower

Cuquemelle E et al. Intensive Care Med 2011
PCT improves the reliability of the clinical diagnosis of sepsis

Patients with SIRS and suspicion of infection (n=78)

Pattern calculation for ICU patients with SIRS with / without PCT on a clinical model

S. Harbarth et al. Am J Respir Crit Care Med 2001;164:396-402
Differentiation between Sepsis and SIRS

Plasma levels of PCT, IL-6 y IL-8

Medical and surgical ICU pat. (n=78), with SIRS and suspicion of infection

S. Harbarth et al. Am J Respir Crit Care Med 2001;164:396-402
Meta-Analyses
Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: A systematic review and meta-analysis.

- Global diagnostic OR for PCT
  - 2966 pts
  - 25 studies
  - 15.7 (9.1-27.1)
  - Risk for positive PCT test in infected pts was 16-fold higher than in non-infected pts.

- Global diagnostic OR for CRP
  - 1322 Pts
  - 15 studies
  - 5.4 (3.2-9.2)
  - \( Q^* \) 0.78 vs. 0.71  \( p = .02 \)

*Uzzan et al.*

*Crit Care Med 2006; 34:1996–2003*
Meta-analysis: Limitations

- Heterogeneity (Uzzan)
  - Age
  - Medical vs. Surgical
    - Uzzan: No medical pts
  - Co-morbidities
  - Underlying illness
    - Cardiac surgery, peritonitis, meningitis, burns

- Single sample (Uzzan)
  - Timing of sample

- Consecutive patients
- Publication bias
  - Negative studies
  - Sample size
- Level of sepsis
  - Critically ill or not
- Diagnostic Accuracy of OR
  - 0-25: Test of little use
  - 25-100: intermediate value
  - >100: excellent performance

Tang B. Crit Care Med 2007
Accuracy of Procalcitonin for Sepsis Diagnosis in Critically Ill Patients: Systemic Review and Meta-Analysis

Sensitivity and specificity: 71% (95% CI 67–76)
AUC 0.78 (95% CI 0.73–0.83).

Tang BM et al Lancet Infect Dis 2007;7:210-17
Procalcitonin for diagnosis of bacterial pneumonia in critically ill patients during 2009 H1N1 influenza pandemic: a prospective cohort study, systematic review and individual patient data meta-analysis

Pfister et al. Critical Care 2014, 18:R44
http://ccforum.com/content/18/2/R44
Limitations
Procalcitonin depending on etiology

Serum procalcitonin measurement contribution to the early diagnosis of candidemia in critically ill patients

Charles PE et al Intensive Care Med 2006; 32: 1577-1583

Retrospective study
50 episodes of sepsis with positive blood culture: 15 candidemias (11 patients) and 35 bacteremias (33 patients)
Elevated procalcitonin without sepsis

- Severe trauma: Meisner et al. Crit Care 2006
- Burns: Carsin et al. Burns 1997
- Cardiogenic Shock: Lecharny et al. CCM 2002
- Pancreatitis: Ammori et al. Pancreas 2003
Lack of a clear diagnostic cutoff

Procalcitonin in septic shock

<table>
<thead>
<tr>
<th>Author</th>
<th>Refer.</th>
<th>N</th>
<th>Estad</th>
<th>PCT</th>
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</thead>
<tbody>
<tr>
<td>Harbarths S</td>
<td>AJRCCM 2001</td>
<td>25</td>
<td>Median</td>
<td>21,3 (1,2-654)</td>
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<tr>
<td>Giamarelllos EJ</td>
<td>ICM 2002</td>
<td>10</td>
<td>Mean</td>
<td>38,76 IC 0,15-77,38</td>
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<td>Claeys R</td>
<td>CCM 2002</td>
<td>53</td>
<td>Median</td>
<td>5,9 (1,6-26,9)</td>
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<tr>
<td>Clec Nh R</td>
<td>CCM 2004</td>
<td>62</td>
<td>Median</td>
<td>14 (0,3-767)</td>
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<tr>
<td>Clec Nh R</td>
<td>CCM 2006</td>
<td>36</td>
<td>Median</td>
<td>8,40 (3,63-24,70)</td>
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<tr>
<td>Cheval F</td>
<td>ICM 2000</td>
<td>16</td>
<td>Mean</td>
<td>89 ▫ 154</td>
</tr>
<tr>
<td>Brunkhorst FM</td>
<td>ICM 2000</td>
<td>39</td>
<td>Mean</td>
<td>12,89 ▫ 4,39</td>
</tr>
</tbody>
</table>
Static vs. Dynamic Measurements
Biomarkers of infection prediction

Procalcitonin as a prognostic and diagnostic tool for septic complications after major trauma*

Gian Paolo Castelli, MD; Claudio Pognani, MD; Massimo Cita, MD; Rolando Paladini, MD

Castelli et al. Crit Care Med 2009
Survival & Concentration Changes over time for PCT and CRP after Multiple Trauma

Correlation of procalcitonin and C-reactive protein to inflammation, complications, and outcome during the intensive care unit course of multiple-trauma patients

Michael Meisner¹, Heide Adina² and Joachim Schmidt³

PCT<0.8 ng/ml: 94 % Probability of survival (NPV)
24 % Probability of non-survival (PPV)

Crit Care 2006;10:R1
Procalcitonin. Conclusion

- Measurement of procalcitonin can aid in the diagnosis and stratification of sepsis.
- Serial determinations could help in predicting sepsis and in determining prognosis.
- Several limitations in sensitivity and specificity, and also cost.
IL-6: Utility

- Cytokines such as IL-6 and IL-8 of limited use clinically because of short half life and rapid receptor binding/antagonism.

- IL-6 and IL-8 levels are closely related to the severity of the physiologic response to infection and systemic inflammation.

- Thus, non-specific: elevated in major surgery, severe trauma, burns, autoimmune disorders, viral infection.
High-mobility group box 1 (HMGB1)

- HMGB1 is released from myeloid cells exposed to lipopolysaccharide.
- HMGB1 has delay kinetics and remains in the circulation for extended periods of time.
- HMGB1 may be an important mediator and therapeutic target in sepsis and related conditions.
HMGB1 as a predictor of organ dysfunction and outcome in patients with severe sepsis

Circulating high-mobility group box 1 (HMGB1) concentrations are elevated in both uncomplicated pneumonia and pneumonia with severe sepsis.

Derek C. Angus, MD, MPH; LiHong Yang, PhD; Lan Kong, PhD; John A. Kellum, MD; Russell L. Delude, PhD; Kevin J. Tracey, MD; Lisa Weissfeld, PhD; for the GenIMS Investigators
Triggering receptor expressed on myeloid cells-1 (TREM-1)

- Immunoglobulin up-regulated in response to infection.
- Soluble TREM-1 is shed from membranes of activated phagocytic cells and can be quantified.

Soluble Triggering Receptor Expressed on Myeloid Cells and the Diagnosis of Pneumonia

Sébastien Gibot, M.D., Aurélie Cravoisy, M.D., Bruno Levy, M.D., Ph.D., Marie-Christine Bene, M.D., Ph.D., Gilbert Faure, M.D., Ph.D., and Pierre-Edouard Bollaert, M.D., Ph.D.

Independent predictors for diagnosing pneumonia

<table>
<thead>
<tr>
<th>Predictor</th>
<th>P Value</th>
<th>Odds Ratio (95% Confidence Interval)</th>
</tr>
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<tbody>
<tr>
<td>Clinical pulmonary infection score &gt;6</td>
<td>0.002</td>
<td>3.0 (1.5–5.9)</td>
</tr>
<tr>
<td>Tumor necrosis factor $\alpha &gt; 150$ pg/ml of BAL fluid</td>
<td>0.004</td>
<td>2.4 (1.8–5.8)</td>
</tr>
<tr>
<td>Interleukin-1$\beta &gt; 75$ pg/ml of BAL fluid</td>
<td>0.003</td>
<td>2.7 (2.0–13.2)</td>
</tr>
<tr>
<td>sTREM-1 $&gt; 5$ pg/ml of BAL fluid</td>
<td>&lt;0.001</td>
<td>41.5 (20.9–77.6)</td>
</tr>
</tbody>
</table>

Time-course of sTREM (soluble triggering receptor expressed on myeloid cells)-1, procalcitonin, and C-reactive protein plasma concentrations during sepsis

Sébastien Gibot, MD, PhD; Aurélie Cravoisy, MD; Marie-Nathalie Kolopp-Sarda, PharmD, PhD; Marie-Christine Béné, PharmSci, PhD; Gilbert Faure, MD, PhD; Pierre-Edouard Bollaert, MD, PhD; Bruno Levy, MD, PhD

Crit Care Med 2005; 33:792–796
A prospective, multicenter derivation of a biomarker panel to assess risk of organ dysfunction, shock, and death in emergency department patients with suspected sepsis

Nathan I. Shapiro, MD, MPH; Stephen Trzeciak, MD, MPH; Judd E. Hollander, MD; Robert Birkhahn, MD; Ronny Otero, MD; Tiffany M. Osborn, MD; Eugene Moretti, MD, MHSc; H. Bryant Nguyen, MD; Kyle J. Gunnerson, MD; David Milzman, MD; David F. Galeski, MD; Munish Goyal, MD; Charles B. Cairns, MD; Long Ngo, PhD; Emanuel P. Rivers, MD, MPH

- **Design:** Prospective multicenter observational study.
- **Patients:** 971 patients septic enrolled.
- **Nine biomarkers were assayed.**
- **Multivariable logistic regression was used to identify an optimal combination of biomarkers to create a panel.**
- **Derived formula for weighting biomarker values was used to calculate a “sepsis score”**

Crit Care Med 2009;37:96-104
## Biomarker Panel in Severe Sepsis

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Overall Population</th>
<th>Severe Sepsis</th>
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<tbody>
<tr>
<td></td>
<td>Mean (sd)</td>
<td>Yes (n = 506)</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Mean (sd)</td>
</tr>
<tr>
<td>Macrophage inhibitory protein-3, ng/mL</td>
<td>0.76 (0.91)</td>
<td>1.02 (0.89)</td>
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<td></td>
<td>0.74 (0.12–1.39)</td>
<td>1.06 (0.41–1.62)</td>
</tr>
<tr>
<td>D-dimer, ng/mL</td>
<td>2200 (1733)</td>
<td>2736 (1730)</td>
</tr>
<tr>
<td></td>
<td>1591 (719–3719)</td>
<td>2513 (1159–4852)</td>
</tr>
<tr>
<td>C-reactive protein, µg/mL</td>
<td>81 (62)</td>
<td>95 (61)</td>
</tr>
<tr>
<td></td>
<td>61 (31–131)</td>
<td>84 (44–146)</td>
</tr>
<tr>
<td>Neutrophil gelatinase-associated lipocalin, ng/mL</td>
<td>267 (322)</td>
<td>381 (373)</td>
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<tr>
<td></td>
<td>128 (50–325)</td>
<td>249 (103–565)</td>
</tr>
<tr>
<td>Protein C, µg/mL</td>
<td>2.58 (1.08)</td>
<td>2.26 (1.02)</td>
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<tr>
<td></td>
<td>2.51 (1.75–3.32)</td>
<td>2.12 (1.49–2.89)</td>
</tr>
<tr>
<td>Interleukin-1 receptor antagonist (interleukin-1ra), pg/mL</td>
<td>2371 (4228)</td>
<td>3625 (5196)</td>
</tr>
<tr>
<td></td>
<td>700 (288–2075)</td>
<td>1267 (499–4339)</td>
</tr>
<tr>
<td>Tumor necrosis factor receptor Ia (tumor necrosis factor-R1a), ng/mL</td>
<td>17.5 (24.7)</td>
<td>24.3 (29.6)</td>
</tr>
<tr>
<td></td>
<td>8.7 (4.8–17.7)</td>
<td>13.1 (7.4–27.3)</td>
</tr>
<tr>
<td>Peptidoglycan recognition protein, ng/mL</td>
<td>119 (153)</td>
<td>153 (183)</td>
</tr>
<tr>
<td></td>
<td>61 (40–134)</td>
<td>83 (48–178)</td>
</tr>
<tr>
<td>Brain natriuretic peptide, pg/mL</td>
<td>287 (761)</td>
<td>401 (877)</td>
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<tr>
<td></td>
<td>42 (5–211)</td>
<td>94 (18–375)</td>
</tr>
</tbody>
</table>

IQR, interquartile range; CI, confidence interval.

The values used for quartile transformations for each individual marker are defined in the table above as follows: quartile 1: (0, beginning IQR range), quartile 2: (lower IQR, median); quartile 3: (median, upper IQR); quartile 4: (>upper IQR).
BIOMARKER PANEL IN SEVERE SEPSIS

Crit Care Med 2009;37:96-104
MULTIVARIATE LOGISTIC REGRESSION AND SEPSIS SCORE

Probability of severe sepsis:

Raw Score = $-8.7 + 0.63 \times \text{NGAL quartile} + 0.41 \times \text{IL-1ra quartile} + 0.50 \times \text{PC quartile}$

Crit Care Med 2009;37:96-104
Which is the useful marker for Risk Stratification of sepsis?

- IL-6
- CRP
- suPAR
- PCT

[Image of a doctor pointing to a question mark]
Several studies have indicated that soluble urokinase-type plasminogen activator receptor (suPAR) concentrations may reflect the severity of infection and have reported that they are associated with a worse outcome.

PRELIMINARY RESULTS


Significant variables were introduced in a linear regression model: only suPAR maintained statistical significance (p=0.02)

The cutoff point for a specificity of 96% and sensitivity of 75% for suPAR was 6 ng/ml
soluble CD14 subtype (sCD14-ST) PRESEPTIN

TLR4

MD2

mCD14

LPS

LBP

Signal transduction

sCD14-ST

Phagocytosis

Phagosome

Phagolysosome

Cathepsin D

Lysosome

CD14

Presepsin

Digested with Cathepsin D

sCD14-ST or Preseptin

- Produced from granulocytes. (PCT is from systemic organs and monocytes.)
- Molecular weight: about 13kDa
- The amino acid sequence: 64 of the N-terminus of CD14
- Can not bind LPS
- Non biological activity
- Half-life in blood: 4-5hr (PCT: 20-24hr)
  - Blood level Increases 2hr after the onset of sepsis, reaches a peak in 3hr, and decreases in 4-8hr
  - IL-6 increases 3hr after the onset.
Presepsin (soluble CD14 subtype) and procalcitonin levels for mortality prediction in sepsis: data from the Albumin Italian Outcome Sepsis trial

<table>
<thead>
<tr>
<th></th>
<th>ICU survival</th>
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<tbody>
<tr>
<td></td>
<td>AUC (95% CI)</td>
<td>Optimal cutoff</td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
<td>PPV (%)</td>
<td>NPV (%)</td>
<td>LR</td>
<td>LR</td>
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<tr>
<td>Presepsin</td>
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<tr>
<td>Day 1</td>
<td>0.69</td>
<td>1631</td>
<td>66.7</td>
<td>74.0</td>
<td>71</td>
<td>70</td>
<td>2.56</td>
<td>0.45</td>
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<td></td>
<td>(0.58 to 0.79)</td>
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<tr>
<td>Day 2</td>
<td>0.70</td>
<td>1718</td>
<td>69.4</td>
<td>73.5</td>
<td>72</td>
<td>71</td>
<td>2.62</td>
<td>0.42</td>
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<td>(0.59 to 0.87)</td>
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<tr>
<td>Day 7</td>
<td>0.74</td>
<td>1606</td>
<td>72.0</td>
<td>70.0</td>
<td>71</td>
<td>71</td>
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<td>(0.64 to 0.84)</td>
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<td>Procalcitonin</td>
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<tr>
<td>Day 1</td>
<td>0.56</td>
<td>14.27</td>
<td>60.4</td>
<td>58.0</td>
<td>58</td>
<td>60</td>
<td>1.44</td>
<td>0.68</td>
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<td>(0.44 to 0.68)</td>
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<td>Day 2</td>
<td>0.55</td>
<td>8.88</td>
<td>60.4</td>
<td>55.1</td>
<td>57</td>
<td>59</td>
<td>1.35</td>
<td>0.72</td>
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<td>(0.44 to 0.67)</td>
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<tr>
<td>Day 7</td>
<td>0.64</td>
<td>1.51</td>
<td>56.0</td>
<td>74.0</td>
<td>68</td>
<td>63</td>
<td>2.15</td>
<td>0.59</td>
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<td></td>
<td>(0.54 to 0.75)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SOFA score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>0.69</td>
<td>9</td>
<td>65.3</td>
<td>68.8</td>
<td>68</td>
<td>66</td>
<td>2.09</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>(0.59 to 0.80)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>0.67</td>
<td>8</td>
<td>73.9</td>
<td>54.2</td>
<td>61</td>
<td>68</td>
<td>1.61</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>(0.56 to 0.78)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>0.75</td>
<td>7</td>
<td>59.6</td>
<td>83.0</td>
<td>78</td>
<td>67</td>
<td>3.50</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>(0.65 to 0.85)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Two-way ANOVA

- Survival: p=0.004
- Time: p=0.22
- Interaction: p=0.03

• non-survivors
Which is the useful marker for Guiding Therapy of sepsis?

- IL-6
- CRP
- EAA
- PCT
- TNF-α
# Biomarker guided antibiotic therapy

<table>
<thead>
<tr>
<th>Trial</th>
<th>Sample size, N</th>
<th>Rate of exclusion, N(%)</th>
<th>Infections Community/nosocomial, N/N</th>
<th>Pneumonia, N</th>
<th>PCT assay</th>
<th>Minimum duration AB therapy</th>
<th>Decision to start antibiotics (no AB), PCT/control</th>
<th>Duration of antibiotic therapy, PCT/control, days</th>
<th>Overruling PCT algorithm, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Svoboda, 2007 [47]</td>
<td>72</td>
<td>381 (84)</td>
<td>0/72</td>
<td>NA</td>
<td>PCT-Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nobre, 2008 [45]</td>
<td>ProSEP 79</td>
<td>203 (72)</td>
<td>53/26</td>
<td>52</td>
<td>TRACE</td>
<td></td>
<td></td>
<td>8(4-27)/14(6-39)</td>
<td>19%</td>
</tr>
<tr>
<td>Schroeder, 2009 [48]</td>
<td>27</td>
<td>98 (78)</td>
<td>0/27</td>
<td>8</td>
<td>PCT LIA</td>
<td>yes</td>
<td></td>
<td>6.6±1.1/8.3±0.7</td>
<td></td>
</tr>
<tr>
<td>Stolz, 2009 [49]</td>
<td>ProVAP 101</td>
<td>63 (38)</td>
<td>0/101</td>
<td>101</td>
<td>TRACE</td>
<td>yes</td>
<td></td>
<td>10(6-16)/15(10-23)</td>
<td>16%</td>
</tr>
<tr>
<td>Hochreiter, 2009 [50]</td>
<td>ProSICU 110</td>
<td>285 (72)</td>
<td>0/110</td>
<td>43</td>
<td>PCT LIA</td>
<td></td>
<td></td>
<td>5.9±1.7/7.9±0.5</td>
<td></td>
</tr>
<tr>
<td>Bouadma, 2010 [46]</td>
<td>PRORATA 601</td>
<td>685 (52)</td>
<td>326/275</td>
<td>354</td>
<td>TRACE</td>
<td>yes</td>
<td></td>
<td>4(1.7%)/15(4.8%)</td>
<td></td>
</tr>
<tr>
<td>Jensen, 2011 [51]</td>
<td>PASS 1200</td>
<td>3 (0.3)</td>
<td>480/720</td>
<td>666</td>
<td>TRACE</td>
<td></td>
<td></td>
<td>6(3-11)/4(3-10)</td>
<td>17.3%</td>
</tr>
</tbody>
</table>
PCT guidance in patients with severe sepsis and septic shock? (ProSEP study)

Inclusion criteria

• Patient suspected of severe sepsis or septic shock
• Initiation of antibiotic therapy < 48hr

Exclusion criteria

• High-risk bacteria (P. aeruginosa, A. baumanii)
• Infection known to require prolonged antibiotic therapy (e.g. endocarditis, deep abscesses, osteomyelitis)
• Severe immune suppression/neutropenia

Nobre et al. Am J Respir Crit Care Med 2008
Suspicion of severe sepsis or septic shock*

Antibiotic therapy

PCT D1

PCT D5

PCT decrease < 90% on D5

Daily PCT measurement

PCT decrease > 90% on D5**

PCT decrease > 90% on Dx**

STOP antibiotics

STOP antibiotics

• in non-complicated infections
** and patient stable
Probability to have antibiotics stopped

Time to antibiotic discontinuation (days)

% patients without antibiotics

PCT
controls

n=68
HR: 1.9 (1.2-3.1)
p=0.009

Nobre et al. Am J Respir Crit Care Med 2008
PCT-guided shortening of antibiotic treatment duration does not affect outcome

<table>
<thead>
<tr>
<th></th>
<th>Control (n=37)</th>
<th>PCT (n=31)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-day mortality</td>
<td>16.2%</td>
<td>16.1%</td>
<td>0.74</td>
</tr>
<tr>
<td>Clinical cure</td>
<td>83.8%</td>
<td>90.3%</td>
<td>0.33</td>
</tr>
<tr>
<td>Nosocomial infection</td>
<td>29.7%</td>
<td>22.6%</td>
<td>0.20</td>
</tr>
<tr>
<td>Infection replace, %</td>
<td>2.7%</td>
<td>3.2%</td>
<td>0.70</td>
</tr>
</tbody>
</table>
Use of procalcitonin to reduce patients’ exposure to antibiotics in intensive care units (PRORATA trial): a multicentre randomised controlled trial


Overruling 53%
1315 patients assessed for eligibility

630 randomized

311 assigned to Procalcitonin Group
4 withdrew consent
307 Included in analysis (1 lost to follow-up on day 15)

319 assigned to Control Group
4 withdrew consent 1 randomized twice
314 Included in analysis (1 lost to follow-up on day 22)

685 ineligible
158 had expected ICU stay <3 days
138 had SAPS II >65
104 had received AB for >24 hours
99 required prolonged therapy
63 not enrolled for logistic reasons
46 had do-not-resuscitate orders
31 were neutropenic
15 had no medical insurance
12 had been enrolled in other studies
10 refused consent
9 excluded for other reasons

Bouadma et al. Lancet 2010
Use of procalcitonin to shorten antibiotic exposure in ICU patients: the ProRata trial

- **Control**
- **PCT-guided**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Control</th>
<th>PCT-guided</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>9.9</td>
<td>6.1</td>
</tr>
<tr>
<td>CAP</td>
<td>10.6</td>
<td>5.6</td>
</tr>
<tr>
<td>VAP</td>
<td>9.4</td>
<td>7.3</td>
</tr>
<tr>
<td>Abdominal infection</td>
<td>10.8</td>
<td>8.1</td>
</tr>
<tr>
<td>UTI</td>
<td>14.5</td>
<td>7.4</td>
</tr>
<tr>
<td>(+) Blood cultures</td>
<td>12.8</td>
<td>9.8</td>
</tr>
</tbody>
</table>

- **N**
  - All patients: 314 (Control), 307 (PCT-guided)
  - CAP: 101 (Control), 79 (PCT-guided)
  - VAP: 66 (Control), 75 (PCT-guided)
  - Abdominal infection: 20 (Control), 14 (PCT-guided)
  - UTI: 18 (Control), 24 (PCT-guided)
  - (+) Blood cultures: 53 (Control), 55 (PCT-guided)

Bouadma et al. Lancet 2010
Use of procalcitonin to shorten antibiotic exposure in ICU patients: the ProRata trial

Bouadma et al. Lancet 2010

Probability of survival, %

Days after inclusion

Control group (n=319)
Procalcitonin (n=311)

p = n.s.
PCT guidance: duration of antibiotic therapy

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>WMD (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bouadma et al</td>
<td>2010</td>
<td>-3.80 (-4.83, -2.77)</td>
<td>21.08</td>
</tr>
<tr>
<td>Hochreiter et al</td>
<td>2009</td>
<td>-2.00 (-2.46, -1.54)</td>
<td>24.07</td>
</tr>
<tr>
<td>Schroeder et al</td>
<td>2009</td>
<td>-1.70 (-2.39, -1.01)</td>
<td>23.06</td>
</tr>
<tr>
<td>Stolz et al</td>
<td>2009</td>
<td>-5.00 (-6.13, -3.87)</td>
<td>20.44</td>
</tr>
<tr>
<td>Nobre et al</td>
<td>2008</td>
<td>-4.00 (-6.64, -1.36)</td>
<td>11.36</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>-3.15 (-4.35, -1.95)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

NOTE: Weights are from random effects analysis
28-day mortality, PCT guidance vs. control

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>OR (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jensen et al</td>
<td>2011</td>
<td>0.97 (0.76, 1.24)</td>
<td>59.98</td>
</tr>
<tr>
<td>Bouadma et al</td>
<td>2010</td>
<td>1.05 (0.71, 1.55)</td>
<td>22.70</td>
</tr>
<tr>
<td>Hochreiter et al</td>
<td>2009</td>
<td>0.99 (0.43, 2.32)</td>
<td>4.87</td>
</tr>
<tr>
<td>Schroeder et al</td>
<td>2009</td>
<td>0.91 (0.15, 5.58)</td>
<td>1.11</td>
</tr>
<tr>
<td>Stolz et al</td>
<td>2009</td>
<td>0.59 (0.22, 1.59)</td>
<td>4.65</td>
</tr>
<tr>
<td>Nobre et al</td>
<td>2008</td>
<td>0.99 (0.27, 3.63)</td>
<td>2.09</td>
</tr>
<tr>
<td>Svoboda et al</td>
<td>2007</td>
<td>0.58 (0.21, 1.57)</td>
<td>4.60</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>0.96 (0.79, 1.15)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

A strategy with escalation of broad-spectrum antimicrobials guided by daily procalcitonin measurements

“Alert procalcitonin”:
1. At baseline, PCT ≥ 1.0 ng/mL.
2. PCT ≥ 1.0 ng/mL that was not decreasing at least 10% from the previous day.

Overruling 17.9%
Procalcitonin-guided interventions against infections to increase early appropriate antibiotics and improve survival in the intensive care unit: A randomized trial


<table>
<thead>
<tr>
<th>Consumption of Antimicrobials</th>
<th>Standard-of-Care-Only (n = 596)</th>
<th>Procalcitonin-Guided (n = 604)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin/tazobactam used within 28 days (DDD)</td>
<td>1893</td>
<td>2925</td>
<td>—</td>
</tr>
<tr>
<td>Proportion of days² followed when piperacillin/tazobactam was used</td>
<td>0.00 (0.00–0.33)</td>
<td>0.11 (0.00–0.56)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Meropenem used within 28 days (DDD)</td>
<td>2174</td>
<td>2480</td>
<td>—</td>
</tr>
<tr>
<td>Proportion of days² followed when meropenem was used</td>
<td>0.00 (0.00–0.00)</td>
<td>0.00 (0.00–0.07)</td>
<td>.23</td>
</tr>
<tr>
<td>Cefuroxime used within 28 days (DDD)</td>
<td>4369</td>
<td>3390</td>
<td>—</td>
</tr>
<tr>
<td>Proportion of days² followed when cefuroxime was used</td>
<td>0.11 (0.00–0.39)</td>
<td>0.04 (0.00–0.29)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Ciprofloxacin used within 28 days (DDD)</td>
<td>6210</td>
<td>8382</td>
<td>—</td>
</tr>
<tr>
<td>Proportion of days² followed when ciprofloxacin was used</td>
<td>0.21 (0.00–0.71)</td>
<td>0.33 (0.04–0.88)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Number (%) intensive care unit days spent with at least three antimicrobials</td>
<td>2721 (57.7%)</td>
<td>3570 (65.5%)</td>
<td>.002</td>
</tr>
</tbody>
</table>

ICU stay

P< 0.004

Overall survival (%)

Hazard ratio: 0.98 (95% CI: 0.83-1.16)
Procalcitonin vs. Standard-of-care-only

Days since enrolment
Use of low procalcitonin levels or similar biomarkers to assist the clinician in the discontinuation of empiric antibiotics in patients who initially appeared septic, but have no subsequent evidence of infection (grade 2C).

... no evidence demonstrates that this practice reduces the prevalence of antimicrobial resistance.

... clinical experience with this strategy is limited and the potential for harm remains a concern.
Sepsis Trials: Precision Medicine

• Use current knowledge to reduce the heterogeneity and noise.
• Better genotype of sepsis: Pharmacogenomic approach.
• Better phenotype of sepsis:
  – Theragnostic approach
  – Immunological status
  – Specific organ dysfunction
Beyond single-marker analyses: mining whole genome scans for insights into treatment responses in severe sepsis

Effect of Genetic Combination Markers on Response to treatment

Man M et al. The Pharmacogenomics Journal (2012), 1-9
Theragnostics

• Treatment strategy for individual patients, that associates both a **diagnostic test** that identifies patients most likely to be helped or harmed by a new medication, and **targeted drug therapy** based on the test results.

• The key application is identification of subgroups of patients presenting a profile likely to give a positive response to a given treatment.
Evaluating the Use of Polymyxin B Hemoperfusion in a Randomized controlled trial of Adults Treated for Endotoxemia and Septic shock
Polymyxin B Immobilized Fiber Column:
No systemic side effects since PMX does not enter circulation
Endotoxin

Diagnostic and Prognostic Implications of Endotoxemia in Critical Illness: Results of the MEDIC Study

John C. Marshall,† Debra Foster,‡ Jean-Louis Vincent,† Deborah J. Cook,§ Jonathan Cohen,‖ R. Phillip Dellinger,§∥ Steven Opal,§ Edward Abraham,§ Stephen J. Brett,‖ Terry Smith,§ Sangeeta Mehta,§ Anastasia Derzko,§ and Alex Romanschin§

JID 2004

<table>
<thead>
<tr>
<th>Test</th>
<th>Low (&lt;0.40)</th>
<th>Intermediate (0.40–0.60)</th>
<th>High (≥0.60)</th>
<th>Levels for trends of association, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>APACHE II, mean ± SD (IQR)</td>
<td>13.3 ± 8.7 (7.0, 17.0)</td>
<td>15.3 ± 9.6 (8.0, 21.0)‖</td>
<td>17.6 ± 9.9 (10.0, 24.0)§</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Admission MOD, mean ± SD (IQR)</td>
<td>3.4 ± 3.2 (1, 5)</td>
<td>3.8 ± 3.3 (1.0, 5.3)</td>
<td>4.6 ± 3.6 (2.0, 7.0)§</td>
<td>.0001</td>
</tr>
<tr>
<td>Admission SOFA, mean ± SD (IQR)</td>
<td>4.3 ± 3.6 (2.0, 6.0)</td>
<td>4.9 ± 3.9 (2.0, 7.0)</td>
<td>5.7 ± 4.1 (3.0, 8.0)§</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Shock, %</td>
<td>11.6</td>
<td>20.5‖</td>
<td>22.7§</td>
<td>.0004</td>
</tr>
<tr>
<td>PaO2:FiO2 ratio, mean ± SD (IQR)</td>
<td>253 ± 111 (185, 322)</td>
<td>215 ± 98 (145, 293)§</td>
<td>205 ± 102 (121, 280)§</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>WBC &lt;4 or &gt;12 ×10³ cells/mm³, %</td>
<td>41.9</td>
<td>48.1</td>
<td>56.5§</td>
<td>.0021</td>
</tr>
</tbody>
</table>
What makes EUPHRATES unique?

- Theranostic trial
  - biomarker positivity + clinical criteria required for enrollment
- Blinded-device trial
- Small number of highly productive sites
- No SIRS criteria
- Relatively small number of patients compared to other trials
Patient selection - EAA™

Personalised medicine: future vision

- Diagnostic test positive: likely to benefit from treatment
- Diagnostic test negative: Unresponsive to therapy
Reduced mHLA-DR expression as a global biomarker of sepsis-associated immunosuppression
![Graph A: Monocytic HLA-DR (mAb/cell) vs. Study Day](image)

![Graph B: TNF-α (pg/ml) vs. Study Day](image)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>GM-CSF Group (n = 19)</th>
<th>Placebo Group (n = 19)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>64.0 ± 13.6</td>
<td>63.3 ± 14.2</td>
<td>NS*</td>
</tr>
<tr>
<td>Sex, male (%)</td>
<td>16/19 (84)</td>
<td>15/19 (79)</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index</td>
<td>27.4 ± 6.3</td>
<td>26.5 ± 4.8</td>
<td>NS*</td>
</tr>
<tr>
<td>Septic shock at baseline (%)</td>
<td>11/19 (58)</td>
<td>10/19 (53)</td>
<td>NS</td>
</tr>
<tr>
<td>Major source of sepsis at baseline (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>11/19 (58)</td>
<td>10/19 (52)</td>
<td>NS</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>6/19 (32)</td>
<td>5/19 (26)</td>
<td>NS</td>
</tr>
<tr>
<td>Other</td>
<td>2/19 (11)</td>
<td>4/19 (21)</td>
<td>NS</td>
</tr>
<tr>
<td>Mortality rate at study Day 28 (%)</td>
<td>3/19 (16)</td>
<td>4/19 (21)</td>
<td>NS</td>
</tr>
<tr>
<td>Days on ICU until study inclusion</td>
<td>6.0 ± 3.3</td>
<td>8.47 ± 8.9</td>
<td>NS*</td>
</tr>
<tr>
<td>Length of ICU stay, days</td>
<td>40.9 ± 26.1</td>
<td>52.1 ± 39.6</td>
<td>NS*</td>
</tr>
<tr>
<td>Total intrahospital stay, days</td>
<td>38.8 ± 32.6</td>
<td>68.9 ± 45.6</td>
<td>NS*</td>
</tr>
<tr>
<td>Need for RRT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARF at baseline (%)</td>
<td>12/19 (63)</td>
<td>11/19 (58)</td>
<td>NS</td>
</tr>
<tr>
<td>Days on RRT</td>
<td>14.4 ± 10.2</td>
<td>11.5 ± 10.2</td>
<td>NS*</td>
</tr>
<tr>
<td>Time on ventilator, Days 1–9, hours</td>
<td>147.9 ± 102.8</td>
<td>207.2 ± 57.5</td>
<td>0.037*</td>
</tr>
</tbody>
</table>
A Randomized, Double-Blind, Placebo-Controlled, Phase 2b Study to Evaluate the Safety and Efficacy of Recombinant Human Soluble Thrombomodulin, ART-123, in Patients With Sepsis and Suspected Disseminated Intravascular Coagulation*  

Jean-Louis Vincent, MD, PhD, FCCM¹; Mayakonda K. Ramesh, MS²; David Ernest, MBBS³;  

No major safety concerns  

p= 0.17
A Randomized, **Double-Blind**, Placebo-Controlled, Four-Arm, Parallel-Group, Proof of Concept, and Dose-Finding Adaptive **Phase 2a/2b Study** to Investigate the Safety, Tolerability and Efficacy and Effect on Quality of Life of **Human Recombinant Alkaline Phosphatase** in the Treatment of Patients With **Sepsis-Associated Acute Kidney Injury**

**Sepsis Trial Of** alkaline **Phosphatase in Acute Kidney Injury**

Protocol version 1.0_27May2014
Study Notification Letter version 1.0_30Sep2014

AM-PHARMA and PPD
Conclusiones

- Los biomarcadores complementan al juicio clínico en el diagnóstico y estratificación de riesgo en la sepsis.
- Los algoritmos de decisión clínica que incluyen biomarcadores pueden tener utilidad para reducir el uso de ABX.
- El fenotipado de cada episodio de sepsis mediante biomarcadores es un requisito en los nuevos estudios.
THANK YOU

rferrer@mutuaterrassa.es