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P1

Urokinase receptor is necessary for bacterial defense against Gram-negative sepsis (melioidosis) by facilitating phagoctytosis

W Joost Wiersinga[1,2], JWR Hovius[1,2], GJW van der Windt[1,2], JCM Meijers[3], JJ Roelofs[4], A Dondorp[5], M Levi[1], NP Day[5,6], SJ Peacock[5,6], T van der Poll[1,2]
[1]Center for Infection and Immunity Amsterdam, [2]Center for Experimental and Molecular Medicine, [3]Department of Vascular Medicine and [4]Department of Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; [5]Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [6]Center for Clinical Vaccinology and Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, UK
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Introduction Urokinase receptor (uPAR, CD87), a glycosylphosphatidylinositol-anchored protein, is considered to play an important role in inflammation and fibrinolysis. The Gram-negative bacterium *Burkholderia pseudomallei* is able to survive and replicate within leukocytes and causes melioidosis, an important cause of pneumonia-derived community-acquired sepsis in Southeast Asia. We here investigated the expression and function of uPAR both in patients with septic melioidosis and in a murine model of experimental melioidosis.
Methods Using a translational approach we conducted a patient study in patients with culture-confirmed sepsis caused by *B. pseudomallei*, *in vitro* experiments using wild-type (WT) and uPAR knockout (KO) cells, and mouse studies using WT and uPAR KO mice inoculated with *B. pseudomallei.*
Results uPAR mRNA and surface expression was increased in patients with septic melioidosis in/on both peripheral blood monocytes and granulocytes as well as in the pulmonary compartment during experimental pneumonia-derived melioidosis in mice. uPAR-deficient mice intranasally infected with *B. pseudomallei* showed an enhanced growth and dissemination of *B. pseudomallei* when compared with WT mice, corresponding with increased pulmonary and hepatic inflammation. uPAR KO mice demonstrated significantly reduced neutrophil migration towards the pulmonary compartment after inoculation with *B. pseudomallei*. Further *in vitro* experiments showed that uPAR-deficient macrophages and granulocytes display a markedly impaired phagocytosis of *B. pseudomallei*. Additional studies showed that uPAR deficiency did not influence hemostatic and fibrinolytic responses during severe melioidosis.
Conclusions These data suggest that uPAR is crucially involved in the host defense against sepsis caused by *B. pseudomallei* by facilitating the migration of neutrophils towards the primary site of infection and subsequently facilitating the phagocytosis of *B. pseudomallei.*

P2

A comparison of acute lung inflammation in *Klebsiella pneumoniae* B5055-induced pneumonia and sepsis in BALB/c mice

V Kumar, S Chibber
Department of Microbiology, Panjab University, Chandigarh, India
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Introduction Lungs play an important role in the body's defense against a variety of pathogens, but this network of immune system mediated defense can be deregulated during acute pulmonary infections.
Objective The present study compares the acute lung inflammation (ALI) occurring during *Klebsiella pneumoniae* B5055-induced pneumonia and sepsis in BALB/c mice.
Methods Pneumonia was induced by intranasal instillation of bacteria (10^4 CFU) while sepsis was developed by placing the fibrin–thrombin clot containing a known amount of bacteria (10^2 CFU) into the peritoneal cavity of animals. Various cytokine (TNFα and IL-1α) levels were estimated using ELISA and the degree of lung inflammation (that is, inflammatory cell infiltration) was evaluated by histopathological analysis. The other markers of inflammation (that is, nitric oxide (NO), malondialdehyde (MDA) and myeloperoxidase (MPO)) were estimated by standard biochemical methods.
Results Mice with sepsis showed 100% mortality within 5 post infection days whereas all the animals with pneumonia survived. In animals suffering from *K. pneumoniae* B5055-induced pneumonia all the inflammatory parameters (TNFα, IL-1α, MPO, MDA and NO) were found to be maximum until the third post infection day, after that a decline was observed, whereas in septic animals all the above-mentioned markers of inflammation kept on increasing until death of the animals. Histopathological study showed that inflammatory damage to the lungs in pneumonia was not very severe as lesser neutrophil infiltration and pulmonary damage (that is, alveolitis, bronchiolitis, endothelitis and perivascular congestion) was seen as compared with lungs taken from septic animals. This can be further strengthened by the presence of alternatively activated alveolar macrophages (AAMacs) or foam cells in lungs of mice with pneumonia after the third post infection day and their number kept on increasing until the seventh post infection day, which might have contributed to the induction of resolution of inflammation and clearance of the infection. But no such AAMacs or foam cells were seen in lungs of septic mice on histopathological examination, lungs were seen to be infiltrated with only neutrophils on all experimental days.
Conclusions Hence, during pneumonia controlled activation of AAMacs or foam cells led to the resolution of inflammation and infection as well.

P3

Performance evaluation and further development of the PCR and microarray-based Prove-it™ Sepsis assay

P Tissari[1], E Tarkka[1], S Mero[1], L Savolainen[1], M Vaara[1], A Zumla[2], J Huggett[2], C Carder[2], V Gant[2], A Aittakorpi[3], S Laakso[3], M Lindfors[3], P Piiparinen[3], N Kumlin[3], H Piiparinen[3], M Mäki[3]

[1]Division of Clinical Microbiology, Helsinki University Hospital Laboratory HUSLAB, Helsinki, Finland; [2]Department of Clinical Microbiology, University College London Hospitals NHS Foundation Trust, and University College London Medical School, Centre for Infectious Diseases and International Health, London, UK; [3]Mobidiag Ltd, Helsinki, Finland

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Introduction The Prove-it™ Sepsis assay is a rapid, broad-range PCR and microarray-based assay designed to identify the majority of sepsis-causing bacteria from positive blood cultures. The pathogen panel covers 50 Gram-negative and Gram-positive bacterial species (Table 1). It also reports methicillin resistance by detecting the mecA gene. The assay time is 3 hours. Our objective was to conduct a performance evaluation study for Prove-it™ Sepsis according to the EN 13612-standard (Performance evaluation of *in vitro* diagnostic medical devices) and to compare obtained results with those of current culture-based diagnostics. We evaluated the sensitivity, specificity and time to result of Prove-it™ Sepsis in two major teaching hospitals in Helsinki and London.

Materials A total of 3,318 blood samples from patients with suspected sepsis were collected. Blood culture bottles of BacT/ALERT 3D (bioMérieux) and BACTEC 924 (Becton Dickinson) were incubated for a total of 6 days or until flagged as positive.

Methods DNA was extracted from blood cultures using the automated DNA extraction instrument easyMAG (bioMérieux) prior to the Prove-it™ Sepsis assay. Conventional blood culture was conducted in parallel and results were only revealed for comparison at the statistical analysis stage. Discordant results were studied by DNA sequencing and case-by-case review of original microbiology laboratory data.

Results Of the analyzed 3,318 blood cultures, 2,107 yielded a pathogen by conventional techniques. Of these, 302 samples contained microbes not covered by Prove-it™ Sepsis, and an additional 137 cultures contained more than one organism. Sensitivity and specificity for Prove-it™ Sepsis were 94.7% and 98.7%, respectively. Of particular significance was the assay's faultless ability to differentiate MRSA from MSSA and from CNS. Furthermore, it provided results on average 1 day earlier than reference methods.

Conclusions Prove-it™ Sepsis was considered to be a fast, robust, and high-performance diagnostic platform, which is easily implemented into everyday laboratory workflow. Both study sites identified cases where timely information provided by Prove-it™ Sepsis would have significantly improved patient management. Examples include more rational management and antibiotic choice subsequent to earlier differentiation of Gram-positive cocci in clumps into MRSA, MSSA, or CNS, and earlier speciation of Gram-negative organisms. Prove-it™ Sepsis is further configured for detection of *Candida* spp. and new bacterial targets. The assay now identifies 60 out of the 302 samples not covered during the evaluation, increasing the pathogen coverage from 86% to 89%. The earlier speciation provided by Prove-it™ Sepsis could contribute to faster, more evidence-based patient management and, thus, positive outcomes.

Table 1 (abstract P3)

Bacteria and an antibacterial resistance marker identified by the Prove-it™ Sepsis assay

Gram-negative	Gram-positive	Antibacterial resistance
Neisseria meningitidis	*Staphylococcus aureus*	Methicillin resistance marker mecA
Enterobacter aerogenes	*Staphylococcus epidermidis*	
Enterobacter cloacae	Coagulase-negative *Staphylococcus*[d]	
Escherichia coli	*Streptococcus pyogenes*	
Klebsiella oxytoca	*Streptococcus agalactiae*	
Klebsiella pneumoniae	*Streptococcus dysgalactiae* subsp. *equisimilis*	
Proteus mirabilis	*Streptococcus pneumoniae*	
Proteus vulgaris	*Enterococcus faecalis*	
Salmonella enterica subsp. *enterica*[a]	*Enterococcus faecium*	
Serratia marcescens	*Listeria monocytogenes*	
Enterobacteriaceae family[b]	*Clostridium perfringens*	
Acinetobacter baumannii		
Pseudomonas aeruginosa		
Stenotrophomonas maltophilia		
Haemophilus influenzae		
Campylobacter jejuni/coli		
Bacteroides fragilis group[c]		

[a]*Salmonella enterica* subsp. *enterica* covers at least the following serovars: *Enteritidis*, *Oranienburg*, *Othmarschen*, *Paratyphi*, *Stanley*, *Typhi*, *Typhimurium*, Virchow, Group A, B, C, D. [b]Enterobacteriaceae covers at least the following species: *Citrobacter amalonaticus*, *Citrobacter freundii*, *Citrobacter koseri*, *Citrobacter braakii*, *Enterobacter hormaechei*, *Enterobacter sakazakii*, *Kluyvera intermedia*, *Morganella morganii*, *Pantoea agglomerans*, *Providencia rettgeri*, *Providencia stuartii*, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis.* [c]*Bacteroides fragilis* covers at least the following species: *B. fragilis*, *B. vulgatus*, *B. thetaiotaomicron.* [d]Coagulase-negative *Staphylococcus* covers at least the following species: *S. capitis*, *S. lugdunensis*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, *S. warneri*, *S. xylosus*.

P4

Nandrolone abuse aggravates septic shock

YF Hsu[1], C Lin[1,2], D-R Chen[1,2]
[1]Department of Research, and [2]Department of Surgery, Changhua Christian Hospital, Changhua, Taiwan
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Introduction One million individuals in the United States alone are estimated to be current or past users of anabolic-androgenic steroid.
Methods To investigate the effects of nandrolone, an anabolic-androgenic steroid, 108 6-week-old male BALB/c inbred mice were used (minimal lethal dose $n = 30$, high-dose vs. low-dose $n = 24$, screening surrogates $n = 6$, and surrogates $n = 48$). Mice were sacrificed at 0, 3 or 6 hours after septic shock induction. The serum levels of malondialdehyde, liver TNFα and spleen IFNγ in mice with septic shock were analyzed. The gene expression of insulin-like growth factor 1, insulin-like growth factor type 1 receptor and insulin-like growth factor binding proteins was also studied.
Results Nandrolone significantly increased serum malondialdehyde at 0, 3 and 6 hours ($P = 0.004$, 0.006 and 0.004), and liver TNFα at 0 and 6 hours ($P = 0.04$ and 0.016). It also increased the spleen IFNγ level at 0 and 6 hours ($P = 0.031$ and 0.01). Compared with 0 hours, the data indicated that nandrolone increases lung insulin-like growth factor type 1 receptor, insulin-like growth factor binding protein 1 and insulin-like growth factor binding protein 2 mRNA expression at 6 hours ($P < 0.05$). These indicated changes due to nandrolone.
Conclusions Nandrolone abuse hastens mortality due to septic shock and increases serum malondialdehyde, liver TNFα, spleen IFNγ level and lung insulin-like growth factor type 1 receptor mRNA, as well as lung insulin-like growth factor binding proteins. Nandrolone abuse may aggravate septic shock.

P5

Use of a screening authorization and randomization center for severe sepsis patient qualification and real-time enrollment in a phase 2 trial of eritoran tetrasodium (E5564), a TLR4 antagonist

J Schentag[1], S Opal[2], M Lynn[3], A Wittek[3], J Wheeler[3]
[1]University at Buffalo School of Pharmacy and Pharmaceutical Sciences, Buffalo, NY, USA; [2]Brown University Medical School, Providence, RI, USA; [3]Eisai Medical Research, Inc., Ridgefield Park, NJ, USA
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Introduction Trials of many promising sepsis modifiers have often failed to demonstrate benefits because of, among other reasons, poor characterization of enrolled patients.
Materials Advantages of utilizing a screening authorization and randomization center (SAC) method to characterize patients in trials for sepsis modifiers are presented.
Methods A central SAC on call 24 hours per day was employed in a phase 2, double-blind, randomized comparison of eritoran 45 and 105 mg versus placebo. SAC activities were conducted (January 2002 to April 2005) by six clinical pharmacists. Severe sepsis was defined with at least three systemic inflammatory response syndrome criteria within 12 hours before onset of ≥1 new organ dysfunction. Patients were randomized and treated within 8 to 12 hours. The 8-hour to 12-hour window for qualification and start of treatment was the primary challenge to the SAC and study sites. Each site used two sequences of drug assignment based on Acute Physiology and Chronic Health Evaluation (APACHE) II predicted mortality to yield a balanced allocation of high and low APACHE II predicted mortality within the three treatment groups: one sequence for subjects with APACHE II predicted mortality 20 to 50%, and another for subjects with predicted mortality 51 to 80% as calculated by the SAC.
Results The SAC screened 1,025 patients from 78 sites; 300 patients from 54 sites were randomized, and 293 were treated. The Independent Clinical Evaluation Committee subsequently qualified 229/293 (78%). Calls to the SAC averaged 24 patients/month; eight patients/month were randomized (33%). Enrollments ranged from 0 to 39 patients/site; 35 sites randomized ≥3 patients; nine sites randomized ≥10 patients. Of 35 sites with ≥3 patients, 6 to 100% led to randomization.
Conclusions Advantages of utilizing the SAC included a high evaluable rate of enrolled patients, correct Predicted Risk of Mortality calculations, timing of qualifying organ failures, drug preparation advice, verification of key clinical data, and eligibility with sponsor prior to enrollment. SAC activities ensured an informative phase 2 trial and will be utilized for the phase 3 trial.

P6

Influence of severity of illness on the effects of eritoran tetrasodium (E5564), a TLR4 antagonist, in patients with severe sepsis

SM Opal[1], AC Kalil[2], SP LaRosa[1], J Gogate[3], M Lynn[3], AE Wittek[3], and the Eritoran Sepsis Study Group
[1]Brown University, Providence, RI, USA; [2]University of Nebraska Medical Center, Omaha, NE, USA; [3]Eisai Medical Research, Inc., Ridgefield Park, NJ, USA
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Introduction Disease severity varies widely in patients with severe sepsis. Previous trials (IL-1RA, TNF-sR p55, antithrombin, and drotrecogin alfa activated (DAA)) suggest that more severely ill patients benefit most from treatment.
Objective The aim of this study was to evaluate the efficacy of eritoran for interaction effects with baseline illness severity.
Methods Prospective covariates from a randomized, double-blind, placebo-controlled, phase 2 trial were analyzed for treatment interaction measured by 28-day mortality. Breslow–Day and multiple logistic regression (LR) were used to assess categorical (CAT) and continuous (CONT) treatment by severity-of-illness interactions.
Results Modified intent-to-treat population ($n = 292$) all-cause 28-day mortality was: placebo, 33.3% (32/96); total eritoran 45 mg/105 mg, 29.6% (58/196). LR analysis identified Acute Physiology and Chronic Health Evaluation (APACHE) II scores, Predicted Risk of Mortality (PROM) scores, IL-6, age, sex, race, and eritoran as associated with survival outcomes. Significant treatment interactions were observed (eritoran vs. placebo) for baseline covariates: APACHE II (CAT, $P = 0.059$; CONT, $P = 0.035$); PROM scores (CAT, $P = 0.028$; CONT, $P = 0.008$); number of organ failures (CAT, $P = 0.079$); international normalized ratio (CAT, $P = 0.05$); and acute physiology score (CONT, $P = 0.039$). No significant treatment interactions were observed with age, sex, shock, DAA use, infection site, microorganism type, platelets, IL-6, or endotoxin levels. Interaction results were similar for eritoran 105 mg only versus placebo.
Conclusions Potential survival benefits of eritoran in severe sepsis patients may be associated with high severity of illness. Treatment by disease severity interaction will be further explored in a phase 3 trial.

P7

Faster differentiation of *Staphylococcus aureus* versus coagulase-negative *Staphylococci* from blood culture material: a comparison of different bacterial DNA isolation methods

AJM Loonen[1], WLJ Hansen[1], A Jansz[2], H Kreeftenberg[2], CA Bruggeman[1], PFG Wolffs[1], AJC van den Brule[3]
[1]Department of Medical Microbiology, Maastricht University Medical Center, Maastricht, the Netherlands; [2]Department of Intensive Care, Catharina Hospital, Eindhoven, the Netherlands; [3]Department of Molecular Diagnostics, Catharina Hospital, Eindhoven, the Netherlands
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Introduction Frequent usage of medical devices, such as intravenous lines, often results in sepsis, which is characterized by high morbidity and mortality. Rapid and reliable detection and differentiation between *Staphylococcus aureus* and coagulase-negative *Staphylococci* (CNS) is therefore clinically relevant to be able to provide adequate early treatment. Blood culture is still the gold standard method in identifying these pathogens but is time consuming. Molecular diagnostics might be a promising alternative to reduce this time-to-result delay.
Objective This study aims to compare different DNA extraction methods from two commonly used blood culture materials, BACTEC (BD) and Bact/ALERT (Biomerieux), to accelerate differentiation between *S. aureus* and CNS.
Methods Two fast real-time PCR duplex test assays, targeting the Tuf gene, to differentiate *S. aureus* from CNS, were developed in order to select the most sensitive one. This Tuf RT-PCR was used to compare three different DNA isolation methods on two different blood culture systems. Negative blood culture material was spiked with *S. aureus*; bacterial DNA was isolated with: automated extractor EasyMAG (Biomerieux), automated extractor MagNA Pure (Roche), and a manual kit MolYsis Plus (MolZyme).
Results The best Tuf RT-PCR method appeared to have a sensitivity of 100 CFU/ml. Approximately 50 positive blood cultures containing Gram-positive cocci in clusters were tested in the Tuf RT-PCR and all were identified correctly. Bacterial DNA isolation, from spiked blood culture material, with the EasyMAG showed the highest analytical performance with a detection limit of 10^3 CFU/ml in Bact/ALERT material, whereas using BACTEC resulted in a detection limit of 10^4 CFU/ml. Hand-on time, for 26 samples, was lowest for the EasyMAG (10 minutes) and highest for the manual kit of MolZyme (2 hours). Total handling time was highest for the MolYsis Plus kit (3.5 hours) and lowest for the automated extractor EasyMAG (50 minutes).
Conclusions A sensitive RT-PCR was developed for detection and differentiation of *S. aureus* versus CNS. Bacterial DNA isolation from Bact/ALERT blood culture material seems to show better reproducibility compared with isolation from BACTEC blood culture material. In this preliminary study the EasyMAG performed better when compared with MolYsis Plus and the MagNA Pure system. In future work this method will be further evaluated with reduced culture times.

P8

Effect of canine hyperimmune plasma on TNFα and inflammatory cell levels in a lipopolysaccharide-mediated rat air pouch model of inflammation

B Essien, M Kotiw, H Buttler, D Strunin
Centre for Systems Biology, University of Southern Queensland, Toowoomba, Queensland, Australia
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Introduction Unregulated elevated levels of serum TNFα have been associated with proinflammatory cytokine cascades that are characteristic in diseases such as septic shock. Endotoxic shock, which has a poorer prognosis than found with other forms of septic shock, is mediated by lipopolysaccharide (LPS), a molecule that is released from the outer membrane of Gram-negative bacteria. LPS is a potent stimulator of TNFα secretion by serum monocytes and tissue macrophages. Whilst the use of monotherapeutic TNFα antagonists has been trialed, none have been registered for use in patients with sepsis.
Objective The purpose of this study was to test the effect of canine hyperimmune frozen plasma (HFP), which is known to contain elevated levels of soluble TNFα receptor 1 (sTNFR1), on TNFα and inflammatory cell levels in a LPS-mediated rat air pouch model of inflammation.
Methods A dorsal air pouch in 175 to 200 g Sprague–Dawley rats was formed by 20 ml subcutaneous infusions of sterile air. Prophylactic subcutaneous injections of canine HFP, canine fresh frozen plasma (FFP) or carprofen were administered daily for 3 days into the lateral flank of the right foreleg at doses recommended by the manufacturers ($n = 10$ for each treatment group). Pouch fluid was harvested by syringe at 1, 6, 12, 24 and 48 hours post LPS administration and subjected to histological and cytokine/cytokine receptor analysis. TNFα and sTNFR1 levels were determined by ELISA and an immunofluorescent dot blot assay.
Results Pouch fluid analysis: maximal effects were detected at 6 hours post LPS administration. TNFα levels were significantly depressed in animals dosed with HFP, but not in animals treated with FFP or carprofen ($P < 0.05$). sTNFR1 levels were significantly elevated in HFP, but not in FFP or carprofen dosed animals ($P < 0.05$). Neutrophil numbers were significantly depressed in HFP dosed but not in FFP or carprofen treated animals ($P < 0.05$).
Conclusions There appears to be a correlation between elevated levels of sTNFR1 and depression of TNFα and neutrophil levels in the pouch fluid of HFP dosed rats ($r = -0.73$, $P < 0.0001$). The data suggest that canine HFP, which has been demonstrated to contain elevated levels of sTNFR1 compared with FFP, has a direct effect on depressing TNFα levels and neutrophil sequestration in the rat air pouch model of inflammation. These data suggest that HFP may be worthy of further investigation to determine whether such preparations have a therapeutic potential for treatment of acute inflammatory diseases in which TNFα is implicated.

P9

Clinical impact of a PCR-based assay for pathogen detection in critically ill patients with evidence of infection

F Bloos[1], A Kortgen[1], S Sachse[2], M Lehmann[3], E Straube[2], K Reinhart[1], M Bauer[1]
[1]Department of Anesthesiology and Intensive Care Medicine, University Hospital Jena, Germany; [2]University Hospital Jena, Institute of Medical Microbiology, Jena, Germany; [3]SIRS-Lab GmbH, Jena, Germany
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Introduction Blood cultures are often negative even in patients with clinical signs of severe sepsis. Furthermore, the long time to result of culture-based methods does not allow the results to guide empiric antimicrobial therapy. PCR-based pathogen detection promises a higher rate of positivity and a faster time to result.
Objective To report the performance of PCR-based pathogen detection compared with blood culture in ICU patients with evidence of infection, and the impact of this test on the antimicrobial therapy.
Methods Patients treated on an interdisciplinary ICU were included into this observational study if a blood culture (BC) was

Figure 1 (abstract P9)

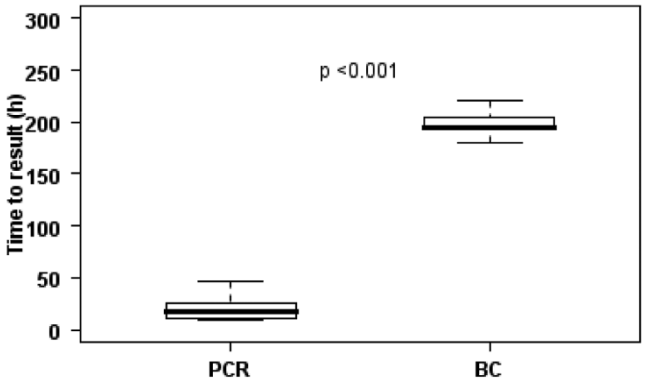
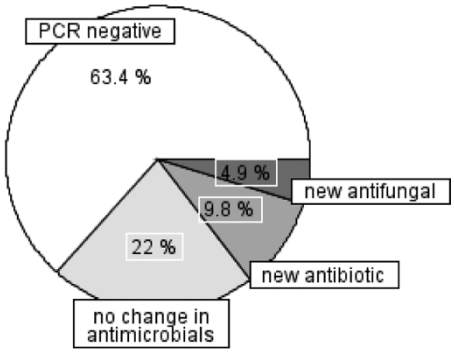


Figure 2 (abstract P9)



drawn on discretion of the treating physician. Blood cultures and EDTA-blood were taken by sterile venous puncture. The EDTA-blood was processed with a PCR-based assay (VYOO®; SIRS-Lab GmbH, Jena, Germany), which detects a panel of 34 bacterial and six fungal pathogens as well as five antibiotic resistances. Data are given as median and interquartile range.

Results Sixty-three patients were included into this study. Age was 68.0 (55.5 to 74.0) years, APACHE II score was 17 (13 to 23), SOFA score at study inclusion was 10.0 (7.50 to 11.0), and ICU mortality was 33.3%. In 54 patients (84.3%) infection was either microbiologically confirmed or clinically proven. The baseline procalcitonin was 2.4 (0.8 to 8.0) ng/ml. Eighty-two pairs of BCs and PCRs have been drawn. Ten (12%) BCs and 30 (36.6%) PCRs were positive (P <0.001). Time to test result was significantly shorter in the PCR than in the BC (Figure 1). Twelve positive PCR results (Figure 2, grey areas) prompted a change in antibiotic or antimicrobial therapy.

Conclusions The PCR-based assay resulted in a considerably higher amount of positive results within a shorter time to result than the BC in this group of high-risk patients with evidence of infection. These data demonstrate that the shorter time to result may guide adjustment of antimicrobial therapy earlier than culture-based methods. Further studies are necessary to prospectively investigate the impact of the PCR technique on antimicrobial therapy and infection control.

P10

Abstract withdrawn

P11

Abstract withdrawn

P12

Abstract withdrawn

P13

Comparison of commercial DNA extraction kits for the detection of bacterial genomic DNA from whole-blood samples using a broad-range PCR

B Krulova, E Nemcova, B Zaloudikova, P Nemec, T Freiberger
Centre for Cardiovascular Surgery and Transplantation, Molecular Genetic Laboratory, Brno, Czech Republic
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Introduction Blood culture is still considered a gold standard for diagnosis of bloodstream infections. Early pathogen detection is a prerequisite for the successful treatment. Nucleic acid based techniques offer a rapid and sensitive option particularly in blood culture negative samples. Efficient bacterial DNA extraction is crucial for following PCR assays. The aim of this study was to determine the detection limit of bacterial genomic DNA using different extraction protocols.

Methods We evaluated five commercially available kits for the extraction of bacterial genomic DNA from whole-blood samples (QIAamp DNA Blood Mini kit, UltraClean DNA BloodSpin Kit, Chemagic DNA Blood Kit, ZR Genomic DNAII Kit, NucliSens miniMAG). Whole-blood samples were spiked with *Escherichia coli* CCM 3988 and *Staphylococcus aureus* CCM 7111. Tenfold dilution series containing concentrations from 10^7 to 10^0 CFU/ml were prepared under sterile conditions and immediately used. A broad-range 16S rDNA end-point PCR was performed for the detection of *E. coli* and *S. aureus* DNA.

Results The sensitivity of each kit was determined as a minimum rate of CFU providing the positive result in the PCR assay. Our results showed the extraction by the QIAamp DNA Blood Mini Kit (supplemented with enzymatic pre-treatment) as the most efficient and sensitive method. This extraction protocol allowed the reproducible detection of *E. coli* and *S. aureus* at concentrations of 10^3 CFU/ml. All kits showed positive results in samples at concentrations from 10^7 to 10^5 CFU/ml.

Conclusions Extraction kits should be capable to recover nucleic acids and remove inhibitors from diverse clinical materials simultaneously. All of the tested kits were able to recover bacterial genomic DNA from whole-blood samples, but the sensitivity of PCR-based detection depends on the DNA extraction protocol used.

P14

Toll-like receptor 9-dependent gene expression *in vivo* is regulated by inductive and suppressive networks

S Klaschik[1,2], D Tross[2], DM Klinman[2]
[1]Department of Anesthesiology and Intensive Care Medicine, University of Bonn, Germany; [2]Cancer and Inflammation Program, NCI Frederick, MD, USA
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Introduction Synthetic oligodeoxynucleotides (ODN) expressing CpG motifs mimic the immunostimulatory activity of bacterial DNA. CpG ODN interact with Toll-like receptor 9 to stimulate an innate immune response characterized by the production of Th1 and proinflammatory cytokines, and the functional maturation of

immune cells. Changes in gene expression mediated by the *in vivo* administration of CpG ODN were identified using microarrays.
Objective We predicted that microarrays could be used to identify reproducible changes in gene expression induced by CpG ODN activation in mice treated *in vivo* over time, and that network analysis would allow us to identify regulators of gene expression.
Materials cDNA was generated from total RNA isolated from spleen cells of mice 30 minutes to 3 days after *in vivo* treatment with 400 μg CpG or control ODN.
Methods Dual-color hybridizations (Biomicro) were performed on murine genome microarrays (NCI). Analyses were conducted on four independently derived RNA samples for each time point. R^2 was 0.90 ± 0.04 for all matched samples. Network analysis was performed using Ingenuity Pathway Analysis.
Results Differential gene activation (P <0.00001) was observed within 30 minutes of CpG ODN treatment (25 genes), peaked at 3 hours (529 upregulated genes), and fell to near background levels after 72 hours. TNFα, IL-1β, NF-κB and IFNγ played central roles in upregulating the early expression of immune-related genes. Of interest, two distinct patterns of gene expression were observed. One subset of genes was activated shortly after CpG ODN administration and remained upregulated for a prolonged period, while unique subsets of additional genes were activated at specific time points, and were rapidly downregulated. Several genes responsible for this downregulation (MYC, IL1RN, SOCS1) were identified.
Conclusions This analysis identifies two distinct patterns of gene regulation associated with CpG-induced activation of the innate immune system of mice. A small number of regulatory genes triggers the patterned upregulation of immune related genes from 30 minutes through 72 hours. A separate set of downregulatory genes subsequently dampens what would otherwise be a continuous positive feedback loop.

P15

Fluid therapy in severe sepsis: results from a representative survey of German ICUs

C Hartog[1], FM Brunkhorst[1], F Bloos[1], C Engel[2], H Bogatsch[3], K Reinhart[1], K Sengebusch[4], M Ragaller[4]
[1]Department of Anesthesiology and Intensive Care Medicine, Jena University Hospital, Germany; [2]Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Germany; [3]Clinical Trial Centre Leipzig, University of Leipzig, Germany; [4]Department for Anesthesiology and Intensive Care Therapy, University Hospital Carl Gustav Carus, Medical Faculty, TU Dresden, Germany
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Introduction Fluid resuscitation is a mainstay of sepsis management.
Objective To describe the practice of fluid therapy in German ICUs.
Methods Analysis of data derived from the SepNet cross-sectional 1-day point-prevalence study of patients with sepsis [1]. ICU directors were asked about their fluid preferences. Data on days after start of sepsis were adjusted to account for overestimation of long-stayers in a point-prevalence survey. SPSS 15.0.1 (SPSS, Chicago, IL, USA) was used; the chi-square test or Kruskal–Wallis H test was applied where appropriate.
Results On operative ICUs, more patients received synthetic colloids (41.4 vs. 21.2%, P <0.001) and crystalloids (74.6 vs. 65.0%, P <0.05), while albumin use did not differ. ICU directors of operative ICUs stated using synthetic colloids more frequently and albumins less frequently than their colleagues from nonoperative ICUs. Stated and actual fluid use did not differ by hospital size. Of 415 patients with severe sepsis, 71.6% received crystalloids. 35.2% synthetic colloids (HES; gelatin, dextran), 16.4% received 5% glucose, and 4.1% albumin solutions. HES was the most frequently used colloid. It was administered to 29.4% of patients as HES 10% in 10.4% (mean dose, 787.8 ± 420.0 ml/24 hours) and as HES 6% in 20.7% (mean dose, 769.1 ± 403.1 ml/24 hours). Patients receiving HES had a higher mean SOFA score than patients without HES (9.92 ± 4.12 vs. 8.00 ± 4.07, P <0.001), tended to more frequent acute renal failure (ARF) (defined as serum creatinine >1.5 mg/dl (132.6 μmol/l) and diuresis ≤500 ml/day, no chronic replacement therapy, RRT), more RRT, lower thrombocyte counts and more frequently received RBCs, but this was non-significant. In total, 29.5% of patients with ARF and 30.3% of patients with RRT received HES. HES was administered to 115 patients between day 0 and day 63 after the start of severe sepsis.
Conclusions Fluid therapy in septic patients varied by type of ICU. About 30% of patients received HES regardless of renal dysfunction. HES was applied for many days after the start of severe sepsis.

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P16

Identification of cathepsin G in the generation of elastase-resistant fragment of vascular endocan: involvement in the regulation of LFA-1-dependent cascade

N De Freitas Caires[1,2,3], M Barrier[1,2,3], S Sarrazin[4], F Depontieu[1,2,3], H Ghamlouch[1,2,3], W Morelle[5], H Drobecq[6], M Delehedde[1,2,3], H Lortat-Jacob[4], C Duez[1,2,3], A Scherpereel[1,2,3,7], P Lassalle[1,2,3]
[1]Inserm, U774, Lille, France; [2]Institut Pasteur de Lille, France; [3]Université Lille Nord de France, Lille, France; [4]CNRS-CEA-UJF, Institut de Biologie Structurale, Grenoble, France; [5]UMR-CNRS 8576, Villeneuve Ascq, France; [6]UMR-CNRS 8161, Lille, France; [7]CHRU Lille, Hôpital Calmette, Lille, France
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Introduction The migration of polymorphonuclear neutrophils (PMN) into inflamed tissue requires fine interactions with the endothelial cell surface. The PMN serine proteases cathepsin G (CG), neutrophil elastase (NE) and proteinase 3 (PR3) were originally thought to play a role by the cleavage of endothelial cell proteins that control the PMN firm adhesion and the transendothelial cell migration. However, how these proteases participate in leukocyte adhesion and transmigration remains controversial. Vascular endocan, also called esm1, is a restricted endothelial cell-secreted proteoglycan constituted by a protein core of 20 kDa and by a unique glycosaminoglycan chain of dermatan sulphate (DS). Endocan is preferentially expressed in lung and kidney tissues. Endocan binds to its leukocytic receptor, the LFA-1 integrin, with an affinity of 18 nM, and inhibits the LFA-1–ICAM-1 interactions. Its expression is upregulated by the proinflammatory mediators TNF, IL-1, and lipopolysaccharide. In human sepsis, the serum endocan increases from fivefold to 30-fold the normal value and correlates with bad prognosis. Here, we examined the role of PMN-derived proteases in the degradation of endocan.

Methods Human endocan is produced by overexpressing HEK 293 cell lines and purified by anion exchange and affinity chromatographies. Proteolysis is performed by addition of endocan with PMA-activated PMN supernatants, purified CG, NE, or PR3. Endocan degradation is evaluated by ELISA, western blot and MALDI-TOF mass spectrometry. Binding assay on Jurkat cells is performed as described previously. Endocan-derived p14 fragments in human serum were detected by immunoprecipitation.
Results We demonstrate that CG, NE but not PR3 degrade endocan. We show that the degradation profiles of endocan by CG or NE are different. We interestingly identify a novel peptide fragment of endocan of 14 kDa, named p14, which represents the main endocan degradation product. This p14 results from the specific cleavage of the full-length endocan by CG. The generation of this particular fragment strictly required the presence of the DS chain on full-length endocan. Furthermore, this p14 fragment becomes resistant to NE. We also demonstrate that p14 inhibits the binding of endocan to Jurkat cells. Finally, we present evidence that p14 could be detected in patient serum suffering from an acute PMN-mediated disease like sepsis.
Conclusions The results suggest that the expression of CG by PMN shortly after their activation, by modifying the micro-environment in degrading vascular endocan, may participate in the complex network that controls tissue infiltration of leukocytes during sepsis.

P17

A review of central venous catheter-related infections in neurointensive care patients in a tertiary referral centre

C-H Tan, P Nair, A Sule, M Rathbone
Walton Centre for Neurology & Neurosurgery (WCNN), Liverpool, UK
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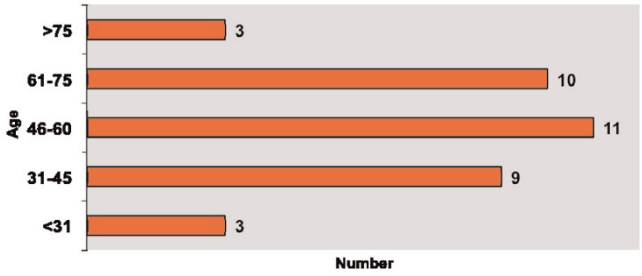
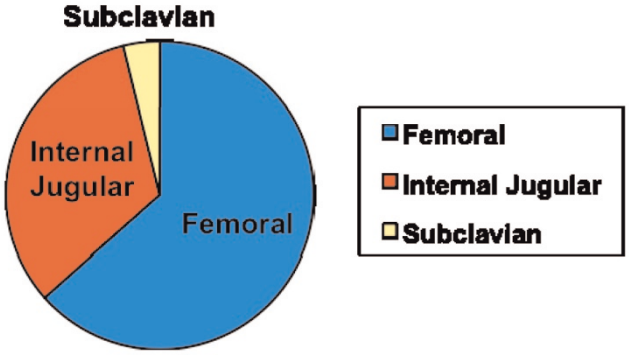
Introduction Intravenous catheter-related bloodstream infections (ICR-BSI) are a major contributing factor to in-hospital mortality and morbidity extending inpatient stay by 10 days and expenditure per patient by £2,000 to £30,000 [1].
Objective A prospective survey was conducted in our unit on all patients with central venous catheters to ascertain the incidence of ICR-BSI, identify the organisms and determine the occurrence of infection from the various sites – femoral, internal jugular and subclavian lines.
Methods The survey was carried out over a period of 13 weeks. Data collected from patients' case notes included site of central line insertion, length of line *in situ*, reason for line removal and positive blood culture reports.
Results During the study period, 104 patients were treated on the unit. Fifty-two central venous lines were inserted in 36 patients (Figure 1): 63.5% femoral ($n = 33$), 32.7% internal jugular ($n = 17$) and 3.9% subclavian lines ($n = 2$) (Figure 2). The lines were reviewed daily and removed if indicated clinically (pyrexia or raised white cell count) or if not required. A total 51.5% of femoral lines ($n = 17$) were removed due to clinical indications, as were 29% ($n = 5$) of internal jugular and 50% ($n = 1$) of subclavian lines. The average duration of a line remaining *in situ* was 4.5 days for femoral, 6 days for internal jugular and 5 days for subclavian lines (Figure 3). Blood cultures were taken at the time of line removal. These yielded positive results in eight femoral, seven internal jugulars and one subclavian line (Figure 4). Our survey indicated that the incidence of ICR-BSI in our unit is 30.8%, (of this 62.5% coagulase-negative staphylococci (CNS), 12.5% *Escherichia coli* and *Pseudomonas* each, and 6.25% MSSA and MRSA each) (Figure 5).

Figure 1 (abstract P17)



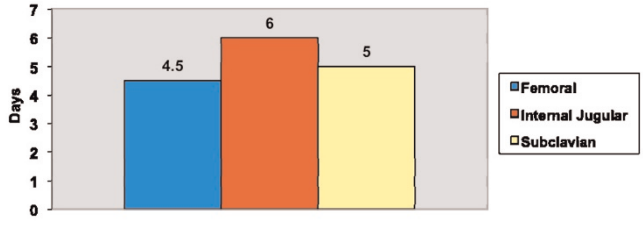
Age distribution of patients in WCNN intensive therapy unit.

Figure 2 (abstract P17)



Distribution of central venous catheters in WCNN intensive therapy unit.

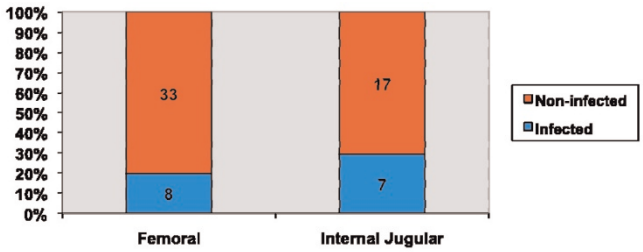
Figure 3 (abstract P17)



Average duration of central venous catheters in WCNN intensive therapy unit.

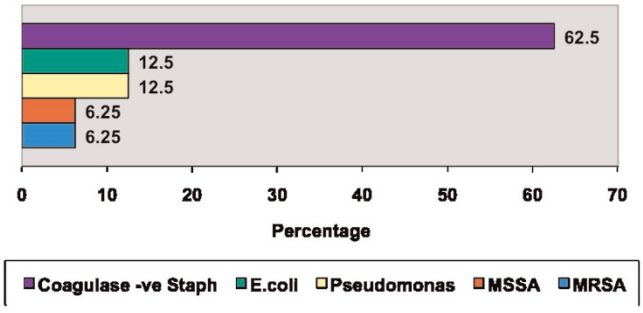
Conclusions The distribution of microorganisms causing bacteraemia is broadly similar in our unit to that in other teaching hospitals in the UK [2], in that CNS was the commonest organism isolated. However, *E. coli* and *Pseudomonas* were the next common organisms, unlike other units where *Staphylococcus aureus* was the second most prevalent organism. The incidence of bacteraemia from femoral lines (53.7/1,000 catheter-days) was lower than that from internal jugular lines (68.6/1,000 catheter-days) possibly due to a higher index of suspicion in the case of femoral lines and earlier removal (Figure 6). Our study highlights

Figure 4 (abstract P17)



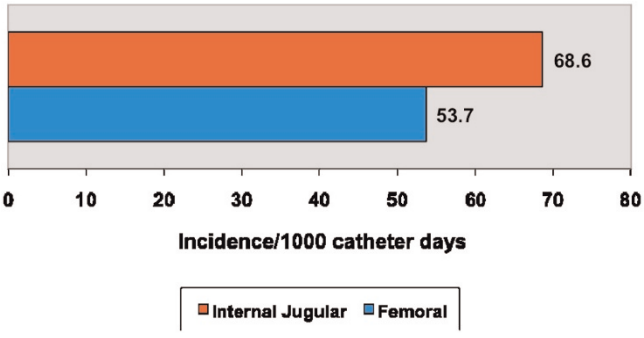
Distribution of ICR-BSI in WCNN intensive therapy unit.

Figure 5 (abstract P17)



Distribution of ICR-BSI organisms in WCNN intensive therapy unit.

Figure 6 (abstract P17)



Incidence of ICR-BSI in WCNN intensive therapy unit.

the fact that femoral lines, which are often the safest option for unstable patients with head injury, can be effectively managed with strict adherence to guidelines to reduce ICR-BSI.

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P18

Heart rate variability, cytokine, and brain responses to infection: insights from a mouse model

K Fairchild, R Gaykema, L Goehler
Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA, USA
Critical Care 2009, **13(Suppl 4):**P18 (doi: 10.1186/cc8074)

Introduction Continuous heart rate variability (HRV) monitoring can detect early sepsis in certain high-risk patient populations, but the mechanisms by which sepsis depresses HRV are not well understood. Our prior studies in rodents have shown that endotoxin causes cytokine-related depression of HRV and activation of specific neuronal networks. The aim of the current studies was to identify pathogen-specific patterns of cytokine expression, HRV changes, and activation of central autonomic pathways in mice.

Materials and methods Adult male C57BL/6 mice implanted with radiotelemetry probes for continuous ECG and temperature monitoring were inoculated intraperitoneally with *Klebsiella pneumoniae* (KP, $n = 21$), methicillin-resistant *Staphylococcus aureus* (MRSA-COL, $n = 9$) or *Candida albicans* (CA-SC5314, $n = 2$). Heart rate variability (standard deviation of RR intervals) was measured continuously for up to 3 days in *K. pneumoniae* and MRSA mice and up to 3 weeks in CA mice. Blood was obtained at two or more time points for culture and measurement of G-CSF, KC, MIP-1β, IFNγ, TNFα, IL-6, and IL-10. Neuronal activation was assessed in multiple brain regions of *K. pneumoniae*-infected mice by c-Fos staining.

Results Compared with sham-treated mice, infected mice had increases in multiple cytokines at 18 hours and 42 hours post inoculation. Cytokine profiles were similar among the three organisms except that CA-infected mice expressed less KC. Bacteria-inoculated mice with adverse outcome (positive blood culture and/or death, $n = 8$ of 21 *K. pneumoniae* and 3 of 9 MRSA) had significantly higher levels of all cytokines compared with mice with good outcome. Substantial depression of HRV was seen in all 11 bacteria-infected mice with adverse outcome and in only one of 19 mice with good outcome. Levels of G-CSF and IL-6 were negatively correlated with HRV (Spearman correlation coefficient = −0.36 and −0.37, respectively, $P = 0.05$ for each) and there was a trend toward a correlation with KC (−0.35, $P = 0.07$). Immunohistochemical studies revealed that, compared with sham-treated controls ($n = 2$), *K. pneumoniae* infection ($n = 3$) was associated with c-Fos induction in the dorsal vagal complex and ventrolateral medulla, paraventricular hypothalamic nucleus, preoptic area, subfornical organ, bed nucleus of the stria terminalis, and medial prefrontal and insular cortices. c-Fos immunoreactivity also occurred in ventricular ependymal cells and in cells associated with large blood vessels.

Conclusions Infection with Gram-positive or Gram-negative bacteria invokes similar changes in cytokines and HRV in mice, whereas preliminary studies suggest Candida infection results in different patterns. *K. pneumoniae* infection causes widespread neuronal activation within the central autonomic network.

P19

Muscimol increases the survival rate and inhibits the inflammatory response in endotoxemic mice

D-Z Hsu, Y-H Li, P-Y Chu, M-Y Liu
Department of Environmental and Occupational Health, National Cheng Kung University Medical College, Tainan, Taiwan
Critical Care 2009, **13(Suppl 4):**P19 (doi: 10.1186/cc8075)

Introduction Affecting the γ-amino butyric acid (GABA) pathway results in an alteration of inflammatory response in various animal

models. However, its mechanism is still unclear. The aim of this study was to determine the effects of muscimol, a GABAA receptor agonist, on lipopolysaccharide-induced mortality and inflammation in mice.
Materials C57BL6 mice, lipopolysaccharide (derived from *Escherichia coli*, serotype O55:B5), and muscimol were used in this study.
Methods Mice endotoxemia was induced by 10 mg/kg lipopolysaccharide intraperitoneally. Muscimol ranging from 0 to 3 mg/kg was given subcutaneously 30 minutes before lipopolysaccharide administration. Serum TNFα, IL-1β, IL-10, and IL-12 were determined using ELISA.
Results Muscimol significantly increased the survival rate in sublethal dose of lipopolysaccharide-treated mice (from 7% to 100%) ($P < 0.0001$) within 72 hours. Muscimol inhibited serum TNFα, IL-1β, and IL-12 production in a dose-dependent manner. Furthermore, muscimol significantly increased serum IL-10 levels ($P < 0.001$) in lipopolysaccharide-treated mice.
Conclusions Muscimol potently increased the survival rate and inhibited inflammatory response in endotoxemic mice.

P20

Sesamol attenuates septic hypotension through peroxisome proliferator-activated receptor activation after the onset of systemic inflammatory response

P-Y Chu, D-Z Hsu, M-Y Liu
Department of Environmental and Occupational Health, National Cheng Kung University Medical College, Tainan, Taiwan
Critical Care 2009, **13(Suppl 4):**P20 (doi: 10.1186/cc8076)

Introduction Hypotension is well relative to the high mortality of sepsis. Sesamol increases the survival rate of septic mice. However, the effect of sesamol on septic hypotension after the onset of systemic inflammation has never been studied. The aim of the study is to investigate the effect of sesamol on septic hypotension.
Materials Wistar rats, lipopolysaccharide (LPS) (derived from *Escherichia coli*, serotype O55:B5), and sesamol were used in this study.
Methods Hypotension was induced by injecting LPS intravenously. Mean arterial pressure was measured using an invasive blood pressure system. Serum nitrite and cytokine levels were determined using the Griess reaction and ELISA, respectively. Peroxisome proliferator-activated receptor (PPAR) activation was measured using a PPAR assay kit.
Results LPS administration significantly increased the serum TNFα level at 1 hour. Sesamol treated 1 hour after LPS administration inhibited the LPS-associated blood pressure decrease. Sesamol failed to decrease the LPS-induced nitrite production, but decreased the LPS-induced TNFα and IL-1β production after the onset of systemic inflammation. Sesamol enhanced the IL-10 production in serum and the PPAR activation in white blood cells.
Conclusions Sesamol may attenuate septic hypotension through alternating cytokine production by PPAR activation after the onset of systemic inflammatory response.

P21

3,4-Methylenedioxyphenol attenuates systemic inflammation and oxidative stress in septic rats

Y-H Li, D-Z Hsu, M-Y Liu
Department of Environmental and Occupational Health, National Cheng Kung University Medical College, Tainan, Taiwan
Critical Care 2009, **13(Suppl 4):**P21 (doi: 10.1186/cc8077)

Introduction Sepsis is one of the major causes of mortality in ICUs. Systemic inflammation and oxidative stress are involved in the pathogenesis and development of sepsis. 3,4-Methylenedioxyphenol (sesamol), one of the lignans in sesame oil, protects against endotoxin-induced oxidative stress and organ failure. However, the effects of sesamol on systemic inflammation and oxidative stress in septic rats have never been investigated.
Objective To investigate the effects of sesamol on systemic inflammation and oxidative stress in septic rats.
Methods Septic rats were induced by cecal ligation and puncture (CLP). Rats received sesamol (10 mg/kg, subcutaneously) 0 and 6 hours after CLP. IL-1β, lipid peroxidation, hydroxyl radical, superoxide anion, xanthine oxidase activity, and nitrite levels in blood were determined 12 hours after CLP.
Results IL-1β, lipid peroxidation, hydroxyl radical, superoxide anion, xanthine oxidase activity, and nitrite levels were significantly increased in CLP-treated rats compared with those in the sham-operation group (all $P<0.05$). Sesamol significantly reduced IL-1β, lipid peroxidation, hydroxyl radical, superoxide anion, xanthine oxidase activity, and nitrite levels compared with the saline group in CLP-treated rats (all $P<0.05$).
Conclusions Sesamol might attenuate systemic inflammation and oxidative stress by inhibiting proinflammatory cytokine and reactive oxygen species generation in septic rats.

P22

Central venous catheter-related infection: a cohort study evaluating dedicated central venous catheter packs

S Mukerji, R Daniels, K Maung, A Mattin
Good Hope Hospital, Worcester, UK
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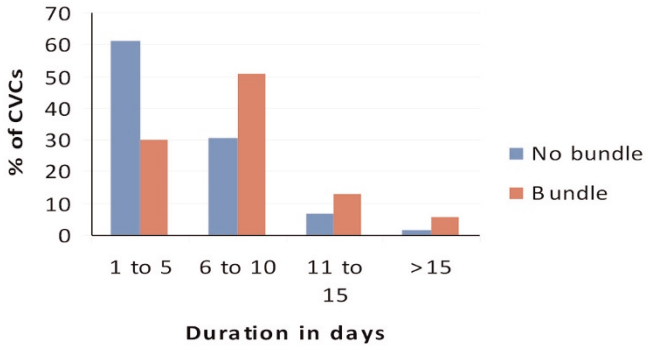
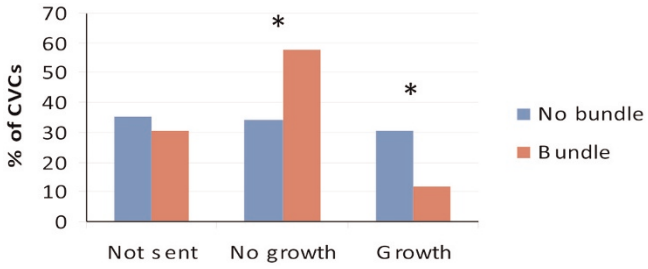
Introduction Central venous catheter (CVC)-related bloodstream infections (CRBSI) are the third most common healthcare-associated infection (HAI) in ICUs, associated with significant morbidity, mortality, increased length of stay and costs [1,2]. Several care bundle studies have suggested that utilising various strategies together (such as training, regular line monitoring and using dedicated line insertion trolleys) can have a positive impact on CRBSI rates [3-6]. However, the impact solely attributable to the provision of a dedicated, stand-alone CVC insertion pack has not been evaluated. We therefore investigated the impact of a new EPIC2 compliant CVC pack, introduced in Good Hope Hospital in 2007, on CVC tip colonisation rates.
Methods Data were collected prospectively between June 2007 and December 2008. Patients were divided into two cohorts: patients whose CVCs were inserted using the CVC packs (B), and those receiving CVCs prior to the introduction of the packs (nB). Data were collated from questionnaires as well as patients' notes on: patient's age and sex; type and site of CVC inserted; location at the time of insertion; the grade of practitioner; and the duration CVCs remained i*n situ*. Using the hospital's patient information system and patient notes, data on CVC tip cultures were obtained. Data are presented as percentages and analysed using multivariate analysis.
Results Complete data were obtained for 347 patients: 246 patients in group nB, 101 patients in group B. Male/female ratio, average age, site of insertion, clinical area of insertion and grade of practitioner were similar in both cohorts. There was a significantly higher number of 5-lumen catheters inserted in group B compared with group nB (81% compared with 44%, $P<0.05$), reflecting change in hospital practice. More B catheters (51%) were left *in situ* longer, for 6 to 10 days, compared with nB catheters (31%) (Figure 1). Thirty-one per cent of nB tips grew colonies of at least one pathogen. There was a significant reduction in the number of B tips growing colonies (12% compared with 31%, $P<0.05$)

Figure 1 (abstract P22)



Number of days CVCs were left *in situ* in both cohorts.

Figure 2 (abstract P22)



Differences in colony growth in the two cohorts. *P <0.05.

(Figure 2). The bundle cohort had no MRSA growth compared with four incidences in the nB group.

Conclusions Our results indicate that use of dedicated CVC packs was associated with a significant reduction in the colonisation rate of CVCs, despite lines being left *in situ* for longer periods and the more frequent use of quin-lumen catheters in the intervention group. There was also a trend toward prevention of MRSA colonisation.

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P23

TLR4 on hematopoietic cells is crucial for host defense against *Klebsiella pneumonia* but TLR2 is needed when bacterial numbers are high

C Wieland[1,2,3], MH van Lieshout[3,4], AJ Hogendijk[3,4], T van der Poll[3,4]
[1]Laboratory of Experimental Intensive Care and Anesthesiology, [2]Department of Intensive Care, [3]Center of Experimental and Molecular Medicine, and [4]Center of Infection and Immunity, Academic Medical Center Amsterdam, the Netherlands
Critical Care 2009, **13(Suppl 4):**P23 (doi: 10.1186/cc8079)

Introduction *Klebsiella* species are opportunistic pathogens that can give rise to severe infections including pneumonia and sepsis. Typically, *Klebsiella* infections are nosocomial and mainly caused by *Klebsiella pneumoniae*, the medically most important species of the genus.

Objective We set out to validate and extend our previous data using C3H/HeJ mice that demonstrated an important role for TLR4 in *K. pneumoniae* pneumonia. Moreover, we were interested in the relative roles of cells from hematopoietic origin and parenchymal cells.

Methods Using TLR2 and TLR4 single and TLR2x4 double knockout (KO) mice on a C57BL/6 background, the roles of TLR2 and TLR4 were investigated independently and together. We intranasally inoculated C57BL/6 wild-type (WT) and KO mice with *K. pneumoniae* (4 x 10^{-3} CFU per mouse) and studied host defense. Moreover, we performed bone marrow transplantation (BMT) experiments in which we transplanted KO bone marrow into irradiated WT mice and *vice versa*.

Results Shortly after infection, both TLR4 and TLR2x4 KO mice demonstrated an attenuated proinflammatory response in the lungs. This was associated with higher bacterial counts 24 hours after infection in the lungs, liver and spleens of both TLR4 and TLR2x4 KO animals. Interestingly, although no differences in antibacterial host defense of TLR2 KO animals were observed, TLR2x4 KO animals were more susceptible to *K. pneumoniae* infection than the single TLR4 KO mice: after 44 hours of infection, 0/8 WT, 0/8 TLR2 KO mice, 5/8 TLR4 KO mice and 8/8 TLR2x4 KO mice had succumbed. Moreover, when infecting all strains with a high dose of *K. pneumoniae* (10^{-4} CFU), no differences in outgrowth were detected between WT, TLR2 and TLR4 KO animals, whereas double KO animals suffered from higher bacterial burdens in the lungs, liver, spleen and blood. BMT of WT bone marrow into irradiated TLR2x4 KO mice resulted in a reversed phenotype with similar bacterial growth compared with syngenic transplanted WT mice.

Conclusions These data confirm our previous research that, during low-dose infections, TLR4 is of primary importance in host defense against *K. pneumoniae*. Nevertheless, when high numbers of bacteria are present, TLR2 acts together with TLR4 to orchestrate the immune response, a protective effect that is primarily mediated by hematopoietic cells.

P24

Effect of the novel influenza A (H1N1) virus in the human immune system

A Antonopoulou, M Raftogiannis, F Baziaka, P Koutoukas, A Savva, T Kanni, M Georgitsi, A Pistiki, EJ Giamarellos-Bourboulis
4th Department of Internal Medicine, University of Athens, Medical School, Athens, Greece
Critical Care 2009, **13(Suppl 4):**P24 (doi: 10.1186/cc8080)

Introduction The pandemic by the novel H1N1 virus has created the need to study any probable effects of that infection in the immune system of the host.

Methods Blood was sampled within the first 2 days of the presentation of signs of infection from 10 healthy volunteers; from 18 patients of flu-like syndrome (FLS); and from 30 patients of infection by H1N1 confirmed by reverse RT-PCR. Absolute counts of subtypes of monocytes and of lymphocytes were determined after staining with monoclonal antibodies and analysis by flow cytometry. Peripheral blood mononuclear cells (PBMCs) were isolated and stimulated with various bacterial stimuli. Concentrations of TNFα, of IL-1β and of IFNγ were estimated in supernatants by an enzyme immunoassay.
Results Mean absolute counts of CD14 monocytes of healthy volunteers, of FLS and of patients infected by the H1N1 virus were 271.1, 464.4 and 607.2%, respectively (P <0.0001 compared with other groups). Respective values of $CD4^+/CD25^+/CD127^-$ cells were 2.0, 4.7 and 9.0% (P <0.0001 compared with other groups). Respective values of CD19 cells were 297.9, 151 and 137.9%. No differences between the three groups were found regarding CD3/CD4 lymphocytes, CD3/CD8 lymphocytes, natural killer (NK) cells and NKT cells. No differences were also found regarding the rate of apoptosis of the above subtypes. Six patients had H1N1-related pneumonia. Mean T-regulatory cells (Tregs) of H1N1-infected patients without pneumonia and with pneumonia were 6.7 and 17.8%, respectively (P = 0.034). Mean release of TNFα by phytohemagglutin-stimulated PBMCs of healthy volunteers, of FLS and of patients infected by H1N1 was 3,658.5, 1,877.3 and 874.4 pg/ml, respectively (P <0.0001 compared with other groups). Respective release of TNFα by *Streptococcus pneumoniae*-stimulated PBMCs was 1,836.9, 949.9 and 478.0 pg/ml (P <0.0001 compared with other groups). Mean respective release of IFNγ by PHA-stimulated PBMCs was 1,651.8, 1,235.1 and 1,114.3 pg/ml (P = 0.010 compared with FLS). Mean respective release of IFNγ by *S. pneumoniae*-stimulated PBMCs was 1,085.7, 748.1 and 709.7 pg/ml (P = 0.024 compared with FLS). No effect of other stimuli was shown on release of TNFα and of IFNγ. Release of IL-1β was not affected.
Conclusions Infection by the H1N1 virus is accompanied by a characteristic impairment of the innate immune responses characterized by defective cytokine responses to *S. pneumoniae*. Alterations of the adaptive immune responses are predominated by an increase of Tregs. These findings signify a predisposition for pneumococcal infections after infection by H1N1 influenza.

P25

Clarithromycin reverses sepsis-induced immunoparalysis of monocytes

M Raftogiannis, A Antonopoulou, F Baziaka, P Koutoukas, T Tsaganos, A Pelekanou, A Spyridaki, M Mouktaroudi, EJ Giamarellos-Bourboulis
4th Department of Internal Medicine, University of Athens, Medical School, Athens, Greece
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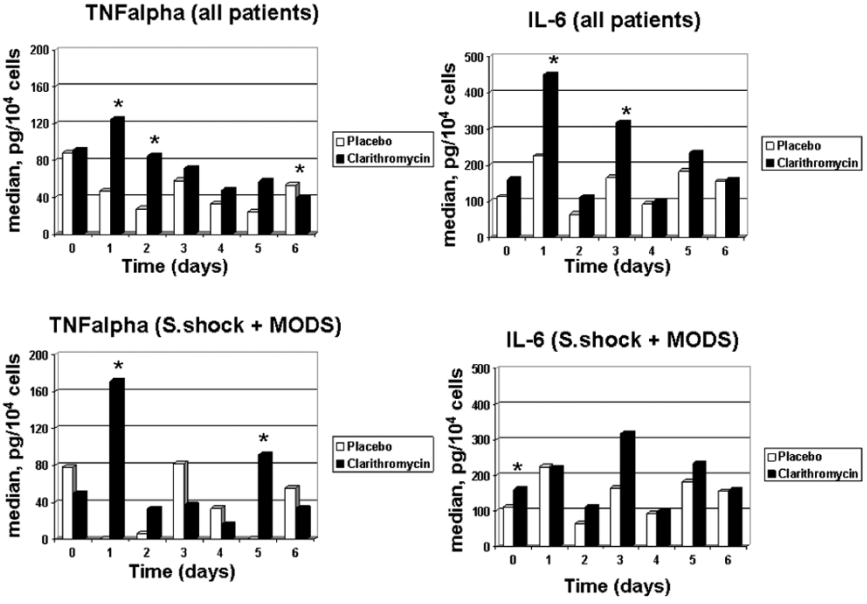
Introduction In a recently published double-blind, randomized trial conducted by our study group, clarithromycin was intravenously administered in patients with ventilator-associated pneumonia (VAP) and sepsis for three consecutive days [1]. An earlier resolution of VAP and a fivefold decrease of the risk for death by septic shock and multiple organ failure (MODS) compared with placebo were shown.
Objective To investigate the mode of action of clarithromycin.
Methods Blood was sampled before administration of the investigational product and on six consecutive days. Peripheral blood mononuclear cells (PBMCs) were isolated after gradient centrifugation over Ficoll. PBMCs were incubated and adherent monocytes were harvested and stimulated for 24 hours with

Figure 1 (abstract P25)



*Statistically significant differences between groups at the indicated time intervals. Day 0 corresponds to time before start of the investigational drug.

10 ng/ml LPS of *Escherichia coli* O55:B5. Concentrations of TNFα and of IL-6 were estimated in supernatants by an enzyme immunoassay.

Results One hundred patients were treated with placebo and another 100 patients with clarithromycin. Median concentrations of TNFα and of IL-6 in monocyte supernatants of all patients and separately of those with septic shock and MODS are shown in Figure 1.

Conclusions Administration of clarithromycin was accompanied by a considerable improvement of the response of monocytes to *ex vivo* stimulation with the release of TNFα and IL-6. These results signify that clarithromycin effectively reverses sepsis-induced immunoparalysis of monocytes.

References

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P26

Plasma-derived human antithrombin attenuates ventilator-induced coagulopathy in a *Streptococcus pneumoniae* pneumonia model in rats

H Aslami[1,2], JJ Haitsma[1], JJ Hoffstra[2], M Levi[2], H Zhang[1], AS Slutsky[1], MJ Schultz[1]

[1]Interdepartmental Division of Critical Care Medicine, Keenan Research Center, Li Ka Shing Knowledge Institute, St Michael's Hospital, Toronto, Ontario, Canada; [2]Departments of Intensive Care and Internal Medicine, Laboratory of Experimental Intensive Care and Anesthesiology, Academic Medical Center, University of Amsterdam, the Netherlands

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Introduction Pneumonia is characterized by local activation of coagulation leading to alveolar fibrin deposition. Lung injurious mechanical ventilation (LI-MV) with conventional tidal volumes (V_T) and no positive end-expiratory pressure (PEEP) aggravates pulmonary coagulopathy. We hypothesized administration of antithrombin (AT), a natural anticoagulant, to attenuate ventilator-induced coagulopathy and inflammation in a rat model of *Streptococcus pneumoniae* pneumonia.

Methods Rats challenged intratracheally with bacteria were ventilated 40 hours later (V_T = 12 ml/kg/no PEEP) after systemic administration of plasma-derived human AT (250 U/kg) or placebo for 3 hours. Endpoints: BALF levels of thrombin–antithrombin complexes (TATc), fibrin degradation products (FDP), AT, plasminogen activator activity (PAA), plasminogen activator inhibitor-1 (PAI-1), pulmonary cytokines and blood cultures. Data are presented as the mean ± SD. Statistics: one-way ANOVA with Dunn's multiple comparison test.

Results *S. pneumoniae* pneumonia was characterized by activation of coagulation (TATc: in pneumonia vs. healthy control, 3.7 ± 0.3 vs. 1.2 ± 0.5 ng/ml; FDP: 291 ± 40 vs. 15 ± 5 ng/ml; AT: 3.7 ± 0.3 vs. 20.0 ± 5.2 IU/ml – all $P < 0.05$) and inhibition of fibrinolysis (PAA: 99.2 ± 5.9 vs. 73.3 ± 5.7% of normal, $P < 0.05$; PAI-1: 1.9 ± 0.6 vs. 10.0 ± 1.4 ng/ml, $P < 0.05$). Systemic administration of AT resulted in supraphysiologic levels of BALF AT levels (25.4 ± 4.9 vs. 5.3 ± 1.0 IU/ml) and prevented further activation of coagulation by MV (TATc: in LI-MV with AT vs. placebo, 3.9 ± 0.3 vs. 6.5 ± 0.8 ng/ml – $P < 0.05$). No changes in pulmonary cytokines were observed between the infected and mechanically ventilated animals (TNF: LI-MV with AT vs. placebo, 1.6 ± 0.4 vs. 1.9 ± 1.3 ng/ml and IL-6: 2.5 ± 1.3 vs. 2.4 ± 1.4 ng/ml – P = NS). The infected animals showed similar numbers of bacteria in blood samples at the start of the experiment, which did not change during the experiment.

Conclusions Systemic administration of AT attenuated ventilator-induced coagulopathy but not inflammation.

P27

Recombinant human tissue factor pathway inhibitor exerts anticoagulant, anti-inflammatory and antibacterial effects in murine pneumococcal pneumonia

F van den Boogaard[1,2,3], X Brands[1,2], M Schultz[3], M Levi[4], J Roelofs[5], C van 't Veer[1,2], T van der Poll[1,2]

[1]Center for Experimental and Molecular Medicine, [2]Center for Infection and Immunity Amsterdam, [3]Laboratory of Experimental Intensive Care and Anesthesiology, [4]Department of Internal Medicine, and [5]Department of Pathology, Academic Medical Center, University of Amsterdam, the Netherlands

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Introduction *Streptococcus pneumoniae* is the most common causative pathogen in community-acquired pneumonia and a major cause of sepsis. Pneumonia elicits a procoagulant state in the lung resulting from activation of coagulation, downregulation of anticoagulant pathways and concurrent inhibition of fibrinolysis. Tissue factor is the main initiator of coagulation. Recombinant human tissue factor pathway inhibitor (rh-TFPI) attenuates sepsis-induced coagulation and has been evaluated in clinical trials involving patients with sepsis and community-acquired pneumonia.

Objective To examine the effect of rh-TFPI on coagulation, inflammation and bacterial outgrowth in *S. pneumoniae* pneumonia in mice, either alone or with concurrent antibiotic treatment.

Methods Pneumonia was induced by intranasal inoculation with *S. pneumoniae*. Four groups of mice ($n = 8$) were treated intraperitoneally with (1) placebo, (2) rh-TFPI every 8 hours, (3) ceftriaxone twice daily or (4) rh-TFPI in combination with ceftriaxone. Early (8 hours) and late (24 hours) initiated treatments were evaluated. Bronchoalveolar lavage fluid (BALF), lungs and plasma were obtained 24 hours (for groups in which treatment was started after 8 hours) or 48 hours (treatment started after 24 hours) after infection. Statistical analysis was performed by Mann–Whitney U test.

Results Pneumonia resulted in local and systemic activation of coagulation (as reflected by increased thrombin–antithrombin complexes) and inhibition of fibrinolysis (as reflected by increased plasminogen activator inhibitor-1 and decreased plasminogen activator activity). Both early and late treatment with rh-TFPI reduced pneumonia-induced coagulation in lungs and plasma; rh-TFPI given with ceftriaxone further attenuated coagulation relative to ceftriaxone only. No effects of rh-TFPI on pneumonia-inhibited fibrinolysis were observed. Cell recruitment in BALF did not differ between groups. Remarkably, rh-TFPI reduced levels of several cytokines and chemokines not only in lung homogenates, but also in BALF (IL-6, IFNγ, MCP-1, LIX) and plasma (IL-6, TNFα, IFNγ). The attenuated host inflammatory response was not reflected by differences in total histopathology scores between treatment groups. In mice not treated with ceftriaxone, rh-TFPI decreased bacterial loads in lung homogenates ~10-fold ($P < 0.01$ vs. placebo) at 48 hours, while leaving bacterial loads in BALF and the systemic compartment unaltered.

Conclusions rh-TFPI attenuates local and systemic coagulopathy, the local and systemic inflammatory response and pulmonary bacterial growth during *S. pneumoniae* pneumonia in mice.

P28

Toll like receptor 1 polymorphisms and susceptibility to invasive candidiasis

T Plantinga[1], M Johnsson[2], B Scott[3], E van de Vosse[4], D Velez[3], JWM van der Meer[1], J van Dissel[4], J Perfect[2], B-J Kullberg[1], MG Netea[1]

[1]Radboud University Nijmegen, the Netherlands; [2]Duke University Medical Center, Durham, NC, USA; [3]University of Miami Miller School of Medicine, Miami, FL, USA; [4]Leiden University Medical Center, Leiden, the Netherlands

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Introduction Invasive candidiasis is a severe systemic fungal infection with *Candida* spp. affecting immunocompromised hosts, which is responsible for the highest mortality rate of all nosocomial infections. Although several clinical predisposing factors are known, the individual risk for developing invasive candidiasis varies significantly. Recognition of fungi such as *Candida albicans* is mediated through receptors of the innate immune system, such as Toll-like receptors (TLRs), that in turn activate innate immune system and antifungal defense.

Objective To assess whether polymorphisms in fungal innate immune receptors such as TLRs and dectin-1 influence susceptibility to invasive candidiasis.

Methods Frequencies of mostly nonsynonymous polymorphisms in several innate immune receptors were genotyped in a total of 331 patients that developed invasive candidiasis and compared with a total of 341 matched control patients that had the same predisposing factors. These included neutropenia, mucosal barrier injury and treatment with immunosuppressive regimens. Furthermore, *in vitro* studies with healthy volunteers were conducted to assess the functional consequences of these polymorphisms regarding cytokine responses.

Results Genotyping for polymorphisms in innate immune receptor genes revealed a higher frequency of three independent non-synonymous TLR1 polymorphisms in the affected group patients that developed invasive candidiasis. These polymorphisms were also demonstrated to be associated with impaired cytokine responses upon stimulations of immune cells, including IL-1β, IL-6 and IL-8.

Conclusions Polymorphisms in TLR1, which is known to dimerize with TLR2 and TLR6, are associated with impaired immune recognition through these receptors and predispose to invasive candidiasis in humans.

P29

Challenges to implementation of sepsis guidelines

S Patel[1], E Wise[2], J Hartin[1], D Walker[1], M Noursadeghi[2,3]

[1]Critical Care, [2]Acute Assessment Unit, and [3]Infection and Immunity, University College London Hospitals NHS Foundation Trust, London, UK

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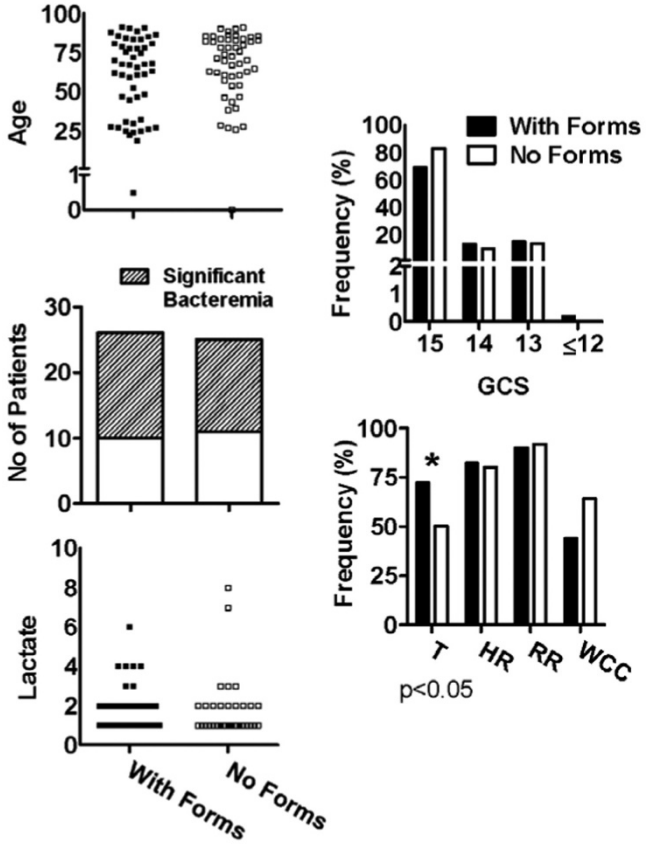
Introduction International surviving sepsis guidelines identified an important role for acute medicine in early management of severe sepsis, but local and multicentre international audits show poor adherence to these guidelines.

Materials We evaluated the use of a Sepsis Case Record (SCR) supported by a systematic educational programme to improve standards. A one-page SCR was derived from surviving sepsis guidelines, to prompt recognition of sepsis syndromes, comprehensive secondary assessment, initiation of resuscitation and antibiotic treatment bundles, and appropriate specialist consultations. The SCR was introduced in the emergency and acute assessment units in our teaching hospital setting within central London, accompanied by a seminar-based educational programme for medical and nursing staff.

Figure 1 (abstract P29)



Methods Two months after its introduction, the use of the SCR form was audited in all acute medical admissions who met the clinical criteria for sepsis. One hundred sequential patients were assessed in a 6-week period over the winter.

Results One-half of the audit sample had SCR forms completed. Specificity of the sepsis criteria was good, with <10% of patients subsequently judged not to have had sepsis. The patients with and without audit forms had comparable demographics, severity of illness and microbiology (Figure 1). Frequency of abnormal temperature was significantly higher in patients with the SCR, suggesting fever remains an important prompt for physicians to consider sepsis. The use of the SCR was also associated with significantly improved assessment of GCS, lactate, travel history and the need for isolation, as well as significantly greater number of specialist consultations (Table 1), albeit still inadequate, ~10% (without SCR) to ~20% (with SCR). The SCR had no effect on frequency of clinical review by senior resident physicians, recording of FiO_2, antibiotic guidelines compliance or blood/urine culture requests, all evident in 40 to 60% of patients. Approximately 75% of all patients received antibiotics within 2 to 6 hours, and a trend for earlier antibiotics was associated with use of the SCR (Figure 2).

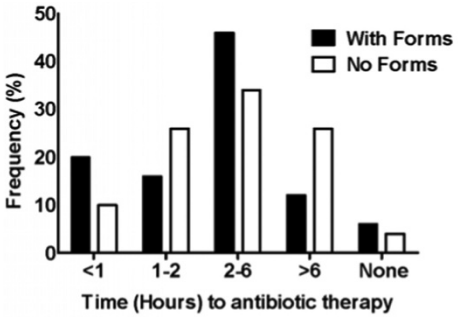
Conclusions The SCR was well received but not used consistently. The lack of abnormal temperature may contribute to this. The use of the SCR did improve early management of sepsis,

Table 1 (abstract P29)

Parameter	With Forms	No Forms	*P* value
SpR review	24/50	25/50	ns
FiO_2	29/50	25/50	ns
Blood culture	42/50	35/50	ns
Urine culture	29/50	23/50	ns
Abx guide compliant	31/50	25/50	ns
ID consult	9/50	8/50	ns
ITU consult	11/50	6/50	ns
GCS	40/50	29/50	<0.05
Lactate	45/50	35/50	<0.05
Micro consult	6/50	0/50	<0.05
Any consult	20/50	8/50	<0.05
Travel history	23/50	9/50	<0.05
Need for isolation	18/50	2/50	<0.05

ns, not significant.

Figure 2 (abstract P29)



but a number of deficiencies persisted. Implementation of sepsis guidelines remains a major challenge in clinical practice. Succinct guidelines were helpful in this setting but need additional educational and feedback support to improve standards of practice.

P30

Differential effects of IL-17 pathway in disseminated candidiasis and zymosan-induced multiple organ failure

FL van de Veerdonk[1,2], BJ Kullberg[1,2], IC Verschueren[1,2], T Hendriks[3], JWM van der Meer[1,2], LAB Joosten[1,2,4], MG Netea[1,2]

[1]Department of Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; [2]Nijmegen Institute for Infection, Inflammation and Immunity, Nijmegen, the Netherlands; [3]Department of Surgery, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; [4]Department of Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands

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Introduction The role of the IL-17 pathway in fungal sepsis remains controversial. Several studies suggested that IL-17 is crucial for the protection against *Candida* sepsis, while other studies reported that IL-17 may contribute to inflammatory pathology and worsening of fungal disease. To address these discrepancies, we assessed the differential role of IL-17 pathway in two models of fungal sepsis: intravenous infection with live *Candida albicans*, in which fungal growth is the main cause of mortality, and zymosan-induced multiple organ failure in which the inflammatory pathology drives the mortality.

Methods IL-17 receptor-deficient (IL-17RA$^{-/-}$) and control mice were intravenously infected with 2 x 10^5 CFU live *C. albicans* UC820 per mouse. Mortality, fungal loads in the kidneys, neutrophil recruitment and phagocytosis and killing were assessed. IL-17RA$^{-/-}$ and control mice were also assessed for mortality in a multiorgan failure sepsis model induced by the fungal component zymosan.

Results On the one hand, IL-17RA$^{-/-}$ mice showed increased mortality and higher fungal loads in the kidneys in the model of disseminated candidiasis. On the other hand, the absence of IL-17RA in the knockout mice did not protect the mice against the multiorgan failure induced by zymosan. Furthermore, no reduction in neutrophil recruitment and defects in phagocytosis and killing in the first few hours of *Candida* infection were found. A significantly lower TNF production in response to *Candida* in cells from IL-17RA$^{-/-}$ mice was observed.

Conclusions These data demonstrate that the IL-17 pathway does not have a major contribution to the inflammatory pathology leading to organ failure in fungal sepsis, and support the concept that the IL-17 pathway is protective during fungal sepsis. In addition, IL-17 deficiency does not appear to reflect a pure innate defect, since it did not result in loss of neutrophil recruitment and function during the first few hours of fungal sepsis. Furthermore, the lower TNF production in response to *Candida* in cells from IL-17RA$^{-/-}$ mice could contribute to susceptibility to disseminated candidiasis.

P31

Caspase-1 and ASC but not NLRP3 mediate antifungal defense in candidiasis sepsis

F van de Veerdonk[1,2], LAB Joosten[2], P Shaw[1], S Smeekens[2], JWM van der Meer[2], B-J Kullberg[2], MG Netea[2], T-D Kanneganti[1]

[1]Department of Immunology, St Jude Children's Research Hospital, Memphis, TN, USA; [2]Department of Medicine, Radboud University Nijmegen Medical Centre, and Nijmegen Institute for Infection, Inflammation and Immunity (N4I), Nijmegen, the Netherlands

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Introduction IL-1β plays an important role in antifungal defense. The inflammasome is thought to be required for caspase-1 activation and processing of the inactive precursor pro-IL-1β into its active form. Contradictory data have been reported regarding the role of the inflammasome in *Candida* sepsis. In order to address these discrepancies, we investigated host defense against disseminated candidiasis in knockout mice defective in the various components of the inflammasome.

Methods Mice defective in caspase-1, ASC, NLRP3 or P2X7 were infected intravenously with *Candida albicans*. Survival, fungal outgrowth in the organs, histology, and cytokine production were compared in these mouse strains with the wild-type C57/Bl6 control mice. PBMCs from healthy volunteers with or without reactive oxygen species (ROS) inhibitor and PBMCs from patients with chronic granulomatous disease (CGD) that are deficient in ROS production were stimulated with *C. albicans*.

Results Caspase-1$^{-/-}$ mice and ASC$^{-/-}$ mice had a decreased survival during disseminated candidiasis (50%) compared with the control mice (100%). Caspase-1$^{-/-}$ mice had a 100-fold increase in fungal loads in the kidneys of the deficient animals (P <0.05)

and histological assessment revealed preferential growth of hyphae in the pyelum of the caspase-1$^{-/-}$ mice. In contrast, ASC$^{-/-}$ mice did not have higher fungal loads, but they showed a significant stronger inflammatory reaction in the kidneys. On days 3 and 7 of infection, the ASC$^{-/-}$ mice splenocytes that were restimulated with *Candida* specifically showed a higher TNF production. NLRP3$^{-/-}$ and P2X7$^{-/-}$ did not display an increased susceptibility to disseminated candidiasis, as shown by normal survival and fungal loads in the organs. Local IL-1β production was lower in caspase-1$^{-/-}$ mice, but not in the ASC$^{-/-}$, NLRP3$^{-/-}$ or P2X7$^{-/-}$ animals. Experiments using the NADPH inhibitor diphenyleneiodonium, or in monocytes isolated from CGD patients who have defective capacity to form ROS, demonstrated that ROS did not mediate inflammasome activation and *C. albicans* induced IL-1β production.

Conclusions Caspase-1-dependent processing of IL-1β is an important step in antifungal host defense during *Candida* sepsis. However, this process is not dependent on the inflammasome components NLRP3, the ATP receptor P2X7, or ROS. These data confirm previous studies in human monocytes showing that IL-1β processing during *Candida* infection did not require pathogen-mediated inflammasome activation, due to the constitutive activation of caspase-1. ASC also plays an important role in *Candida* sepsis, but unexpectedly seems to have a different function, specifically by regulating TNF production and local inflammation in the organs.

P32

Early recognition and management of sepsis at West Middlesex University Hospital

Z Aboud, T Peters
ICU Department, West Middlesex Hospital, London, UK
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Introduction Mortality associated with severe sepsis remains high at 30 to 50% and rises to 50 to 60% when shock is present. The Surviving Sepsis Campaign (SSC) recommends two bundles for severe sepsis management to achieve 25% reduction in mortality; the Initial Resuscitation Bundle (within the first 6 hours) and the Management Bundle (within 24 hours). West Middlesex University Hospital set up a severe sepsis management protocol based on the SSC initial resuscitation and management bundles. It is a 350-bed hospital with an emergency department. Five hundred patients (medical and surgical) are admitted to the critical care unit per year.
Objective To assess the early recognition of sepsis and the application of the initial resuscitation bundle according to SSC guidelines at West Middlesex University Hospital.
Methods Retrospective data collection of all patients with severe sepsis or septic shock who were admitted to the ITU over 3 months (December 2008, January and February 2009). All patients who developed sepsis before admission to the ITU/HDU were included.
Results Thirty-three patients were admitted to the ITU at West Middlesex Hospital with either severe sepsis or septic shock. Median age was 72 years. The overall mortality rate was 50%. Patients with septic shock had a mortality rate of 52%. The results of the initial resuscitation of the patients are summarized in Table 1. In septic shock patients, only 35% had ITU intervention within 6 hours (had CVP insertion and/or started on vasopressor and/or inotropic support). Central venous oxygen saturation or mixed venous oxygen saturation was not measured for these patients.
Conclusions Early recognition and the initial resuscitation of sepsis at this District General Hospital were assessed for the first time. Patients with severe sepsis or septic shock were not resuscitated appropriately and the SSC guidelines were not implemented, resulting in a high mortality rate. The results showed that there is a delay in recognizing sepsis at early stages resulting in inadequate management of patients. In septic shock patients, this resulted in delayed CVP measurement and administration of vasopressors and/or inotropic support. Therefore, we have suggested an educational programme running throughout the year to educate medical and nursing teams about the early recognition and management of sepsis, with emphasis on the strict implementation of all tasks of sepsis protocol according to SSC guidelines to reduce the mortality rate by 25%. We also suggest setting up critical care beds on each ward that will be supported by ITU outreach for CVP insertion and level 1 monitoring.

P33

The selective V_{1a} receptor agonist FE 202158 does not cause von Willebrand factor release in sheep unlike arginine vasopressin

S Rehberg[1], P Enkhbaatar[1], R Laporte[2], J Rehberg[1], E La[2], K Wisniewski[2], LD Traber[1], CD Schteingart[2], PJM Riviere[2], DL Traber[1]
[1]Investigational Intensive Care Unit, Department of Anesthesiology, The University of Texas Medical Branch, Galveston, TX, USA; [2]Ferring Research Institute, Inc., San Diego, CA, USA
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Introduction The mixed V_{1a}/V_2 receptor agonist arginine vasopressin (AVP) is recommended by the guidelines of the Surviving Sepsis Campaign as an adjunct vasopressor in norepinephrine-resistant septic shock. However, AVP may be procoagulant

Table 1 (abstract P32)

Breakdown of tasks of the initial resuscitation bundle achieved within 6 hours

Initial resuscitation tasks (within 6 hours)	Number of patients achieved/total number of patients	% of patients where SSC recommendation was followed
Serum lactate measured	25/33	76
Obtaining blood cultures prior to antibiotic administration	6/33	18
Broad-spectrum antibiotics within 3 hours from time of presentation for Emergency Department admissions	6/9	67
1 hour for non-Emergency Department ICU admissions	5/24	21
In patients with septic shock or serum lactate >4 mmol/l (36 mg/dl)		
Fluid challenges	7/23	30
Vasopressors	17/23	74
CVP >8 mmHg in nonmechanically ventilated patients (12 to 15 in mechanically ventilated patients)	2/23	9

through V_2 receptor-mediated effects (for example, von Willebrand factor (vWF) release).
Objective We hypothesized that the selective V_{1a} receptor agonist FE 202158, which lacks the activity at the V_2 receptor, might not have the procoagulant effects of AVP. This hypothesis was tested by measuring vWF antigen (vWF:Ag) activity in plasma of healthy sheep during administration of either FE 202158, AVP, the selective V_2 receptor agonist desmopressin, or vehicle.
Methods After measurements of vWF:Ag activity and hemoglobin concentration in blood over a 1-hour baseline period, 24 female sheep were randomly assigned to receive either an intravenous bolus of the selective V_2 receptor agonist desmopressin (1 nmol/kg) or a continuous intravenous infusion of AVP (3 pmol/kg/min), the selective V_{1a} receptor agonist FE 202158 (10 pmol/kg/min) or vehicle (0.9% NaCl, $n = 6$ each). The infusion rates were representative of the requirements for the treatment of sepsis-induced vasodilatory hypotension in sheep. vWF:Ag activity and hemoglobin concentration were measured 60, 90 and 120 minutes after initiation of treatment. Because of the V_2 receptor-mediated fluid retention, vWF:Ag activity was corrected for plasma volume changes by calculating the ratio of vWF:Ag activity/hemoglobin concentration (vWF:Ag/Hb). Data are expressed as a percentage of the mean baseline value and presented as mean ± SEM.
Results Whereas there were no significant changes in vWF:Ag/Hb in vehicle-treated animals over time, desmopressin and AVP caused an immediate increase in vWF:Ag/Hb after 60 minutes (129 ± 6% and 121 ± 2% of baseline (100%), respectively; $P < 0.01$ each). At each time point during the 120-minute study period, vWF:Ag/Hb was significantly higher in desmopressin-treated and AVP-treated animals than in vehicle-treated animals ($P < 0.001$ each). In contrast, there was no significant difference between FE 202158-treated and vehicle-treated animals ($P = 0.225$). Notably, vWF:Ag/Hb in the FE 202158 group (maximum 108 ± 2% at 120 minutes) was significantly lower than the AVP group (maximum 123 ± 2% at 60 minutes; $P \leq 0.005$) and the desmopressin group (maximum 138 ± 6% at 120 minutes; $P < 0.001$) at every time point.
Conclusions Unlike AVP, the selective V_{1a} receptor agonist FE 202158 did not increase vWF:Ag/Hb ratios in plasma compared with vehicle-treated animals. Therefore, a selective V_{1a} receptor agonist such as FE 202158 might be superior to AVP or other mixed V_{1a}/V_2 receptor agonists under conditions that produce activation of the coagulation system, such as severe sepsis and septic shock.

P34

Increased asymmetrical dimethyl-arginine suppresses nitric oxide production in *Pseudomonas aeruginosa* sepsis

L Sousse, C Jonkam, D Traber, S Rehberg, L Traber, D Herndon, P Enkhbaatar
Department of Experimental Pathology and Department of Anesthesiology, University of Texas Medical Branch, Galveston, TX, USA
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Introduction More than 750,000 patients in the United States develop sepsis annually. Previously, we have shown that plasma nitric oxide (NO) levels were approximately sevenfold higher and that arginase activity is significantly lower in methicillin-resistant *Staphylococcus aureus* (MRSA) sepsis than in *Pseudomonas aeruginosa* sepsis. In the present study, we hypothesize that increased asymmetrical dimethyl-arginine (ADMA), an endogenous inhibitor of NO synthase, is responsible for the suppressed NO production in *Ps. aeruginosa* sepsis.
Methods Ewes were operatively prepared and randomized after a 7-day recovery period into control, MRSA, and *Ps. aeruginosa* groups ($n = 6$). Injury consisted of instillation of (2 to 5) x 10^{11} CFU live MRSA or *Ps. aeruginosa* into the airway, and the sheep were sacrificed after 24 hours. In addition, groups of C57Bl/6J wild-type mice and iNOS knockout mice ($n = 6$) were nasally inoculated with (2 to 5) x 10^8 CFU live MRSA or *Ps. aeruginosa* and were sacrificed after 8 hours.
Results *Ps. aeruginosa*-treated sheep had a significantly higher ADMA (1.79 ± 0.14 vs. 1.16 ± 0.24 μM, $P < 0.05$), lower plasma NOx (6.83 ± 0.17 vs. 9.63 ± 0.64 μM, $P < 0.05$), and higher arginase activity (1.55 ± 0.16 vs. 1.07 ± 0.11 μM urea/μg protein, $P < 0.05$) compared with MRSA-treated sheep. These changes were associated with more pronounced lung injury in *Ps. aeruginosa* sepsis compared with MRSA sepsis (PaO_2/FiO_2: 205 ± 72 vs. 319 ± 82, $P < 0.05$). *Ps. aeruginosa*-treated mice had a significantly higher arginase activity (0.60 ± 0.12 vs. 0.16 ± 0.0038 μM urea/μg protein, $P < 0.05$) and higher protein oxidation with carbonyl groups (3,223 ± 440.7 vs. 1,124 ± 140.1, $P < 0.05$) compared with MRSA-treated mice. iNOS knockout mice treated with MRSA had significantly lower arginase activity than wild-type mice (0.21 ± 0.01 vs. 0.36 ± 0.04 μM urea/μg protein, $P < 0.05$).
Conclusions Suppressed NO production in *Ps. aeruginosa* sepsis is caused by the increased expression of ADMA. Increased arginase activity is most probably caused by augmented oxidative stress, which results in a more severe lung injury.

P35

Disseminated intravascular coagulation during human septic shock: relation with lactate levels

KJ Hartemink[1,2], CE Hack[3], ABJ Groeneveld[1]
[1]Department of Intensive Care and the Institute for Cardiovascular Research, [2]Department of Surgery, and [3]Department of Clinical Chemistry, VU University Medical Center, Amsterdam, the Netherlands
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Introduction The exact pathogenic role of disseminated intravascular coagulation (DIC) during septic shock is incompletely understood.
Objective We studied the relation between sensitive and specific markers for DIC and lactate levels in the course of time, to evaluate whether DIC could contribute to microvascular obstruction and tissue hypoxygenation.
Methods We prospectively studied 14 consecutive septic shock patients with a pulmonary artery catheter in place. For 3 days after admission, hemodynamic variables, and plasma levels of lactate, thrombin–antithrombin complexes (TAT), tissue plasminogen activator (tPA), plasminogen activator inhibitor (PAI) and plasmin–α_2-antiplasmin complexes and TNFα, IL-6 and complement activation product C3a were measured 6-hourly.
Results Of the 14 patients, eight died in the ICU. Patients had a hyperdynamic circulation with tachycardia, mild hypotension and increased cardiac index. The course of TAT, tPA and particularly of PAI predicted the course of lactate levels, independently of hemodynamic and inflammatory factors. Lactate and PAI elevations persisted in nonsurvivors versus survivors.
Conclusions Our observations show that, in the course of human septic shock, activation of coagulation and, particularly, inhibition of activated fibrinolysis are independently associated with hyperlactatemia. This suggests a contribution of DIC resulting from a coagulation/fibrinolysis imbalance to microvascular obstruction, tissue hypoxygenation and thereby to ultimate demise.

P36

Monitoring of procalcitonin, IL-6 and brain natriuretic peptide for sepsis diagnosis in cardiac surgery

R Barchetta, C Alessandrini, C Di Corato, F Candidi, F Turani, M Falco
Department of Anaesthesia and Intensive Care, European Hospital, Rome, Italy
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Introduction Procalcitonin (PCT) and IL-6 are markers used in the evaluation of systemic inflammation (SIRS) and septic states. The purpose of this study is to analyse changes in plasma concentrations of PCT and IL-6 in patients undergoing cardiac surgery on-pump and assess its reliability in the early detection of post-operative infectious complications. In all patients the variation of brain natriuretic peptide (BNP) was also evaluated in order to stratify the clinical condition of patients.
Methods We measured serum levels of PCT, IL-6 and BNP in adult patients undergoing myocardial revascularization and/or valve surgery performed in extracorporeal circulation. The measurements were performed on the day before the intervention (T0), at the end of surgery (T1) and then until the third and fourth postoperative day (T2 to T4). We also recorded the onset of cardiac, respiratory, neurological, renal and septic complications. The diagnosis of sepsis was confirmed retrospectively on the basis of clinical, radiological and microbiological data. All data are expressed as mean and standard deviation. The Kruskal–Wallis test was used to assess changes over time of variables. $P < 0.05$ was considered statistically significant.
Results There have been enrolled 60 patients undergoing cardiac surgery in extracorporeal circulation. Among these, nine patients developed septic complications. The results of temporal changes and the significance are presented in Table 1.

Table 1 (abstract P36)

Results of temporal changes and significance

	T0	T1	T2	T3	T4
PCT (ng/ml)					
Nonseptic	0.04	0.04	0.58	0.34	0.34
Septic	0.04	0.15	2.63	1.87	0.74
P	NS	<0.001	<0.001	<0.001	<0.01
IL-6 (pg/ml)					
Nonseptic	12	160	129	78	75
Septic	18	184	145	261	92
P	NS	NS	NS	<0.01	NS
BNP					
Nonseptic	159	154	347	428	492
Septic	373	627	731	756	798
P	<0.01	< 0.01	<0.01	<0.05	<0.05

Conclusions In patients who develop septic complications, changes in PCT occur earlier than changes in IL-6. Furthermore, BNP performs in the same fashion as PCT and correlates better than IL-6 with the clinical data of the infection status. In conclusion, monitoring PCT seems to be useful in early diagnosis of septic complications in patients undergoing cardiac surgery and more sensitive on the variations in IL-6. The combined study of variations in PCT and BNP could improve the diagnostic accuracy in these patients.

P37

T-cell-specific peroxisome proliferator-activated receptor gamma depletion inhibits T-cell apoptosis and improves survival of septic mice via an IL-2-dependent mechanism

MV Schmidt[1], P Paulus[2], A-M Kuhn[1], V Meilladec-Jullig[1], K Zacharowski[2], B Bruene[1], A von Knethen[1]
[1]Institute of Biochemistry I, Faculty of Medicine, Goethe-University Frankfurt, Germany; [2]Department of Anaesthesia, Intensive Care Medicine & Pain Therapy, University Hospital Frankfurt, Germany
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Introduction Immune paralysis with massive T-cell apoptosis is a central pathogenic event during sepsis and correlates with septic patient mortality. Previous observations implied a crucial role of peroxisome proliferator-activated receptor gamma (PPARγ) during T-cell apoptosis.
Methods To elucidate mechanisms of PPARγ-induced T-cell depletion, we used an endotoxin model as well as the caecal ligation and puncture sepsis model to imitate septic conditions in wild-type versus conditional PPARγ knockout (KO) mice.
Results PPARγ KO mice showed a marked survival advantage compared with control mice. Their T cells were substantially protected against sepsis-induced death and showed a significantly higher expression of the pro-survival factor IL-2. Since PPARγ is described to repress nuclear factor of activated T cells (NFAT) transactivation and concomitant IL-2 expression, we propose inhibition of NFAT as the underlying mechanism allowing T-cell apoptosis. Corroborating our hypothesis, we observed up-regulation of the pro-apoptotic protein BIM and downregulation of the anti-apoptotic protein Bcl-2 in control mice, which are down-stream effector proteins of IL-2 receptor signaling. Application of a neutralizing anti-IL-2 antibody reversed the pro-survival effect of PPARγ-deficient T cells and confirmed IL-2-dependent apoptosis during sepsis.
Conclusions Apparently antagonizing PPARγ in T cells might improve their survival during sepsis, which concomitantly enhances defence mechanisms and possibly provokes an increased survival of septic patients.

P38

Induction of severe *Staphylococcus aureus* sepsis in pigs

TM Iburg[1], PS Leifsson[1], M Kjelgaard-Hansen[2], P Heegaard[3], B Wiinberg[2], B Aalbaek[1], AE Olsson[1], MGS Hansen[1], LB Thomsen[1], HE Jensen[1], JS Agerholm[1], OL Nielsen[1]
[1]Department of Veterinary Disease Biology, and [2]Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark; [3]Department of Veterinary Diagnostics and Research, National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark
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Introduction Organ dysfunction is an integrated part of severe sepsis, and severe sepsis is one of the major causes of death in ICUs. Lately Gram-positive bacteria accounted for more than one-half of the overall sepsis cases reported in the USA, with *Staphylococcus aureus* being the most commonly isolated bacterium. Effective treatment of sepsis is still not optimal and good animal models are needed for research in pathogenesis and treatment. *S. aureus* infections are also common in pigs and are isolated from approximately 40% of embolic lesions found in slaughter-pigs.
Objective To establish a porcine model of severe sepsis.

Methods Twelve pigs in four groups were inoculated intravenously once or twice with 1 x 10^8 *S. aureus*/kg body weight and euthanized consecutively from 6 to 48 hours after inoculation. Mock-inoculated pigs served as controls. Body temperature was measured and blood samples were taken at regular intervals for bacteriology, haematology, clinical chemistry, and acute phase reactant determinations. Full necropsy was done and tissue samples were collected for bacteriology and histology. Apoptosis was measured in the spleen.
Results Onset of clinical disease (fever and lethargy) was seen at 7 to 8 hours after inoculation. Blood bacterial counts remained low throughout the study. SIRS characterized by fever, leukocytosis, increased levels of CRP, IL-6, IL-1β, TNFα, and decreased level of serum iron was detected after 12 hours. Both CRP and IL-6 levels peaked at 36 hours. Platelet numbers declined slightly and were lower than in the controls at 48 hours. Thromboelastography showed increased hypercoagulability over time. Levels of serum aspartate aminotransferase and bilirubin were elevated at 24 and 36 hours. Blood urea nitrogen levels had increased at 36 hours; however, no difference was seen in serum creatinine levels. Disseminated microabscesses were found in the lung at 6 hours, but had disappeared at 48 hours. In the bones, the presence of microabscesses progressed until 48 hours. Other histopathological signs related to inoculation were limited to a renal microabscess at 12 hours, splenic microabscesses at 24 hours and centrilobular hepatic necrosis with thrombosis in one animal at 48 hours. In the liver and kidneys, various degrees of fibrinous exudation were found. The number of apoptotic cells in the splenic white pulp was increased at 48 hours.
Conclusions All infected pigs developed sepsis with metastatic abscesses and at 48 hours severe sepsis was present with signs of dysfunction of the liver and the coagulation system. The splenic apoptotic response indicates reduced function and immunosuppression.

P39

Cyclin-dependent kinase inhibitor r-roscovitine reduces lipoteichoic acid lung inflammation and improves the resolution of antibiotic-treated *Streptococcus pneumoniae* pneumonia

AJ Hoogendijk[1,2], JJTH Roelofs[3], MHP van Lieshout[1,2], DC Blok[1,2], T van der Poll[1,2], CW Wieland[2,4]
[1]Center for Infection and Immunity Amsterdam, [2]Center for Experimental and Molecular Medicine, [3]Department of Pathology, and [4]Laboratory of Experimental Intensive Care and Anesthesiology, Academic Medical Center, Amsterdam, the Netherlands
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Introduction *Streptoccocus pneumoniae* pneumonia remains associated with high morbidity and mortality. Antibiotic treatment frequently is insufficient in limiting lung damage due to inflammation. Therefore, additional treatment strategies are needed. The drug r-roscovitine, a cyclin-dependent kinase (CDK) inhibitor, was demonstrated to reduce inflammation in several models of inflammation.
Objective We studied the potential of r-roscovitine to modulate host defense during sterile inflammation and bacterial infection of the lung.
Methods Isolated neutrophils were treated with 20 μM r-roscovitine and CDK and caspase 3 activity were determined by western blot analysis. Sterile lung inflammation was induced by intranasal administration of 100 μg lipoteichoic acid (LTA), a prominent cell wall component of Gram-positive bacteria. Simultaneously 70 mg/kg r-roscovitine or vehicle was injected intraperitoneally. Twenty-four hours later bronchoalveolar lavage (BAL) was performed and differential cell counts were determined. Bacterial pneumonia was induced by inoculation of 5 x 10^4 CFU *S. pneumoniae*. r-Roscovitine (70 mg/kg) or vehicle was administered 24 hours later in combination with antibiotic therapy (ceftriaxon; 20 mg/kg). Mice were sacrificed after 48 hours. In a second experiment, mice were infected and treated at 24 and 72 hours and sacrificed 96 hours post infection.
Results r-Roscovitine treatment significantly reduced phosphorylated CDK substrate and increased cleaved caspase 3 levels in isolated neutrophils. During LTA-induced lung inflammation, r-roscovitine treatment significantly reduced the amount of PMNs in the BAL fluid and cytokines in lung homogenates. After 48 hours of bacterial pneumonia, r-roscovitine-treated animals displayed enhanced pulmonary bacterial outgrowth. Cytokine production and lung damage scores were higher in the r-roscovitine-treated group as compared with vehicle. Interestingly, when studying the animals at 96 hours post infection, r-roscovitine treatment resulted in lower bacterial outgrowth and chemokine levels in the lung.
Conclusions With this study, we reproduced earlier findings that r-roscovitine treatment reduces CDK activity and induces apoptosis in neutrophils; we demonstrated that r-roscovitine diminishes inflammatory responses in sterile inflammation; and we found that r-roscovitine treatment in bacterial pneumonia is detrimental early in infection but beneficial at later time points. We believe that the negative effect of r-roscovitine reflects the importance of neutrophil antibacterial defense early in infection. Yet, during resolution of infection, apoptosis of neutrophils induced by r-roscovitine could present a way of damage control as opposed to unwanted side effects of neutrophil function.

P40

Honey as an immunomodulator during sepsis in animal model

M Kassim[1], M Mansor[1], M Achoui[2], OS Yan[1], S Devi[3], KM Yusoff[4]
[1]Department of Anesthesiology, [2]Department of Pharmacology, [3]Department of Medical Microbiology, and [4]Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
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Introduction Malaysian honey (Gelam) has antibacterial activity and it also has a high antioxidant capacity and free radical scavenger activities. Honey extracts showed potent activity against TNFα in L929 cell and NO in RAW 264.7 macrophage as well as inhibitory effects on the prostaglandin E_2 and nitric oxide (NO) in inflammatory tissues of rat. Sepsis is mediated in part by bacterial endotoxin, which stimulates macrophages/monocytes to sequentially release early (for example, TNF, IL-1) cytokines and inducible enzymes such as inducible nitric oxide (iNOS) synthase and heme oxygenase 1 (HO-1) and late such as high-mobility group box 1 (HMGB1).
Objective To investigate the role of honey as an immunomodulator in sepsis induced by LPS in rats.
Methods Four groups (n = 6) of rats were used. The treatment group received honey with LPS, the positive control group were given LPS (5 mg/kg), the negative control group were given saline only, while the fourth group were only given honey; all doses were 1 ml by intravenous route. Blood samples were collected 4 hours later and all rats were sacrificed after 24 hours. TNFα, IL-1β, IL-6, IL-10, NO, HO-1 and HMGB1 were quantified using ELISA. The effect of honey on coagulation (PT and APPT) in whole blood *ex vivo* from healthy volunteers (n = 10) was measured.

Results After 4 hours of treatment, the cytokines, NO and HO-1 were measured in all groups. Honey showed evidence of immunomodulatory effects with reduced cytokines (TNFα ($P < 0.001$), IL-1β ($P < 0.001$), IL-10 ($P < 0.001$)) and NO ($P < 0.037$) in the treatment group, while the change in IL-6 was not significant between all groups, HO-1 ($P < 0.001$) was increased in the treatment group, but only slightly increased in the honey group. After 24 hours of treatment, HMGB1 ($P < 0.025$) and IL-1β ($P < 0.001$) were reduced in the treatment group as well. HO-1 ($P < 0.013$) continuously increased in all groups. Curiously, honey alone induced TNFα ($P < 0.001$) and IL-1β ($P < 0.03$) at 4 hours, and HO-1 ($P < 0.028$) at 24 hours compared with saline. Honey prolonged the time of PT and APPT in a dose-dependent manner.
Conclusions Honey behaves as immunomodulator by acting in two ways, by inducing HO-1, TNFα, and IL-1β and at the same time inhibiting cytokines, NO and HMGB1 that is induced by LPS. However, the exact mechanism remain unclear, but our suggestion is that since honey induces HO-1, TNFα and IL-1β this may cause changes or inhibition in the signaling of cytokines and NF-κB. Honey could therefore be used as a pharmacological tool in sepsis in the future.

P41

Are phenylcarboxylic acids really markers in severe sepsis?

NV Beloborodova, AS Khodakova, AJ Olenin
Bakoulev Scientific Center of Cardiovascular Surgery, Moscow, Russian Federation
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Introduction Laboratory diagnostics of sepsis need to be improved. There is no evidence in the literature whether microbial metabolites could be used as sepsis markers. As a result of large-scale screening of microbial compounds we showed that levels of some phenylcarboxylic acids (PCAs) were increased in blood of septic patients. The content of *p*-hydroxyphenyllactic acid (HPLA), phenyllactic acid (PLA) and *p*-hydroxyphenylacetic acid (HPAA) was significantly higher in blood of patients with severe sepsis compared with control groups. The aim of the present study is to evaluate the sensitivity and specificity of PLA, HPLA and HPAA as markers for severe sepsis diagnostics.
Methods In total, 264 blood samples from 200 adults were included to research. All persons were divided into groups with infectious complications after cardiosurgery (35 severe sepsis, 35 local infection complication) and others (33 non-infection complication after surgery, 30 smooth recovery after surgery, 42 before surgery and 25 healthy volunteers). Clinical characteristics and procalcitonin (PCT), a well established biomarker of sepsis, were assessed in all patients. Severe sepsis was diagnosed according to consensus criteria, also a level of PCT ≥2 ng/ml was an additional criterion. Blood concentrations of PCAs were determined by gas chromatography–mass spectrometry.
Results The levels of PCA in two control groups (healthy people and patients before surgery) were HPAA 0.4 to 0.8 x 10^{-6} M, HPLA 1.2 to 1.5 x 10^{-6} M, PLA 0.3 to 0.4 x 10^{-6} M and were not significantly different. Otherwise the levels of HPAA, HPLA and PLA as 11.6 (3.3 to 33.6) x 10^{-6} M, 7.5 (3.0 to 14.4) x 10^{-6} M and 1.8 (1.1 to 4.9) x 10^{-6} M in all severe sepsis patients were significantly increased versus control groups and versus all other groups ($P < 0.0001$). In addition the levels of PCAs from surviving and nonsurviving severe sepsis patients were compared with each other. Nonsurviving sepsis patients had a significantly higher content of PLA (3.6 (1.5 to 6.4) x 10^{-6} M vs. 1.2 (0.8 to 1.6) x 10^{-6} M for survivors); the same trend was observed for HPLA (12.5 (5.6 to 34.7) x 10^{-6} M vs. 2.8 (2.2 to 5.0) x 10^{-6} M correspondingly), but not for HPAA.
Conclusions The following levels of PCAs are appropriate for diagnostic of sepsis: HPAA 8 x 10^{-6} M (sensitivity 64.3%, specificity 88.9%), HPLA 3 x 10^{-6} M (sensitivity 75%, specificity 66.4%), PLA 1 x 10^{-6} M (sensitivity 75%, specificity 71.6%). Obtained data indicate that quantitative measurement of PLA, HPLA and HPAA in blood could be used for sepsis diagnostics in clinical practice and also as a predictor of outcome in high-risk surgery.

P42

Impact of community-based education on sepsis

K Choy, CA Agcaoili, K Halimi
Washington Hospital Healthcare System, Fremont, CA, USA
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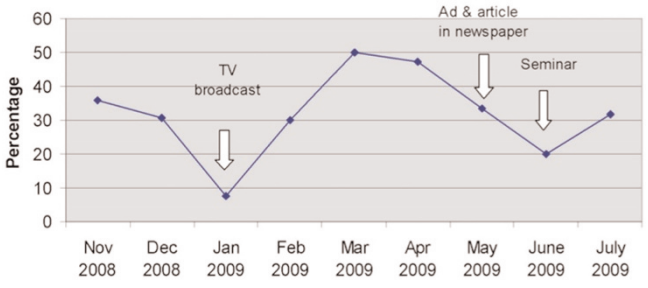
Introduction Sepsis is an uncontrolled infection that can develop very quickly throughout the body. Sepsis can strike anyone at any age and people with pre-existing medical conditions may be at greater risk. Patients with sepsis often present to the emergency department from home. Community education programs focus on raising public awareness of sepsis, its signs and symptoms, and can positively impact outcomes.
Objective A multidisciplinary public health campaign was developed to educate a local urban community about recognizing the signs and symptoms of sepsis, preventing infection, and seeking treatment early. Targeting education efforts at the community level engages consumers to become involved in the care of their health.
Methods A multimedia approach including print, television broadcast, hospital website on the Internet, news article and advertisement in the newspaper were widely distributed to maximize the ability to reach citizens throughout the area. A live seminar presented by two physicians and a clinical nurse specialist was strategically used to enhance learning, and participants completed an evaluation upon completion of the seminar.
Results Ninety-six percent of the participants who attended the live seminar rated the program as excellent. The participants shared that the topic was very educational, informative, and felt that their questions were answered. Because of the education media campaign that targeted 320,000 households and the hospital-wide implementation of the sepsis bundles, the mortality for severe sepsis decreased from 40% at baseline to 32.8%, which was a 18% relative risk reduction (Figure 1).

Figure 1 (abstract P42)



Severe sepsis mortality.

Conclusions This community-based education program on sepsis demonstrated that education programs offered to the community will improve overall outcomes and promote quality care of sepsis patients. In the pursuit of evaluating the effectiveness of the program, there will be ongoing monitoring of its impact. Future education programs will continue to sustain improvements.

P43

IL-33 protects mice from sepsis by inhibiting TLR4 signaling

JC Alves-Filho[1,2], F Sonego[1], FO Souto[1], A Freitas[1], WA Verri Jr[1], D Xu[2], FQ Cunha[1], FY Liew[2]
[1]Department of Pharmacology, School of Medicine of Ribeirao Preto, University of São Paulo, Brazil; [2]Division of Immunology, Infection and Inflammation, Glasgow Biomedical Research Centre, University of Glasgow, UK
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Introduction Sepsis is an acute systemic inflammation following infection, with a high mortality rate and limited therapeutic options. IL-33 is a recently identified member of the IL-1 family that binds to ST2 receptor, which is preferentially expressed on Th2 and mast cells. Accordingly, the IL-33/ST2 pathway is closely associated with the activation and production of type-II cytokines (IL-4, IL-5 and IL-13). However, ST2 has been implicated in inhibiting macrophage-dependent inflammation in response to LPS by negatively regulating Toll-like receptor-4 (TLR4) activation. Although TLRs have been implicated as an important element of host defense against infections, evidence indicates that these receptors may also play a detrimental role in the pathophysiology of sepsis.
Objective To investigate the role of the IL-33/ST2 pathway in experimental sepsis.
Methods and results We show that IL-33 treatment (1 μg/mouse, intravenously) markedly reduced mortality in WT mice (50%, $P<0.01$) undergoing experimental sepsis induced by the cecal ligation and puncture (CLP) model. We did not detect any differences between ST2 KO and WT mice in peritoneal bacterial load and mortality rate after CLP, suggesting that the endogenous IL-33 does not participate in the pathophysiology sepsis. However, while the exogenous injection of IL-33 markedly reduced the CLP-induced mortality in WT mice, IL-33 failed to do so in $ST2^{-/-}$ mice, indicating the critical role of ST2 on the protective effect of IL-33. Notably, we found that IL-33-treated mice developed significantly increased neutrophil infiltration in the peritoneal cavity (fourfold) and more efficient bacterial clearance than untreated mice ($n = 10$) after CLP. Moreover, IL-33 treatment leads to marked reduction (30 to 70%) of systemic proinflammatory cytokines (TNFα, IL-6 and CXCL2) but not a shift toward a Th2 immune response (IL-4 and IL-13). The chemokine receptor CXCR2 plays a central role in the recruitment of neutrophils into the site of infection. Flow cytometry analysis showed that direct activation of TLR4 in neutrophils downregulates the expression of CXCR2 and, consequently, impaired CXCL2-driven and CLP-driven neutrophil migration, *in vitro* and in *vivo* respectively. Notably, IL-33 prevented the downregulation of CXCR2 on circulating neutrophils during CLP *in vivo* or LPS-treated neutrophils *in vitro*. Finally, we demonstrated that IL-33 reversed the TLR4-induced reduction of CXCR2 via the inhibition of LPS-induced G-protein-coupled receptor kinase-2 (GRK2) expression, a potent negative regulator of CXCR2.
Conclusions Altogether we provide here a novel mechanism of action of IL-33 and establish a potential therapeutic role of this new cytokine in sepsis.
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P44

Comparison of microscopic, phenotypic and molecular techniques for the rapid identification and susceptibility testing of *Staphylococci* from positive blood culture bottles

A Shah, NC Gordon, L Pheel, DW Wareham
St Bartholomew's and The London NHS Trust, London, UK
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Introduction The ability to rapidly identify and determine the antimicrobial susceptibility of bacterial pathogens is an undisputed requirement for strategies aimed at improving the management of sepsis. *Staphylococci* are amongst the most common organisms isolated from blood cultures but it is difficult to rapidly distinguish between those representing contamination of the blood culture with harmless skin commensals (*Staphylococcus epidermidis*) and those that contain pathogenic species (*Staphylococcus aureus*).
Objective A number of phenotypic and genotypic tests with a vast range of complexity, speed and cost have been proposed to help distinguish these organisms. We evaluated the sensitivity, specificity and speed of a range of these tests compared with the standard laboratory identification protocol which takes up to 48 hours.
Methods Positive blood culture sets (BACT/Alert 3D) ($n = 113$) in which Gram-positive cocci in clusters were seen on the initial film were included. Further identification as *S. epidermidis* versus *S. aureus* was attempted using the following techniques directly from the positive bottles: (1) morphology and organization of the cocci on Gram stain (10 minutes), (2) rapid tube coagulase (2 hours), (3) direct DNAse test using toluedine blue agar (4 hours), (4) species-specific multiplex PCR (4 hours), (5) direct inoculation of selective commercial chromogenic media (18 hours), and (6) direct inoculation of mannitol-supplemented Mueller–Hinton agar (MMHA) combined with disc diffusion susceptibility testing (18 hours). Identifications were compared with the standard laboratory identification at 36 hours.
Results Definitive laboratory identification revealed *S. epidermidis* 87% ($n = 98$), *S. aureus* 11% (methicillin-sensitive *S. aureus* (MSSA) $n = 9$, methicillin-resistant *S. aureus* (MRSA) $n = 3$), *Micrococcus* spp. 2% ($n = 2$); one culture contained a mixture of *S. aureus* and *S. epidermidis*. The sensitivity and specificity of each of the direct techniques was calculated as follows: (1) microscopy 53% and 98%, (2) rapid coagulase 92% and 100%, (3) direct DNAse 69% and 98%, (4) multiplex PCR 88% (no amplification in 12 samples) and 99%, (5) chromogenic *S. aureus* media 90% and 100%, and (6) MMHA 100% and 78%.
Conclusions All the methods had specificity >95%, except for MMHA, although this has the added advantage of providing susceptibility results. Chromogenic agar had the highest sensitivity and specificity, although it provided a result only 12 hours quicker than the standard protocol. Rapid tube coagulase was highly specific and one of the most rapid tests. This may be the most useful, inexpensive technique providing preliminary results to guide empirical therapy, especially in resource-poor settings.

P45

Evidence that simvastatin prevents induction of nitric oxide synthase by LPS in the porcine isolated coronary artery

S Al-Shalmani[1], S Chinnah[2], R Mahajan[2], V Wilson[1]
[1]School of Biomedical Sciences, and [2]Academic Division of Anaesthesia and Intensive Care, University of Nottingham Medical School, Nottingham, UK
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Introduction Several retrospective studies suggest that prior use of statins can reduce hospital mortality in patients diagnosed with

either bacteraemia or sepsis. In an accompanying abstract we demonstrated that pre-treatment with simvastatin prevents LPS-induced hyporesponsiveness of the porcine isolated coronary artery (PCA); an observation consistent with the clinical data. Although this effect of simvastatin is qualitatively similar to that of a known inhibitor of inducible nitric oxide synthase (1400W), it unlikely that direct inhibition of the enzyme is implicated. We have investigated whether the beneficial effect of simvastatin on LPS-induced changes in the PCA involves alteration in the induction of nitric oxide synthase (iNOS).

Methods Segments of the PCA were dissected from hearts and incubated (2 x 5 mm) in DMEM at 37°C in the presence of an antibiotic mixture (60 μg/ml benzylpenicillin, and 20 μg/ml streptomycin sulphate), with or without 1 μg/ml LPS, 3 μM simvastatin or a combination of the two (simvastatin added 60 minutes before LPS). The medium also contained 1 mM L-arginine. After 24 hours, segments were removed and weighed (mg wet weight). The nitrite/nitrate content (nmol) of the bathing medium was determined by spectrophotometry using the Griess reaction. In a separate experiment, segments were prepared for immunohistochemical determination of the presence of iNOS and CD31. Differences between mean values were assessed by ANOVA (post-hoc Dunnett test).

Results Under control conditions the coronary artery segments produced 25.9 ± 3.7 nmol/mg wet weight nitrite/nitrate (n = 8) over 24 hours. Exposure to 1 μg/ml LPS caused a sevenfold increase in nitrite/nitrate production (199.0 ± 40.6 nmol/mg wet weight, n = 8). Although 3 μM simvastatin did not affect basal nitrite/nitrate production, it inhibited the response to LPS (35.6 ± 7.4 nmol/mg wet weight nitrite/nitrate) by 94.7 ± 5.7% (n = 8). Immunohistochemical assessment of four arteries revealed the presence of CD31 on endothelial cells under control conditions. Exposure to 1 μg/ml LPS was associated with an increase in endothelial CD31 and the appearance of iNOS in the adventitia. Co-incubation of segments with 3 μM simvastatin and LPS produced a profile similar to that of control segments (CD31-positive, iNOS-negative, n = 4).

Conclusions Simvastatin suppressed the induction of nitric oxide caused by LPS and the associated increase in nitrite/nitrate production. This finding helps to explain our observation that simvastatin prevented LPS-induced hyporesponsiveness of the coronary artery, and is also consistent with clinical studies suggesting that prior use of statins may afford protection against bacterial sepsis.

P46

Pre-treatment with simvastatin prevents LPS-induced hyporesponsiveness of porcine isolated coronary artery

S Al-Shalmani[1], S Chinniah[2], R Mahajan[2], V Wilson[1]
[1]School of Biomedical Sciences, and [2]Academic Division of Anaesthesia and Intensive Care, University of Nottingham Medical School, Nottingham, UK
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Introduction Several retrospective studies suggest that prior use of statins can reduce hospital mortality in patients diagnosed with either bacteraemia or sepsis. Simvastatin has been shown to modify the proinflammatory effect of lipopolysaccharide (LPS) on neutrophils and endothelial cells. However, it is not clear whether these effects are also manifest on vascular smooth muscle, which becomes hyporesponsive to vasoconstrictor agents due to the induction of nitric oxide synthase. We have investigated the effect of pre-treatment with simvastatin on LPS-induced changes in contractions of porcine isolated coronary artery (PCA).

Methods Segments (5 mm) of the PCA were dissected from hearts and incubated in Krebs–Henseleit (K–H) solution at 37°C in the presence of an antibiotic mixture (60 μg/ml benzylpenicillin and 20 μg/ml streptomycin sulphate), with or without 1 μg/ml LPS, 3 μM simvastatin or a combination of the two (simvastatin added 60 minutes before LPS). After 16 to 18 hours, segments were prepared for isometric tension recording in K–H solution. The segments were then exposed to cumulatively increasing concentrations of KCl and then U46619. In some experiments, some segments were exposed to 10 μM 1400W, a selective inhibitor of inducible nitric oxide synthase prior to the addition of the agonists. Responses are shown as gram weight or calculated as the concentration causing 50% of the maximum effect (–log EC_{50}). Differences between mean values were assessed by ANOVA (post-hoc Dunnett test).

Results KCl and U46619 caused concentration-dependent contraction of the PCA. Table 1 shows that treatment with 1 μg/ml LPS overnight (and subsequent removal) significantly reduced the maximum response to KCl and U46619 in the PCA by 32.0 ± 4.5% (n = 12) and 28.9 ± 12.3% (n = 12), without changing the potency of either agent. These effects of LPS on vasoconstrictor responses were not observed when 10 μM 1400W was added to the organ bath, after removal of the endotoxin (Table 1). LPS also failed to impair constrictor responses if 3 μM simvastatin was present during the incubation period and was subsequently removed (Table 1).

Table 1 (abstract P46)

Effect of LPS, 1400W and simvastatin on maximum response and potency of vasoconstrictor agent on the PCA

	KCl		U46619	
Incubation conditions	Maximum (g wt)	–log EC_{50}	Maximum (g wt)	–log EC_{50}
Control (n = 12)	10.23 ± 0.71	1.56 ± 0.04	10.39 ± 0.71	7.52 ± 0.08
LPS	6.83 ± 0.48*	1.60 ± 0.04	6.77 ± 0.80**	7.64 ± 0.06
LPS then 1400W	9.35 ± 1.21	1.64 ± 0.06	9.41 ± 0.46	7.54 ± 0.04
Control (n = 18)	12.13 ± 0.58	1.55 ± 0.01	13.46 ± 0.51	7.83 ± 0.01
LPS	8.41 ± 0.58**	1.51 ± 0.02	9.90 ± 0.47**	7.75 ± 0.06
Simvastatin and LPS	11.82 ± 0.34	1.53 ± 0.03	13.92 ± 0.38	7.73 ± 0.04

Data presented as mean ± SEM (n = 12 to 18). *P <0.05, **P <0.01, denotes a statistically significant difference from control.

Conclusions Prolonged exposure to LPS caused hyporesponsiveness of the PCA by a mechanism that appears to involve the induction of nitric oxide synthase. Since pre-treatment of the PCA with simvastatin reduced LPS-induced changes in vasoconstrictor responses, it unlikely that the effect of the statin involves direct inhibition of NOS (compare 1400W). These findings are consistent with clinical studies suggesting that prior use of statins may afford protection against bacterial sepsis.

P47

Effects of statins on postoperative sepsis, systemic inflammatory response syndrome and mortality after colorectal surgery

A Khan[1], D Yeung[1], B Wyatt[1], T Rafai[1], J Byant[1], A Coates[1], E Fitzgerald[2], A Acheson[2], V Wilson[1]
[1]School of Biomedical Sciences, and [2]Division of Surgery, University of Nottingham Medical School, Nottingham, UK
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Introduction Colorectal surgery carries significant risks of postoperative morbidity and mortality. One of the major hazards is an increased risk of sepsis; an important component of which is systemic inflammatory response syndrome (SIRS). Several recent studies suggest that the noncholesterol-related, pleiotropic effects of statins may limit the development of sepsis and associated inflammation. This study investigates the impact of prior statin therapy on the incidence and outcome of postoperative sepsis and SIRS in colorectal surgery patients.
Methods A retrospective cohort analysis of 577 patients who underwent curative surgery for colorectal cancer was conducted to evaluate postoperative morbidity and mortality (within 30 days of surgery). The primary endpoints were: 30-day in-hospital mortality, admission to intensive care (ICU), and a positive diagnosis of SIRS or sepsis.
Results Prior to admission, 21.7% of patients were taking either simvastatin, atorvastatin, fluvastatin, pravastatin or rosuvastatin. Patients on statins were significantly older than those not on statins (statin – 74.7 years (SD = 6.5) vs. nonstatin – 69.2 years (SD = 13.4), $P = 0.022$), more likely to have pre-existing co-morbidities and in receipt of antidiabetic agents and other cardiovascular drugs. Table 1 shows there was no difference in mortality rate between the two groups. Furthermore, the incidence of nosocomial infection and sepsis did not differ between the statin and nonstatin groups. Despite being more likely to be admitted to the ICU, the statin group was significantly less likely to develop either SIRS (in or out of the ICU) or postoperative wound infection or be admitted to the ICU for infective/inflammatory sequelae.

Table 1 (abstract P47)

Outcomes for statin and nonstatin patients following colorectal surgery

Parameter	Statin	Nonstatin	Sig.
Overall mortality	9/125 (7.2%)	27/452 (6.4%)	0.77
Nosocomial infections	42/125 (33.6%)	132/452 (29.2%)	0.40
Sepsis	10/75 (13.3%)	42/203 (20.7%)	0.22
SIRS	16/125 (12.8%)	148/452 (32.7%)	<0.001
Wound infection	11/125 (8.8%)	66/452 (14.6%)	0.04
Admitted to the ICU	34/125 (27.2%)	63/452 (13.9%)	<0.001

Sig,. significance.

Conclusions Statin patients were older than nonstatin patients, and had a greater burden of co-morbidities, yet the mortality rate did not differ between the two groups. The possibility that prior use of statins may influence inflammatory or infective events associated with this surgical procedure is supported by the significantly lower incidence of wound infection, SIRS and sepsis in statin-treated patients, illustrating the potential for statins to confer protection against these insults in the most critically ill patients.

P48

Multiple organ dysfunction syndrome: the scapegoat? Assessment of organ dysfunction between surviving and dying mice in the acute phase of polymicrobial sepsis

M Osuchowski[1], K Weixelbaumer[1], P Raeven[1], D Remick[2], K Reise[1], A Kozlov[1], M van Griensven[1], H Redl [1], S Bahrami[1]
[1]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology in the Trauma Research Center of AUVA, Vienna, Austria; [2]University of Boston, School of Medicine, Boston, MA, USA
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Introduction Multiple organ dysfunction syndrome (MODS) frequently complicates sepsis contributing to poor outcome. Yet the evolution of MODS in the early septic mortality (ESM) is unclear. To delineate the ESM–MODS relationship, we compared the development and magnitude of organ injury between dying and surviving mice in the acute phase (days 1 to 5) of polymicrobial sepsis.
Methods Female OF-1 mice were subjected to cecal ligation and puncture (CLP). In the first mouse subset, 20 µl blood was collected daily for 5 days or until death (mice followed for 28 days). To define the pre-lethal changes in circulating parameters, mice were retrospectively divided into two groups based on outcome in the acute sepsis: DEAD (all died within 5 days, $n = 39$) and survivors (SUR; alive at day 28, $n = 40$). In the second subset, mice were sacrificed within 24 hours of projected death (based on the body T <28°C, 100% specificity, $n = 7$) and matched with SUR (body T >35°C, 100% sensitivity) from the same post-CLP day and controls.
Results In the first subset, significant difference was observed between SUR and DIED in the circulating urea, ALT, LDH and glucose during the 1-day to 5-day time course but the magnitude of these changes varied among post-CLP days. Therefore, we used the day of death as a reference point clustering all pre-lethal parameter values as the 72, 48 and 24 hours prior-to-death (on any 1 to 5 post-CLP day) time points for comparison with the SUR values. A significant separation between SUR and DIED occurred generally at 24 hours prior to death: pre-lethal urea increased to 78 (vs. 40 mg/dl in SUR), ALT to 173 (vs. 106 U/l) and LDH to 798 (vs. 445 U/l), while pre-lethal glucose declined to 41 (vs. 74 mg/dl). In the second subset (only 24 hours prior-to-death time point), acute deaths were not preceded by a significant rise in creatinine (DIED 6.8 vs. 4.3 µM/l in SUR) and troponin I (239 vs. 119 pg/ml). Similarly, respiratory function of mitochondria in the liver and kidney was not impaired in either DIED and SUR compared with controls. Injury scores in the liver, kidney, heart and lung showed no apparent morphological disparity between moribund, surviving or control mice. Incidence of cell apoptosis (TUNEL) in organs was not increased in either of the groups.
Conclusions The increase of selected organ function/metabolic markers was manifested at least 24 hours prior to death. Despite statistical significance, the relatively small magnitude of these changes questions organ failure as a direct cause of death in the early phase of CLP sepsis.

P49

Mathematical modeling of community-acquired pneumonia patients

J Sarkar[1], DD Marathe[1], AM Inglis[1], KW Hurst[1], JA Kellum[2], DC Angus[2], Y Vodovotz[3], S Chang[1]
[1]Immunetrics Inc., Pittsburgh, PA, USA; [2]Department of Critical Care Medicine, University of Pittsburgh, PA, USA; [3]Department of Surgery, University of Pittsburgh, PA, USA
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Introduction Sepsis is defined by the systemic response to an infection, governed by dynamic interactions between the tissues, immune cells and inflammatory mediators. We used the Immunetrics platform to build a large-scale mathematical model that encompasses these biological components. The model incorporates a virtual clinician, an automated system to examine simulated patients' status at clinically relevant intervals and administer standard of care interventions as necessary, thereby altering the dynamics of the disease state. The model reproduces many characteristics of systemic response to an infection, including the time course of cytokines, coagulation factors, clinical markers, early and late organ failure, and early versus late deaths.
Methods The ordinary differential equation-based model was used to simulate the progression of sepsis over a 30-day hospital stay. This model was fit to published human endotoxemia data as well as data from severely septic community-acquired pneumonia (CAP) patients from the GenIMS study. The model was fit to 15 biomarkers and clinical markers, including mean arterial pressure, PaO_2, creatinine, TNFα, IL-6, and PAI-1.
Results Figure 1 illustrates simulated output as compared with the median time course data for surviving CAP patients without co-morbidities and for CAP patients without co-morbidities who died between 4 and 8 days after admission. After incorporating changes in physiological and immune function due to patient demographics and co-morbidities (for example, COPD, cardiovascular disease), a handful of parameters were changed to fit the model to median time course data from 14 subgroups. These parameters included pathogen virulence, effectiveness of antibiotics, baseline status of patients at study inclusion, and occurrence of secondary infection. By overlaying variations in the above parameters about the median model, the model can encompass the entire spectrum of patients observed in the GenIMS study. The virtual clinician administers individualized treatment for each simulated patient. The model simulations provide clear evidence of changes in disease progression as a function of differences in treatment.
Conclusions The ability of our model to reproduce a large variety of patients with a relatively small number of parameter changes illustrates the robustness of the underlying biological processes being modeled. The model may help identify real signals in immensely variable and noisy multidimensional sepsis patient data, and distinguish real patient responses from clinical study-site-related variability. This model is currently undergoing further validation. Future capabilities include assessment of risk and benefit of new drugs for sepsis or new treatment strategies (for example, early goal-directed therapy) in different patient cohorts.

P50

Heart rate variability in the early resuscitation of septic shock

RC Arnold[1], DJ Lundy[1], L Glaspey[1], G Green[2], AJE Seely[2]
[1]Cooper University Hospital, UMDNJ-Robert Wood Johnson Medical Center, Camden, NJ, USA; [2]The Ottawa Hospital and Research Institute, Ottawa, ON, Canada
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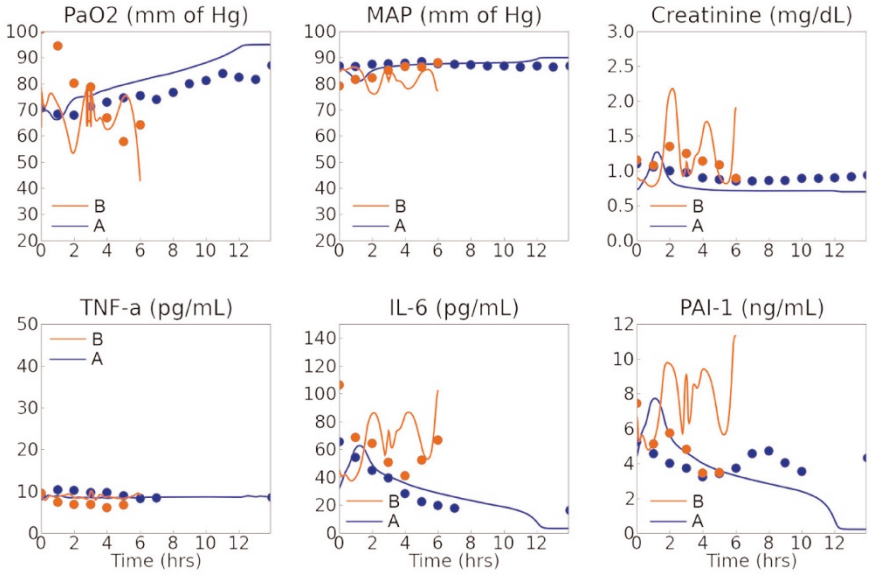
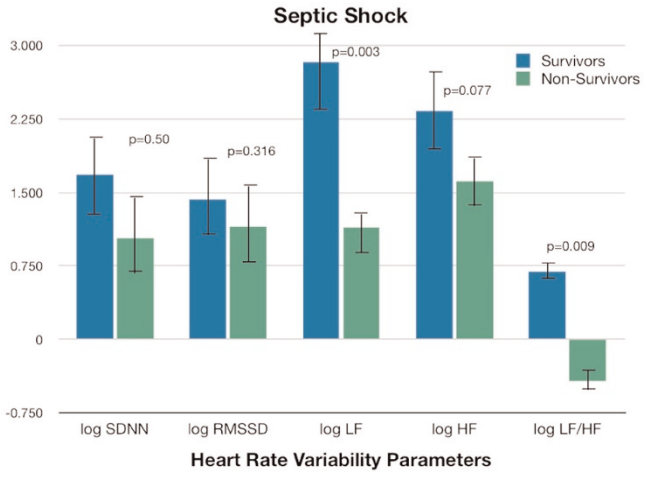
Introduction The assessment of heart rate variability (HRV) has provided valuable insight in sepsis. Impaired HRV has been shown

Figure 1 (abstract P49)



Comparison of time course data from model simulation (solid line) and median of patients (filled circles) from **(a)** surviving CAP patients without co-morbidities and **(b)** CAP patients without co-morbidities dying between 4 and 8 days after admission.

Figure 1 (abstract P50)



Heart rate variability parameters measured during the initial resuscitation of patients with septic shock. Nonsurvivors showed significantly impaired variability as measured by LF and the HF/LF ratio. Data presented as logarithmic transformation to control for scale and allow visual comparison. Differences between survivors and nonsurvivors were calculated using raw averages without logarithmic conversion. Time domain factors: SDNN = standard deviation of the normal R–R interval; RMSSD = root mean square of the difference of successive R–R intervals. Frequency domain factors of HRV spectrum: LF = low frequency (0.04 to 0.15 Hz); HF = high frequency (0.18 to 0.4 Hz).

to be diagnostic of sepsis, heralding its onset, and prognostic of its impact, correlating with the development of sepsis-induced organ failure and death.

Objective To analyze the ability of HRV to act as a prognostic aid in the early resuscitation of septic shock, and to study the effect that early fluid resuscitation will have on the direction and magnitude of subsequent measurements of HRV in patients with septic shock.

Methods Subjects were prospectively identified within the emergency department of an urban-based tertiary-care medical center during their initial evaluation and treatment for septic shock, defined as a systemic infection with a systolic blood pressure <90 mmHg after intravenous fluid or a serum lactate >4.0 mmol/l. Continuous cardiac telemetry was obtained for the assessment of HRV using a standardized set of multiple parameters including variables of frequency and time domain analyses. Fluid administration was recorded during the initial resuscitation. A composite endpoint of increasing organ failure and in-hospital mortality was measured.

Results Prospective analysis of 15 patients with septic shock was made. The in-hospital mortality rate was 67% (10/15). Nonsurvivors had a significantly impaired HRV compared with survivors when measured through multiple parameters ($P < 0.01$), as seen in Figure 1. There was no difference between fluid administration between survivors and nonsurvivors (4,475 vs. 5,220 ml, P = NS). There was no relationship seen between intravenous fluid administration and the change in 2-hour HRV seen in the early resuscitation of septic shock.

Conclusions In the early resuscitation of septic shock, HRV assessment can differentiate survivors from nonsurvivors, independent of organ severity measurement. While the use of fluid administration in the early resuscitation of shock did not correlate with changes in HRV measured at 2 hours, this relationship may exist when measured at longer time points allowing for the physiologic response to manifest in the HRV trend. The assessment of HRV in patients with infection can identify those at high risk for clinical deterioration and can potentially serve as an endpoint of resuscitation in patients with septic shock.

P51

Changes in MIF and thyroxine, in a clinically relevant large animal model of sepsis

E Miller[1], S Rehberg[2], H Linge[1], P Enkhbaatar[2], L Traber[2], D Traber[2], Y Al-Abed[1]

[1]The Feinstein Institute for Medical Research, Manhasset, NY, USA; [2]The University of Texas Medical Branch, Galveston, TX, USA

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Introduction Critically ill patients often have abnormally low plasma thyroxine (T4) concentrations even in the absence of thyroidal illnesses, with the lowest T4 values being observed in patients (particularly older individuals) with sepsis. Staphylococcal infection of the lung is the most common presentation of sepsis in the medical ICU setting, and is associated with a much higher mortality in older individuals than in younger. In studies where the lungs of rats are challenged with staphylococcal components lipoteichoic acid and peptidoglycan, we have noted negative correlations between plasma MIF and T4.

Objective To examine the dynamics of MIF and T4 in a clinically relevant, large animal model of sepsis.

Methods Adult female sheep (30 to 40 kg) with age approximately equivalent to humans of 55 years were surgically prepared for chronic study. After 5 days recovery, approximately 2.5 x 10^{11} CFU methicillin-resistant *Staphylococcus aureus* (ATCC 4300, a clinical isolate) were instilled into the lung via a bronchoscope. The animals were ventilated, and hemodynamics were monitored continuously for 24 hours post instillation. Blood and lymph samples were collected at baseline and at 3, 6, 12, 18, and 24 hours. MIF was measured by semiquantitative western blot and free-T4 by ELISA.

Results The plasma concentration of MIF increased from 24.9 ± 4.5 ng/ml at baseline to 30.1 ± 2.5 ng/ml in the first 6 hours post instillation of the bacteria. During this time period, free-T4 concentration decreased from 1.6 ± 0.5 ng/dl to 0.4 ± 0.3 ng/dl. The concentration of MIF in the pulmonary lymph was approximately 10% of the plasma level, and showed no time-dependent decrease, although the lymph flow increased approximately fourfold over the course of the study.

Conclusions We have found that intratracheal instillation of staphylococcal cell wall components in rats results in an imbalance of plasma MIF and free-T4, and that pulmonary-derived MIF in sepsis induces cardiocirculatory depression. Here we show that, in a clinically relevant, large animal model of sepsis induced by pulmonary infection with methicillin-resistant *S. aureus*, increased plasma concentration of MIF and decreased free-T4 occurred in a similar manner to our rat studies, and that the total amount of MIF in the lymph also increased. Since strong negative correlations exist between prognosis and levels of T4 or MIF in patients with sepsis, our findings underscore interest in possible interactions between the MIF and T4 molecules that may be critical for the outcome of the disease.

P52

Discovery of a natural antagonist of macrophage migration inhibitory factor

Y Al-Abed, C Metz, KF Cheng, B Aljabari, H Linge, M Ochani, X Lin, V Pavlov, T Coleman, K Tracey, EJ Miller
The Feinstein Institute for Medical Research, Manhasset, NY, USA
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Introduction Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that plays a critical role in the pathogenesis of sepsis. Plasma MIF concentrations are significantly higher in nonsurvivors than survivors of severe sepsis, and administration of antibodies that neutralize MIF activity improves survival in an experimental model of sepsis. Three-dimensional X-ray crystallography shows MIF has a homotrimeric conformation and we have determined that the hydrophobic cavity formed between two adjacent subunits of the homotrimer is required for the pro-inflammatory activity of the molecule. We have designed several small molecules that fit into the site critical for the proinflammatory action of MIF, and confirmed the interaction by the crystal structure of the MIF complex. Binding of MIF in this way inhibits its proinflammatory activity, improves the clinical outcome in sepsis, and recapitulates immunotherapy and gene deletion. However, no natural soluble ligand of MIF has been reported previously.
Hypothesis MIFnl1 binds to the hydrophobic cavity of MIF. Increased concentrations of MIF in sepsis deplete plasma MIFnl1 and lead to a critical MIF:MIFnl1 imbalance.
Results We have discovered a natural ligand, designated MIFnl1, that binds the proinflammatory site of MIF with high affinity, and effectively modulates its activity. We examined several classes of endogenous small molecules and their metabolites and observed that MIFnl1 binds to, and inhibits, the hydrophobic cavity of MIF in a dose-dependent manner with an IC_{50} of 15.8 μM. Importantly, MIFnl1 was a more potent inhibitor of MIF than ISO-1 (IC_{50} = 25 μM), the gold standard synthetic inhibitor of MIF. In addition, in plasma from patients with sepsis, we found an inverse correlation between the increased level of MIF and the decreased concentration of MIFnl1. Therefore, we hypothesized that supplementation of this ligand during sepsis should compensate for its dramatic reduction and improve survival in our peritonitis model of sepsis in C57/Bl6 mice. Administration of MIFnl1 improved the 7-day survival rate to 60% compared with 20% observed for the vehicle-treated mice.
Conclusions Our data identify for the first time the presence of a natural, ligand antagonist of MIF in plasma. This suggests that, during severe sepsis, increased production and release of MIF leads to an imbalance of the MIF:MIFnl1 regulatory mechanism resulting in the development of an overwhelming systemic inflammatory response leading to cardiovascular collapse and death. A better understanding of the kinetics of MIF/ligand regulation in patients with sepsis may lead to improved outcome in this devastating disease.

P53

Age-associated increased inflammatory response to pulmonary bacterial challenge

HM Linge, K Lin, Y Al-Abed, EJ Miller
Cardiopulmonary Research, Center for Heart and Lung Research, The Feinstein Institute for Medical Research, Manhasset, NY, USA
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Introduction Sepsis, the systemic inflammation following infectious insult, is a major cause of morbidity and mortality particularly in older individuals. While most animal models of sepsis have studied polymicrobial or Gram-negative sepsis from an abdominal origin in young animals, the most common presentation in the medical ICU is in older individuals (median age 64 years), with Gram-positive bacteria (predominantly *Staphylococcus aureus*) infection in the lung. Our recent studies reveal a critical role for macrophage migration inhibitory factor (MIF) in the pathogenesis of sepsis, and thyroxine (T4), two molecules with major implications in sepsis. Therefore, we reasoned that the age-related severity of sepsis may be due to an exaggerated imbalance between MIF and its inhibitor.
Objective To examine age-dependent differences in inflammatory responses following pulmonary staphylococcal challenge.
Methods Lipoteichoic acid (LTA) and peptidoglycan (PGN) are major inflammatory components of the staphylococcal cell wall known to induce shock. Male Fischer 344 rats either Young (6 months) or Old (>18 months), approximately equivalent to humans of 18 and 60 years respectively, were anesthetized and LTA and PGN (1.5/5 mg/kg in 200 μl) were administered intratracheally. Six hours later, blood was collected from the heart, and post mortem, the lungs were lavaged for analysis of the epithelial lining fluid. Young, untreated cage control animals (Control) were also assessed.
Results The plasma MIF concentration significantly increased with LTA/PGN challenge (Control, 24.9 ± 0.6 ng/ml; Young, 38.8 ± 5.9 ng/ml; Old, 64.2 ± 12.1 ng/ml) and the mean concentration in the old animals was significantly higher than in the young; $P = 0.004$. Conversely, plasma free-T4 significantly decreased with LTA/PGN challenge (Control, 2.3 ± 0.9 ng/dl; Young, 1.7 ± 0.7 ng/dl; Old, 1.0 ± 0.4 ng/dl) and the mean concentration in the old animals was significantly lower than in the young; $P < 0.05$. Significant age-specific differences were also noted in the response with respect to neutrophils and IL-6 within the lavage fluid.
Conclusions Typically, critically ill patients have abnormally low plasma T4 concentrations even in the absence of thyroidal illnesses (the so-called euthyroid sick phenomenon) with the lowest values being observed in septic and/or elderly individuals, suggesting a strong negative correlation between prognosis and T4 concentration. The data suggest that an increased early inflammatory response in older compared with young animals results in an exaggerated imbalance between MIF and T4, possibly leading to development of an uncontrolled systemic response.

P54

Neutrophil recruitment by macrophage migration inhibition factor and CXCL1 to the lung following staphylococcal stimulation is significantly elevated in advanced age

HM Linge, K Takahashi, E Miller
Cardiopulmonary Research Center for Heart and Lung Research, The Feinstein Institute for Medical Research, Manhasset, NY, USA
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Introduction Mortality from sepsis is greater in the elderly than in the young although incidence only increases slightly. Pulmonary infections caused by *Staphylococcus aureus*, which progress into sepsis, are a major cause of death in elderly patients. Adiponectin, multifaceted adipokine with anti-inflammatory properties, is secreted primarily from adipose tissue. It is increasingly acknowledged that the tissue microenvironment changes with old age. With increasing age, ectopic fat accumulates, increasing the possibility of elevated levels of adipose-derived mediators in the older individual.
Objective To investigate age-dependent changes in the intra-pulmonary response to staphylococcal challenge. We hypothesized

that older animals have higher levels of adiponectin due to adipose tissue accumulation and that these levels will neutralize the proinflammatory consequences of staphylococcal stimulation of the lung.

Methods Young (6 months) and old (>18 months) male Sprague–Dawley rats were challenged intratracheally with *S. aureus* cell wall components lipoteichoic acid (LTA, 0.15 mg) and peptidoglycan (PGN, 0.5 mg) or saline alone. After 24 hours, plasma was collected and lungs lavaged post mortem. Concentrations of total protein, chemokines and MIF were assessed. *In vitro* studies examined the accumulation of adiponectin in the culture medium of the adipocyte cell line 3T3-L1 following direct challenge with LTA/PGN (3 μg/ml; 100 μg/ml) for 6 hours.

Results The older age group, compared with the young group receiving the same stimulus, showed significantly elevated alveolar levels of MIF and CXCL1 (KC), both involved in neutrophil recruitment. Neutrophils (controls: 0.029 ± 0.033, young: 13.9 ± 7.8, old: 29.3 ± 10.5, x 10^6) and the total number of cells (controls: 0.72 ± 0.26, young: 16.4 ± 9.7, old: 32.9 ± 11.2, x 10^6) within the alveolar space were significantly and age-dependently increased, following pulmonary insult. Plasma adiponectin did not change significantly. However, instillation of LTA/PGN significantly elevated the levels of adiponectin found within the alveolar space (controls: 3.9 ± 2.4, young: 22.5 ± 8.8, old: 29 ± 10.8 ng/ml, $P<0.01$). Interestingly, following LTA/PGN challenge of adipocytes, there was a significant decrease in adiponectin concentration in the culture medium (control: 20.3 ± 0.78, LTA/PGN: 18.23 ± 1.14 pg/ml, $P = 0.016$).

Conclusions The proinflammatory but not anti-inflammatory components of the immune response (assessed by neutrophil recruitment and adiponectin concentrations, respectively) differed significantly between the age groups. Since adiponectin decreased on direct stimulation of adipocytes, the increased adiponectin within the lungs may reflect increased lung permeability, and/or production by other cells within the lung. These findings are important in understanding the response to pulmonary infections in the older patient and may lead to the identification of novel targets for age-dependent therapeutic strategies.

P55

Naturally acquired anti-high mobility group box 1 antibodies during septic shock

B Sauneuf[1], D Grimaldi[1,2,3], C Rousseau[1], J-D Chiche[1,2,3], C Desgranges[1], J-P Mira[1,2,3]

[1]Department of Cellular Biology, Host–Pathogen Interactions, Cochin Institute, University Paris Descartes, INSERM U567, CNRS (UMR 8104), Paris, France; [2]Medical Intensive Care Unit, Cochin Hospital, Paris, France; [3]University Paris Descartes, Paris, France

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Introduction High mobility group box 1 (HMGB1) is a pleiotropic cytokine, implicated in the pathophysiology of sepsis. This alarmin, usually located in the nucleus, is released after tissue injury and activates various innate immunity receptors, leading to sustained inflammatory response. Inhibition of HMGB1 by anti-HMGB1 antibodies has been reported to decrease mortality in experimental models of sepsis. In the present work, we analyse whether HMGB1 secretion during septic shock leads to the production of naturally acquired anti-HMGB1 antibodies during septic shock.

Methods All patients with septic shock criteria and no immunosuppression were included during a 6-month period of time. After informed consent, blood samples (200 μl) were drawn on the day of shock and at D3, D7, and D14. Plasma HMGB1 levels were measured, using a commercial ELISA kit (Sino-Test Corporation, Sagamihara, Japan). Auto anti-HMGB1 antibodies were detected in serum by a homemade ELISA test.

Results Forty-two septic shock patients were included. Median age was 70 (59 to 78) years, SAPS 2 was 68 (51 to 83), and the mortality rate was 29%. HMGB1 was undetectable in the plasma of a control population. In contrast, high levels of HMGB1 were found in all septic patients (median = 5.71 ng/ml at D7). Thirteen patients (38%) presented a significant production of auto anti-HMGB1 IgG antibodies during the course of sepsis. The age, sex ratio, median HMGB1 level and mortality rate were similar in patients producing (PPAb) and not producing antibodies (PNPAb). However, as compared with the PNPAb patients, the PPAb group had significant higher APACHE II ($P = 0.027$) and SOFA scores ($P = 0.02$) at the onset of shock and had a more important but nonsignificant decrease of the cardiovascular SOFA score between day 1 and day 7 ($P = 0.063$).

Conclusions Naturally acquired anti-HMGB1 antibodies are produced during septic shock. The presence of antibodies is associated with higher severity scores and might be associated with an improvement of the haemodynamic dysfunction. The neutralizing capacity of these autoantibodies and their physiological role remain to be investigated.

P56

Delayed increased S100A9 mRNA predicts hospital-acquired infection after septic shock

M Fontaine[1], A Pachot[2], A Larue[2], B Mougin[2], C Landelle[1], C Allombert[2], F Venet[3], M-A Cazalis[2], G Monneret[3], A Lepape[1]

[1]Intensive Care Units, Hospices Civils de Lyon, CH Lyon-Sud, Lyon, France; [2]Joint Unit Hospices Civils de Lyon 'bioMerieux, Hôpital E. Herriot, Lyon, France; [3]Immunology Laboratory, Hospices Civils de Lyon, Hôpital E. Herriot, Lyon, France

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Introduction Septic shock (SS) remains a serious disease with high mortality and increased risk of hospital-acquired infection (HAI). Access to biomarkers assessing prognosis of these outcomes is of utmost importance in order to select patients for future therapeutic strategies. Alarmins are normal cell constituents that can be released into the extracellular milieu during states of cellular stress and subsequently activate the innate immune system. Several alarmins of the S100 family are released by phagocytes in response to cell stress, recognized by RAGE and/or TLR4 on monocytes and therefore highlighted as relevant mediators in sepsis pathophysiology. Among them, S100A8 and S100A9 exist mainly as a heterodimer called calprotectin. However, several studies suggest independent functioning of S100A9, making it an interesting candidate biomarker in septic syndromes.

Objective To assess capacity of S100A9 mRNA in whole blood from SS patients to predict survival and the occurrence of HAI.

Methods The authors conducted a cohort study. This study was conducted in two ICUs in Lyon University Hospital. The study included 166 SS patients and 44 healthy volunteers. PAXgene blood samples were obtained regularly in the course of the syndrome for S100A9 gene expression analysis using qRT-PCR.

Results The overall mortality was 38% and the mean SAPS II on shock onset was 52. Thirty-seven patients (23%) experienced at least one HAI after septic shock. We found that S100A9 mRNA levels at days 1 to 3 after the onset of shock were significantly higher in SS patients compared with healthy volunteers (median: 1,460 vs. 16,620; $P<0.0001$) but not significantly different in nonsurviving versus surviving patients (median: 18,070 vs. 16,310; $P = 0.1278$). In contrast, systemic S100A9 mRNA levels measured at days 7 to 10 were significantly higher in the group of

patients that were going to develop HAI compared with patients that were not (median: 10,140 vs. 7,160; $P = 0.009$). Multivariate analysis showed that the S100A9 mRNA level at days 7 to 10 after the onset of septic shock significantly increases the probability of HAI with odd ratios of 1.12 per unit ($P = 0.0054$).

Conclusions Our results showed that S100A9 mRNA is overexpressed in blood from SS. We showed that its persistent overexpression over time is associated with the occurrence of secondary HAI. This biomarker may be of major interest in identifying patients at increased HAI risk who could benefit from either targeted therapy aimed at restoring immune functions (in case of associated immunosuppression) or reinforced antibiotherapy and measures against cross-transmission.