Increasing number of organ dysfunctions is an excellent predictor of in-hospital mortality in emergency department patients with suspected infection: an internal and external prospective validation study

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POSTER PRESENTATIONS

P1
Validation of a novel surveillance paradigm for ventilator-associated events

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Background: Reliable surveillance methods are indispensable for benchmarking of healthcare-associated infection rates. The National Healthcare Safety Network (NHSN) recently introduced surveillance of ventilator-associated events (VAE), including ventilator-associated conditions (VAC) [1]. This new algorithm is amenable to automated implementation and strives for more consistent interpretation. We assess the feasibility and reliability of automated implementation.

Methods and tools: Retrospective analysis of an ICU cohort with acceptable assessment of ventilator-associated pneumonia (VAP) in two academic medical centers (January 2011 to June 2012). The algorithm was electronically implemented as specified by the NHSN using minute-to-minute ventilator data. Two minor modifications were developed to improve stability and comparability with manual surveillance (10th percentile and intermittent ventilation). Concordance was assessed between the algorithms and prospective surveillance. Attributable mortality of VAC was estimated by multivariable competing-risk survival analysis.

Results: Two thousand and eighty patients contributed 2,296 episodes of mechanical ventilation (MV). VAC incidence was 10.0/1,000 MV days. Prospective surveillance identified 8 VAP cases/1,000 MV days. The original VAC algorithm detected 32% (38/115) of patients affected by VAP; positive predictive value was 25% (38/152). Using the 10th percentile identified the same number of VAC cases, but only 116 were identical. VAC incidence was 24.9/1,000 MV days with the intermittent ventilation modification. Concordance between the original algorithm and the modified versions was suboptimal. Estimates of attributable mortality varied by implementation: original VAC subdistribution hazard ratio (sdHR) = 4.33, 10th percentile sdHR = 6.26 and intermittent ventilation sdHR = 2.40.

Conclusions: Concordance between manual VAP surveillance and the VAE algorithm was poor. Although electronic implementation of the VAE algorithm was feasible, small variations considerably altered the events detected and their effect on mortality. Using the current specifications, comparability across institutions using different electronic or manual implementations remains questionable.

Reference

P2
Acute kidney injury decreases long-term survival over a 10-year observation period

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Background: We hypothesized that one single episode of acute kidney injury (AKI) reduces long-term survival compared with no acute kidney injury (No AKI) following recovery from critical illness.

Materials and methods: A prospective cohort of 2,010 patients admitted to the ICU between 2000 and 2009 at a provincial referral hospital was followed to determine whether AKI influences long-term survival.

Results: Of the 1,844 eligible patients, 18.4% had AKI stage 1, 12.1% had stage 2, 26.5% had stage 3, and 43.0% had No AKI, using the KDIGO classification. The mean and median follow-up time was 8.1 and 8.7 years. The 28-day, 1-year, 5-year and 10-year survival rates were 59.6%, 44.9%, 37.4%, and 33.4%, in patients with any AKI (stage 1, stage 2, stage 3), which was significantly worse compared with the critically ill patients with no AKI at any time (P < 0.01). The adjusted 10-year mortality risk associated with AKI was 1.44 (95% CI = 1.2 to 1.7) among 28-day survivors. Patients who had mild AKI (stage 1) had significantly worse survival at 28 days, 1 year, 3 years, 5 years and 10 years compared with No AKI (P < 0.01) (Figure 1A). Patients with sepsis and AKI who survived 28 days had significantly poorer 5-year and 10-year survival compared with nonseptic AKI (P < 0.01) (Figure 1B).

Conclusions: Patients with one episode of mild (stage 1) AKI have significantly lower survival rates over 10 years than critically ill patients without AKI. The causes and mechanisms of this association warrant further careful study. Close medical follow-up of these patients may be warranted and mechanistic research required understanding how AKI influences distant events.

P3
Heparin-binding protein improves prediction of severe sepsis in the emergency department

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Critical Care 2013, 17(Suppl 4):P3; doi:10.1186/cc12904

Background: Septic shock and sepsis are major causes of death worldwide, and patients with severe sepsis have a mortality rate of 18-30%. The pathophysiological mechanisms that lead to the development of sepsis are not fully understood. Recent studies have demonstrated the crucial role of microbial products in the induction of systemic inflammatory response syndrome (SIRS) and sepsis, and the heparin-binding protein (HBP) has been shown to modulate the ability of neutrophils to respond to bacterial lipopolysaccharides (LPS) by up-regulating the expression of adhesion molecules. The aim of this study was to investigate whether HBP improves prediction of severe sepsis in the emergency department.

Methods: A prospective cohort study was conducted at a university hospital emergency department. Patients admitted with severe sepsis were included. The diagnosis of severe sepsis was based on the criteria of the International Sepsis Forum. Blood samples were collected upon admission, and the plasma levels of HBP were measured using an enzyme-linked immunosorbent assay (ELISA). The area under the receiver operating characteristic (ROC) curve was used to compare the diagnostic performance of HBP with other biomarkers.

Results: A total of 100 patients were included in the study, of whom 60 had severe sepsis. The plasma levels of HBP were significantly higher in patients with severe sepsis compared with those without severe sepsis. The area under the ROC curve for HBP was 0.85, which was significantly higher than that for other biomarkers, such as C-reactive protein and procalcitonin. The optimal cut-off value for HBP was 50 ng/mL, with a sensitivity of 85% and a specificity of 80%.

Conclusions: The plasma levels of HBP are significantly higher in patients with severe sepsis compared with those without severe sepsis. HBP improves prediction of severe sepsis in the emergency department, and is a potential biomarker for the early diagnosis of severe sepsis.

Reference

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Background: The early identification of risk of developing severe sepsis in patients with suspected infection remains a difficult challenge. We hypothesized that an elevated plasma level of heparin-binding protein (HBP), a neutrophil-secreted mediator of vascular leakage, would be a predictor of delayed clinical deterioration and progressive organ dysfunction in emergency department (ED) sepsis patients.

Materials and methods: A prospective, multicenter study in Sweden and the US was conducted of 763 patients presenting to an ED with suspected infection and signs of systemic inflammation. Based on recorded clinical and laboratory parameters and final diagnoses, patients were classified into various groups depending on the severity of the infection and inflammatory response. Plasma levels of HBP were measured and compared with levels of other standard sepsis biomarkers including procalcitonin, lactate, WBC, and C-reactive protein.

Results: The final diagnoses were severe sepsis with organ failure in 338 patients, nonsevere sepsis without organ failure in 340 patients, and no infection in 85 patients. One-hundred and forty-three patients (19%) presented without signs of severe sepsis, but developed delayed circulatory failure and/or organ dysfunction within 72 hours of enrolment. In this patient group, an elevated HBP level could predict the delayed clinical deterioration and progressive organ dysfunction. The 10-year survival from ICU admission for patients classified as having any stage of AKI according to the KDIGO classification using serum creatinine was significantly lower for patients with AKI compared to those without AKI.
Impact of the Surviving Sepsis Campaign clinical guideline of in sepsis mortality in a public health institution in Brazil

Suellen C de Aguiar, Guilherme F Garcia, Daniela N Ferreira, Francisco C de Souza, Valda MF Mendonça, Vaneza F Ribeiro, Lívia M Ferreira, Flávio D Capanema, Luana C de Carvalho, Marina F de Gomes

Comissão Central de Protocolos Clínicos, Fundação Hospitalar do Estado de Minas Gerais, Belo Horizonte, MG, Brazil

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Background: Sepsis is the principal cause of mortality in intensive therapy units (ITUs) around the world [1]. Several international organizations created in 2002 the Surviving Sepsis Campaign (SSC), targeting the reduction of sepsis mortality in 25% during 5 years [2]. The Fundação Hospitalar do Estado de Minas Gerais (FHEMIG), Brazil, was incorporated in this campaign with eight hospitals (four general hospitals, one trauma hospital, one oncologic center, one infectious diseases center, one maternity hospital). The aim of this study is to evaluate the impact of using the SSC sepsis protocol in severe sepsis and sepsis shock lethality in the FHEMIG net hospitals.

Materials and methods: This is a retrospective cohort study based on eight ITU public hospitals. The inclusion criteria were patients with severe sepsis and sepsis shock according to the SSC protocol, from January 2010 to December 2012, aged older than 18 years, which had a final outcome of hospital discharge or death. The sepsis lethality was compared annually from 2010 to 2012. Since 2010, the implementation of educative and managerial measures was based on the SSC guidelines: auditing of medical charts; education in sepsis care; issue of booklet and posters about sepsis; inclusion of sepsis information in the medical residence program; and collaboration of hospital directors in monitoring and giving information about sepsis; inclusion of sepsis in the hospital committee. Data were collected and analyzed on EPIINFO software, using ANOVA test for comparisons with precision of 95%.

Results: In the period of 3 years, 1,698 severe sepsis and sepsis shock patients were registered and 1,152 (67.84%) died. We verified a reduction of 12% (P = 0.0073) on lethality global. Hospitals 2 and 6 had a significant reduction on lethality, of 35% (P < 0.0001) and 17% (P = 0.0073) respectively (Table 1).

Conclusions: The sepsis lethality is still high in this institution (64.1%), compared with the Public Hospitals in Brazil (59.6%) and the world rate (30.8%) [3]. After the adoption of managerial measures based on the SSC protocol, there was a significantly reduction in lethality, but only one hospital reached the target reduction of 25% on lethality. This heterogeneity could be explained by different engagements of the professional board and directory and different patient’s profiles. The sepsis mortality is a major challenge in the world [4], and application of the SSC protocol led to a significant reduction in sepsis lethality.

Acknowledgements: The authors would like to acknowledge the assistance of the staff and local protocol team of the participant hospitals: Hospital João XXIII, Hospital Alberto Cavalcanti, Hospital Geral de Barbacena, Hospital Júlia Kubitschek, Hospital Eduardo de Menezes, Maternidade Odete Valadares, Hospital Regional João Penido and Hospital Regional Antônio Dias.

References
3. Instituto Latino Americano de Sepse. [http://www.sepsisnet.org]

P5
Passive immunotherapy of extended peritonitis as abdominal sepsis prevention
Oleandr Butyrsky*, Viktor Starosek
Department of Surgical Diseases, Crimean State Medical University, Simferopol, Ukraine

Critical Care 2013, 17(Suppl 4):P5; doi:10.1186/cc12906

Background: The outcome of extended peritonitis is determined by many factors including antimicrobial defense. Microbial invasion, surgery, and intensive therapy cause secondary immunity deficiency associated with septic complication incidence and post-surgery lethality. The great importance in initialization and supporting these processes belongs to Escherichia coli endotoxin that participates in digestive tract immunity and general immunoresistance.

Table 1(abstract P4) Severe sepsis and sepsis shock death in the eight FHEMIG hospitals, from 2010 to 2012

<table>
<thead>
<tr>
<th>Hospital</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>P value</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>Death (n)</td>
<td>Death (%)</td>
<td>Death (n)</td>
<td>Death (%)</td>
<td>Death (n)</td>
</tr>
<tr>
<td>1</td>
<td>103</td>
<td>62</td>
<td>60.2</td>
<td>120</td>
<td>66</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>52</td>
<td>88.1</td>
<td>114</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>22</td>
<td>91.7</td>
<td>33</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>68</td>
<td>57</td>
<td>83.8</td>
<td>71</td>
<td>63</td>
</tr>
<tr>
<td>5</td>
<td>64</td>
<td>50</td>
<td>78.1</td>
<td>63</td>
<td>51</td>
</tr>
<tr>
<td>6</td>
<td>94</td>
<td>75</td>
<td>79.8</td>
<td>92</td>
<td>54</td>
</tr>
<tr>
<td>7</td>
<td>39</td>
<td>31</td>
<td>79.5</td>
<td>56</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td>15</td>
<td>30.6</td>
<td>38</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>364</td>
<td>72.8</td>
<td>587</td>
<td>397</td>
</tr>
</tbody>
</table>
Thirty-two patients ages 15 to 86 (male:female = 1:1) were included in the study. Introducing hyperimmune plasma (specific immunotherapy) in low immunity level patients with peritonitis after sandoglobulin H injection was effective. Growth of anti-LPS antibody titer was associated with positive dynamics of inflammation progress, and prevents abdominal sepsis. Efficacy of specific immunotherapy of abdominal sepsis was evaluated. We investigated activity of immunogenesis (production of specific antibodies) in AS in 50 patients under impact of hyperimmune plasma infusion obtained from the donors who recently endured acute inflammatory abdominal diseases. The control group was made up of 10 healthy people.

**Results:** Our investigations demonstrated that the donors’ titer of specific antibodies is evidently more according to indexes of anti-Escherichia coli, anti-Pseudomonas aeruginosa, anti-Staphylococcus aureus, anti-bacteroids, anti-peptococci immunity. Introducing hyperimmune plasma obtained from such donors evidently increases specific antibody titer in AS patients.

**Conclusions:** Introducing hyperimmune plasma (specific immunotherapy) increases titer of specific antibodies, and increased concentration of specific antibodies improves forecast of survival in AS.

**Materials and methods:** Thirty-two patients ages 15 to 86 (male:female = 1:1) were included in the study. Introducing hyperimmune plasma (specific immunotherapy) in low immunity level patients with peritonitis after sandoglobulin H injection was effective. Growth of anti-LPS antibody titer was associated with positive dynamics of inflammation progress, and prevents abdominal sepsis.

**Conclusions:** The majority of peritonitis patients have decreased competent anti-LPS antibodies, which determines the severity of the post-surgery period. Low immunity level patients need passive nonspecific immunotherapy that stimulates protective functions, blocks mechanisms of inflammation progress, and prevents abdominal sepsis.

**Efficacy of specific immunotherapy of abdominal sepsis**

Olexandr Butynsky"1, Iryna Butynsky"2

1Department of Surgical Diseases, Crimean State Medical University, Simferopol, Ukraine; 2Department of Hygiene, Crimean State Medical University, Simferopol, Ukraine

**Background:** According to the 2004 WHO Annual Report, abdominal sepsis (AS) is one of the most dangerous diseases of the 21st century. But the issue of its treatment including immunotherapy is very far from being completely solved. Our aim was the demonstration of specific immunotherapy efficacy in AS.

**Materials and methods:** We investigated activity of immunogenesis (production of specific antibodies) in AS in 50 patients under impact of hyperimmune plasma infusion obtained from the donors who recently endured acute inflammatory abdominal diseases. The control group was made up of 10 healthy people.

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**Conclusions:** Introducing hyperimmune plasma (specific immunotherapy) increases titer of specific antibodies, and increased concentration of specific antibodies improves forecast of survival in AS.
Background: Previous studies have identified that nearly 30% of patients with severe sepsis and septic shock lack a definitive microbial etiology. The characteristics and outcomes of culture negative septic shock are not well defined despite large epidemiologic studies on septic shock.

Materials and methods: Retrospective nested cohort study of 2,651 patients with culture-negative septic shock and 6,019 culture-positive septic shock patients derived from a trinational, 8,760-patient database of patients with septic shock between 1989 and 2008.

Results: In total, 30.6% of cases of septic shock cases were identified as culture-negative within the database. Patients with culture-negative septic shock (CNSS) experienced similar ICU mortality as did those with culture-positive septic shock (CPSS) (41.7% vs. 40.5%, P = 0.221) and individual overall hospital mortality (51.9% vs. 51.9%, P = 0.008) but similar mortality at 6 hours the CNSS group (odds ratio, 2.87; 95% CI, 1.78 to 4.65) and identical APACHE II scores (median 19 (IQR 6 to 54) vs. 19 (IQR 6 to 54), P = 0.237) (Tables 1 and 2).

Table 1(abstract P7): Comparison of variables of culture-positive and culture-negative septic shock (Continued)

<table>
<thead>
<tr>
<th>Variable</th>
<th>CPSS (%)</th>
<th>CNSS (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital LOS</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Missing (%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>26.3 (34.1)</td>
<td>23.1 (31.1)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>15.0</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.5 to 3,700</td>
<td>0.3 to 314.0</td>
<td></td>
</tr>
<tr>
<td>APACHE</td>
<td></td>
<td></td>
<td>0.4450</td>
</tr>
<tr>
<td>Missing (%)</td>
<td>375 (62)</td>
<td>179 (68)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>25.7 (8.1)</td>
<td>25.7 (8.3)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>25.0</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>4 to 70</td>
<td>6 to 54</td>
<td></td>
</tr>
<tr>
<td>Days to extubation</td>
<td></td>
<td></td>
<td>0.2414</td>
</tr>
<tr>
<td>Mean</td>
<td>6.5 (9.5)</td>
<td>6.2 (9.2)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>4.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.0 to 117.0</td>
<td>0.0 to 100.0</td>
<td></td>
</tr>
<tr>
<td>Days on pressors</td>
<td></td>
<td></td>
<td>0.1927</td>
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<tr>
<td>Missing (%)</td>
<td>1,878</td>
<td>892</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.6 (3.7)</td>
<td>3.3 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.0 to 54.0</td>
<td>0.0 to 34.0</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Missing (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate - baseline</td>
<td></td>
<td></td>
<td>0.2377</td>
</tr>
<tr>
<td>Missing (%)</td>
<td>5,328 (88.5)</td>
<td>2,328 (87.8)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.3 (3.8)</td>
<td>4.4 (4.5)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3.1</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.3 to 26.4</td>
<td>0.3 to 26.7</td>
<td></td>
</tr>
<tr>
<td>Lactate - 6 hours</td>
<td></td>
<td></td>
<td>0.2214</td>
</tr>
<tr>
<td>Missing (%)</td>
<td>5,132 (85.3)</td>
<td>2,204 (83.1)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.1 (3.7)</td>
<td>4.0 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2.8</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.1 to 25.8</td>
<td>0.4 to 23.1</td>
<td></td>
</tr>
<tr>
<td>Lactate - 24 hours</td>
<td></td>
<td></td>
<td>0.4919</td>
</tr>
<tr>
<td>Missing (%)</td>
<td>5,164 (85.8)</td>
<td>2,236 (84.3)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.7 (4.1)</td>
<td>4.1 (5.0)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>2.4</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.3 to 54.4</td>
<td>0.2 to 37.6</td>
<td></td>
</tr>
</tbody>
</table>

Similar to our previous findings, we identified by the second hour after onset of persistent/recurrent hypotension that the in-hospital mortality rate was significantly increased relative to receiving therapy within the first hour (odds ratio, 1.62; 95% CI, 1.21 to 2.15; P < 0.001) in the CPSS group. Following increasing delays in the administration of appropriate antimicrobial therapy over the first 6 hours after the onset of hypotension, patients in both groups experienced nearly congruent, significant increases in hospital mortality; at 6 hours the CNSS group (odds ratio, 2.87; 95% CI,
1.72 to 4.78; \( P < 0.0001 \)) and the CPSS group (odds ratio, 3.44; 95% CI, 2.17 to 5.48; \( P < 0.0001 \)) (Figure 1). Survival differences between these time intervals are not significantly different in patients with CNSS and CPSS. Conclusions: Patients with CNSS behave similarly to CPSS patients in nearly all respects. As with bacterial septic shock, early appropriate antimicrobial therapy appears to improve mortality. Earlier recognition of infection is the most obvious effective strategy to improve hospital survival. Optimal duration of therapy is not well defined among patients with CNSS. In addition to early, appropriate antimicrobial therapy, use of de-escalation strategies such as serial procalcitonin levels may be useful to determine the length of empiric broad-spectrum antimicrobial use in this population.

Table 2 (abstract P7) Comparison of major sites of infection

<table>
<thead>
<tr>
<th>Site of Infection</th>
<th>Culture-positive</th>
<th>Culture-negative</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiratory infection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n ) (%)</td>
<td>2,172 (63.9)</td>
<td>1,228 (36.1)</td>
<td></td>
</tr>
<tr>
<td>ICU LOS (median, IQR)</td>
<td>8.0 (4, 16)</td>
<td>6.7 (3, 12.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hospital LOS (median, IQR)</td>
<td>16.0 (6, 32)</td>
<td>13.0 (5, 26)</td>
<td>0.0024</td>
</tr>
<tr>
<td><strong>Gastrointestinal infection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n ) (%)</td>
<td>1,476 (58.9)</td>
<td>1,030 (41.1)</td>
<td></td>
</tr>
<tr>
<td>ICU LOS (median, IQR)</td>
<td>6.5 (3, 13)</td>
<td>5.0 (3, 11)</td>
<td>0.0153</td>
</tr>
<tr>
<td>Hospital LOS (median, IQR)</td>
<td>15 (5.7, 33)</td>
<td>11.1 (3, 29)</td>
<td>0.0059</td>
</tr>
</tbody>
</table>

Figure 1 (abstract P7) Odds ratio of death by antibiotic delay in culture-positive and culture-negative septic shock.
study (EUDRACT # 2011-002266-20) was to determine the effect of SRT2379 on the inflammatory responses in normal healthy male subjects after exposure to LPS.

Materials and methods: This single-blind, placebo-controlled study consisted of four treatment arms (n = 8 per arm): (1) oral SRT2379 50 mg; (2) oral SRT2379 250 mg; (3) oral SRT2379 1000 mg; and (4) placebo. All subjects received a single dose of study drug on day 1 followed by intravenous LPS 4 hours later. Laboratory parameters of inflammation along with assessment of clinical signs, safety assessments, and pharmacokinetic measurements were recorded at baseline and after LPS administration.

Results: SRT2379 was well tolerated. Adverse events were similar across all treatment groups and were predominantly as expected with LPS administration. Pharmacokinetic exposures increased in a dose-dependent manner. SRT2379 did not significantly impact cytokine release as compared with placebo. TNFα (183.52, 177.57, 123.84 vs. 195.30 pg/ml for groups 1, 2, 3, vs. group 4, respectively, P > 0.05), IL-6 (195.25, 237.51, 180.26 vs. 250.08 pg/ml, respectively, P > 0.05), IL-17 (3.88, 2.59, 6.42 vs. 8.09 pg/ml, respectively, P > 0.05), IL-8 (126.11, 105.25, 110.56 vs. 108.77 pg/ml, respectively, P > 0.05), and IL-10 (12.61, 13.03, 40.40 vs. 11.90 pg/ml, respectively, P > 0.05). SRT2379 also had no impact on vital signs, leukocyte counts, or coagulation activation markers compared with placebo.

Conclusions: Although SRT2379 suppresses inflammatory markers in preclinical experiments, we were unable to demonstrate a similar impact in this human model of endotoxemia. This may be due to potency or exposure issues, with the compound. SRT2379 terminated for further clinical development. More promising candidates are being identified for future clinical exploration.

Reference

P11

**Bacteriological profile and antimicrobial sensitivity pattern of blood culture isolates among septicemia-suspected children at Tikur Anbessa Specialized Hospital and Yekatit 12 Hospital, Addis Ababa, Ethiopia**

Background: Septicemia is a systemic disease caused by the spread of microorganisms and their toxins in the blood. These bloodstream infections are a major cause of morbidity and mortality in children in developing countries [1-4]. It has been confirmed by culture that is associated with clinical manifestation and systemic response [5-7]. It is crucial to continuously monitor any change in the local patterns of infection and susceptibility to various antibiotics. The aim of this study was to determine the bacteriological profile and antimicrobial sensitivity patterns among children suspected of having septicemia.

Materials and methods: A cross-sectional study involved about 201 study subjects, 110 (54.7%) were male. The majority (147, 73.1%) of them were neonates (≤28 days). The mean length of hospitalization was 11.24 days. Out of the 201 tested blood samples, blood cultures were positive in 56 (27.9%) cases (Figure 1). Gram-negative and Gram-positive bacteria constituted 51.8% and 46.4%, respectively. The most frequent pathogen found was *Staphylococcus aureus* (23.2%), followed by *Serratia marcescens* (21.4%), CoNS (19.6%), Klebsiella (16%), and *Salmonella* spp. (5.4%) and Enterobacter cloacae (3.6%) (Figure 2). The majority of bacterial isolates showed high resistance to ampicillin, penicillin, co-trimoxazole, gentamicin and tetracycline. Ciprofloxacin and nalidixic acid were the most effective antimicrobial agents for Gram-negative bacteria, while vancomycin and clindamycin for Gram-positive bacteria (Table 1). Deaths occurred in 25 (12.4%) children, out of which 13 (23.2%) had bacteremia.

Conclusions: The present study revealed that both Gram-positive and Gram-negative bacteria were responsible for bloodstream infections and the majority of the isolates were multidrug resistant. *S. aureus* and *S. marcescens* were the most common isolated bacteria from blood cultures. The alarmingly higher percentages of multidrug-resistant isolates urge us to take infection prevention measures and to conduct other large studies for appropriate empiric antibiotic choice.

Acknowledgements: The authors would like to acknowledge the technical support provided by the members of the Departments of Microbiology and Pediatrics of Tikur Anbessa Specialized and Yekatit 12 Hospitals. They also thank Mr. Joseph Kenea for his excellence statistical support. This work was supported by AHRI/ALERT and AAU.

References


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Figure 1 (abstract P11) Distribution of 56 blood culture isolates by age interval and gender.

Figure 2 (abstract P11) Distribution of blood culture isolates in children with suspected of having sepsis.
Table 1 (abstract P11) Antimicrobial resistance pattern of bacteria isolated from blood culture

<table>
<thead>
<tr>
<th>Antimicrobial drugs</th>
<th>S. aureus (n = 13)</th>
<th>S. marcescens (n = 12)</th>
<th>CoNS (n = 11)</th>
<th>Klebsiella spp. (n = 9)</th>
<th>Salmonella spp. (n = 3)</th>
<th>E. cloacae (n = 2)</th>
<th>Other GNB* (n = 3)</th>
<th>Other GPB** (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pencillin</td>
<td>92.3</td>
<td>ND</td>
<td>81.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>100</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>84.6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cindamycin</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>30.8</td>
<td>ND</td>
<td>54.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Chloramph</td>
<td>7.7</td>
<td>25</td>
<td>36.4</td>
<td>44.4</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30.8</td>
<td>0</td>
<td>18.2</td>
<td>44.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>15.4</td>
<td>ND</td>
<td>18.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>38.5</td>
<td>33.3</td>
<td>54.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>66.7</td>
<td>50</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>30.8</td>
<td>91.7</td>
<td>36.4</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>53.8</td>
<td>91.7</td>
<td>45.5</td>
<td>55.5</td>
<td>100</td>
<td>100</td>
<td>66.7</td>
<td>100</td>
</tr>
<tr>
<td>SXT</td>
<td>61.5</td>
<td>91.7</td>
<td>81.8</td>
<td>77.8</td>
<td>100</td>
<td>100</td>
<td>66.7</td>
<td>0</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>46.2</td>
<td>16.7</td>
<td>27.3</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>44.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not done; SXT, sulphamethafoxazol/trimethoprim. *Gram-negative bacteria (Acinetobacter baumannii, Enterobacter cloacae, and Pseudomonas aeruginosa). **Gram-positive bacteria (Enterococcus spp. and Streptococcus spp.).

P13
Development of a new monoclonal antibody-based point-of-care testing assay for the quantification of procalcitonin in whole blood for a rapid sepsis diagnostic
Martin Rieger, Daniela Rascher, Anton Hartmann
Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Department of Environmental Science, Research Unit Microbe-Plant Interactions, Neuherberg, Germany

Background: After recent studies of the BMBF (SepNet), sepsis causes about 150 deaths per day in Germany, making it the third leading cause of death in Germany. In acute sepsis, rapid diagnosis and rapid medication is crucial. Both as a reliable parameter for diagnosis of sepsis and for guiding the antibiotic therapy, procalcitonin (PCT) is a very sensitive available biomarker [1] and is recommended in the current guidelines [2] to be quantified under sepsis suspicion. Although there are a couple of commercially available fast analytical devices for the quantification of PCT, none of these devices completely fulfill all requirements for a point-of-care testing (POCT) device which are: bedside testing; no sample preparation (whole blood testing); simple handling with ready-to-use and single-use cartridges; and short turnaround time between analysis and medical treatment in the clinical necessary concentration range. Whereas most devices fulfill the latter requirements they are still too big for bedside testing or cannot handle whole blood.

Materials and methods: Based on newly developed monoclonal antibodies (mAbs) [3], a fast and sensitive immunoassay for the quantification of PCT in whole blood was developed and transferred to a commercially developed (not available on market) POCT device (respons®IQ) from pes diagnostysysteme GmbH.

Results: With the newly developed mAbs the achieved limit of detection for PCT in plasma and whole blood is 0.04 ng/ml and 0.05 ng/ml respectively, which is within the clinical necessary range (<0.05 ng/ml). The now established assay shows high reproducibility within 9 minutes, independent of different plasma samples due to the selection of suitable additive compounds. In a first set of leftover patient samples, the PCT-POCT assay showed good correlation (R² = 0.986, n = 14, m = 2) with the state-of-the-art technology Kryptor (BRAHMS) (D Rascher, M Rieger, HMGU, AMP, unpublished data). Moreover, in cooperation with Dr A Geerlof (HMGU), technology Kryptor (BRAHMS) (D Rascher, M Rieger, HMGU, AMP, unpublished data). This hrPCT will replace expensive (5 k€/mg) and batch-to-batch varying commercial available hrPCTs as standard reference material.

Conclusions: The assay shown here for the quantification of PCT fulfills all requirements for POCT. Within 9 minutes, PCT can be quantified near the patient’s bed in whole blood without sample preparation.

Acknowledgements: The authors thank Dr A Geerlof (HMGU) for producing recombinant PCT, Dr E Kremmer (HMGU) for producing the mAbs and Dr P Miethe from the Forschungszentrum für Medizintechnik und Biotechnologie (fzmb GmbH) for the delivery of the patient plasma samples.

References

P14
Pentoxifylline therapy among preterm neonates <1,500 g in reducing mortality from neonatal sepsis: a double-blind, randomized placebo-controlled trial
Jessamine C Sareno*, Jacinto Blas V Mantaring
Department of Pediatrics, Section of Newborn Medicine, Philippine General Hospital, Metro Manila, Philippines
Critical Care 2013, 17(Suppl 4):P14; doi:10.1186/cc12914

Background: Pentoxifylline, a xanthine derivative, has raised new interest in neonatal research due to its immunomodulatory functions and its potential role in reducing mortality from sepsis. Two small studies on a intention-to-treat basis have determined whether the use of pentoxifylline as an adjunctive therapy for sepsis in preterm neonates (~36 weeks) weighing <1,500 g will truly result in a reduction in the all-cause mortality.

Materials and methods: Preterm infants ≤1,500 g with suspected infection admitted to the NICU of a large tertiary, training, government hospital were eligible for inclusion in the study. After informed consent,
they were randomized to receive either pentoxifylline at a dose of 6 mg/kg/hour or placebo. Patients with major congenital malformations, congenital infections and severe hemorrhage were excluded from the study. Pentoxifylline was administered as a 6 ml infusion for 6 hours for 6 days. The control group received normal saline in the same manner as the pentoxifylline infusion. Patients, parents and physicians (outcome assessors) were blinded to the treatment assignments. The primary outcome was analyzed on an intention to treat basis. The primary outcome measured in the study is the occurrence of all-cause mortality between the two groups. Secondary outcomes measured include mortality from sepsis, adverse drug reactions and length of hospital stay.

Results: A total of 312 neonates are included in this interim analysis: 156 in the pentoxifylline group and 156 in the control group. Baseline characteristics were comparable between the two groups. In this analysis, there is no difference in the occurrence of death among patients in the pentoxifylline group versus the placebo group (RR: 1.08 (0.83, 1.41)). There is no statistical difference in the risk of death from septic shock (RR: 1.03 (0.67, 1.59), \( P = 1.0 \)). There was also no significant difference in the length of hospital stay in the two groups (36 days in treatment group vs. 35 days in control group, \( P = 0.910 \)). No significant adverse drug reactions were noted with pentoxifylline use.

Conclusions: Pentoxifylline as an adjunct therapy for sepsis did not show a decrease in the all-cause mortality. There is also no difference in the occurrence of death from sepsis and length of hospital stay. No adverse drug reactions were noted with pentoxifylline use.

Acknowledgements: The authors thank the neonatology fellows of the Philippine General Hospital and Ms Carmi Pitajen, RN, research assistant.

Background: Sepsis is a serious clinical condition with a considerable morbidity and mortality. Procalcitonin (PCT) is a good biomarker for early diagnosis and infection monitoring. A semi-quantitative PCT assay can be performed at the bedside and has good diagnostic value [1,2]. The present study aimed to investigate the effect of a semi-quantitative PCT test on the empirical antibiotic initiation time, the appropriateness of empirical antibiotics and mortality in septic patients.

Materials and methods: The study design was a randomized diagnostic trial, which was also a pragmatic trial. Septic patients more than 18 years old with and without signs of organ hypoperfusion or dysfunction who were admitted to Cipto Mangunkusumo Hospital emergency department in the internal medicine unit were eligible. Subjects were randomly assigned to either a semi-quantitative PCT-examined group (study group) or a control group. Semi-quantitative PCT test results will be informed to the physicians taking care of the patients. The primary outcome was 14-day mortality. Secondary outcomes were the time of initiation and appropriateness of empirical antibiotics. A Tropical Infection Consultant will assess the appropriateness of empirical antibiotics based on Pedoman Umum Penggunaan Antibiotik Departemen Kesehatan Republik Indonesia.

Results: Two hundred and five patients met the inclusion criteria. Ninety-five of 100 subjects from the study group and 102 of 105 subjects from the control group were included in the analysis (Figure 1). Both groups have equal baseline characteristics (Table 1). The mortality risk was lower in the study group (RR 0.53; 95% CI 0.36 to 0.77). The study group had greater probability to have a first dose of empirical antibiotic in less than 6 hours compared with the control group (RR 2.48; 95% CI 1.88 to 3.26). No effect was seen in appropriateness of empirical antibiotics between groups (RR 0.99; 95% CI 0.92 to 1.08) (Table 2).

Conclusions: Semi-quantitative PCT examination affects the empirical antibiotic initiation time and mortality in septic patients, but not the appropriateness of empirical antibiotics.

Figure 1 (abstract P15) Enrollment of patients and completion of the study.
Is Strongyloides stercoralis a risk factor for sepsis severity?
Patrícia Terra Alves1, Marcelo Arantes Levenhagen1, Fabiana de Almeida Araujo Santos1, Omar Pereira de Almeida Neto1, Liliane Barbosa da Silva Passos3, Cezar Augusto dos Santos1, Julia Maria Costa Cruz2, Luz Ricardo Goulart1
1Genetics and Biochemistry Institute, Federal University of Uberlândia, Brazil; 2Biomedical Sciences Institute, Federal University of Uberlândia, Brazil; 3Clinical Hospital, Federal University of Uberlândia, Brazil

Background: Sepsis is a complex disease with an initial proinflammatory profile triggered by an infection process, which is typically followed by a compensatory anti-inflammatory response, leading to immunosuppression. There are few cases in literature relating sepsis with opportunistic infections, such as strongyloidiasis, which may lead to severe clinical consequences due to hyperinfection. Human strongyloidiasis is a neglected tropical disease of major worldwide distribution, affecting millions of people. Despite of the fact that infection with Strongyloides stercoralis is usually self-limited and with low morbidity in immunocompetent individuals, it may become lethal in cases of immunosuppression, such as AIDS, corticosteroid treatment and transplantation. Our aim in this work was to investigate the presence of S. stercoralis antigens and anti-parasitic IgG in sepsis patients in a highly endemic area of strongyloidiasis.

Materials and methods: Serum samples from 27 individuals with strongyloidiasis and 27 healthy subjects were used as positive and negative controls, respectively, according to their parasitological analyses. Additionally, 27 sepsis patients were also investigated. We have used ELISA tests to detect S. stercoralis antigens and IgG anti-S. stercoralis in all three groups. The cutoff value was determined by the ROC curves obtained by Prism 5.0 software.

Results: IgG anti-S. stercoralis was detected in six patients; five under septic shock and one with sepsis. Among them, four were positive for the parasite antigen-antibody immune complex; three under septic shock and one with sepsis, demonstrating that 15% of sepsis patients were infected by the parasite, which may have significantly contributed with the hyperinfection presented by septic-shock patients (10%).

Conclusions: There are only two reports of an association between S. stercoralis infection and immunosuppression, which led to lethal sepsis cases. However, our preliminary analysis through antigen-antibody

Table 1(abstract P15) Baseline characteristics of the patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Semi-quantitative PCT-examined group, n (%)</th>
<th>Control group, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;60 years</td>
<td>28 (29.5)</td>
<td>23 (22.5)</td>
</tr>
<tr>
<td>≤60 years</td>
<td>67 (70.5)</td>
<td>79 (77.5)</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>51.4 ± 15.7</td>
<td>48.6 ± 46.0</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>42 (44.2)</td>
<td>40 (39.2)</td>
</tr>
<tr>
<td>Female</td>
<td>53 (55.8)</td>
<td>62 (60.8)</td>
</tr>
<tr>
<td>Sepsis severity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepsis</td>
<td>57 (60.0)</td>
<td>54 (52.9)</td>
</tr>
<tr>
<td>Severe sepsis and septic shock</td>
<td>38 (40.0)</td>
<td>48 (47.1)</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without comorbidities</td>
<td>20 (21.1)</td>
<td>20 (19.6)</td>
</tr>
<tr>
<td>With comorbidities</td>
<td>75 (78.9)</td>
<td>82 (80.4)</td>
</tr>
<tr>
<td>Source of infection</td>
<td></td>
<td></td>
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<tr>
<td>One source</td>
<td>82 (86.3)</td>
<td>86 (84.3)</td>
</tr>
<tr>
<td>≥2 sources</td>
<td>13 (13.7)</td>
<td>16 (15.7)</td>
</tr>
<tr>
<td>14-day mortality</td>
<td>26 (27.4)</td>
<td>53 (52.0)</td>
</tr>
</tbody>
</table>

Table 2(abstract P15) Effect of semi-quantitative procalcitonin assay on adequacy of empirical antibiotics and mortality in septic patients

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Semi-quantitative PCT assay, n (%)</th>
<th>RR (95% CI)</th>
<th>P value</th>
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</thead>
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<tr>
<td></td>
<td>Examined</td>
<td>Not examined</td>
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</tr>
<tr>
<td>Empirical antibiotic initiation time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6 hours</td>
<td>83 (87.4)</td>
<td>36 (35.3)</td>
<td>2.48 (1.88 to 3.26)</td>
</tr>
<tr>
<td>&gt;6 hours</td>
<td>12 (12.6)</td>
<td>66 (64.7)</td>
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</tr>
<tr>
<td>Appropriateness of empirical antibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appropriate</td>
<td>88 (92.6)</td>
<td>95 (93.1)</td>
<td>0.99 (0.92 to 1.08)</td>
</tr>
<tr>
<td>Inappropriate</td>
<td>7 (7.4)</td>
<td>7 (6.9)</td>
<td></td>
</tr>
<tr>
<td>14-day mortality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26 (27.4)</td>
<td>53 (52.0)</td>
<td>0.53 (0.36 to 0.77)</td>
</tr>
<tr>
<td>No</td>
<td>69 (72.6)</td>
<td>49 (48.0)</td>
<td></td>
</tr>
</tbody>
</table>

References

P16

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immune complex demonstrated that this parasitic infection might be more common in sepsis than expected. The correct diagnosis of the causal infection in sepsis may support the correct therapeutic choice, which is fundamental to avoid the continuous spread of specific pathogens/parasite triggers that will eventually lead to hyperinfection, and consequently to severe sepsis.

Acknowledgements: The authors would like to thank the patients and their families for the direct collaboration in this work, the medical staff from the ICU of the university hospital for providing the biological samples and the clinical parameters, and financial support by CNPq, CAPES, and FAPEMIG.

Conclusions: In intermediate care setting patients, the combination of FDP and PCT could be useful for an early discrimination of sepsis from non-infective SIRS. PRO-ADM, sCD14-ST, and lactate should be considered as early indicators of more intensive ward care and precocious ICU admission.

References

Table 1 (abstract P17)

<table>
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<tr>
<th>Biomarker</th>
<th>Cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>PLR</th>
<th>NLR</th>
<th>Accuracy</th>
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<tr>
<td>PRO-ADM</td>
<td>0.2 nmol/l</td>
<td>83</td>
<td>37</td>
<td>80</td>
<td>41</td>
<td>1.3</td>
<td>0.5</td>
<td>72</td>
</tr>
<tr>
<td>PCT</td>
<td>0.1 ng/ml</td>
<td>80</td>
<td>74</td>
<td>90</td>
<td>54</td>
<td>3.0</td>
<td>0.2</td>
<td>78</td>
</tr>
<tr>
<td>sCD14-ST</td>
<td>0.407 ng/ml</td>
<td>90</td>
<td>50</td>
<td>84</td>
<td>62</td>
<td>1.8</td>
<td>0.2</td>
<td>80</td>
</tr>
<tr>
<td>FDP</td>
<td>180 ng/ml</td>
<td>80</td>
<td>70</td>
<td>89</td>
<td>53</td>
<td>2.6</td>
<td>0.2</td>
<td>77</td>
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<tr>
<td>FDP + PCT</td>
<td>180 + 0.1 ng/ml</td>
<td>90</td>
<td>68</td>
<td>90</td>
<td>81</td>
<td>3</td>
<td>0.075</td>
<td>88</td>
</tr>
</tbody>
</table>

Background: More than 50% of all septic patients admitted to intensive care departments derive from intermediate care units (ICU). Biomarkers represent the most promising tool for early diagnosis of sepsis; but their accuracy in ICU has been largely disregarded. Moreover, given the complexity of the septic pathophysiology, a panel of biomarkers could be more effective than a single one. For this reason we tested acute phase protein, cell surface, vasotonous related, coagulation system, and tissue hypoxia markers in early ruling in/out of sepsis in patients suffering from systemic inflammatory response syndrome (SIRS) [2-5].

Materials and methods: This prospective observational study included all SIRS [5] patients newly admitted to a medical ward from February to May 2012. Cases were diagnosed as sepsis or non-infective SIRS by clinical examination, cultures of the biological fluid, and imaging during a 7-day follow-up. Investigators were blinded to biomarker results. Survivors at 7 and 30 days were also assessed. Samples for procalcitonin (PCT), presepsin (sCD14-ST), pro-adrenomedullin (PRO-ADM), fibrin degradation products (FDP) and lactate were collected within 4 hours of admission. Their role in predicting diagnosis and survival, alone or in combination, have been investigated by receiver operating characteristic (ROC) curve, Youden index, relative risk and binary logistic regression.

Results: Among the 60 sepsis patients (microbiological and clinical sepsis), the most common sites of infection were the lung (67%), urinary tract (17%), abdomen (5%), and skin (8%). The sepsis group had significantly higher levels of PCT, sCD14-ST and FDP than the non-infective SIRS group. The area under the ROC was 0.80, 0.78, and 0.67 for FDP, PCT, and sCD14-ST respectively. Main results are reported in Table 1: the combination of FDP and PCT detected correctly 10 more cases, the combination of FDP and PCT detected correctly 10 more cases, and the combination of FDP and PCT detected correctly 10 more cases.

Background: Sepsis still represents the leading cause of mortality among children and its etiology changes according to age, immune status and geographic location [1-4]. Prevention of this disease has key role in reducing morbidity and mortality and includes development and application of vaccines [5-7]. In 2010, pneumococcal and meningococcal C vaccines were introduced in the basic immunization schedule in Brazil. The application of these may already be influencing the etiologic profile of sepsis in childhood [7]. The evaluation of this profile, as well as the clinical manifestations and course of sepsis in the post vaccine, becomes essential for better clinical decision and effective therapeutic approach in hospitalized patients. The objective was to determine clinical manifestations, etiology and outcome of sepsis in patients admitted to a pediatric ICU.

Materials and methods: A retrospective cohort study, by collecting data from medical records of patients diagnosed with sepsis admitted to the pediatric ICU of Hospital Municipal Dr. Mario Gatti, Campinas, SP, from January 2011 to December 2012. The variables studied were: age, sex, vaccination schedule, etiologic agent identified in cultures and clinical outcome.

Results: Eighty-seven patients were included in the study (56 male, 31 female) with a mean hospital stay of 8.16 days, vasoactive drug use of 2.82 days and 5.33 days of mechanical ventilation. In total, 57/87 cultures were negative. Among the positive, the majority (21/30) was collected less than 48 hours after admission and the most frequent etiologies were: Gram-negative bacteria (10), Staphylococcus aureus (7) and Neisseria meningitidis (4). Two cultures were positive for Streptococcus pyogenes and only one for Strepctococcus pneumoniae. Twenty-four (16.1%) patients died.
Mortality was higher in patients with incomplete immunization ($P = 0.047$). Among the cases with meningococcal etiology, 3/4 were not vaccinated.

Conclusions: The clinical group of patients diagnosed with sepsis showed short time of hospitalization, use of vasoactive drugs and mechanical ventilation. Mortality was high and higher in the group of patients with incomplete immunization. Among the causative agents, it was predominantly Gram-negative bacteria and $S$. aureus, no vaccine-preventable etiologies.

References

P19
Difficulties in implementation of the project ‘HUPE against sepsis’: speaking of people who watch
Sérgio da Cunha1, Mário Castro Alvare Pérez2, Elisabete Novello Ferreira2, Luana Ferreira de Almeida3, Elane Passos Pereira Assumpção3, Paulo Vieira Damasco4, Jorge da Silva Motta5, Rogério Marques de Souza5, Viviane Silva e Silva6, Elizabeth de Andrade Marques7, Wagner Ismerim Lobão7, Irene de Souza e Silva8, Ana Alice de A Trani9, Jessica Bernardes Almeida Borges da Silva1, Julio Cesar Delgado Correia10, Catherine Valdez10, Jessica Oliveira10
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Introduction: The project ‘HUPE against sepsis’ seeks to emphasize the importance of early recognition of sepsis, in order to accelerate the implementation of measures associated with decreased mortality for severe sepsis. It is therefore important that the professionals involved in healthcare are attentive to quick detection of signs and symptoms associated with the condition. The objective was to identify the difficulties for the implementation of the protocol advocated by the Surviving Sepsis Campaign and adopted by the project ‘HUPE against sepsis’.

Materials and methods: The study was conducted in clinical medical and surgical wards, DIP, general duty, cardiac and ICUs of the Pedro Ernesto University Hospital (HUPE), totaling 11 inpatient units. In January 2013, a questionnaire was applied to doctors, nurses and nurse technicians, including effective, contractors and residents. This instrument contained closed questions, professional profile and was related to the topic in question.

Results: Fifty-one professionals participated in the study: 22 (43%) medical staff and 29 (57%) nurses. Of these, 12 were medical residents, and eight were nursing residents, all in the first year (approximately 78% of workers investigated). Most physicians (55%), 38% of nurses and 40% of nurses claimed to have greatest difficulty administering the first dose of antibiotics within up to 1 hour after the diagnosis. About 45% of doctors and 31% of nurses also reported difficulty in the distribution of materials to acquire the sepsis kit (which contains materials for deep venous puncture, invasive hemodynamic monitoring and collecting blood cultures). Physicians (41%) and nurses (40%) still reported as a problem going to the pharmacy to get the first dose of the antibiotic. Other limiting factors were also appointed: obtaining the vesical catheterization of delay (for hourly diuresis control); rapid identification of severe sepsis; printed data record of the protocol; samples of blood culture for aerobic; and peripheral venous access puncture.

Conclusion: The difficulties pointed out by the professionals investigated are common and include factors that prevent the correct and early implementation of the protocol, be they of institutional and/or professional responsibility. Seeking solutions to the problems raised allows a targeting of future actions to be developed, among them the constant updating and training of professionals involved in assistance for the patients investigated. This allows, also, the search for better institutional infrastructure appropriate to meeting the demands of the patient with severe sepsis.

Background: Neutrophils as a part of nonspecific immunity factors play a crucial role in antimicrobial resistance. Reactive oxygen species (ROS) are an important compound of the neutrophils' microbialidal action. Analysis of neutrophils' ROS production could provide valuable data on a phagocyte link of immunity [1]. A chemiluminescent (CL) assay being highly sensitive allows evaluating oxidative output of the cells in dynamics. Many studies on neutrophil CL in humans with different diseases have been published [2,3]. However, the results often vary between authors because of the lack of standardized method of CL analysis. So we have developed a methodology of neutrophils' CL analysis according to the principles of evidence-based medicine.

Materials and methods: One hundred and twenty healthy donors and 17 ICU patients with second-third-degree burns participated in this study. We held an assay on the 1st, 8th and 15th day after injury and later; 37 observations in total. To dilute blood samples we used Hank's balanced salt saline (HBSS) with glucose, pH 7.4. Luminol (Sigma-Aldrich) was dissolved in double-distilled water at 1 mM. N-formyl-methionyl-leucyl-phenylalanine (FMLP; Sigma-Aldrich) and 4-phorbol-12-myristate-13-acetate (PMA; Sigma-Aldrich) were diluted in dimethyl sulfoxide (MP Biomedicals, LLC) to make stock solutions that were dissolved in HBSS on the day of experiment. CL was evaluated by means of a chemiluminometer Lum-12 (Department of Biophysics, Moscow State University) [4].

Results: We substantiate an optimal experiment design in the context of obtaining the highest intensity of analytic signal and reproducible findings. Thus we have developed a method for evaluation of a neutrophil function, based on a step-by-step stimulation of the cells by PMA and FMLP. Using our approach, we investigated the distributions of CL characteristics for the population of 80 healthy donors. We obtained reproducible kinetic profiles with intensive flash and absent glow phase of emission in all of the samples. Profiles of ICU patients' samples showed high intensity of both flash and glow phase of emission (Figure 1). Insufficient glow phase indicated subsequent development of severe septic complications.

Figure 1 (abstract P20) Kinetics of CL response in ICU patient and donor
Conclusions: As a result we suggest a reliable and replicable method for the evaluation of a neutrophil function. Investigation of the glow phase of the emission is promising to forecast risks of septic complications; we constructed a range of values of adjusted CL glow amplitude at different neutrophil counts that indicates a low probability of SIRS and septic complications that could be useful for correction of intensive treatment tactics.

Acknowledgements: The author would like to express deepest appreciation to all those who provided the possibility to perform this research. The author wishes to thank Prof. YA Vladimirov and the team of Department of Biophysics at Moscow State University (Russia) and Dr MA Godkov for assistance and guidance with this study and for submitting of equipment and reagents. Also the author would like to thank Dr EN Kobzeva and Prof. Dr SV Smirnov for the opportunity to work with blood donors and ICU patients. Furthermore, the author would also like to acknowledge with much appreciation Dr VV Kulabukhov and the staff of the Department of Burn Resuscitation at Vishnevsky Institute of Surgery (Moscow, Russia) for their suggestions and encouragement.

References

Materials and methods: This was a retrospective matched cohort study, set in a large urban academic tertiary ED at Aarhus University Hospital, Aarhus, Denmark with approximately 56,000 patient visits annually. Adult ED patients with blood cultures obtained from 1 January through 31 December 2011. ED patients with blood culture-confirmed bacteremia were matched 1:3 to patients with negative cultures. The outcome was true bacteremia. Features of the clinical history, co-morbid illnesses, physical observations and laboratory tests were used to evaluate the performance of the clinical decision rule including calculation of the total score (Table 1). We report operating characteristics and the summary statistic for the decision rule.

Results: Among 1,526 patients, 105 (6.9%) patients were classified with true bacteremia. The sensitivity of the prediction rule was 94% (95% confidence interval (CI) 88 to 98%) and specificity 48% (95% CI 42 to 53%). Positive and negative predictive values were 37% (95% CI 32 to 44%) and 96% (95% CI 92 to 99%), respectively. The area under the receiver-operating characteristics curve was 0.83 ± 0.02 standard error (Figure 1).

Conclusions: The clinical decision rule performed well in our ED setting and is likely to be a useful supplement to clinical judgment.

Acknowledgements: The CONSIDER Sepsis Network is a collaboration of clinical researchers with an interest in sepsis at Aarhus University Hospital, Aarhus, Denmark.

Reference

P24
Increasing number of organ dysfunctions is an excellent predictor of in-hospital mortality in emergency department patients with suspected infection: an internal and external prospective validation study

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Background: Conscious assessment for organ dysfunction in infected patients is not uniformly performed since the prognostic performance of organ dysfunction has not been validated. We hypothesize that the number of organ dysfunctions is a prognostic marker in emergency department (ED) patients with suspected infection and that an increasing number of organ dysfunctions correlates with in-hospital mortality.

Materials and methods: A prospective observational study of adult (18+ years) ED patients with suspected infection presenting to one of two urban, academic medical center EDs. The inclusion criterion was a time-critical diagnosis, identification of emergency department (ED) patients at risk of bacteremia is therefore a priority. The study objective was to validate a previously published clinical decision rule for predicting a positive blood culture in ED patients with suspected infection based on minor criteria, major criteria and a total score [1].

Table 1(abstract P23) Decision rule

<table>
<thead>
<tr>
<th>Major criteria</th>
<th>Minor criteria (1 point each)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected endocarditis (3 points)</td>
<td>Age &gt;65 years</td>
</tr>
<tr>
<td>Temperature &gt;39.4°C (103.0°F) (3 points)</td>
<td>Temperature 38.3 to 39.3°C</td>
</tr>
<tr>
<td>Indwelling vascular catheter (2 points)</td>
<td>Chills</td>
</tr>
<tr>
<td></td>
<td>Vomiting</td>
</tr>
<tr>
<td></td>
<td>Hypotension (systolic blood pressure &lt;90 mmHg)</td>
</tr>
<tr>
<td></td>
<td>White blood cell count &gt;18,000 cells/mm³</td>
</tr>
<tr>
<td></td>
<td>Bands &gt;5% (in our setting, immature cells &gt;0.5%)</td>
</tr>
<tr>
<td></td>
<td>Platelets &lt;150,000 cells/mm³</td>
</tr>
<tr>
<td></td>
<td>Creatinine &gt;2.0 mg/dl (177 µl)</td>
</tr>
</tbody>
</table>

A blood culture is indicated by the rule if at least one major criterion or two minor criteria are present. Otherwise, cultures may be omitted. Points used to calculate the total score.
Figure 1 (abstract P23) Receiver operating characteristics curve (ROC) for external validation of the bacteremia prediction rule, calculated using the total score.

Area under ROC curve = 0.8319

Figure 1 (abstract P24)
Logistic regression was performed to determine the independent mortality odds. Results: Four thousand, nine hundred and fifty-two patients were enrolled at BIDMC and 483 patients at AUH. Overall mortality rates were 4% and 11% with mean ages of 58 ± 21 and 69 ± 16 years, respectively. The mortality rate increased with increasing number of organ dysfunctions: BIDMC: 0 organ dysfunctions, 0.6% mortality; 1 dysfunction, 3.3%; 2 dysfunctions, 7.8%; 3 dysfunctions, 15.9%; and ≥ 4 dysfunctions, 34.3%; and AUH: 2.2%, 6.7%, 17%, 41%, and 57% mortality (Figure 1). The number of organ dysfunctions remained an independent predictor after adjustment for age and Charlson Index (Table 1). The AUCs for the models were 0.82 and 0.87, respectively (Figure 2). The effect of specific types of organ dysfunction on mortality was largest for respiratory dysfunction (OR 3.57 (95% CI 2.5 to 5.1)) in the internal and for hematologic dysfunction (OR 33.57 (8.56 to 127.3)) in the external validation set (Table 2).

Conclusions: Using readily available criteria in the ED to assess the number of organ dysfunctions is a reliable tool in predicting in-hospital mortality in both validation sets and could assist in risk prognostication and aid with earlier, targeted therapy.

Table 1(abstract P24) Effect of number of organ dysfunctions on in-hospital mortality adjusted for age and Charlson Comorbidity score

<table>
<thead>
<tr>
<th>Number of organ dysfunctions</th>
<th>Internal validation set in-hospital mortality</th>
<th>External validation set in-hospital mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5 (2.3 to 8.6)</td>
<td>3.1 (0.9 to 10.4)</td>
</tr>
<tr>
<td>2</td>
<td>9.3 (4.8 to 18.1)</td>
<td>7.3 (2.1 to 24.7)</td>
</tr>
<tr>
<td>3</td>
<td>18.0 (8.8 to 36.9)</td>
<td>33.6 (8.56 to 127.3)</td>
</tr>
<tr>
<td>4</td>
<td>50.5 (22.0 to 115.8)</td>
<td>450.0 (8.56 to 236.2)</td>
</tr>
<tr>
<td>5</td>
<td>39.0 (8.9 to 170.7)</td>
<td>285.9 (16.9 to 483.2)</td>
</tr>
</tbody>
</table>

Data presented as OR (95% CI).

Table 2(abstract P24) Effect of organ dysfunctions on mortality adjusted for age and Charlson Index

<table>
<thead>
<tr>
<th>Variable</th>
<th>Internal in-hospital mortality</th>
<th>External in-hospital mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematologic</td>
<td>3.4 (2.2 to 5.2)</td>
<td>29.0 (7.1 to 116.9)</td>
</tr>
<tr>
<td>Respiratory</td>
<td>3.6 (2.5 to 5.1)</td>
<td>1.4 (0.8 to 2.6)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>3.2 (0.9 to 11.5)</td>
<td>18.5 (7.3 to 46.8)</td>
</tr>
<tr>
<td>Metabolic</td>
<td>3.0 (2.2 to 4.0)</td>
<td>6.7 (2.9 to 11.2)</td>
</tr>
<tr>
<td>Neurologic</td>
<td>2.4 (1.7 to 3.4)</td>
<td>8.9 (4.7 to 17.1)</td>
</tr>
<tr>
<td>Renal</td>
<td>2.1 (1.4 to 3.0)</td>
<td>5.4 (2.7 to 10.9)</td>
</tr>
<tr>
<td>Hepatologic</td>
<td>2.2 (1.4 to 3.4)</td>
<td>4.5 (1.1 to 18.2)</td>
</tr>
</tbody>
</table>

Data presented as OR (95% CI).

Lactate levels in emergency department patients across all causes of physiologic instability

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Background: Physiologic instability (PI) is a common, critical problem in the emergency department (ED) [1,2], and can have different underlying causes. The ability to determine the underlying cause of instability is paramount for early treatment and risk stratification [3]. Lactate has been shown to have prognostic value in some categories of unstable patients [4,5]. The objective of this study was to investigate how serum lactate concentrations differ across categories of PI and the association of lactate concentrations with clinical deterioration for each category.

Materials and methods: A prospective observational study of adult patients with PI at a university ED. PI was defined as lactate ≥4 mmol/L, or >5 minutes of heart rate (HR) ≥130, or respiratory rate (RR) ≥24, or shock index ≥1, or systolic blood pressure <90 mmHg. We excluded patients with no lactate measurements, isolated atrial tachycardia, seizure, intoxication, psychiatric agitation, or tachycardia due to pain. A physician retrospectively
Figure 1 (abstract P25) Lactate levels across groups of physiological instability.

Figure 2 (abstract P25) Levels of lactate across groups of physiologic instability stratified by deterioration. no/yes = deterioration present or not.

(*) Significant differences between groups (p < 0.05).
categorized PI cause. Categories were defined as septic, cardiogenic, hemorrhagic, hypovolemic, or other. The primary outcome was deterioration, defined as acute renal failure (elevated creatinine to >2x baseline levels), intubation, vasopressors, or in-hospital mortality.

Results: We identified 1,156 patients with PI and excluded 324. Of the remaining, 304 did not have lactate measurements, leaving 528 for the analysis: 302 septic, 46 cardiogenic, 29 hemorrhagic, 57 hypovolemic, and 94 with another cause of instability. The differences in lactate levels between groups were not statistically significant (Figure 1). The lactate levels were statistically different between patients who deteriorated when compared with patients who did not deteriorate in the sepsis group (3.05 mmol/l vs. 1.91 mmol/l, P < 0.0001) and the other group (2.89 mmol/l vs. 1.94 mmol/l, P = 0.002). No statistically significant differences were demonstrated for the cardiogenic, the hemorrhagic or the hypovolemic groups (Figure 2).

Conclusions: Lactate levels were not significantly different between the five groups with PI. However, in patients in the sepsis or other group, elevated lactate predicted deterioration. This was not demonstrated for the other causes of PI. This study suggests that in unstable patients lactate has the same likelihood of elevation between different causes of instability, but it may not have the same prognostic value for deterioration across underlying causes.

Acknowledgements: CONSIDER Sepsis Network is a collaboration of clinical researchers with an interest in sepsis at Aarhus University Hospital, Aarhus, Denmark.

References

P26 Rapid molecular test (SeptiFast®) reduced time for adjustment of antibiotic treatment in comparison with conventional blood cultures in critically ill sepsis patients: a randomized controlled clinical trial (preliminary results)

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Background: Sepsis is the main cause of death in ICUs all over the world. Early detection of the pathogen is essential for appropriate antimicrobial treatment.

Materials and methods: To evaluate the reduction in time of antimicrobial adjustment therapy in patients with sepsis comparing a rapid molecular test (SeptiFast®) with conventional blood cultures, a randomized controlled clinical trial was conducted between October 2012 and May 2013 in a cardiology hospital. Adult patients staying more than 48 hours in hospital with clinical suspicion of sepsis were included in the study. Blood samples were collected for cultures (Bact/ALERT® and Septifast® test immediately prior to initiation of antibiotic therapy. Patients were allocated into two groups. In the Intervention Group (GI), Septifast® results were communicated to the medical researcher and antimicrobials were adjusted. In the Control Group (GII), Septifast® results were not informed and therapy adjustment was based on the blood culture. Registered in Clinical trials.gov (NCT 01450538).

Results: Forty-six patients were included, 17 in GI and 29 in GII. Key data are shown in Table 1. In GI therapy adjustment was done in 580 minutes compared with 3,007 minutes in GII (P = 0.004).

Conclusions: The rapid molecular test (SeptiFast®) reduced the time for adjustment of antibiotic treatment in comparison with conventional blood cultures in critically ill sepsis patients.

Table 1(abstract P26) Distribution of characteristics in the two groups

<table>
<thead>
<tr>
<th></th>
<th>Intervention group (n=17)</th>
<th>Control group (n=29)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63 (46 to 75)</td>
<td>66 (39 to 85)</td>
<td>0.340</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>5 (30%)</td>
<td>10 (34%)</td>
<td>0.999</td>
</tr>
<tr>
<td>Hospital stay (mean, days)</td>
<td>32 (9 to 118)</td>
<td>31 (3 to 112)</td>
<td>0.632</td>
</tr>
<tr>
<td>APACHE II (mean)</td>
<td>17 (8 to 29)</td>
<td>17 (8 to 29)</td>
<td>0.730</td>
</tr>
<tr>
<td>Ejection fraction &lt;40%</td>
<td>8 (47%)</td>
<td>17 (58%)</td>
<td>0.545</td>
</tr>
<tr>
<td>Receiving antibiotics prior to study</td>
<td>11 (65%)</td>
<td>11 (38%)</td>
<td>0.126</td>
</tr>
<tr>
<td>Septic shock</td>
<td>9 (53%)</td>
<td>16 (55%)</td>
<td>0.999</td>
</tr>
<tr>
<td>Patients with adjustment therapy based on SeptiFast®</td>
<td>6 (35%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Patients with adjustment therapy based on blood culture</td>
<td>-</td>
<td>7 (21%)</td>
<td></td>
</tr>
<tr>
<td>Mean time (minutes) of adjustment therapy</td>
<td>580</td>
<td>3,007</td>
<td>0.004</td>
</tr>
<tr>
<td>Pathogens detected in SeptiFast®</td>
<td>5. aeruginosa (2), K. pneumonia/oxytoca (1), E. aerogenes/clonae (1), S. marcescens (1), A. baumannii (1)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pathogens detected in blood culture</td>
<td>-</td>
<td>S. aureus (4), K pneumonia (2), M. morgani (1)</td>
<td></td>
</tr>
<tr>
<td>28-day mortality</td>
<td>9 (53%)</td>
<td>17 (59%)</td>
<td>0.765</td>
</tr>
</tbody>
</table>
Comparison between inflammatory biomarkers procalcitonin, IL-6 and C-reactive protein for infection diagnosis and fever evolution in neutropenic patients, submitted to hematopoietic stem cell transplantation

Karin Schmidt Rodrigues Massaro1, Rodrigo Macedo2, Maria Aparecida Shikanai-Yasuda1, Silvia Figueiredo Costa1
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Background: Biomarkers were assessed during neutropenic fever in hematopoietic stem cell transplantation (HSCT). The objective was to assess serum values of C-reactive protein (CRP), procalcitonin (PCT) and IL-6 to identify infection in HSCT and risk factors for death.

Materials and methods: Prospective study with 296 patients submitted to autologous or allogeneic HSCT. PCT, CRP and IL-6 dosed at the following moments: febrile neutropenia, fever, 24 hours upon fever, 72 hours upon fever and long-lasting fever. Patients were classified into groups I (afebrile), II (fever of unknown origin) and III (clinically or microbiologically proven fever). ROC curves, sensitivity, specificity, and multivariate analysis were used to evaluate factors associated with death.

Results: One hundred and ninety patients had fever. Mean and median values of IL-6 at fever onset in group I with regard to group II (P = 0.013) presented significantly higher values. Levels of CRP in group I differed significantly from those found in group III (P < 0.05). Groups differed in levels of IL-6 and CRP at fever onset. Group II presented IL-6 and CRP concentrations significantly lower than group III. Cutoff values of PCT: fever onset, 24 hours upon fever, 72 hours of fever, and long-standing fever were: 0.32; 0.47; 0.46 and 0.35 µg/l. At fever onset, sensitivity was 52.3 and specificity 52.6 for infection diagnosis. Best cutoff values of CRP for fever onset, 24 hours upon fever, 72 hours upon fever and long-standing fever were: 79, 120, 108 and 72 mg/l. At fever onset, sensitivity was 55.4 and specificity was 55.1. Best cutoff values of IL-6 for fever onset, 24 hours upon fever, 72 hours upon fever and long-standing fever were: 34, 32, 16 and 9 pg/ml. At fever onset, sensitivity and specificity were: 59.8 and 59.7. In the autologous group, IL-6 presents significant values at initial moments. Independent risk factors identified in the multivariate analysis were: related donor, unrelated donor, Gram-negative infection, DHL ≥390 UI/l, urea ≥25 mg/dl and CRP ≥120 mg/l.

Conclusions: IL-6 and CRP are associated with the early diagnosis of clinically or microbiologically confirmed infection in post-HSCT febrile neutropenia. The association of the three biomarkers did not present any advantage, nor did it improve diagnostic accuracy. IL-6 was the only biomarker significantly associated at an early stage of infection when assessed only in patients submitted to autologous HSCT. The independent variables associated with death were: allogeneic transplantation, Gram-negative infection, DHL ≥390 UI/l at fever onset and urea ≥25 mg/dl at fever onset and CRP ≥120 mg/l.

P29
Sepsis-associated brain dysfunction in critically ill patients

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Background: Delirium is a common occurrence in critically ill patients and is associated with an increase in morbidity and mortality [1]. Some evidence suggests that septic patients with delirium may differ from a general critically ill population. In a subgroup analysis of the MENDS study, a benefit of dexmedetomidine sedation over lorazepam was only evident in septic patients [2]. The aim of our study was to investigate the relationship between systemic inflammation and the development of delirium in septic and nonseptic critically ill patients.

Materials and methods: We performed a cohort study in a 20-bed mixed ICU that included consecutive patients admitted for more than 24 hours. Delirium was diagnosed using the Confusion Assessment Method for the Intensive Care Unit (CAM-ICU). Coma was defined as a Richmond Agitation Sedation Scale (RASS) score of -4 or -5. Blood samples were collected within 12 hours of enrollment for determination of TNFα, soluble TNF receptor (STNFRI)-1 and STNFRI-2, IL-1β, IL-6, IL-10 and adiponectin.

Results: Seventy-eight patients were included in the study: 26 nonseptic/nondelirium (control), 13 nonseptic/delirium (delirium), 21 septic/nondelirium (septic) and 18 septic/delirium (sepsis-associated delirium) (SAD). From all analyzed biomarkers only STNFR1, STNFR2 and adiponectin were independently associated with delirium occurrence, but none of these biomarkers had a significant interaction with sepsis. In contrast, there was significantly interaction between sepsis and IL-1β suggesting that this cytokine is differentially modulated when comparing septic and nonseptic patients with delirium.
Conclusions: The association between IL-1β and delirium is different in septic versus nonseptic patients, suggesting that mechanisms which drive SAD may differ from that of nonseptic ICU delirium.

Acknowledgements: This work was funded by the NENASC project (PRONEX program CNPq/FAPESC), INCT-TM, PROCAD Sepse - CAPES and FAPESC.

References:

P30
Clinical features and prognosis of patients co-infected with HIV and tuberculosis in the ICU
Ana Carla Fecchio, Carla Ribeiro da Silva, Rodrigo T Amâncio, Denise Medeiros, Emerson C Mesquita, André M. Japiassú, Fernando Bozza
Instituto de Pesquisa Clínica Evandro Chagas, Fiocruz, Rio de Janeiro, Brazil
Critical Care 2013, 17(Suppl 4):P30; doi:10.1186/cc12930

Background: Despite advances in treatment, tuberculosis (TB) remains a global threat and is the leading co-infection among Brazilian HIV-infected patients [1-4]. Mortality of TB in the presence of sepsis and shock septic can be as high as 67%, with respiratory failure being the leading cause of ICU admission [2,3,5]. Clinical factors that enhance mortality among HIV-TB patients are yet to be explored.

Materials and methods: We retrospectively accessed data of HIV/AIDS critically ill patients from January 2007 until May 2012, at a referral infectious diseases hospital. All patients admitted to the ICU with laboratory-confirmed TB were included in the analysis. Demographic and clinical data were categorized for survivors and nonsurvivors and the results were displayed as frequency (%), median values and interquartile range.

Results: Fifty patients were included. Hospital mortality was 48%. Age was 31.5 years (26.5 to 42.75) in the survivors group versus 33.5 years (30 to 45.25) in nonsurvivors (P = 0.25), and the SAPS II score was 45.3 (37.25 to 55.75) versus 47.25 (38.75 to 54.25) (P = 0.58). The most common TB presentation was disseminated disease (56%) followed by pulmonary (44%), with no difference according to survival. The delta of days between ICU admission and beginning of TB treatment was not different between groups. Rifampicin (94%), pyrazynamide (94%), isoniazide (92%) and ethambutol (76%) were administered in the majority of patients, while fluoroquinolones and aminoglycosides were administered in 64% and 54% respectively. Nonsurvivors presented with more elapsed time since HIV diagnosis (11 ± 7) vs. 26.5 (5 to 72), P = 0.10; lower nadir CD4 count (72.5 (27.25 to 133.5) vs. 24 (14.5 to 63), P = 0.03); and HAART initiated within 30 days after admission (62% vs. 29%, P = 0.03, odds ratio 3.9 (95% CI 1.2 to 12.71)). The main reason for ICU admission was respiratory failure (70%). Nonsurvivors needed mechanical ventilation (88% vs. 48%, P = 0.006) and vasopressors (71% vs. 41%, P = 0.05) more frequently. Neurological dysfunction was more common in nonsurvivors (79% vs. 41%, P = 0.01, odds ratio 5.2 (95% CI 1.5 to 18.2)). After multivariate analysis, neurological dysfunction was associated with higher mortality, while HAART in the first 30 days of hospitalization was a protective associated factor.

Conclusions: The disseminated form was the most common presentation of TB in HIV/AIDS critically ill patients. Nonsurvivors were more prone to multiple organ dysfunction syndrome, with neurological dysfunction associated with hospital mortality. The administration of HAART within 30 days of hospitalization was associated with survival.

References:

Table 1 (abstract P31) Demographic and clinical characteristics observed in patients with severe sepsis and septic shock

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (years; IQR)</td>
<td>53 ± 19</td>
</tr>
<tr>
<td>Male gender</td>
<td>64%</td>
</tr>
<tr>
<td>Type of admission</td>
<td></td>
</tr>
<tr>
<td>Medical</td>
<td>33 (47%)</td>
</tr>
<tr>
<td>Surgical</td>
<td>39 (53%)</td>
</tr>
<tr>
<td>ICU admission source</td>
<td></td>
</tr>
<tr>
<td>Emergency room</td>
<td>34 (47%)</td>
</tr>
<tr>
<td>Surgical room</td>
<td>38 (53%)</td>
</tr>
<tr>
<td>Interval between hospital admission and ICU</td>
<td></td>
</tr>
<tr>
<td>&gt;24 hours</td>
<td>20 (28%)</td>
</tr>
<tr>
<td>&lt;24 hours</td>
<td>52 (72%)</td>
</tr>
<tr>
<td>Physical examination</td>
<td></td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>46 (63.9%)</td>
</tr>
<tr>
<td>Capillary refill time reduced</td>
<td>45 (62.5%)</td>
</tr>
<tr>
<td>Sedation</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>55 (76.4%)</td>
</tr>
<tr>
<td>No</td>
<td>17 (23.6%)</td>
</tr>
</tbody>
</table>
### Table 1 (abstract P31): Demographic and clinical characteristics observed in patients with severe sepsis and septic shock (Continued)

<table>
<thead>
<tr>
<th>Comorbidities</th>
<th>Value (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>23 (31.9%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>11 (15.3%)</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>30 (41.6%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>29 (40%)</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>7 (9.7%)</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>18 (25%)</td>
</tr>
<tr>
<td>Blood products</td>
<td>39 (54.2%)</td>
</tr>
<tr>
<td>Cardiac arrest rate during ICU stay</td>
<td>4 (2.88%)</td>
</tr>
<tr>
<td>APACHE II, median (IQR)</td>
<td>28 (18 to 34)</td>
</tr>
<tr>
<td>SOFA score</td>
<td></td>
</tr>
<tr>
<td>Initial, median (IQR)</td>
<td>3 (2 to 8)</td>
</tr>
<tr>
<td>Media, median (IQR)</td>
<td>6 (5 to 10)</td>
</tr>
<tr>
<td>Maximum, median (IQR)</td>
<td>11 (7 to 13)</td>
</tr>
<tr>
<td>Severe sepsis</td>
<td>15 (20.8%)</td>
</tr>
<tr>
<td>Septic shock</td>
<td>57 (79.2%)</td>
</tr>
<tr>
<td>ICU stay, median (days; IQR)</td>
<td>8 (4 to 15)</td>
</tr>
<tr>
<td>Hospital stay, median (days; IQR)</td>
<td>20 (8 to 40)</td>
</tr>
<tr>
<td>Positive cultures</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>12 (17%)</td>
</tr>
<tr>
<td>Sterile tissue or cavity</td>
<td>11 (15%)</td>
</tr>
<tr>
<td>ICU mortality</td>
<td>13 (18%)</td>
</tr>
<tr>
<td>Hospital mortality</td>
<td>13 (18%)</td>
</tr>
</tbody>
</table>

Demographic and clinical characteristics observed in patients with severe sepsis and septic shock in the ICU/HRTN between April 2011 and October 2012. IQR, interquartile range.

### Table 2 (abstract P31): Clinical and laboratory variables related to mortality in patients with severe sepsis and septic shock

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>0.248</td>
<td>0.05</td>
</tr>
<tr>
<td>Age</td>
<td>0.309</td>
<td>0.01</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>0.478</td>
<td>0.01</td>
</tr>
<tr>
<td>SOFA score</td>
<td>0.572</td>
<td>0.01</td>
</tr>
<tr>
<td>Fluid balance (24 hours)</td>
<td>0.350</td>
<td>0.01</td>
</tr>
<tr>
<td>Fluid balance (7 days)</td>
<td>0.590</td>
<td>0.01</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>0.548</td>
<td>0.01</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>0.266</td>
<td>0.01</td>
</tr>
<tr>
<td>PaO2/FiO2</td>
<td>0.320</td>
<td>0.01</td>
</tr>
<tr>
<td>Vasopressor agent (24 hours)</td>
<td>0.445</td>
<td>0.01</td>
</tr>
<tr>
<td>MAP (24 hours)</td>
<td>0.485</td>
<td>0.01</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.548</td>
<td>0.01</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.375</td>
<td>0.01</td>
</tr>
<tr>
<td>Steroid use</td>
<td>0.337</td>
<td>0.01</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>0.404</td>
<td>0.01</td>
</tr>
<tr>
<td>NT-pro-BNP</td>
<td>0.269</td>
<td>0.05</td>
</tr>
<tr>
<td>PCT</td>
<td>0.320</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Clinical and laboratory variables related to the mortality observed in patients with severe sepsis and septic shock in the ICU/HRTN between April 2011 and October 2012 (n = 72). Data expressed as Spearman r and P value. MAP, mean arterial pressure; NT-pro-BNP, N-terminal prohormone; PCT, procalcitonin.

### Table 3 (abstract P31): Univariate analysis of variables associated with mortality in patients with severe sepsis and septic shock

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>3.24</td>
<td>1.13 to 22.40</td>
<td>0.06</td>
</tr>
<tr>
<td>Age</td>
<td>1.05</td>
<td>0.92 to 13.70</td>
<td>0.00</td>
</tr>
<tr>
<td>MAP 24 hours</td>
<td>0.79</td>
<td>0.71 to 0.90</td>
<td>0.00</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>1.03</td>
<td>1.00 to 1.06</td>
<td>0.03</td>
</tr>
<tr>
<td>Fluid balance (24 hours)</td>
<td>1.00</td>
<td>1.00 to 1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Vasopressor use (24 hours)</td>
<td>1.18</td>
<td>0.84 to 2.80</td>
<td>0.00</td>
</tr>
<tr>
<td>Steroid use</td>
<td>5.28</td>
<td>2.30 to 11.80</td>
<td>0.00</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>5.36</td>
<td>0.97 to 29.6</td>
<td>0.05</td>
</tr>
<tr>
<td>Mechanical ventilation (I)</td>
<td>0.762</td>
<td>0.60 to 0.96</td>
<td>0.12</td>
</tr>
<tr>
<td>PEEP</td>
<td>1.24</td>
<td>0.70 to 1.70</td>
<td>0.08</td>
</tr>
<tr>
<td>PIP</td>
<td>1.22</td>
<td>1.07 to 1.39</td>
<td>0.04</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>6.29</td>
<td>2.05 to 19.2</td>
<td>0.00</td>
</tr>
<tr>
<td>PCT</td>
<td>1.14</td>
<td>0.90 to 1.30</td>
<td>0.06</td>
</tr>
<tr>
<td>Lactate</td>
<td>9.55</td>
<td>1.12 to 73.96</td>
<td>0.03</td>
</tr>
<tr>
<td>Creatinine</td>
<td>4.07</td>
<td>0.99 to 22.32</td>
<td>0.01</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>1.09</td>
<td>0.97 to 1.23</td>
<td>0.00</td>
</tr>
<tr>
<td>SOFA score</td>
<td>1.24</td>
<td>1.06 to 1.44</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Univariate analysis of variables associated with mortality observed in patients with severe sepsis and septic shock in the ICU/HRTN between April 2011 and October 2012 (n = 72). MAP, mean arterial pressure; PCT, procalcitonin; PEEP, positive end expiratory pressure; PIP, peak inspiratory pressure; t, time (days).

### Table 4 (abstract P31): Multivariate analysis of variables associated with mortality in patients with severe sepsis and septic shock

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (24 hours)</td>
<td>0.74</td>
<td>0.64 to 0.85</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fluid balance (24 hours)</td>
<td>1.00</td>
<td>1.00 to 1.01</td>
<td>0.002</td>
</tr>
<tr>
<td>Male gender</td>
<td>5.35</td>
<td>1.10 to 26.15</td>
<td>0.038</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>60.85</td>
<td>4.97 to 74</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Multivariate analysis of variables associated with mortality observed in patients with severe sepsis and septic shock in the ICU/HRTN between April 2011 and October 2012 (n = 72). CI, confidence interval; HR, hazard ratio; MAP, mean arterial pressure.

the emergency room (ER). The same intensivist team assists all patients in the ER and during ICU permanence. The principal investigator did not perform any orientation or intervention in the treatment of selected patients. Clinical (age, sex, infection focus, fluid balance, hemodialysis, use of corticosteroids, antibiotic therapy, APACHE II, SOFA), laboratory (blood cell counting, lactate, creatinine, bilirubin, glucose, cortisol, NT-proBNP, C-reactive protein (CRP), procalcitonin (PCT), Troponin I), hemodynamic (blood pressure, heart rate, left ventricular systolic function (echocardiography)) and respiratory parameters (respiratory rate, PaO2/FiO2, PEEP and peak inspiratory pressure (PIP)) were analyzed from ICU admission until discharge or death. Echocardiography was performed at 48 hours and on the 10th day after ICU admission.

**Results:** Seventy-two patients (64% male), mean age 52 ± 19 years, were consecutively included, 21% (15/72) with severe sepsis and 79% (57/72) with septic shock. Mortality was 18% (13/72), of these 21% (3/13) for severe sepsis and 79% (10/13) for septic shock. Median APACHE II score was 28 (16 to 37) and SOFA score 6 (5 to 10) (Table 1). There was positive correlation between mortality with: male gender, APACHE II, SOFA, positive 24-hour fluid balance, hemodialysis indication, corticosteroid use, leukopenia, lactate, NT-proBNP and PCT levels (Table 2). From univariate analysis, practically the same significant association with mortality was observed (Table 3). In addition, the final multivariate Cox model showed
that male gender, hypotension (first 24 hours), leukopenia and positive fluid balance (first 24 hours) had an impact on mortality (Table 4). Glycemic control and early antibiotic use were not relevant.

Conclusions: Precocious treatment, judicious fluid management and individualized care showed benefit in the treatment of patients with severe sepsis, septic shock.

P32 Risk factor for mortality associated with carbapenem-resistant Enterobacteriaceae infections
Cláudia MDM Carlinho,1 Cintia MC Giron,1 Marcos T Tanita,1 Jamile Vale1, Larissa Oliveira2, Ana P Marchi,2 Silvia F Costa2
1Universidade Estadual de Londrina, PR, Brazil; 2Universidade de São Paulo, SP, Brazil
Critical Care 2013, 17(Suppl 4):P32; doi:10.1186/cc12932

Background: Antimicrobial resistance has emerged and increased in the last 25 years, complicating the treatment of nosocomial infections, especially for extended-spectrum beta-lactamate Enterobacteriaceae (ESBL). Furthermore, disseminated use of invasive procedures, particularly in ICU patients favors the emergence of multiresistant pathogens. Moreover, Enterobacteriaceae has developed a new mechanism of antimicrobial resistance and became resistant to carbapenems. Among Enterobacteriaceae resistant to carbapenem, Klebsiella pneumoniae is the most common. These pathogens manifest resistance to most of antimicrobials tested and are associated with high mortality rates. The aim of this study is to describe epidemiologic data about nosocomial infections due to carbapenem-resistant Enterobacteriaceae and identify risk factors for death.

Materials and methods: Longitudinal study evaluating patients with infections caused by carbapenem-resistant Enterobacteriaceae, isolated from blood, urine, tracheal secretions, skin and soft tissues, treated accordingly to the Brazilian Society of Infectious Diseases guidelines, from March 2011 to December 2012. Acute Physiology and Chronic Health Evaluation (APACHE II) was calculated to evaluate severity of disease and Sequential Organ Dysfunction Assessment (SOFA) to measure organ dysfunction. Comorbidities were classified according to Charlson comorbidities index list. Patients were followed until hospital discharge.

Results: During the study period, 174 nosocomial infections caused by carbapenem-resistant Enterobacteriaceae were identified in 148 patients. All infections were microbiologically documented and 136 (78.2%) occurred in patients who were admitted to the ICU. Sepsis (17.8%), polytrauma (14.4%), cardiovascular disease (13.8%) and respiratory disease (11.5%) were the most common diagnosis. Most of the patients (78%) had one or more comorbidities according to Charlson criteria, and 57/148 (43.2%) patients had three or more comorbidities. Median APACHE II was 20.7 (7 to 38) and median SOFA at ICU admission was 7 (0 to 14). Median length of hospital stay was 43 (6 to 230) days. K. pneumoniae was the most common enterobacteria (86.8%), followed by Enterobacter spp. (8%). The mechanism of resistance was identified as Klebsiella pneumonia carbenapenemase (KPC) present in 72.2% of K. pneumoniae infections. Shock was present in 81/148 (46.6%) patients and dialysis was used in 62/148 (35.6%). Hospital mortality was 62.6% and associated mortality was 33.3%. Multivariate analysis identified dialysis and pneumonia as independent risk factors for death.

Conclusions: Many patients infected with carbapenem-resistant Enterobacteriaceae were identified, and most of them were caused by carbapenem producing bacteria. These infections were associated with high mortality rate. Shock, dialysis, pneumonia, SOFA discharged >6 and APACHE >20 were identified as independent risk factors for mortality.

P33 Rapid response team: the early identification of septic patients
Andrea Pardini1, Michele Jaures, Sandra Christina Pereira Lima Shiramizo
Hospital Israelita Albert Einstein, São Paulo, Brazil
Critical Care 2013, 17(Suppl 4):P33; doi:10.1186/cc12933

Background: Rapid response teams (RRTs) represent an intuitively simple concept: when a patient demonstrates signs of imminent clinical deterioration, a team of providers is summoned to the bedside to immediately assess and treat the patient with the goal of preventing ICU transfer, cardiac arrest, or death [1]. Patients whose condition deteriorates acutely while hospitalized often exhibit warning signs (such as abnormal vital signs) in the hours before experiencing adverse clinical outcomes. Sepsis is an illness in which the body has a severe response to bacteria or other germs. This response may be called systemic inflammatory response syndrome (SIRS) [2]. The criteria for calling the RRT are the same as/similar to symptoms of sepsis. We aimed to describe the various criteria for calling the RRT for patients who developed sepsis, initial treatment before transfer to the ICU or step-down unit and outcomes.

Materials and methods: This retrospective study was conducted in 2012 in the ICU of Hospital Israelita Albert Einstein, a general, private tertiary hospital. During the study period, the hospital had 614 beds, 6.7% (41/614) of which were in the ICU and 13.5% (83/614) were the step-down unit. We included patients 18 years of age or older diagnosed with severe sepsis and septic shock treated by the RRT and transferred to the ICU or step-down unit for study retrospectively. We excluded patients who had contraindications to cardiac resuscitation. Severe sepsis and septic shock were defined according to the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference definitions [3]. Data regarding age, gender, Simplified Acute Physiology Score II (SAPS II) [4], presence of the following comorbidities, criteria for calling the RRT, initial treatment for sepsis, length of ICU and total stay, and patient outcome were recorded.

Results: Sixty-five of 41 (63.1%) were males, 23 (35.4%) were transferred to the step-down unit and 42 (64.6%) were transferred to ICU. Their age was 64.7 ± 17.8 years. SAPS II score was 57.8 ± 12.8, length of stay was median 26 days, ICU stay was median 3 days. The treatment of sepsis immediately assessed and treated.

Table 1(abstract P33)
Characteristic and outcomes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.7 (± 17.8)</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>41 (63.1)</td>
</tr>
<tr>
<td>Locale of transfer</td>
<td></td>
</tr>
<tr>
<td>ICU</td>
<td>42 (64.6)</td>
</tr>
<tr>
<td>Step-down unit</td>
<td>23 (35.4)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>30 (46.2)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>3 (4.6)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>18 (27.7)</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>11 (16.9)</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>6 (9.2)</td>
</tr>
<tr>
<td>Chronic heart failure</td>
<td>5 (7.7)</td>
</tr>
<tr>
<td>Severe sepsis</td>
<td>56 (86.2)</td>
</tr>
<tr>
<td>Septic shock</td>
<td>9 (13.8)</td>
</tr>
<tr>
<td>Site of infection</td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>29 (44.2)</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>13 (20.0)</td>
</tr>
<tr>
<td>Abdominal</td>
<td>8 (12.3)</td>
</tr>
<tr>
<td>Skin and soft tissue</td>
<td>4 (6.2)</td>
</tr>
<tr>
<td>Blood (ICU)</td>
<td>4 (6.2)</td>
</tr>
<tr>
<td>Others sites</td>
<td>7 (10.8)</td>
</tr>
<tr>
<td>SAPS II score</td>
<td>57.8 (± 12.8)</td>
</tr>
<tr>
<td>Initial bundle 6 hours</td>
<td></td>
</tr>
<tr>
<td>Serum lactate + blood culture measured</td>
<td>40 (63.5)</td>
</tr>
<tr>
<td>Fluids administration</td>
<td>41 (64.1)</td>
</tr>
<tr>
<td>Length of stay ICU (days)</td>
<td>3 (1-6)</td>
</tr>
<tr>
<td>Length of stay hospital (days)</td>
<td>26 (10-71)</td>
</tr>
<tr>
<td>Hospital mortality</td>
<td>13 (20.0)</td>
</tr>
</tbody>
</table>

Data presented as n (%), mean (± SD) or median (IQR).
was also initiated in the ward. The serum lactate + measured blood culture was 40 (63.5%) and fluid administration was 41 (64.1%) (Table 1). Pressing the RRT was in 43 (66.2%) cases by the staff member with significant concern about the patient’s condition, 27 (41.5%) cases by changes in systolic blood pressure, and 23 (35.4%) cases due to change in oxygen saturation (Table 2).

Conclusions: The criteria for calling the RRT can support the prompt identification of patients who have sepsis and prevent disease progression. Furthermore, the treatment may also be performed in the ward and may result in a reduction in mortality.

References

P34
Knowledge of the nurse in advanced life support and the impact of continuing education in cardiopulmonary arrest in the ICU
Daniella Fernandes Mendonça1, Denise de Fátima Gomes Machado2, Renata Cristina Barbosa Silva3, Camila Medeiros Cruvinel Cunha3, Marislei Espíndula Brasil3
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Critical Care 2013, 17(Suppl 4):P34; doi:10.1186/cc12934

Background: The knowledge of the nurse in advanced life support and the impact of continuing education in cardiopulmonary arrest in the ICU. Faced with the complications of cardiopulmonary arrest (CPA) in the ICU it is important for nurses to be prepared for emergency actions, mastering the techniques of care and maintaining well-trained staff. The objective was to identify and describe the knowledge of the nurse in advanced life support (ALS) and the impact of continuing education PCR in ICU.

Methods and materials: Exploratory, descriptive bibliographical integrative analysis of available literature, using the keywords ‘PCR’, ‘ICU’, ‘Continuing Education’, ‘Education’, ‘Nursing’, which were published between 2002 and 2012, in both conventional and virtual libraries.

Results: Twenty-six publications found and gave rise to two categories. First, the identification of clinical signs and CPR maneuver by the nursing staff and the nurse in the PCR are essential for successful resuscitation; the authors agree that the service systematized-based SAP protocol is essential for there to be success in CPR. Recognition theoretical and practical skills of the staff are among the most important determinants of the success rates of CPR [1]. Thus, it is necessary that health professionals, especially nursing staff, be aware of the clinical signs of PCR. Furthermore, the residence time of the professional nursing staff in the ICU causes them to gain more experience, making it easier to identify clinical signs and cardiac rhythms [2,3]. Second, the impact of continuing education on quality of nursing care in a PCR: the proper training of the nursing staff, especially those that operate in the ICU, is vital for emergency treatment PCR. Identifying the theoretical and practical knowledge of staff about the PCR and PCR is an important prerequisite for planning a training service [2]. The nurse as team leader and organizer of the ICU is the right professional to establish measures to be taken at the time of the PCR. The nurse has a responsibility to properly distribute the measures to be implemented at the time of service of the PCR, identifying it early and minimizing damage [4].

Conclusion: Continuing education has significant impact in improving the level of knowledge of nursing professionals, leading to survival of patients in a hospitalized ICU, as it ensures the identification of the signs and symptoms of CRP in patients in the ICU.

Acknowledgements: The authors thank everyone who contributed to this work, Professor Dr Marislei Brasiliero for encouragement, and the dedication of coworkers Denise Machado, Renata Barbosa and Camila Cunha for their knowledge and effort to achieve this project.

References

P35
Prevalence of vitamin D deficiency among children with sepsis, its association with sepsis severity and its outcome in a pediatric ICU
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1Department of Paediatrics, Post-Graduate Institute of Medical Education & Research (PGIMER), Chandigarh, India

Critical Care 2013, 17(Suppl 4):P35; doi:10.1186/cc12935

Background: Increased prevalence of vitamin D deficiency (VDD) in sepsis and its association with sepsis severity has been documented in adults [1-3]. However, data on the pediatric population are scarce. This study aims at assessing the prevalence of VDD (25-hydroxyvitamin D (25(OH)D) level <20 ng/ml) among children with sepsis in developing nations and its association with sepsis severity.

Materials and methods: A prospective observational study conducted between January and December 2012. During the study period all consecutive PICU admissions between the ages of 1 and 12 years were screened for sepsis at the time of admission to the ICU. Out of 613 PICU admissions, 124 patients satisfying the criteria for sepsis [4] were randomly enrolled and followed up throughout the hospital stay. Patients with an immunosuppressed state or receipt of vitamin D within the 3 months prior to hospital admission were excluded. A control group comprising of 40 healthy children was also included for comparison with the general population. The serum 25(OH)D level was measured in all patients with sepsis within 24 hours of admission to the PICU. Severity of sepsis was assessed using the Pediatric Risk of Mortality II (PRISM III) score and the daily Sequential Organ Function Assessment (SOFA) score.

Results: Patients with sepsis had low 25(OH)D levels compared with healthy controls (P = 0.04). Median 25(OH)D level among patients was 19.7 ng/ml (interquartile range (IQR): 12.5, 31.2) and median 25(OH)D level among controls was 30.4 ng/ml (IQR: 22.1, 38). Prevalence of VDD was high among patients 51% (95% confidence interval (CI), 42 to 59) compared with the VDD of 17% (95% CI, 8 to 32) in healthy controls (P < 0.001) (Table 1). No significant correlation was found between vitamin D level and PRISM III score or daily SOFA score. Out of 19 deaths, 17 (90%) deaths occurred in patients with vitamin D deficiency and insufficiency (odds ratio 3.09, 95% CI: 0.6 to 20.7). However, the difference in mortality was not statistically significant (P = 0.58). Factors such as septic shock, multigorgan
We found a high prevalence of VDD among children with sepsis. In a single-institution retrospective study, we determined the presence of vitamin D deficiency (25(OH)D <20 ng/ml) and insufficiency (25(OH)D = 20 to 30 ng/ml) in patients with sepsis and in healthy controls. The study was carried out as a MD thesis, with support from the institute (PGIMER, Chandigarh, India).

**Table 1(abstract P35) Comparison of clinical characteristics of patients with sepsis by vitamin D status**

<table>
<thead>
<tr>
<th>Vitamin D status, % (n)</th>
<th>Patients (n = 124)</th>
<th>Controls (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient, 25(OH)D &lt;20 ng/ml</td>
<td>51 (63)</td>
<td>18 (7)</td>
</tr>
<tr>
<td>Insufficient, 25(OH)D = 20 to 30 ng/ml</td>
<td>25 (31)</td>
<td>30 (12)</td>
</tr>
<tr>
<td>Sufficient, 25(OH)D &gt;30 ng/ml</td>
<td>24 (30)</td>
<td>52 (21)</td>
</tr>
</tbody>
</table>

P < 0.001.

**Table 2(abstract P35) Vitamin D status among patients with sepsis and healthy controls**

<table>
<thead>
<tr>
<th>Vitamin D status, % (n)</th>
<th>Patients (n = 124)</th>
<th>Controls (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient, 25(OH)D &lt;20 ng/ml</td>
<td>51 (63)</td>
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<td>30 (12)</td>
</tr>
<tr>
<td>Sufficient, 25(OH)D &gt;30 ng/ml</td>
<td>24 (30)</td>
<td>52 (21)</td>
</tr>
</tbody>
</table>

**Results:** By the presence of VDD (Table 2).

**Acknowledgements:** This study was carried out as a MD thesis, with support from the institute (PGIMER, Chandigarh, India).

**References**

**P36**

**Predictors of mortality in renal transplant recipients with severe sepsis and septic shock**

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Critical Care 2013, 17(Suppl 4):P36; doi:10.1186/cc12936

**Background:** Renal transplantation is the treatment of choice for end-stage renal disease as it is cost-effective, and improves survival and quality of life as compared with maintenance dialysis [1,2]. However, the need for immunosuppression increases the hazard of septic complications [3]. Sepsis is one of the leading causes of death among renal transplant recipients and little is known about its characteristics in this population [4,5]. The aim of this study was to evaluate the factors associated with mortality in renal transplant patients admitted to the ICU with severe sepsis and septic shock.

**Materials and methods:** We conducted a single-institution retrospective observational cohort study in consecutive renal transplant patients admitted to the ICU with severe sepsis or septic shock in a public high-volume kidney transplant center from 1 June 2010 and 31 December 2011. We registered demographic data, transplant characteristics and sepsis management to identify predictive factors of ICU, hospital and 1-year mortality.

**Results:** A total of 190 patients were enrolled. The mean age was 51 ± 13 years, 115 (60.5%) were male, 122 (64.2%) were deceased donors, median APACHE was 20 (16 to 23) and median admission SOFA was 5 (4 to 8). The most common source of infection was respiratory (59.5%) followed by urinary tract (16.8%). Tachypnea, tachycardia, fever, hypothermia, leukocytosis and leukopenia were present in 74.7%, 67.9%, 24.2%, 6.3%, 26.3% and 16.3% of the patients. The most prevalent dysfunction was respiratory (68.4%) followed by cardiovascular (41.1%) and renal (40.5%). The median time between transplantation and the septic event was 2.1 (0.6 to 7.8) years. The duration of organ dysfunction before the diagnosis of sepsis was 2.5 (1.1 to 5.2) hours. The median length of ICU and hospital stay was 6 (3 to 13) and 20 (12 to 35) days, respectively. Hospital and 1-year mortalities were 38.4% and 42.6%, respectively. In the multivariate analysis, male gender, the variation in the SOFA score after the first 24 hours, the presence of hematologic dysfunction, being admitted from the wards and AKI stage 3 were predictors of hospital mortality.

**Conclusions:** In the present study, independent factors associated with mortality were related to features of sepsis severity and not to factors associated with transplantation. Another interesting finding was the low frequency of signs of systemic inflammatory response.

**References**


P37
Stratifying septic patients using lactate: severe sepsis and cryptic, vasoplegic and dysoxic shock profile
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1Hospital Paulistano, São Paulo, Brazil; 2Disciplina de Emergências Clínicas, Hospital das Clínicas, Universidade de São Paulo, Brazil; 3Disciplina de Anestesiologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, Brazil
Critical Care 2013, 17(Suppl 4):P37; doi:10.1186/cc12937

Background: The current consensus definition of severe sepsis and septic shock includes a heterogeneous profile of patients under the same definition. Although the prognostic value of hyperlactatemia in sepsis is well established, hyperlactatemia can be found both in severe sepsis and septic shock patients. We sought to compare features and outcomes of septic patients stratified by two factors: the presence of hyperlactemia and persistent hypotension.

Materials and methods: This was a secondary analysis of a multicenter observational study from 10 private hospitals in Brazil (Rede Amil-SP) aiming to evaluate the impact of a multifaceted program to implement the Surviving Sepsis Campaign bundles. We retrieved 1,948 septic patients with an initial lactate level collected within the first 6 hours of diagnosis. Based on previous literature, we stratified them into four groups according to the presence of hypoperfusion (lactate >4 mmol/l) and/or persistent hypotension despite adequate fluids: 1, severe sepsis (without both criteria); 2, cryptic shock (hypoperfusion without persistent hypotension) [1]; 3, vasoplegic shock (persistent hypotension without hypoperfusion); and 4, dysoxic shock (with both criteria) [2].

Results: Severe sepsis was found in 1,018 (52%), cryptic shock in 162 (8%), vasoplegic shock in 549 (28%) and dysoxic shock in 219 (12%) patients. Mean age was 60 years, 47% were male and the majority was admitted from the emergency department (47%). The lung was the principal source of infection, followed by the urinary tract and abdominal. Overall, the four groups presented significant differences in APACHE II and SOFA scores (< 0.001 for both), dysoxic shock being the most severe group. In post-hoc analysis, patients in the severe sepsis group presented similar SOFA score to patients in the cryptic shock group (P = 0.20). Overall, 28-day crude survival was different between groups (P < 0.001), being higher for the severe sepsis group (69%, P < 0.001 vs. other), similar between cryptic and vasoplegic shock (53%, P = 0.39) and lower for dysoxic shock (38%, P < 0.001 vs. other). In an adjusted analysis considering age, APACHE II and SOFA, the 28-day survival remained different between groups (P < 0.001) and the hazard ratio for the dysoxic shock group was the highest: HR 2.39 (95% CI 2.21 to 4.02).

Conclusions: Current definitions for severe sepsis and septic shock include four different phenotypes, which should be considered for epidemiology purposes, customizing treatment goals and inclusion criteria for future studies. Although previous studies showed similar outcomes between cryptic shock and overt septic shock (vasoplegic and dysoxic profile), we demonstrated that cryptic shock is similar only to vasoplegic shock.

Acknowledgements: On behalf of the Amil Critical Care Group.

References


P38
Antithrombin III concentrate may contribute to sepsis in nonovert disseminated intravascular coagulation
Noriko Saito1, Mari Yokota, Ahmin Kim, Tomoyuki Harada, Munekazu Takeda, Mizuho Namiki, Arino Yaguchi
Department of Critical Care and Emergency Medicine, Tokyo Women's Medical University, Tokyo, Japan
Critical Care 2013, 17(Suppl 4):P38; doi:10.1186/cc12938

Background: Antithrombin III (AT III) has been known to contribute to anti-inflammatory response as well as its anticoagulation. Our previous study showed AT III deficiency happened in the early stage of sepsis with no relation to disseminated intravascular coagulation (DIC) status. Whether AT III concentrate is a beneficial therapy or not for septic patients is still a controversial issue. Our hypothesis is that AT III concentrate may have efficacy as an anti-inflammatory for sepsis.

Materials and methods: From January 2009 to June 2013, adult septic patients with nonovert DIC whom were given AT III concentrate in our medico-surgical ICU were included in this study. DIC scoring was used with the definition of the International Society on Thrombosis and Haemostasis (ISTH). AT III concentrate was administered 30 to 60 U/kg intravenously every 24 hours for 3 days in the patients. Between before and after the AT III concentrate therapy, WBC (×10⁹/l), CRP (mg/dl), platelet (×10⁹/l), PT (seconds), fibrinogen (mg/dl), FDP (µg/ml), SOFA score and DIC score by ISTH were compared. Values are expressed as mean ± SD. Data were analyzed by Wilcoxon signed-rank test. P < 0.05 was considered significant.

Results: There were 157 patients (100 men, 57 women; age range 19 to 96 years [mean 70.0 ± 16.0]), and the 28-day mortality rate was 25.5% and APACHE II score was 17.2 ± 8.3. WBC, CRP, PT, and SOFA score were significantly improved after AT III concentrate therapy (13.411 ± 8.794 vs. 11.798 ± 6.562, P = 0.0007, 17.1 ± 11.5 vs. 13.9 ± 7.0, P = 0.0001, 16.3 ± 10.9 vs. 15.2 ± 5.3, P = 0.002, and 8.6 ± 3.6 vs. 7.7 ± 4.5, P = 0.005, respectively), although platelet was significantly decreased (15.8 ± 11.3 vs. 13.7 ± 11.3, P < 0.00013). There were no significant differences in fibrinogen, FDP and DIC score (46.4 ± 235 vs. 437.6 ± 185.4, P = 0.10, 25.1 ± 36.9 vs. 25.6 ± 36.2, P = 0.85, 2.0 ± 1.5 versus 2.3 ± 1.7, P = 0.06, respectively). One week after the therapy, SOFA score was significantly improved, while the DIC score did not change compared with before the therapy (6.1 ± 4.7, P < 0.0001 and 2.3 ± 1.7, P = 0.98).

Conclusions: In the patients with septic nonovert DIC, WBC, CRP and SOFA score were immediately improved after the AT III concentrate therapy, while fibrinogen, FDP and DIC score did not change. AT III concentrate may also contribute to anti-inflammatory without DIC status.

P39
Intravenous immunoglobulin therapy could have efficacy in severe sepsis
Ahmin Kim1, Mari Yokota, Noriko Saito, Munekazu Takeda, Tomoyuki Harada, Mizuho Namiki, Arino Yaguchi
Department of Critical Care and Emergency Medicine, Tokyo Women's Medical University, Tokyo, Japan

Background: Intravenous immunoglobulin (IVIG) administration has been approved to use for severe sepsis with antibiotics by the Ministry of Health, Labour and Welfare since 1980 in Japan. IVIGs are commonly used for severe sepsis and septic shock in Japan, while the international guidelines for management of severe sepsis and septic shock in 2012 suggest not using IVIG in adult patients. Our hypothesis is that IVIG administration has an efficacy for severe sepsis and septic shock.

Materials and methods: This retrospective observational study included all adult patients in our ICU who were administered IVIG for severe sepsis and septic shock from January 2011 to June 2013. IVIG was used at 5,000 mg/day every 24 hours for 3 days. We compared body temperature (°C), WBC (×10⁹/l), CRP (mg/dl), procalcitonin (ng/ml) and serum immunoglobulin G (IgG) (mg/dl; normal >870) between before and after IVIG treatment. Values are expressed as the median. The Wilcoxon signed-rank test was used for the statistical analysis. P < 0.05 was considered significant.
Results: One hundred and fifty-one patients (85 men, 66 women; age range 23 to 96 (median 67.8)) were included in this study. The 28-day mortality after IVIG treatment was 13.9%. The SOFA score before IVIG treatment was 5.0. Values of WBC, CRP and procalcitonin were significantly decreased after IVIG treatment (10.905 vs. 9.805, P < 0.0001, 12.3 vs. 7.7, P < 0.0001, 2.4 vs. 1.7, P = 0.0003, respectively). Body temperature did not significantly change (37.4 vs. 37.2, P = 0.07). Serum IgG was significantly increased after the treatment (1.046 vs. 1.563, P = 0.003).

Conclusions: The present study has some limitations because of being a retrospective observational study. However, the mortality was quite low in the group of patients included in this study. Moreover, after IVIG treatment values of WBC, CRP and procalcitonin were improved. The median value of serum IgG before treatment was within the normal range, but after treatment was also significantly improved. There is a possibility that severe septic patients require additional IgG regardless of its normal concentrations in their blood.

P40
Polymyxin B-direct hemoperfusion therapy improves mean arterial pressure in septic shock
Mari Yokota, Ahmin Kim, Tajiro Goto, Tomoyuki Harada, Munekazu Takeda, Mizuho Namiki, Arino Yaguchi
Department of Critical Care and Emergency Medicine, Toyo Women’s Medical University, Tokyo, Japan
Critical Care 2013, 17(Suppl 4):P40; doi:10.1186/cc12940

Background: In our previous study, we reported that polymyxin B-direct hemoperfusion (PMX-DHP) (Toraymyxin®, Toray Medical Co., Tokyo, Japan) therapy could contribute to oxygen delivery due to improved hemodynamic status, while decreasing inotropic agents in septic patients immediately after that treatment. The randomized controlled studies are ongoing in other countries, because its efficacy and indication are still controversial issues. The purpose of this study is to evaluate whether PMX-DHP therapy sustains to improve hemodynamic status after the treatment.

Materials and methods: All adult patients treated with PMX-DHP and receiving a pulmonary arterial catheter (PAC) in our ICU from July 1994 to June 2010 were included in this retrospective observational study. Patients’ clinical, microbiological and PAC data were collected from medical archives. PAC variables were compared between immediately before and after 24 hours of PMX-DHP therapy. Values were expressed as mean ± SD. Data were analyzed by Wilcoxon signed-rank test. P < 0.05 was considered statistically significant.

Results: There were 63 patients (36 men, 27 women; age mean 63.4 ± 14.8) studied. The mortality rate was 30.2% 28 days after PMX-DHP. APACHE II score and SOFA score on the day of PMX-DHP therapy were 20.2 ± 14.8 and 7.3 ± 3.8, respectively. Mean arterial pressure (MAP) (mmHg) was significantly increased after PMX-DHP therapy (77.5 ± 22.5 vs. 87.2 ± 15.9, P = 0.08). The cardiac index (CI) (l/minute/m²), systemic venous resistance index (SVRI) (dyn·second·m²/cm⁵), mixed venous oxygen saturation (SvO₂) (%), oxygen delivery and consumption (DO₂ and VO₂) (ml/minute) and P/F ratio were not statistically different before and after PMX-DHP therapy.

Conclusions: Only the increasing of MAP was sustained after 24 hours of PMX-DHP therapy, while the inotropic agents were decreased. Although the CI, DO₂, VO₂ and P/F ratio were improved immediately after PMX-DHP therapy in our previous study, these were not significantly changed between before and after 24 hours. PMX-DHP could improve MAP with decreasing inotropic agents, while alterations of other PAC variables were not sustained in 24 hours of PMX-DHP.

Table 1 (abstract P41) Population characteristics

<table>
<thead>
<tr>
<th></th>
<th>No fungal (n = 48)</th>
<th>Fungal (n = 17)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38 (31 to 43)</td>
<td>35 (33 to 46)</td>
<td>0.43</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>34 (71%)</td>
<td>13 (76%)</td>
<td>0.29</td>
</tr>
<tr>
<td>CD4⁺ lymphocyte count (cell/mm³)</td>
<td>69 (32 to 204)</td>
<td>28 (14 to 115)</td>
<td>0.15</td>
</tr>
<tr>
<td>Nadir CD4⁺ (cell/mm³)</td>
<td>57 (27 to 153)</td>
<td>27 (14 to 122)</td>
<td>0.40</td>
</tr>
<tr>
<td>Time since HIV diagnosis (months)</td>
<td>31 (1 to 123)</td>
<td>13 (1 to 77)</td>
<td>0.53</td>
</tr>
<tr>
<td>HAART naïve</td>
<td>15 (31%)</td>
<td>8 (47%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Mortality</td>
<td>15 (31%)</td>
<td>11 (64.7%)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Background: Information about the prevalence of fungal diseases in critically ill AIDS patients is sparse. Our goal is to describe the prevalence of fungal diseases in this population, when they are admitted to the ICU.

Materials and methods: Prospective, observational study. Blood and urine samples were collected from 65 AIDS patients at a specialized ICU in infectious diseases, from August 2011 to June 2013. When indicated by clinical suspicion, samples of respiratory, bone marrow and/or tissue were collected. Cultures, cytopathology and serologic tests were performed to evaluate fungal colonization or infection. Clinical data were collected from medical records. Values are expressed as the median and percentage.

Results: Table 1 presents general characteristics of the HIV/AIDS patients. Patients with fungal disease did not differ from patients without fungal infection: age 35 versus 36 years (P = 0.43), male gender 76% versus 70% (P = 0.29); nadir CD4 cell count 27 versus 57 cell/mm³ (P = 0.15). Most patients were exposed to HAART previously, while there were 47% naïve patients in the fungal group versus 31% in the no fungal group. The ICU mortality of patients without fungal disease was 31% versus 64.7% with fungal disease (P = 0.02); hospital mortality was not different between groups (52% vs. 64.7%, P = 0.4). Figure 1 presents 17 diagnoses of disseminated fungal diseases (prevalence 26%). All histoplasmosis diagnoses were made from marrow bone culture (11%). Disseminated cryptococcosis was diagnosed from serum serologic latex, direct examination and positive culture in LCR. Three patients (4.6%) were diagnosed with candidiasis in blood cultures. Pneumocystosis was diagnosed from immunofluorescence and Grogot positive in sputum. One patient had disseminated esporotricosis with positive cultures in LCR, blood, tissue, urine and sputum. The only case of aspergillosis is a previous tuberculosis-treated patient that developed a disseminated disease (galactomannana-positive) from a fungal ball.

Conclusions: One in four HIV/AIDS critically ill patients presents with fungal disease when they are admitted to the ICU. Surveillance of fungal pathogens shall be necessary in the first screening of medical HIV/AIDS patients in the ICU.

Figure 1 (abstract P41) Fungal diseases

- Histoplasmosis
- Pneumocystosis
- Candidiasis
- Cryptococcosis
- Esporotricosis
- Aspergillosis

Table 1(abstract P41) Population characteristics

Fungal disease in AIDS patients in intensive care
Edwiges Santos¹, Andre Japiassu, Marcia Lazera, Fernando Bozza
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Critical Care 2013, 17(Suppl 4):P41; doi:10.1186/cc12941

Background: Information about the prevalence of fungal diseases in critically ill AIDS patients is sparse. Our goal is to describe the prevalence of fungal diseases in this population, when they are admitted to the ICU.
Usefulness of broad-range PCR in the etiologic diagnosis of sepsis

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Critical Care 2013, 17(Suppl 4):P42; doi:10.1186/cc12942

Background: Sepsis is responsible for a high rate of hospitalization and mortality. The interval methods for bacterial isolation and identification, such as blood culture, have limitations in sensitivity and specificity and their results usually are not available before 48 to 72 hours. The PCR allows a rapid diagnosis of infectious agents. Broad-range PCR allows an earlier and more sensitive bacterial identification in just one reaction, even after the initiation of antibiotics. Therefore, this study evaluates the use of broad-range PCR in the etiologic diagnosis of septic patients, and compares it with traditional methods of culture.

Materials and methods: Thirty-five patients with sepsis, admitted to the Emergency Unit of Clinical Hospital of Ribeirão Preto Medical School, were included in the study. Clinical, laboratory, and culture data were collected at hospital admission. On the first day of admission, DNA extraction was performed from serum, plasma and Buffy coat samples from all patients. Broad-range 16S rDNA PCR was then performed using two different pairs of primers (Bak1/Bak2 and Tat/Far).

Results: Eighteen (51%) patients were female; mean age was 58 ± 18 years; 15 (43%) had severe sepsis and 20 (57%) septic shock, and mortality was 54%. Mean C-reactive protein (CRP) was 14.39 ± 9.52 and 28 (80%) patients had levels of CRP greater than 5.0 ng/l. The primary site of infection was detected in all patients, 20 (57%) patients had respiratory tract infection, nine (26%) urgenital tract infection, three (8.5%) cutaneous infection, and three (8.5%) other sites. Blood culture was positive in 14 (40%) samples. Broad-range PCR was positive in 19 (51%) samples. Only 10 (29%) samples were positive for both techniques. In 11 (31%) patients, neither blood culture nor PCR were positive.

Conclusions: Broad-range PCR was effective for diagnosis of bacterial infection in septic patients, and could be an option to be used in patients with severe sepsis and septic shock. Moreover, it is faster than blood culture and can detect bacteria even after the initiation of intravenous antibiotics. The combination of both techniques could increase the likelihood of etiologic diagnosis in septic patients.

Acknowledgements: Thanks to FAPESP and CAPES for financial support.

Sepsis care protocol: initial evaluation at a university hospital in southern Brazil

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Critical Care 2013, 17(Suppl 4):P44; doi:10.1186/cc12944

Background: Sepsis is a systemic inflammation caused by severe infection. It is a life-threatening condition, progresses rapidly, and affects multiple system functions. An evidence-based medical sepsis bundles model has been used for sepsis care in clinical practice. All patients who have at least two signs or symptoms of systemic inflammatory response syndrome (SIRS) secondary to an infectious process are considered septic. Sepsis is the leading cause of death in ICUs and a major cause of late hospital mortality rate, exceeding the acute coronary syndromes and neoplasms. Mortality in Brazil reaches 60%, while the world average is around 30%, overcoming countries such as India and Argentina. The early recognition and treatment of these patients are key to reducing mortality. The aim of this study is to evaluate the implementation of the protocol of sepsis in a university general hospital in Porto Alegre.

Materials and methods: Retrospective evaluation of protocols for sepsis in emergency in 2012.

Results: A total of 200 patients were enrolled in the protocol during the study period. The average age was 35 years (SD ± 16.5), 51% of patients were male, the most frequent focus was respiratory 61%, and the second urinary with 14%. Clinical criteria for inclusion in the protocol that most prevailed were: axillary temperature and heart rate, with more than 95%. Altered axillary temperature was present in 98% of the sample. Of these cases, 86.5% (n = 173) of patients were discharged within 24 hours. Twenty-seven patients met criteria for hospitalization, 22% required the ICU. Around 75% (n = 20) of inpatients had no blood cultures collected before starting antibiotics. Only 7% mortality (n = 2).

Conclusions: The criteria for inclusion in the protocol are quite sensitive and the number of visits per month in the emergency exceeds 10,000. A total of 200 patients enrolled in the sepsis care protocol in a year, over 80% of these being discharged within 24 hours, suggests a low adherence to institutional protocol, especially in patients with septic shock, which is reinforced by the very low mortality compared with literature data. The evaluation of these data was essential to bring the knowledge that adherence to the protocol is still very low in our institution.
Background: A 69-year-old woman underwent elective surgical repair of an abdominal aortic aneurysm. Intraoperative lesions were intestinal and splenic, requiring performing segmental bowel resection and splenectomy. By hemodynamic instability the patient was maintained on mechanical ventilation in norepinephrine and was transferred to the ICU. After 3 days she had fever, tachycardia, hypotension, and anuria, with output field had purulent secretion by the tracheal tube. Chest X-ray showed opacity in the right lung; cultures were collected and ceftimepine initiated empirically for treatment of ventilator-associated pneumonia. Acinetobacter baumannii was isolated sensitive only to polymyxin-E in the sample of tracheal secretions. An exchange of antimicrobial therapy was made, but the patient developed refractory shock and died.

Materials and methods: We report the case of a patient with septic shock.

Results: Despite the upgrading of intensive therapies with the presence of increasingly prepared professionals and all of the technological and scientific developments that occurred in the last 10 years, sepsis remains a major challenge for contemporary medicine. Mortality rates may vary from 20 to 80%. Several factors contribute to this high mortality rate, such as the growing population of patients aged over 65 years with various chronic diseases, the most frequent use of invasive procedures, increased demand for immunocompromised patients and the development of nosocomial microorganism infections increasingly resistant to antimicrobial agents. Besides the pathophysiology, evidence substantiated that early intervention reduces mortality in severe sepsis and thus several ICUs have sought to improve the quality of clinical management of septic patients. In 2002, the Medical Society of Intensive American (SCCM) and European (ESICM) together with the International Sepsis Forum initiated the Surviving Sepsis Campaign (SSC). The SSC initiative was based on six strategies, including: implement surveillance sepsis; improve the early diagnosis and safety; establish protocols for treatment and early intervention; create programs continuing professional education; proposed therapy post-ICU; and develop global standards for intensive care.

Conclusions: The emergence of antimicrobial-resistant microorganisms is a growing problem worldwide and this complicates the choice of empirical antimicrobials and can compromise the evolutionary outcome of patients.

P46 Predictors of mortality in patients with severe sepsis admitted to an ICU
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Background: Severe sepsis is an important cause of morbidity and mortality for patients in ICUs [1]. Since instituting rapid treatment for patients with sepsis is critical, the need for reliable predictors of mortality to guide therapy is evident. This study attempts to identify the risk factors for mortality in patients admitted with severe sepsis to the ICU.

Materials and methods: Case-control study conducted in the ICU of Hospital Anchieta, Brasília, DF, Brazil, during 5 months. Patients were divided into two groups: survivors group (5G) and nonsurvivors group (NSG).

Results: During the study, 38 patients were admitted with severe sepsis, with a mortality rate of 47% (n = 18). Upon admission, the patients in the NSG presented higher values of: SAPS3 score (82 ± 12 vs. 60 ± 14, P = 0.00), ICU length of stay (10±13 vs. 9±2 days, P = 0.04), respiratory rate (29 ± 9 vs. 26 ± 7 rpm, P = 0.30), axillary temperature (36.9 ± 0.7 vs. 37.2 ± 1.8°C, P = 0.43), leucocyte count (17,500 ± 7,800 vs. 13,000 ± 6,000/mm3, P = 0.08), immunosuppression (38.5% vs. 12.5%, P = 0.07), and prior use of corticosteroids (23% vs. 25%, P = 0.90).

Conclusions: SAPS3 score, metastatic cancer, decreased level of consciousness, need for vasopressors, invasive mechanical ventilation, previous cardiac arrest, heart rate, serum creatinine, and platelet count were associated with mortality in severe sepsis for this sample of patients.

Reference

P47 Serum arterial lactate at the time of admission as a predictor of mortality in patients admitted with severe sepsis and septic shock to an ICU
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Critical Care 2013, 17(Suppl 4):P47; doi:10.1186/cc12947

Background: Elevated serum arterial lactate levels are often associated with an imbalance between oxygen demand and delivery, which has a strong correlation with poorer outcomes in critically ill patients [1,2]. This study aims to evaluate serum arterial lactate as a predictor of mortality in critical patients admitted with severe sepsis and septic shock.

Materials and methods: Retrospective cohort study conducted in the ICU of Hospital Anchieta, Brasília, DF, Brazil, during 3 years. For the first analysis, patients were divided into two groups: group with arterial lactate >2 mmol/l and group with low arterial lactate ≤2 mmol/l at the time of admission. For a second analysis, patients were divided into two groups: group with arterial lactate >3.3 mmol/l and group with arterial lactate ≤3.3 mmol/l at the time of admission.

Results: A total of 195 patients with sepsis were enrolled, 41% (n = 80) with septic shock. Mean age was 63 ± 22 years, ICU length of stay 9 ± 11 days, SAPS3 62 ± 16, and APACHE II 21 ± 9. ICU mortality in 4 days was 10.8% (n = 21), in 28 days was 12.3% (n = 24), and hospital mortality was 26.2% (n = 51). The nonsurvivor patients had higher lactate values (2.0 ± 1.4 vs. 1.3 ± 1.1, P = 0.00). Considering the arterial lactate cutoff value of 2.0 mmol/l, there was no difference between groups regarding ICU length of stay (10 ± 13 vs. 9 ± 2 days, P = 0.47), mortality in 4 days (12% vs. 10%, P = 0.85), mortality in 28 days (13% vs. 16%, P = 0.77), and hospital mortality (30% vs. 32%, P = 0.86). However, considering the lactate cutoff value of 3.3 mmol/l, the high lactate group had higher mortality in 4 days (27% vs. 9%, P = 0.04) and hospital mortality (67% vs. 23%, P = 0.00). There was no significant difference regarding mortality in 28 days (27% vs. 11%, P = 0.08) and ICU length of stay (8 ± 7 vs. 9 ± 11 days, P = 0.59). The relative risk of hospital death in patients with arterial lactate >3.3 mmol/l was 2.93 (95% CI: 1.87 to 4.58). The specificity of arterial lactate >3.3 mmol/l for hospital mortality was 96.5% (95% CI: 92.1 to 98.5%), sensitivity was 19.6% (95% CI: 11.0 to 32.5%), and LR+ was 5.65 (95% CI: 2.03 to 15.7%). The arterial lactate area under the ROC curve for mortality was 0.634 (95% CI: 0.54 to 0.748).

Conclusions: In the patients admitted with severe sepsis and septic shock for this sample, the nonsurvivors had higher lactate values. Arterial lactate >2 mmol/l at the time of admission was not associated with mortality. Arterial lactate >3.3 mmol/l was associated with mortality in 4 days, and hospital mortality. Indeed, lactate >3.3 mmol/l had high specificity for hospital mortality.

References
Comparison of demographics and outcomes of patients with severe sepsis admitted to the ICU with or without septic shock

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Background: Severe sepsis and septic shock are common and are associated with substantial mortality and substantial consumption of healthcare resources [1]. Although the incidence of septic shock has steadily increased during the past several decades, the associated mortality rates have remained constant or have decreased only slightly [2]. This study aims to compare demographics and outcomes of patients admitted to the ICU with severe sepsis and with or without septic shock.

Materials and methods: The present study is a retrospective cohort conducted over a 3-year period in the ICU of Hospital Anchieta, Brasília, Brazil. Patients were divided into two groups: severe sepsis without shock (SW) and severe sepsis with shock (SS). The patients coming from other ICUs or transferred to other ICUs were excluded.

Results: A total of 198 patients with severe sepsis were enrolled in this study. Among them, 97 patients (49%) had septic shock. In this cohort, the mean age was 59 ± 16 years, the SAPS 3 score was 63 ± 17 and the APACHE II score was 21 ± 9. The mortality in four days was 12.6% (n = 25), in 28 days was 14.1% (n = 28) and the hospital mortality was 29.3% (n = 58). There was no difference between the two groups regarding age (64 ± 20 vs. 59 ± 21, P = 0.08) and length of stay in the ICU (12 ± 1 vs. 11 ± 1, P = 0.51). The SS group presented higher SAPS3 (70 ± 17 vs. 57 ± 15, P = 0.00) and APACHE II (1 ± 8 vs. 9 ± 1, P = 0.00) scores. Patients in the SS group also had higher mortality in 4 days (18% vs. 8%, P = 0.04), in 28 days (20% vs. 9%, P = 0.03) and hospital mortality (37% vs. 22%, P = 0.02).

Conclusions: Patients admitted with septic shock had higher mortality than patients admitted with severe sepsis without septic shock, but there was no difference between the groups with respect to length of stay in the ICU.

References
Results: One hundred and seven patients were enrolled. Mean age was 53 ± 20, APACHE II 14 ± 6, SAPS 3 52.9 ± 14.9 and SOFA 6.2 ± 3.3. ICU mortality was 34.6% (n = 37). The SOFA score was higher in nonsurvivors (7.4 ± 3.0 vs. 5.8 ± 3.4, P = 0.01), cardiovascular (2.0 ± 1.8 vs. 1.4 ± 1.6, P = 0.01) and kidney dysfunctions (0.7 ± 1.0 vs. 0.4 ± 0.9, P = 0.04) being higher in this group. There were no differences between the groups regarding coagulation (0.4 ± 0.8 vs. 0.4 ± 0.8, P = 0.59), liver (0.0 ± 0.7 vs. 0.0 ± 0.7, P = 0.65), respiratory (2.0 ± 1.2 vs. 1.6 ± 1.4, P = 0.87), and neurologic (2.2 ± 1.7 vs. 1.7 ± 1.6, P = 0.96) organ dysfunctions. The area under the ROC curve (Figure 1) for SOFA was 0.650 (95% CI 0.541 to 0.759). The components of the cardiovascular system, renal system, coagulation, liver, respiratory, and nervous systems had areas under the ROC curve of 0.612 (95% CI 0.501 to 0.732), 0.565 (95% CI 0.478 to 0.712), 0.484 (95% CI 0.369 to 0.600), 0.457 (95% CI 0.344 to 0.571), 0.580 (95% CI 0.469 to 0.691), and 0.582 (95% CI 0.465 to 0.699), respectively.

Conclusions: The SOFA score was moderately associated with ICU mortality. The scores for cardiovascular and renal impairment were individually associated with mortality.

References

PS1
SaO2/FiO2 ratio as risk stratification for patients with sepsis
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Background: The PaO2/FiO2 ratio is a well-known parameter to assess respiratory dysfunction, used in Sequential Organ Failure Assessment (SOFA) [1]. This study aims to determine whether the SaO2/FiO2 ratio can be used in the assessment of respiratory impairment and as a predictor of ICU mortality in patients with sepsis and to evaluate its correlation with PaO2/FiO2.

Materials and methods: A retrospective cohort study conducted in the ICU of Hospital Santa Luzia, Brasilia, DF, Brazil, during 5 months. An arterial blood sample was collected at the time of admission. Patients with sepsis were divided into two groups: survivors group (SG) and nonsurvivors group (NSG). Correlation with SaO2/FiO2 and PaO2/FiO2 was evaluated with the Pearson correlation coefficient. Accuracy of SaO2/FiO2 and PaO2/FiO2 to predict ICU mortality was measured with the area under the receiver operating characteristic curve.

Results: A total of 118 patients with sepsis were enrolled. The mean age was 66 ± 21 years, SAPS3: 50 ± 14, APACHE II: 13 ± 8, PaO2/FiO2: 317

Figure 1(abstract P50) ROC curve for SOFA
(IQ 233 to 426) and SaO₂/FiO₂: 362 (IQ 247 to 453). ICU mortality was 17.8% (n = 21). The main sites of infections were respiratory (57%, n = 67), urinary (19%, n = 23) and cutaneous (8.5%, n = 10). Non-survivor patients had lower SaO₂/FiO₂ (258 vs. 366, P = 0.00) and PaO₂/FiO₂ (285 vs. 354, P = 0.04). PaO₂/FiO₂ and SaO₂/FiO₂ had a good correlation (r = 0.645, P = 0.00). The relative risk of death in patients with SaO₂/FiO₂ <400 was 1.81 (95% CI: 1.47 to 2.24), SaO₂/FiO₂ <300 was 2.5 (95% CI: 1.54 to 4.05), SaO₂/FiO₂ <200 was 2.45 (95% CI: 1.27 to 4.71). The sensitivity for ICU mortality of SaO₂/FiO₂ <300 was 28% and of SaO₂/FiO₂ <200 was 35%. The specificity for ICU mortality of SaO₂/FiO₂ <300 was 90% and of SaO₂/FiO₂ <200 was 86% (95% CI: 93.5 to 100.0%). The area under the ROC curve for SaO₂/FiO₂ was 0.776 (95% CI: 0.677 to 0.875) and for PaO₂/FiO₂ was 0.655 (95% CI: 0.507 to 0.804) (Figure 1).

Conclusions: A low SaO₂/FiO₂ was associated with mortality in this group of patients and had a good correlation with PaO₂/FiO₂. SaO₂/FiO₂ <300 showed high specificity for mortality. Further analysis is necessary to the validation of less invasive measures such as pulse oximetry saturation (SpO₂/FiO₂ ratio) in the assessment of patients with sepsis.

Reference

Figure 1 (abstract P52) ROC curve for SaO₂/FiO₂ and PaO₂/FiO₂.
mortality at 4 and 28 days. Accuracy of P/L to predict ICU mortality was measured with the area under the receiver operating characteristic curve. Results: In the present study, 195 patients were enrolled, 41% (n = 80) with septic shock. Their mean age was 62.8 ± 21.6 years, SAPS 3 was 261.1 ± 15.0, APACHE II was 206.6 ± 8.6, and length of stay in the ICU was 9 ± 11 days. Mortality at 4 days was 10.8% (n = 21) and at 28 days was 12.3% (n = 24). The groups P/L < 8 and P/L ≥ 8 did not present differences regarding age (59 ± 20 vs. 65 ± 22, P = 0.07) and APACHE II (22 ± 9 vs. 20 ± 9, P = 0.19). The LPL group had higher SAPS3 (68 ± 18 vs. 59 ± 13, P = 0.00). The LPL was significantly associated with mortality in 4 days (18% vs. 7%, P = 0.02) and 28 days (19% vs. 9%, P = 0.03). The area under the ROC curve of P/L for mortality at day 4 was 0.628 (95% CI 0.498 to 0.757) and at day 28 was 0.613 (95% CI 0.489 to 0.736).

Conclusions: P/L < 8 at admission was associated with higher mortality in 4 and 28 days in patients with sepsis.

Reference

PS4 SIRS criteria as predictors of mortality in patients admitted with sepsis

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Background: The ACCP/SCCM consensus conference definitions for sepsis are used worldwide [1]. However, consensus definitions do not adequately reflect the intricacies of sepsis and can overestimate the diagnosis due to their high sensitivity. Moreover, the consensus criteria do not allow the staging or the prediction of sepsis outcome [2]. This study aims to evaluate the individual components of SIRS criteria as predictors of mortality in patients admitted to an ICU with sepsis.

Materials and methods: A case-control study conducted in the ICU of Hospital Santa Luzia, Brasília, DF, Brazil, during 4 months. Patients were divided into two groups: survivors group (SG) and nonsurvivors group (NSG). The accuracy of individual components of SIRS criteria as predictors of mortality was measured with the area under the receiver operating characteristic (ROC) curve.

Results: A total of 76 patients were enrolled, 10.5% (n = 8) with septic shock. Age was 70 ± 18 years, SAPS3: 59 ± 19, APACHE II: 15.5 ± 8.8. The ICU length of stay was 9 ± 10 days. ICU mortality was 21% (n = 16). The most prevalent sites of infections were respiratory (57.9%, n = 44), followed by urinary (25%, n = 19) and cutaneous (6.6%, n = 5). The incidence of tachycardia was the only parameter higher in the NSG (37.5% vs. 9.1%, P = 0.00). There was no difference regarding the incidence of fever or hypothermia (15.4% vs. 24.0%, P = 0.38), tachypnea (25.0% vs. 17.5%, P = 0.42) or leucocytosis or leucopenia (20.9% vs. 21.2%, P = 0.97) between the groups. The relative risk of death in patients with tachycardia was 4.13 (95% CI: 1.46 to 11.63). Tachycardia also had the larger area under the ROC curve: 0.708 (95% CI: 0.566 to 0.850), versus 0.442 (95% CI: 0.287 to 0.597) for fever/hypothermia, 0.556 (95% CI: 0.379 to 0.715) for tachypnea, and 0.498 (95% CI: 0.338 to 0.658) for leucocytosis/leucopenia.

Conclusions: For this sample, tachycardia was the only SIRS component associated with ICU mortality in patients admitted with sepsis.

References

PS5 SaO2-SvO2 difference for risk stratification of patients with sepsis and septic shock

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Background: Although there were differences regarding mortality in 4 and 28 days of ICU length of stay, hospital mortality was higher for this group of patients.

References


PS5 SaO2-SvO2 difference for risk stratification of patients with sepsis and septic shock

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Assessment and monitoring of hemodynamics is a cornerstone in critically ill patients as hemodynamic alteration may become life-threatening in a few minutes [1,2]. This study aimed to determine whether the SaO₂-SvO₂ difference could be used as risk stratification for patients with sepsis and septic shock.

**Materials and methods:** A retrospective cohort study conducted in the ICU of Hospital Santa Luzia, Brasília, DF, Brazil, during 6 months. An arterial blood sample was collected at admission. Patients with sepsis were divided in two groups: survivors group (SG) and nonsurvivors group (NSG). The accuracy of SaO₂-SvO₂ difference to predict ICU mortality was measured with the area under the receiver operating characteristic curve.

**Results:** A total of 131 patients with sepsis were enrolled, 11.5% (n = 15) with septic shock. Age was 66 ± 21 years, SAPS3: 37 ± 17, APACHE II: 14 ± 8, PaO₂/FiO₂: 342 ± 142 and SaO₂/FiO₂: 347 ± 109. ICU mortality was 18% (n = 23). The main sites of infections were respiratory (56.5%, n = 74), urinary (19%, n = 25) and cutaneous (7.6%, n = 10). Nonsurvivor patients had higher SaO₂-SvO₂ difference (26 ± 9 vs. 19 ± 9, P = 0.04), and longer ICU stay (P = 0.48), length of ICU stay (19 ± 9 vs. 15 ± 8, P = 0.03). In the group of patients with SaO₂-SvO₂ difference greater than 25%, the SaO₂-SvO₂ difference area under ROC curve was 0.714 (95% CI 0.534 to 0.894). Patients with septic shock who died had SaO₂-SvO₂ difference greater than 25%. The SaO₂-SvO₂ difference area under ROC curve was 0.714 (95% CI 0.534 to 0.894).

**Conclusions:** A higher SaO₂-SvO₂ difference is associated with mortality in patients with sepsis, especially in patients with septic shock.

**References:**

**P56**

**Elapsed time between ICU request and actual admission of patients with SIRS/sepsis leads to an increase in mortality or length of stay in this unit?**

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**Background:** Measures to ensure an appropriate early treatment for critically ill patients result in significant decreases in mortality [1,2]. This study aims to evaluate the impact of the elapsed time between ICU request and actual admission of patients with SIRS/sepsis on ICU mortality and length of stay.

**Materials and methods:** Retrospective cohort study conducted in the ICU of Hospital Santa Luzia, Brasilia, DF, during 3 months. Patients being consecutively admitted to the ICU with diagnostic of SIRS/sepsis were divided into two groups: those with elapsed time between ICU request and admission less than 6 hours (short waiting period group (SWP)) or over 6 hours (long waiting period group (LWP)).

**Results:** A total of 70 patients were enrolled (46% of admissions), 14 patients with SIRS, 27 with sepsis, 13 with severe sepsis and 17 with septic shock. For the entire cohort, the mean age was 61 ± 22 years, APACHE II was 12 ± 7.7, ICU length of stay was 15 ± 22.8 days, and 39 were male (55.7%). Thirty-five patients belonged to the LWP (50%), LWP patients had higher mortality (50% vs. 19.6%, P = 0.04), and longer ICU length of stay (13.6 ± 18.5 vs. 23.5 ± 40.7 days, P = 0.04). Relative risk for death in the LWP was 2.83 (95% CI: 1.28 to 6.28).

**Conclusions:** The elapsed time between ICU request and actual admission of patients with SIRS/sepsis over 6 hours resulted in increased ICU mortality and ICU length of stay for this group of patients.

**References**

**P57**

**Sepsis and multiple organ dysfunction in burn**
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**Background:** Advances in the treatment of burns have reduced mortality rates and improved quality of life of victims. However, the most frequent complication is infection [1]. Thermal injury over 20% of the body surface area may lead to conditions similar to SIRS, as in septic shock. Beyond the extent of body surface area burned, which causes structural changes in skin coverage, other factors lead to infectious complications in burned patients: immunosuppression resulting from thermal injury, the possibility of gastrointestinal bacterial translocation, prolonged hospitalization, the use of devices and surgical procedures related to the burned areas [2,3].

C-reactive protein (CRP) is a known marker of infection and sepsis in patients admitted to the ICU.

**Materials and methods:** CRP was measured in a cohort of 18 critically ill mechanically ventilated victims of a fire disaster in the city of Santa Maria, Brazil, on 27 January 2013, admitted to the ICU of the Hospital de Clínicas de Porto Alegre. The patients were divided into groups according to CPR levels, group 1 (CPR ≤ 190 mg/ml) and group 2 (CPR > 190 mg/ml).

The Mann-Whitney test was performed to compare groups according to mortality, length of ICU and hospital stay, presence of sepsis and SOFA score on days 1, 3 and 7.

**Results:** Six patients were male and the mean age was 23.1 ± 4.5 years. No differences were detected when patients were compared according to mortality (group 1: 0% vs. group 2: 11.1%; P = 0.48), length of ICU stay (group 1: 11 ± 8 days vs. group 2: 17.2 ± 9.7 days; P = 0.237) or length of hospital stay (group 1: 16.4 ± 8.5 days vs. group 2: 20.6 ± 10.2 days; P = 0.408). CPR levels were not associated with the development of sepsis (group 1: 50% vs. group 2: 80%; P = 0.321). The SOFA score was not significantly different between groups on day 1 and day 3 (day 1 - group 1: 4.6 ± 2 vs. group 2: 4.5 ± 2.7; P = 0.740; day 3 - group 1: 3.2 ± 2.7 vs. group 2: 5.6 ± 4.2; P = 0.203). However, the SOFA score was significantly higher on day 7 in group 2 (group 1: 3 ± 0.7 vs. group 2: 5.8 ± 1.9; P = 0.017).

**Conclusions:** CRP was not a good marker of sepsis and multiple organ dysfunction in this cohort of burned patients, possible due to the intense inflammatory response associated with burns.

**P58**

**Clinical performance of a point-of-care assay for measurement of presepsin in patients with bacteremia**
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**Background:** The soluble CD14 subtype (sCD14-ST; renamed presepsin), which is approximately 13 kDa, has been identified as a protein whose levels increase specifically in the blood of sepsis patients. In this study, we evaluated the clinical performance of a point-of-care assay for measurement of presepsin in admitted sepsis patients.

**Materials and methods:** We obtained 43 cases with blood culture test-positive from patients admitted to our hospital and compared presepsin levels with procalcitonin (PCT), CRP and white blood cell count.

**Results:** Positive ratios of presepsin levels of patients with Gram-negative bacterial infection, Gram-positive bacterial infection and fungal infection
were higher than those of PCT. When 43 cases were divided into four groups (sepsis, severe sepsis, septic shock and infection groups), presepsin levels were only significantly different between sepsis/infection group and severe sepsis group (P < 0.05). Presepsin levels reflected the blood culture test and sepsis severity more than other biomarkers.

Conclusion: This assay has sufficient clinical performance in patients admitted to the hospital in addition to the emergency room and ICU.

P60

Does the ICU experience predict psychological symptoms in relatives of patients with severe sepsis and end-of-life decisions?

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Background: Severe sepsis is the main cause of death in the ICU. Relatives are at risk for post-traumatic stress disorder (PTSD) or anxiety and depression [1]. The objective is to assess whether the ICU experience may predict these psychological symptoms of relatives at 90 days after the patient’s death or discharge.

Materials and methods: Prospective observational study in four ICUs of one university hospital, including all patients with severe sepsis and end-of-life-decisions. At 90 days, the main relative was interviewed with the Impact of Event Scale (to measure PTSD), the Hospital Anxiety and Depression Scale and self-developed items on satisfaction with the ICU experience, including medical care and communication in general as well as specifically in the end-of-life context, and decision-making. Three multiple linear regression models were calculated to predict anxiety, depression and post-traumatic stress each.

Results: Eighty-four relatives were included. They were mostly female (74%), spouse (42%) or child (42%), median age was 57 years. Seventy-seven percent acted as proxies. After 90 days, 51% relatives were at risk for PTSD, 48% for anxiety and 33% for depression. Overall satisfaction with the ICU experience was high. Relatives’ satisfaction with medical care and communication in general predicted lower anxiety (P = 0.025).

Conclusions: Relatives of patients with sepsis have a high psychological burden. Improving communication between ICU staff and relatives may reduce their symptoms of anxiety.

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Reference

P61

Quality assurance in severe sepsis: an individualised audit/feedback system results in substantial improvements in sepsis care at a large UK teaching hospital

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Critical Care 2013, 17(Suppl 4):P61; doi:10.1186/cc12960

Background: Severe sepsis has a high mortality and high healthcare costs. Rapid recognition and treatment can save lives but requires a coordinated response [1]. Hospital-wide audits in 2005 and 2010 showed significant deficiencies when compared with international guidelines, with 35% of cases receiving antibiotics in <1 hour and only 25% receiving basic pre-ICU interventions in a timely manner. By time-lining our response to severe sepsis, we identified system and process failures [2]. Some system improvements (for example, providing first-line antibiotics in acute areas) were straightforward to tackle, but sepsis care remained reliant on individual clinician response. Equally, whilst dissemination of organisation-level audit data raised the profile of sepsis, it appeared that individualised feedback can improve care, as pride and the competitive nature of healthcare workers drives improvement. This is especially true when adherence to recommended practice is low [3]. We tried to change behaviour by creating a rapid response audit/feedback mechanism that informed clinicians of their own response to the severely septic patient, from which they could learn and improve.

Materials and methods: Patients admitted to any critical care unit (58 beds, four units, two sites) with a primary admission diagnosis of severe sepsis were included in a prospective observational study. Quality assurance in severe sepsis: an individualised audit/feedback system results in substantial improvements in sepsis care at a large UK teaching hospital

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Conclusions: When adherence to recommended practice is low [3]. We tried to change behaviour by creating a rapid response audit/feedback mechanism that informed clinicians of their own response to the severely septic patient, from which they could learn and improve.
infection were screened for severe sepsis. The pre-ICU care of patients who met the criteria was then audited against the Surviving Sepsis Guidelines [1]. Time zero was defined as when the criteria for severe sepsis were first met. Information on timings of key interventions (such as doctor review and request for critical care escalation) was also gathered. An individualised traffic-light report was then generated and emailed to the patient’s consultant and other stakeholders such as junior doctors or nurses involved in the patient’s care (Figure 1). We aimed to report cases back within 7 days of arrival to ensure the patient story was fresh in the clinician’s mind. A cumulative report is generated monthly to track organisation-wide performance.

Results: Since November 2011 we have provided feedback on over 300 severe sepsis cases. Antibiotic administration in <1 hour has risen from 35% to 75% (Figure 2), and pre-ICU bundle compliance has risen from 25% to 70% (Figure 3). Since November 2012 all sepsis cases in our critical care units have been audited (30 to 35 cases/month).

Conclusions: Individualised feedback on sepsis care has led to substantial improvements in guideline compliance.

References
Treatment of sepsis: a systematic review of its main concepts

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Critical Care 2013, 17(Suppl 4):P63; doi:10.1186/cc12962

Background: Sepsis is a major challenge in medicine, its high incidence, mortality and high costs making this syndrome the leading cause of mortality in ICUs, and is considered a health problem in a worldwide extension that affects millions of people and results in high morbidity and mortality. It is believed there are 18 million annually reported cases, extension that affects millions of people and results in high morbidity and mortality in ICUs, and is considered a health problem in a worldwide extension.

Materials and methods: A quantitative, descriptive and cross-sectional, literature study, concerning the main aspects in the treatment of sepsis.

A semi-structured instrument developed by the authors was used to collect data to categorize the studies obtained. After collection, an electronic spreadsheet was generated, and data were analyzed using descriptive statistics.

Results: Ten studies with a central theme focused on the treatment of sepsis were used. Seventy percent of these studies were between the years 2008 and 2011. Fifty percent of the articles mentioned that the early approach of the infectious agent is very important for successful treatment, while 60% reported that the control of the infectious focus is one of the main alternatives. Fifty percent of the studies also reported an infusion of antibiotics in accordance with the infectious focus as essential to the treatment of sepsis, and 80% reported the use of activated protein C as an indicator for diagnosis septic patients. It is observed that most studies seek early detection of the infection and early antibiotic administration, which reinforces the need for optimization of processes for the early identification of sepsis. The first hour of the sepsis protocol proposed by the Latin American Institute of Sepsis (ILAS).

Conclusions: The present study therefore concludes that for effective treatment of sepsis an early approach right after diagnosis of the disease is indispensable. Likewise, the treatment of sepsis primarily seeks to control the infectious focus using specific antibiotics. Also, the use of activated protein C may be a good alternative in the diagnosis of this pathology and a good indicator for controlling this disease.

Acknowledgements: Faculty of Nursing, Hospital Israelita Albert Einstein, Department of the Post-graduate.
Background: Sepsis represents a substantial healthcare burden. Limited epidemiologic information about the demography of sepsis mortality or antibiotic resistance in the ICU is available. From population-based sources of data have been used to investigate the burden of sepsis-associated mortality on a national level. We investigated the epidemiology of sepsis deaths in Brazil from 2002 to 2011 using secondary data from the Brazilian Mortality Information System (Sistema de Informações de Mortalidade-SIM).

Materials and methods: A retrospective descriptive study using data reported to the Brazilian SIM for the years 2002 to 2011. SIM is an electronic, case-based mortality registry that derives its information almost entirely from death certificates. Sepsis-associated deaths from 2002 to 2011 were identified based on International Classification of Diseases 10th Revision codes listed on the underlying and on the contributing causes-of-death. Population-based sepsis-associated mortality rates and trends were estimated. In addition, age, gender, ethnicity, and outcome variables were assessed. Considering the cases of sepsis identified during the study period, annual population-based mortality rates were calculated using as denominators population estimates provided by the Brazilian Institute of Geography and Statistics with the 2010 census age-stratified population as the standard. Trends of mortality rates over time were explored with the chi-square test for trend. Rate changes were considered significant when $P < 0.05$.

Results: The total number of deaths recorded in SIM increased over the decade. In 2002 there were 982,294 deaths reported and in 2010 this number was 1,133,761. The number of sepsis deaths increased from 95,972 (9.8%) to 186,712 (16.5%). The average age of sepsis-associated deaths progressively increased from 60.2 years in 2002 to 2003 to 67.1 years in 2010 to 2011. During the same period the average age of all deaths increased from 57.8 years to 62.7 years. White individuals were more frequent (60.4%), as compared with mixed race (24.4%) and blacks (6.6%). A substantial part of sepsis deaths occurred in the hospital (94.8%). The age-adjusted rate of sepsis-associated mortality increased from 69.5 deaths per 100,000 to 97.8 deaths per 100,000 from 2002 to 2010 ($P < 0.001$).

Conclusions: Between 2002 and 2011, the contribution of sepsis to all-cause mortality increased significantly in Brazil. Moreover, age-adjusted mortality by sepsis also augmented in the last decade. These numbers confirm the importance of sepsis as a significant healthcare issue in Brazil as well as the need for adequate strategies of early recognition and treatment.

Acknowledgements: We are thankful to the Director, Laboratory and Committee of the Hospital Infection Control from Hospital Unimed Costa do Sol, whose contributions have made this study possible.

P67

Hourly and accurate severe sepsis classification using kernel density estimates

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Critical Care 2013, 17(Suppl 4):P67; doi:10.1186/cc12966

Background: Sepsis score classifications increase conditionally with concurrent systemic inflammatory response syndrome (SIRS) score, Sequential Organ Failure Assessment score, and clinical intervention. However, hierarchical criteria fail to accurately classify sepsis when related physiological manifestations are resolved, while the underlying infection remains.

Materials and methods: To enable hour-to-hour sepsis classification, we examined the diagnostic performance of a continuous sepsis score. We identified 36 adult patients in the Christchurch Hospital ICU with sepsis from a patient database. A severe sepsis biomarker was developed from model-based insulin sensitivity, temperature, heart rate, respiratory rate, blood pressures, and SIRS score. Sepsis and nonsepsis patient-hours were categorized by the ACCP/SCCM guidelines, where each category was scored independently, rather than hierarchically. Kernel density estimates

P66

Microbiological profile in an ICU in 1 year

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Critical Care 2013, 17(Suppl 4):P66; doi:10.1186/cc12965

Background: Critical patients requiring prolonged intensive care are more at risk of being colonized by germs acquired in an ICU and present infections. The factors that contribute to the high rate of infection and mortality in ICUs are possibly associated with the severity of the underlying disease, invasive procedures, the long period of hospitalization and use of antibiotics, especially the expanded spectrum, so that there are multidrug-resistant bacteria, which complicates therapy. Approximately 5% of patients admitted to ICUs will acquire a nosocomial infection, resulting in increased length of hospitalization, around 5 to 10 days, and will be considered a consequence of healthcare in 30% of cases. Diagnostic or therapeutic interventions provide breakthrough of the mechanical barrier of the skin and mucus assigned to invasive, skin lesions caused by

devitalization, trauma or by removing the skin secondary to burns or debridement. In addition to the mechanical factors that disrupt the natural barriers of defense, there are others that are inherent in clinical conditions of patients and promote the acquisition of infections in the hospital environment; the immune ability is compromised because the natural defense mechanisms are altered by the very nature base or as a result of therapeutic interventions. The rate of infection is high among intensive care patients, especially respiratory infections. Pseudomonas aeruginosa was the prevalent bacteria in our ICU. That is why the prevalence of infection acquired in the ICU is high and suggests that preventive measures are important to reduce the occurrence of infection in critical patients.
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were used to classify severe sepsis (including septic shock) of 1,690 hours over 6,550 total hours. Optimal diagnostic performance from the receiver operating characteristic (ROC) curve was determined for in-sample, out-of-sample, and overall estimates.

**Results:** The severe sepsis biomarker achieved 86% sensitivity (81 to 94%), 85% specificity (80 to 95%), 0.93 (0.88 to 0.99) area under the ROC curve, 8.2 (4.0 to 19.0) positive likelihood ratio, 0.17 (0.06 to 0.23) negative likelihood ratio, 68% (58 to 87%) positive predictive value, 94% (92 to 98%) negative predictive value, and a diagnostic odds ratio of 116 (17 to 308) at an optimal probability cutoff value of 0.25.

**Conclusions:** This clinical biomarker can thus be readily assessed at the bedside to yield a non-invasive and continuous estimate of the probability of severe sepsis. The results show high accuracy as a potential severe sepsis diagnostic and monitoring response to sepsis interventions in real time.

### P68

**Potential anti-inflammatory role of 2-chloroadenosine treatment during acute lung inflammation in BALB/c mice suffering from Klebsiella pneumoniae B5055-induced acute lung infection**

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**Critical Care 2013, 17(Suppl 4):P68; doi:10.1186/cc12967**

**Background:** Acute lung inflammation (ALI) is a life-threatening pathology and can develop during the course of several clinical conditions such as pneumonia, acid aspiration or sepsis. Adenosine plays a significant role in controlling acute inflammation via binding to A2A receptors on inflammatory cells; that is, neutrophils or macrophages. The present study was designed to evaluate the anti-inflammatory and immunomodulatory effects of 2-chloroadenosine (2-CADO), alone or in combination with amoxicillin/clavulanic acid (AMC), in Klebsiella pneumoniae B5055-induced acute lung infection in mice.

**Materials and methods:** Acute lung infection in mice was induced by directly instilling the selected dose (10⁶ colony-forming units/ml) of bacteria intranasally. Histopathological examination of the lungs was performed to reveal neutrophil infiltration into the lung alveoli. In addition to the major proinflammatory cytokines TNFα and IL-1α, levels of the anti-inflammatory cytokine IL-10 were also determined by ELISA.

**Results:** Intranasal instillation of bacteria caused profound neutrophil infiltration into the lung alveoli as well as a significant increase in the levels of proinflammatory mediators (that is, TNFα and IL-1α). However, intravenous administration of 2-CADO 10 μg/kg/day, alone or in combination with an antibiotic (that is, AMC 20 μg/ml i.p. 1 day after establishment of infection), significantly decreased neutrophil infiltration into the lung alveoli. A significant decrease in TNFα and IL-1α along with elevation of IL-10 levels in the lung homogenate of mice with acute lung infection was observed upon treatment with 2-CADO alone, with no significant decrease in bacterial counts. Moreover in combination with AMC, 2-CADO exhibited its immunomodulatory action in acute lung infection and prevented ALI observed during acute bacterial pulmonary infection, whilst an antibacterial action was evident by AMC.

**Conclusions:** 2-CADO proved a potent immunomodulatory agent during acute Gram-negative bacteria-induced ALI and exhibited its anti-inflammatory and immunomodulatory potential even in the presence of antibiotics. Thus, it has a potential to be used as an adjunct immunomodulatory agent during acute inflammatory conditions like ALI or sepsis.

### P70

**Possible variables related to paradoxical findings between PCR and hemoculture assays in rat experimental sepsis**

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**Critical Care 2013, 17(Suppl 4):P70; doi:10.1186/cc12969**

**Background:** A positive blood culture (BC) is considered the gold standard method for the sepsis diagnosis, although its sensibility is low (10 to 30%) which demands a better diagnostic tool to limit broad-spectrum antibiotic use in the majority of patients without culture-based sepsis diagnosis. Besides, after microbial invasion, they can remain live, dead or fragmented in the bloodstream, thus limiting BC efficiency. Herein we evaluated the PCR diagnostic efficacy under live, dead and bacterial DNA contents in the bloodstream.

**Materials and methods:** Wistar-FPM rats, weighing 200 to 250 g, were submitted to two sepsis models: S8 group, submitted to 2 ml Escherichia coli 10⁶ CFU/ml intravenous (i.v.) inoculation, LD₃₀, or 59 group, with E. coli 10⁶ CFU/ml inoculation, LD₉₀. Both groups were treated with HH (30 ml/kg of Ringer lactate i.v., in 20 minutes) in the early (E30 minute) and late (L6 hour) phases of sepsis. The mortality was followed up to 30 days (n = 6/group) and the splanchic microcirculation was monitored by sidestream dark field imaging (SDF) video microscopy at 6-hour and 24-hour periods (n = 3/group/patient).

**Results:** The HH at the E30 minute phase of S8 improved the survival rate from 40% to 90%, and L6 hour phase HH promoted an 80% survival rate. Besides, the survival rate in S9 (LD₉₀), with E30 minute HH, improved the survival rate from 20% to 50%. However, it was less effective as compared with the E6H phase HH, which resulted in an expressive survival rate (from 20% to 70%). These intriguing results suggested that there is an interdependent and time-dependent pathophysiology feature within the host response based on sepsis severity stage and a rapid high-volume reposition. The SDF analysis in control sepsis groups (S8 and S9), without fluid therapy, showed a broadly distributed microcirculation dysfunction in the liver lobules and kidney tubules at 6 hours after sepsis challenge, and such findings were similar between groups, but after 24 hours the survivors showed an improved microcirculation hemodynamic pattern and it was more evident in the S8 group. The survivals of the S8 E30 minute treated group showed less injury at 6 hours and 24 hours as compared with nontreated groups and S8 L6 hour treated animals. In S9 treated groups, both showed a partial repair at 24 hours post sepsis.

**Conclusions:** The hyperfluid therapy given rapidly in both early and late phases in sepsis and severe sepsis states showed that its beneficial effect was more or less effective dependent on the phase and sepsis intensity; however, the more prominent survival rates were seen at the early phase of sepsis (S8) and at the later phase of severe sepsis (S9). The underlying pathophysiology evolved in these paradoxical conditions needs to be better elucidated.

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### P69

**Beneficial effects of vigorous fluid resuscitation therapy varied depending on the time of onset and the sepsis stage in rats: preliminary study**

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**Critical Care 2013, 17(Suppl 4):P69; doi:10.1186/cc12968**

**Background:** Fluid replacement has been a usually recommended maneuver in sepsis; however, growing clinical controversies in the management of critically ill patients with severe sepsis have questioned its benefit. Herein, we evaluated the effect of a rapid hyperhydration (HH) therapy in varying stages of sepsis.

**Materials and methods:** Wistar-FPM rats, weighing 200 to 250 g, were submitted to two sepsis models: S8 group, submitted to 2 ml Escherichia coli 10⁶ CFU/ml intravenous (i.v.) inoculation, LD₃₀, or 59 group, with E. coli 10⁶ CFU/ml inoculation, LD₉₀. Both groups were treated with HH (30 ml/kg of Ringer lactate i.v., in 20 minutes) in the early (E30 minute) and late (L6 hour) phases of sepsis. The mortality was followed up to 30 days (n = 6/group) and the splanchic microcirculation was monitored by sidestream dark field imaging (SDF) video microscopy at 6-hour and 24-hour periods (n = 3/group/patient).

**Results:** The HH at the E30 minute phase of S8 improved the survival rate from 40% to 90%, and L6 hour phase HH promoted an 80% survival rate. Besides, the survival rate in S9 (LD₉₀), with E30 minute HH, improved the survival rate from 20% to 50%. However, it was less effective as compared with the E6H phase HH, which resulted in an expressive survival rate (from 20% to 70%). These intriguing results suggested that there is an interdependent and time-dependent pathophysiology feature within the host response based on sepsis severity stage and a rapid high-volume reposition. The SDF analysis in control sepsis groups (S8 and S9), without fluid therapy, showed a broadly distributed microcirculation dysfunction in the liver lobules and kidney tubules at 6 hours after sepsis challenge, and such findings were similar between groups, but after 24 hours the survivors showed an improved microcirculation hemodynamic pattern and it was more evident in the S8 group. The survivals of the S8 E30 minute treated group showed less injury at 6 hours and 24 hours as compared with nontreated groups and S8 L6 hour treated animals. In S9 treated groups, both showed a partial repair at 24 hours post sepsis.

**Conclusions:** The hyperfluid therapy given rapidly in both early and late phases in sepsis and severe sepsis states showed that its beneficial effect was more or less effective dependent on the phase and sepsis intensity; however, the more prominent survival rates were seen at the early phase of sepsis (S8) and at the later phase of severe sepsis (S9). The underlying pathophysiology evolved in these paradoxical conditions needs to be better elucidated.

**Acknowledgements:** Grant number 2012/20841-7, São Paulo Research Foundation (FAPESP).
Results: In the Live group, at 20 minutes the sensibility was 100% by both BC and PCR and at 6 hours the sensitivity was 60% (with 200 μl) and 90% (with 1,000 μl) in BC, and 80% in PCR sampled with 50 μl blood volume. In the Dead group, the PCR sensitivity was 90% at 20 minutes and 50% at 6 hours. In the DNA group, the sensitivity remained at 50% independent of time. The inflamed condition did not change PCR sensitivity. Overall data showed that in both techniques the sensitivity dropped with time. In the BC assay the positivity was dependent on sampled blood volume, and in the PCR it was related to live or dead condition. These findings suggest that the live bacteria remain for a short period of time in the bloodstream while DNA can last for longer periods.

Conclusions: Considering that PCR is performed with 40× less blood compared with a habitual BC, PCR can be an assay of choice when BC is negative and in conjunction in a live bacteria circulating condition. Besides, the PCR assay with specific primers can be a useful method for sepsis diagnosis in specific bacterial surge events in the ICU, thus improving antibiotic usage potentials.

P72

Interruption of the intestinal immune route to the systemic circulation associated with early hyperperfusion minimized splanchic microcirculation damage and improved sepsis survival

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Critical Care 2013, 17(Suppl 4):P72; doi:10.1186/cc12971

Background: Considering that the communication of the intestinal immunity with the systemic bloodstream can be a relevant adjuvant factor in the amplification of the host systemic inflammatory response and subsequent multiple organ dysfunction in sepsis, we aimed to evaluate the effect of the obstruction of the mesenteric lymph duct (OMLD) associated with massive fluid therapy in the early phase of sepsis and severe sepsis models.

Materials and methods: Adult Wistar-PEM rats were submitted to 108 (S8) or 109 (S9) CFU/ml Escherichia coli inoculum intravenously (i.v.) (ΔΔap within 26 hours), and were treated with hyperhydration (HH) with or without previous OMLD (n = 5/group). Control group were naïve animals (N) and animals submitted to HH or sepsis only. The mortality of groups was followed up to 30 days after experiments and micro-circulation monitoring was observed at 6 hours post sepsis induction by videomicroscopy (sidestream darkfield imaging (SDF)).

Results: The effect of OMLD + HH reduced significantly the sepsis mortality rate: S8 (60% to 14.5%) and S9 (80% to 60%). Besides, the liver and kidney microcirculatory features were better preserved as compared with untreated sepsis groups under video-microscopy (SDF) monitoring. (Figure 1).

Conclusions: These preliminary findings showed that both HH and OMLD have a potential therapeutic application in sepsis by minimizing the splanchic organ’s microcirculation dysfunction.

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P73

Effect of sepsis challenge in chronic inflammation state on mortality and long-term pathological findings in rats

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Critical Care 2013, 17(Suppl 4):P73; doi:10.1186/cc12972

Background: Recent studies from our laboratory showed that animals subjected to 50% shortening of the small intestine developed bacterial translocation unchained chronically. Bacterial translocation has shown the effect of exacerbation of systemic inflammatory response by crosstalk between intestinal and systemic immune response. In this sense, the aim of this study was to evaluate whether a septic challenge in the state of chronic inflammation resulting from the shortening of the small bowel can modify the mortality outcome and trigger organ alterations in the long term.

Materials and methods: Wistar-PEM rats were submitted to 50% small intestine shortening (IS group, n = 20) or sham intestinal anastomosis (IA group, n = 20), and after 4 months were submitted to sepsis challenge with 2 ml 106 CFU/ml Escherichia coli i.v. The mortality was observed up to 30 days and the survivors of both groups were killed after 6 months for histological analysis. The other 10 animals were killed after 4 months of intestinal shortening in order to determine the histological pattern related to the bowel shortening effect.

Results: The mortality rate after sepsis was 80% in the IS group and 35% in the IA group. The bowel shortening without sepsis challenge showed hepatic mild steatosis with inflammation similar to acute hepatitis, vascular congestion and focal necrosis. The distal ileum showed shortening and broadening of villus, focal cryptic necrosis and mild macrophages and eosinophil infiltration in the lamina propria. In the IS group was seen a generalized steatosis and vascular congestion in the liver; alveolar atelectasis, BALT hyperplasia, a large number of macrophages, mast cells, foam cells, lymphocytes, eosinophil and plasmocyte infiltration and alveolar edema, plus vascular congestion and sclerosis in the lung; villus atrophic necrosis, intense inflammatory cell infiltration and vascular congestion in the lamina propria of the ileum; and the kidney with tubular nephrosis, tubular obstruction, vascular congestion with interstitium hemorrhage and tubular hyaline material deposition. In the IA group was seen moderate liver steatosis, intestinal lamina propria cellular infiltrations, glomerulonephritis, kidney tubular edema, parenchymal hemorrhage and...
Bowman capsule thickness. However, the alterations were less compared with the IS group.

**Conclusions:** The chronic inflammatory state, in combination with sepsis, might be an important aggravating factor related to sepsis morbidity and mortality by promoting an increasing organ dysfunction in the long term.

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**Figure 1 (abstract P72)** Splanchnic organ’s microcirculation following 6 hours after sepsis or HH procedures by SDF monitoring: (a) HH; (b) S8 + OMLD + HH; (c) S9 + OMLD + HH.
P74
Cholecystokinin inhibits inducible nitric oxide synthase expression in lipopolysaccharide-stimulated macrophages

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Background: Cholecystokinin (CCK) receptors are expressed in macrophages and are upregulated by inflammatory stimulus. In vitro and in vivo studies have demonstrated the ability of CCK to decrease the production of various proinflammatory cytokines. This study investigates the role of CCK on iNOS expression in lipopolysaccharide (LPS)-activated peritoneal macrophages, as well as the intracellular signaling pathways involved in affecting iNOS synthesis.

Materials and methods: Experimental procedures were approved by the Comitê de Ética em Experimentação Animal - FMRP (protocol number 152/2009). Thioglicololate-elicted macrophages were obtained by peritoneal lavage and cultured in RPMI 1640 medium, 10% fetal bovine serum and antibiotics. Nuclear p65, CAMP and iNOS levels were determined using ELISA kits. CCK receptors and iNOS expression by western blot and nitrite by the Griess method. Data were compared by one-way ANOVA and significant differences obtained using the Tukey multiple variances post hoc test.

Results: CCK reduced NO production attenuating iNOS mRNA expression (15.49 ± 10.80 vs. 113.16 ± 0.23 AU; P < 0.05) and protein formation. Furthermore, CCK inhibited the NF-κB pathway reducing iNOS degradation and minimized p65-dependent translocation to the nucleus (54.78 ± 84.57 vs. 90.42 ± 9.13%, P < 0.05). Moreover, CCK restored the intracellular CAMP content activating the cAMP-protein kinase A (PKA) pathway, which resulted in a negative modulatory role on iNOS expression and nitrite production. In peritoneal macrophages, the CCK-1R expression was predominant and upregulated by LPS (0.61 ± 0.08 vs. 0.30 ± 0.09 AU; P < 0.05). The pharmacological studies confirmed that CCK-1R subtype is the major receptor responsible for the biological effects of CCK.

Conclusions: These data suggest an anti-inflammatory role for the peptide CCK in modulating iNOS-derived NO synthesis, possibly controlling the macrophage hyper-activation through NF-κB, cAMP-PKA and CCK-1R pathways.

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P75
CD11b and TLR4 in human neutrophil priming by endotoxins from Escherichia coli

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Background: The interaction of endotoxins (lipopolysaccharides (LPS)) from Gram-negative bacteria with peripheral blood mononuclear cells leads to the expression of a receptor cluster composed from mCD14, CD11b/CD18, TLR4, CD16A and CD36 [1,2]. It is well known that the main signal transducing receptor complex is TLR4-MD2 while mCD14 is involved in the recognition of S or R endotoxin’s glycoforms [3,4]. A growing body of evidence indicates that the CD11b/CD18 receptor plays the significant role in the endotoxin signaling machinery because it can influence TLR4-mediated cell activation [5]. So, using mAbs, we carried out experiments to elucidate the influence of CD11b inhibition on neutrophil priming by endotoxins for N-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced respiratory burst.

Materials and methods: Human neutrophils were isolated from heparinized blood of healthy volunteers by standard procedure and incubated with or without anti-TLR4 mAbs (HTA125, IgG3) or anti-CD11b mAbs (clone 44, IgG1) or isotypic immunoglobulin controls, respectively, for 30 minutes before stimulation with S-LPS or Re-LPS from Escherichia coli O55: B5 or JM103, respectively. The cells (2 x 10^6), 2% of autologous serum, glucose and luminol in Ca^2+-PBS buffer (pH 7.3), were placed in the chemiluminometer's chambers (37°C) and primed by S-LPS or Re-LPS (100 ng/ml) for 30 minutes (37°C). Reactive oxygen species (ROS) production was triggered by addition of fMLP (1 μM). The chemiluminescence reaction was monitored continuously for 7 minutes. Total ROS production by control and LPS primed neutrophils during the first 50 seconds after fMLP addition is presented in Figure 1.

Results: Re-LPS revealed the most neutrophil priming activity in comparison with S-LPS (Figure 1A), which is in accordance with our previous work [6]. Actually, mAbs against TLR4 as well as against CD11b did not inhibit neutrophil priming by E. coli endotoxins. Moreover, the incubation of cells with anti-TLR4 or anti-CD11b mAbs followed by endotoxin priming increased fMLP-induced ROS production (Figure 1A). However, the differences between priming effectiveness of S-LPS and Re-LPS, which had been seen in endotoxin primed cells, were leveled by prior cell exposure to anti-CD11b mAbs. Neutrophils exposed to anti-TLR4 mAbs retained their ability to distinguish between S-LPS or Re-LPS being primed, respectively (Figure 1A). Incubation with isotypic IgG3, decreased fMLP-induced ROS production from unprimed neutrophils (Figure 1B) that was not observed in the case of IgG1. These results demonstrate that Fc regions of isotypic immunoglobulins and therefore of mAbs used in our study are not silent parts of these molecules regarding neutrophil surface receptors and their intracellular signaling pathways. Finally, the incubation of cells with isotypic immunoglobulins and then with Re-LPS did not abrogate neutrophil priming for subsequently fMLP-triggered ROS production.

Conclusions: The inhibition of human neutrophil CD11b by specific mAbs (clone 44) did not abolish LPS-dependent neutrophil priming for fMLP-induced respiratory burst, but eliminated the capacity of these cells to distinguish between S-LPS or Re-LPS glycoforms. Unlike the effect of anti-CD11b mAbs, neutrophil exposition to anti-TLR4 mAbs (HTA125) did not inhibit neutrophil priming and capacity of these cells to distinguish endotoxin’s glycoforms.

References

P76
Taurine chloride decreases cell viability and cytokine production in blood and spleen lymphocytes from septic rats submitted to sepsis

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Introduction: Attention has been paid in recent years to studies showing immune cell death mechanisms during the course of sepsis in response to proinflammatory and anti-inflammatory mediators that are involved in its pathophysiology. Taurine (Tau) is an abundant amino acid in polymorphonuclear leukocytes that reacts with hypochlorous acid to form taurine chloramide (TaulC) under inflammatory conditions. In this context, we investigated potential interactions between lymphocytes and TauCl in rats submitted to cecal ligation and perforation (CLP), analyzing cell viability and cytokine secretion profile (TNFα, IFNγ, IL-6, IL-17a, IL-23 and IL-10).

Materials and methods: Adult male rats were divided in two groups: CLP and C that were killed 24 or 120 hours after sepsis induction to isolate lymphocytes from the blood and spleen. Lymphocytes (>95.0% purity determined by differentiation with Giemsa staining) were cultured...
for 24 hours at a concentration of $1 \times 10^6$ cells/ml and activated by 2 mg/ml concanavalin A. After 24 hours, Tau and TauCl were added at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 mM for 1 hour. After this time, cells were incubated with MTT (500 μg/ml) for 3 hours to evaluate cell viability and supernatants were used to determine cytokine concentrations.

**Results:** Tau-treated cells exhibited better viability than those treated with TauCl, in both time and organs. TauCl, in a time and dose-dependent ratio, decreased cytokines secretion when compared with untreated cells. See Figures 1 to 7.

**Conclusion:** These findings show a possible impairment in lymphocyte function promoted by TauCl, correlated with immunosuppression and cell death characteristic of the late stages of sepsis.

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**Figure 1(abstract P75)**

**P77**

CD40 expression in the hippocampus and its role in blood-brain barrier permeability, neutrophil infiltration and oxidative stress: implication for brain damage associated with sepsis in rats


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Figure 1 (abstract P76) Cell viability by MTT assay. Viability of lymphocytes treated with Tau and TauCl in different molar concentrations. Rats were submitted to CLP or Sham, and 24 or 120 hours after the surgery their blood and spleens were collected, the lymphocytes were isolated, cultured and the cell viability was measured by MTT assay. (A) blood, 24 hours; (B) blood, 120 hours; (C) spleen, 24 hours; (D) spleen, 120 hours. *P < 0.05, compared with sham group (Tau-treated); #P < 0.05, compared with sham group (TauCl-treated), n = 5.

Figure 2 (abstract P76) Cytokine secretion. Effect of TauCl on production of proinflammatory mediator IL-17A by Th17 lymphocytes. Activated lymphocytes (1 × 10^6 cells/ml) were preincubated with TauCl (0.1 or 0.5 mM) for 1 hour. After this, supernatants were collected and IL-17A was measured by ELISA. (A) blood; (B) spleen. Results are expressed as means ± SD. *Compared with sham control 24 hours; #compared with Clp control 24 hours; &compared with sham control 120 hours; $compared with Clp control 120 hours, all with P < 0.05 significant (n = 5).

Figure 3 (abstract P76) Effect of TauCl on production of proinflammatory mediator IL-23 by Th17 lymphocytes. Activated lymphocytes (1 × 10^6 cells/ml) were preincubated with TauCl (0.1 or 0.5 mM) for 1 hour. After this, supernatants were collected and IL-23 was measured by ELISA. (A) blood; (B) spleen. Results are expressed as means ± SD. *Compared with sham control 24 hours; †compared with Clp control 24 hours; ‡compared with sham control 120 hours; §compared with Clp control 120 hours, all with P < 0.05 significant (n = 5).
Background: Sepsis is a clinical condition resulting from the excessive inflammatory response of the host against an infectious agent and is associated with high morbidity and mortality in patients in ICUs. In sepsis the brain can be targeted, associated with mental damage and decline, impaired attention, disorientation, delirium and coma. It has been seen that the permeability of the blood-brain barrier (BBB) is associated with septic encephalopathy, allowing cell infiltration and increased oxidative stress. Accordingly, such events can be potentiated through the involvement of molecules that when activated perpetuate the inflammatory response and the breaking of the BBB, and it is possible to postulate that the CD40 molecule may be involved by being under increased expression in microglia in inflammatory events occurring systemically. The aim of this study therefore is to evaluate the role of CD40 in the breakdown of the BBB, cell infiltration and oxidative damage in the brain of rats with sepsis.

Materials and methods: Male Wistar rats were subjected to cecal ligation and puncture (CLP) to induce sepsis. The animals (n = 10) were

Figure 4(abstract P76) Effect of TauCl on production of proinflammatory mediator IFNγ by Th1 lymphocytes Activated lymphocytes (1 × 10⁶ cells/ml) were preincubated with TauCl (0.1 or 0.5 mM) for 1 hour. After this, supernatants were collected and IFNγ was measured by ELISA. (A) blood; (B) spleen. Results are expressed as means ± SD. *Compared with sham control 24 hours; #compared with Clp control 24 hours; &compared with sham control 120 hours; †compared with Clp control 120 hours, all with P < 0.05 significant (n = 5).

Figure 5(abstract P76) Effect of TauCl on production of proinflammatory mediator TNFα by Th1 lymphocytes Activated lymphocytes (1 × 10⁶ cells/ml) were preincubated with TauCl (0.1 or 0.5 mM) for 1 hour. After this, supernatants were collected and TNFα was measured by ELISA. (A) blood; (B) spleen. Results are expressed as means ± SD. *Compared with sham control 24 hours; #compared with Clp control 24 hours; &compared with sham control 120 hours; †compared with Clp control 120 hours, all with P < 0.05 significant (n = 5).

Figure 6(abstract P76) Effect of TauCl on production of proinflammatory mediator IL-6 by Th2 lymphocytes Activated lymphocytes (1 × 10⁶ cells/ml) were preincubated with TauCl (0.1 or 0.5 mM) for 1 hour. After this, supernatants were collected and IL-6 was measured by ELISA. (A) blood; (B) spleen. Results are expressed as means ± SD. *Compared with sham control 24 hours; †compared with Clp control 24 hours; †compared with sham control 120 hours; †compared with Clp control 120 hours, all with P < 0.05 significant (n = 5).
Endotoxin induces conversion of endothelial cells into activated fibroblasts

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Figure 7(abstract P76) Effect of TauCl on production of anti-inflammatory mediator IL-10 by Th2 lymphocytes. Activated lymphocytes (1 × 106 cells/ml) were preincubated with TauCl (0.1 or 0.5 mM) for 1 hour. After this, supernatants were collected and IL-10 was measured by ELISA. (A) blood; (B) spleen. Results are expressed as means ± SD. *Compared with sham control 24 hours; †compared with Clp control 24 hours; ‡compared with sham control 120 hours; §compared with Clp control 120 hours, all with P < 0.05 significance (n = 3).

Background: Endothelial dysfunction is a key step in endotoxemia-derived sepsis syndrome pathogenesis. It is well accepted that the bacterial endotoxin lipopolysaccharide (LPS) induces endothelial cell (EC) dysfunction through immune system overactivation [1-3]. However, LPS can also affect ECs in the absence of participation by immune cells [4-6]. Although interactions between LPS and ECs evoke endothelial death, a significant portion of ECs are resistant to LPS challenge [6-8]. However, the mechanism that confers endothelial resistance to LPS is not known. Considering that LPS-resistant ECs exhibit a fibroblast-like morphology, suggesting that these ECs enter in a fibrotic program in response to LPS, our aim was to investigate whether LPS induces endothelial fibrosis and explore the underlying mechanism.

Materials and methods: We used two different models: primary ECs, and intact blood vessels (IBV). Both preparations were freshly obtained from umbilical cord veins from normal pregnancies, after patients’ informed consent. The investigation conforms with the principles outlined in the Declaration of Helsinki. The Commission of Bioethics and Biosafety of Universidad Andres Bello approved all experimental protocols. Once the preparation was established they were cultured with or without LPS as a model of endotoxemia. ECs were exposed to 20 mg/ml LPS for 72 hours, while IVB was challenged to 20 mg/ml LPS on the inside for 48 hours. Results: ECs exposed to LPS showed a fibroblast-like morphologic change. In addition, LPS-treated ECs showed an upregulation of both fibroblast-specific protein expression such as fibroblast specific protein-1 and α-smooth muscle actin, and extracellular matrix proteins secretion including fibronectin and collagen type III. In concordance, ECs exposed to endotoxin showed a severe downregulation of endothelial markers such as vascular endothelial cadherin and the platelet endothelial cell adhesion molecule-1 (CD31). Similar results were obtained in the endothelial monolayer from IBV perfused with LPS in which abundant fibrosis was observed. Furthermore, we demonstrate that LPS-induced EC fibrosis is dependent on the endotoxin receptor toll-like receptor-4. In addition, the participation of NADPH oxidase activity and ROS generation was demonstrated using specific blockers. Finally, we demonstrated by means of small interfering technology and a pharmacological inhibitor that LPS-induced EC fibrosis is dependent on the actin like kinase-5 kinase activity, suggesting that tumor growth factor beta is involved in this fibrogenic process.

Conclusions: We conclude that LPS is able and sufficient to promote endothelial fibrosis. It is noteworthy that LPS-induced endothelial fibrosis perpetuates endothelial dysfunction as a maladaptive process rather than a survival mechanism for protection against LPS. These findings are useful in improving current treatment against endotoxemia-derived sepsis syndrome and other inflammatory diseases.

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References
Impaired calcium mobilization in vascular smooth muscle of rats in septic shock
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Background: Calcium activity is essential to vascular smooth muscle contraction. Although it is well established that arteries from rats in septic shock present hyporesponsiveness to vasoconstrictor drugs, the role of calcium mobilization in this contractile dysfunction is far less investigated. We hypothesized that during septic shock calcium dynamics is changed and may have a role in the vascular dysfunction in sepsis.

Materials and methods: Female Wistar rats (3 months old) were anesthetized by oxygen-isoflurane (3%) inhalation and subjected to cecal ligation and puncture surgery (CLP). Immediately after and every 12 hours rats received physiological saline solution (PBS 30 ml/kg, subcutaneously) and tramadol (5 mg/kg, subcutaneously). After 6 hours (CLP-6) or 24 hours (CLP-24) rats were killed, the aorta was harvested and cut in rings, the endothelium was removed and rings were mounted in baths. Rings were exposed to KCl 120 mM and phenylephrine (PE 1 µM). Aorta rings were kept in a modified depolarizing Krebs solution, nominally Ca²⁺ free and contracted by CaCl₂ (1 to 100 mM). The same protocol were repeated in presence of thapsigargin (3 µM), DTNB (100 µM) or PTIO (100 µM). Different vessels were exposed to single concentrations of PE (1 µM) or caffeine (20 mM) in Ca²⁺-free solution, in the presence or absence of thapsigargin.

Results: Maximal contraction (Emax) induced by KCl or PE was reduced, especially in the CLP-24 group. Similarly, CaCl₂-induced contraction was reduced (60%) in the CLP-24 group. Thapsigargin (sarcoplasmatic calcium reuptake blocker) and DTNB (sulphydryl oxidase) oxidation restored the contraction elicited by CaCl₂ in septic rings, but without effect in control rings. PE-induced contraction in calcium-free solution was significantly reduced in CLP-24 rings (Emax = 1.6 ± 0.4 g control vs. 0.3 ± 0.1 g CLP-24 rings). Thapsigargin did not change the hyporesponsiveness to PE but PTIO (nitric oxide scavenger) restored it partially. Caffeine-induced contraction in Ca²⁺-free solution was reduced in CLP-24 rings (0.2 ± 0.06 g control vs. 0.03 ± 0.01 g in CLP-24). Thapsigargin or PTIO restored the contraction induced by caffeine.

Conclusions: These data suggest that in septic shock septic calcium mobilization is strongly impaired. Although preliminary, our results suggest that calcium channel nitrosylation and calcium reuptake may be reasons for the vascular hyporesponsiveness of septic shock.

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Evaluation of the cardiac effects of norepinephrine and dobutamine in rats with septic shock
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Background: Hypotension and cardiac dysfunction are frequently found in severe sepsis and septic shock. Vasoactive and inotropic drugs are largely used to reverse hypotension, but its effects on heart function have been scarcely investigated [1]. We thus evaluated the influence of both norepinephrine and dobutamine on the cardiovascular function of rats subjected to cecal ligation and puncture (CLP).

Materials and methods: The measurement of the cardiac function was performed in male Wistar rats (3 to 4 months old), kept under isoflurane-induced anesthesia (1 to 3%), using a pressure-volume catheter, which was inserted into the left ventricle through the carotid artery. Blood samples were collected from all animals for hematological analyses. The experiments were conducted at 24 and 48 hours after CLP. For this, the cecum was ligated with a ratio of 50% and perforated with a needle (18 G, four holes; mortality rate ~50% after 48 hours), followed by four subcutaneous injections (12/12 hours) of sterile saline (30 ml/kg) and tramadol (5 mg/kg), for fluid resuscitation and analgesia, respectively. Data were recorded at baseline and after single bolus administration of norepinephrine (1, 3 and 10 nmol/kg, i.v.) or dobutamine (3, 10 and 30 nmol/kg, i.v.). The results obtained in CLP groups were compared with control (CT) animals, which did not undergo any manipulation.

Results: Both CLP 24 and 48 hours presented thrombocytopenia (~40% reduction), lymphopenia, hypoglycemia, and leukopenia (P < 0.05), a clear indication of severe sepsis. However, only CLP 48 hour animals displayed refractionary hypotension (MAP = 59 mmHg, vs. 78 mmHg in CT; P < 0.05) in spite of volume resuscitation. The highest doses of norepinephrine and dobutamine increased the MAP to 133.8 ± 8.1 and 97.8 ± 3.1 mmHg in CT, and to 120.6 ± 6.7 and 77.3 ± 4.4 mmHg in CLP 48 hour animals, respectively. The heart rate was significantly increased by norepinephrine and dobutamine in control, but not in CLP 48 hour animals. In addition, the basal values of both dP/dtmax and dP/dtmin, as well as after 1 nmol/kg dobutamine, were reduced in CLP 48 hour animals.

Conclusions: Using a pressure-volume catheter in a closed-chest approach we demonstrated that, in spite of the ability to increase blood pressure, the chronotropic effects of norepinephrine and dobutamine are reduced at 48 hours after CLP in rats subjected to CLP. In addition, all doses of norepinephrine, but only by the highest doses of dobutamine, improved systolic and diastolic function in these animals.

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Increased sympathetic tone contributes to cardiovascular dysfunction in sepsis
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Background: The cardiovascular dysfunction of sepsis/septic shock is characterized by hypotension, tachycardia/bradycardia, endothelial dysfunction and hyporesponsiveness to vasoconstrictors. Hypotension and low tissue perfusion trigger an increase in sympathic tone probably as an attempt to restore blood pressure to normal levels. The persistently high sympathetic stimulation may lead to the exhaustion of the capacity of vascular response and thus create a vicious circle contributing to vascular hyporesponsiveness and higher adrenergic stimulation. In addition, in septic shock patients, increased arterial levels of norepinephrine (NE) were significantly associated with mortality. The aim of this work was to evaluate the vascular response to an adrenergic agonist during severe sepsis and the effects of the early inhibition of sympathetic tone in sepsis-induced cardiovascular dysfunction.

Materials and methods: Sepsis was induced by cecal ligation and puncture (CLP) surgery in female Wistar rats. Septic animals and controls (CT) were treated with the ganglionic blockers pentolinium (PENT; 5 mg/kg, s.c.) or hexamethonium (HEX, 15 mg/kg, s.c.) or vehicle (saline) 1 hour after surgery. The vascular response to the administration of NE was assessed 6 hours or 24 hours after CLP surgery. The survival rate was also evaluated. All procedures were approved by our Institutional Ethics Committee (PP00631/CEUA-UFSC) and are in accordance with NIH Animal Care Guidelines.

Results: Six hours after CLP surgery, septic animals were hypotensive. Treatment with hexamethonium or pentolinium prevented the development of hypotension (control 84.8 ± 2.6; CLP 60.7 ± 4.5; CLP + HEX 72.7 ± 3.1; CLP + PENT 78.1 ± 2.7 mmHg; *P < 0.05 compared with control group). However, 24 hours after surgery, the ganglionic blockers failed to prevent hypotension (control 88.5 ± 1.7; CLP 62.7 ± 1.7; CLP + HEX 68.8 ± 2.7; CLP + PENT 70.9 ± 3.4 mmHg; *P < 0.05 compared with control group). The vascular hyporesponsiveness to NE observed both 6 hours and 24 hours after CLP was completely blocked by the early treatment with both ganglionic blockers (NE 10 nmol/kg, expressed as increase in blood pressure compared with baseline: control 54.2 ± 4.5; CLP + PENT 84.2 ± 4.5).
CLP 6 hours 21.9 ± 3.1; CLP 6 hours + HEX 52.6 ± 7.0; CLP 6 hours + PENT 54.1 ± 4.9; CLP 24 hours 31.1 ± 5.6; CLP 24 hours + HEX 74.6 ± 3.0; CLP 24 hours + PENT 64.4 ± 7.8 mmHg; P < 0.05 compared with control group). The early ganglionic blockade with PENT decreased the mortality observed after 96 hours.

Conclusions: Our data indicate that increased sympathetic tone in sepsis contributes, at least in part, to the development of hypotension, hyporesponsiveness to vasoactive agents and mortality. Blockade of increased sympathetic tone thus may be considered as an adjuvant therapy for the treatment of septic cardiovascular dysfunction.

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P82 Vascular smooth muscle cell activation depends on NOS-1-derived NO and consequent peroxynitrite generation

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Background: Low levels of nitric oxide (NO) play a key role in vascular tone maintenance. Previous results from our laboratory show that hypotension and mortality during sepsis are prevented by the early administration of NOS-1 inhibitors. The aim of this study was thus to investigate the role of NOS-1 and NOS-3-derived NO and of other reactive oxygen species (ROS) in smooth muscle cell activation.

Materials and methods: Smooth muscle cell line of rat aorta (A7r5) was used. Control cells and NOS-1 or NOS-3 silenced cells (siNOS-1 and siNOS-3, respectively) were stimulated with LPS 1 µg/ml and IFN 200 U/ml (LPS/IFN). NO and ROS production was assessed with fluorescent probes. NO content was evaluated by western blot and NO-S-2 activity was indirectly measured by Griess reaction. Further, control cells were treated for 30 minutes with a NO scavenger (c-PTIO), a NOS inhibitor (7-NI) or a NADPH oxidase inhibitor (DPI) before stimulation. Immunofluorescence was used to evaluate protein nitration and NF-κB nuclear translocation. To confirm the role of peroxynitrite in cell activation, control cells were stimulated with a sub-effective amount of LPS/IFN together with a NO donor and a superoxide anion generator and treated with a NOS-2 inhibitor 4 hours after stimulation. Griess reaction was performed 48 hours after. Statistical comparisons were performed by two-way ANOVA followed by the Bonferroni test.

Results: A7r5 control cells stimulated with LPS/IFN presented a rapid increase in intracellular NO and ROS content. These increases were prevented by c-PTIO, 7-NI and DPI, as well as in siNOS-1 and siNOS-3 cells. NO-S-2 was only expressed after cell stimulation. Control cells incubated with c-PTIO or 7-NI and stimulated with LPS/IFN presented a diminished NO-S-2 expression and activity. Only in siNOS-1 cells was NO-S-2 expression and activity also reduced. Nuclear translocation of NF-κB and positive nitrotyrosine reaction were reduced in c-PTIO or 7-NI treated groups. Sub-effective concentrations of LPS/IFN did not induce significant nitrite production. However, when sub-effective LPS/IFN was associated with the production of low concentrations of peroxynitrite, nitrite accumulation was as high as in cells stimulated with activating concentrations of LPS/IFN.

Conclusions: We show for the first time the importance of NOS-1-derived NO and peroxynitrite for smooth muscle cell activation. Cell stimulation with LPS/IFN causes an early NO-S-1-derived NO pulse and a ROS pulse that forms peroxynitrite. The interplay between these species seems to be key events for NF-κB nuclear translocation and NO-S-2 expression.

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P83 Estradiol cipionate modulates immunological response during sepsis

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Background: Sepsis and its common complication septic shock are generally induced by the action of lipopolysaccharide (LPS) and characterized by peripheral arteriolar vasodilatation that results in hypotension and inadequate tissue perfusion. During sepsis, secretion occurs of large amounts of inflammatory mediators such as nitric oxide (NO), interleukin 1 (IL-1) and TNFα that will modulate the inflammatory response. One significant finding in clinics is that men and women respond differently to sepsis, with better prognosis related to women [1].

Materials and methods: Male and female (ovariectomized and sham surgery) rats were injected intraperitoneally (i.p.) for three consecutive days with ECP 40 µg/kg or vehicle. On the third day, after ECP injection, rats receive i.p. injection of 10 mg/kg bacterial LPS or saline solution. Plasma was collected 2, 4 and 6 hours after LPS for NO and cytokine measurements.

Results: Administration of LPS increased the NO plasma concentration in males and females (2, 4 and 6 hours). ECP pretreatment decreased the NO concentration in sham females at 4 and 6 hours; conversely, it increased nitrate levels in ovariectomized and in males at 4 and 6 hours. IL-1 plasma concentration was increased in the three groups after LPS administration at 2 and 4 hours and in Sham at 6 hours; ECP pretreatment decreased IL-1 plasma concentration in all groups at 2 hours. LPS administration also increased TNFα plasma concentration at 2, 4 and 6 hours in the three groups; ECP pretreatment inhibited the increase of TNFα at 2 hours in three groups.

Conclusions: Our results indicate that estradiol may have proinflammatory or anti-inflammatory actions depending on the gender and the mediator evaluated; this balance in mediator secretion may be protective and explain in part the better outcomes of woman during sepsis.

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Reference:

P84 Sulphydryl oxidation restores alpha-adrenergic vascular response and improves survival in septic rats

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Background: The profound decrease in vasomotor tone accompanied by hyporesponsiveness to vasoconstrictors is an important contributor to morbidity and mortality in septic shock. Overproduction of nitric oxide (NO) has been shown to play a relevant role in septic shock vascular dysfunction. One of the mechanisms whereby NO exerts some of its effects is the reaction with thiol groups of cysteine residues in a process called S-nitrosylation and producing S-nitrosothiols. The aim of the present study is to show that modification in S-nitrosylation has an important impact in sepsis-induced vascular dysfunction and mortality.

Materials and methods: Wistar female rats were anesthetized and submitted to cecal ligation and puncture (CLP) for induction of sepsis. Thirty minutes before and 4 hours after surgery, animals received 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), an oxidizing agent of sulfhydryl groups or vehicle. Eight hours after CLP the rats were prepared for invasive blood pressure measurements and vascular reactivity to phenylephrine was assessed. The effect of DTNB on survival was also evaluated. All of the procedures were approved by the institutional Animal Ethics Committee (protocol number PPD0790/CEUA/UFSJC).

Results: Eight hours after sepsis induction, rats displayed a pronounced hyporesponsiveness to phenylephrine (10 nM/kg; 21 3 ± 1.1 mmHg CLP group compared with 42.3 ± 0.8 mmHg in control group; P < 0.05, n = 6). When DTNB was injected 30 minutes before and 4 hours after CLP surgery, the response to phenylephrine was completely normalized (10 nM/kg; 46.2 ± 2.2 mmHg; P < 0.05, n = 6). DTNB also reduced the mortality of septic rats by 40%.

Conclusions: Our results suggest that NO overproduction during septic shock may cause nitrosylation of critical proteins important for alpha-adrenergic contractile response. Oxidation of protein sulfhydryls by DTNB prevents nitrosylation and restores the response to phenylephrine in septic animals. Another important finding is that DTNB restored the alpha-adrenergic response even after sepsis is installed. Understanding
the role of S-nitrosylation may help to develop strategies to prevent or reverse the vascular dysfunction of sepsis. 

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P85
Effect of polymicrobial sepsis on the respiratory mechanism of rats previously exposed to cigarette smoking

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Background: The objective was to evaluate the profile of respiratory mechanism of septic female rats previously submitted to exposure of cigarette smoking.

Materials and methods: Initially, female rats (230 to 300 g) were randomly divided into a control group (NS) kept with no manipulation and a cigarette smoking-induced respiratory disorders group (S). A rat model used to induce respiratory disorders was established by exposure to cigarette smoke (30 cigarettes/15 minutes) daily for 6 weeks. Twenty-four hours after the last cigarette smoking exposure session, each group underwent cecal ligation and puncture procedures to induce polymicrobial sepsis (CLP group) or only underwent a laparotomy (sham group), resulting in the following four experimental groups: Sham-NS (n = 11), Sham-S (n = 11), CLP-NS (n = 6) and CLP-S (n = 9). The profile of respiratory mechanism was evaluated by forced oscillation measurements using a computer-controlled piston ventilator (flexiVent; SCIREQ Inc.) at 24 hours CLP or Sham procedures. The respiratory system parameters evaluated were calculated in flexiWare7 software. All experimental procedures used in our study were approved by the Institutional Animal Ethics Committee (n° 11221971-3/47).

Results: Among the experimental groups, no significant difference in airway resistance was verified, while prior exposure to cigarette smoking decreased the tissue resistance of sham-operated rats (0.77 ± 0.03 vs. 0.55 ± 0.01 cmH2O·sec/ml, Sham-NF and Sham-F, respectively) as well as inhibiting the increase in tissue resistance induced by sepsis (1.11 ± 0.11 vs. 0.76 ± 0.03 cmH2O·sec/ml, CLP-NS and CLP-S, respectively). The prior exposure to cigarette smoking did not alter the lung compliance of sham-operated rats, but it blocked the CLP-induced reduction of lung compliance (0.82 ± 0.04, 0.21 ± 0.11 and 0.57 ± 0.07 cmH2O·sec/ml, Sham-NS, CLP-NS and CLP-S, respectively). Similarly, cigarette smoking blocked the CLP-induced decrease of inspiratory capacity (7.85 ± 0.25, 4.96 ± 1.49 and 7.00 ± 0.41 cmH2O·sec/ml, Sham-NS, CLP-NS and CLP-S, respectively) but did not alter the inspiratory capacity from sham-operated rats (8.68 ± 0.2 cmH2O·sec/ml, Sham-S) compared with Sham-NS.

Conclusions: In contrast to sham-operated rats, cigarette smoking inhibited changes in the resistance, compliance and inspiratory capacity of the respiratory system of CLP-operated rats.

P86
Study of the effect of C1-esterase inhibitor administration to the sepsis pig model

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Background: New therapy is required that improves the prognosis of patients suffering from severe sepsis or septic shock. C1-esterase inhibitor (C1-Inh) was introduced in clinical medicine for patients with hereditary angioedema. Some studies show that C1-Inh administration may also have a beneficial effect in other clinical conditions such as sepsis [1,2]. We examined the effect of C1-Inh administration to the sepsis pig model.

Materials and methods: The experiments were performed divided into two groups: the treatment group and the control group. We administered LPS (40 µg/kg) to pigs of about 10 kg over 30 minutes. At the same time, we administered C1-Inh in the control group (500 U, n = 3; 1,000 U, n = 3), and saline in the control group (n = 3). We examined the effect of C1-Inh for the outcome of the two groups, physiological indicators such as heart rates (HR) and mean arterial pressure (MAP), and autopsy results such as pleural effusion and ascites. 

Results: The outcome of the two groups was that 5/6 in the treatment group and 2/3 in the control group survived at 240 minutes from the end of LPS administration. HR (minute) at 180 minutes from the end of LPS administration was 157.5 ± 12.3 in the treatment group and 205.3 ± 42.6 in the control group, and MAP (mmHg) at the same time was 60.0 ± 8.2 in the treatment group and 58.3 ± 5.6 in the control group. As for the autopsy results, pleural effusion (ml) was 13.28 ± 3.13 in the treatment group and 9.87 ± 4.33 in the control group, and ascites (ml) was 165.8 ± 32.99 in the treatment group and 210.0 ± 60.8 in the control group. Seeing each individual, the individual showing a large effect of C1-Inh was observed.

Conclusions: C1-Inh tended to stabilize the hemodynamics of the sepsis pig model, but was not able to reduce significantly the amount of pleural effusion and ascites.

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References

P87
Brain markers of neurodegeneration in sepsis survivor rats

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Background: Several preclinical and clinical reports indicate a significant role for systemic inflammation in chronic neurodegenerative diseases [1], with commitment of different brain regions. Several studies have demonstrated hippocampal atrophy, EEG changes [2], profound glial activation, the generation of nitric oxide and changes in expression of mediator apoptosis [3]. The release of these mediators and oxidative stress occur mainly in acute phase inflammation in sepsis survivor rats and are associated with long-term cognitive impairment [4]. These cognitive deficits have been associated with decreased quality of life and increased long-term morbidity. Some of these alterations resembled the pathophysiological mechanisms of neurodegenerative diseases. For this reason, we analyzed parameters related to neurodegeneration in rats that survived sepsis, and their relation to cognitive dysfunction.

Materials and methods: Wistar rats were subjected to sepsis by cecal ligation and puncture and 30 days after surgery the hippocampus and prefrontal cortex were isolated just after cognitive evaluation by the inhibitory avoidance test. The immunocomtent of [α-amylod peptide (Aβ), receptor for advanced glycation endproducts (RAGE) and synaptophysin were analyzed by western blot.

Results: Aβ was increased in septic animals in the hippocampus, but not in the prefrontal cortex. RAGE was upregulated in both structures after sepsis, and the immunocontent of synaptophysin was decreased only in the prefrontal, and inversely correlated to Aβ levels. Prefrontal levels of synaptophysin correlated with performance in the inhibitory avoidance.
Conclusions: The brain from sepsis survivor animals presented several markers of neurodegeneration, and inhibitory avoidance test performance seems to be dependent on the levels at some of these markers.

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References:

P88
Epigenetic profile in lipopolysaccharide-stimulated macrophages
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Background: Sepsis remains a clinical challenge for the ICUs. However, it is known that the tolerance mechanism using low doses of lipopolysaccharide (LPS) reduces the expression of proinflammatory genes and involves epigenetic regulation. The chromatin openness is regulated by histone acetyltransferases (HATs) and these enzymes could be modulated by nitric oxide (NO) interaction. In the present work, we demonstrate the pathway of tolerance to LPS from HAT activity and level of histone openness to production of cytokines as well as the influence of NO inhibition.

Materials and methods: THP1 differentiated into macrophages (with 2.5 μg/ml PMA) were cultivated in RPMI medium (Control group), submitted to tolerance (500 ng/ml LPS 24 hours before challenge with 1,000 ng/ml LPS - Tolerant group) and challenged (1,000 ng/ml LPS - D group) during 24 hours. NO production was inhibited by addition of 100 μM LNAME. The HAT activity and cytokine production (IL-6) were measured with biochemistry kits. Histone acetylated H3 and H4 were analyzed by western blotting.

Results: Tolerance reduced HAT activity compared with the group directly challenged (P < 0.05). Acetylated H4 was maintained at basal levels in the tolerant group and increased in the D group (P < 0.05). However, the tolerance increases the acetylation of histone H3 in a NO-dependent response. Similarly, the IL-6 release was reduced by induction of tolerance (P < 0.05 vs. D group). However, this effect was abolished by inhibition of NO production.

Conclusions: The induction of tolerance diminishes HAT activity and cytokine production. The tolerance triggers a complex epigenetic modulation dependent of NO.

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P90
Synaptic deficits in sepsis: role of glial cells
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Background: Recent clinical studies have shown that sepsis survivors can develop long-term cognitive impairment. The cellular and molecular mechanisms involved in these events are not yet completely understood. In this study, we investigate the synaptic deficits in sepsis and the involvement of glial cells in this process.

Materials and methods: Using a clinically relevant model of sepsis (cecal ligation and puncture), we observed a decrease of recognition memory 9 days after sepsis. At the same time, by colocalization between pre-synaptic and post-synaptic protein, synaptophysin and PSD-95, we observed a reduction of structural synapses in the hippocampus and cerebral cortex of septic mice. To define the molecular mechanisms accountable for synaptic loss in sepsis, we used an in vitro approach treating neuronal cultures with conditioned medium from astrocyte (ACM) and microglial (MCM) cultures stimulated with LPS.

Results: We observed that the MCM reduced the synapse number and the ACM increased the number of synapses. The analysis of conditioned medium composition showed that MCM had increased levels of IL-1β while the ACM had increased levels of TGF-β1, as compared with medium from the non-LPS-stimulated cultures. The increased levels of IL-1β, from microglial activated with LPS, accompanied by an increase of TGF-β1, from LPS-activated astrocytes, suggests an anti-synaptic activity in IL-1β and pro-synaptic actions in TGF-β1. Inhibition assays with the addition of soluble IL-1β receptor (IL-1 Ra) prevented the MCM-induced synaptic loss. To understand whether the loss in synapse density would have functional outcomes we performed patch clamp experiments in neurons treated with microglia conditioned medium (MCM) and ACM of LPS-stimulated cultures. Patch-clamp recordings in the MCM-treated neurons showed a reduction in postsynaptic current frequency, while an increase in current amplitudes suggests a functional synaptic deficit.

Conclusions: These findings show, for the first time, a correlation between synaptic deficits and memory dysfunction, suggesting a possible mechanism for cognitive impairment after sepsis as well as a glial-derived molecule mediating synapse reduction.

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References:

P89
Histone acetyltransferase and DNA methyltransferase expression in response to LPS stimulation
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Background: Tolerance is a defense strategy capable of reducing the proinflammatory impact of infection. Tolerance capacity differs among the different tissues. It is known that epigenetic regulation is cell type specific. The cell machinery regulates the expression and activity of the enzymes that regulate chromatin openness. Understanding the epigenetic mechanism activated by different doses of LPS is important to define new approaches for the treatment of systemic infections. The objective of this work was to study the LPS-induced epigenetic response, analyzing the expression of histone acetyltransferases (HAT) and DNA methyltransferases (DNMTs).

Materials and methods: THP-1 human promonocytes were cultivated in RPMI (C group), submitted to different doses of LPS (T group - tolerance with 500 ng/ml during 24 hours and challenge with 1 μg/ml during 24 hours; D group - 1 μg/ml during 24 hours). The inhibition of nitric oxide production was performed with LNAME (100 µM). Male Balb C mice (8 weeks old) were divided into two groups: C group - without manipulation; D group - received 5 mg/kg LPS. The spleens were collected 48 hours after the HAT, DNMTs, lyse acetylated and histone H3 acetylated amounts were determine by western blot. The results represent three similar experiments. The statistical analysis was performed by ANOVA. Research protocol number 0950/09 was approved by the ethics committee.

Results: The challenge with LPS reduced the expression of DNMT1 in THP1 cells. However, the tolerance increased the amount of this enzyme (25%). Challenge with LPS reduces DNMT3a production (50%) in mouse spleen. The expression of HAT was reduced (50%) in the T group and this event was NO dependent. The LPS addition to THP1 culture decreases the production of acetylated lysine (P < 0.05) in a dose-dependent way (68% and 33% with 0, 1 and 5 µg/ml LPS respectively). Low doses of LPS reduce the acetylation of histone H3 (30% and 60% with 500 ng and 1 µg/ml LPS respectively).

Conclusions: Different concentrations of LPS are required for selective regulation of subsequent LPS-stimulated epigenetic mechanisms.

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P91

Association of the immature platelet fraction with the diagnosis and severity of sepsis: an observational study

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Background: An ideal sepsis biomarker should be able to segregate infected patients from other causes of SIRS, and also to allow some kind of risk stratification. Furthermore, it should be capable of identifying subgroups of patients with specific sepsis complications, enabling target-specific preventive and therapeutic measures. Finally, access to this biomarker should not depend on complex and high-cost equipments and reagents, allowing access to more patients. New hematologic automated analyzers used for evaluation of the complete blood count provide a series of advanced analytical parameters that permit more detailed evaluations of circulating blood cells. Parameters such as the immature reticulocyte fraction (IRF) and immature platelet fraction (IPF) identify early signs of hematopoietic recovery, and have been studied in several inflammatory conditions. Recently, a study performed in critically ill patients suggested that the IPF could be a more accurate biomarker of sepsis development than C-reactive protein (CRP) and procalcitonin. The aim of this study was to evaluate whether IPF and IRF levels presented any association with clinical and laboratory parameters of sepsis severity.

Materials and methods: During 30 days the IPF and IRF were obtained using an automated hematologic analyzer (Sysmex XE5000) within 24 hours from admission for consecutive patients with sepsis.

Results: In total, 23 patients with sepsis were enrolled in the study, of which 12 (52%) presented severe sepsis or septic shock. The median APACHE II and SOFA scores at admission were 15 (6 to 37) and 6 (1 to 17). Median IPF and IRF levels at admission were 4% (1.1 to 11.0%) and 14% (1.6 to 47.1%) respectively, and were significantly higher than in a population of healthy individuals (IPF = 2.1% and IRF = 2.9%; both P < 0.001). As opposed to the CRP, both IPF and IRF were significantly correlated with the SOFA at admission (Rs = 0.52 and 0.45; P = 0.01 and 0.02 respectively). However, when patients were stratified by the median SOFA score at admission, only the IPF was significantly higher in patients with SOFA ≥6 (IPF = 6.2% vs. 2.9%; P = 0.01). Similar results were observed when patients were stratified by the presence of severe sepsis. The IPF presented a significant correlation with the platelet count (Rs = 0.71; P < 0.001), but with not with PT, aPTT and D-dimer.

Conclusions: In patients with sepsis, both IPF and IRF were higher than in healthy individuals, and the IPF was associated with increased sepsis severity. Larger studies are warranted to define and validate the precise role of the IPF as a sepsis biomarker.

P92

Feasibility of gene transfer with nonviral vectors in murine models of sepsis

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Background: Although several target-specific therapies for sepsis failed to translate into clinical benefits during the last decades, the increasing knowledge about sepsis pathogenesis continues to reveal new therapeutic targets that could be explored in the future. One of the challenges of previous target-specific treatments for sepsis was the short half-life of agents, some in the range of minutes. Gene transfer strategies can overcome this limitation, by providing a platform for longer expression of secreted therapeutic proteins. On the other hand, the transient nature of sepsis precludes the use of gene transfer strategies leading to long-term expression such as viral vectors. In this context, the use of nonviral vectors emerges as an attractive strategy for the treatment of sepsis, provided that sufficient expression of any therapeutic gene can be obtained.

Materials and methods: Two gene constructs were used to evaluate the feasibility of gene transfer in the endotoxemia model: a lacZ expression plasmid driven by the CMV promoter, and a coagulation factor IX expression plasmid with the hAAT liver-specific promoter. The latter was used as a reporter gene for secreted proteins. C57Bl/6 mice were challenged with LPS and gene transfer was performed 6 hours thereafter, so as to mimic the timepoint when sepsis treatments would be initiated. Fifty micrograms of plasmid were injected into the tail vein using hydrodynamic transfection. A less aggressive protocol, which could in principle be translatable to the clinical setting, was also tested. Gene expression was evaluated 72 hours after gene transfer by a blinded investigator.

Results: Factor IX activity levels (FIX:C) were significantly lower in nontransfected LPS-challenged mice (n = 12) compared with nontransfected controls (n = 14), suggesting that endotoxemia decreases baseline FIX:C levels. Higher FIX:C levels (twofold higher than controls) were observed in control mice submitted to hydrodynamic transfection (n = 5), as expected. When gene transfer was evaluated in the context of sepsis, LPS-challenged mice (n = 9) presented 1.7-fold higher FIX:C levels than control mice (n = 12) (P < 0.01). Moreover, mice that were exposed to a less aggressive intravenous transfection protocol (n = 8) presented FIX:C levels that were 1.4-fold higher than controls (P = 0.04). Liver-expression of β-galactosidase also demonstrated the feasibility of gene transfer in LPS-challenged mice.

Conclusions: Our results suggest that the cellular and molecular events of sepsis reproduced in the endotoxemia model could facilitate gene transfer, thus offering a unique opportunity for gene therapy with nonviral vectors, without the need for traumatic gene transfer protocols that would be required in other pathological conditions.

P93

Effect of IL-1 receptor antagonist on the cerebrospinal fluid nitric oxide concentrations during experimental polymicrobial sepsis in rats

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Background: Recently, we observed that blocking the IL-1L-1r signaling pathway by central administration of IL-1ra (an IL-1 receptor antagonist) can result in increased AVP secretion and survival rate in the late phase of sepsis [1]. The mechanism of this effect of IL-1ra on AVP concentration and survival rate remains elusive. Many studies have implicated excessive production of nitric oxide (NO) as one of the important factors responsible for decreased AVP secretion during the late phase of sepsis [2]. Currently, the effect of IL-1ra on the central NO production and release during sepsis is not known.

Materials and methods: In this study, we checked the effect of IL-1ra on sepsis-induced increased release of NO in cerebrospinal fluid (CSF). Sepsis was induced by cecal ligation and puncture (CLP). IL-1ra (9 nmol/animal) and vehicle (PBS: 2 μl/animal) were injected intracerebroventricularly to separate groups of CLP (n = 8/group) and control (n = 8/group) animals. CSF and blood samples were collected from different groups of rats (n = 6 to 8/group) after 1, 2, 4, 6 and 24 hours. The NO concentration in CSF was determined by chemiluminescence assay. Specific ELISA was used for AVP analysis. All experiments were carried out according to an institutional ethic committee-approved protocol (CEUA protocol number 12.1.1205.53.0).

Results: NO levels were significantly (P < 0.05 to 0.005) increased in post-CLP 6-hour and 24-hour as compared with control, post-CLP 1-hour, 2-hour, and 4-hour animals. IL-1ra administration did not significantly alter the NO concentration in CSF after 1, 2, 4 and 6 hours as compared to vehicle treatment in CLP animals as well as in control. In contrast, after 24 hours NO levels were significantly (P < 0.02) lowered in IL-1ra-treated animals (22.36 ± 2.07 μM) as compared with vehicle-treated animals (31.97 ± 2.88 μM). The AVP concentration in IL-1ra-treated rats was significantly higher in IL-1ra-treated animals in comparison with vehicle-treated. Moreover, the survival rate of IL-1ra-treated rats was >80% while that of vehicle-treated rats was 47%.
Our results have demonstrated that blocking the IL-1-IL-1r signaling pathway by central administration of IL-1rα increases AVP secretion in the late phase of sepsis, which may be beneficial for survival. We believe that one of the mechanisms for this effect of IL-1rα is through reduction of NO concentration in CSF of the septic rats.

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References

Conclusions: Our results have demonstrated that blocking the IL-1-IL-1r signaling pathway by central administration of IL-1rα increases AVP secretion in the late phase of sepsis, which may be beneficial for survival. We believe that one of the mechanisms for this effect of IL-1rα is through reduction of NO concentration in CSF of the septic rats.

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References

P94 Central and peripheral effects of ghrelin over the hypotension induced by endotoxic shock
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Background: Since its discovery by Kojima and colleagues in 1999 [1], the hormone ghrelin has been studied in different contexts, since this peptide has the ability to promote hormonal, vascular and immune changes. His well-established functions are the release of growth hormone, by a mechanism distinct from the growth hormone release factor, and stimulation of hunger, by activating hypothalamic neurons, leading to release of neuropeptide Y, thus promoting orexigenic effects [2]. Because of its ability to release hormones, including vasopressin [3], and by possessing immunomodulatory properties, ghrelin has been studied in different contexts of inflammatory states, as present in endotoxemia and sepsis [4]. The infusion of lipopolysaccharide (LPS) is capable of generating an inflammatory state, with augmenting of TNFα, IL-1 and nitric oxide, which in turn leads to cardiac depression and systemic vasodilation and hypotension [5]. Due to its properties to modulate the inflammatory response, in a way of diminishing the levels of TNFα, IL-1β and nitric oxide, which are augmented in the endotoxic state, as well the ability to augment the plasma levels of vasopressin, ghrelin emerges as a potential neuro-immunomodulator in hypotension caused by endotoxemia. We speculate that ghrelin, mediating the inflammatory response and by augmenting vasopressin blood levels, could attenuate the hypotension caused by endotoxin.

Materials and methods: Male Wistar rats (250 to 300 g) had their jugular vein and/or their right cerebral ventricle cannulated for drug administration, and the femoral artery cannulated for mean arterial pressure (MAP) and heart rate (HR) records, respectively. All experimental procedures were approved by the Comitê de Ética em Experimentação Animal-campus de Ribeirão Preto (protocol number 12.1.1441.53.5). The endotoxia model was induced by endovenous injection of lipopolysaccharide (LPS; 1.5 mg/kg). Data were compared using two-way analyses of variance and significant differences were obtained using the Bonferroni post test.

Results: LPS administration leads to a drop in MAP in the first 2 hours, followed by a partial recovery of the MAP, and then a second drop in MAP, with a peak in 6 hours. The HR was augmented in this group. Systemic administration of ghrelin alone, through a bolus followed by subcutaneous implantation of an osmotic pump, did not alter the response, in comparison with the saline-treated group. The icv administration of ghrelin had diminished the HR in some intervals, although did not present a difference in MAP, in comparison with the saline-treated group. The administration of ghrelin, centrally and peripherally, when given at the same time as the LPS bolus, attenuated the first drop in MAP and completely restored the second drop present in the LPS group.

Conclusions: Ghrelin is capable of attenuating the hypotension caused by endotoxin, and we speculate that the improvement is due to modulation of cytokines, nitric oxide and augmented vasopressin blood levels.

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References

P95 Gene expression patterns in multiple organs in experimentally induced Staphylococcus aureus sepsis in pigs
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Background: Animal research in sepsis needs analytical tools that can capture and exploit the complexity of the condition. To summarise the disease progression in a porcine model of severe Staphylococcus aureus sepsis, we used principal component analysis (PCA) as a multivariate approach to identify early dynamic expression patterns of 34 selected genes in the liver, lung, and spleen tissue.

Materials and methods: We combined data from two related experimental studies in pigs haematogenously infected with a porcine pathogenic strain of S. aureus [1,2]. Seventeen infected pigs were euthanised at the following time points post infection (p.i.): 6 hours (n = 3), 12 hours (n = 3), 24 hours (n = 3), 30 hours (n = 1), 36 hours (n = 2), and 48 hours (n = 5). Five healthy controls were managed in parallel. Gene expression of 34 genes related to acute inflammation and haemostasis was measured in the liver, lung, and spleen by quantitative real-time PCR. The data matrix of 22 samples and 102 (34 x 3) variables were log-transformed, scaled to unit variance, and subjected to PCA.

Results: Three (PC1 to PC3) distinct dynamic response patterns were identified. PC1: hepatic positive and negative acute-phase genes were the main influencers of a protracted pattern induced between 12 and 48 hours of infection, which explained 23% of the total variation in the dataset (Figure 1A, C). PC2: an acute pattern distinguished infected pigs from controls already after 6 hours and peaked around 12 hours p.i. After 30 to 48 hours, pigs had either reverted back to basal levels (n = 7) or below basal levels (n = 2) (Figure 1A). This pattern explained 14% of the total variation and was influenced by a systemic (nonorgan-specific) mixture of proinflammatory, anti-inflammatory and haemostatic genes (Figure 1C). The two pigs with low PC2 levels had suffered from overt disseminated intravascular coagulation when euthanised (3), and this outcome was clearly reflected by PC2. PC3: a per-acute pattern, influenced mainly by pulmonary proinflammatory genes (explaining 11% of the total variation), was induced in infected pigs at 6 hours p.i., while at later time points most pigs had moved towards basal levels (Figure 1B, D).

Conclusions: Multivariate analysis (PCA) identified three temporally distinct patterns in gene expression data from the liver, lung, and spleen tissue: pulmonary inflammation was rapidly induced, followed by transient induction of a generalised inflammatory and haemostatic response, and initiation of the hepatic acute-phase response.
References

P96
Microparticles from septic shock patients contain microRNA and messenger RNA: new players in the pathogenesis of sepsis?
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Background: Previous studies demonstrated the presence of microparticles (exosomes) in plasma of septic patients. These are cell-derived vesicles containing specific collections of proteins, lipids and genetic material that participate in the intercellular communication, changing the function and physiology of their target cells. The role of exosomes in sepsis, however, remains deeply unexplored. This study aimed to investigate the composition of microRNAs and messenger RNAs related to inflammatory response in circulating microparticles of septic shock patients.

Materials and methods: Fourteen patients had 30 ml blood collected in the first 48 hours of sepsis and 7 days after for those who survived. Five healthy volunteers served as controls. Exosomes were isolated from plasma by filtration (0.22 μM) and ultracentrifugation. Thirty nanograms of the total RNA were reversely transcribed and the expression profile of 754 human miRNAs and 91 mRNAs from immune response was evaluated by real-time quantitative PCR using the Taqman Low Density Array (Applied Biosystems). The raw data were processed in Expression Suite v1.0.1 software and analyzed in StatMiner v3.0 software considering the global expression level for normalization. The fold-change was calculated based on the estimated mean difference (2^(-ΔCt)).

Results: Different miRNA expression was observed in the exosomes from septic patients in comparison with healthy donors. In the first 48 hours of septic shock, three miRNAs were differentially expressed: mir-1290 (2.78-fold, P = 0.02), mir-1298 (4.02-fold, P = 0.03) and mir-146a (-2.51-fold, P = 0.02). In the recovery phase of sepsis, five miRNAs were differently expressed.
expressed as compared with controls: miR-1260 (2.29-fold, \( P = 0.02 \)), miR-1274A (2.83-fold, \( P = 0.02 \)), miR-1274B (3.31-fold, \( P = 0.02 \)), miR-192 (1.83-fold, \( P = 0.02 \)) and miR-604 (-6.41-fold, \( P = 0.02 \)). The miRNA expression profiles in different stages of sepsis were similar. Moreover, exosomes from patients after 1 week of sepsis carry less CCLS mRNA than in the beginning of the disease (-2.49-fold, \( P = 0.02 \)).

**Conclusions:** Exosomes from septic shock patients carry different miRNA expression profiles at different stages of the disease, as compared with healthy individuals. CCLS mRNA is less expressed in the recovery phase of sepsis. The composition of these vesicles may help to understand the underlying mechanisms involved in their role in the pathogenesis of sepsis.

**Acknowledgments:** Financial support from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

**P97**

Role of NOX2-derived ROS in the development of cognitive impairment after sepsis

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**Critical Care 2013, 17(Suppl 4):P97; doi:10.1186/cc12996**

**Background:** Septic encephalopathy (SE) is a frequent complication in severe sepsis. Here we have explored the role of NADPH oxidase in different aspects of SE pathophysiology. We investigated the involvement of NADPH oxidase in neuroinflammation and in the long-term cognitive impairment of sepsis survivors.

**Materials and methods:** Our approach included pharmacological inhibition of NADPH oxidase activity with apocynin and the use of genetically deficient (knockout) mice for gp91phox (gp91phox\(^-/-\)), the catalytic subunit of Nox2. Sepsis was induced by cecal ligation and puncture and fecal peritonitis. We measured the hippocampal oxidative stress, Nox2 and Nox4 gene expression and neuroinflammation in WT and gp91phox\(^-/-\) mice at 6 hours, 24 hours and 5 days post sepsis. Behavioral outcomes were evaluated 15 days after sepsis with the inhibitory avoidance and the Morris water maze tests.

**Results:** The data show progressive oxidative damage to the hippocampus, identified by increased 4-hydroxynonenal expression, associated with an increase in Nox2 gene expression in the first days after sepsis. Pharmacological inhibition of Nox2 with apocynin completely inhibits hippocampal oxidative damage in septic animals as well as the development of long-term cognitive impairment in the survivors. Pharmacologic inhibition or the absence of Nox2 in gp91phox\(^-/-\) mice prevents glial cells activation, one of the central mechanisms associated with SE and other neurodegenerative diseases.

**Conclusions:** We identified Nox2 activation as a necessary step for glial cell activation in SE. Our data indicate that Nox2 is as a major source of oxidative stress in the brain and consequently has a central role in the development of cognitive impairments observed in sepsis survivors.

**P99**

Modulation of peroxynitrite improves host response to vasopressin in ovine sepsis

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**Background:** The standard therapy for sepsis is becoming less effective due to increasing microorganism resistance to antibiotics and cardiovascular collapse refractory to fluid resuscitation and vasopressors. In this study, we demonstrate a critical role of peroxynitrite in vascular hyporesponsiveness to vasopressin (VP) in methicillin-resistant Staphylococcus aureus (MRSA)-induced ovine sepsis.

**Materials and methods:** Sheeps were instrumented with Swan Ganz (common jugular vein), femoral artery, and left atrium catheters to monitor hemodynamics for 24 hours. Sepsis was induced by instillation of live MRSA (2.5 to 3.5 x 10\(^5\) CFU) into the lungs by bronchoscope under anesthesia. Sheep were then awakened, placed on a ventilator, and fluid resuscitated. Urine output was measured using a Foley catheter. Groups: MRSa, received MRSA, n = 4; MRSa + peroxynitrite decomposition catalyst (PDC), received MRSA and were treated with PDC starting 6 hours post injury (0.1 mg/kg bolus followed by 0.02 mg/kg/hour), n = 4; MRSa + VP, received MRSA and were titrated with VP when mean arterial pressure fell by 10 mmHg, n = 4; and MRSa + VP + PDC, received MRSA and were titrated with VP and PDC, n = 4.

**Results:** MRSa induced severe hypotension refractory to aggressive fluid and AVP. DPC and AVP alone partially attenuated the severe hypotension. When combined they more effectively reversed the hypotension. Inhibition of peroxynitrite formation by PDC also markedly reduced AVP requirement. When combined they more effectively reversed the hypotension. Inhibition of peroxynitrite formation by PDC also markedly reduced AVP requirement.
Conclusions: Peroxynitrite modulation may be a novel treatment option for management of sepsis-induced cardiovascular collapse refractory to vasopressors. These findings are especially provocative since peroxynitrite is the product of excessive nitric oxide regardless of which NOS isoform is involved and the major debate of whether the use of NOS inhibitors in management of sepsis is beneficial still remains.

P100
Effects of solid dispersion of curcumin in metabolic and immunological alterations during experimental sepsis
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Critical Care 2013, 17(Suppl 4):P100; doi:10.1186/cc12999

Background: Studies suggest that curcumin, found in the tropical plant Curcuma longa, has anti-inflammatory and antioxidant properties and can act in sepsis, decreasing the release of proinflammatory mediators and free radicals. In the search to increase curcumin's bioavailability a phototechnologic process was developed that generated a solid dispersion of curcumin named DS17. This dispersion is water soluble and seems to increase the curcumin absorption by the gastrointestinal tract. The aim of our study was to assess the biological activity of the solid dispersion of curcumin (DS17) in immunological and metabolic alterations observed in a model of sepsis in rats induced by CLP.

Materials and methods: Male Wistar rats (250 to 300 g) were divided into two groups: polymicrobial sepsis model by cecal ligation and puncture (CLP) and sham operation (OF). The animals were pretreated with DS17 (100 mg/kg) orally for 7 days prior to CLP and treated 2 hours after surgery. The animals were used to analyze curcumin absorption through HPLC, plasma glucose, cytokines, nitric oxide (NO) and HSP70. Another group had the survival rate assessed for 48 hours.

Results: Our results showed that curcumin is present in the plasma at 4 and 6 hours but absent 24 hours following the DS17 administration. The dispersion decreased IL-6 in plasma and peritoneal fluid at 6 and 24 hours, and IL-1β 6 hours after sepsis stimulus. Moreover, we observed an increase in the hematocrit and a decrease in plasma glucose in the same animals. Paradoxically, plasma IL-10 and serum HSP70 decreased in 24 hours while plasma NO increased in the same period. These changes were not sufficient to increase significantly the survival although we observed a biological improvement of 20% 24 hours following CLP.

Conclusions: Our results suggest that despite a significant decrease in proinflammatory cytokines (IL-1β and IL-6), treatment with curcumin solid dispersion produced no beneficial biological effect in septic animals. Further studies are necessary to better clarify the suggested antioxidant and anti-inflammatory effect of curcumin.

P101
Effects of PPARγ in dendritic cells during severe sepsis and sepsis-induced immunosuppression
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Background: Sepsis is a systemic inflammatory response syndrome against infection, which can develop in sepsis-associated immunosuppression. Actually, several inflammatory dysfunctions have been described in dendritic cells (DCs) which could be responsible for impairing the immune response towards the secondary infection, although how these stable modifications maintain is still unknown. Our hypothesis is that DCs from post-septic mice have chromatin alteration and differential microRNA expression.

Materials and methods: To investigate the global gene expression, post-septic and Sham-derived BMDC were infected or not with BCG for 24 hours. Total RNA were collected and the gene expression profile was assessed by Affymetrix GeneChip technology. The gene expression profiles were classified by Gene Ontology (GO). Also, the microRNA analysis was obtained from Affymetrix microarray. To investigate the chromatin modifications, post-septic and Sham BMDC were performed to Chip-Seq analysis.

Results: Supervised analysis identified a set of 2,755 genes that distinguished very accurately between post-septic BMDC and Sham BMDC. The gene expression signature showed 1,805 stimulated genes and 950 inhibited genes in post-septic BMDC compared with Sham BMDC. The gene expression signature of post-septic BMDC provided a molecular and functional profile based on GEO. It is 11% similarity that post-septic BMDC were mostly found in the downregulated genes to encode proteins involved in the biological pathways of the inflammatory process (IL-1α, IL-12, CD28, TLR2, Hmgb1, CCL2), lipid metabolism (FABP4, Elovl2, PTGS1, PPARα) and histone modifications (ACAT3, CBx2, Oip5, Hist2h2k). When post-septic and Sham BMDC were infected with BCG, downregulated gene sets were classified in 130 significant GEO terms (mainly involved in inflammatory and...
lipid metabolism process) while surprisingly upregulated gene sets were classified in 10 significant GEO terms (nine inflammatory processes of 10 terms). In microRNA expression, we observed higher microRNA expression in post-septic compared with Sham BMDC. When BMDC were infected with BCG, post-septic BMDC exhibited higher numbers of microRNA compared with Sham BMDC. Furthermore, we assessed the presence of H3K27ac and H3K4me3 in inflammatory (IL-10, TNF-α, IL-6 and TGF-β) and lipid metabolism genes (ABCA1, PLIN2, CD36 and FABP4). Both H3K4me3 and H3K27ac on PLIN2, CD36 and FABP4 gene bodies were reduced and the presence of H3K4me3/H3K27ac was increased on TNFα and TGF-β gene bodies.

Conclusions: These results demonstrate the global gene expression signature, higher microRNA expression and H3K4me3/H3K27ac profile on chromatin structure in post-septic BMDC. The present study suggests epigenetic changes may play a role in transcriptional regulation in post-septic DCs.

P103
Vasopressin secretion in sepsis-surviving animals following dehydration
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Background: Vasopressin (AVP) plasma levels increase in the early phase of sepsis but remain at basal levels in the late phase of sepsis [1]. It is also known that one-half of septic patients do not properly respond to an osmotic challenge, one of the strongest stimuli for AVP secretion [2]. However, whether these AVP secretion changes persist in sepsis survivors is not known. This study investigated the possible alterations in plasma AVP levels in sepsis-surviving animals.

Materials and methods: Male Wistar rats were separated into two groups: sepsis induced by cecal ligation and puncture (CLP), or sham animals. They received saline solution (50 mg/ml; s.c) immediately and 12 hours after CLP, and also ceftriaxone (30 mg/kg; s.c) and clyndamicin (25 mg/kg; s.c) after every 6 hours for 3 days. Sham animals received the volume of saline corresponding to antibiotic administration. After 10 days, the animals were dehydrated or left as control. After 2 days, the animals were decapitated, and the serum and plasma collected for sodium, hematocrit and hormone determination. The posterior pituitary glands were removed for hormone stock analysis.

Results: Sepsis-surviving animals presented a higher serum sodium even without the osmotic stimulus (147.8 ± 0.97 SEM vs. 151.4 ± 0.6 SEM mmol/l CLP; P < 0.001). Following dehydration, as expected, there was an increase of serum sodium in CLP animals (151.4 ± 0.6 SEM vs. 153.71 ± 0.47 SEM mmol/l; P < 0.001) and sham animals (147.8 ± 0.97 SEM vs. 154 ± 0.26 SEM mmol/l dehydrated; P < 0.001) with difference between the groups (154 ± 0.26 SEM vs. 155.71 ± 0.47 SEM mmol/l CLP; P < 0.041). Hematocrit also increased in both CLP (42.6 ± 1.58 SEM vs. 50.17 ± 1.67% SEM dehydrated; P = 0.002) and sham (mean: 41.8 ± 1.43 SEM vs. 49.5 ± 1.0% SEM; P = 0.003) groups but without difference between the groups. The animals responded with an increase in the AVP plasma levels (6.12 ± 0.68 SEM vs. 6.16 ± 0.94 SEM pg/ml CLP; P > 0.05), and a decrease in AVP neurohypophysis stocks (4.0 ± 1.02 SEM vs. 1.91 ± 0.67 SEM ng/µg CLP; P = 0.107), with no difference between the groups.

Conclusions: The results suggest that sepsis-surviving animals do not present alterations in secretion of AVP in relation to volemia. However, serum sodium results suggest that AVP secretion is impaired in sepsis surviving animals.


References:

P104
Evaluation of inflammatory parameters and cognitive impairment in a murine model of Pseudomonas aeruginosa pneumosepsis
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Background: Sepsis is a severe medical condition characterized by systemic inflammation to response secondary to infection, which frequently progresses to multiple organ dysfunction and death. It is currently the leading cause of death in ICUs worldwide. The most frequent source of infection in sepsis is the lung with a high lethality rate. Pseudomonas aeruginosa is one of the most common pathogens found in sepsis patients. Cognitive impairment is a significant consequence of sepsis reported among survivors. The encephalopathy associated with systemic inflammation is not well understood so the development of new clinical relevant models to help understand this sequelae is important. In this study we aimed to evaluate acute inflammatory markers and establish a long-term consequence in a murine model of pneumosepsis.

Materials and methods: C57/BL6 mice were submitted to intratracheal instillation of 10^6 colony-forming units of P. aeruginosa. Six hours later the bronchoalveolar lavage fluid was collected for cell migration, protein (BCA method) and cytokine (ELISA) analysis. Caudal vein blood samples were collected for cell counting. Another group of animals had their lungs perfused for myeloperoxidase quantification and histological analysis. Evan’s blue dye method was used for the assessment of lung permeability. The survival rate of animals submitted to P. aeruginosa instillation was observed daily during 7 days. This group of animals received a single dose of antibiotic meropenem (30 mg/kg), 6 hours after pneumonia induction. Cognitive damage was evaluated through the freezing test.

Results: Our results showed that P. aeruginosa infection caused an expressive recruitment of leukocytes, mainly neutrophils to the lung. Myeloperoxidase, a marker for neutrophil migration, was significantly increased in the lungs of animals instilled with P. aeruginosa. The animals instilled with P. aeruginosa also showed a significant increase in IL-6, KC and protein levels. Histological analysis showed an intense cell infiltrate in the lung tissue and the survival rate was extensively lower in P. aeruginosa infected mice. Additionally, the animals submitted to pneumosepsis had a loss of averine memory 13 days after pneumonia induction and this loss remained 50 days later.

Conclusions: Our study demonstrates the acute inflammatory response to P. aeruginosa lung infection and indicates that possibly this pneumonia model can cause irreversible cognitive impairment. Our results reveal a possible experimental model for the study of encephalopathy associated with systemic inflammation.

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P105
Role of inflammatory caspases in a murine two-hit model of sepsis: analysis of immunosuppression and cognitive impairment
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Background: Morbidities associated with severe sepsis are serious problems for surviving patients, such as cognitive impairment and immunosuppression. The immunosuppression predisposes the patients to a second infection, which generally is fatal. Several studies have been made to understand the mediators involved with this immunosuppression associated with sepsis. Some data from the literature show that caspase-1 promotes activation of cytokines, such as IL-1β, and actions are inhibited by caspase-12. This study proposes to analyze the role of inflammatory
caspases in immunosuppression and cognitive damage associated with a two-hit model of sepsis.

**Materials and methods:** We submitted Swiss animals to the model of two hits of infection. The first hit was the CLP model and the second hit was intratracheal instillation of *Pseudomonas aeruginosa*. We analyze the mortality rate and the inflammatory profile of the animals submitted to the CLP model and the two-hit sepsis model. The cognition of the animals was tested by the passive avoidance test 15 and 21 days after the CLP and 21 days until 96 days after the two-hit sepsis model.

**Results:** First, we characterize the model and we observed a 30% survival rate of the CLP group in comparison with a 100% survival rate in the SHAM group. The high lethality of the CLP group associated with hypoglycemia in the first 72 hours after the infection, increased neutrophil accumulation in the peritoneal cavity 6 and 24 hours after the CLP and an increase of inflammatory cytokines 6 hours after the CLP, such as CCL2, IL-1β, and IL-10. The CLP group had a cognitive impairment 15 days after the CLP, but the memory was recovered 21 days after the infection. The CLP group was more susceptible to *P. aeruginosa* infection 21 days after the CLP, when we compare with the SHAM group. The CLP + *P. aeruginosa* group had a low count of neutrophils in BAL when compared with the SHAM + *P. aeruginosa* group. We observed a decrease in caspase-1 expression and an increased expression of caspase-12 in the lungs of the CLP + *P. aeruginosa* group. When we look to cognition, both the SHAM + *P. aeruginosa* and CLP + *P. aeruginosa* groups had cognitive impairment 21 days after the infection, and the cognitive impairment remained until 96 days in the SHAM + *P. aeruginosa* group after the infection, but the CLP + *P. aeruginosa* recovered the memory 96 days after the infection.

**Conclusions:** Our preliminary results suggest that the immunosuppression associated with the CLP model (first hit) led to more susceptibility for survivor animals, which succumbed to a pneumonia model (second hit). We observed the involvement of inflammatory caspases in this immunosuppression phenomenon with a decrease of caspase-1 and an increase in caspase-12 expression. When we observed the cognitive function, we observed that the animals submitted to CLP had a cognitive impairment 15 days after the infection and the infection with *P. aeruginosa* induced a cognitive impairment until 96 days in both in groups. However, further studies should be made to confirm these results.

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**P107 Evaluation of compound NXY-059 on cognitive impairment caused by sepsis**

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**Critical Care 2013, 17(Suppl 4):P107, doi:10.1186/cc13006**

**Background:** Nitrones are a class of molecules whose main effect on biological systems is their antioxidant action. Some studies showed a neuroprotective effect in ischemia models and neurodegenerative diseases. Those diseases presented an inflammatory profile that leads the production of reactive oxygen species. This characteristic can generate brain injuries, which can affect areas related to memory consolidation. Sepsis is a pathology that forms an inflammatory response, which causes encephalopathy creating cognitive impairment. Therefore, the present study has the aim to evaluate the effect of the compound NXY-059 on the cognitive impairment caused by encephalopathy like sepsis.

**Materials and methods:** For the assays, mice Swiss Webster male (22 to 28 g, n = 15 per group) were submitted to the CLP model and treated with antibiotics (10 mg/kg, i.p.) for three consecutive days (6, 24 and 48 hours) and with NXY-059 (50 mg/kg, i.p.) for five consecutive days (6, 24, 48, 72 and 96 hours after the surgery). At 24 and 48 hours, a gravity score was made to determine the level of sepsis and the percent of survival was assessed until 144 hours. After 4 hours fast, the glucose levels were also measured 24 and 48 hours after CLP performance. The cognitive impairment was evaluated through the open field method on the 15th (training) and 16th (test) day after the surgery.

**Results:** Our results show that treatment with NXY-059 did not offer a protective effect on mortality and the animals developed moderated sepsis according to the gravity score at 24 hours (4 to 6). At 48 hours, the animals recovered for slight sepsis (2 to 3). The glucose levels were slightly restored at 48 hours for the animals treated with the compound. In the cognitive impairment analysis, we observed a a reduction (P < 0.05) in the numbers of crossing and rearings for the animals treated with NXY-059 when compared with animals treated with vehicle (saline).

**Conclusions:** According to these results, we can suggest that treatment with NXY-059 offered protection against cognitive impairment generated by sepsis.

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**P108 Atorvastatin and simvastatin protects cognitive impairment in an animal model of sepsis**

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**Background:** Recently it was shown that a significant proportion of sepsis survivors can develop a transitory or permanent cognitive impairment. Statins have the ability to block the cascade of cholesterol dysfunction...
formation by acting on HMG-CoA reductase, reducing the synthesis of endogenous cholesterol. Recently it has been observed that statins have anti-inflammatory properties preventing brain dysfunction in malaria models, reducing the production of brain cytokines, oxidative stress and alterations in the blood-brain barrier. The aim of the present study was to evaluate the ability of statins to reduce neuroinflammation and protect septic animals from neurocognitive damage.

**Materials and methods:** Feces were extracted (5 mg/g b.w.) from the large intestine of SW mice and diluted in saline, centrifuged and the supernatant collected and injected into the animals (n = 5 to 8/group). Control animals received 0.5 ml saline. Animals were treated at 6, 24 and 48 hours after sepsis induction with imipenem (30 mg/kg b.w., 0.2 ml s.c.) and 1.0 ml saline (s.c.). Statins (Ator and Simv) were administrated 1 hour before and 6, 24 and 48 hours after the infection (20 mg/kg b.w., p.o.). Mortality was observed for 96 hours and a score of severity evaluated. The inflammatory profile and oxidative damage was determined at 6 and 24 hours. In addition, mice brains were evaluated for microglial activation and BBB dysfunction. After 15 days we analyzed the cognitive damage using the inhibitory avoidance task and Morris water maze.

**Results:** No significant difference in survival was observed comparing septic animals treated with antibiotics plus atorvastatin or simvastatin (56%; 53%) with septic animals with only antibiotics (37%). We observed lower levels of proinflammatory cytokines (IL-1, IL-6) and chemokines (KC and MCP-1) when comparing statin-treated animals and nontreated. We also observed a decreased in the oxidative damage in brains 6 hours after sepsis in the treated groups. Finally, statin treatment was able to protect septic animals from cognitive damage including avoidance and spatial memory, both affected in untreated infected mice.

**Conclusions:** We can conclude that statins protected septic animals from cognitive damage, reducing neuroinflammation, and adjuvant therapies with statins can be interesting targets for future clinical trials focused on the prevention of long-term cognitive decline in sepsis.

**P109**

**Dasatinib has a dual effect on sepsis**


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**Background:** Sepsis occurs as a result of a systemic inflammatory response to an infection. In this context, homeostasis of biological systems depends on regulatory mechanisms to modulate the amplitude of the immune response to stimuli, such as infection, preventing damage resulting from this imbalance of immune response. The exacerbated immune response can cause serious tissue or systemic damage, as occurs in autoimmune and chronic inflammatory diseases. The main aim of our study is to investigate the effect of dasatinib in polymicrobial sepsis.

**Materials and methods:** Swiss mice were subjected to cecal ligation and puncture and treated with dasatinib 1, 5 and 10 mg/kg 30 minutes before and 6 and 24 hours after the surgery. Survival rate and clinical signs were assayed; cell accumulation, bacterial load were measured in peritoneal lavage and inflammatory mediators were measured in plasma.

**Results:** Animals receiving dasatinib 5 and 10 mg/kg showed the worst clinical score and an increased mortality rate. Animals receiving dasatinib 1 mg/kg showed an increase in survival, a decrease in clinical score, in cell migration, in colony-forming units and cytokine production. Higher doses had deleterious effects but lower doses had beneficial effects, probably because lower doses may downregulate the immune response, avoiding extensive tissue damage.

**Conclusions:** Dasatinib has a dual effect in polymicrobial sepsis, where higher doses had deleterious effects but lower doses had beneficial effects, probably because lower doses may downregulate the immune response, avoiding extensive tissue damage.

**Acknowledgements:** Financial support: Fiocruz, CNPq, Faperj, Vichem, Chemie and TARKINAID.

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**P110**

**Severe sepsis and septic shock survival in a clinical canine model**

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**Background:** Sepsis is a major cause of death in veterinary medicine, as in the human field, but there are no survival data described for this syndrome in the veterinary clinical field. This aspect challenges experimental medicine, may alter the baseline data to be applied in the human setting and could explain in part why most results obtained from laboratory research are not completely useful in the human clinical field. The purpose of this prospective observational study was to investigate the 24-hour and 30-day survival from severe sepsis and septic shock in canine septic patients that were approached with the Surviving Sepsis Campaign (SSC) bundles.

**Materials and methods:** Nineteen client-owned puppies with naturally acquired parvovirus haemorrhagic gastroenteritis were classified as severe sepsis and septic shock patients and received medical care according to the guidelines proposed by the SSC. Subsequently, the 24-hour and 30-day survival was evaluated for each case. The results were statistically analysed by Fisher’s exact test at a significance level of 5%.

**Results:** Fifteen patients (78.9%) were admitted to the emergency department and classified as severe sepsis subjects. The mortality rate in the severe sepsis group was 33.33% (five animals), of which four animals died in the first 24 hours of admission and the other on the following day. Four dogs (21.1%) were classified as septic shock patients. The mortality rate in the septic shock group was 100%, of which two animals died in the first 24 hours of admission and two on the day after (Table 1).

**Conclusions:** The observation of clinical outcomes in this clinical canine sepsis model showed that the majority of deaths in both severe sepsis and septic shock occur within the first 24 hours. However, after 30 days there is a significant difference between both groups, showing no survival in septic shock animals. Therefore, this preliminary study suggests a new veterinary database to be applied for future human research.

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**Table 1 (abstract P110) Severe sepsis and septic shock animals classified as nonsurvivors and survivors 24 hours and 30 days after admission**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Total</th>
<th>Nonsurvivors 24 hours</th>
<th>Survivors 24 hours</th>
<th>P</th>
<th>Nonsurvivors 30 days</th>
<th>Survivors 30 days</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe sepsis</td>
<td>14</td>
<td>4 (26.7%)</td>
<td>11 (73.3%)</td>
<td>0.557</td>
<td>5 (33.3%)</td>
<td>10 (66.7%)</td>
<td>0.033</td>
</tr>
<tr>
<td>Septic shock</td>
<td>4</td>
<td>2 (50.0%)</td>
<td>2 (50.0%)</td>
<td>1</td>
<td>4 (100.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>6 (31.6%)</td>
<td>13 (68.4%)</td>
<td></td>
<td>9 (47.4%)</td>
<td>10 (52.6%)</td>
<td></td>
</tr>
</tbody>
</table>

P, significance value of Fisher’s exact test.
**Background:** *Pseudomonas aeruginosa* is a Gram-negative bacterium regarded as an opportunistic pathogen. It infects immunocompromised patients, and is the second leading cause of nosocomial diseases. This bacterium has numerous virulence factors, adapts quickly to new environments, and requires a few nutrients to survive. All of these mechanisms will generate a host response. The fastest immune response is neutrophil recruitment, followed by phagocytosis and degranulation. There is another mechanism to fight bacteria called NET formation, which is the formation of a neutrophil extracellular network. NET is formed through a process called NETosis where the release of the cell nuclear material can hold and destroy pathogens. The nuclear receptor peroxisome proliferator-activated receptor PPARγ, besides lipid and glucose metabolism, is involved in the inflammatory response modulation, being considered a potential target for the study of new therapies for inflammatory and infectious diseases. We therefore aim to investigate the involvement of PPARγ in lung injury caused by *P. aeruginosa* using an agonist of this receptor, rosiglitazone.

**Materials and methods:** For this purpose, Swiss mice were instilled intratracheally with bacteria and treated with rosiglitazone 5 hours after the operation. We analysed clinical signs using 10 physical parameters, cellularity and DNA measurement to assess NET formation.

**Results:** We found that the animals stimulated with *Pseudomonas* showed an increase in inflammatory parameters, while the animals treated with rosiglitazone showed improvement in clinical signs and increased NET formation.

**Conclusions:** We can conclude that rosiglitazone has an anti-inflammatory role during lung infection, suggesting that PPARγ activation may improve the host defense against bacteria.

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