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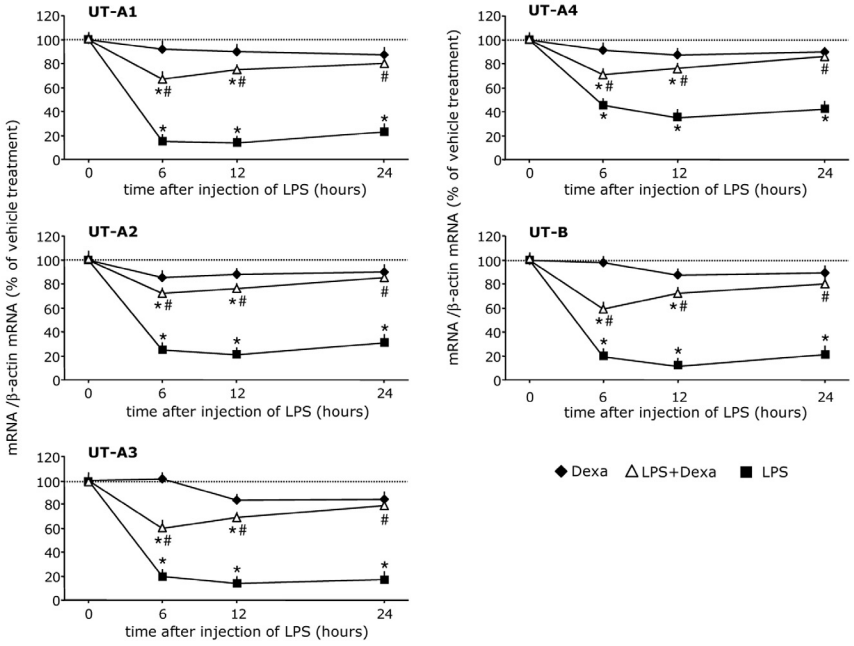
P1

Cytokine-mediated regulation of renal urea transporters during sepsis

Christoph Schmidt, Klaus Hoecherl, Michael Bucher
Anaesthesiology Department, Hospital of the University of Regensburg, Regensburg, Germany
Critical Care 2007, **11(Suppl 4):**P1 (doi: 10.1186/cc5980)

Background The pathogenesis of endotoxemic tubular dysfunction with failure in urine concentration is poorly understood. Urea plays an important role in the urinary concentrating mechanism and expression of the urea transporters UT-A1, UT-A2, UT-A3, UT-A4 and UT-B is essential for tubular urea reabsorption. The present study attempts to assess the regulation of renal urea transporters during severe inflammation *in vivo*.
Materials and methods By agreement of the animal protection committee C57BL/6J, mice were injected with lipopolysaccharides (LPS, 10 mg/kg) or proinflammatory cytokines. Hemodynamic, renal parameters and the expression of renal urea transporters were investigated. To clarify the role of cytokines and renal ischemia in the regulation of renal urea transporters, experiments were performed with cytokine knockout mice, mice treated with low-dose LPS (1, 5 mg/kg) as a sepsis model without induction of hypotension, glucocorticoid-treated mice, and mice with renal artery clipping serving as a model for renal ischemia.
Results and discussion LPS-injected mice (10 mg/kg) presented with reduced glomerular filtration rate, fractional urea excretion and inner medulla osmolality associated with a marked decrease in expression of UT-A1, UT-A2, UT-A3, UT-A4 and UT-B (Figure 1). Similar alterations were observed after application of TNFα, IL-1β, IFNγ or IL-6. LPS-induced downregulation of urea transporters was not affected in knockout mice with deficient TNFα, IL-receptor-1, IFNγ or IL-6. Glucocorticoid treatment inhibited LPS-induced increases of tissue TNFα, IL-1β, IFNγ or IL-6 concentration, diminished LPS-induced renal dysfunction and attenuated the downregulation of renal urea transporters. Injection of low-dose LPS (1, 5 mg/kg) also led to renal dysfunction paralleled by a downregulation of renal urea transporters without alterations in blood pressure. Renal ischemia induced by renal artery clipping did not influence the expression of urea transporters.
Conclusion Our findings demonstrate downregulation of renal urea transporters that probably accounts for tubular dysfunction during sepsis. Furthermore, they suggest that downregulation of

Figure 1 (abstract P1)



Effect of lipopolysaccharide (LPS) (10 mg/kg), dexamethasone (10 mg/kg) and the combination of both on UT-A1, UT-A2, UT-A3, UT-A4 and UT-B mRNA in the kidney 6, 12 and 24 hours after intraperitoneal injection. Values are related to signals obtained for β-actin mRNA and presented as the percentage of vehicle control. Mean ± SEM of six animals per group. $^{*}P < 0.05$ versus control, $^{\#}P < 0.05$ versus LPS treatment.

renal urea transporters during LPS-induced acute renal failure is mediated by proinflammatory cytokines and is independent from renal ischemia due to sepsis-induced hypotension.

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P2

The role of regulatory T cells in the resistance of CCR4 knockout mice during severe sepsis

Raphael Molinaro[1], Alessandra Monteiro de França[1], Josi Alves-Filho[2], Fernando de Queiroz Cunha[2], Marcelo Bozza[3], Steven Kunkel[4], Claudia Benjamim[1]
[1]Department of Pharmacology, UFRJ, Rio de Janeiro, RJ, Brazil; [2]Department of Pharmacology, USP, Ribeirão Preto, SP, Brazil; [3]Microbiology Institute, UFRJ, Rio de Janeiro, RJ, Brazil; [4]Department of Pathology, UMMS, Ann Arbor, MI, USA
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Background Studies reveal that regulatory T (T_{reg}) cells control immune responses; therefore these responses must be controlled to enable effective protection against infections and cancer. CCR4 knockout ($CCR4^{-/-}$) mice are more resistant to lipopolysaccharide shock. So, our aim is to study the mechanisms involved in the resistance of $CCR4^{-/-}$ mice subjected to severe sepsis by cecal ligation and puncture (CLP) and how T_{reg} cells modulate this effect.
Methods C57/BL6 mice were subjected to a CLP model, whereby the cecum was partially ligated and punctured nine times with a 21 G needle. Sham-operated mice were used as control. Mice subjected to CLP and sham surgery were treated with antibiotic from 6 hours after surgery until 3 days.
Results $CCR4^{-/-}$ mice subjected to CLP presented an increase in the survival rate (78%) compared with wild-type mice (17%), and presented a marked improvement in the innate response concerning neutrophil migration to the peritoneum and lung, bacterial load and cytokine levels compared with wild-type mice. Besides, T_{reg} cells from $CCR4^{-/-}$ CLP mice did not inhibit proliferation of T effector cells as observed for T_{reg} cells from wild CLP mice, at a proportional ratio of T effector:T_{reg} cells. Interesting, T_{reg} cells from $CCR4^{-/-}$ CLP mice inhibit 30% of neutrophil migration to bronchoalveolar lavage when co-injected with fungal challenge as secondary infection in sham recipient mice, while the T_{reg} cells from wild CLP mice inhibit 80%, much more than expected.
Conclusion These results suggest that T_{reg} cells from $CCR4^{-/-}$ mice did not present a suppressive response and this could be an important factor in their survival. These results are strong evidence for the role of T_{reg} cells in immunosuppression following severe sepsis.

P3

Abdominal sepsis: efficacy of passive immunotherapy

Ruslan Knut, Peter Fomin, Oleg Sydorchuk, Ruslan Sydorchuk, Olexii Kolomoiets, Larysa Sydorchuk, Igor Sydorchuk, Mohhamad Daraghmeh
General Surgery Department, Bukovina State Medical University, Ukraine
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Background Owing to immune disorders playing a key role in development of systemic inflammatory response syndrome, passive immune therapy is considered a method of choice for abdominal sepsis (AS) patients. Existing remedies (specific hyperimmune serum, specific antibodies and immunoglobulins) are expensive and require exact validation of pathogens. The aim of the study was to evaluate the efficacy of using the AS reconvalescent donor plasma for passive immunotherapy of AS.
Materials and methods The study was conducted experimentally on 775 Wistar line rats and 38 inbreed dogs. A total of 296 patients with AS were also involved in the study; 58 formed the control group; 26 patients were selected as reconvalescent donors of plasma. Serum concentrations (ELISA) of major antibodies were determined against most significant pathogens (*Escherichia coli*, *Staphylococcus* spp., *Staphylococcus aureus*, *Bacteroides* spp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*).
Results Changes of serum antibody concentrations were time dependent and fluctuating during the current of AS forming the waveform curve. Most remarkable decreases were found during 24–72 hours of AS. Serum antibody titers to the main pathogens were slightly higher due to antibiotics and detoxification therapy. Operation by itself decreased titers from 4.42 ± 0.28 to 3.49 ± 0.25 (*E. coli*), and from 5.41 ± 1.02 to 3.0 ± 0.58 (*P. aeruginosa*). Antistaphylococcal antibody titers decreased from 7.22 ± 0.9 before surgery to 4.83 ± 0.47 after. Repeated operations alter antibody concentrations even more significantly. The highest levels of antibodies were found in patients who underwent successful treatment of AS 1–2 months prior to investigation. Their plasma was used in treatment of AS patients. Intravenous administration of two-dose 100–200 ml hyperimmune plasma per day prevented a following decrease of antibody levels, and in 98% of cases increased them (21.39 ± 1.47%). The cost of treatment was 15–37% lower if compared with traditional methods (control group).
Conclusion There is exact evidence of efficacy for using hyperimmune plasma in patients with abdominal sepsis; it is more cost-effective if compared with traditional methods of immunotherapy.

P4

The modified light chain of inter-alpha inhibitor/antibody fusion protein, MR1007, improves survival in the rabbit sepsis models

Masaki Nakamura, Takashi Takeuchi, Yukiko Yatagai, Katsuki Naito, Kamon Shirakawa, Yoshitaka Hosaka, Shoji Furusako
Discovery Research, Mochida Pharmaceutical Co., Ltd, Gotemba, Japan
Critical Care 2007, **11(Suppl 4):**P4 (doi: 10.1186/cc5983)

Background Inter-alpha inhibitor is an endogenous serine protease inhibitor and is markedly reduced in severe sepsis. Therapeutic inter-alpha inhibitor replacement showed a survival advantage in several animal models. The light chain is responsible for the serine protease inhibitory activity of inter-alpha inhibitor. Since procoagulant and proinflammatory proteases as well as innate immune cells are activated in sepsis, we genetically engineered a novel fusion protein, MR1007, which consists of the modified light chain of inter-alpha inhibitor and the anti-CD14 antibody, and evaluated the potential of MR1007 as an anti-sepsis agent.
Methods Inhibitory activity against serine proteases was assayed using purified enzymes and chromogenic substrates. Anticoagulant activity was measured using human or rabbit plasma. The inhibitory effect on endothelial cell injury was assessed using human umbilical vein endothelial cells. Binding to CD14 and leukocytes was analysed using Biacore or radiolabeling. The survival benefit was evaluated in the endotoxin shock model and the cecal ligation and puncture (CLP) model.
Results MR1007 inhibited the thromboplastin-induced thrombin generation by inhibiting activities of coagulation factors Xa and XIa at 10–100 μg/ml. It also prevented the contact pathway generation of bradykinin at 10–30 μg/ml. Additionally, it inhibited the

leukocyte elastase-induced endothelial cell injury at 10–100 μg/ml. MR1007 had a high affinity for CD14 and bound to leukocytes, but did not block lipopolysaccharide binding to CD14. In the rabbit endotoxin shock model, MR1007 (3 mg/kg, i.v.) even when given 8 hours after the injection of endotoxin improved the survival ($n = 12$, $P < 0.05$), whereas both antithrombin and prednisolone exhibited less efficacy. Moreover, MR1007 (10 mg/kg, i.v.) given at 2 hours post-CLP improved the survival ($n = 9$, $P < 0.05$) in the CLP model.

Conclusions These results suggest that the modified light chain of inter-alpha inhibitor fusion protein, MR1007, can effectively suppress not only the serine protease-mediated coagulation, but also leukocyte-induced inflammation, so that MR1007 may become a promising anti-sepsis agent.

P5

Microbial metabolites in the blood of patients with sepsis

Anastasia Khodakova, Natalia Beloborodova
Bakulev Scientific Center for Cardiovascular Surgery, Moscow, Russia
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Background Molecular mechanisms of the pathophysiology of sepsis remain unknown. Preliminary results allow one to suppose that investigation of biological effects of microbial metabolites, particularly aromatic acids, is one of the most promising methods in elucidation of the problem. These compounds can be produced during microbial fermentation of aromatic amino acids. But little is known of which microorganisms participate in such processes. The aim was to assess the comparative level of aromatic acids in serum of cardiosurgical patients with documentary sepsis and to clarify the *in vitro* metabolic profile of aromatic acids in spent growth medium of main clinical blood isolates.

Materials and methods Serum samples were collected from 83 adult subjects (mean age 52 (42–58) years). The main group of investigation consisted of 12 cardiosurgical patients with documentary sepsis, with mortality of 42% (5/12). The comparison groups were: 16 clinically healthy volunteers, 36 patients with acquired heart diseases before surgery, nine patients with smooth recovery on the third day after surgery, 10 patients with pneumonia after surgery. The cultures ($n = 32$) of *S. aureus*, *S. epidermidis*, *E. faecalis*, *K. pneumonia*, *S. marcesceus*, *E. coli*, *E. cloacae*, *A. baumanii*, *P. aeruginosa*, *C. albicans* and *C. parapsilosis* were isolated from the blood of cardiosurgical patients and identified. Concentrations of aromatic acids were determined by gas chromatography–mass spectrometry. Data were compared by Mann–Whitney U-test, $P < 0.05$ considered significant.

Results Significant differences were observed among the groups (Table 1). 3-Phenylpropionic and 1-indolacetic acids were found to be prevalent in groups of healthy volunteers and patients before surgery. Increased levels of phenyllactic acid (PLA), *p*-hydroxyphenylacetic acid (HPAA), *p*-hydroxyphenyllactic acid (HPLA) and 3-indolacetic acid were revealed in the group of sepsis compared with other groups. Moreover, the highest concentrations of PLA, HPAA and HPLA were in serum of nonsurvivors ($n = 5$) compared with survivors ($n = 7$): PLA, 1,651 (656–1,959) versus 233 (122–360) ng/ml, $P = 0.02$; HPAA, 5,976 (2,689–6,667) versus 1,108 (461–2,121) ng/ml, $P = 0.02$; HPLA, 3,313 (2,409–6,098) versus 564 (446–718) ng/ml, $P = 0.005$. Gas chromatography–mass spectrometry analysis of spent growth medium showed that Gram-negative enterobacteria produced increased amounts of PLA and HPLA acids. Particularly, *K. pneumonia* had the highest level of acids PLA = 100 r.u. (r.u. – the ratio of substance content in sample to uninoculated media) and HPLA = 60 r.u., *E. coli* had PLA = 35 r.u. and HPLA = 20 r.u., and *S. marcesceus* and *E. cloacae* had PLA = 4 r.u. and HPLA = 6 r.u. The culture of Gram-positive cocci produced increased level of the same acids, for *S. aureus* PLA = 20 r.u. and HPLA = 17 r.u., and for *S. epidermidis* PLA = 6 r.u. and HPLA = 3 r.u., except for *E. faecalis*, which had the only PLA = 6 r.u. Gram-negative nonfermented bacteria produced increased levels of 1-indolacetic acid and 3-indolacetic acid, but no PLA and HPLA. The level of aromatic acids in the medium after cultivation of fungi was equal to control.

Conclusions Increased levels of PLA, HPAA and HPLA in serum patients with sepsis, especially with fatal outcome, are associated with development of infectious complications. These compounds are produced by clinically important bacteria, such as *K. pneumonia* > *E. coli* > *S. aureus* > *S. marcesceus*, *E. cloacae*, *S. epidermidis*, but not by fungi. The results can denote biological activity of these microbial metabolites and their influence on pathogenesis of sepsis.

P6

The impact of protocolized sepsis order set on the process of care in patients with severe sepsis/septic shock

Bekele Afessa, John Mullon, Andrew Badley and Ognjen Gajic
Mayo Clinic College of Medicine, Pulmonary and Critical Care Division, Rochester, MN, USA
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Background Based on the available evidence, professional societies have published practice guidelines on severe sepsis and septic shock. We started using a paper order set based on the guidelines in our medical intensive care unit (ICU) in October

Table 1 (abstract P5)

Concentration of aromatic acids in serum sepsis patients and subjects of comparison groups

Aromatic acid (ng/ml)	Patients with sepsis ($n = 12$)	Volunteers ($n = 16$)	Patients before surgery ($n = 36$)	Patients with smooth recovery ($n = 9$)	Patients with pneumonia ($n = 10$)
p-Hydroxyphenylacetic acid	2,140 (631–3,516)	72 (62–93)***	114 (53–220)***	263 (109–313)**	456 (344–667)*
3-Phenylpropionic acid	0	35 (20–54)**	4 (0–29)	0	0 (0–3)
Phenyllactic acid	367 (217–1,098)	47 (37–64)**	58 (37–93)**	89 (68–126)*	112 (89–177)*
p-Hydroxyphenyllactic acid	1,543 (564–2,731)	195 (159–371)***	254 (134–373)***	288 (269–772)*	465 (314–836)
1-Indolacetic acid	0	262 (113–385)***	47 (0–218)*	275 (228–499)***	0 (0–27)
3-Indolacetic acid	246 (183–628)	93 (56–141)*	181 (60–542)	57(30–74)*	231 (149–375)

Data presented as median (25th–75th percentile range). *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$, compared with sepsis patients.

2005. This prospective study aims to describe the impact of the order set on the process of care.

Materials and methods We included patients with severe sepsis/septic shock treated in our ICU between October 2005 and April 2007. We collected Acute Physiology and Chronic Health Evaluation (APACHE) III derived severity data, compliance with six elements of early goal-directed therapy and hospital mortality. Compliance with each element was defined as the use of the following within 6 hours of severe sepsis/septic shock: use of central venous pressure, central venous oxygen saturation measurement, adequate fluid resuscitation and appropriate use of vasopressors, inotropes and transfusion of red blood cells. The ICU admission severity of illness and sepsis stage (severe or shock) were entered in a logistic regression model to determine the independent impact of the order set on mortality. $P < 0.05$ was considered significant.

Results Of 561 patients (168 severe sepsis and 373 septic shock), 31 were excluded for not authorizing research. The order set was utilized in 328 (61.9%) of 530 patients. There were no significant differences in gender, age, race, and severity of illness at ICU admission between the order set and nonorder set groups. The order set was more likely to be used in patients with septic shock than in those with severe sepsis (67.3% versus 51.4%; $P = 0.0004$). Compliance with all six elements occurred in 130 (39.6%) of the order set group compared with 50 (24.8%) of the nonorder set group ($P = 0.0004$). Although mortality did not change, compliance with five of the six elements improved significantly with the order set. Logistic regression analysis showed that shock (odds ratio (OR) = 2.384, 95% confidence interval (CI) = 1.431–3.970; $P = 0.0008$) and predicted APACHE III mortality (%) (OR = 1.040, 95% CI = 1.031–1.050; $P < 0.0001$) were associated with mortality, not the order set (OR = 0.742, 95% CI = 0.476–1.157; $P = 0.1881$).

Conclusions This study showed that a protocolized order set improves compliance with the standard of care in patients with severe sepsis and septic shock. However, it did not resolve some of the noncompliance problems and did not improve survival.

P7

Impact of autologous centrifuged shed mediastinal blood on procalcitonin, C-reactive protein levels and postoperative complications during the early period following cardiac surgery

Judita Andrejaitiene[1], Audrone Veikutiene[2], Edmundas Širvinskas[1], Rimantas Benetis[3]

[1]Institute for Biomedical Research, Kaunas Medical University, Kaunas, Lithuania; [2]Kaunas University of Medicine Hospital, Department of Cardiothoracic and Vascular Surgery, Kaunas, Lithuania; [3]Kaunas University of Medicine, Institute for Cardiology, Kaunas, Lithuania

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Background Cardiac surgery with cardiopulmonary bypass (CPB) is associated with a number of adverse effects due to systemic inflammatory response syndrome, a physiologic reaction to tissue injury. Because of this response, conventional clinical and biological signs may be misleading in the diagnosis of postoperative complications, particularly infections. The aim of the study was to evaluate the impact of autologous centrifuged shed mediastinal blood procedures in attitude of infection, estimating the predictive role of procalcitonin (PCT) and C-reactive protein (CRP) changes during the postoperative period.

Materials We have analysed data on 90 patients, who had been subjected to cardiac surgical procedures on CPB: there are 41 patients in Group I, who were reinfused with the centrifuged autologous mediastinal blood 4 hours after the end of surgery; and 49 patients in Group II, whose shed mediastinal blood was not reinfused (control group).

Methods We studied the quantity of haemoglobin, haematocrit and leucocyte counts, and the value of CRP and PCT concentrations before the surgery (baseline), 4 and 20 hours after the end of surgery and during 5 days after surgery. Preoperative patient conditions, intraoperative and postoperative periods, were recorded. Statistical significance was accepted at a level of $P < 0.05$.

Results In Group I, patients who were reinfused with the centrifuged autologous shed mediastinal blood, requirement for the allogenic blood transfusion procedures was significantly lower (14.6% versus 38.8%, $P < 0.05$). The CRP concentration was greater, but there were no significant differences between the groups in all postoperative periods. At 20 hours after the end of surgery and the second postoperative day, the increase of the PCT concentration was significant and often observed in group II (33.3% versus 58.3%), where there were significantly more complications of infection (2.4% versus 10.2% $P < 0.05$) and a significantly longer length of postoperative hospital stay (9.32 ± 2.55 versus 14.38 ± 4.27 days, $P < 0.05$).

Conclusions Our data suggest that the early reinfusion of autologous centrifugated shed mediastinal blood procedures did not increase bleeding and statistically significantly reduced the requirement of allogenic blood transfusion procedures, reduced the number of infection complications, and significantly shortened the length of postoperative hospital stay. In evaluation of postoperative infection rates, PCT is highly suggestive as a marker of postoperative complications.

P8

System approach to the diagnosis and treatment of septic arthritis in newborns

Gennadiy Khanes[1], Svitlana Bidnenko[2], Volodymyr Grygorovskyy[3], Iryna Maksakova[1], Valeriy Romashko[4]

[1]Neonatal Surgery Department, Ukrainian Specialized Pediatric Hospital OkhMatDyt, Kyiv, Ukraine; [2]Bacteriologic Laboratory of Scientific Research Institute of Traumatology and Orthopedics of the Academy of Medical Sciences of Ukraine, Kyiv, Ukraine; [3]Morphologic Laboratory of Scientific Research Institute of Traumatology and Orthopedics of the Academy of Medical Sciences of Ukraine, Kyiv, Ukraine; [4]Immunologic Laboratory, Ukraine Specialized Pediatric Hospital OkhMatDyt, Kyiv, Ukraine

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Background Bone and joint sepsis occurs in 20% of newborns having perinatal sepsis. The feature of septic arthritis against the background of age-specific functional immunodeficiency gives us a reason to consider this decease as the manifestation of immunodeficiency. Septic arthritis in newborns is lethal in 10% of cases and gives orthopedic complications in 20% of cases. The starting moments of septic arthritis development are: mother's intrauterine infection and nosocomial infecting in maternity hospitals and intensive care departments, and purulent omphalitis.

Materials and methods We present the experience of diagnostics and treatment of 180 newborns aged 3 days and older having septic affection of the hip, knee, shoulder and other joints. The crucial role in diagnostics of septic arthritis is played by: cytology

of smear, ultrasonic examination of a joint, X-ray examination of a joint, bacteriological, serological, PCR and PCT-Q. The bacteriological monitoring in 30% of newborns among the pathogens showed CoMRSA, often mixed with fungi and *Pseudomonas aerogenes* or *Klebsiella pneumonia*, and in 10% there was PCR of *Toxoplasma gondi*, *Chlamidia trachomatis* or cytomegalovirus. Immunological monitoring allowed one to determine the patients who needed substitute therapy by immunoglobulin and a number of immunomodulators. The degree of infection process severity and the adequacy of antibacterial therapy are determined by serologic investigations, PCR and PCT-Q. The treating complex included joint lavage, antibiotics, anticytokine, antifungal agents, probiotics, and magnetic-laser therapy.
Results The lethality was reduced to zero, transition into the chronic form was up to 2.5%, and orthopedic complications were presented in 10% of cases.
Conclusion The after-history of treatment was researched and showed that when the diagnosis was made in less than 3 days, complications occurred in 3.3% of cases, and after 6–7 days the complications occurred in 22.4% of patients.

P9

Protease-activated receptor 2 blocking peptide counteracts endotoxin-induced inflammation and coagulation and ameliorates glomerular fibrin deposition in a rat model of acute renal failure

Satoshi Gando, Subrina Jesmin, Sohel Zaedi, Shamsul Haque Prodhan, Atsushi Sawamura, Takashi Miyauchi, Naoto Yamaguch
Division of Acute and Critical Care Medicine, Department of Anesthesiology and Critical Care Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan
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Background Glomerular and microvascular thrombosis due to the activation of the inflammation and coagulation pathway contribute to the occurrence of acute renal failure in sepsis. The protease-activated receptors (PARs) have been shown to play an important role in the interplay between the inflammation and coagulation.
Materials and methods We hypothesized that PAR2 blocking would improve glomerular and vascular thrombosis by attenuating the inflammation and coagulation, leading to the prevention of acute renal failure, and we assessed the effects of the PAR2 blocking peptide (PAR2 BP) in a rat model of lipopolysaccharide (LPS)-induced acute renal failure.
Results Levels of TNFα were significantly expressed 1 hour after LPS administration, followed by: (i) an increase in levels of tissue factor, factor VIIa, factor Xa, thrombin and plasminogen activator inhibitor-1; (ii) unchanged levels of tissue factor pathway inhibitor; and (iii) subsequent deposition of fibrin in kidney tissues, which led to the elevation of creatinine and blood urea nitrogen. Time-dependent PAR2 expression was observed at both the gene and protein levels. Immunoreactivities of PAR2 and fibrin were co-localized in the glomerulus and the other kidney tissues. PAR2 BP suppressed TNFα elevation, and attenuated activation of the coagulation, thus leading to a decrease in fibrin formation and its deposition in the glomerulus. However, the levels of creatinine and blood urea nitrogen remained unchanged.
Conclusions These results show that PAR2 plays a crucial role in the inflammatory and coagulation process of LPS-induced renal failure and may in part participate in the pathogenesis of the disease.

P10

Resistin in severe bacterial infections

Linda Johansson, Anna Linnér, Jonas Sundén-Cullberg, Carl-Johan Treutiger, Anna Norrby-Teglund
Center for Infectious Medicine, Institutionen för Medicin Huddinge, F59 Karolinska Institutet, Stockholm, Sweden
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Background Resistin has recently been recognized to act as a proinflammatory cytokine in humans. Patients with severe sepsis or septic shock had significantly elevated systemic levels of resistin, which correlated with severity of disease. Here we have further characterized the release of resistin during severe bacterial infections.
Materials and methods Acute phase sera collected from patients with septic shock caused by Gram-negative ($n = 19$) or Gram-positive ($n = 19$) bacteria were analyzed for resistin by ELISA. Tissue biopsies ($n = 12$) from patients with *Streptococcus pyogenes* severe soft tissue infections were stained for resistin and cell markers, and were analyzed by confocal microscopy. Human neutrophils were stimulated with lipopolysaccharide or streptococcal superantigens, and resistin was assessed in the supernatants.
Results Serum resistin levels were significantly elevated in patients with Gram-positive, as compared with Gram-negative, septic shock ($P = 0.004$). Analyses of tissue biopsies revealed that resistin was highly expressed at the local site of infection. Dual-staining for cell markers confirmed published findings that monocytes are a source of resistin in humans, but importantly the stainings revealed that the majority of resistin-producing cells were negative for the monocytic marker CD68. Further analyses identified these cells as neutrophils. A positive correlation between resistin levels and neutrophil counts was found in blood of septic shock patients ($P = 0.005$). *In vitro* cell cultures revealed resistin release by neutrophils stimulated with lipopolysaccharide or superantigens.
Conclusions This study demonstrates that the systemic resistin levels in septic shock differ depending on the causative microorganisms. The data also reveal that, at the local tissue site of infection, resistin is produced mainly by neutrophils, and systemic resistin strongly correlates with circulating levels of neutrophils. The systemic and local hyper-resistinemia noted is likely to contribute to the pathogenesis of acute invasive bacterial infections.

P11

Mortality rate reduction associated with severe sepsis and septic shock management protocol implementation

Constantino José Fernandes Junior, Alexandre Gonçalves de Sousa, Gisele de Paula Dias Santos, Eliezer Silva, Nelson Akamine
Hospital Israelita Albert Einstein Division of Medical Practice – Department of Protocols, São Paulo, Brazil
Critical Care 2007, **11(Suppl 4):**P11 (doi: 10.1186/cc5990)

Background The Surviving Sepsis Campaign is an international effort to reduce severe sepsis and septic shock associated mortality by 25% in 5 years. We developed a management protocol in our institution 2 years ago in order to follow the proposed recommendations of this campaign, and describe the clinical impact of assuming this critical pathway on the mortality rate.
Materials The study was conducted within the emergency department and intensive care unit of a tertiary hospital. A management protocol for severe sepsis and septic shock was based on the Surviving Sepsis Campaign guidelines and was implemented

Table 1 (abstract P11)

Proceedings concerning 'before' versus 'after' groups

Proceedings	'Before' group (%)	'After' group (%)	*P* value
Cultures obtained before antibiotics	68.1	84.3	0.01
Antibiotics in a due time (2-hour interval)	61.7	80.0	0.009
Corticosteroids	56.4	74.4	0.01
Activated protein C	1.0	12.2	0.002

by a 'sepsis' team including emergency department and critical care physicians, intensive care nurses and pharmacists, chaired by a full-time coordinator.

Methods We performed a 'before and after' evaluation of the critical pathway concerning 184 critically ill patients sequentially admitted throughout a 16-month period.

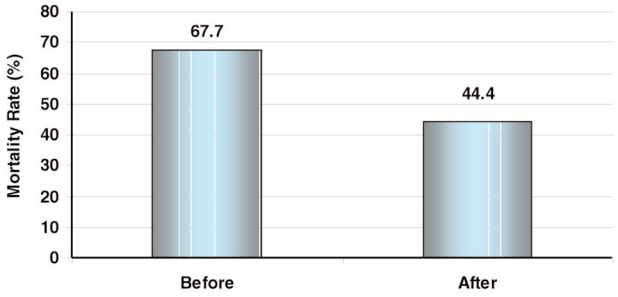
Results A total of 184 patients with severe sepsis or septic shock entered the study. Ninety-four patients had their analysis performed before the implementation of the standardized protocol (the 'before' group), and 90 patients were managed following the implementation of the protocol (the 'after' group). Basal demographic variables and the severity of illness score (APACHE II) were similar for both groups.

Patients in the 'after' group had statistically more cultures obtained before institution of antibiotics and more patients received antibiotics in a due time (2 hours from diagnosis). In addition, those patients received more corticosteroids and activated protein C (Table 1).

The intensive care unit length of stay and the hospital length of stay were similar in both groups. Remarkably the hospital mortality rate was significantly lower (34.4%) in the 'after' group in septic shock patients (67.7% versus 44.4%, $P < 0.04$) (Figure 1).

Conclusions The implementation of the Surviving Sepsis Campaign guidelines through a standardized protocol was associated with a 34% reduction in septic shock-related hospital mortality.

Figure 1 (abstract P11)



Mortality rate reduction in septic shock patients (42 deaths in 'before' group versus 32 deaths in 'after' group). $P < 0.04$.

P12

Neonatal sepsis due to multidrug-resistant *Klebsiella terrigena* in the neonatal intensive care unit in Gaza, Palestine

Farid Abu Elamreen
Medical Microbiology Department, AlShifa Hospital Central Laboratory & Blood Bank, Ministry of Health, Gaza, Palestine
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Background Bloodstream infection (BSI) is a major cause of morbidity and death encountered in the neonatal intensive care units (NICUs). The rates of BSIs vary significantly in NICUs across the nation. *Klebsiella* spp. led to serious concern about septicaemic neonates in NICUs due to high resistance against commonly used antimicrobial agents. The objective of the study was to report the prevalence and resistance pattern of *Klebsiella terrigena* isolated from cases of neonatal septicaemia at Alshifa Hospital, the largest tertiary hospital in Palestine.

Methods Blood taken from newborn babies admitted to the NICU at Alshifa Hospital, Gaza, Palestine with a clinical diagnosis of neonatal sepsis was cultured. A total of 355 positive blood cultures isolated from January to December 2005 were studied. Antimicrobial susceptibility was determined by disc diffusion method.

Results A total of 355 blood cultures positive were studied; the most common organism found were *Klebsiella* spp. 202/355 (56.9%), and 56/202 (25.5%) were *K. terrigena*. *K. terrigena* showed a high degree of resistance to commonly used antibiotics (Ampicillin, Piperacillin, Cephalexin, Cefuroxime, Cefaclor and Gentamicin), a moderate degree of resistance to Cefotaxime, Ceftazidim, Ceftriaxone and Amikacin, and most of the isolates were sensitive to Meropenem.

Conclusion Neonatal sepsis remains one of the leading causes of neonatal admission, morbidity, and mortality in developing countries. *Klebsiella* spp. were the major cause of neonatal sepsis in Gaza in 2005. The rare Klebsiella species (*K. terrigena*) have developed multidrug resistance, and management of patients infected with them is becoming a problem in developing countries. There is a need to carefully formulate therapeutic strategies to control infections in NICUs.

P13

The identification and use of common physiologic monitoring parameters in the care of critically ill patients at risk for sepsis

Karen Giuliano, Erica Cummings
Philips Medical Systems, Andover, MA, USA
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Background Sepsis is a common source of morbidity and mortality among critically ill patients. Targeting measures to reduce the incidence of and to promote early recognition and treatment of sepsis is at the forefront of many critical care initiatives. Advances in the management of severe sepsis have evolved over recent years in an attempt to combat the spiraling mortality trends. The Surviving Sepsis Campaign (SSC) is a worldwide initiative promoting the evidence-based treatment of sepsis, with the explicit goal of reducing both the morbidity and mortality associated with sepsis. This study was conducted to assess the clinical relevance of the early physiologic screening criteria advocated by early goal-directed therapy for sepsis, and the Surviving Sepsis Campaign guidelines.

Materials and methods The Project IMPACT® database was used to obtain a sample of patients ($n = 363$) with an ICU admission diagnosis of sepsis and a random acuity-matched comparison sample of patients with an admission diagnosis ($n = 364$) other than sepsis.

Results Significant group differences were found on all physiologic monitoring variables tested (high temperature, $P = 0.000$; low

temperature, $P = 0.001$; heart rate, $P = 0.004$; respiratory rate, $P = 0.005$; and mean arterial pressure, $P = 0.000$). In the logistic regression model, high temperature and mean arterial pressure functioned as significant predictors, with odds ratios of 2.12 for temperature at or above 38°C and 3.87 for MAP less than 70 mmHg. The odds ratio of having sepsis was 4.63 if both of these predictors were present.

Conclusions It is important to understand the value of common monitoring parameters in the early identification of sepsis, since those parameters are continuously monitored and readily available. It is the responsibility of bedside clinicians to assure that the parameters chosen for monitoring provide the most accurate reflection of the patient's clinical status. These results provide some support for the use of the currently recommended criteria for physiologic monitoring in the early identification of patients at risk for developing sepsis. Furthermore, if this could be done automatically, it would probably shorten the recognition time and thus speed up the initiation of sepsis treatment. *ProtocolWatch* is a tool that offers an electronic version of the SSC guidelines, screens the physiologic criteria automatically, and is resident on a bedside patient monitor. Development of tools such as *ProtocolWatch* will probably be an important adjunct to sepsis identification and treatment in the future.

P14

The role of CC-chemokine receptor 4 in murine polymicrobial sepsis

Stefan Maier[1], Tobias Traeger[1], Wolfram Keßler[1], Pia Körner[1], Hendrik Mehmcke[1], Yolande Chvatchko[2], Marlene Mikulczak[1], Tobias Ebker[1], Claus-Dieter Heidecke[1]

[1]Department of Surgery, Universität Greifswald, Germany; [2]Serono Pharma, Geneva, Switzerland

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Background Chemokines and chemokine receptors are crucially involved in the mechanisms leading to septic shock after severe systemic infections. The CC-chemokine receptor 4 (CCR4) is predominantly expressed on T cells driving the immune response in a Th2 direction. Interestingly, CCR4 knockout (KO) mice show no phenotype in allergy models. In contrast, they are highly protected in the lipopolysaccharide shock model. We analyzed the role of CCR4 in a murine model or polymicrobial sepsis, colon ascendens stent peritonitis (CASP).

Materials and methods In the CASP model, a stent is surgically inserted into the ascending colon of experimental mice. This leads to a persistent leakage of the gut with defined size and – depending on the stent size – to a lethal or sublethal polymicrobial sepsis. We performed 16 G CASP operations in CCR4 KO mice or wild-type (WT) controls. For *ex vivo* analysis, organ expression of CCR4 and its ligands, CCL17 and CCL21 were detected by real-time PCR. The bacterial loads of various organs were analyzed. Additionally, tissue cytokine levels were detected by cytometric bead array. Finally, adoptive transfer experiments from CCR4 KO mice to WT animals with or without CASP-induced peritonitis were performed.

Results Similarly to the lipopolysaccharide shock, CCR4 KO mice are protected in the CASP model. After sepsis induction, CR4 is massively downregulated, whereas expression of the ligands seems to be not severely affected. The absence of CCR4 signals improves bacterial clearance in the investigated organs. In organs of septic CCR4 KO mice significantly reduced IL-6 and monocyte chemoattractant protein-1 levels were found as compared with the WT controls. Astonishingly, adoptive transfer of CCR4 KO splenocytes from CASP mice in WT animals resulted in a strongly reduced susceptibility of these mice to the CASP procedure, whereas transfer of WT splenocytes did not affect the outcome.

Conclusion We report a significant role of CCR4 signals in a clinically relevant polymicrobial sepsis model.

P15

LL-37 at the local site of streptococcal skin and soft-tissue infections

Pontus Thulin[1], Linda Johansson[1], Parham Sendi[1], Erika Hertzén[1], Adam Linder[2], Per Åkesson[2], Bertil Christensson[2], Malak Kotb[3], Donald Low[4], Birgitta Agerberth[1], Anna Norrby-Teglund[1]

[1]Karolinska Institutet, Stockholm, Sweden; [2]Lund University, Lund, Sweden; [3]Veterans Affairs Medical Center, Memphis, TN, USA; [4]University of Toronto, Toronto, ON, Canada

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Background As part of the innate immune system, antimicrobial peptides (defensins and cathelicidins) are produced by both circulating and epithelial cells. Cathelicidins have been reported as an essential component against group A streptococcal (GAS) skin infections. However, bacterial factors such as streptococcal pyrogenic exotoxin B (SpeB) may inactivate these peptides. We have studied the interaction between GAS and the human cathelicidin LL-37, by use of patient tissue material.

Methods and materials Thirty-seven biopsies from 17 patients suffering from GAS skin and soft-tissue infection were obtained and graded according to disease severity (erysipelas, cellulitis, necrotizing fasciitis). Three additional biopsies served as negative controls. Tissue sections were immunostained for LL-37, GAS, SpeB and specific cell markers. Sections were investigated by light and confocal microscopy, and results were quantified by *in situ* imaging.

Results High expression of LL-37 was detected in erysipelas and severe soft-tissue infections, and showed a significant positive correlation to bacterial load ($P < 0.001$ and $P = 0.042$, respectively). Confocal microscopy identified neutrophils as the main source of LL-37 at the epicenter of infection, and the degree of neutrophil infiltration showed a significant positive correlation to LL-37 levels ($P < 0.001$). LL-37 and SpeB were detected in the same biopsy areas, and colocalization was confirmed by confocal microscopy.

Conclusions Despite the high expression of LL-37 in close proximity to streptococci at the local site of infection, there seems to be a significant lack of antimicrobial effect, as evident by the bacterial load. The colocalization of SpeB and LL-37 suggests that this streptococcal factor probably contributes significantly to a resistance mechanism towards antimicrobial peptides at the local tissue site.

P16

HMGB1 expression in streptococcal soft-tissue infections correlates with disease severity

Parham Sendi[1], Linda Johansson[1], Pontus Thulin[1], Adam Linder[2], Per Åkesson[2], Bertil Christensson[2], Donald Low[3], Anna Norrby-Teglund[1], Carl-Johan Treutiger[1]

[1]Karolinska Institutet, Stockholm, Sweden; [2]Lund University, Lund, Sweden; [3]University of Toronto, Toronto, ON, Canada

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Background High mobility group box-1 (HMGB1) is an intracellular protein that is secreted by activated immune cells during inflammation, or is passively released by cells undergoing necrosis. HMGB1 acts both as a proinflammatory cytokine and a chemokine,

and has been identified as a late mediator of sepsis. We have studied HMGB1 expression in streptococcal skin and soft-tissue infections, and its relation to bacterial load as well as to infiltration of inflammatory cells.

Materials and methods Thirty-seven biopsies from 17 patients suffering from streptococcal skin and soft-tissue infection were obtained and graded according to disease severity (erysipelas, cellulitis, necrotizing fasciitis). Three additional biopsies served as negative controls. Tissue sections were immunostained for HMGB1, group A streptococcus, and specific cell markers. Sections were investigated by light microscopy, and results were quantified by *in situ* imaging.

Results HMGB1 was found both intracellularly and secreted in the tissue. Its expression increased in parallel to disease severity and was significantly higher in necrotizing fasciitis than in erysipelas ($P = 0.023$). HMGB1 showed a positive correlation to neutrophils ($P < 0.01$) in erysipelas, but not in severe infections. A correlation to bacterial load was not found.

Conclusion In contrast to erysipelas, large amounts of necrotic tissue are present in severe skin infections, which probably contribute considerably to the expression of HMGB1. The high values may disturb a statistical correlation to the degree of inflammatory cell infiltration in the tissue. However, our results suggest that the massive HMGB1 expression at the local site of infection is probably an important mediator and enhancer of inflammation in skin tissue and soft-tissue infections, as evident by its expression in correlation to disease severity.

P17

Can procalcitonin reflect the etiology of the bacteremia?

Ekaterina Chernevskaya, Natalia Beloborodova, Tatyana Vostrikova
Bakoulev Scientific Center for Cardiovascular Surgery, Moscow, Russia
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Background An early diagnosis of bacteremia is crucial to facilitate adequate treatment of severe infections. We analyzed 5,564 blood cultures for a 3-year period (2004, 2005, 2006) with a 20% rate of positive blood culture, and observed the increasing prevalence of Gram-positive bacteremia (45%/48%/63%, respectively). Classical clinical inflammatory signs of Gram-negative and Gram-positive infection are often similar, while some biomarkers may help in early diagnostic of the nature of pathogen before obtaining the blood culture results. The objective of the study was to estimate the value of procalcitonin (PCT) as a discriminate marker of Gram-positive and Gram-negative infection in suspected bacteremia patients.

Materials and methods During 3 years monitoring of PCT and blood culture in a total of 150 episodes (113 cardiac patients with postoperative complication (systemic inflammatory response syndrome)) of positive blood culture with simultaneous PCT-test, results were registered and retrospectively analyzed. For blood culture we used the BacT/ALERT 3D system (bioMerieux, France) and the BBL CRYSTAL Identification Systems Enteric/Nonfermenter ID Kit (Becton Dickinson, USA). PCT concentrations were measured by immunoluminometric method (PCT LIA; B.R.A.H.M.S Aktiengesellschaft GmbH, Germany). The data were compared by Mann–Whitney U-test, and $P < 0.05$ was considered statistically significant. The data are expressed as the median and 25th and 75th percentiles.

Results In our study, 101/150 (67%) clinically important bacteremia were caused by Gram-negative bacteria and 49 (33%) by Gram-positive pathogens. The serum PCT concentration (median) was significantly higher in the group of Gram-negative bacteremia patients than in the group of patients with Gram-positive bacteremia (5.40 versus 0.86 ng/ml, $P < 0.001$) (Table 1). A PCT level > 2 ng/ml was reported in 72/101 (71%) cases in Gram-negative bacteremia patients, whereas in patients with Gram-positive bacteremia this level of PCT was reported twofold lower (16/49 (32.6%) cases). The analysis of mortality in patients with systemic infection (PCT > 2ng/ml + bacteremia) has shown comparable data in groups of patients with Gram-positive and Gram-negative bacteremia (8/14 (57.1%) and 31/55 (56.3%), respectively).

Conclusion A high PCT level in patients with suspected infection may be indicative of Gram-negative infection before obtaining the culture results.

P18

Control of hyperglycemia among septic and nonseptic patients in the general intensive care unit

Alexander Samokhvalov, Fabio Zveibil, Nicola Makhoul
General and Respiratory Intensive Care Units, Western Galilee Medical Center, Nahariya, Israel
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Background Hyperglycemia is common among patients admitted to intensive care units (ICUs) and carries risk for various complications, especially sepsis, and prolonged ICU stay. Some studies have demonstrated that intensive insulin control of blood glucose reduced morbidity and mortality. The objective was to determine whether intensive or conventional insulin control of blood glucose in hyperglycemic septic and nonseptic ICU patients is correlated with the prognosis.

Materials and methods Septic and nonseptic ICU patients with hyperglycemia were randomly assigned to a group treated intensively with insulin targeting glucose at 6.6–8.4 mmol/l or to a conventional insulin therapy group where glucose upon exceeding 12 mmol/l was controlled at 8.4–12 mmol/l. Rates of morbidity and mortality, hypoglycemic episodes and required insulin dosage were compared.

Results A total of 89 patients were enrolled, including 27 patients with sepsis: 11 patients were treated with insulin intensively with a

Table 1 (abstract P17)

Procalcitonin and clinical inflammatory signs in groups of patients with Gram-negative and Gram-positive bacteremia

	Gram-negative bacteremia patients	Gram-positive bacteremia patients	*P*
Number of cases	101	49	
Procalcitonin (ng/ml)	5.4 (1.78–12.21)	0.86 (0.28–2.19)	<0.001
White blood cell count (× 10^9/l)	15.4 (11.1–23.8)	14.2 (11–22)	Not significant
Temperature (°C)	37.5 (37–38)	37 (37–38)	Not significant
Multiple organ failure (number of patients (%))	16/80 (20%)	7/32 (21.8%)	Not significant

mean glucose of 8.3 mmol/l, while 16 patients received conventional insulin treatment with a mean of 10.3 mmol/l. Thirty nonseptic patients received intensive insulin treatment with a mean of 8.46 mmol/l and 32 nonseptic patients were treated conventionally with a mean of 10.4 mmol/l. Among septic patients, both groups were similar with respect to age and Acute Physiology and Chronic Health Evaluation scores. There was no significant difference between groups in the morbidity, including rates of new infection, renal and hepatic damage. There was a somewhat shorter ICU stay in the intensive treatment group. Both groups had similar ICU, inhospital and 28-day follow-up mortalities and similar rates of hypoglycemic episodes. The daily dosage of insulin was higher with the conventional treatment. Similar results were obtained among nonseptic patients between both groups, but septic patients had a longer total ICU stay and higher mortality.
Conclusions Intensive insulin control of blood glucose at 8.4 mmol/l does not affect the mortality or morbidity of septic and nonseptic patients in intensive care, except for a somewhat shorter ICU stay. An increased insulin dosage in the conventional treatment group was attributed to the group's higher initial blood glucose, probably due to a higher prevalence of diabetes and associated insulin resistance and toxicity hyperglycemia.

P19

Effects of vasopressin and terlipressin in ovine septic shock on mesenteric blood flow and survival

Sebastian Rehberg[1], Christian Ertmer[1], Matthias Lange[1], Andrea Morelli[2], Hugo Van Aken[1] Martin Westphal[1]
[1]Department of Anaesthesiology and Intensive Care, University of Münster, Münster, Germany; [2]Department of Anesthesiology and Intensive Care, University of Rome, 'La Sapienza', Rome, Italy
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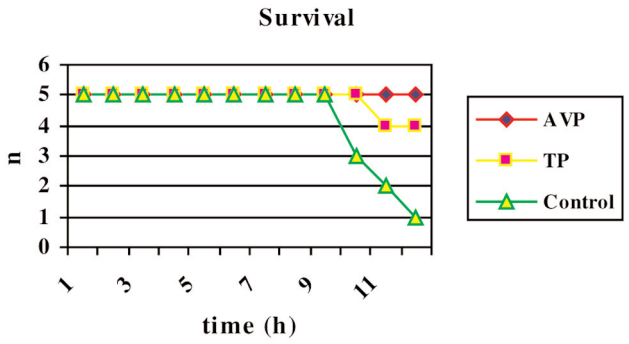
Background Vasopressin agonists, such as arginine vasopressin (AVP) and terlipressin (TP), are increasingly used to stabilize haemodynamics in catecholamine refractory hyperdynamic septic shock. However, it is still not fully understood if and how low-dose infusion of both drugs impacts on mesenteric blood flow (Qma) and outcome. The present study was conducted as a prospective, randomized, controlled laboratory experiment to compare the effects of AVP and TP on Qma and mortality in an established model of ovine septic shock.
Materials and methods Fifteen ewes were anaesthetized and instrumented for chronic haemodynamic monitoring. A median laparotomy was performed to place a flow-probe around the superior mesenteric artery and to take faeces from the caecum under sterile conditions. After the gut and abdomen had been closed and baseline measurements (BL1) taken, the faeces were injected into the peritoneal cavity. After the onset of septic shock (defined as mean arterial pressure (MAP) < 60 mmHg), a second set of measurements (BL2) was taken. The animals were then randomly assigned to receive either AVP (0.5 mU/kg/min) or TP (1 µg/kg/hour). The control group received only the vehicle (normal saline). Norepinephrine was titrated to maintain MAP at 70 ± 5 mmHg in all groups. Systemic haemodynamics, global oxygen transport including arterial lactate concentrations, gas exchange, electrolytes and Qma were determined at baseline and following every hour after the onset of septic shock. Animals surviving the 12-hour study period were deeply anaesthetized and killed by an overdose of saturated potassium solution. Mortality was analyzed by the Kaplan–Meier survival analysis. All the other variables were compared using two-way analysis of variance with appropriate post-hoc comparisons.
Results The Qma and electrolytes were similar between groups. However, systemic haemodynamics and global oxygen transport were stabilized more effectively in both treatment groups versus control animals. Notably, continuous infusion of AVP and TP significantly prolonged survival as compared with the control group ($P < 0.05$ each; Figure 1). There were no differences between the treatment groups.
Conclusions In this clinically relevant large animal model of septic shock, low-dose infusion of AVP and TP did not impair the Qma, but stabilized systemic haemodynamics and prolonged survival. Our data suggest that early infusion of AVP or TP may be beneficial in catecholamine-refractory septic shock.

Figure 1 (abstract P19)



Survival of animals over time.

P20

Neutrophil CD64 expression, a marker of sepsis/infection, can be performed on a hematology blood counter and has variable correlation to C-reactive protein, procalcitonin and soluble CD163

Bruce Davis[1], Karen Becker[1], Henry Rinder[2], Kathleen Davis[1]
[1]Trillium Diagnostics, Brewer, ME, USA; [2]Department of Laboratory Medicine, Yale University, New Haven, CT, USA
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Background Neutrophil CD64 expression (PMN CD64) has been proposed as an improved laboratory indicator of severe infection and sepsis. Little is published on the interrelationship between PMN CD64 and the soluble phase indicators of inflammation, such as C-reactive protein (CRP), procalcitonin (PCT), and soluble CD163.
Methods We studied PMN CD64 in three clinical groups: neonates (390 specimens), hospitalized patients (236 specimens), and ambulatory outpatients (96 specimens). PMN CD64 was measured as an index using the Leuko64 (Trillium Diagnostics). Samples were also processed in parallel for the measurement of CD64 using the Leuko64 assays for flow cytometry on a FACScan (Becton Dickinson) and the blood cell counter Cell Dyn Sapphire (Abbott Diagnostics). Data were analyzed using Leuko64 software (Trillium Diagnostics). Results from both platforms were expressed as the PMN CD64 index, the monocyte CD64 index, and the monocyte CD163 index. CRP was measured in parallel. Plasma samples were stored at −70°C for subsequent measurement of procalcitonin (B.R.A.H.M.S.) and soluble CD163 (Trillium Diagnostics).
Results PMN CD64 correlated best with CRP, closely followed by PCT, but the degree of correlation varied among the clinical groups. The correlation was best in neonates ($r = 0.592$ for CRP and $r = 0.391$ for PCT), followed by hospitalized patients

($r = 0.345$ for CRP and $r = 0.349$ for PCT), and less so in outpatients ($r = 0.251$ for CRP and $r = 0.257$ for PCT). The correlation between PMN CD64 and the soluble markers was higher than that between CRP and PCT ($r = 0.331$ for hospitalized patients, $r = 0.305$ for neonates, and $r = 0.196$ for ambulatory patients). Soluble CD163 levels only weakly correlated with PMN CD64, CRP and PCT. The Sapphire results were highly correlated with flow cytometry ($r = 0.99$). The measured level of imprecision of both assays was <12% CV for PMN CD64, monocyte CD64, and monocyte CD163 indices. The assay results were available in <1 hour.

Conclusions This study shows a moderate correlation of PMN CD64 with the 'acute phase reactants' CRP and PCT. Soluble CD163 is only weakly correlated with the other parameters and may independently define further subsets of patients based upon different anti-inflammatory responses to the clinical condition. The interrelationship of these parameters varies in different clinical situations. We demonstrate it is feasible to automate cellular assays for infection/sepsis in a routine hematology laboratory providing access to a larger patient population.

P21

Tolerance to lipopolysaccharide regulates apoptosis in B lymphocytes

Edielle de Sant Anna Melo, Renata Gorjao, Ruy Curi, Irineu Tadeu Velasco, Francisco Garcia Soriano
University of São Paulo, Medicine School, Medical Clinical Department, Clinical Emergency – LIM 51, São Paulo, Brazil
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Background The most important event determining sepsis evolution is immune system cell apoptosis, the immune cell elimination compromises the host effective response, and prevention of apoptosis events improve survival in sepsis models. Our objective was to identify whether lipopolysaccharide (LPS) tolerance regulates apoptotic genes and caspase pathway.

Materials and methods Male Balb-C mice received LPS (1 mg/kg), a tolerant dose, and controls received 0.9% physiological serum during 5 days, both receiving on day 7 a LPS lethal dose (20 mg/kg). Control, 2 and 4 hours after lethal dose, IL-10, IL-6, IL-1β, TNFα and MIP2 were measured by ELISA. Splenic B lymphocytes were separated through magnetic beads and genes were analyzed by microarray, comparing control and tolerant groups. The tolerant and control groups were followed during 5 days to analyze survival.

Results See Table 1. The mRNA of caspases 2, 7, 8 and 11, Bid, Apaf-1 and FAS genes were reduced in the tolerant mice. The IL-6 levels reduced in the tolerant mice (724 ± 15 pg/ml) versus control mice (1,488 ± 96 pg/ml) in 2 hours. IL-1β was reduced at 0 hours and at 4 hours in the tolerant group (657 ± 25 pg/ml) versus control (1,117 ± 20 pg/ml). MIP2 also showed a reduction at 4 hours in tolerant (1,803 ± 159 pg/ml) versus control mice (2,173 ± 252 pg/ml). The tolerant animals had 100% survival, controls had zero survival. In all mentioned data, $P < 0.05$.

Conclusion Tolerance was able to reduce cytokine plasma levels, immune cell apoptosis and mortality to LPS lethal doses.

Table 1 (abstract P21)

Genic expression of apoptosis in tolerance to lipopolysaccharide

Genebank analysis number	Name	Ratio[a]
X92346	TNF receptor-associated factor 4 (TRAF4)	3.8
U06948	Fas antigen ligand (FASL)	4.5
U37522	TNF-related apoptosis-inducing ligand (TRAIL)	3.9
M83649	Fas l receptor	4.8
U88990	Inhibitor of apoptosis protein 3	Down
D28492	Caspase 2 precursor	Up
U39613	Caspase 7, apoptosis-related cysteine protease	Up
U59463	Caspase 11	3.4
U13021	Caspase 2, apoptosis-related cysteine protease	Up
AF064071	Apoptotic protease activating factor 1	Up
U39643	Fas-associated factor 1	Up
X67914	Programmed cell death 1 protein precursor	Up
NM022684	Bid, apoptotic protease	4.6
XM232860	Caspase 8, apoptosis-related cysteine protease	Up

[a]Ratio = controls/tolerants: down, down to 0.05; up, up to 5.0.

P22

Physiological parameters, location of infection and organ failure are significant predictors of misdiagnosing severe sepsis

Diane Chamberlain[1], Christine Wilson[1], Tamara Hunt[2]
[1]Flinders University, Faculty of Health Sciences, Adelaide, Australia; [2]Flinders Medical Centre, Adelaide, Australia
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Background Severe sepsis and septic shock are common disease processes in the critically ill and are associated with substantial morbidity and mortality. The importance of the early identification and diagnosis of severe sepsis has been highlighted by the Surviving Sepsis Guidelines with the aim to provide early and aggressive management in order to improve outcome. In contemporary practice, all clinicians have the responsibility of identifying severe sepsis. Therefore the objectives of this study were to determine whether emergency department and intensive care clinicians could identify and diagnose severe sepsis in those patients in their care within the first 24 hours of admission, and to identify predictors of failing to diagnose sepsis.

Methods The patient cohort were prospectively screened and enrolled on admission to intensive care within the first 24 hours. Severe sepsis was defined as new-onset acute organ dysfunction, using consensus criteria. Clinical data and physiological parameters were collected prospectively. Diagnosis was based on microbiologically confirmed clinical findings. Clinicians caring for each patient were prospectively surveyed.

Results All 402 subjects had infection. Infection sites included 52% pneumonia, 17% urinary, 15% abdominal, 6% wound and skin, and 10% isolated organs and bone. Single-organ failure was evident in 21%, 42% had two-organ failure, 29% had three-organ failure and 8% had four-organ failure. Nurses identified sepsis in 141 of the 402 patients ($P < 0.001$) whereas physicians did so in 265 of the 402 patients ($P < 0.05$). Misdiagnosis of severe sepsis by the attending nurse or physician was more likely to be associated with pneumonia (odds ratio (OR) = 4.2 (95% confidence interval (CI) = 3.6–4.2), $P < 0.01$), urinary sepsis (OR = 2.9 (95% CI = 2.6–3.4), $P < 0.5$), less than three-organ failure (OR = 3.1 (95% CI = 2.4–3.7), $P < 0.01$), Gram-negative infection (OR = 2.3 (95% CI = 1.6–3.5), $P < 0.5$) and presenting without fever (OR = 3.5 (95% CI = 3.1–3.9), $P < 0.05$). Thirty-two percent of clinicians did not know the criteria for severe sepsis and 57% missed the patient diagnosis in their care at that time.

Conclusion In this study, misdiagnosis of severe sepsis is still an acknowledged problem in meeting the goals of early resuscitation.

Protocols and monitoring tools may assist the early identification of severe sepsis so appropriate care can be prioritised and resuscitation implemented early in their admission.

P23

In vitro and *in vivo* determination of anti-TNFα activity in canine plasma from donors subject to preconditioning with endotoxin

Michael Kotiw[1], Michael Morgan[2], Jesus Lopez[1], Steven Taylor[2], Ian Shiels[2]
[1]Centre for Systems Biology, University of Southern Queensland Toowoomba, Queensland, Australia; [2]School Biomedical Sciences, University of Queensland, St Lucia, Australia
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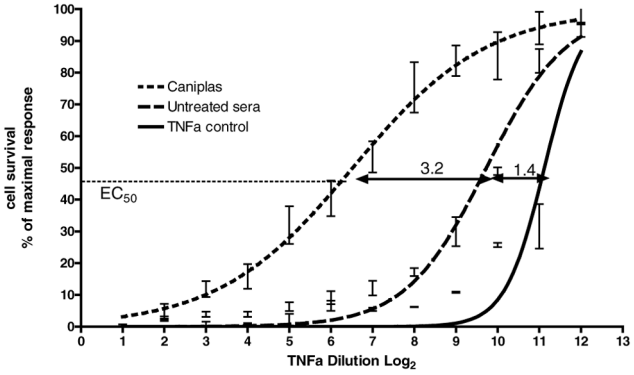
Background Septic shock is characterized by cardiovascular and vasomotor failure that is induced by an uncontrolled cascade of inflammatory mediators such as TNFα, IL-1β and IL-6. In dogs, systemic bacterial infections, haemorrhage, trauma, gastric dilatation/volvulus and pancreatitis are the major causes of septic shock. While endotoxin is a recognized effector molecule that can initiate an inflammatory cascade, it has been reported that preconditioning with endotoxin can downregulate inflammatory cytokine responses to subsequent endotoxin challenge. This study reports the effect of endotoxin preconditioning on anti-TNFα activity present in plasma from canine donors.

Figure 1 (abstract P23)



	Caniplas (n=3)	Untreated sera (n=3)	TNFa control (n=3)
LOGEC50	6.523	9.729	11.12
HILLSLOPE	0.2697	0.4502	0.9225

	Caniplas (n=3)	Untreated sera (n=3)	TNFa control (n=3)
95% Confidence Intervals			
LOGEC50	6.212 to 6.834	9.547 to 9.912	10.94 to 11.30
HILLSLOPE	0.2228 to 0.3166	0.3727 to 0.5277	0.5727 to 1.272

	Global (shared)
Comparison of Fits	
Null hypothesis	LOGEC50 same for all data sets
Alternative hypothesis	LOGEC50 different for each data set
P value	P<0.0001
Conclusion (alpha = 0.05)	Reject null hypothesis
Preferred model	LOGEC50 different for each data set
F (DFn, DFd)	220.8 (2,102)

	Caniplas (n=3)	Untreated sera (n=3)	TNFa control (n=3)
Goodness of Fit			
Degrees of Freedom	34	34	34
R^2	0.9480	0.9629	0.8884
Absolute Sum of Squares	2464	1406	3002
Sy.x	8.513	6.432	9.396

In vitro TNFα dose-response to canine sera.

Materials and methods Plasma from preconditioned (Caniplas®) and normal dogs (FFP) was provided blind to the study by a commercial supplier (Plasvacc Pty Ltd). *In vitro* anti-TNFα activity in canine donor plasma was determined by a L929 murine cell TNFα inhibition bioassay using recombinant murine TNFα. *In vivo* effects were tested by a rat subcutaneous skin pouch model. Rats were pretreated for 3 days with either Caniplas®, FFP, saline (2 ml/day, s.c.) or carprofen (5 mg/kg, s.c.) and inflammation was induced by injecting monosodium urate crystals into the pouch (5 mg/ml in 5 ml saline). Fluid was taken from pouches at specified intervals for cell count, TNFα and IL-6 analysis. Data analysis: normalized data were fitted to a four-parameter logistic curve. The fitted midpoints were compared statistically for datasets using an F-test and calculated fitted hill slopes.
Results In the rat skin pouch model, both Caniplas® and FFP reduced TNFα levels and Caniplas® was a more potent antagonist. The heightened anti-TNFα activity of Caniplas® compared with FFP was confirmed in the *in vitro* cell bioassay (Figure 1). Neither Caniplas® nor FFP reduced inflammatory cell infiltration or the levels of IL-6.
Conclusion While we need to confirm the mechanism, we report that preconditioning with endotoxin does illicit specific anti-TNFα activity and that this observation has been confirmed in both *in vitro* testing and *in vivo* animal models. It is plausible that preconditioning animals with endotoxin induces an increase in the concentration of soluble TNFα receptors I and II in donor plasma, and that this is the probable source of TNFα antagonism. This report suggests that preconditioned plasma may be a beneficial treatment where inflammation causes increased expression of TNFα.

P24

The human antimicrobial peptide LL-37 induces endothelium-dependent vascular smooth muscle relaxation mediated via the lipoxin A4 receptor

Ingrid Berkestedt, Axel Nelson, Mikael Bodelsson
Section of Anaesthesiology and Intensive Care, Department of Clinical Sciences, Lund University, Lund, Sweden
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Background Septic shock includes blood vessel dilatation and activation of innate immunity. The activation causes release of antimicrobial peptides such as LL-37. It has been shown that LL-37 can attract leukocytes via the lipoxin A4 receptor (ALX). ALX is also present in vascular endothelial cells. To explore possible ways of pharmacological intervention in septic shock, we investigated whether LL-37 can affect vascular tone.
Materials and methods Human omental arteries and veins were obtained during abdominal surgery. The circular smooth muscle activity, in the wall of the vessel segments, was studied in organ baths. Gene expression was studied using reverse transcriptase PCR.
Results LL-37, at micromolar concentrations, induced a concentration-dependent and endothelium-dependent relaxation in vein segments but not in artery segments precontracted by endothelin-1. The relaxation was profoundly reduced by potassium chloride (30 mM) to inhibit endothelium-derived hyperpolarizing factor (EDHF), while it was less affected by the NOS inhibitor L-NAME and not at all by indomethacin. The ALX agonist, WKYMVm, did also induce a relaxation, and both the relaxations induced by LL-37 and WKYMVm were inhibited by the ALX antagonist WRWWWW. ALX was expressed in the endothelium.
Conclusion We demonstrate for the first time that the human antimicrobial peptide LL-37 induces endothelium-dependent

relaxation in human omental veins mediated via an effect on endothelial ALX. The relaxation involves the release of nitric oxide and EDHF but not prostanoids. LL-37 released from white blood cells could contribute to the blood vessel dilatation during sepsis and treatment with ALX antagonists such as WRWWWW might be successful.

P25

Human protein C concentrate in the treatment of hemolytic uremic syndrome

Burkhard Wermter[1], Harald Koeditz[1], Kathrin Seidemann[1], Thomas Jack[1], Bernadette Brent[1], Lars Pape[2], Armin Wessel[1], Michael Sasse[1]
[1]PICU Interdisciplinary Paediatric Intensive Care Unit and [2]Paediatric Nephrology, MHH Medizinische Hochschule Hannover, Germany
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Background Human protein C (PC) concentrate may anticipate thrombotic microangiopathy and facilitate fibrinolysis in the severe hemolytic uremic syndrome (HUS).
Materials and methods We report the effects of PC in six HUS patients. HUS is characterized by a simultaneous occurrence of hemolytic anemia, thrombocytopenia and acute renal failure. Post-diarrheal HUS is often based on an infection with enterohemorrhagic *Escherichia coli* producing Shiga toxins. Our current pathogenetic understanding is that Shiga toxins cause endothelial injury, leading to thrombotic microangiopathy. There is still a 5% rate of mortality particularly caused by cerebral involvement. We treated six children with a severe cerebral manifestation, five of them suffered from a multiple organ dysfunction syndrome (MODS), of HUS with PC over 7–10 days. All patients suffered peritoneal dialysis, one patient a plasmapheresis. In addition to the treatment of the MODS, all of the patients received 100–200 U/day PC.
Results All of the patients showed signs of disseminated intravascular coagulation. We found typical hypodense lesions in basal ganglia and edema of the brain in computed tomography. During the therapy with PC, MODS was remarkably improved and abnormal D-dimer and plasminogen activator inhibitor 1 levels could be normalized. All of the patients recovered a nearly normal kidney function. Two patients persisted in a severe reduced neurological status. The others showed only slight or no neurological disabilities on discharge. No adverse effects were observed with the PC concentrate administration.
Conclusion There is no generally accepted therapy regimen to treat HUS in case of neurological involvement. Mortality in HUS accompanied with cerebral microangiopathy is high and difficult to alter. This is the first trial of human PC concentrate administration to anticipate thrombotic microangiopathy in HUS. All of our patients showed rapid clinical improvement under PC administration. Four of six patients were discharged in a healthy condition despite their severe disease. The containment of the severe neurological involvement and the lack of side effects in the treatment with human PC concentrate administration in our patients yield hope that PC treatment may be an effective therapy regimen in the treatment of severe HUS.

P26

Biofilm forming *P. aeruginosa* induces an enhanced inflammatory response in human monocytes

Cristina Ciornei[1], Christophe Beloin[2], Alexey Novikov[3], Martine Caroff[3], Catherine Fitting[1], Jean-Marc Ghigo[2], Jean-Marc Cavaillon[1], Minou Adib-Conquy[1]
[1]Pasteur Institut, Cytokines & Inflammation, Paris, France; [2]Pasteur Institut, Genetics of Biofilms, Paris, France; [3]Equipe 'endotoxines', CNRS UMR 8619, Paris-Sud University, Orsay, France
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Background The clinical picture of sepsis is varying, and the severity of the disease is influenced by numerous factors including the infectious agent and the host genetics. *P. aeruginosa* and *S. aureus*, the frequently sepsis-causing agents, can switch from a planktonic to a biofilm lifestyle. The cell wall of *P. aeruginosa* contains lipopolysaccharide (LPS), which can detach and trigger the immune response in the patient. We investigated the effects of live planktonic or biofilm bacteria on human monocytes in terms of the production of proinflammatory cytokines, such as TNF, in order to determine whether these two bacterial lifestyles influenced the immune response during sepsis.
Materials and methods Clinical isolates of *P. aeruginosa* and *S. aureus*, bacterial culture plates and media (Luria-Bertani and tryptic soy broth), RPMI media, ELISA kits, a spectrophotometer and biofermentors were used. The same bacterial concentration of planktonic and biofilm forming *P. aeruginosa* and *S. aureus* strains was obtained by optical density measurements and confirmed by colony counting. Blood samples were collected from healthy donors and monocytes were isolated by adherence and further incubated together with live planktonic or biofilm forming strains of *P. aeruginosa* or *S. aureus* for 5 hours; the cytokine content was measured by ELISA. LPS was extracted from *P. aeruginosa* in order to investigate the structural differences between planktonic and biofilm derived LPS. The limulus amebocyte lysate test, SDS-PAGE gel electrophoresis and mass spectrometry of LPS were performed to analyze the LPS structure.
Results The production of TNF, IL-6 and IL-1α was increased in monocytes incubated with biofilm forming *P. aeruginosa* as compared with planktonic ones, whereas no difference between cytokine responses was observed in monocytes incubated together with planktonic or biofilm *S. aureus*. Two predominant forms of rough LPS were detected in planktonic *P. aeruginosa* by SDS-PAGE and one of the rough LPS bands was absent in the biofilm. Fatty acids differed by their level of hydroxylation in the two bacterial growth conditions as seen by mass spectrometry.
Conclusions Biofilm forming *P. aeruginosa* induces an enhanced inflammatory response in human monocytes compared with the planktonic bacteria, and LPS structures were found to be different. No difference was seen in response to *S. aureus* planktonic or biofilm bacteria. The biofilm *P. aeruginosa* was more immunostimulatory than the planktonic form. LPS from biofilm forming bacteria may increase the immune response during sepsis.

P27

Laboratory markers to determine clinical significance of coagulase-negative staphylococci in blood cultures

Piret Mitt[1], Siiri Kõljalg[2], Krista Lõivukene[2], Epp Sepp[2], Irja Lutsar[2], Matti Maimets[1], Paul Naaber[2]
[1]Tartu University Hospital, Department of Infection Control, Tartu, Estonia; [2]University of Tartu, Departmentt of Microbiology, Tartu, Estonia
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Background Coagulase-negative staphylococci (CoNS) are important causes of bloodstream infection, especially in immunocompromised patients, but they are also the most common contaminants of blood cultures; discrimination between these two is often difficult. The time necessary for microbial growth to appear (time to positive) has been used as a laboratory marker for assessing clinical significance of CoNS bacteremia. However, with the use of continuously monitoring blood culture systems, the previous study results are controversial. The aim of the present study was to assess microbiological laboratory markers that are suggestive of true CoNS bacteremia.
Materials and methods All blood cultures positive for CoNS between 1 October 2006 and 30 April 2007 in Tartu University Hospital were included in this analysis. Blood specimens were monitored with the BACTEC 9240 system. Microbes were identified using the VITEK 2 system and antibacterial susceptibility pattern was tested according to CLSI standards. The CDC definition for bloodstream infection to determine the clinical significance of CoNS was used.
Results A total of 109 CoNS blood isolates from 86 patients (51 male; median age 22 years, range 0–94 years) were identified. According to the CDC criteria, 81 isolates were contaminants and 28 were true causes of bacteremia. Fifteen of the patients with infection were from the intensive care unit. The time to positive for blood cultures in infected patients was shorter than that in contaminated subjects: median 30 hours (range 20–68 hours) versus 42 hours (range 16–116 hours), respectively ($P = 0.029$). A total of 10 different CoNS species were identified; *S. epidermidis* was most commonly isolated in both groups – 21/28 in infection and 49/81 in contamination (OR, 1.87; 95% CI, 0.77–4.56). *S. hominis* comprised a higher proportion in the contamination group than in the infection group (17/81 versus 2/28 respectively; OR, 2.59; 95% CI, 0.84–8.01).
Conclusions The majority of CoNS isolated from blood cultures were contaminants. The time necessary for microbial growth is an important laboratory marker in differentiating between true bacteremia and contamination.

P28

Inhibition by telithromycin of systemic and respiratory inflammation induced by endotoxin in mice

Magdalena Leiva, Alfonso Ruiz-Bravo, Maria Jimenez Valera
University of Granada, Department of Microbiology, Granada, Spain
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Background Lower respiratory tract infections are the cause of septic shock in 25% of patients. Telithromycin (TEL), the first ketolide antibiotic, is used for treatment of respiratory infections. TEL is a semisynthetic derivative of the macrolide erythromycin. Beyond their antimicrobial activity, macrolides display immunomodulatory effects, including the inhibition of inflammatory reactions. In the present study, we demonstrate the anti-inflammatory effects of TEL on a septic shock model and a respiratory inflammation model in mice.

Table 1 (abstract P28)

Effect of telithromycin on cytokine levels in bronchoalveolar lavage and plasma in a mouse respiratory inflammation model

Treatment	TNFα in BAL (pg/ml)	MIP-2 in BAL (pg/ml)	MIP-2 in plasma (pg/ml)
None	11 ± 6	41 ± 3	1 ± 4
TEL	9 ± 4	28 ± 2	0 ± 0
LPS	2846 ± 369	1637 ± 254	40 ± 10
TEL + LPS	1279 ± 285[a]	1020 ± 240[b]	24 ± 5[c]

[a]$P < 0.005$, significantly different from mice treated with LPS alone.
[b]$P < 0.001$, significantly different from mice treated with LPS alone.
[c]$P < 0.05$, significantly different from mice treated with LPS alone.

Materials and methods TEL was a gift from Aventis Pharma (Neuville-sur-Saonc, France). Lipopolysaccharide (LPS) from *Escherichia coli* O26:B6 was purchased from Sigma Chemical Co. (St Louis, MO, USA). BALB/c female mice (10–12 weeks old) were maintained in the facilities of the University of Granada. To induce septic shock, mice were injected intraperitoneally with a dose of 50 mg LPS/kg body weight. To induce respiratory inflammation, mice were exposed for 20 minutes to aerosolized LPS (500 μg/ml saline) in a chamber connected to an air nebulizer (Miko, CA-MI s.n.c., Italy). To investigate the effects of TEL, mice received a single dose of 20 mg ketolide/kg body weight by intraperitoneal injection, 1 hour prior to the administration of LPS. Heparinized blood samples were centrifuged, and plasma was stored at −20°C until cytokine determination. To obtain bronchoalveolar lavage (BAL) fluids, the lungs were lavaged with 1 ml phosphate-buffered saline through an intratracheal catheter. Enzyme immunoassays kits were use to determine TNFα (Pierce Endogen, Rockford, IL, USA) and macrophage inflammatory peptide 2 (MIP-2) (R&D Systems, Minneapolis, MN, USA). The differences in cytokine levels were analyzed using Student's *t* test. $P < 0.05$ was considered significant.
Results When mice were intraperitoneally challenged with a lethal dose of LPS, TEL protected 50% of animals and significantly reduced the plasma levels of TNFα at 2 hours after LPS administration. In the respiratory inflammation model, the treatment with TEL significantly reduced the BAL levels of TNFα and MIP-2 at 4 hours post endotoxin (Table 1).
Conclusions TEL exerts anti-inflammatory activity *in vivo* that may contribute to its pharmacological effectiveness in the treatment of respiratory infections and their possible progression to septic shock.

P29

Study of procalcitonin as a marker of sepsis in patients of a general hospital during a 2-year period

Caterina Petrochilou, Myrianthi Pouyouka, Vasiliki Skandami, Charika Kontou, Melpomeni Kalderi, Alkipi Nitsa, Marina Toutouza
Department of Microbiology, 'Hippokration' General Hospital, Athens, Greece
Critical Care 2007, **11(Suppl 4):**P29 (doi: 10.1186/cc6008)
Background The aim of this study was to determine the number of specimens with abnormal procalcitonin (PCT) values in the total of specimens that were examined during a 2-year period.
Materials and methods We tested 1,114 patients who were hospitalized in various clinics of Hippokration General Hospital of

Athens during the period 2005–2006. PCT was measured because there was clinical suspicion of sepsis. The PCT value in serum was determined using the immunoluminometric assay method (Liaison-BRAHMS PCT) with a normal range of 0.10–0.50 ng/ml.
Results In the total of 1,114 specimens, which corresponded to the same number of patients, 178 patients (15.98%) had abnormal PCT values that ranged between 1 and 167 ng/ml. These patients were *a posteriori* clinically and by laboratory examination proven to be in systemic inflammatory response syndrome and sepsis.
Conclusion According to the results, PCT is a useful marker for the early diagnosis of systemic bacterial infection when clinical and laboratory signs are nonspecific for this determination.

P30

Lung-derived macrophage migration inhibitory factor in adult patients with septic shock, and its role in cardiocirculatory depression

Hans Lee[1], Kaie Ojamaa[1], Greg Ruskin[1], Erfan Hussain[1], Maowen Hu[1], Xinchun Lin[1], Christine Metz[1], Yousef Al-Abed[1], Edmund Miller[1,2]
[1]Laboratory of Cardiopulmonary Research, The Feinstein Institute, Manhasset, NY, USA; [2]Albert Einstein College of Medicine Bronx, NY, USA
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Background Migration inhibitory factor (MIF), a critical proinflammatory mediator in sepsis, has a profound affect on cardiovascular function. Our animal studies show that the lungs release MIF into the systemic circulation during late sepsis. MIF released in this way has direct and immediate access to cardiac cells. The purpose of our study was to assess the lung as a source of MIF in human septic shock patients and to further study the MIF-associated pathways involved in cardiovascular depression.
Materials and methods Nine adult patients with septic shock in the medical intensive care unit were studied. Blood samples were collected pre-lung (pulmonary artery or central venous catheter) and post-lung (arterial line catheter) at 12, 24 and 48 hours from the time of diagnosis. MIF was measured using an ELISA, and differences in plasma MIF concentration pre-lung versus post-lung were assessed using a paired *t* test on signed ranks. Furthermore, since inhibition of caspase 3 activity during sepsis reduces myocardial apoptosis and cardiac dysfunction, we examined *in vitro* the effect of MIF on cardiomyocyte apoptosis.
Results The mean age of patients was 57.6 years (range 25–82 years). Bloods from six patients were culture positive. There was a wide variation in plasma MIF concentrations in both pre-lung (0.2–64.7 ng/ml) and post-lung (0.2–76.4 ng/ml). However, there was a significant increase in the median MIF level of the post-lung blood (3.9 ng/ml) compared with pre-lung blood samples (2.9 ng/ml, $P = 0.005$). At 48 hours post diagnosis, eight out of nine individuals had increased MIF concentration post-lung, with a mean increase of 64 ± 49%. These findings were independent of the source or nature of the infection. The basal level of apoptosis in primary cardiomyocytes cultured in defined, serum-free medium was 3.7 ± 0.9% (% TUNEL positive of total nuclei). This increased significantly ($P < 0.001$) to 15.5 ± 3.9% and 26.0 ± 7.1% when treated with MIF 20 and 30 ng/ml, respectively. Simultaneous treatment of cardiomyocytes with MIF and specific MIF inhibitor ISO1 (50 mM) resulted in significant attenuation of the apoptotic effect, returning apoptosis to a basal level at 4.1 ± 0.6%.
Conclusion The study demonstrates for the first time in humans that the lung is a major source of MIF in septic shock. The study further suggests that MIF released from the lung may reduce cardiac function by increasing cardiomyocyte apoptosis, and that blocking MIF released from the lung to prevent cardiocirculatory deterioration may provide new strategies for the treatment of sepsis.

P31

Inhibition of central leukotrienes and nitric oxide production affects vasopressin secretion induced by polymicrobial sepsis

Maria José Alves da Rocha, Letícia Antunes Athayde, Gabriela Ravanelli de Oliveira-Pellegrin
Departmento de Morfologia, Estomatologia e Fisiologia, Faculdade de Odontologia e de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil
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Background Sepsis induces an increase in vasopressin (AVP) secretion and massive production of inflammatory mediators, including leukotrienes (LTs) and nitric oxide (NO), which are also produced in the brain and may affect the neuroendocrine system. Our aim was to study the role of central LTs and NO in AVP secretion during polymicrobial sepsis induced by cecal ligation and puncture (CLP).
Methods Male Wistar rats (250–280 g) received an intracerebroventricular injection of MK-886 (1 μg/kg), an LT biosynthesis inhibitor, or L-NAME (250 μg), an NO synthase inhibitor. Controls received a carrier injection before CLP, or were only sham operated. In one group of animals, survival was monitored for 5 days. In another group, the animals were decapitated at 4, 6 or 24 hours after surgery and blood was collected for measurements of hematocrit, plasma nitrate (NOx) and AVP levels.
Results CLP caused an increase in plasma AVP levels in the initial phase of sepsis, which was blocked by the central administration of MK-886 and was elevated by intracerebroventricular-injected L-NAME ($P < 0.05$). This contrasted with the final phase of sepsis, when plasma AVP remained at basal levels and was not altered by the administration of LT and NO blockers. The gradual increase in NOx levels was reduced by MK-886 and blocked by L-NAME. The increase in hematocrit caused by CLP was diminished ($P < 0.05$) by L-NAME injection, but was not modified by central administration of MK-886. The low survival rates observed after CLP were improved by the central administration of both drugs ($P < 0.05$).
Conclusion These results suggest that central LTs and NO differentially affect AVP secretion during CLP-induced experimental sepsis.
Acknowledgement Supported by FAPESP.

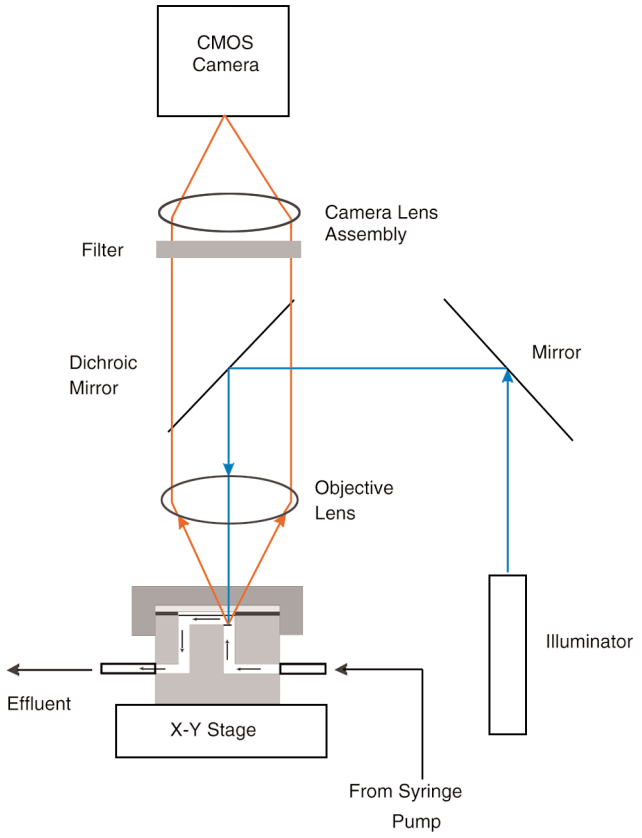
P32

Real-time, low-cost detection of individual fungal cells in blood using Fountain Flow™ cytometry

Paul E Johnson[1,2], Christopher M Havens[1,2], Joseph F Johnson[1,2]
[1]Department of Physics & Astronomy, University of Wyoming, Laramie, WY, USA; [2]SoftRay, Inc., Laramie, WY, USA
Critical Care 2007, **11(Suppl 4):**P32 (doi: 10.1186/cc6011)

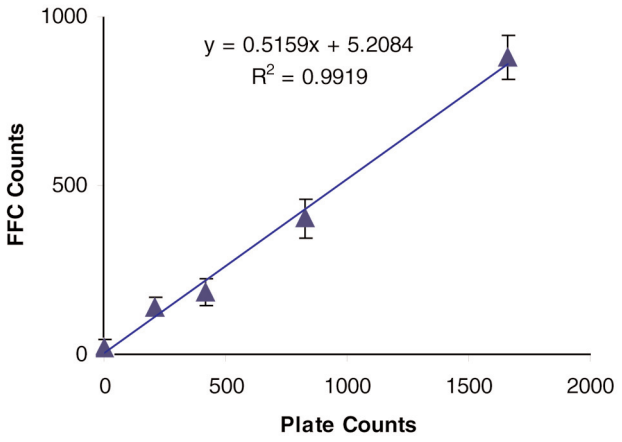
Background As fungi are considerably more slow growing than bacteria and are not affected by broad-spectrum antibiotics, too often conventional diagnostics fail to identify fungal bloodstream infection and confirmation is made postmortem. We describe the initial tests of a Fountain Flow™ cytometer (FFC) to detect pathogenic fungi in human blood, rapidly and cost-effectively. This innovative technique is based on fluorescence in which a stream of solution containing the cells, labeled with a fluorescent stain, is illuminated with an LED (Figure 1). The resulting fluorescence is detected with a CMOS imager.

Figure 1 (abstract P32)



The Fountain Flow™ cytometer. Fluorescently stained cells flow through the flow cell toward the CMOS camera, illuminated by an LED or laser. Cell(s) in the CMOS camera focal plane are imaged by the camera through the flow cell window at the wavelength of fluorescence.

Figure 2 (abstract P32)



Fountain Flow™ cytometer counts of *C. albicans* in 0.1 ml red blood cells. Comparison of Fountain Flow™ cytometer (FFC) counts and plate counts of *C. albicans* spiked into 1:20 diluted red blood cells (RBCs). The line of best fit gives a 52% counting efficiency.

Materials and methods The FFC used here was used by us in previous published research to detect individual bacteria and amoebae in river water at concentrations ≥200 bacteria/ml and 0.06 amoebae/ml. An optical stimulant of blood was made by mixing red blood cells (RBCs) in saline to obtain a RBC concentration comparable with that of human blood. This was further diluted 1:20 in distilled water to reduce the opacity. Then the solution was inoculated with *Candida albicans* from the American Type Culture Collection to obtain samples of varying concentration. To label the *Candida*, FUN1, a fluorescent, fungus-specific dye (Invitrogen), was used. The dye Trypan was used to suppress background, especially from RBCs, which absorb small amounts of FUN1.

Three 0.1 ml samples at each concentration were flowed into the FFC in 200 seconds (although a much greater rate was possible), while 500 images were taken continuously. The images were then analyzed (in minutes) with custom software to count the fluorescent spots in the images corresponding to *Candida*. Plate counts of *Candida* on YM agar (18 hours incubation) were used for comparison.

Results Figure 2 shows the comparison of the FFC and plate counts.

Conclusions The FFC has potential as a system for low-cost, real-time detection of low concentrations of fungi in human blood. Further work is required to lower the false detection rate, to increase the detection efficiency, and to perform tests on human blood with its additional complexity.

P33

Recurrence of Gram-negative nosocomial pneumonia in the critically ill patient following short-course antibiotic therapy

Richard Pugh, Richard Cooke, Ged Dempsey
University Hospital Aintree, Department of Critical Care, Liverpool, UK
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Background The optimal duration of antibiotic therapy for hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) is not clear. A multicentre randomised controlled trial indicated similar clinical efficacy for 8 versus 15 days of antibiotic therapy for VAP, with less emergence of multiresistant organisms following the shorter course [1]. *Pseudomonas aeruginosa* is difficult to eradicate, however, and American Thoracic Society guidelines for treatment of HAP due *to P. aeruginosa* recommend 14 days of therapy [2]. Our aim was to study the rate of recurrence following treatment of Gram-negative HAP in a critically ill population with short-course (typically 5 days) antibiotic therapy.

Materials and methods We retrospectively reviewed 50 patients treated consecutively with short-course (typically 5 days) antibiotic therapy for Gram-negative HAP in a UK teaching hospital critical care unit from 2004 to 2007. Pneumonia was defined as semi-quantitative respiratory culture (≥2+) of a single Gram-negative isolate, clinical pulmonary infection score ≥6 and initiation of antibiotic therapy. Recurrence of HAP was defined either as relapse (pure growth of the organism causing the initial infection) or reinfection (due to a different organism). Patients were studied until hospital discharge or death.

Results Demographic and outcome data are summarised in Table 1. The commonest causative organisms were *P. aeruginosa*

Table 1 (abstract P33)

Demographic and outcome data	
Demographic data at initial diagnosis	
Median age (range) (years)	69.5 (19–84)
Sex (% male)	78
Mechanical respiratory support (%)	78
Median APACHE II score	19
Outcome of patients surviving initial course of antibiotics	
Nonresolution (%)	2.1
Recurrence (%)	10.4 (all relapse)
Intensive care unit mortality (%)	41.7
Hospital mortality (%)	60.4

(42%), Enterobacter species (14%) and Klebsiella species (14%). Two patients died before completing the initial course of antibiotics.

Conclusions Treatment of Gram-negative HAP in the critically ill patient with short-course antibiotic therapy is associated with a low rate of recurrence (10.4%). This compares favourably with reported recurrence rates of 18–26% following ≥2 weeks of antibiotic therapy for VAP [1,3,4].

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P34

Fibrinolysis in early recognition of sepsis in patients with severe trauma

Lev Gerasimov, Viktor Moroz
Research Institute of General Reanimatology, Russian Academy of Medical Sciences, Moscow, Russia
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Background Early diagnosis of sepsis improves outcomes for trauma patients. The aim of this study was to detect additional markers that help to recognize sepsis before manifestation in patients with severe multiple trauma and blood loss. It is known that depletion of fibrinolysis promotes microvascular alterations after massive blood loss. During inflammation, C-reactive protein may inhibit fibrinolysis by inducing plasminogen activator inhibitor 1. These alterations are correlated with an increased risk of multiple organ failure and death. In accordance with the above-mentioned purpose, we investigated fibrinolysis at the early post-traumatic period in patients with and without sepsis.

Materials and methods We examined a database of 58 intensive care unit patients with trauma and shock, Acute Physiology and Chronic Health Evaluation II score (first day in intensive care unit) of 27.4 ± 0.5. Group 1 consisted of 23 patients who developed sepsis (day after admission, when severe sepsis or septic shock manifested, was 6.1 ± 0.28). Group 2 consisted of 35 patients without sepsis within the early post-traumatic period (1–14 days after injury). The plasma fibrinolytic potential was evaluated by the euglobulin lysis time, determined by a semiautomatic method.

Results Data are presented as the mean ± median (Table 1). It was established that fibrinolytic potential was significantly lower in patients with sepsis versus patients without sepsis since the fifth day after admission. At the same time, for white blood cells, a significant difference was observed just after the seventh day following admission. Platelets did not differ between groups.

Conclusion Plasma fibrinolytic potential is closely related to inflammation and may be used as a marker of sepsis. Moreover, plasma fibrinolytic potential may be a useful test for early identification of the high-risk sepsis patient.

P35

A functional microsatellite in the macrophage migration inhibitory factor gene influences susceptibility to meningococcal sepsis

Pascal Renner[1], Thierry Roger[1], Saul Faust[2], Anne-Laure Schiesser[1], Marlies Knaup-Reymond[1], Helen Betts[3], Pierre-Yves Bochud[4], Murielle Bochud[5], Robert Read[6], Michael Levin[3], Thierry Calandra[1]
[1]Infectious Diseases Service, CHUV, Lausanne, Switzerland; [2]Division of Infection, Inflammation and Repair, University of Southampton, Southampton, UK; [3]Department of Paediatrics, St Mary's Hospital, Imperial College, London, UK; [4]Institute for Systems Biology, Seattle, USA; [5]Division of Genetic Epidemiology, Case Western Reserve University, Cleveland, USA; [6]Division of Genomic Medicine, Royal Hallamshire Hospital, Sheffield, UK
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Background The cytokine migration inhibitory factor (MIF) has recently emerged as an important effector molecule of innate immune responses and sepsis. Two functional MIF promoter polymorphisms, a 5–8 CATT tetranucleotide repeat at −794 (CATT5–8) and a −173*G/C single nucleotide polymorphism, have been associated with susceptibility to and/or severity of rheumatoid arthritis, atopy and ulcerative colitis. Our objective was to study the impact of MIF gene polymorphisms on susceptibility to *Neisseria meningitidis* sepsis and to analyze the functional and biological effects of MIF polymorphisms *in vitro*.

Table 1 (abstract P34)

Fibrinolysis, white blood cells and platelets in groups during the early post-traumatic period

	Group 1 (with sepsis, $n = 23$)			Group 2 (without sepsis, $n = 35$)		
Days after admission	3	5	7	3	5	7
Fibrinolysis (6–12 min)	76.2 ± 2.8	86.5 ± 2.9*	90.3 ± 3.9**	74.8 ± 3.2	76.3 ± 3.2	62.5 ± 4.5
White blood cells	9.2 ± 0.32	9.9 ± 0.28	10.9 ± 0.45*	9.3 ± 0.19	9.6 ± 0.25	9.5 ± 0.36
Platelets	218 ± 14.4	192 ± 16	184 ± 15.5	209 ± 15.1	208 ± 14.6	218 ± 14.5

*$P_w < 0.05$ versus Group 2. **$P_w < 0.001$ versus Group 2.

Methods A case–control study of 1,106 children and adults with meningococcal sepsis and 626 control subjects and a family-based study (106 families with one afflicted child) to analyze transmission of MIF alleles using the transmission disequilibrium test. The −794 CATT5–8 microsatellite and −173*G/C single nucleotide polymorphism were detected by PCR. MIF promoter alleles cloned into a luciferase reporter construct were tested in resting and stimulated THP-1 human monocytic cells. DNA binding activity was assessed by EMSA.

Results Compared with control subjects, the frequency of the CATT5–5 genotype was markedly reduced in meningococcal sepsis patients (5.3% versus 8.8%; OR = 0.6, 95% CI = 0.4–0.9, $P = 0.01$ for CATT5–5 versus all other CATT*x*–*x*). The frequencies of the −173*G/G, −173*G/C or −173*C/C genotypes was comparable in meningococcal sepsis patients and control subjects ($P = 0.75$). The transmission disequilibrium test in families with one afflicted child revealed that the CATT5 allele was preferentially not transmitted ($P = 0.002$) to meningogoccal sepsis offspring, while the opposite situation occurred for the CATT6 allele ($P = 0.024$). *In vitro* studies showed lower activity of CATT5 promoter compared with all other CATT promoters in resting and *N. meningitidis*-stimulated THP-1 cells. Consistently, DNA binding activity to the CATT region of the MIF promoter increased with an increasing number of the CATT repeats.

Conclusion The frequency of the −794 CATT5–5 genotype was reduced in children with meningococcal sepsis and was less frequently transmitted from parents to affected offspring, suggesting that it may confer protection against severe sepsis due to *N. meningitidis*. Reduced transcription factor DNA-binding activity to CATT5 and weaker CATT5 promoter transcriptional activity provided a functional relevance of the CATT5 polymorphism. MIF polymorphisms might help to identify patients who may benefit from anti-MIF treatment strategies.

P36

Methicillin-resistant *Staphylococcus aureus*-induced vascular leakage is associated with excessive production of nitric oxide and vascular endothelial growth factor

Perenlei Enkhbaatar, Esechie Aimalohi, Lillian Traber, Collette Joncam, Rhykka Connelly, Matthias Lange, Atsumori Hamahata, D Herndon, Daniel Traber

Department of Anesthesiology, University of Texas Medical Branch, and Shiners Hospital for Children, Galveston, TX, USA

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Background Methicillin-resistant *Staphylococcus aureus* (MRSA)-related pneumonia and/or sepsis are a frequent serious menace. The aim of the study was to establish a standardized and reproducible model of MRSA-related ovine septic pneumonia and to compare the pathophysiological responses to those seen in a previously established *Pseudomonas aeruginosa*-induced ovine sepsis model.

Materials and methods Twenty-four sheep were operatively prepared for chronic study. After 5 days recovery, tracheostomy was performed under anesthesia and injury was given. Sheep were randomly allocated into four groups: (1) Sham group, no injury ($n = 6$); (2) Smoke group, exposed to smoke inhalation ($n = 6$); (3) PS group, exposed to smoke inhalation and instilled with *P. aeruginosa* (2.5×10^{11} CFU) into the lungs by bronchoscope ($n = 6$); and (4) MRSA group, exposed to smoke inhalation and instilled with MRSA (2.5×10^{11} CFU) into the lungs by bronchoscope ($n = 6$). Smoke inhalation injury was induced by inhalation of cotton smoke (48 breaths, <40°C). After the injury, animals were awakened and maintained on mechanical ventilation by 100% O_2 for first 3 hours, and thereafter the O_2 concentration was adjusted according to blood gases. The sheep, including the Sham group, were resuscitated by lactated Ringer's solution with an initial rate of 2 ml/kg/hour that was further adjusted according to hematocrit and filling pressure. The experiment lasted 24 hours.

Results The mean arterial pressure was severely depleted in the MRSA and PS groups, while it was stable in the Sham and Smoke groups. The fluid net balance was significantly higher in the MRSA group compared with the other groups, including the PS group. Plasma nitrite/nitrate was unchanged in the Sham and Smoke groups compared with baseline values. There was an approximately sevenfold increase in Plasma nitrite/nitrate in the MRSA group versus an approximately 2.5-fold increase in the PS group compared with the Sham group 12 hours post-injury (Table 1). The excessive nitric oxide in the MRSA group was associated with a significant increase in lung tissue vascular endothelial growth factor mRNA and its protein expression.

Conclusion The severe vascular leakage syndrome seen in the MRSA group may be due to excessive production of nitric oxide and vascular endothelial growth factor, a potent permeability factor. MRSA is largely responsible for these pathological changes rather than smoke inhalation alone.

P37

Epidemiology of community-acquired bacteremia patients admitted to the intensive care unit

Guiomar Castro[1], Rui Carneiro[1], Orquidea Ribeiro[2], Altamiro Ribeiro[2], António Carneiro[1], Teresa Cardoso[1]

[1]Unidade de Cuidados Intensivos Polivalente, Hospital Geral de Santo António, Porto, Portugal; [2]Serviço de Bioestatística e Informática da Faculdade de Medicina do Porto, Porto, Portugal

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Background Community-acquired bacteremia (CAB) is a common cause of hospital and intensive care admission, with a case fatality rate of 20–30%. Its early identification and association with the probable source of infection and agents will permit an early and effective antibiotic therapy, and would probably contribute to a decrease in the morbidity and mortality.

Materials and methods A prospective, observational study of all the patients with community-acquired sepsis (CAS) admitted to a tertiary, mixed, 12-bed, intensive care unit (ICU), at a University Hospital, between 1 December 2004 and 30 November 2005. In this study the CAB subgroup was analyzed. CAB was defined by an infection present at hospital admission or within the first 48 hours with a positive blood culture obtained in the same period.

Results Throughout the study period, 347 patients were admitted; 149 (43%) with CAS. Blood cultures were obtained in 137

Table 1 (abstract P36)

	Sham group	Smoke group	PS group	MRSA group
Fluid net balance (ml/kg), 24 hours	−0.01 ± 6.0	12.7 ± 8.8	64.3 ± 14.8*†	167.0 ± 7.0*†#
Plasma nitrite/nitrate (μM), 24 hours	5.12 ± 0.7	4.85 ± 0.5	12.09 ± 0.7*†	22.3 ± 3.4*†#

Data expressed ±SEM.*$P < 0.05$ versus Sham group; †$P < 0.05$ versus Smoke group; #$P < 0.05$ versus PS group.

patients (93%), and 24 patients (17.5%) had CAB. From these, 67% were male, with a median age of 57 years (interquartile range = 47–71). The median SAPS II score was 51. Distribution by focus of infection was as follows: respiratory (35%); endovascular (21%); intraabdominal (17%); urinary (13%); central nervous system (8%) and skin and soft tissue (8%). All patients admitted with skin and soft-tissue CAS had CAB. Gram-positive microorganisms represented 50% of all isolations, followed by Gram-negative (33%). No fungus was isolated. *Streptoccocus pneumoniae* and *Escherichia coli* were the more frequent, representing 45% of all isolations. Patients with CAB had more septic shock than patients with negative blood cultures (75% versus 49%, $P = 0.039$) and higher ICU (50% versus 28%, $P = 0.049$), 28-day (54% versus 28%, $P = 0.014$) and hospital (57% versus 33%, $P = 0.041$) mortalities, although the median SAPS II score was not significantly different between both groups (51 versus 45, $P = 0.112$).

Conclusions CAB represented 7% of all ICU admissions, with an associated ICU crude mortality of 50%. Respiratory and endovascular focuses of infection were the two most frequent, with Gram-positive microorganisms representing one-half of all isolations.

P38

Time course of granulocyte–macrophage colony-stimulating factor and IL-8 in severe sepsis: are the initial low levels of IL-8 a consequence of immunodepression?

Simona Mera, Doina Tatulescu, Cristina Cismaru, Mirela Flonta, Dumitru Carstina
Clinic of Infectious Diseases, Cluj-Napoca, Romania
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Background Neutrophil proliferation, activation and recruitment are key events in the pathogenesis of sepsis. Granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-8 are cytokines regulating the proliferation, differentiation and maturation of polymorphonuclear cells and mononuclear phagocytic progenitors, enhancing their functions, adhesion and chemotaxis.

Materials and methods In 11 patients fulfilling the ACCP/SCCM criteria for severe sepsis, we analyzed serum levels of GM-CSF and IL-8 at days 1, 2 and 7 after admission. Patients (10 males/one female) had a median age of 68 years (range 22–87 years). The primary infection had urinary (seven patients) and respiratory (four patients) origins. Seven healthy volunteers served as controls.

Results Four out of 11 patients had positive blood cultures (Gram-negative). At admission, the median procalcitonin was 2 ng/ml (0.5–10 ng/ml). All patients survived except one patient that died 5 days after admission following acute myocardial infarction. GM-CSF was always below the detection limit (7.8 pg/ml) in septic patients and controls. In seven patients, serum IL-8 was below the detection limit (31.2 pg/ml) at all time points. The median value for IL-8 for the group of patients was 0 pg/ml (0–264 pg/ml), but while IL-8 was absent in the sepsis of respiratory origin it had a median value of 32 pg/ml in urinary sepsis. Surprisingly, IL-8 was detectable in five out of seven controls, with a median of 68.7 pg/ml (0–145.2 pg/ml).

Conclusions We failed to identify detectable circulating levels of GM-CSF in healthy individuals or in severe sepsis. The circulating levels of IL-8 were lower in septic patients than in healthy controls. As previously suggested, locally produced GM-CSF might have a role in this condition. Initial low levels of IL-8 found might be a component of the immunodepression seen in severe sepsis. Also, it is possible that the IL-8 levels correlate with the primary site of infection and the severity of the immunodepression related to that.

P39

Severe sepsis and septic shock in pregnancy and puerperium: an 11-year review in a maternity intensive care unit

Spyridon Pactitis, Grammati Koutra, Charalambos Charalambidis, Zoi Psomadaki, Athanasia Karouzou, Charalambos Ladakis
IASO Maternity Hospital, ICU, Athens, Greece
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Background To estimate the incidence, the etiology and the outcome of severe sepsis and septic shock during pregnancy and puerperium.

Materials and methods Retrospective collection of data for all obstetric patients with severe sepsis and septic shock admitted to the intensive care unit over an 11-year period (May 1996–May 2007) in a maternity hospital (IASO). Data collected include the characteristics of severe sepsis and septic shock [1], the source of infection, the responsible infectious organisms, and the outcome.

Results In the 11-year period, 1,321 women required intensive care unit admission (0.80% of all deliveries) and 52 of them (4%) had severe sepsis or septic shock. The most common infection was chorioamnionitis (23 patients, 41%) and endomyometritis (primarily after cesarean delivery) (11 patients, 21%). Other common infections were septic abortion, pneumonia and pyelonephritis. The most common etiologic agents were Gram-negative rods, followed by Gram-positive bacteria. One patient had fungal (*Candida albicans*) infection and another *Clostridium* spp. No deaths were recorded.

Conclusions Sepsis is an infrequent yet important cause of morbidity and possible death in gravitas. Early recognition of sepsis may prevent maternal complications. The factors that contribute to a decrease rate of severe sepsis, septic shock and death in pregnant women are young age and few comorbid conditions, and the organisms responsible for infections in these women are usually responsive to common broad-spectrum antimicrobial agents.

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P40

The kinetics of IL-17 production in the lungs and plasma of mice after intratracheal infection with *Klebsiella pneumoniae*

Vanja Vasiljev Marchesi[1], Tomislav Rukavina[1,2]
[1]Department of Microbiology and Parasitology, Medical Faculty, University in Rijeka, Croatia; [2]Department of Microbiology, Public Health Institute, Rijeka, Croatia
Critical Care 2007, **11(Suppl 4):**P40 (doi: 10.1186/cc6019)

Background IL-17 is a proinflammatory cytokine predominantly produced by T cells, which is involved in the innate immune responses to various physiologic and pathophysiologic processes including bacterial host defense. The neutralisation experiments showed that the lack of IL-17 leads to decreased neutrophil emigration and systemic granulopoietic responses to pulmonary bacterial pathogens and allergens. The aim of our study was to determine the kinetics of IL-17 in plasma and lungs of animals intratracheally infected with *Klebsiella pneumoniae*.

Materials and methods In our experiments we used 8–12-week-old BALB/c male mice. Mice were intratracheally inoculated with 150 CFU *K. pneumoniae* strain Caroli. At different time points, mice were sacrificed and the lungs and blood were aseptically

removed and prepared for the cytokine determination. Cytokine determination was performed by commercial ELISA kit (Bender-Med Systems, Vienna, Austria).
Results The IL-17 concentration in lung homogenates slightly increased in the first 2 hours of infection. Then it slightly decreased and again started to increase 24 hours after the infection. The concentration in the lungs reached the maximal value 48 hours post infection. These results are consistent with data previously published by others. On the other hand, we also found increased plasma values of IL-17. Its concentration in plasma started to increase 12 hours after the infection and reached the peak value 24 hours post infection. These results are in contrast with the results of others who reported no changes in systemic IL-17 production after the intratracheal *K. pneumoniae* challenge.
Conclusions IL-17 in local host defenses against the Gram-negative pathogens is undoubtedly important for the clearance of microorganisms, but its importance in the systemic host response is still not resolved. Its maximal concentration in plasma correlates with the appearance of the bacteria in the blood after 24 hours, so we speculate that its role is also to stimulate systemic proinflammatory cytokines to combat release of bacteria and/or their toxic products into the blood system.

P41

Microbiological diagnosis of sepsis: comparison between real-time polymerase chain reaction and blood culture techniques

Simona Barnini, Carlotta Dodi, Mario Campa
Dipartimento di Patologia Sperimentale, Biotecnologie Mediche, Infectivologia ed Epidemiologia, Unità Operativa di Microbiologia, Università di Pisa, Pisa, Italy
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Background A rapid microbiological diagnosis permits one to undertake an appropriate therapy against bacterial sepsis and contributes both to improve the healing possibilities and to lower the high costs of hospitalisation.
Materials and methods Blood samples from hospitalised patients were inoculated into Bactec Plus Aerobic/F and Anaerobic bottles (standard inoculum; Becton Dickinson) and into an EDTA tube (3 ml). The *Septifast* Lys kit, *Septifast* Prep kit and *LightCycler Septifast* kit (Roche) were used in disrupting bacterial walls, and in extracting and amplifying bacterial DNA, respectively. Blood agar plates were from Becton Dickinson. Strains were identified by Vitek2 (Biomerieux) panels. Real-time PCR results were analysed by *Septifast* identification software. Blood cultures were incubated into a Bactec 9240 instrument and, if positive, aliquots were taken, plated onto blood agar plates and identified according to routine procedures. EDTA–blood samples were processed according to the *LightCycler Septifast* test.
Results Among 147 samples from 51 patients with sepsis clinical signs, 30 were positive by Bactec and 27 by *Septifast* (which did not contain probes for three species isolated by cultural method). Concordance between the two methods was 76% for species identification. Different results between the two methods included 10 Bactec-positive and *Septifast*-negative samples (*Acinetobacter baumannii* was not amplified seven times, Gram-positive cocci were not determined by *Septifast* identification software three times) and 12 Bactec-negative and *Septifast*-positive samples (five Gram-positive cocci, three *Pseudomonas aeruginosa*, three *Escherichia coli*, one *Klebsiella* spp.), while four samples were positive by both methods but gave different species. The time response was evaluated: *Septifast* was faster than the traditional method in 97% cases.
Conclusions Although the impossibility to determine antibiotic susceptibility represents a great limit of the *Septifast* technique, the short response time may contribute to undertake a rapidly tuned therapeutic regimen. *Septifast* Test appears to be a valid help in an early assessment of sepsis, along with cultural method.

P42

Community-acquired bacteraemia with sepsis in adults admitted to the intensive care unit: a prospective multicentre study

Teresa Cardoso[1], António Carneiro[1], Orquídea Ribeiro[2], Altamiro Pereira[2]
[1]Unidade de Cuidados Intensivos Polivalente do Hospital Geral de Santo António, Porto, Portugal; [2]Serviço de Bioestatística e Informática Médica da Faculdade de Medicina da Universidade do Porto, Porto, Portugal
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Background The main objectives of this study were to describe the epidemiology and microbiology of community-acquired bacteraemia (CAB) in patients with sepsis and to determine the associated crude mortality.
Materials and methods A prospective, multicentre, cohort, study, was performed on community-acquired bloodstream infections with sepsis admitted to Portuguese intensive care units (ICUs), from 1 December 2004 to 30 November 2005, with a follow-up until discharge. CAB was defined as an infection that was present on admission or within the first 48 hours, with positive blood cultures drawn in the same period. Bacteraemia was considered to be healthcare related (HCRB) if: (a) patients had wound dressing or intravenous treatments in the previous 30 days, (b) patients were observed at a hospital or haemodialysis centre or had chemotherapy in the previous 30 days, (c) patients were admitted to an acute care hospital for 2 days or more in the previous 90 days, (d) patient lives at a nursing home or institution.
Results Seventeen units entered the study from north to south Portugal, corresponding to 41% of all mixed national ICU beds. Over this period 4,142 patients were admitted to the study – 897 (22%) had community-acquired sepsis, and of these 804 (90%) had blood cultures done in the first 48 hours. Significant isolates were grown from samples of 160 patients (20%). The following focus of infection was identified: respiratory (37%), intra-abdominal (19%), urinary (16%), endovascular (14%), skin and soft tissue (7%), central nervous system (5%) and other (2%). Of these 31% were health-care related (HCRB). Comparing patients without HCRB with those who had HCRB, an inversion in the micro-biological profile was found with Gram-positive dominating the first (55% versus 29%) and Gram-negative dominating in the latter (29% versus 54%). When compared with patients without bacteraemia, the 28-day crude mortality was higher for patients with positive blood cultures (39% versus 29%, $P = 0.014$); however, when adjusted to other variables, it was not found to be an independent determinant of 28-day mortality.
Conclusion CAB with severe sepsis accounts for 4% of all ICU admissions with a crude 28-day mortality rate of 39%, although not representing an independent risk factor for 28-day mortality. The microbiological profile is quite different in healthcare-associated bacteraemia, making their identification extremely important to prompt an initial adequate antibiotic approach.
Acknowledgement Presented on behalf of the SACiUCI study group.

P43

Clinical experience with lipopolysaccharide adsorber in cardiac surgery

Sten Blomquist, Victoria Gustafsson, Teo Manolopolos, Leif Pierre
Department of Cardiothoracic Anaesthesia, Heart–Lung Division, Lund University Hospital, Lund, Sweden
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Background Endotoxemia is common in cardiac surgery using extracorporeal circulation (ECC) and is correlated to the time of bypass, aortic clamping and postoperative complications. Adsorption of lipopolysaccharide (LPS) has been used in septic patients with positive results.
Materials and methods The Alteco LPS Adsorber contains polyethylene discs with a specific polypeptide that binds LPS. The priming volume is 80 ml and the recommended blood flow is 100–150 ml/min. Heparin is used for anticoagulation. Fifteen patients scheduled for elective surgery for coronary artery disease and/or valvular surgery were included in the study. Nine patients had the LPS adsorber included in the ECC circuit whereas six patients served as controls with no adsorber. Blood flow through the adsorber was initiated at the time of aortic clamping, adjusted to 150 ml/min, and terminated when weaning from ECC started. Samples for LPS, TNFα, IL-6, and IL-1β were taken before anaesthesia, 10 minutes after aortic clamping, at skin suture and 6 hours after skin suture.
Results There were no differences between the groups in age, perfusion time and time of aortic clamping (Table 1). The time of adsorber treatment is also shown. The use of the adsorber was uneventful and flow was easily maintained at 100–150 ml/min. LPS was found in two patients, one in each group at skin suture. Both patients had a long clamping time, 113 versus 123 min. Cytokines and complement results are presented in Tables 2 and 3. There were no significant changes in TNFα or IL-1β while IL-6 increased in both groups. Complement factors C3 and C4 decreased in the control group while C1q increased in the adsorber group.
In vitro studies have shown that the Alteco LPS adsorber can reduce LPS in whole blood. LPS was found in only two patients in our study, probably due to the short duration of perfusion and clamping. Thus the possibility to evaluate the efficacy of the adsorber in this setting was limited. Only IL-6 increased during the study but no difference between the groups was seen. The decrease in C3 and C4 seen in the control group may indicate activation of inflammatory system, more pronounced in the control group.
Conclusion The adsorber can be used safely in the extracorporeal circulation. Further studies in patients with more pronounced endotoxic response are needed to evaluate the effects of the adsorber on clinical outcome.

Table 1 (abstract P43)

Demographics and technical data

	Age (years)	Perfusion time (min)	Clamping time (min)	Adsorption time (min)
Adsorber	104 (35–80)	104 (46–198)	71 (30–139)	85 (41–160)
Controls	73 (59–86)	118 (50–239)	83 (24–167)	

Data presented as the mean (range).

Table 2 (abstract P43)

Concentrations of cytokines

	Preanaesthesia	10 min declamp	Skin suture	6 hours postoperative	ANOVA *P* value
TNFα (pg/ml)					
Adsorber	7.18 ± 4.3	11.6 ± 4.4	11.9 ± 4.7	5.3 ± 1.6	Not significant
Controls	11.88 ± 10.1	4.0 ± 0.5	5.4 ± 0.9	5.4 ± 1.2	Not significant
IL-6 (pg/ml)					
Adsorber	8.88 ± 2.9	46.0 ± 19.6	38.6 ± 16.1	87.3 ± 19.0	<0.05
Controls	2.47 ± 0.6	38.7 ± 20.0	51.4 ± 25.3	117.6 ± 36.9	<0.05
IL-1β (pg/ml)					
Adsorber	0.19 ± 0.1	1.10 ± 0.6	1.50 ± 0.9	0.36 ± 0.31	Not significant
Controls	0.20 ± 0.1	0.02 ± 0.02	0.11 ± 0.01	0.01 ± 0.01	Not significant

Data presented as the mean ± SEM.

Table 3 (abstract P43)

Concentrations of complement factors

	Preanaesthesia	6 hours postoperative	*t*-Test *P* value
C3 (g/l)			
Adsorber	1.45 ± 0.06	1.11 ± 0.15	Not significant
Controls	1.16 ± 0.05	0.97 ± 0.16	<0.05
C4 (g/l)			
Adsorber	0.32 ± 0.03	0.26 ± 0.05	Not significant
Controls	0.30 ± 0.03	0.25 ± 0.02	<0.05
C1q (%)			
Adsorber	113.9 ± 7.4	92.3 ± 7.4	<0.05
Controls	94.8 ± 5.0	83.1 ± 9.2	Not significant

Data presented as the mean ± SEM.

P44

Oxidant-induced TLR4 translocation in murine macrophages is Src kinase dependent

Patrick Tawadros, Zhanfei Wang, Simone Birch, Katalin Szaszi, Andras Kapus, John Marshall, Ori Rotstein
St Michael's Hospital, Department of Surgery, University of Toronto, Toronto, ON, Canada
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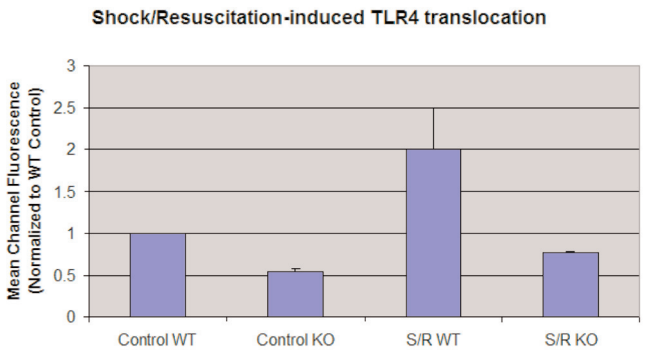
Background Multiorgan failure is a major cause of late mortality following trauma. Oxidative stress generated during shock/resuscitation (S/R) contributes to tissue injury by priming the immune

Figure 1 (abstract P44)



Shock/resuscitation (S/R)-induced TLR4 translocation is inhibited in Src knockout (KO) mice. WT, wild type.

system for an exaggerated response to subsequent inflammatory stimuli such as lipopolysaccharide (LPS): the so-called 'two-hit hypothesis'. The mechanisms of oxidant-induced cell priming, however, remain poorly elucidated. Our group has previously reported a role in this priming process for translocation of the LPS receptor TLR4 to the plasma membrane [1]. We have also previously shown that oxidant priming reprograms LPS signaling in macrophages to a Src-dependent pathway leading to PI3k activation [2]. Taken together, we hypothesized that Src activation may play a role in oxidant-induced TLR4 translocation.

Materials and methods Wild-type (WT) and triple Src (hck/fgr/lyn$^{-/-}$) knockout (KO) mice were subjected to hemorrhagic shock and resuscitation to generate oxidative stress *in vivo*. Alveolar macrophages (AMs) were then retrieved by bronchoalveolar lavage and analyzed for TLR4 translocation by immunofluorescence staining and flow cytometry. In a separate *in vitro* experiment, AMs from WT and KO mice were exposed to 200 μM hydrogen peroxide for 60 minutes and similarly analyzed by flow cytometry for surface expression of TLR4.

Results *In vivo*, S/R induced visible translocation of TLR4 to the plasma membrane of AMs from WT mice as seen by immunofluorescence. This effect was completely inhibited in the Src KO animals, and the observation was corroborated quantitatively by flow cytometry (see Figure 1). In order to confirm the role of oxidative stress in S/R-induced TLR4 translocation, AMs from WT and KO mice were exposed to hydrogen peroxide *in vitro*. Hydrogen peroxide caused an increase in surface expression of TLR4 in WT AMs by a ratio of 1.23 compared with WT control. This effect was once again inhibited in the Src KO AMs, where the ratio was 0.95 compared with WT control.

Conclusion Oxidative stress induces TLR4 translocation to the cell surface of macrophages in a Src-dependent manner. Since cellular responsiveness to LPS is known to correlate with surface levels of TLR4, this novel finding may direct future therapies in modulating oxidant-induced cellular priming and subsequent organ failure.

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P45

Reduction of gap junction proteins and intercalated disc structural remodeling in the hearts of mice submitted to sepsis

Mara Rubia Nunes Celes, Diego Torrez-Dueñas, Jose Carlos Alves-Filho, Djane Braz Duarte, Fernando Queiroz Cunha, Marcos Antonio Rossi
Departments of Pathology and Pharmacology, Faculty of Medicine of Ribeirao Preto, University of São Paulo, Ribeirao Preto, São Paulo, Brazil
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Background Cardiac dysfunction due to impaired myocardial contractility has been recognized as an important factor contributing to high mortality in septic patients. A recent study from our laboratory gives support to the opinion that myocardial structural change, classifiable as inflammatory cardiomyopathy, could be responsible for sepsis-induced myocardial dysfunction. The present study describes intercalated disc remodeling under both protein expression and structural features in experimental severe sepsis induced by cecal ligation and puncture (CLP) in mice.

Materials and methods Male C57Bl/6 mice weighing between 18 and 22 g were subjected to sham-operated surgery, moderate or severe septic injury, both induced by CLP.

Results Severe septic injury was accompanied by large number of bacteria in the peritoneal cavity and blood, high levels of TNFα and MIP-1α in the septic focus and serum, marked hypotension, and high mortality rate. Western blot analysis and immunofluorescence showed a marked decrease of key gap and adherens junction proteins (connexin43 and N-cadherin, respectively) in mice subjected to severe septic injury. These changes may result in the loss of intercalated disc structural integrity characterized in the electron microscopic study by partial separation or dehiscence of the gap junction and adherens junctions.

Conclusion Our data provide important insight regarding the alterations in intercalated disc components resulting from severe septic injury. The intercalated disc remodeling under both protein expression and structural features in experimental severe sepsis induced by CLP may be partly responsible for myocardial depression in sepsis/septic shock. The abnormal parameters may emerge as therapeutic targets and their modulation might provide beneficial effects on future cardiovascular outcomes and mortality in sepsis.

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P46

The critical role of heme oxygenase in neutrophil migration impairment in polymicrobial sepsis

Andressa Freitas, Jose Carlos Alves-Filho, Marina Moreira Suavinha, Fernando Spiller, Daniela Dal-Secco, Fernando Queiroz Cunha
Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of São Paulo, Ribeirao Preto, São Paulo, Brazil
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Background Sepsis is a systemic inflammatory response resulting from the inability of the host to restrict local infection. During severe sepsis occurs a marked failure of neutrophil migration into the infectious focus, which is associated with dissemination of infection resulting in high mortality. Recently, we showed that heme oxygenase (HO) products, carbon monoxide and biliverdin, downregulate neutrophil recruitment by reducing the neutrophil/

endothelium rolling and adhesion in a noninfectious inflammatory model. This study aimed to investigate a possible role of the HO-1 pathway on the failure of neutrophil recruitment in mice subjected to severe (S-CLP) polymicrobial sepsis induced by cecal ligation and puncture (CLP).

Materials and methods To evaluate the role of HO-1 in S-CLP, the Balb/c mice were pretreated with a specific HO-1 inhibitor, zinc protoporphyirin IX (ZnPPIX 30 mg/kg, subcutaneously) 30 minutes before severe sepsis induction. Mice were killed 6 hours after CLP and HO-1 expression in mesenteric tissue and in blood neutrophils were determined. In another set of experiments, mice were killed 6 hours and 12 hours after CLP, and neutrophil migration to the peritoneal cavity, bacteremia, lung neutrophil sequestration assessed by myeloperoxidase activity and lung histology, cytokines, liver, kidney and cardiac function and mean arterial pressure were determined. The survival rate of animals was assessed every 12 hours up to 120 hours after surgery.

Results A significant increase in HO-1 expression was observed in mesenteric tissue and in blood neutrophils after S-CLP. The ZnPPIX pretreatment prevents the failure of neutrophil endothelium rolling, adhesion and migration observed in vehicle-pretreated mice subjected to S-CLP. As consequence, these mice presented reduced bacteremia, low levels of seric TNFα and lung neutrophil sequestration, reduced liver, kidney and cardiac injury and improved mean arterial pressure, resulting in an increase of the survival rate.

Conclusions These data suggest that during an infectious process HO-1 displays a crucial role on the failure of neutrophil migration to infectious focus and consequently on the susceptibility in severe sepsis.

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P47

TLR2 signaling downregulates chemokine receptor CXCR2 and impairs neutrophil migration in severe polymicrobial sepsis

Jose Carlos Alves-Filho, Andressa Freitas, Fernando Spiller, Fabricio Souto, Heitor Paula-Neto, Joao Santana Silva, Fernando Queiroz Cunha

Department of Pharmacology and Immunology, School of Medicine of Ribeirao Preto, University of São Paulo, São Paulo, Brazil

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Background There is a marked defect in neutrophil migration into the infectious focus during severe sepsis, which is associated with the severity of disease. We recently demonstrated that this phenomenon is a consequence of the downregulation of chemokine receptor CXCR2 on the surface of circulating neutrophils. Toll-like receptors (TLRs) are pattern-recognition receptors that are important in innate immune responses to bacterial infection. TLR activation in phagocytes produces proinflammatory cytokines and chemokines that contribute directly to elimination of infectious agents. However, a sustained inflammatory response can result in tissue damage and sepsis. Here, we address the role of TLR2 in the downregulation of CXCR2 and establishment of neutrophil migration impairment in severe sepsis.

Materials and methods TLR2-deficient ($TLR2^{-/-}$) and C57BL/6 (WT) mice were subjected to severe polymicrobial sepsis by the cecal ligation and puncture (CLP) model, and neutrophil migration, bacteremia, CXCR2 expression and cytokines levels were evaluated.

Results It was observed that TLR2 is critical for downregulation of CXCR2 expression on circulating neutrophils during severe sepsis, since this event was prevented in $TLR2^{-/-}$ mice. In accordance, $TLR2^{-/-}$ mice did not present failure of neutrophil migration into the infectious focus and, consequently, they presented lower bacteremia and did not display systemic inflammation determined by reduced levels of circulating cytokines, showing an improved survival rate. Furthermore, *in vitro*, TLR2 agonist (lipoteichoic acid) was able to downregulate CXCR2 expression and to markedly inhibit neutrophil chemotaxis induced by CXCR2-ligand. The downregulation of CXCR2 was associated with enhanced expression of G-protein-coupled receptor kinase-2 (GRK-2), which is known to play an important role in the desensitization and internalization of this chemokine receptor. Finally, we showed that *in vitro* lipoteichoic-acid-stimulated neutrophils adoptively transferred into naïve WT mice display a significantly reduced competence to migrate into the perinoneal cavity in response to thioglycolate.

Conclusion Altogether, these findings suggest that TLR2 through GRK-2 signaling downregulates CXCR2 expression on the surface of circulating neutrophils, which is a critical determinant of impairment of neutrophil migration into the infection focus during severe sepsis.

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P48

The activity of the endothelial Tie-2 receptor modulates ventilation-induced lung injury in septic mice

Shirley Mei[1,2], Jack Haitsma[3], Daniel James[3], Colleen Parker[2], Arthur Slutsky[3], Duncan Stewart[1,2]

[1]Institute of Medical Science, University of Toronto, Toronto, ON, Canada; [2]Terrence Donnelly Heart Centre, St Michael's Hospital, Toronto, ON, Canada; [3]Interdepartmental Division of Critical Care Medicine, University of Toronto, Toronto, ON, Canada

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Background Patients requiring mechanical ventilation, particularly in the presence of sepsis, are at risk for ventilation-induced lung injury (VILI), which is characterized by increased pulmonary vascular permeability and inflammation. Inflammation in VILI is initiated through interactions between endothelial and inflammatory cells. The vasoprotective protein angiopoietin-1, a ligand for the endothelial-selective tyrosine kinase receptor Tie-2, has recently been recognized to play a critical role in regulating endothelial inflammation and vascular permeability. In contrast, angiopoietin-2, which binds with equal affinity, has been described as a Tie-2 antagonist, inhibiting receptor activation in response to angiopoietin-1. We therefore examined the role of the angiopoietins/Tie-2 pathway in mechanically ventilated, septic mice.

Materials and methods Wild-type (WT) or $Tie\text{-}2^{+/-}$ mice were anaesthetized and sepsis was induced by cecum ligation and puncture (CLP). After 24 hours, animals were ventilated for 6 hours to induce VILI. Animals were ventilated in a pressure-controlled mode, at a fractional inspired oxygen concentration of 0.5, an inspiration to expiration ratio of 1:2, a peak inspiratory pressure of 14 cmH_2O and a positive end-expiratory pressure of 2 cmH_2O. Animals were sacrificed and bronchoalveolar lavage (BAL) was performed for total cell count, differential, and permeability parameters. Inflammatory cytokine IL-6 levels were measured in BAL and plasma.

Results There was no difference between sham-operated or CLP-operated WT mice in total cell numbers in BAL. Sham operated $Tie\text{-}2^{+/-}$ mice had similar cell numbers, while ventilation in CLP-operated $Tie\text{-}2^{+/-}$ mice resulted in higher cell numbers ($P = 0.06$ versus WT), with an increase mainly in macrophages. IL-6 plasma levels were increased in septic $Tie\text{-}2^{+/-}$ mice subjected to mechanical ventilation compared with the WT mice, whereas IL-6 levels in BAL were not significantly different. Despite the higher total cell counts, permeability parameters measured in $Tie\text{-}2^{+/-}$

mice were lower than in WT mice (albumin, $P = 0.08$; total proteins, not significant; IgM, $P = 0.05$).
Conclusions Pulmonary vascular permeability in mice with knockdown of endothelial Tie-2 receptor is preserved in ventilated septic mice. However, there was an increase in the number of inflammatory cells and systemic inflammation (IL-6) in these animals. These results further emphasize the importance of Tie-2 in regulating vascular permeability in the lungs.

P49

Pharmacological implications of the spleen in sepsis

Bolin Cai, Fei Chen, Jared Huston, Luis Ulloa
Laboratory of Surgical Immunology, University of Medicine and Dentistry of New Jersey, Newark, NJ, USA
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Background Our previous studies indicated that nicotine attenuated systemic inflammation, and improved survival in both lethal endotoxemia and cecal ligation and puncture [1].
Materials and methods Here we analyzed the pathophysiological and pharmacological implications of the spleen in experimental sepsis. Acetylcholine and nicotine inhibit the production of TNF and HMGB1 from lipopolysaccharide-treated RAW264.7 macrophage-like cells in a concentration-dependent manner. Nicotine inhibits the production of these cytokine macrophage-like cells through a mechanism dependent on the α_7-nicotine acetylcholine receptor (nAChR). From a physiological perspective, our studies indicate that the spleen has a significant expression of the α_7nAChR. To study the implications of the splenic α_7nAChR in the therapeutic potential of nicotine in sepsis, we analyzed the effects of treatment with nicotine in splenectomized mice. Adult male 8–12-week-old BALB/c mice (25–30 g) were splenectomized and 7 days later subjected to cecal ligation and puncture to induce polymicrobial peritonitis. Control sham animals underwent laparotomy without splenectomy. Administration of nicotine (400 mg/kg, i.p.) or vehicle injection (1 × PBS, i.p.) began 24 hours after surgery, and thereafter twice a day for 3 days. Animals were euthanized after 44 hours for serum cytokine analysis.
Results Treatment with nicotine improved survival in sham mice but it worsened the survival of splenectomized mice. Nicotine inhibited the production of TNF and HMGB1 from lipopolysaccharide-treated macrophages differentiated from peripheral blood mononuclear cells. However, *in vivo*, nicotine attenuated serum TNF levels in sham mice but not in splenectomized mice with polymicrobial peritonitis. There were no differences in serum TNF, IL-12, IFNγ or IL-10 levels between vehicle-treated or nicotine-treated splenectomized animals. Unlike systemic TNF, circulating HMGB1 levels correlated with the increased mortality induced by nicotine in splenectomized mice. Treatment with nicotine increased circulating serum HMGB1 levels in splenectomized mice with experimental sepsis.
Conclusion These results suggest that the spleen has major pharmacological implications for the treatment of sepsis.
Acknowledgements Supported by grants of the AHA and the USAMRMC Log#05308004 to LU.
Reference
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P50

Peroxynitrite mediates neutrophil migration failure in severe polymicrobial sepsis

Daniela Dal-Secco, Diego Torres-Dueñas, Andressa Freitas, Mara Rubia Nunes Celes, José Carlos Alves-Filho, Fernando Spiller, Vitor Faeda Dalto, Marcos Antonio Rossi, Sérgio Henrique Ferreira, Fernando Queiroz Cunha
Departments of Pharmacology and Pathology, Faculty of Medicine of Ribeirao Preto, University of São Paulo, Ribeirao Preto, São Paulo, Brazil
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Background Sepsis is a systemic inflammatory response resulting from the host inability to restrict local infection. The failure of neutrophil migration to the infectious focus is one of the mechanisms involved in this process. Recently, it was demonstrated that this event is mediated by nitric oxide. The present study addressed the possibility that peroxynitrite ($ONOO^-$), a nitric oxide-derived powerful oxidizing and nitrating compound, can be also involved in the neutrophil migration failure.
Materials and methods Male C57Bl/6 mice were pretreated, subcutaneously, with saline or uric acid (UA; 100 mg/kg) 1 hour before induction of severe sepsis injury (SSI) or were treated with Tetrakis (FeTPPS; 5.0 mg/kg) 15 minutes after SSI, induced by cecal ligation and puncture. Intravital microscopy was used to evaluate leukocyte rolling and adhesion in the mesenteric microcirculation. TNFα and MIP-1α levels were evaluated by ELISA, lung neutrophil influx by myeloperoxidase activity and nitrotyrosine by immunofluorescence.
Results The mice pretreated with UA (an $ONOO^-$ scavenger) and subjected to SSI did not present failure of neutrophil rolling, adhesion and migration to the infectious focus. As a consequence, they presented low bacteremia, diminished TNFα and MIP-1α levels in circulation and reduced myeloperoxidase activity. Moreover, increased nitrotyrosine labeling detected in leukocytes present in mesenteric tissues and neutrophils obtained from SSI peritoneal exudate were reduced by UA pretreatment. Finally, the UA pretreatment enhanced significantly the survival rate of the SSI mice. Similarly, treatment with Tetrakis (FeTPPs), a more specific $ONOO^-$ scavenger, reestablished neutrophil migration and increased the survival rate.
Conclusion Together, the results indicate that $ONOO^-$ partially mediates the reduction of neutrophil/endothelium cell interaction and consequently the neutrophil migration failure to the infectious focus and susceptibility in severe sepsis. Therefore, these results identify $ONOO^-$ inhibitors as potential targets for novel sepsis therapies.
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P51

Selective V1a receptor agonists in experimental septic shock

Daniel Traber
Department of Anesthesiology, University of Texas Medical Branch, Galveston, TX, USA
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Background Septic shock is caused by hypotension secondary to vasodilation. This hypotension is resistant to fluid resuscitation and requires the use of vasopressor agents. Recently, it was shown that it may be due to a deficiency in the vasopressor hormone arginine–vasopressin (AVP), a mixed V1a/V2 receptor agonist, leading to the clinical use of AVP in septic shock. We tested the hypothesis that the selective V1a receptor agonist FE 202158 is at

least as effective as AVP in a sheep model of pneumonia-induced septic shock.

Materials and methods Sheep were surgically instrumented ahead of the study. After obtaining baseline data, they were anesthetized and insufflated with 48 breaths of cotton smoke and 1,011 colony forming units of *Pseudomonas aeruginosa* instilled into their airways via a tracheostomy. The sheep were then placed on a ventilator and awakened for the study. They were resuscitated with Ringer's solution to maintain left atrial and central venous pressures and hematocrit at baseline levels. If, despite fluid management, the mean arterial pressure (MAP) fell by 10 mmHg from baseline, a continuous intravenous infusion of AVP or FE 202158 was initiated and titrated to keep MAP within this limit, except in a septic control group and in a nonseptic sham group.

Results In the septic control group, MAP fell by ~30 mmHg and the systemic vascular resistance index (SVRI) by ~50% over the 24-hour study. In the AVP group, although the MAP and SVRI were maintained above septic control group levels, they could not be brought back to sham group levels. In contrast, in the FE 202158 group, the MAP and SVRI were maintained at sham group levels. The septic control group accumulated ~7 l fluid over the 24-hour study (~20% body weight), while this was reduced by ~50% in the AVP group, and was totally prevented in the FE 202158 group. The fluid accumulation was associated with a constant hematocrit, suggesting that it was extravascular. It was also correlated with a fall in plasma total protein and oncotic pressure, suggesting protein leakage from the vasculature and probably reflecting sepsis-induced vascular leak syndrome. The full prevention of this fluid and protein leakage with FE 202158 was reversed to AVP group levels by infusing the selective V2 receptor agonist desmopressin together with FE 202158.

Conclusion FE 202158 was not only fully effective at preventing a sepsis-induced decrease in MAP and SVRI, but was superior to AVP at reducing vascular leak syndrome because of its V1a receptor selectivity.

P52

Gender, infection, critical illness, and death: superior survival for female trauma patients admitted to the intensive care unit treated for infection in a dedicated, prospective, multicenter study

Brian Swenson, Hugo Bonatti, Lesly Dossett, Addison May, Robert Sawyer

University of Virginia Health System, Department of Surgery, Charlottesville, VA, USA

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Background Multiple studies have suggested gender-based differences exist in infection incidence and outcome in critically ill patients, but these relationships have not been clearly defined. We present the largest prospective multicenter study designed to measure the impact of gender on infection incidence and outcome in critically ill surgical and trauma patients to date.

Methods Patients admitted for at least 3 days to surgical or trauma intensive care units (ICUs) at two academic tertiary care centers from October 2001 to May 2006 were prospectively followed for the occurrence of infection and clinical outcomes. Logistic regressions were performed to assess independent risk of gender on the acquisition of an ICU infection and inhouse mortality in patients with ICU-acquired infections. Demographic and clinical variables that were significant by univariate analyses were included in the models.

Results The final cohort followed 2,291 patients (trauma $n = 1,407$, nontrauma $n = 884$) with 383 (27.2%) female trauma patients and 413 (46.7%) female nontrauma patients. The proportion of

Table 1 (abstract P52)

Logistic regression analysis assessing predictors of mortality in patients with ICU-acquired infections

	Infected trauma patients ($n = 652$)			Infected nontrauma patients ($n = 397$)		
Variable	Odds ratio	95% confidence interval	*P* value	Odds ratio	95% confidence interval	*P* value
Female gender	**0.45**	**0.22–0.95**	**0.036**	1.15	0.696–1.911	0.58
Age, per year	1.02	0.10–1.04	0.087	**1.04**	**1.019–1.062**	**<0.001**
White race	1.00	–		1.00	–	–
Black race	0.62	0.24–1.62	0.33	1.08	0.44–2.59	0.87
Other race	1.03	0.29–3.67	0.97	**3.61**	**1.08–12.1**	**0.037**
Any comorbidity	0.89	0.40–2.00	0.77	0.76	0.29–1.98	0.57
Chronic corticosteroid use	0.48	0.05–5.0	0.54	1.35	0.66–2.74	0.41
Hepatic insufficiency	**4.25**	**1.33–13.6**	**0.015**	1.61	0.78–3.33	0.20
Cardiac disease	1.18	0.49–2.82	0.72			
Baseline serum creatinine ≥2.0 (units)	**7.46**	**1.11–50.1**	**0.039**			
Malignancy	0.17	0.018–1.56	0.12	1.39	0.80–2.44	0.24
Body mass index, per kg/m^2				0.98	0.96–1.01	0.24
Admission from home				1.03	0.41–2.60	0.95
McCabe score at ICU admission, per point	1.21	0.60–2.44	0.59	1.35	0.91–2.01	0.14
APACHE II score at ICU admission, per point	**1.07**	**1.02–1.12**	**0.0081**	**1.08**	**1.04–1.12**	**<0.001**
WHO functional status at ICU admission, per point	1.46	0.89–2.39	0.14			
Multiple Organ Dysfunction score at ICU admission, per point	**1.12**	**1.04–1.22**	**0.0040**			
Probability of trauma survival	0.49	0.23–1.02	0.055			
ICU length of stay, per day	1.02	0.97–1.07	0.42			
Hospital length of stay, per day	**0.95**	**0.91–0.98**	**0.032**			

Bold data are statistically significant.

patients with at least one ICU-acquired infection was 47.6% for trauma males and 43.1% for trauma females ($P = 0.13$); and was 44.2% for nontrauma male patients and 45.8% for nontrauma female patients ($P = 0.63$). The inhospital mortality of patients with at least one ICU-acquired infection was 12.5% for trauma males and 7.3% for trauma females ($P = 0.06$); and was 30.8% for nontrauma male patients and 28.0% for nontrauma female patients ($P = 0.55$). Logistic regression analysis did not show gender to be a significant variable in the acquisition of an ICU infection. The results of a logistic regression analysis of predictors of inhospital mortality in patients with ICU-acquired infections are presented in Table 1. Only variables significant by univariate analysis were entered into logistic models. Empty cells represent variables that were significant for only one group (for example – only trauma, but not nontrauma).

Conclusion Gender does not appear to play a role in acquisition of an ICU infection in either trauma or nontrauma patients. In contrast, female gender appears to provide a strong survival advantage in trauma patients with ICU-acquired infections. This phenomenon was not observed in the nontrauma group. Further investigation into the hormonal and cytokine differences between the genders in critically ill trauma patients and their response to infection may prove beneficial.

P53

Effects of antifungal agents on the production of cytokines from macrophages stimulated by *Aspergillus fumigatus* conidia

Jung-Hyun Choi[1], Eun-Young Kwon[2], Chulmin Park[2], Sun Hee Park[1], Su-Mi Choi[1], Dong-Gun Lee[1], Jin-Hong Yoo[1], Wan-Shik Shin[1]

[1]Department of Internal Medicine, Division of Infectious Diseases, College of Medicine, Catholic University of Korea, Korea; [2]Catholic Research Institutes of Medical Science, Catholic University of Korea, Korea

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Background We evaluated the immunomodulatory effect of three antifungal agents, amphotericin B (AmB), micafungin (MF) and itraconazole (ITZ), in the aspect of cytokine production and expression of NF-κB in *Aspergillus fumigatus* conidia-treated RAW264.7 cells, a murine alveolar macrophage cell line.

Materials and methods We evaluated the cytotoxic effect of antifungal agents using a commercial cell proliferation assay. TNFα and IL-10 production according to stimulation (control, *A. fumigatus* conidia only, conidia + antifungal drug, conidia + antifungals + GM-CSF) was evaluated and each compared using a commercial ELISA method. NF-κB activation was evaluated by western blot analysis.

Results AmB, MF and ITZ showed a dose-dependent cytotoxic effect on the tested cells. Stimulation of cells by *A. fumigatus* conidia induced TNFα production. Pretreatment of AmB at concentrations not affecting cellular survival did not change the production of TNFα compared with conidia-treated cells, but pretreatment of MF or ITZ showed a reduced amount of TNFα production compared with conidia-treated cells. AmB also showed a synergistic effect on TNFα production when simultaneously treated with GM-CSF. IL-10 production was markedly increased when the cells were treated with AmB with conidia. MF and ITZ induced a smaller increase of IL-10 production. AmB also showed a synergistic effect on the production of IL-10 when treated with GM-CSF simultaneously. *A. fumigatus* conidia enhanced expression of NF-κB. The degree of NF-κB expression was associated with the amount of TNFα and IL-10 produced.

Conclusion The antifungal agents we used in this experiment showed decreased TNFα production and increased IL-10 production from the RAW264.7 cells stimulated by *A. fumigatus* conidia after pretreatment of antifungal agents. But more studies such as the association between the immunomodulatory effect and antifungal activity and the difference of the signal pathway of cellular activation according to drugs should be performed.

P54

Time to positivity as a novel predictor of outcome in intensive care unit patients with sepsis

Susie Jerwood[1], Sonia Hudson[1], Matthew Hankins[2], John Paul[1], Owen Boyd[1], Jonathan Cohen[2]

[1]Brighton Sussex County Hospital, Eastern Road, Brighton, Sussex, UK; [2]Brighton and Sussex Medical, Falmer, Sussex, UK

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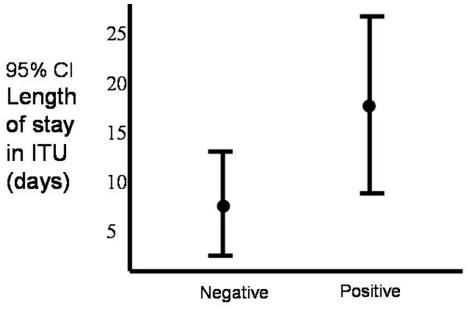
Background Inadequate antibiotic therapy predicts poor outcome from sepsis, but there is no simple test of adequacy. We suggested that a time-to-positivity assay (T_{pos}) might act as a surrogate for antimicrobial activity and predict outcome from sepsis in the intensive care unit (ICU) [1]. We are conducting a prospective clinical trial to test this hypothesis.

Methods We studied 35 sequential ICU patients with onset of sepsis who had not had antibiotics for at least 3 days. Sepsis was defined as clinical evidence of infection plus at least three criteria for systemic inflammatory response syndrome. All patients received standard empiric therapy. Sera taken at 24 hours post antibiotics were inoculated into blood culture bottles containing standardised bacteria, incubated in an automated microbial detection system and the time to positivity noted. The primary clinical endpoint was days in the ICU.

Results Cultures that are negative after 5 days of incubation indicate adequate antimicrobial therapy; cultures that become positive in <5 days indicate inadequate antimicrobial therapy. Patients with negative T_{pos} ($n = 11$) were associated with a stay in the ICU of less than 6.5 days ($P = 0.052$). See Figure 1.

Figure 1 (abstract P54)



Average length of stay for patients with negative and positive time-to-positivity 1 (taken at 24 hours after first dose of antibiotics) with 95% confidence intervals.

Conclusion These data show that some septic patients on standard antimicrobial regimens are receiving inadequate therapy. The T_{pos} is a new, simple assay that might be used effectively to monitor antibiotic use.

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