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# Sepsis 2008

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## P1

### Medical simulation for severe sepsis: improving both factual knowledge and crisis management skills

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*Critical Care* 2008, **12(Suppl 5):**P1 (doi: 10.1186/cc7034)

**Background** The morbidity and mortality from severe sepsis depends largely on how quickly and comprehensively evidence-based therapies are administered. As such, a huge opportunity exists. However, optimal care requires not only factual knowledge, but also numerous practical strategies including the ability to recognize a disease, to identify impending crises, to communicate effectively, to run a team, to work under stress and to simultaneously coordinate multiple tasks. Medical simulation offers a way to practice these essential crisis management skills, and without any risk to patients.
**Methods** Following a didactic lecture on the key components of the Surviving Sepsis Campaign Guidelines, we trained 20 emergency medicine residents on a portable Laerdal Patient Simulator. Pre-programmed sepsis scenarios were developed following a needs assessment and modified Delphi technique. To maximize realism, this was performed in the acute care area of the Emergency Department and included a pre-briefed respiratory therapist and nurse. We videotaped resident performance and provided nonpunitive feedback, focusing on the comprehensiveness of therapy (for example, whether broad-spectrum antibiotics were given) and crisis resource management strategies (for example, whether help was asked for and tasks were appropriately allocated).
**Results** Evaluation using a five-point Likert scale demonstrated that participants found this very useful (4.5/5), that lessons were complementary and supplementary to those learned from lectures (4.5/5) and that medical simulation was realistic (4/5). In addition, despite prior sepsis lectures, comparison of pre-tests and post-tests showed that more emergency medicine residents would: administer broad-spectrum antibiotics as soon as possible following hypotension (14/20 pre-test, compared with 16/20 post-test), administer low-dose corticosteroids for those with refractory shock (10/20 pre-test, compared with 13/20 post-test), and would favour norepinephrine as a vasopressor (8/20 pre-test, compared with 12/20 post-test). Participants specifically valued the chance to observe and practice crisis resource management skills, which they felt had not been previously addressed (19/20).
**Conclusion** Medical simulation appears to be an effective way to change both knowledge and behaviours in the treatment of severe sepsis. Many education and licensing boards also expect trainees to become not only content experts, but also effective communicators, collaborators, resource managers and advocates. These laudable goals are difficult to capture with traditional lectures but are comparably easy using medical simulation. We hope others will consider medical simulation as a complementary teaching and quality-assurance strategy in the fight against sepsis.

## P2

### Protective potential of 2-chloroadenosine in *Klebsiella pneumoniae* B5055 induced sepsis in BALB/c mice

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**Background** The incidence of sepsis or systemic inflammatory response syndrome in both developing countries as well as in the developed countries is rising despite the extensive research in understanding the molecular basis of sepsis pathogenesis. Sepsis is currently treated with antibiotics along with various adjunctive therapies. However, current existing therapies do not provide much efficacy in terms of patient survival and development of multiorgan dysfunction during sepsis.
**Methods** In the present study, we have developed the murine model of sepsis by placing *Klebsiella pneumoniae* B5055 entrapped in fibrin-thrombin clot in the peritoneal cavity of BALB/c mice. The mice were subsequently treated with adenosine analog 2-chloroadenosine (10 μg/kg/day intravenously). The efficacy of 2-chloroadenosine was investigated in terms of survival of animals and various inflammatory parameters (that is, malondiadehyde, myeloperoxidase, nitric oxide) in the lungs, liver and serum. Also the levels of proinflammatory cytokines (that is, TNFα and IL-1α) were determined.
**Results** The 2-chloroadenosine treatment significantly improved the survival of animals over a period of 5 days and increased the survival of animals to 70% as compared with the control group where 100% mortality was observed. The 2-chloroadenosine treatment significantly ($P<0.05$) decreased the production of TNFα, IL-1α and malondiadehyde, myeloperoxidase, and nitric oxide production in the serum, lung and liver of mice.
**Conclusion** The 2-chloroadenosine protected the mice from sepsis by increasing their survival and by decreasing the production of inflammatory markers investigated in the study.

## P3

### Use of clinical decision support to improve compliance with the Surviving Sepsis Campaign

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**Background** Evidence suggests that early, timely and aggressive resuscitation for patients with septic shock can have a significant impact on both morbidity and mortality. However, even with the widespread awareness of the Surviving Sepsis Campaign (SSC) guidelines, adherence varies widely. It has been shown that clinical decision support systems can help clinicians improve various

**Table 1 (abstract P3)**

**Mean values for age, Acute Physiology and Chronic Health Evaluation (APACHE) II score, resuscitation/management bundle completion and antibiotic administration for initial subjects**

| | Age (years) | APACHE II score | Resuscitation bundle completion (time) | Management bundle completion (time) | Time to antibiotic administration (minutes) |
|---|---|---|---|---|---|
| Group 1 ($n = 39$), prior to PW | 67.6 | 21.2 | 61.5% (11.6 hours) | 85.2% (21.1 hours) | 201 |
| Group 2 ($n = 46$), after PW implementation | 61.7 | 21.6 | 72.1% (9.1 hours) | 85.3 (16.3 hours) | 144 |

aspects of clinical practice, particularly when they are integrated into clinical practice and present at the point of care. *Protocol Watch* (PW) was developed as a bedside tool to assist clinicians with both implementation of and compliance with the SSC guidelines. The purpose of this research was to measure the impact that using PW had on adherence to the SSC guidelines.

**Methods** Participants were critically ill patients in two large university-affiliated teaching hospital intensive care units in the United States. Prior to the installation of PW, implementation of the SSC guidelines was done using a paper-based system of standing orders. Baseline data on compliance with the SSC guidelines were collected. PW, which offers an electronic version of the guidelines and is resident on the bedside patient monitor, was then installed in all critical care beds. The post PW installation data collection is currently being collected.

**Results** Preliminary results show improvements in compliance with the resuscitation bundle, improved compliance with antibiotic administration, and a decreased time for completion of the resuscitation and management bundles and antibiotic administration (see Table 1). In addition, the feedback from the clinical users has been extremely positive.

**Conclusion** If the final data analysis supports the preliminary findings, PW could emerge as an important method for assisting in the implementation of the SSC guidelines, thus making a valuable contribution in the care of critically ill patients with sepsis.

### P4

### Prognostic value of amino terminal pro-b-type natriuretic peptide in septic patients

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**Background** Amino terminal pro-B-type natriuretic peptide (NT proBNP) has been demonstrated high in septic patients. Our objective was to analyze the behavior of this biomarker and its prognostic value in a cohort of septic patients admitted to the ICU.

**Methods** A prospective cohort study was carried out in septic patients admitted to the ICU. The analyzed variables were demographic characteristics, severity scales, empiric antibiotic treatment, determination of NT proBNP and mortality. A univariate analysis and a multivariate logistic regression on mortality using SPSS 12.0 were conducted.

**Results** We included 98 patients, 37% female, age 64 years (standard deviation, 16). The median Acute Physiology and Chronic Health Evaluation II score was 18 (standard deviation, 7) and the median Sepsis-related Organ Failure Assessment score was 7 (standard deviation, 5). Occurrence of medical sepsis was 55%. The most common focus was abdominal (40%), followed by pneumonia (32%) and urine (16%). The ICU mortality was 22.7% and the hospital mortality was 30%. The values of NT proPNB were higher in patients with septic shock ($P = 0.001$) and with acute kidney failure (creatinine >2 mg/l) ($P < 0.001$). There was a linear correlation between the values of NT proPNB and creatinine ($r^2 = 0.33$, $P < 0.001$). The values of NT proPNB at admission were significantly higher in patients who died in the ICU ($P = 0.027$). In the multivariate analysis, the variables significantly associated with mortality were Acute Physiology and Chronic Health Evaluation II score, the female sex, inadequate empiric antibiotic treatment, fungal infection or Gram-positive infection and the presence of mechanical ventilation. In the analysis of those 66 cases with NT proPNB values seriated, the variables independently associated with mortality in the ICU were inadequate empirical antimicrobial treatment, Sepsis-related Organ Failure Assessment score at 24 hours, acute kidney failure and deterioration in the values of NT proPNB.

**Conclusion** The admission values for NT proPNB of septic patients in the ICU do not add significant information for prognosis, but are indicators of cardiovascular and renal dysfunction. The worsening of these values during admission appears to be associated with increased mortality in the ICU.

### P5

### Compliance with vasopressor use of early goal-directed therapy is not associated with decreased mortality in severe sepsis/septic shock

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**Background** Early goal-directed therapy (EGDT) reduces mortality of severe sepsis/septic shock by 16%. The elements of EGDT include central venous pressure (CVP) and central venous oxygen saturation ($ScvO_2$) monitoring, administration of intravenous fluid to achieve CVP of 8 to 12 mmHg, administration of vasopressors, transfusion of red cells (RBC), and administration of inotropes. This prospective observational study aims to determine the impact of complying with each of these six EGDT elements.

**Methods** The study included patients with severe sepsis/septic shock treated in our ICU. We collected demographics and the Acute Physiology and Chronic Health Evaluation III probability of death, compliance with each of the six EGDT elements within 6 hours of severe sepsis/septic shock onset and hospital mortality. The probability of hospital death at ICU admission, sepsis stage (severe or shock), and compliance with each EGDT element were entered into a multivariate logistic regression model. $P < 0.05$ was considered statistically significant.

**Results** Excluding 31 patients who did not authorize the research, 530 patients, 355 (67%) with septic shock, were included in the study. The compliance rates with the six elements were: inotrope

use 50%, $ScvO_2$ measurement 60%, adequate fluid resuscitation 69%, RBC transfusion 83%, CVP monitoring 83%, and vasopressor use 89%. The observed and predicted hospital rates were 33% and 36%, respectively. Shock (odds ratio (OR), 95% confidence interval (CI) = 2.13, 1.27 to 3.58; $P = 0.004$) and predicted mortality (%) (OR, 95% CI = 1.04, 1.03 to 1.05; $P < 0.001$) and compliance with use of vasoactive drugs (OR, 95% CI = 2.93, 1.25 to 6.86; $P = 0.013$) were independently associated with mortality. There was no independent association between hospital mortality and inotrope use, $ScvO_2$, adequate fluid resuscitation, RBC transfusion, and CVP monitoring.

**Conclusion** Compliance with vasopressor use of EGDT may not improve survival in severe sepsis/septic shock. Further studies are needed to determine which elements of EGDT improve outcome.

### P6

### *Pseudomonas aeruginosa* elastase induces IL-8 production in the lung cells via the epidermal growth factor/extracellular signal-regulated proteins/NFκB pathway

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**Background** The induction of chemokine secretion by fibroblasts is crucial for the migration of leukocytes into the parenchyma of the injured lung. Several bacterial products activate the lung's structural as well as immune cells to produce proinflammatory cytokines and chemokines. We report that elastase from *Pseudomonas aeruginosa* (PE) evokes IL-8 mRNA expression and protein secretion in nonmalignant culture of human lung fibroblasts by activating the receptor for epidermal growth factor (EGFR) and downstream mitogen-activated protein kinases (MAPK) pathway.

**Methods** We utilized western blot analysis to detect phosphorylation of EGFR and signal transduction intermediates. Northern blot and ELISA analyses were used to determine IL-8 RNA expression and cytokine secretion.

**Results** We found that the enzymatically active PE enhances IL-8 mRNA and protein secretion but does not increase IL-10 or TNF expression. PE induces phosphorylation of the EGFR and the extracellular signal-regulated proteins (ERK1/2) of the MAPK pathway. Pretreatment of the cells with neutralizing antibody to EGFR or the EGFR-specific tyrphostin AG1478 markedly attenuated the PE-induced ERK1/2 activation. PE-induced IL-8 expression is also abolished in the presence of the MEK inhibitor U0126, indicating the involvement of ERK1/2 in this process.

**Conclusion** Taken together, the results show PE could modulate lung inflammation by exploiting the EGFR/ERK/NFκB pathway and enhancing IL-8 production by lung fibroblasts.

### P7

### Significant decrease of central venous catheter-associated bloodstream infection rates in 38 German intensive care units

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**Background** Central venous catheter (CVC)-associated bloodstream infections (BSI) remain a major complication in ICUs. The aim of the study was to evaluate the influence of a structured multimodal intervention programme on the CVC-BSI rate of 38 ICUs in Germany.

**Methods** ICUs of the 'Krankenhaus Infektions Surveillance System' showing a CVC-BSI rate above the median were asked to implement a 12-month intervention programme starting in April 2007. The intervention included specific evidence-based recommendations for CVC insertion and use, and involved nurses and physicians. Modules were posters, script and advanced training. The modules' content was composed and distributed according to the 'train the trainer' principle by the National Reference Center for Surveillance of Nosocomial Infections. Infection rates were calculated before (January 2005 to June 2006) and during the intervention (May 2007 to March 2008).

**Results** Thirty-eight ICUs participated in the study. The ICUs had a median of 11 beds and eight ventilator beds. The majority of ICUs (47%) were affiliated to teaching hospitals; 30% were affiliated to university hospitals. The CVC utilization rate before implementation of the intervention was 69.4 CVC-days per 100 patient-days. The pooled mean CVC-BSI rate was 2.9 CVC-BSI per 1,000 CVC-days. A preliminary analysis of the data obtained during the intervention period showed a decrease of the mean CVC-BSI rate in the participating ICUs (2.2 CVC-BSI per 1,000 CVC-days; relative risk = 0.77, 95% confidence interval = 0.63 to 0.94, $P = 0.011$), whereas the CVC utilization rate remained almost unchanged (69.0 CVC-days per 100 patient-days).

**Conclusion** A structured multimodal intervention programme in addition to ongoing surveillance activities led to a significant decrease of CVC-associated BSI rates.

### P8

### LightCycler SeptiFast assay as a tool for the rapid diagnosis of sepsis in patients receiving antimicrobial therapy

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**Background** We analysed the clinical utility of the standardised, Conformite Europeanne-certified, multiplex real-time PCR assay for the molecular diagnostics of sepsis that was approved for *in vitro* diagnostics use (LightCycler SeptiFast assay; Roche Diagnostics, Pleasanton, CA, USA). The SeptiFast assay enables detection of DNA from 25 human pathogens (Gram-positive and Gram-negative bacteria as well as fungi).

**Materials** The study enrolled 50 patients with clinical diagnosis of sepsis that received medical care at the University Hospital for Infectious Diseases, Zagreb and Zagreb University Clinical Center in Croatia. Ten patients were treated at the Department of Haematology following bone marrow or peripheral blood stem cell transplantation; 30 patients were hospitalised in the ICU and 10 patients outside the ICU. All patients enrolled in the study were already receiving empirical antimicrobial therapy at the time of testing.

**Methods** Peripheral blood samples from the patients were analysed using the LightCycler SeptiFast assay and cultivation.

**Results** Fifteen out of 50 (30%) samples tested positive with the SeptiFast assay for bacterial or fungal DNA. Gram-negative bacteria were detected in 13 of 15 samples (*Klebsiella pneumoniae/oxitoca*, $n = 6$; *Escherichia coli*, $n = 3$; *Pseudomonas aeruginosa*, $n = 4$). Gram-positive bacterial DNA (*Enterococcus faecium*, $n = 1$ and *Streptococcus pneumoniae*, $n = 1$) was detected in two patients with polymicrobial sepsis (in combination with *K.*

*pneumoniae/oxitoca* in both patients). *Aspergillus fumigatus* DNA was detected in two patients. Six out of 50 samples (12%) were positive by both SeptiFast assay and culture. Additional SeptiFast-positive results (negative by cultivation) were obtained in nine of 50 patients (18%). Four out of 50 samples (12%) tested negative by the SeptiFast assay but were positive by culture. Those results were interpreted as false negative molecular testing. The remaining 31 samples tested negative by both SeptiFast assay and culture. In the group of 10 haematological patients, SeptiFast results were positive for six of the 10 patients (60%), whereas blood cultures were positive in only two out of 10 patients (20%).
**Conclusion** We conclude that the SeptiFast assay is a clinically valuable add-on to conventional culture methods for rapid aetiological diagnosis of sepsis in patients where the empirical antimicrobial therapy has already been started and pretreatment blood cultures were negative.

## P9

### Eosinophilia as a marker of adrenal insufficiency in critically ill patients with severe septic shock: 1-year prospective study

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**Background** Adequate adrenocortical function is essential to survive critical illness. The number of circulating eosinophils has been proposed as a marker of adrenocortical function. The goal of the present study was to determine whether eosinophilia could serve as a useful and early marker of adrenal insufficiency in critically ill patients with severe septic shock.
**Methods** During a 1-year period, we studied prospectively all 294 patients admitted to our ICU. Sixteen patients (13 male/three female, 5.4% of admissions) with eosinophilia defined as more than 3% of the white blood cell count and severe septic shock, refractory to fluid and vasopressor resuscitation, were included. A high-dose (250 μg, intravenously) corticotropin stimulation test was performed in all included patients.
**Results** The mean age was 47.2 ± 18.7 years, the Acute Physiology and Chronic Health Evaluation II score on admission day was 18.6 ± 6.8 and the Sepsis-related Organ Failure Assessment score was 10.3 ± 2.7 on eosinophilia day. The mean eosinophil count was 6.9 ± 3.5% of white blood cells. Eosinophilia was present 1.9 ± 0.9 days (range 8 hours to 4 days) before the onset of septic shock. Multidrug-resistant Gram-negative bacteria in 14 patients, Gram-positive in three patients and fungi in two patients were isolated and considered responsible for sepsis. Baseline cortisol levels were 19.4 ± 8.1 μg/dl and the adrenal response to the corticotropin stimulation test was 8.3 ± 4.9 μg/dl above baseline. Eleven out of 16 patients failed to respond to the corticotropin stimulation test above the critical level of a 9 μg/dl rise, and two out of 16 patients had baseline cortisol concentration <10 μg/dl. A hydrocortisone infusion (300 mg/day) treatment resulted in haemodynamic improvement in 12 out of 16 patients (75%). The 28-day mortality (following the onset of septic shock) was 43.7%. The only independent predictor of death was age ($P = 0.027$).
**Conclusion** Relative eosinophilia may be considered a useful and early bioassay for adrenocortical function assessment in critically ill patients with severe septic shock and assumed adrenocortical depression.

## P10

### Polymerase chain reaction detection of sepsis-inducing pathogens in blood using SepsiTest™

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**Background** PCR enables the identification of bacterial DNA in culture-negative samples from patients with suspected infection, allowing the confirmation of, for example, meningitis and septic arthritis. Gross discrepancies in the incidence of positive results between culturing and PCR have been reported, the latter corresponding better to bacterial loads observed by immunofluorescence microscopy and inflammatory response measurements. The goals of PCR assaying of clinical samples for pathogens are improved disease surveillance, early guidance on appropriate antibiotic therapy and patient management.
**Methods** SepsiTest™ is a new PCR test for the presence of bacterial and yeast pathogens in whole blood samples. The test combines sample preparation, the directed extraction of pure pathogen DNA from 1 ml blood, with PCR assays for the universal detection of bacteria and yeasts based on the amplification and monitoring of 16S and 18S rDNA sequences, respectively. Blood from septic patients was extracted and analysed, using SepsiTest™ together with sequencing of amplicons from positive samples and online BLASTN analysis for the identification of pathogens.
**Results** The test was validated by the determination of the limits of detecting pathogens in blood (spiking experiments, >95% sensitivity, $n = 6$), including (colony-forming units/ml) *Staphylococcus epidermidis* (20), *Staphylococcus aureus* (40), *Streptococcus pneumoniae* (40), *Escherichia faecalis* (120), *Escherichia coli* (150), *Klebsiella pneumoniae* (110), *Enterobacter aerogenes* (210), *Pseudomonas aeruginosa* (460) and *Candida albicans* (400). In total, samples from 55 patients with systemic inflammatory response syndrome criteria were analysed in an ongoing study. Compared with blood culturing, the preliminary data showed a diagnostic sensitivity of 60%, specificity of 98%, negative predictive value of 91% and positive predictive value of 86%.
**Conclusion** The data are discussed with respect to the significance of the molecular test for the diagnosis of sepsis. Special emphasis is put on the clinical data available supporting the finding of PCR-positive but culture-negative results.

## P11

### Impact of an educational program on the Surviving Sepsis Campaign implementation for sepsis management

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**Background** Severe sepsis and septic shock represent around 10% of the ICU admissions with a mortality rate near 50%. The Surviving Sepsis Campaign (SSC) is an international quality improvement program heading to standardize sepsis management.
**Objective** To evaluate an educational program to implement the SSC strategies in our fourth-level hospital.

**Table 1 (abstract P11)**

**Demographic and clinical characteristics of patients**

| | Period 1 ($n = 36$) | Period 2 ($n = 37$) |
|---|---|---|
| Age (mean (SD)) | 67.63 (14.66) | 65.48 (12.45) |
| Acute Physiology and Chronic Health Evaluation II score (mean (SD)) | 18.42 (14.66) | 19.23 (12.08) |
| Male sex (*n* (%)) | 23 (62.16) | 20 (50.05) |
| Location at sepsis diagnosis (*n* (%)) | | |
| Emergency department | 17 (47.22%) | 22 (59.45%) |
| Hospitalization wards | 13 (36.11%) | 6 (16.21%) |
| ICU | 6 (16.21%) | 9 (24.32%) |
| Type of sepsis (*n* (%)) | | |
| Septic shock | 8 (22.22%) | 13 (35.13%) |
| Severe sepsis | 28 (77.77%) | 24 (64.86%) |
| Origin of infection (*n* (%)) | | |
| Pneumonia | 18 (50%) | 19 (51.35%) |
| Abdominal infection | 6 (16.66%) | 11 (29.72) |
| Urinary tract infection | 4 (11.11%) | 5 (13.51%) |
| Catheter-related infection | 3 (8.33%) | 0 |
| Soft-tissue infection | 2 (5.55%) | 1 (2.70%) |
| Others sites of infection | 2 (5.55%) | 1 (2.70%) |
| Meningitis | 1 (2.77) | 0 |

**Methods** We implemented an educational program for physicians and healthcare professionals to apply the SSC strategies in the Emergency Department and the ICU. The program was evaluated from May to October 2007 (Period 1) in terms of the compliance of the SSC initial resuscitation bundle goals (lactate, blood cultures, antibiotic administration, fluid administration, achieving central venous pressure (CVP) >8 mmHg and central venous oxygen saturation ($ScvO_2$) >70%) and management bundle goals (adhering to policy on corticosteroids and activated C protein administration, glucose control <150 mg/dl, plateau pressure ≤30 $cmH_2O$). The results were evaluated. Six months later the program was repeated to reinforce the SSC concepts. The SSC strategies implementation continued for the next 6 months and the program was evaluated assessing the same indicators (Period 2). The results from the two periods were compared.

**Results** Seventy-three consecutive subjects were included (36 in Period 1 and 37 in Period 2). The demographic characteristics were similar in both groups (Table 1). With the exception of blood cultures (86.1% vs. 57.75%, $P = 0.01$), the initial resuscitation bundle goals' compliance was better achieved during Period 2; lactate measured (66.66% vs. 89.18%, $P = 0.02$), antibiotics administered within indicator timeline (50% vs. 75.67%, $P = 0.03$), appropriate fluid administration (63.88% vs. 94.59%, $P = 0.001$), when indicated achievement of CVP >8 mmHg (52.77% vs. 78.37%, $P = 0.02$) and $ScvO_2$ >70% (58.33% vs. 67.57%, $P = 0.47$). With the exception of the activated C protein administration policy (91.66% vs. 86.48%, $P = 0.1$), the management bundle goals' compliance was also better achieved during Period 2; corticosteroids according to policy (94.44% vs. 97.29%, $P = 0.61$), glucose control <150 mg/dl (52.77% vs. 78.37%, $P = 0.02$), plateau pressure ≤30 $cmH_2O$ (75% vs. 89.18%, $P = 0.13$). In general we found a trend to better compliance in resuscitation bundle goals (33.33% vs. 40.54%, $P = 0.62$) and management bundle goals (63.88% vs. 67.56%, $P = 0.80$) during Period 2 (Table 2).

**Table 2 (abstract P11)**

**Performance of sepsis bundle compliance**

| Measure | Period 1 ($n = 36$) | Period 2 ($n = 37$) | *P* value |
|---|---|---|---|
| Initial resuscitation bundle goals | | | |
| Measure lactate | 24 (66.66) | 33 (89.18) | 0.02 |
| Blood cultures before antibiotics | 31 (86.11) | 21 (5.757) | 0.01 |
| Antibiotics within timeline | 18 (50.0) | 28 (75.67) | 0.03 |
| Fluid and vasopressors | 23 (63.88) | 35 (94.59) | 0.001 |
| CVP >8 mmHg | 19 (52.77) | 29 (78.37) | 0.02 |
| $ScvO_2$ >70% | 21 (58.33) | 25 (67.56) | 0.47 |
| Management bundle goals | | | |
| Low-dose corticosteroids policy | 34 (94.44) | 36 (97.29) | 0.61 |
| Activated protein C policy | 33 (91.66) | 32 (86.48) | 0.1 |
| Glucose control <150 mg/dl | 19 (52.77) | 29 (78.37) | 0.02 |
| Inspiratory plateau pressure control | 27 (75.0) | 33 (89.18) | 0.13 |
| Initial resuscitation bundle completed | 12 (33.33) | 15 (40.54) | 0.13 |
| Management bundle completed | 23 (63.88) | 25 (67.56) | 0.80 |

Data presented as *n* (%).

**Conclusion** An educational program and a retraining process when linked with SSC implementation were effective in producing process change in the management of severe sepsis, achieving better compliance with currently accepted best practice.

## P12

## Automation of Septifast® for molecular diagnosis of infection in septic patients

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**Background** Time is a critical issue in confirming infection in systemic inflammatory response syndrome patients with suspected sepsis. Early diagnosis, followed by prompt implementation of an appropriate treatment, improves the prognosis of these patients. The SeptiFast® test is a new multiplex PCR test for simultaneous detection of sepsis-relevant microorganisms. It technically consists of three well-defined phases: extraction, detection on the LightCycler 2.0 machine and data analysis. The first one is a manual procedure based on filtration that takes 3.5 to 4 hours. The whole procedure including detection and data analysis takes approximately 6 hours. The aim of our study was to automate the current protocol using MagNa pure® compact and to evaluate the potential advantages of reducing the time to perform the method.

**Materials and study design** Inclusion criteria were age ≥18 years and suspected sepsis. The Ethics Committees from our institution approved the study, and patient or relatives' consent was obtained for blood sampling for PCR. Patient population (ICU): 100 Septifast® determinations and correlative blood cultures and microbiological cultures were performed in 64 patients with systemic inflammatory response syndrome and high clinical risk factors for bloodstream infection.

**Table 1 (abstract P12)**

**Comparative values of Septifast® (manual extraction) versus Septifast® (MagNa Pure® compact extraction)**

| | Blood culture vs. Septifast® (manual extraction) | Blood culture vs. Septifast® (MagNa Pure® compact extraction) |
|---|---|---|
| Sensitivity | 50 (27 to 72) | 80 (56 to 94) |
| Specificity | 98.75 (93 to 99) | 95 (87 to 98) |
| Positive predictive value | 90.9 (58 to 99) | 80 (56 to 94) |
| Negative predictive value | 88.76 (80 to 94) | 95 (87 to 98) |
| Prevalence | 20 | 20 |
| Likelihood ratio positive test | 40 (5.43 to 294.5) | 15.99 (6 to 43.63) |
| Likelihood ratio negative test | 0.506 (0.326 to 0.78) | 0.21 (0.087 to 0.5) |
| Proportion agreement (strength of agreement) [1] | 0.89 (moderate) | 0.92 (substantial) |
| Bias index | 0.09 | 0 |
| Prevalence index | –0.69 | –0.6 |
| Kappa (8) | 0.586 | 0.75 |
| Extraction volume | 1,000 µl | 400 µl |
| Time (extraction) | 4 to 3.5 hours | 25 to 30 minutes |

95% confidence interval calculated with binomial expansion.

**Methods** We used a new multiplex PCR-based test, LightCycler® SeptiFast® Test MGRADE (Roche Diagnostics, Penzberg, Germany), including software for simultaneous detection of sepsis-relevant microorganisms directly from blood, according to the manufacturer's recommendations. We followed the standard manufacturer's recommended procedure for extraction and compared it with the procedure using the MagNa pure® compact (Roche Applied Science, Penzberg, Germany) extraction column based on magnetic nanoparticles. Samples for blood culture analysis were drawn from a fresh venipuncture site according to the common guidelines (DGHM). Blood cultures were analyzed using a semi-automated blood system (BacT/ALERT®; bioMerieux, Marcy-Etoile, France). VITEK II (bioMerieux) was the system used to phenotypically identify pathogens growing from blood cultures and other microbiological tests (stool, urine, respiratory samples, catheter, etc.).
**Results** The results were both retrospective and observational. The quality of PCR results versus other laboratory findings and clinical evaluation, using sensitivity and specificity analysis and kappa evaluation to compare the results obtained, was determined. One hundred determinations were studied comparing both methods for extraction. Table 1 presents comparative data for both methods. For sensitivity and specificity analysis, the blood culture data define the disease condition (infection).
**Conclusions** MagNa pure® extraction versus the standard procedure for extraction used in the SeptiFast® test reduces the total test time from 6 to 3 or 3.5 hours. Use of this alternative protocol does not affect the sensitivity or specificity of the method.

**Reference**

1. Landis RJ, Koch GG: **The measurement of observer agreement for categorical data.** *Biometrics* 1977, **33:**159-174.

## P13

## Troponin can discriminate the most severe septic patients and should be included as an early routine test in Surviving Sepsis Campaign patients

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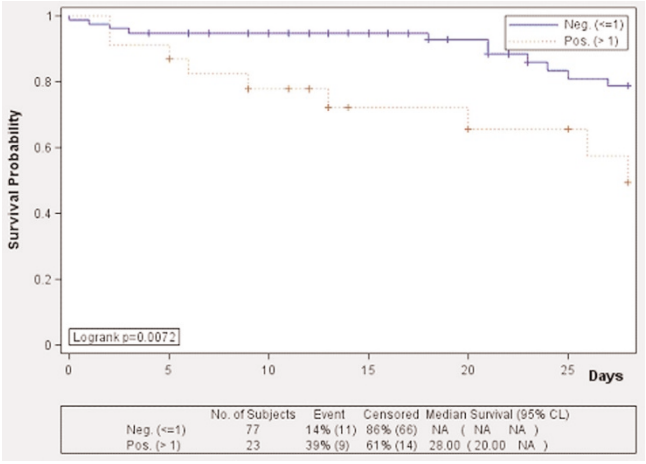
*Critical Care* 2008, **12(Suppl 5):**P13 (doi: 10.1186/cc7046)

**Background** Myocardial injury can now be easily recognized in critically ill septic patients and is associated with increased ICU mortality. Troponin I is recognized nowadays as a highly sensitive and specific serum marker of sepsis-induced myocardial injury/depression and can predict outcome in the critical care setting.
**Materials** In order to confirm this prediction power we included troponin I evaluations in our cohort of 100 patients within 24 hours of admission to our institutional protocol with strict adherence to the Surviving Sepsis Campaign guidelines within a tertiary care hospital, therefore eliminating heterogeneous population bias.
**Methods** Cumulative survival curves were constructed using the Kaplan–Meier method and were compared with the log-rank test. Results were considered significant at $P<0.05$.
**Results** A total of 100 patients with severe sepsis/septic shock met the inclusion criteria and were evaluated during the study period. Demographic characteristics of the study population are presented in Table 1. Except for age, which was slightly greater in

**Figure 1 (abstract P13)**




Kaplan–Mayer 28-day survival curves.

**Table 1 (abstract P13)**

**Demographic and clinical features of the study population**

| Variable | Troponin level | *n* | Mean | Median | *P* value |
|---|---|---|---|---|---|
| Acute Physiology and Chronic Health Evaluation II score | All patients | 98 | 25.20 | 25.00 | 0.2779 |
| | Negative | 75 | 24.75 | 24.00 | |
| | Positive | 23 | 26.70 | 25.00 | |
| Age (years) | All patients | 100 | 72.37 | 77.00 | 0.0185 |
| | Negative | 77 | 70.45 | 74.00 | |
| | Positive | 23 | 78.78 | 82.00 | |
| Organ dysfunctions | All patients | 100 | 2.91 | 3.00 | 0.6028 |
| | Negative | 77 | 2.87 | 3.00 | |
| | Positive | 23 | 3.04 | 3.00 | |
| Mean arterial pressure (mmHg) | All patients | 62 | 60,03 | 58.17 | 0.5152 |
| | Negative | 51 | 60.24 | 58.33 | |
| | Positive | 11 | 59.06 | 56.33 | |
| ICU length of stay (days) | All patients | 100 | 11.42 | 5.32 | 1.000 |
| | Negative | 77 | 11.86 | 5.02 | |
| | Positive | 23 | 9.94 | 6.63 | |
| Lactate (mg/dl) | All patients | 96 | 34.95 | 27.00 | 0.6717 |
| | Negative | 73 | 35.03 | 27.00 | |
| | Positive | 23 | 34.70 | 26.00 | |

the positive troponin group, every other variable was not significantly different, rendering the whole group very homogeneous. Kaplan–Meier survival analysis within 28 days of patient inclusion is shown in Figure 1 as stratified by troponin positivity (>1.0 ng/ml). Troponin-positive patients showed significant increased mortality with a log-rank value of 0.0072.

**Conclusion** The elevations of troponin observed were mostly small to modest, reflecting minor cardiac injury, but they nonetheless presaged increased mortality very early in the course of the disease. Others have postulated that increased-troponin patients can probably benefit most from drotrecogin-α administration with mortality reduction, thereby rendering troponin determination mandatory in critically ill septic patients. Troponin should therefore probably be included as an early routine test in the Surviving Sepsis Campaign.

## P14

### Pulmonary macrophage migration inhibitory factor: an important contributor to age-dependent inflammation in sepsis

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*Critical Care* 2008, **12(Suppl 5):**P14 (doi: 10.1186/cc7047)

**Background** Sepsis, the progressive, systemic host response to infection, kills over 215,000 individuals annually in the United States, and the mortality rate increases greatly with age. A recent ICU survey of sepsis occurrence in acutely ill patients identified the major site of infection as the lung (68%), *Staphylococcus aureus* as the most common infecting agent (30%), and the median patient age as 64 years. We therefore developed a rodent model using *S. aureus* cell wall components lipoteichoic acid (LTA) and peptidoglycan G (PGN) instilled into the lungs to study age-related changes in pulmonary infection and sepsis.

**Materials** Male Fischer 344 rats (Harlan Inc., Indianapolis, IN, USA); C57Bl/6 mice, wild-type ($mif^{+/+}$) and migration inhibitory factor (MIF) gene-deficient ($mif^{-/-}$) (Feinstein Institute for Medical Research, Manhasset, NY, USA); LTA and PGN (Sigma-Aldrich Co., St Louis, MO, USA); ELISA Assays (R&D Systems, Minneapolis, MN, USA); high-density oligonucleotide expression microarrays (GeneChips; Affymetrix Inc., Santa Clara, CA, USA).

**Methods** Saline alone (control) or LTA 1.5 mg/kg and PGN 5 mg/kg were instilled intratracheally into Fischer 344 rats 6, 18, or 24 months old (equating approximately to humans of 18, 45 and 60 years). After 6 hours, the animals were euthanized, blood collected from the left atrium, and the lungs lavaged with saline (bronchoalveolar lavage (BAL)). To further characterize the contributions of MIF following LTA–PGN challenge, we used GeneChips to obtain a description of the global transcriptomic response of lungs from $mif^{+/+}$ and $mif^{-/-}$ mice 6 hours post LTA–PGN.

**Results** There were significant age-related increases in lung compliance and BAL protein content (sham, 0.2 ± 0.01; 6 months, 1.2 ± 1.4; 18 months, 5.2 ± 0.7; 24 months, 4.8 ± 0.4 mg/ml), suggesting a more severe injury in the older age groups. BAL concentrations of GRO-KC (CXCL1) (sham, 0.13 ± 0.03; 6 months, 2.46 ± 0.79; 18 months, 2.89 ± 0.50; 24 months, 4.02 ± 1.54 ng/ml) and IL-6 (sham, 0.1 ± 0.1; 6 months, 5.2 ± 5.6; 18 months, 14.2 ± 2.3; 24 months, 13.0 ± 6.8 ng/ml), and blood MIF concentrations around threefold higher in the older rats suggest an age-dependent increase in inflammatory response. Comparative analysis of lung transcriptomes (~8,500 mRNAs) of the mice suggested a larger response in $mif^{-/-}$ mice of genes regulated by NFκB.

**Conclusion** These observations suggest, for the first time, a role for MIF–NFκB molecular circuitry modulating cardiopulmonary system responses following pulmonary infection. Since MIF can cause cardiac dysfunction, the increased MIF response during sepsis in the older individual may be responsible for an increased mortality in this patient population.

## P15

### Fully human anti-macrophage migration inhibitory factor antibodies as potential therapeutics for sepsis and septic shock

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*Critical Care* 2008, **12(Suppl 5):**P15 (doi: 10.1186/cc7048)

**Background** Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine and counter-regulator of glucocorticoids. Since the 1990s, MIF has been known to be a critical mediator of sepsis and septic shock. Circulating concentrations of MIF are elevated in patients with sepsis, and MIF levels are associated with disease severity and fatal outcome. Mouse monoclonal antibodies and rabbit polyclonal antibodies against MIF were shown to be protective in various animal models of sepsis. MIF therefore emerged as an attractive new target for treatment of patients with severe sepsis and septic shock.
**Methods** A diverse panel of human MIF-specific antibodies was generated by selection from a phage display library. This panel was subjected to extensive *in vitro* testing to identify antibodies that neutralize MIF activity. The antibody showing the highest potential was improved by affinity maturation; that is, by generating modified versions of this antibody by CDR1-2 shuffling and selecting high-affinity variants by phage display. The lead candidate antibody and its affinity maturated variant were tested in experimental mouse models of endotoxic shock and *Escherichia coli* peritonitis sepsis.
**Results** *In vitro* testing of human anti-MIF antibodies enabled the identification of antibodies that neutralize the activity of MIF in a glucocorticoid overriding activity assay and in a proliferation assay. Antibody Bax94 was designated as lead candidate, and affinity maturation of this antibody led to the generation of BaxA10, a variant with a 10-fold higher affinity for MIF. In an endotoxic shock model, pretreatment of mice with Bax94 and BaxA10 reduced circulating concentrations of TNF (control antibody: 4.9 ± 10.3 ng/ml; Bax94: 0.4 ± 0.4 ng/ml, $P < 0.01$; BaxA10: 0.2 ± 0.5 ng/ml, $P < 0.0001$) and of IL-6 (control antibody: 3.1 ± 1.6 ng/ml; Bax94: 2.1 ± 0.78 ng/ml, $P = 0.0003$, Bax10: 1.5 ± 0.7 ng/ml, $P < 0.0001$), and increased survival from 21% (control antibody) to 52% (Bax94; $P < 0.05$) and 58% (BaxA10; $P < 0.05$). The protective effect of the two anti-MIF antibodies was also demonstrated in a live *E. coli* peritonitis sepsis model in which survival rates increased from 10% (control antibody) to 34% (Bax94; $P < 0.05$) and to 56% (BaxA10; $P < 0.01$).
**Conclusion** We have generated fully human anti-MIF antibodies that neutralized the proinflammatory effects of MIF *in vitro* and that showed significant protective effects in experimental sepsis. *In vivo* protection tended to correlate with the affinity of the antibody for MIF.

## P16

### Effects of hydroxyethyl starch and gelatin solutions on renal function in surgical intensive care unit patients

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*Critical Care* 2008, **12(Suppl 5):**P16 (doi: 10.1186/cc7049)

**Background** There is continuing concern regarding adverse renal effects of colloid solutions in ICU patients.
**Objective** To compare two colloids, hydroxyethyl starch (HES 6% 130/.04) and gelatin 4%, on renal function in ICU patients.
**Methods** A before–after study of surgical ICU patients. Consecutive patients admitted from January to June 2005 formed Group HES, with HES as the standard colloid of choice. Patients admitted from January to June 2006 formed Group GEL, with gelatin as the primary colloid administered.
**Results** There were 1,383 patients in Group HES and 1,528 in Group GEL; 118 and 87 patients, respectively, had severe sepsis. There were some differences between groups in comorbidities and surgical procedures for the patients overall; however, these characteristics were more closely matched in the subset of severe sepsis patients. The incidence of renal failure and the ICU and hospital mortalities were similar in the two groups. In multivariate analysis, cumulative doses >33 ml/kg of either HES (odds ratio = 1.85, 95% confidence interval = 1.01 to 3.41, $P < 0.001$) or gelatin (odds ratio = 1.99, 95% confidence interval = 1.05 to 3.79, $P = 0.035$) were associated with higher risk of renal failure. In severe sepsis, hospital mortality tended to be higher in Group HES than in Group GEL (43% vs. 31%, $P = 0.076$). Patients with severe sepsis who received a cumulative dose >33 ml/kg of either colloid had a higher incidence of renal failure, which reached statistical significance for the HES group.
**Conclusion** Moderate cumulative doses of modern HES or gelatin solutions are associated with higher risk for acute renal failure. These solutions should be used with caution, especially in severe sepsis, until their safety can be demonstrated.

## P17

### Daily blood sampling in septic mice: an optimal and effective monitoring tool

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*Critical Care* 2008, **12(Suppl 5):**P17 (doi: 10.1186/cc7050)

**Background** Daily assessment of circulating immune/inflammatory and organ function parameters is strongly advocated in septic patients. In models of murine sepsis, such monitoring becomes challenging given the limited blood volume available for analysis. We studied the influence of daily versus single sampling in acutely (days 1 to 5) septic mice upon their short/long-term survival, organ function and complete blood count (CBC). We additionally tested the reliability of CBC differential in resuspended cell pellet versus whole blood analysis.
**Methods** Seventy-four female OF-1 mice (18 to 21 g body weight) were subjected to cecal ligation and puncture (CLP). Blood sampling volumes (by facial vein puncture) of 35 µl ($n = 40$) and 20 µl ($n = 34$) were tested. The samples were immediately diluted 1:10 to a final volume of either 350 µl (group 1) or 200 µl (group 2). Half of each group was sampled either daily for 5 days or only on day 5 post CLP. For comparison of resuspended versus regular CBCs, 150 µl (of the original 350 µl) was analyzed immediately after sampling. The remaining 200 µl was then spun, plasma removed (180 µl), the cell pellet re-suspended with an equal volume of the diluent and CBC performed.
**Results** Repetitive daily bleeding, regardless of the volume, did not affect either short-term (5 days) or long-term (28 days) CLP mortality. By day 5, changes between groups in the level of circulating IL-6, IL-1 receptor antagonist and organ function/metabolic

parameters (ALT, LDH, glucose and urea) were identical. In group 1 (35 µl), the red blood cell (RBC) count was reduced by 22% while the hemoglobin (Hb) concentration decreased by 23% (both $P<0.05$). However, only a minimal decrease of RBC and Hb by 10% and 11%, respectively (both $P<0.05$), was observed in group 2 (20 µl). In neither group were platelet or white blood cell counts affected by repetitive bleeding. Except for lymphocytes, the comparison of regular and resuspended CBCs displayed a high correlation for all cell types ($r>0.9$, slope $>0.9$). On each post-CLP day, the lymphocytes correlation remained moderate, reaching $r=0.6$ (slope = 0.6) on average (days 1 to 5). This effect was reproduced when tested in non-CLP OF-1 mice ($n=12$) at 1:2 dilution ($r=0.5$, slope = 0.7).

**Conclusion** Although we noted a statistically significant (and inversely proportional) decrease in RBC and Hb after repetitive daily bleeding, its biological impact was probably marginal. Differential blood analysis in resuspended pellet was highly reliable for all (except lymphocytes) cell populations tested. The results indicate that low-volume daily blood sampling allows a multi-directional and minimally invasive monitoring of various immuno-inflammatory parameters in acutely septic mice.

## P18

### Microcirculation changes following acute and chronic portal hypertension and their response towards bacterial translocation challenge

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*Critical Care* 2008, **12(Suppl 5):**P18 (doi: 10.1186/cc7051)

**Background** Patients with cirrhosis have higher risk of infection and multiorgan dysfunction syndrome. Increasing evidence relates them to the higher susceptibility to bacterial translocation (BT) by liver dysfunction and hemodynamic changes. Herein, we evaluated the role of BT in acute and chronic portal hypertension (a-PH and c-PH) states without cirrhoses.

**Methods** Forty-eight Wistar rats were distributed in BT, a-PH, c-PH with/without BT and control groups. a-PH (minimal shunting) and c-PH (extensive shunting) were induced by calibrated portal vein stenosis and were submitted to BT on days 2 and 14, respectively, by oroduodenal inoculation (10 ml *Escherichia coli* R-6, $10^7$ or $10^{10}$ colony-forming units (CFU)/ml) and confinement into the small bowel for 2 hours. BT-sham (saline), PH-sham (without portal stenosis), and PH groups were monitored for splachnic (liver, spleen, ileum) and kidney perfusion by laser Doppler, and mesenteric lymph node (MLN), liver, spleen, lung, blood and peritoneal fluid (PF) samples were cultured.

**Results** In the BT $10^{10}$ group, the culture was 100% positive at the MLN, liver and spleen (5.3 and 3 $\log_{10}$ CFU/g, respectively), while the blood, PF and lung were negative. In a-PH animals, the BT $10^{10}$ pattern was 100% to the MLN, liver and spleen (5.4 and 4 $\log_{10}$ CFU/g, respectively), lung (100%, 3 $\log_{10}$, $P<0.05$) and PF (10%, 0.6 $\log_{10}$). In turn, c-PH-BT$10^{10}$ findings were similar to BT$10^{10}$ alone but there was an increased translocation to PF (40%, 1 $\log_{10}$, $P<0.05$). On the other hand, for BT $10^7$ all cultures were negative, but in PH-BT$10^7$ translocation occurred to the MLN (a-PH 50%, 1 $\log_{10}$ CFU/g; c-PH 25%, 0.7 $\log_{10}$ CFU/g) plus to the PF (a-PH 12.5%, 0.08 $\log_{10}$ CFU/g; c-PH 25%, 0.16 $\log_{10}$ CFU/g), evidencing a change in the gut threshold for BT in the PH state. Bacterial challenge in the a-PH state showed that the liver, spleen and kidney go into a hypoperfusion state (–38, –45.2 and –36 Δ%, respectively), in contrast to the ileum hyperperfusion response (+75 Δ%). Similarly, at c-PH the liver and kidney maintained a hypoperfusion state (–17%, –33% Δ%). Based on both saline-PH groups' data, however, the ileum and liver microcirculation functional adaptation was mostly compromised only at the chronic phase.

**Conclusion** Portal hypertension factor without cirrhosis increases and changes the pattern of BT, especially to the lung at a-PH and to the PF at c-PH states, under small bowel Gram-negative bacterial overgrowth conditions. These findings, in addition to the tissue perfusion impairment, might explain the higher susceptibility to infection in portal hypertension diseases.

## P19

### Contribution of the gut association lymphoid tissue inflammatory response under bacterial translocation and sepsis challenge is the relevant factor for the onset of multiple organ dysfunction syndrome

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*Critical Care* 2008, **12(Suppl 5):**P19 (doi: 10.1186/cc7052)

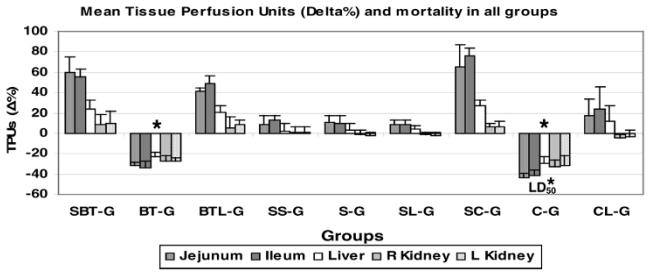
**Background** The gastrointestinal tract has been implicated in sepsis and organ failure by bacterial translocation (BT) and gut-immune system crosstalk with the systemic immunity, but is not yet clearly demonstrated in clinics. Herein we examined the role of gut-associated lymphoid tissue on host inflammatory response by BT as a contributing feature for multiorgan dysfunction in sepsis.

**Methods** Wistar rats were challenged to BT (10 ml *Escherichia coli* R6, $10^{10}$ colony-forming units (CFU)/ml), sepsis (2 ml *Enterobacter cloacae* 89, $10^7$ CFU/ml), and sepsis plus BT, with/without mesenteric lymph flow interruption (MLFI) by canulation and lymph deviation or by mesenteric lymphadenectomy plus lymph duct ligation 5 days before in the following groups ($n=20$/group): BT (BT-G); BT with MLFI (BTL-G); sepsis (S-G); sepsis with MLFI (SL-G); combination of BT to sepsis (C-G); combination with MLFI (CL-G); sham-BT (SBT-G); sham-sepsis (SS-G); and sham-combination (SC-G). Samples (mesenteric lymph node, blood, spleen and liver) were collected 2 hours after and were cultured for bacterial recovery of both sepsis and BT origin. Tissue perfusion (jejunum, ileum, liver, kidneys) and mesenteric microcirculation were monitored at 0 and 2 hours. Systemic blood and intestinal lymph were collected for cell count and phenotyping. The groups' mortality was followed.

**Results** The BT index was not modified by MLFI, but BT alone and sepsis plus BT provoked significant hypoperfusion in all organs plus microcirculation injuries. The MLFI at BTL-G and CL-G completely abrogated tissue hypoperfusion (Figure 1) and microcirculation injury. The lymph-cell count post BT, sepsis and combined challenges was significantly increased compared with controls although the composition was similar (98% to 99% lymphocytes and 1% to 2% others). The blood-leucocyte count was unchanged in all groups (Figure 2). TCD3$^+$ over B lymphocytes (CD45RA$^+$) predominated in both lymph (83%/7%) and blood (68%/3%) ($P<0.05$), and their percentages did not differ between groups. The CD4$^+$/CD8$^+$ ratio also did not differ between groups. Naïve cells (CD4$^+$CD45RC$^+$) predominated in the TCD4$^+$ population in lymph, and memory cells (CD4$^+$CD45RC$^-$) in blood. CD8$^+$CD45RC$^+$ cells predominated in both lymph and blood. The proportion of T-regulatory (CD4$^+$CD8$^+$/CD25$^+$) cells was low in both lymph and blood for all groups (Figures 3 and 4). MLFI completely prevented the death observed in C-G ($LD_{50}$).

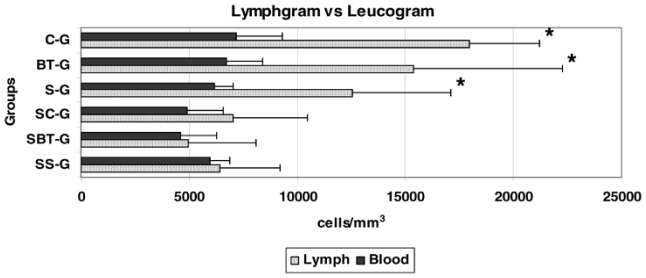
**Conclusion** The gut-associated lymphoid tissue response following bacterial challenge and its crosstalk with the systemic immunity

**Figure 1 (abstract P19)**



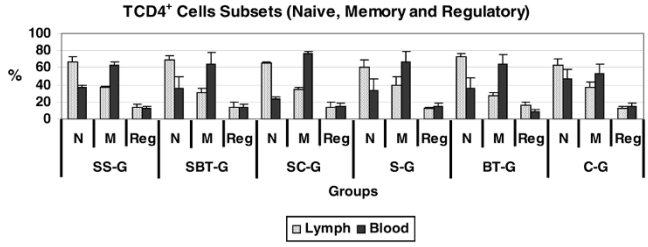

Comparison of the tissue perfusion (jejunum, ileum, liver and kidneys) in Δ% and the mortality index (DL) in all groups. *$P < 0.05$.

**Figure 2 (abstract P19)**



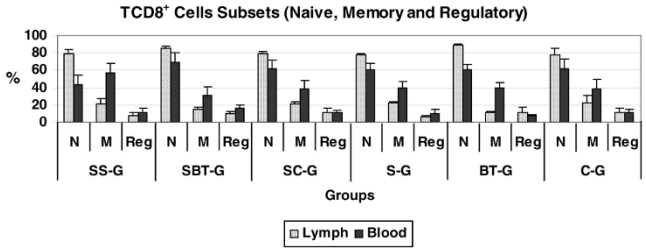

Total number of leukocytes in the mesenteric lymph and blood per $mm^3$ of all groups. *$P < 0.05$.

**Figure 3 (abstract P19)**




CD4-positive T-cell subsets (N, naïve; M, memory; and Reg, regulatory) in the mesenteric lymph and blood of all groups by flow cytometry.

**Figure 4 (abstract P19)**




CD8-positive T-cell subsets (N, naïve; M, memory; and Reg, regulatory) in the mesenteric lymph and blood of all groups by flow cytometry.

via the lymphatic system is the key factor related to the aggravation of systemic inflammation and death in sepsis.

## P20

## Relationship between sepsis intensity, Gram-negative bacterial overgrowth and bacterial translocation in rats

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*Critical Care* 2008, **12(Suppl 5):**P20 (doi: 10.1186/cc7053)

**Background** Some of the enteric microbiota role is linked to the gut immune system, the angiogenic mechanism and colonization resistance, and its disruption has been correlated to local disease and aggravation of a systemic inflammatory state such as in sepsis and critical illness. Herein we examined the role of diverse sepsis intensities on aerobic and anaerobic facultative Gram-negative microbiota of the small bowel (SB) and large bowel (LB) and subsequent bacterial translocation (BT) potentials in rats.
**Methods** Wistar rats (±200 g) were submitted to varying degrees of monobacterial sepsis (S-7, S-8 and S-9 groups, with $10^7$, $10^8$ and $10^9$ colony-forming units/ml/100 g body weight of *Escherichia coli* R6 intravenously, respectively) and samples of the duodenum, jejunum, ileum, cecum, feces, mesenteric lymph nodes (MLN), liver and spleen were harvested at 6, 12 and 24 hours post sepsis and cultured in MacConkey agar medium ($n = 6$/period/group). Control groups were sham with saline injection and naïve without any procedure ($n = 6$/period/group).
**Results** The Gram-negative colonization rise within the SB occurred from the proximal to distal compartments and significant overgrowth onset was seen from 6 hours in the SB and 12 hours in the LB in the sepsis groups, suggesting that LB overgrowth was probably due to SB overgrowth. With severe sepsis (S-8, S-9), the overgrowth was more pronounced and remained for a longer period at the ileum and cecum, but at the duodenum and jejunum the peak growth seen in the 6 to 12 hours period returned to normal level at 24 hours. The maximum overgrowth index comparisons between naïve, S-8 and S-9 were ($\log_{10}$): 0.0 versus 2.8 versus 4.7 (duodenum), 2.5 versus 4.2 versus 6.0 (jejunum), 4.0 versus 7.4 versus 7.5 (ileum), and 5.1 versus 8.0 versus 7.8 (cecum), respectively. Spontaneous BT to the MLN occurred only following sepsis (50% at S-8, 5.6% at both S-7 and S-9) and was 100% *E. coli*. Not only *E. coli* but all other Gram-negatives were overgrown after sepsis stimulus. S-7 and minor trauma (sham) provoked a transient overgrowth but in lesser intensity and endurance.
**Conclusion** Acute sepsis states induced a significant and transient SB and LB Gram-negative microbiota overgrowth directly proportional to the severity of sepsis. The BT event was dependent on both the sepsis degree and overgrowth factors.

## P21

### Activated protein C–protein C inhibitor complex as a prognostic marker in sepsis

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*Critical Care* 2008, **12(Suppl 5):**P21 (doi: 10.1186/cc7054)

**Background** The PROWESS study and later trials of activated protein C (APC) treatment in sepsis have shown only modest reductions in mortality. A recent Cochrane systematic review (CD004388) records doubtful efficacy and serious adverse effects. To optimize the benefit/risk ratio of APC treatment of each patient, a biomarker of protein C (PC) activation is urgently needed and the use of such a marker, activated protein C–protein C inhibitor (APC–PCI), has been investigated in the present study.
**Methods** APC–PCI was measured in acid citrate plasma by means of a newly developed sandwich ELISA (median normal value 0.13 ng/ml, range 0.07 to 0.26, $n = 16$). Levels of APC–PCI and PC were monitored (daily to alternate days) in 135 consecutive critically ill patients, 53 of whom had sepsis during the observation period. The state of PC activation to APC was categorized as nonactivated, moderately activated or highly activated, based on maximum APC–PCI values in relation to the normal range.
**Results** The maximum APC–PCI values were 0.03 to 29 ng/ml, median 0.44 ng/ml. The overall mortality of the 53 sepsis patients was 32% (17/53). The mortality and relative mortality (mortality of activation group/overall mortality) of each activation group are presented in Table 1. A bell-shaped mortality relationship was noted, with high mortalities in both the nonactivated and highly activated groups. Notably, the lowest mortality was recorded in the moderately activated group. Subdividing activation groups by the Acute Physiology and Chronic Health Evaluation (APACHE) II score yielded the highest mortality (5/7 = 71%, relative mortality 227.7%) in the nonactivated subgroup with APACHE II score ≥25, whereas the APACHE II score did not influence mortality in the other activation groups. Minimum PC levels did not correlate with APC–PCI and showed no significant differences between the activation groups.
**Conclusion** Nonactivation of PC in sepsis may represent the failure of an appropriate protective response and is therefore associated with increased mortality, especially when the APACHE II score is elevated. Septic patients without PC activation and a high APACHE II score may be those who are most likely to benefit from APC treatment. PC measurements were not predictive of PC activation as indicated by APC–PCI levels.

## P22

### Which is the worst factor in the sepsis aggravation: translocated bacterial amount or the gut-associated lymphoid tissue activation?

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*Critical Care* 2008, **12(Suppl 5):**P22 (doi: 10.1186/cc7055)

**Background** The intestinal hypothesis of sepsis has been attributed to bacterial translocation (BT) and the aggravation of sepsis is related to the increased vascular permeability state that potentates the BT index. In the present study we examined the BT index during sepsis with or without mesenteric lymph exclusion.
**Methods** Wistar rats (±200 g) were submitted to the BT process (*Escherichia coli* R6, 10 ml of $10^{10}$ colony-forming units (CFU)/ml) and nonlethal sepsis (*Escherichia cloacae* 89, 2 ml of $10^7$ CFU/ml) plus BT, with or without mesenteric lymph interruption by mesenteric lymph node resection and lymph duct ligature 5 days prior to the experiments. Samples (blood, spleen and liver) were collected 2 hours after the inoculation and were cultured to recover bacteria of intestinal origin. One-half of the animals/group was observed to the mortality index. The groups ($n = 20$/group) were: BT group (BT-G); BT with lymphadenectomy (BTL-G); combination (C-G); and combination with lymphadenectomy (CL-G).
**Results** BT was 100% positive in all groups. The BT index was similar between BT-G, BTL-G and CL-G ($P = 0.6$) and mortality was not observed in these groups, although a considerable amount of translocated bacteria could be recovered, particularly at the liver and spleen (Figure 1). When BT was added to the sepsis without lymph exclusion (C-G) the BT index was statistically lower ($P = 0.04$), but 50% ($LD_{50}$) of mortality occurred within 30 hours (Figure 1).

**Figure 1 (abstract P22)**

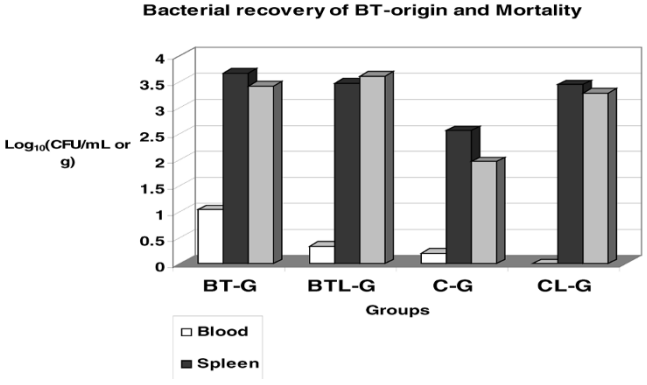


**Table 1 (abstract P21)**

**Mortality and relative mortality (mortality of activation group/overall mortality) of each activation group**

| | PC activation group | | | | | | |
|---|---|---|---|---|---|---|---|
| | Nonactivated, APC–PCI <0.25 ng/ml | | Moderately activated, APC–PCI 0.25 to 0.72 ng/ml | | Highly activated, APC–PCI >0.72 ng/ml | | |
| | Mortality | Relative mortality | Mortality | Relative mortality | Mortality | Relative mortality | *P* value ($\chi^2$) |
| Sepsis mortality (deaths/*n*) | 43.8% (7/16) | 136.4% | 13.0% (3/23) | 40.7% | 50.0% (7/14) | 155.9% | 0.032 |

**Conclusion** These results showed that, more than the amount of translocated bacteria, the gut-associated lymphoid system activation by the BT process played a pivotal role in the worsening of sepsis. Besides, BT occurred independently of mesenteric lymph interruption, showing that the hematological pathway of BT might be the principal route for bacterial dissemination into the bloodstream.

## P23

### Effects of inducible nitric oxide synthase and neuronal nitric oxide synthase inhibition on methicillin-resistant *Staphylococcus aureus* sepsis

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**Background** Methicillin-resistant *Staphylococcus aureus* (MRSA)-induced sepsis/septic shock is a major cause of death in the ICU. We have previously reported that excessive nitric oxide (NO) plays a key role in impaired cardiovascular function during MRSA sepsis. In the present study, we demonstrate a role of inducible (iNOS) and neuronal (nNOS) nitric oxide synthase-derived excessive NO in MRSA-induced cardiovascular morbidity using potent and selective iNOS dimerization inhibitor BBS-2 and selective nNOS inhibitor 7-nitroindazole (7-NI).
**Methods** Ewes were operatively prepared and randomized after a 5-day to 7-day recovery period to the groups: sham (noninjured, nontreated, $n = 6$); control (injured, nontreated, $n = 6$); BBS-2 (injured, treated with BBS-2, $n = 5$); and 7-NI (injured, treated with 7-NI, $n = 3$). Injury consisted of insufflation of 48 breaths of cotton smoke followed by instillation of 2 to 5 x $10^{11}$ colony-forming units of live MRSA into the airways of the sheep. BBS-2 (100 μg/kg/hour) and 7-NI (1 mg/kg/hour) was given starting from 1 hour to the end of the study (24 hours).
**Results** Hemodynamic variables were stable in sham animals. Control sheep developed a hyperdynamic circulatory state as evidenced by a significant increase in cardiac output and a severe fall in mean arterial pressure (Table 1). BBS-2 significantly attenuated hypotension 12 and 18 hours ($P < 0.05$) post injury. In contrary, 7-NI reversed the fall in blood pressure at a later time point. Severe fluid retention seen in control animals was reduced by 7-NI, but not by BBS-2. 7-NI also improved oxygenation. Elevated levels of heart tissue 3-nitrotyrosine (marker of peroxynitrite formation) and poly (ADP)ribose (footprint of DNA damage) were attenuated by both iNOS and nNOS inhibition.
**Conclusion** iNOS inhibition had a partial effect in MRSA sepsis-related cardiovascular morbidity. However, nNOS inhibition had a stronger effect on both severe hypotension and fluid retention following MRSA sepsis. Since nonspecific NOS inhibition is associated with unwanted side effects, more targeted inhibition of NO using specific NOS inhibitors may be a useful tool against the MRSA sepsis-related menace.

**Table 1 (abstract P23)**

**Hemodynamic variables**

| | Sham | Control | BBS-2 | 7-NI |
|---|---|---|---|---|
| Cardiac output (l/min) | | | | |
| Baseline | 4.6 ± 0.4 | 5.2 ± 0.3 | 4.9 ± 0.2 | 5.8 ± 0.1 |
| 24 hours | 4.1 ± 0.3 | 9.2 ± 0.6 | 8.5 ± 0.6 | 7.4 ± 0.8 |
| Mean arterial pressure (mmHg) | | | | |
| Baseline | 99 ± 1 | 92 ± 2 | 102 ± 3 | 98 ± 1 |
| 24 hours | 100 ± 1 | 70 ± 1 | 71 ± 2 | 93 ± 1 |
| Fluid retention at 24 hours (ml/kg) | 0.0 ± 5.9 | 282 ± 17 | 293.0 ± 6.0 | 177.6 ± 58.3 |
| $PaO_2/FiO_2$ | | | | |
| Baseline | 563 ± 19 | 520 ± 30 | 480 ± 14 | 547 ± 15 |
| 24 hours | 619 ± 7 | 176 ± 68 | 157 ± 93 | 212 ± 82 |

## P24

### Use of direct Etest in the management of ventilator-associated pneumonia due to resistant Gram-negative pathogens

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*Critical Care* 2008, **12(Suppl 5):**P24 (doi: 10.1186/cc7057)

**Background** Increasing bacterial antibiotic resistance combined with the increasing incidence of *Clostridium difficile* disease complicate the choice of empirical therapy for ventilator-associated pneumonia (VAP). Etest strips rapidly adsorb a gradient of antimicrobial agent onto agar, allowing determination of minimum inhibitory concentration of cultured organisms. Their use on plates directly inoculated with respiratory samples to provide rapid susceptibility results in VAP has previously been described in a study in which the principal pathogens were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The current study evaluated the technique in a setting where resistant *Enterobacteriaceae* predominate.
**Methods** Chromogenic Mueller–Hinton agar was inoculated with 100 respiratory specimens from patients clinically suspected to have VAP. Vancomycin, cefoxitin, cefotaxime, ceftazidime, piperacillin–tazobactam and meropenem Etest strips were then applied to the inoculated medium strips selected to aid detection of resistant Gram-negative pathogens. In addition, a *P. aeruginosa* diatab was applied to plates to facilitate identification of this organism. The plates were incubated at 37°C in 5% $CO_2$ overnight and subsequently interpreted using a prospectively designed protocol to suggest antimicrobial choice. Specimens were processed using UK standard methods in parallel to allow comparison of speed and accuracy.
**Results** Forty-four out of 100 samples yielded no significant bacterial growth using the standard method. Sixty-three isolates were speciated from the remaining 56 samples (including 37 coliforms and 13 *P. aeruginosa*). Fifty-four of these samples had detectable growth at day 1 by the Etest method. Of the coliforms,

17 possessed extended-spectrum β-lactamase genes, including ampC, all detected by the Etest method. Organisms were confirmed susceptible to the Etest suggested antibiotic in 52/54 cases. Direct results would have led to a change in antibiotics to a more appropriate agent in 11 cases. An early indication of the presence of a multiresistant pathogen would have occurred in four cases. All Etest results were available within 24 hours, compared with a mean time to sensitivity reporting of 2.25 days using the standard method.
**Conclusion** The direct Etest method provides rapid and accurate susceptibility results on patients with VAP, expediting selection of an antibiotic of appropriate spectrum.

### P25

### Evaluation of an agar-gradient minimum-inhibitory-concentration method (the Etest) as a rapid and direct measure of antimicrobial susceptibility in Gram-negative bacteraemia

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**Background** The selection of appropriate antibiotics to treat Gram-negative bacteraemia may be life-saving. Rapid methods of antimicrobial susceptibility testing have sought to guide early antibiotic selection and usage. We sought to evaluate whether a combination of chromogenic agar and six Etest gradient diffusion strips could be used to provide a clinically useful, direct rapid antimicrobial susceptibility test result following 4 hours of incubation.
**Methods** Fifty consecutive Gram-negative blood culture isolates were tested over a 4-month period. Following confirmation of Gram-negative bacilli, 200 μl blood was directly inoculated from the enrichment broth onto a chromogenic MH agar plate. Six Etest strips were directly applied onto the agar using an automated placement device (Simplex C76; Inverness Medical UK (Bio-Stat Division), Stockport, UK). The antibiotics used were cefoxitin, cefotaxime and ceftazidime (to indicate the presence of extended-spectrum and ampC β-lactamase producers), vancomycin (to 'screen' for Gram-positive organisms), and piperacillin–tazobactam and meropenem (based on local prescribing patterns). The plates were incubated at 35 to 37°C and read at 4, 6 and 24 hours. Minimum-inhibitory concentration values were determined and organisms were categorized as susceptible/resistant according to British Society for Antimicrobial Chemotherapy breakpoints. A presumptive identification and susceptibility profile was obtained at 4 hours, based upon which the investigators recorded a decision as to whether the patients' antibiotics could be escalated, de-escalated or remain unchanged. The results were correlated with those at 6 and 24 hours, and with the report issued following routine susceptibility testing, as performed at our institution.
**Results** Forty-five (90%) cultures had Etest susceptibility profiles interpretable at 4 hours. Of the five remaining, three (6%) were read at 6 hours and two (4%) at 24 hours. In three cases there was a mixed growth of organisms. Twelve (24%) organisms had resistance mechanisms identified, of which 10 (83%) were confirmed by our routine antimicrobial susceptibility rest method. At 4 hours, nine (18%) patients were receiving too narrow spectrum antibiotics. Additionally, the investigators felt that antibiotics could have been safely de-escalated in 16 (32%) cases and continued in the remaining 25 (50%) patients.
**Conclusion** Our evaluation of this method has shown that it can provide rapid and reliable antibiotic susceptibility information at 4 hours. This could potentially have a major impact on antibiotic use and may significantly affect patient management.

### P26

### Statin anti-inflammatory therapy in septic patients

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**Background** Statins have a well-known pleiotrophic effect as anti-inflammatory agents, immunomodulators, antioxidants, antithrombotic agents and endothelial stabilizers. Statins, however, have been proposed as a therapeutic tool in septic patients.
**Objective** To investigate possible statin therapeutic uses in the induced sepsis inflammatory process.
**Methods** A prospective, longitudinal, experimental study was performed from November 2007 to March 2008 of 40 consecutive septic patients who were randomly assigned to one of the following groups: treatment group (received 80 mg daily simvastatin for 14 days) or control group (did not receive simvastatin). Inflammatory markers (sedimentation rate (SR), C-reactive protein (CRP), and antitrombin III) were measured on days 0, 5, 10 and 14. Results are expressed as the median (25th–75th interquartile interval) and groups were compared with the Mann–Whitney *U* test.
**Results** The SR diminished from 34 (21 to 45) to 19 (14 to 23) in the treatment group, versus the control group where it increased from 28 (21 to 40) to 36 (27 to 50), with $P < 0.01$ when both groups were compared. CRP behaved in a similar way, diminishing in the treatment group and increasing in the control group. On day 14, the SR and CRP reached normal values: 4 (2 to 6) and 1 (0 to 2), respectively, in the treatment group versus 22 (19 to 36) and 8 (4 to 14), respectively, in the control group ($P < 0.001$). Antithrombin III increased in both groups, from 33 (28 to 50) to 90 (88 to 98) in the treatment group and from 33 (29 to 50) to 50 (48 to 55) in the control group ($P < 0.01$). The length of stay was longer in the control group: 22 (18 to 26) days versus 15 (14 to 16) days in the treatment group ($P < 0.01$).
**Conclusion** The present study demonstrates that statins are able to decrease the systemic inflammatory response and provide endothelial increased stability properties from the fifth treatment day; reducing the mechanical ventilation time rates, and so on, with the patient's long stay.

### P27

### Rapid identification of sepsis-causing pathogens with polymerase chain reaction and microarray-based assay

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**Background** During the course of a bacterial infection, a rapid identification of causative agents is necessary for the determination of effective treatment options. We developed a method based on a modified broad-range PCR and an oligonucleotide microarray for the simultaneous detection and identification of 19 sepsis-causing pathogens at species level, as well as coagulase-negative staphylococci and *Enterobacteriaceae* at taxon level.

**Materials** One hundred and fifty-four positive and 15 negative blood culture samples were collected to evaluate the assay performance. For the analysis, DNA was automatically extracted from the samples.
**Methods** The broad-range PCR primer mixture was designed using conservative regions of topoisomerase gene subunits *gyrB* and *ign* from various bacteria. The primer design allowed the use of a novel DNA amplification method producing labeled, single-stranded DNA suitable for microarray hybridization. The probes on the microarray were designed against species-specific or taxon-specific variable regions of the *gyrB* and *ign* genes flanked by the primers. As a microarray platform for the probes, we applied TubeArray that is a microreaction vial containing a microarray at the bottom. To indicate the detection of antimicrobial resistance, we included *mecA*-specific primers and probes in the same assay. Furthermore, the software for automated data analysis was provided.
**Results** Comparison of the assay results with the gold standard culturing method revealed sensitivity of 98% and specificity of 93%. When the *mecA* identification was correlated with *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus* genus detection by the specific probes, accurate information about the association of the *mecA* gene with staphylococci was provided. The results from these 15 samples were in line with the antimicrobial susceptibility testing (oxacillin susceptibility testing).
**Conclusion** The results from the method were available 3 hours after the positive blood culture result. Up to 24 samples could be processed simultaneously. The assay therefore provides rapid and reliable data, which can guide antimicrobial treatment decisions in a timely manner. We have further broadened the pathogen panel for the detection of 50 bacterial species; 24 at the species level and at least 26 species at the taxon level. The described panel is now commercially available from Mobidiag Ltd (Helsinki, Finland) under the product name Prove-it™ Bacteria.

## P28

## Bacterial flagellin triggers myocardial innate immune responses and acute contractile failure

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*Critical Care* 2008, **12(Suppl 5):**P28 (doi: 10.1186/cc7061)

**Background** Septic shock is associated with severe cardiac dysfunction, whose mechanisms remain partly undefined. Recent data suggested that it might be triggered by the direct action of microorganisms and their products on the heart itself. We previously showed that flagellin, the protein monomer from bacterial flagella, is a potent activator of NFκB-dependent proinflammatory signaling in cultured cardiomyocytes. In the present study, we investigated whether flagellin might induce such an inflammation in the heart *in vivo* and contribute to cardiac dysfunction.
**Methods** Mice were injected intravenously with 1 μg flagellin. At selected timepoints (30 minutes to 4 hours), the effects of flagellin were evaluated by its ability to activate NFκB, mitogen-activated protein kinases and downstream signaling. Expression of the flagellin receptor TLR5 was also investigated. Cardiac function was evaluated after 4 hours using a microtip pressure–volume catheter inserted into the left ventricle. Also, human cardiac tissue was obtained from the right atrium in patients undergoing elective coronary artery bypass grafting surgery, to determine the presence of TLR5 in the human heart.
**Results** Cultured cardiomyocytes, as well as hearts from mice and humans, expressed TLR5 protein at a high level. Flagellin activated NFκB and the mitogen-activated protein kinases p38 and JNK in cardiomyocytes *in vitro* and *in vivo*, and also upregulated the transcription of TNFα and MIP-2. *In vivo*, flagellin also induced the recruitment of neutrophils within the heart. Functionally, flagellin induced significant increases in end-systolic and end-diastolic left ventricle volumes, indicating cardiac dilation, and a significant reduction of end-systolic elastance and maximal elastance, indicating depressed myocardial contractility. In contrast, no change in the slope of the end-diastolic pressure–volume relationship was noted.
**Conclusion** Bacterial flagellin induces a prototypical inflammatory response in cardiomyocytes *in vitro* and in the myocardium *in vivo*. These effects are associated with a profound alteration of the left ventricle systolic function *in vivo*, suggesting that flagellin may represent a critical mediator of cardiac dysfunction in septic shock.

## P29

## Alterations in cardiac hemodynamics following endotoxin are not due to reduced myocardial contractility

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**Background** Impaired cardiac function due to reduced myocardial contractility is a typical manifestation of septic shock, whose mechanisms are poorly defined. Experimentally, the administration of endotoxin (lipopolysaccharide (LPS)) to laboratory animals is classically used to study the mechanisms of septic cardiomyopathy. However, most studies evaluating the effects of LPS on the heart *in vivo* have relied on indirect, load-dependent, indices of cardiac function, and thus could not precisely determine the real consequences of LPS on cardiac contractility. We therefore evaluated the direct effects of LPS on cardiac contractility in mice, using left ventricular (LV) micro-tip pressure–volume (PV) catheters, which provide load-independent measurements of cardiac function, including end-systolic elastance (Ees) and maximal elastance (Emax).
**Methods** Male BALB/c mice received an intraperitoneal injection of *Escherichia coli* LPS (1, 5, 10, or 20 mg/kg). After 2, 6 or 20 hours, selected groups of mice were anesthetized, intubated and mechanically ventilated. A PV catheter was inserted into the left ventricle through the right carotid artery. LV pressure (end systolic (LVSP) and end-diastolic (LVDP)) and volumes (end systolic (ESV) and end-diastolic (EDV)) were recorded, allowing the calculation of the stroke volume, stroke work, cardiac output and ejection fraction. Ees and Emax were computed from the slope of the end-systolic PV relationships of successive PV loops obtained at rapidly reduced preload, by inferior vena cava compression. Mice were sacrificed at the end of the experiments.
**Results** EDV decreased with LPS, mostly after 6 hours, whereas ESV did not change. LVSP was slightly decreased only after 6 hours, and LVDP was not significantly influenced by LPS. The stroke volume, stroke work, ejection fraction and $dp/dt_{max}$ were reduced at all doses of LPS, mostly after 6 hours and slightly recovered after 20 hours. In spite of an increase in heart rate, the cardiac output decreased, especially after 6 hours and at the high doses (10 and 20 mg/kg) of LPS. Most importantly, both Ees and Emax markedly increased after all doses of LPS, mostly after 2 and 6 hours, and returned back to control values after 20 hours.
**Conclusion** In striking contrast with the usual belief, LPS does not induce direct negative inotropic effects in the mouse, but instead markedly enhances contractility. The alterations in cardiac function induced by LPS are only, and entirely, due to altered loading conditions, which are mainly observed 6 hours after the injection of LPS.

**P30**

## The flagellin/Toll-like receptor 5 axis elicits diffuse proinflammatory signaling and innate immune defenses *in vivo*

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*Critical Care* 2008, **12(Suppl 5):**P30 (doi: 10.1186/cc7063)

**Background** The development of septic shock is related to the activation of nonspecific (innate) immune responses, triggered by the interactions between molecules released by pathogens and specific cellular receptors in the host, termed Toll-like receptors (TLRs). Flagellin is a 55 kDa protein isolated from the flagellum of Gram-negative bacteria, which may activate such responses through its recognition by TLR5. The tissue distribution of TLR5, as well as the actions of flagellin on various organs *in vivo*, has not been previously established. We therefore conducted the present study to determine the presence of TLR5 receptor in major organs from mice, and to evaluate whether flagellin could trigger prototypical innate immune responses through activation of NFκB and mitogen-activated protein kinase (MAPK) signaling pathways in these organs.
**Methods** Mice were injected intravenously with 1 μg recombinant Salmonella flagellin. At selected timepoints (30 minutes to 6 hours), the mice were sacrificed and the major organs (lung, liver, gut and kidney) were harvested for expression of the flagellin receptor TLR5; for the activation state of NFκB (monitored by the degree of phosphorylation and degradation of its inhibitor IκBα and by the NFκB-DNA-binding activity); for the activation state of MAPK (monitored by the degree of phosphorylation of JNK, p38 and ERK); for the expression of inflammatory cytokines; and for the activation of apoptotic pathways (monitored by the degree of caspase-3 and poly(ADP-ribose) polymerase cleavage). Plasma was obtained for the measurements of cytokine levels.
**Results** TLR5 protein was constitutively expressed in all organs. The injection of flagellin activated NFκB and MAPKs at 30 minutes, and markedly enhanced the generation of the cytokines TNFα, IL-1β, IL-6, TREM-1, and MIP-2 at 1 hour and 3 hours. Similarly, these cytokines significantly increased in the plasma from 1 hour to 6 hours after flagellin. Flagellin also triggered prototypical apoptotic changes in all organs.
**Conclusion** Bacterial flagellin activates inflammatory signaling and apoptosis in most major organs *in vivo*, and thus may represent a critical mediator of multiple organ failure during Gram-negative septic shock.

**P31**

## A novel therapeutic agent to prevent sepsis-induced acute kidney injury and mortality

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**Background** Acute kidney injury (AKI) occurs in about one-half of the patients who develop septic shock, and the mortality of AKI with sepsis is extremely high. An effective therapeutic intervention is urgently needed. In the present study we tested the ability of a novel tetrapeptide, EA-230, to improve survival and attenuate loss of kidney function in a clinically relevant model of sepsis – cecal ligation and puncture (CLP) in mice.
**Methods** Sepsis was induced in C57BL/6 mice by CLP. Four hours postoperatively, EA-230 was administered. Subsequently, animals were treated twice daily for four consecutive days intraperitoneally. The effects of 20, 30, 40, or 50 mg/kg were compared with those of saline. Survival and renal function were monitored. Inulin clearance and *para*-aminohippuric acid clearance were used to measure the glomerular filtration rate and renal blood flow.
**Results** All saline-treated control animals died within 5 days of CLP, whereas EA-230 treatment improved survival significantly in a dose-dependent manner. The best result was obtained with 50 mg/kg EA-230 (43.8% survival after 2 weeks). Serum creatinine and blood urea nitrogen increased markedly 24 hours after CLP. EA-230 attenuated the increases in creatinine and blood urea nitrogen significantly in the 30 to 50 mg/kg treatment groups. Furthermore, the glomerular filtration rate and renal blood flow were significantly higher ($P<0.05$) 36 hours post CLP in EA-230-treated mice versus those treated with saline.
**Conclusion** EA-230 is a novel and promising therapeutic agent for preventing AKI in sepsis. Its beneficial effect is associated with an improvement in renal hemodynamics.

**P32**

## Identifying sepsis in the emergency room: the best clinical and laboratory variables

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*Critical Care* 2008, **12(Suppl 5):**P32 (doi: 10.1186/cc7065)

**Background** Early diagnosis, antibiotics and supportive therapy are essential in sepsis. The diagnostic value of clinical and laboratory variables was evaluated in a prospective observational study.
**Methods** A cohort of 404 adult patients admitted to the Department of Infectious Diseases from the emergency room for suspected severe infection was studied. A bacterial infection requiring antibiotic treatment was diagnosed in 306 patients (pneumonia 130 patients, urinary tract infection 80 patients, skin/soft tissue 43 patients, other bacterial infections 53 patients) and bacteriemia in 68 patients (most common isolates: pneumococci 19 patients, *Escherichia coli* 18 patients, *Staphylococcus aureus* eight patients, β-haemolytic streptococci seven patients). Nonbacterial infections or noninfectious conditions were diagnosed in 82 patients. The physiological variables recorded were: temperature, heart rate, blood pressure, respiratory rate (RR), oxygen saturation, urine output, and cerebral status. The laboratory variables were: C-reactive protein (CRP), lactate, bicarbonate, creatinine, urea, hemoglobin, white blood cells (WBC), neutrophils, platelets, International Normalized Ratio, D-dimer, albumin, bilirubin, procalcitonin (PCT), IL-6 and lipopolysaccharide binding protein (LBP).
**Results** In a univariate analysis, PCT, IL-6, LBP, CRP, bilirubin and maximum RR during the first 4 hours (RR max 0 to 4 hours) were associated with bacteremia with $P<0.001$ and CRP, PCT, IL-6, LBP, WBC, neutrophils, RR max 0 to 4 hours and hemoglobin were associated with a bacterial infection with $P<0.001$. In a multivariate logistic regression, PCT, RR max 0 to 4 hours, bilirubin and CRP each contributed significantly to the accurate prediction of bacteremia. To predict a bacterial infection, CRP, WBC, hemoglobin and RR max 0 to 4 hours contributed significantly. The diagnostic accuracy of these variables was compared with the

ability of the physicians caring for the patients to prescribe antibiotics appropriately. Of the 306 patients with bacterial infections requiring antibiotics, 76% had actually received antibiotics within 4 hours of arrival; and of the patients not requiring antibiotics, 54% were not on antibiotics after 4 hours. All variables tested had inferior diagnostic accuracy compared with the clinician.

**Conclusion** We conclude that for the clinician, who evaluates patients with a suspected infection, special attention should be directed to the RR, CRP and WBC but the basic evaluation of the patient's medical history and a thorough clinical examination and assessment of the patient's general condition cannot be replaced by any laboratory parameter. Novel markers such as PCT, IL-6, and LBP seem not to give added value in the emergency room.

## P33

### Role of central nitric oxide on hormonal and cardiovascular alterations in experimental polymicrobial sepsis

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*Critical Care* 2008, **12(Suppl 5):**P33 (doi: 10.1186/cc7066)

**Background** Polymicrobial sepsis induced by cecal ligation and puncture (CLP) causes a massive nitric oxide (NO) synthesis and consequently several physiological alterations in cardiovascular and hormonal systems. Central NO is reported to modulate the secretion of vasopressin. Our aim was to study the central effect of an unspecific NO synthase inhibitor (L-NAME) on the mean arterial pressure (MAP), plasma nitrate levels (pNO), plasma arginine vasopressin concentration (pAVP) and hypothalamic arginine vasopressin mRNA content during polymicrobial sepsis induced by CLP.

**Methods** Male Wistar rats received an intracerebroventricular injection of L-NAME (250 µg) or saline (vehicle) and 30 minutes later they were submitted to CLP or to a sham operation. Animals were decapitated 0, 4, 6, 20 or 24 hours after surgery and blood was collected for pNO and pAVP measurements. The brains were removed and the supraoptic and paraventricular nuclei were punched out for vasopressin mRNA determination by real-time PCR. In another set of animals the MAP was measured each 15 minutes 1 hour before and during 24 hours after surgery with intervals.

**Results** CLP caused an increase in pNO after 6 hours, and in pAVP at 4 and 6 hours, while the MAP decreased during 5 hours after surgery. Hypothalamic vasopressin mRNA showed a tendency to decrease in both nuclei. L-NAME pretreatment increased survival (80% versus 67%), blocked pNO increase and MAP decrease and also resulted in an increase in plasma vasopressin concentration in the initial phase of sepsis ($P<0.05$). The vasopressin mRNA content increased at 20 and 24 hours in the paraventricular nucleus and only at 24 hours in the supraoptic nucleus.

**Conclusion** These results demonstrate that central NO plays a role in blood pressure and in vasopressin synthesis and release during polymicrobial sepsis in rats.

**Acknowledgement** Financial support from FAPESP.

## P34

### Blocking central leukotrienes synthesis affects vasopressin release during sepsis

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**Background** Recent studies revealed that vasopressinergic neurons have a high content of LTC4 synthase, a critical enzyme in *cys*-leukotriene synthesis that may play a role in regulating vasopressin secretion. The present study investigates the role of this enzyme in arginine vasopressin (AVP) release during experimentally induced sepsis.

**Methods** Male Wistar rats received an intracerebroventricular injection of MK 886 (1.0 µg/kg), a leukotriene (LT) synthesis inhibitor, or vehicle, 1 hour before cecal ligation and puncture (CLP) or sham operation. In one group of animals the survival rate was monitored for 5 days. In another group, the animals were decapitated 0, 4, 6, 18 and 24 hours after CLP or sham operation, and blood was collected for hematocrit, serum sodium and nitrate, plasma osmolality, protein and arginine AVP determination. The neurohypophysis was removed for quantification of AVP content, and the hypothalamus was dissected for LTC4 synthase analysis by western blot.

**Results** The mortality rate after CLP was reduced by the central administration of MK 886. The increase in plasma AVP levels and hypothalamus LTC4 synthase content in the initial phase of sepsis was blocked, whereas the decrease in neurohypophyseal AVP content was partially reversed. The increase of serum nitric oxide and hematocrit was reduced, and the decrease in plasma protein and osmolality was not affected by the LT blocker. In the final phase of sepsis, the plasma AVP level and the hypothalamic LTC4 synthase content were at basal levels. The central administration of MK 886 increased the hypothalamic LTC4 synthase content but did not alter the neurohypophysis AVP content and plasma AVP levels observed during this phase.

**Conclusion** These results suggest that the central LTs are involved in the vasopressin release observed during sepsis.

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## P35

### Systemic inflammatory response syndrome and severe sepsis definitions outside the intensive care unit: contribution or confusion?

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**Background** The terms systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis and septic shock were defined in 1992 and have been universally accepted. In the present study, the prevalence of SIRS and severe sepsis in patients with significant bacterial infections was assessed.

**Methods** A total of 404 adult patients admitted to the Department of Infectious Diseases from the emergency room (ER) for suspected severe infection was studied prospectively. Laboratory variables defining SIRS and severe sepsis were measured on arrival while physiological variables were recorded on arrival in the ER and every 4 hours for 24 hours.
**Results** Bacterial infections requiring antibiotic treatment were diagnosed in 306 patients. One hundred and fifty of these developed severe sepsis during the first 24 hours. Significant bacteremia was detected in 68 patients. In these three groups 26%, 22% and 21%, respectively, failed to meet two or more of the SIRS criteria on arrival in the ER. In the group of patients that did not have an infection nor did not need antibiotic treatment, 63% had SIRS on arrival. SIRS on arrival correlated significantly with bacterial infection and development of severe sepsis, but not with bacteremia. Of the SIRS criteria, only the respiratory rate and white blood count contributed significantly to this finding; the heart rate and temperature did not. Intensive care was required for 14/150 patients (9%) with severe sepsis and for 6/68 (9%) bacteremic patients. Altogether 11/404 (2.7%) patients died within 28 days.
**Conclusion** As a tool for definition of sepsis and selection of patients for clinical sepsis trials, SIRS lacks acceptable sensitivity and specificity in a selected ER population with a high risk of serious infection. Excluding patients with less than two or even three SIRS criteria may exclude a large cohort of patients with sepsis and result in biased enrollment to clinical trials. It may be time to abandon the SIRS criteria as an entry criterion for sepsis trials and to instead focus on more strict definitions of underlying infections in association with sepsis-related hypoperfusion and organ dysfunction. Many of the patients developed severe sepsis within 24 hours, yet only a small proportion required intensive care, putting the term *severe sepsis* into question. Severe sepsis in the ICU setting is known to be associated with a high mortality, whereas this might not be the case outside the ICU.

## P36

## Circulating myeloid and plasmacytoid dendritic cells are strongly diminished in septic shock

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**Background** Dendritic cells (DCs) play a key role in the initiation and integration of innate and adaptive immune responses to microbial infection. In contrast to neutrophils, macrophages or lymphocytes, there are virtually no data on the time course of circulating DCs in septic shock. Using a novel specific and sensitive assay, we analyzed the evolution of circulating myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) in septic shock.
**Methods** We enrolled immunocompetent adult patients with septic shock (SS, $n = 43$) and with shock from other etiologies (NSS, $n = 29$). Sixteen healthy controls (HC) were also used as reference for mDCs and pDCs. Blood samples (200 µl) were drawn on the day of shock, then after 3 and 7 days. DC analyses were performed using the DC-labelling kit Trucount® assay (BD Biosciences, San Jose, CA, USA). $CD11c^+CD123^-$ cells (mDCs) and $CD11c^-CD123^+$ cells (pDCs) were selected and counted by flow cytometry (FACSCanto™; BD Biosciences). The HLA-DR mean fluorescence index was measured.
**Results** The age, sex ratio, Simplified Acute Physiology Score II, Sepsis-related Organ Failure Assessment score, nosocomial infection (NI) and mortality rates did not statistically differ between SS and NSS patients. On day 1, mDC and pDC counts were significantly lower in both SS and NSS patients as compared with controls ($P < 0.001$). Patients with SS had significantly lower mDC and pDC counts than NSS patients ($P < 0.001$) both at day 1 and day 3. The HLA-DR mean fluorescence index of mDCs and pDCs was lower in SS patients compared with HC ($P = 0.005$ and $P = 0.037$, respectively) but did not differ between other groups. Interestingly, 10 out of the 43 SS patients developed NI after a median time of 9 (7.5 to 11) days in the ICU. Whereas mDCs increased in patients without NI, mDC counts remained low at day 7 in patients who developed NI: mDC counts and their relative variation between day 1 and day 7 were significantly lower in patients who developed NI than in those who did not ($P < 0.05$). Logistic regression analysis indicates that a negative mDC relative variation is associated with an increased risk of nosocomial infection with an odds ratio of 22 (2.53 to 191) ($P = 0.005$).
**Conclusion** Circulating mDC and pDC counts are lower in SS than in NSS as early as day 1. The persistence of a low count of mDCs after SS seems to be associated with the advent of nosocomial infection during the ICU stay.

## P37

## suPARnostic® as a treatment efficacy monitoring tool in systemic inflammatory response syndrome/sepsis patients

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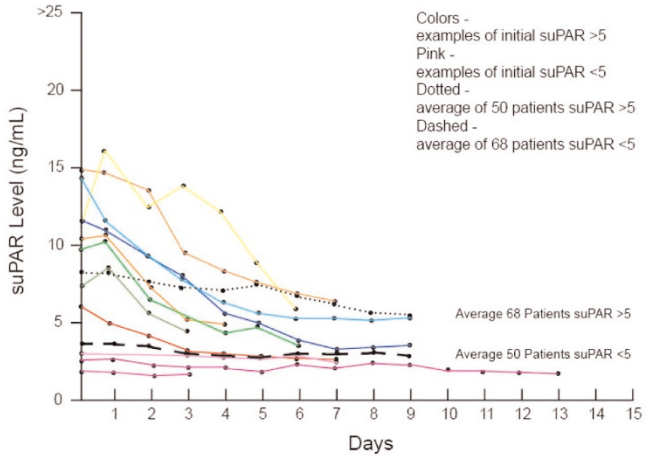
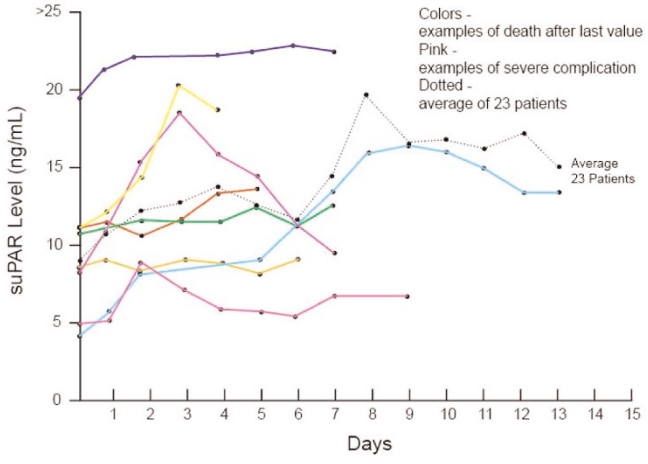
**Background** Biomarkers may aid in risk triaging of systemic inflammatory response syndrome (SIRS) patients at admittance to hospital and in the monitoring of response to medical intervention. The overall aim is reducing mortality in SIRS and sepsis patients.
**Methods** A prospectively collected cohort of patients with SIRS that were admitted to an emergency department and a department of infectious diseases at a Copenhagen University hospital were studied. Samples obtained daily during hospitalization were measured for soluble urokinase plasminogen activator receptor (suPAR) using the CE/IVD-approved (the product complies with the European Directives for In-Vitro Diagnostics) suPARnostic® assay and were compared with various other clinical parameters associated with assessing disease severity, including C-reactive protein, procalcitonin, Simplified Acute Physiology Score II, and Sepsis-related Organ Failure Assessment scores. Survival was assessed using receiver operating curve statistics.
**Results** One hundred and fifty-one patients were included in the study, nine of whom died within 30 days of admission. Admission levels of suPAR were significantly higher in survivors compared with nonsurvivors with an area under the curve of 0.80 and 0.92 when combined with age. Admission levels of procalcitonin and C-reactive protein were not significantly different between survivors and nonsurvivors. Simplified Acute Physiology Score II and Sepsis-related Organ Failure Assessment scores were significant predictors of death in this setting as well. During treatment, survivors showed overall declining suPAR levels (Figure 1) while continuously elevated suPAR levels were observed in nonsurvivors (Figure 2).

**Figure 1 (abstract P37)**




suPAR levels among surviving SIRS patients during treatment. Dotted line, mean suPAR among patients with an inclusion suPAR >5 ng/ml ($n = 50$). Dashed line, suPAR levels among patients with an inclusion suPAR <5 ng/ml ($n = 68$).

**Figure 2 (abstract P37)**




suPAR levels among patients who either had severe complications ($n = 14$) or died ($n = 9$) within 30 days of hospitalization. Dotted line, mean suPAR for the 23 patients.

**Conclusion** The suPARnostic® assay provided significant information on risk of mortality following admission. Continuous elevated suPAR levels during treatment were associated with poor clinical outcome.

## P38

### Methicillin-resistant *Staphylococcus aureus* enhances alveolar epithelial cell permeability through vascular endothelial growth factor and cytoskeletal disruption

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*Critical Care* 2008, **12(Suppl 5):**P38 (doi: 10.1186/cc7071)

**Background** Methicillin-resistant *Staphylococcus aureus* (MRSA) is now a common infection encountered in hospitals and communities. We have previously shown that MRSA causes reactive oxygen and nitrogen (ROS/RNS)-dependent increased vascular permeability, multiorgan system failure and death in our ovine model. Using type II alveolar epithelial cells (A549), we hypothesized that MRSA increases expression of vascular endothelial growth factor (VEGF), a regulator of vascular permeability to water and proteins, and disrupts barrier function by disrupting cytoskeletal integrity.
**Methods** A549 cells were challenged with $10^5$ colony-forming units MRSA over a time course of 24 hours and were visualized for markers of ROS/RNS formation (2,7-dichlorodihydrofluorescein), as well as VEGF and actin expression by confocal imaging and western blot analyses. Cellular permeability was measured by quantifying FITC-dextran flow through a monolayer of A549 cells.
**Results** MRSA caused a significant 7.4-fold increase in 2,7-dichlorodihydrofluorescein fluorescence over unchallenged controls. L-NAME, an inhibitor of nitric oxide formation, blocked this response. Western blot analyses confirmed the confocal observations that MRSA caused an 8.15-fold increase in VEGF expression, versus cells that were pre-incubated with L-NAME (3.4-fold). MRSA also induced formation of actin stress fibers and subsequent cellular contraction. In support of these observations, MRSA caused a 405% increase in cellular permeability to FITC-dextran. However, pre-incubation with L-NAME had no effect on MRSA-induced barrier dysfunction.
**Conclusion** MRSA induces VEGF expression in a ROS/RNS-dependent manner. MRSA also causes alveolar epithelial cell barrier dysfunction by disrupting the actin cytoskeleton independent of nitric oxide synthase activity. Together, the data suggest that MRSA-increased vascular permeability in the lung may be due, in part, to disruption of the cytoskeletal integrity and increased expression of VEGF, but the overall mechanism involves multiple pathways and requires further study.

## P39

### Phosphoinositide-3 kinase gamma kinase activity significantly contributes to the pathophysiology of sepsis and multiorgan failure

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**Background** Sepsis is characterized by systemic inflammation (systemic inflammatory response syndrome (SIRS)), leading to multiple organ failure and death. The lung and liver are both prone to septic-induced damage resulting from leukocyte infiltration, cellular apoptosis, and epithelial/endothelial breakdown. Furthermore, coagulation can potentiate the inflammatory response and

**Table 1 (abstract P39)**

**Portion of results displaying the systemic, lung, liver and coagulation responses of PI3Kγ WT, KO and KD mice to CLP-induced sepsis**

| | WT sham | KO sham | KD sham | WT CLP | KO CLP | KD CLP |
|---|---|---|---|---|---|---|
| Systemic response | | | | | | |
| MIP-2 concentration (pg/ml in plasma x $10^4$) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 13 ± 5** | 2 ± 1* | 2 ± 1* |
| IL-6 concentration (pg/ml in plasma x $10^3$) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 18 ± 5* | 19 ± 10* | 22 ± 8* |
| Lung injury | | | | | | |
| Pathology score | 1.67 ± 0.42 | 1.8 ± 0.49 | 1.71 ± 0.42 | 4.17 ± 0.31* | 2.00 ± 0.63 | 2.00 ± 0.45 |
| Permeability (protein in BAL (μg/ml)) | 194 ± 7 | 199 ± 3 | 195 ± 8 | 234 ± 13* | 191 ± 13 | 192 ± 10 |
| Neutrophil infiltration (cell x $10^3$/ml BAL) | 0.3 ± 0.2 | 0.8 ± 0.4 | 0.2 ± 0.2 | 11.7 ± 5.9* | 0.2 ± 0.1 | 0.8 ± 0.3 |
| MIP-2 concentration (pg/ml in BAL x $10^2$) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 21.6 ± 1.0* | 1.1 ± 0.4 | 1.2 ± 0.5 |
| IL-6 concentration (pg/ml in BAL x $10^2$) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 14 ± 5* | 1 ± 0 | 2 ± 0 |
| Apoptosis (% apoptotic cells) | 0.3 ± 0.1 | 1.2 ± 0.2* | 1.0 ± 0.2* | 7.7 ± 0.8** | 1.3 ± 0.3* | 1.2 ± 0.3* |
| Akt phosphorylation (fold increase over WT sham) | 1.0 ± 0.1 | 0.7 ± 0.0* | 0.7 ± 0.1* | 1.4 ± 0.1** | 0.8 ± 0.1* | 0.8 ± 0.1* |
| Liver injury | | | | | | |
| Pathology score | 0.33 ± 0.21 | 0.20 ± 0.20 | 0.20 ± 0.20 | 2.83 ± 0.17** | 1.20 ± 0.37* | 1.83 ± 0.17* |
| Apoptosis (% apoptotic cells) | 1.0 ± 0.2 | 4.6 ± 1.2* | 4.3 ± 1.0* | 23.9 ± 2.7** | 5.6 ± 0.7* | 4.9 ± 1.0* |
| Coagulation | | | | | | |
| tPA (pg/ng protein) | 404 ± 17 | 369 ± 53 | 423 ± 23 | 1 ± 1** | 104 ± 36* | 61 ± 31* |
| PAI-1 (pg/μg protein) | 2.9 ± 0.2 | 2.7 ± 0.2 | 3.1 ± 0.4 | 84.8 ± 13.4** | 50.5 ± 9.2* | 52.0 ± 9.0* |
| Fibrinogen (ng/mg protein) | 805 ± 35 | 807 ± 40 | 779 ± 33 | 1424 ± 89** | 1043 ± 57* | 1079 ± 58* |

BAL, bronchoalveolar lavage; tPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor 1.

contribute to septic mortality. Phosphoinositide-3 kinase gamma (PI3Kγ) plays a dominant role in the inflammatory response; however, its role in the pathogenesis of sepsis, specifically SIRS, lung/liver inflammation and damage, apoptosis, coagulation and mortality remains unknown. We hypothesized that mice lacking PI3Kγ or possessing a kinase-dead enzyme will be protected against septic-induced injury.

**Methods** PI3Kγ wild-type (WT), knockout (KO) and kinase dead (KD) mice were randomized to cecal ligation and perforation (CLP)-induced sepsis or a sham laporatomy. After 18 hours, plasma and bronchoalveolar lavage and/or lung and liver tissue were collected. Plasma was assessed for inflammatory mediators and the lung/liver was analysed for pathology score, leukocyte infiltration, inflammatory mediators, edema, apoptosis, coagulation and downstream intracellular signalling of PI3Kγ. A separate cohort of WT and KO mice were used for evaluation of 7-day survival following CLP.

**Results** Systemically, KO and KD mice showed a reduction in five of 22 measured cytokines/chemokines (MIP1a, MIP2, RANTES, MCP1 and IL-10) compared with WT controls. In the lungs, KO and KD mice were significantly protected against septic damage, as observed by decreased pathology scores, edema/permeability, leukocyte infiltration, inflammation (all 22 measured mediators), apoptosis and Akt/mitogen-activated protein kinase activation, compared with WT lungs. Similarly, livers of CLP-exposed KO and KD mice had decreased pathology scores, leukocyte infiltration, apoptosis and coagulation derangements compared with WT controls. Furthermore, Kaplan–Meier analysis of 7-day survival following CLP showed KO mice had significantly reduced mortality compared with WT mice. See Table 1.

**Conclusion** The present study demonstrates that while PI3Kγ has a modest effect on SIRS during sepsis, its kinase activity is pivotal to the successive development of coagulation derangement and lung/liver inflammation and damage, probably through the modification of leukocyte recruitment and apoptosis. Furthermore, PI3Kγ is shown to effect CLP-septic-induced mortality, implying that it may be a possible therapeutic target in sepsis and multiple organ failure.

## P40

## Relationship between protein C and antithrombin III deficiencies in sepsis without disseminated intravascular coagulation status

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**Background** Recently, a reciprocal relationship has been known between anti-inflammation and anticoagulation responses to infection. In our previous studies, protein C (PC) deficiency and antithrombin III (AT III) deficiency have been shown in septic patients. Moreover, these AT III deficiencies in sepsis did not relate to their disseminated intravascular coagulation (DIC) status. We hypothesize that PC activity relates to AT III activity in septic patients without DIC status.

**Materials** Fifty ICU patients were included in this study and divided into three groups by primary diagnosis on admission; trauma patients, nonseptic patients, and septic patients. The patients who had already DIC on admission were excluded.

**Methods** Serum PC activity (%) (Diagnostica Stago®, Tokyo, Japan) and serum AT III activity (%) (Sysmex®, Kobe, Japan) were measured on admission. PC and AT III activities were compared between three groups. Values are expressed as the median. Data were analyzed by the Kruskal–Wallis test and the Mann–Whitney U test. Pearson's correlation coefficient was used for correlation. $P<0.05$ was considered statistically significant.

**Results** There were 23 trauma patients, 12 nonseptic patients and 15 septic patients. PC activity was significantly lower in septic patients than in trauma or in nonseptic patients (54.6 versus 85.6,

94.0% respectively, $P = 0.0006$). AT III activity was also lower in septic patients than in other groups (54.2 versus 94.4, 81.2% respectively, $P < 0.001$). There were correlations of PC activity with AT III activity in trauma patients ($r = 0.76$, $P < 0.0001$) and in non-septic patients ($r = 0.61$, $P = 0.048$). Especially, in septic patients, PC activity had significant correlation with AT III activity ($r = 0.91$, $P < 0.0001$).

**Conclusion** Both PC deficiency and AT III deficiency had already been shown in septic patients on admission to the ICU, but nevertheless no DIC status. The relationship between PC activity and AT III activity was found in all patients and there could be a definite correlation in septic patients.

## P41

## ELISpot analysis of lipopolysaccharide-stimulated leukocytes: human granulocytes selectively secrete IL-8, MIP-1β and TNFα

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**Background** Granulocytes or polymorphonuclear cells (PMN) represent the majority of leukocytes in peripheral blood. As terminally differentiated cells, they contain few ribosomes and assist innate immunity mainly through phagocytosis and degranulation. Whether or not they can release proinflammatory cytokines such as TNFα and IL-1β has been a controversial issue. To clarify the role of PMN in this aspect, lipopolysaccharide (LPS)-induced cytokine secretion from PMN was analyzed at the single-cell level with the ELISpot technique.

**Methods** PMN and peripheral blood mononuclear cells (PBMC) from healthy human donors were prepared by gradient-based centrifugation to a purity >98%. ELISpot assays were used for detection of a large panel of inflammatory mediators. Cells were stimulated with endotoxin (LPS, 100 ng/ml) for 20 hours and the numbers of secreting cells were quantified with an ELISpot reader. For comparison, cytokine production was also analyzed by ELISA. In some experiments, PBMC were depleted of monocytes using anti-CD14 magnetic beads.

**Results** Purified PMN secreted IL-8 and MIP-1β and a subpopulation also released TNFα after LPS stimulation. In contrast and different from some earlier reports, we were unable to detect secretion of IL-1β, IL-12, granulocyte–macrophage colony-stimulating factor, IL-6 or IFNγ. Furthermore, granulocytes did not secrete the cytotoxic molecules perforin or granzyme B in response to LPS. Compared with the limited cytokine production by PMN, PBMC secreted significant amounts of all substances investigated and were found to require a 100x lower concentration of LPS than granulocytes to obtain the maximum number of responding cells. In addition, $CD14^+$ monocytes were found to be the primary source of production.

**Discussion** By use of the ELISpot method we could establish the cytokine profiles for both PBMC and PMN based on the frequency and pattern of cytokine secreting cells, rather than the amount of produced cytokine as by ELISA. This way, low levels of contaminating monocytes present in our PMN preparations could be discriminated from the granulocytes. Additionally, we could demonstrate that ELISpot, compared with ELISA, not only provides a more sensitive means of detection but potentially gives biologically more relevant information.

**Conclusion** LPS-stimulated PMN were shown to secrete IL-8, MIP-1β and TNFα but not IL-1β, IL-6, IL-10, IL-12, granulocyte–macrophage colony-stimulating factor, IFNγ, perforin or granzyme B. Our findings suggest that the ELISpot assay may be a suitable tool in further studies of cellular signaling.

## P42

## Prognostic factors of severe sepsis: a result of Korean sepsis registry system

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*Critical Care* 2008, **12(Suppl 5):**P42 (doi: 10.1186/cc7075)

**Background** Severe sepsis is a highly fatal condition, but the prognostic factors of severe sepsis are not yet fully understood.

**Materials** One thousand and twenty-six severe sepsis patients (community-acquired infection only) were registered in the Korean Sepsis Registry System from May 2005 to November 2007. The Korean Sepsis Registry System is a web-based ongoing prospective data collection system from 12 tertiary referral hospitals in Korea.

**Methods** The Acute Physiology and Chronic Health Evaluation II score, Serial Organ Failure Assessment (SOFA) at admission and serial 1 to 4 days after admission, demographic characteristics, comorbidity conditions with the Charlson score, Glasgow coma scale, organ dysfunction index, infection site, organism, and laboratory data at admission of 1,026 severe sepsis patients were analysed and evaluated to determine the association with 7-day mortality respectively. To develop a prognostic model, decision tree analysis was carried out with SAS 9.1.

**Results** The 7-day mortality rate was 13.6/100 patients. Age was an independent risk factor, but the highest mortality (25.3%) was seen in the 60 to 69 years age group. The greater the number of organ dysfunctions, the higher the mortality. The underlying conditions were not statistically significant as a risk factor of 7-day mortality except liver diseases ($P = 0.0015$). The blood pressure, Charlson score, Acute Physiology and Chronic Health Evaluation II score and SOFA score at admission were all significantly associated with mortality. The initial laboratory values of hemoglobin, white blood cells, platelets, fibrinogen, prothrombin time, partial prothrombin time, arterial pH, potassium and albumin at admission were also statistically significant in bivariate analysis. Systemic infection and central nervous system infection showed 26.7% and 25.0% 7-day mortality. In a prognostic model by decision tree analysis, the blood coagulation factors (prothrombin time, platelet) and SOFA at 5 days after admission were the most significant prognostic factors of 7-day mortality. The sensitivity and specificity of this model were 67.5% and 96.8%, respectively.

**Conclusion** The blood coagulation factors and SOFA were the most significant prognostic factors of 7-day mortality.

**P43**

## Nucleic acid amplification-based pathogen detection in the blood of severe sepsis patients

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**Background** Blood culture is an important method for identifying the underlying microorganism causative of sepsis. However, appropriate antibiotic therapy may possibly be delayed due to the long time to results for culture-based methods. Nucleic acid amplification (NAT) of microbial DNA may significantly shorten the time to pathogen detection but clinical data for this technique are missing. The goal of the present study was to compare a new NAT protocol with blood culture results in critically ill patients with evidence of infection.
**Methods** Patients either with severe sepsis (sepsis group) or systemic inflammatory response syndrome without evidence of infection (control group) were included. A blood culture (BC) was obtained and 10 ml ethylenediamine tetraacetic acid blood was simultaneously taken by sterile venous puncture. Microbial DNA was measured using NAT-based pathogen detection with multiplex PCR testing for 45 targets (VYOO®; SIRS-Lab, Jena, Germany).
**Results** Thirty-six samples from 24 septic patients (age 66.0 ± 3.4 years) and 32 samples from 22 control patients (age 64.6 ± 5.1 years) were obtained. The PCRs of all control patients were negative while five BCs from the control group (15.6%, $P = 0.06$) were positive. In sepsis patients, five BCs (13.4%) tested positive compared with 14 positive PCRs (38.9%, $P = 0.03$). Median procalcitonin levels were higher in PCR-positive tested sepsis patients (7.0 ng/ml; interquartile range, 1.5 to 14.5) compared with patients with negative PCR (1.8 ng/ml; interquartile range, 1.0 to 6.2). However, the difference did not reach statistical significance ($P = 0.15$). No similar correlation between procalcitonin and findings of BC were observed. Procalcitonin for negative BCs was 2.4 ng/ml (1.4 to 7.6), and was 2.1 ng/ml (0.4 to 9.2, $P = 0.35$) for positive BCs.
**Conclusion** Multiplex PCR detected pathogens significantly more often than the concomitant BC, while there was no rate of false positive results in patients without evidence of infection. Positive PCRs were associated with higher serum procalcitonin levels, thus suggesting the clinical importance of a positive PCR result. Larger sample sizes are needed to confirm these observations.

**P44**

## HMGB1 is an early mediator of inflammation and may be negatively regulated by soluble receptor for advanced glycation end products in experimental endotoxinemia and in sepsis

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**Background** Cell membrane-bound receptor for advanced glycation end products (RAGE) is a major receptor for HMGB1. RAGE also circulates in soluble form – sRAGE, which binds circulating HMGB1 and inhibits its proinflammatory actions.
**Materials** HMGB1, sRAGE, IL-6, IL-10 and TNFα levels were studied over 5 hours in 16 healthy volunteers exposed to endotoxin, and HMGB1 and sRAGE over 4 days in 45 patients with a diagnosis of severe sepsis or septic shock.
**Methods** HMGB1 and sRAGE were measured using ELISA.
**Results** Eleven out of 16 volunteers had measurable levels of HMGB1 at baseline, before endotoxin injection. Within 2 hours of endotoxin injection, parallel to the rise of IL-6, IL-10 and TNFα, HMGB1 increased more than fourfold in 12/16 volunteers (from baseline 0.52 (0 to 1.05) to 2.3 (1.63 to 4.42) (median, interquartile range)). In four volunteers, levels diminished after endotoxin was injected. sRAGE increased in all volunteers during the studied period. In 11 out of 16 volunteers, HMGB1 and sRAGE were released in patterns that seemed to be inversely related, suggesting mutual negative regulation. We went on to study release patterns of HMGB1 and sRAGE at days 0, 1 and 4 in patients with severe sepsis or septic shock. In 36/45 patients, the increase or decrease of HMGB1, between at least two time points, was opposite to the change in sRAGE levels, again suggesting mechanisms of negative regulation between the two.
**Conclusion** (1) sRAGE and circulating HMGB1 have release patterns that suggest mutual negative regulation. Further studies are needed to evaluate sRAGE as a therapeutic means for reducing harmful HMGB1 levels in various inflammatory conditions. (2) HMGB1 is not only a late mediator of inflammation. It increased fourfold within 2 hours of endotoxin injection in healthy volunteers, parallel to the rise of IL-6, IL-10 and TNFα. (3) HMGB1 circulates at measurable levels in healthy individuals.

**P45**

## Toll-like receptor pathway signaling is differently regulated in neurophils and peripheral mononuclear cells of patients with sepsis, severe sepsis and septic shock

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**Background** Upregulation and downregulation of inflammatory response was described in blood cells from septic patients, according to the stage of sepsis and the cells evaluated. This study aimed to evaluate the Toll-like receptor (TLR) signaling pathway gene expression in peripheral blood mononuclear cells (PBMC) and neutrophils in patients throughout the different stages of sepsis.
**Methods** Septic patients admitted to two emergency rooms and two ICUs in one university and one teaching hospital were enrolled in the study, including five with sepsis, five with severe sepsis and five with septic shock. Five healthy volunteers were enrolled as controls. The Human-TLR Signaling Pathway, which comprises 84 genes related to TLR-mediated signal transduction, was evaluated by real-time PCR in PBMC and neutrophils obtained from patients and controls. Results were expressed as CT and were normalized with the housekeeping gene 18SrRNA (ΔCT). The fold change for each gene ($2^{-\Delta\Delta CT}$) was compared between the groups. Genes with fold changes greater than two and significant changes in ΔCT are reported as differently expressed.
**Results** The fold change ratios in PBMC gene expression between septic patients and healthy controls revealed a dynamic process

according to the stage of sepsis, tending towards downregulation of the TLR signaling pathway in PBMC in the more severe forms of the disease. In patients with sepsis and severe sepsis, fold-change analyses showed upregulated genes mostly in TLR receptors and adaptor or TLR interacting protein groups. The downregulated genes consisted mostly of downstream pathways and target genes, and they included the NFκB, JNK/p38 pathway, and effectors. However, the differential gene expression was restricted to five downregulated genes in septic shock patients, which are found in the effector and downstream pathways. Neutrophils showed a different pattern of adaptation. Patients with sepsis, severe sepsis and septic shock presented a broad gene upregulation, which included all functional groups evaluated and persisted throughout the stages of the disease.
**Conclusion** TLR-signaling pathway genes are differently regulated in PBMC and neutrophils of septic patients, and are dynamically modulated throughout the different stages of sepsis.

## P46

### Downregulation of IL-6 and preserved reactive oxygen species production in human monocytes tolerized by lipopolysaccharide and challenged with Toll-like receptor agonists and whole Gram-negative and Gram-positive bacteria

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**Background** Tolerance to lipopolysaccharide (LPS) occurs when animals or cells exposed to LPS become hyporesponsive to a subsequent challenge of LPS. This mechanism is believed to be involved in the downregulation of cellular responses observed in patients with severe sepsis and septic shock. The aim of the present investigation was to evaluate the induction of tolerance in monocytes of healthy volunteers, in whole blood, after LPS exposition *in vitro*, assessed by intracellular cytokine detection and reactive oxygen species (ROS) generation.
**Methods** Peripheral blood cells were conditioned with small doses of LPS for 18 hours and challenged with different agonists of Toll-like receptors (macrophage-activating lipopeptide-2, flagellin and LPS) and whole Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) killed bacteria. For detection of intracellular IL-6, samples of whole blood were stimulated for 6 hours. Monocytes were identified by forward-scatter and side-scatter parameters and CD14-positive staining. The samples were stained to verify the intracellular production of IL-6 on monocytes by flow cytometry. For induction of ROS, whole blood was stimulated for 30 minutes with LPS, *P. aeruginosa* and *S. aureus*. ROS production was measured by flow cytometry, using 2′,7′-dichlorfluorescein-diacetate detection.
**Results** The conditioning with increasing doses of LPS resulted in lower intracellular detection of IL-6 in monocytes after the challenge with LPS. A similar effect was observed with macrophage-activating lipopeptide-2, *P. aeruginosa*, and *S. aureus*, but not with flagellin. LPS conditioning with 15 ng/ml LPS, on the other hand, resulted in preserved or increased production of ROS in monocytes after challenge with LPS, *P. aeruginosa* and *S. aureus*.
**Conclusion** The phenomenon of tolerance involves a complex regulation, in which the production of proinflammatory cytokines, such as IL-6, is diminished, whereas the production of ROS is preserved or even increased.

## P47

### Validation of polymerase chain reaction in experimental sepsis diagnosis beyond blood culture

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**Background** A positive blood culture (BC) is considered the gold-standard method for 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, bacteria can remain dead or fragmented in the circulation, thus limiting BC efficiency. Herein we evaluated the PCR diagnostic efficacy under live, dead and fragmented bacteria contents in the bloodstream.
**Methods** Wistar rats were distributed into three groups ($n = 20$/group) based on live, dead and DNA inoculations. Another lipopolysaccharide (LPS) + DNA group (1 mg/kg LPS injection plus 4 hours later DNA injection, $n = 10$) was designed for DNA detection under an induced inflammatory state. Live, dead and extracted DNA forms of *Pseudomonas aeruginosa* (ATCC 27853), $10^7$ colony-forming units/ml/100 g body weight, were injected into the tail vein of respective groups. Blood samples were collected after 20 minutes ($n = 10$) and 6 hours ($n = 10$) from all groups except for the LPS + DNA group (6 hours), and were submitted to a nested PCR assay using general and specific primers. BC was performed only in the live group.
**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% to BC and 80% to PCR. In the dead group, the PCR sensitivity was 90% at 20 minutes and 50% at 6 hours. In the DNA group, the sensitivity was 90% at 20 minutes and 40% at 6 hours, and in the LPS + DNA group at 6 hours the sensitivity was 40%.
**Conclusion** The sensitivity of the PCR was as effective as BC in 20 minutes and superior in 6 hours. Besides, the PCR assay was able to detect circulating dead bacteria and bacterial DNA in the blood, which is not possible by the BC method. These findings suggest that the live bacteria remains for a short period of time in the bloodstream as compared with dead and DNA bacteria, and a systemic inflammation state seems to not interfere with the PCR assay. Besides, the PCR tool with specific primers can be a useful method for sepsis diagnosis in the negative blood culture conditions as well as in specific bacterial surge events in the ICU, thus improving the antibiotic usage potentials.

## P48

### A novel multiplex quantitative polymerase chain reaction assay for the early prediction of sepsis in critically ill patients presenting signs of shock and organ dysfunction

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**Background** Clinical signs of sepsis often overlap with other symptoms present in patients after trauma, surgery and chemotherapy, amongst others. The clinical utility of existing biomarkers to detect sepsis, such as procalcitonin (PCT) and C-reactive

protein (CRP), is limited in patients suffering from shock and organ dysfunction. As a result, tests with superior positive and negative predictive values are mandatory in life-threatening infection.

**Methods** We have analyzed the clinical utility of a new class of transcriptomic biomarkers derived from circulating leukocytes. Prospectively collected whole blood samples from 460 patients admitted to the operative ICU of the University Hospital Jena were used in a microarray/quantitative PCR study to identify sensitive and specific biomarkers. Microarrays comprising 5,308 probes corresponding to 3,704 human genes relevant to inflammation, immune response and related processes were used for analysis. The identification of a signature specific for the discrimination between systemic inflammatory response syndrome and sepsis in patients suffering from shock and organ dysfunction was performed in independent training and test phases. The training set of 96 patients was selected by an independent ICU committee. An algorithm was established combining and transforming the gene-expression signals into a continuous, nondimensional score indicating either infectious or noninfectious causes for organ dysfunction. The resulting classificator was validated in a test set comprising 1,784 ICU days of 364 patients. For each marker, a robust quantitative PCR assay was established.

**Results** The final microarray signature could be transferred into a multiplex quantitative PCR format retaining full sensitivity and specificity with a time to result of approximately 5 hours. Moreover, it could be demonstrated that the combination of seven biomarkers possesses the same accuracy compared with the complete biomarker set. The area under the curve in the test group was determined as 0.79 (PCT – 0.65, CRP – 0.67). Moreover, the quantitative PCR assay determined the onset of sepsis up to 48 hours prior to the clinical diagnosis backed by daily CRP and PCT testing.

**Conclusion** With its high predictive value for the differentiation between infectious and noninfectious causes of shock and organ dysfunction, this new class of biomarkers may help to identify patients with life-threatening infections among patients at risk and to guide therapy (for example, with anti-infective agents).

## P49

## A comparative study between conventional and antimicrobial-filled central venous catheters

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**Background** Central venous catheters (CVCs) are very useful in the management of patients hospitalized in the ICU. Among the complications related to the permanence of CVCs, infection stands out. This may increase the morbidity and mortality, cost and length of stay in the ICU. A comparative study between antiseptic-impregnated and standard catheters is therefore of great value.

**Objective** To compare the duration of standard CVCs with those that are antiseptic-impregnated: silver sulfadiazine and chlorhexidine.

**Methods** A prospective study was performed, in a randomized, alternate, and nonblind fashion. The measure of Acute Physiology and Chronic Health Evaluation II score and central venous access were made alternating the type of CVC used in each patient, so the study was randomized. The information recorded on each patient was sex, Glasgow coma score, site of the puncture, reason for withdrawal of the catheter and the type of catheter used. We cultured (qualitatively) the tip of the catheter. The patients were divided into two groups: group I (36 patients, 47 punctures) used the standard CVC, and group II (33 patients, 47 punctures) used the impregnated CVC.

**Results** Length of duration: group II = 14.2 days, group I = 10.2 days. Excluding death in both groups, length of stay: group I = 10.4 days, group II = 15.8 days. Adding all periods of catheterization for each group: group I = 483 days, group II = 670 days. The total duration of group II was 38.71% higher than that of group I. Regarding the reason for withdrawal of the CVC, predominant was suspected infection in 74.6% of standard CVCs and in 48.9% of impregnated CVCs. The culture of the catheter tip was positive on 10 occasions (21.2%) in standard CVCs, against six occasions (12.7%) in impregnated CVCs. Most patients had Glasgow coma score <9. Average Acute Physiology and Chronic Health Evaluation II score: 17.8 in group I, 20.2 in group II. The predominant site of puncture was the subclavian vein (56.3%), and the catheters remained much of the time on this site when compared with the other sites used (jugular and femoral). But when we take only group II into consideration, the catheters located in the jugular vein remained longer.

**Conclusion** The length of stay with the use of impregnated CVCs was higher (15.8 days) than that for the standard CVCs (10.4 days). The rate of infection was higher in the standard CVCs. Patients who require CVCs for long periods have benefited with the use of impregnated CVCs, because they present long-term use, lower rates of infection, and avoidance of successive punctures and risks of the procedure. In view of the clinical benefits already mentioned, the benefit reached by the use of antiseptic-impregnated catheters compensated the initial extra expensive cost of 40%.