Abstracts

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### Abstracts

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Experimental Sepsis Research

017 Infection 2011

The role of thromboxane and leukotrienes in mediation of vasocostrictive effects of platelet activating factor in the isolated perfused rat small intestine

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Introduction: The intestinal circulation is prone to septic disturbances. Platelet activating factor (PAF) is a potent sepsis mediator that impairs microvascular perfusion by inducing vasocostriction.

Objectives: The mechanisms of PAFs vascular effects in the intestine are not yet fully understood, but it is thought that secondary lipid mediators such as eicosanoids are involved. The objective of our study was to clarify their role in the PAF-induced vasocostriction in the model of the isolated perfused rat small bowel [1].

Methods: Small intestines from rats were perfused and challenged with a 0.5 nmol PAF bolus. Thromboxane (TX) and leukotriene (LT) concentrations were quantified in the vascular perfusate by ELISA. The protective effects of TX and LT receptor antagonists (TX-RA: SQ29548; LT-RA: MK571) as well as of cyclooxygenase (COX: acetyl salicylic acid) and lipoxygenase (LOX: AA861) inhibitors and of drugs that increase intracellular cAMP levels (foroskolin; IBMX) were analysed. A PAF receptor antagonist (PAF-RA: ABT491) served as control. The following groups were studied: PAF (n = 5), PAF + TX-RA (n = 4), PAF + TX-RA + LT-RA (n = 6), PAF + COX inhibitor + LOX inhibitor (n = 5), PAF + foroskolin + IBMX (n = 4), PAF + PAF-RA (n = 6). All substances were administered continuously 15 min before the end of a 60 min equilibration period. The vascular pressure responses in the organ were recorded. The maximal pressure amplitude and the area under the pressure response curve were analysed.

Results: PAF administration elicited its typical transitory vasocostrictive effect and led to TX and LT release into the vascular perfusate. Surprisingly, TX-RA ± LT-RA as well as COX and LOX inhibition were ineffective in suppressing the PAF-induced vasocostriction, whereas AC stimulation in combination with PDE inhibition exerted a protective effect. The use of the PAF-RA completely abolished the effects of PAF.

Conclusions: In contrast to the well-described pathways of PAF in other organs [2], TX and LT seem not to play a major role in the mediation of vasocostrictive PAF effects in the intestine as neither TX-RA, LT-RA nor COX/LOX inhibition were protective. Increased intracellular cAMP concentration attenuated the PAF-induced intestinal vasocostriction and could potentially diminish hypoperfusion and ischemic damage during acute intestinal inflammation.

References: [1] Lautenschläger et al. Am J Physiol Gastrointest Liver Physiol 2010; 298:G304–13. [2] Uhlig et al. J Appl Physiol. 1994; 77:262–9.

019 Infection 2011

Human neutrophils specifically respond to the filamentous morphotype of the sepsis pathogen Candida albicans by activation of antifungal effector functions

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Introduction: Human polymorphonuclear neutrophil granulocytes (PMN) represent the most effective phagocytes against Candida albicans, the predominant causative agent of invasive candidiasis. Phenotypic switching is a major virulence factor of this opportunistic fungus as the filamentous form relates to invasive growth.

Objectives: PMN become specifically activated by C. albicans filaments but not by yeast cells (Wozniok et al. 2008). Here we used an in vitro C. albicans infection model to analyze PMN effector mechanisms, i.e. degranulation and cytokine release, upon activation by the two morphotypes.

Methods: Primary neutrophils were co-incubated with short filaments or yeasts of C. albicans SC5314 (wt) or the yeast-lock mutant efg1/cph1” for 10–240 min at 37°C. Using differential FACS staining expression of degranulation markers on PMN was analyzed. To quantify degranulation the supernatant of co-incubation experiments was screened for Lactoferrin, LL-37 and Myeloperoxidase (MPO) release with ELISA. The cytokine pattern was analyzed by Luminex technology.

Results: In response to C. albicans filaments PMN degranulation markers CD11b, CD18, CD66b and CD63 were 2–51× upregulated. The filamentous form had a 8–42% stronger impact on surface marker expression compared to the yeast form. A close contact between PMN and the fungus was essential for the increase of expression with only minor activation of bystander PMNs. Consistently, filaments promoted a ~50% higher release of the granule proteins lactoferrin, LL-37 and MPO than efg1/cph1” yeasts after 4 h of co-incubation. Screening a broad cytokine spectrum, we identified additionally to IL-8 (Wozniok et al. 2008) VEGF, Gro-a, HGF, MIP1-β and IL12(p70) in response to the fungus. Again filaments had a significantly greater impact (2× for VEGF, 4× for IL-8) than efg1/cph1”. Gro-a and HGF release was filament specific. However, MIP1-β and IL12(p70) were induced almost regardless of the morphotype. Currently we further investigate PMN response mechanisms against the two morphotypes, e.g. analyzing phagocytosis and respiratory burst.

Conclusions: These results confirm further that human neutrophils very well discriminate between the two morphotypes of C. albicans. The virulence associated filamentous form rather than the yeast form of the fungus not only activates PMN but also induces effector mechanisms like degranulation and cytokine secretion specifically.

Reference: Wozniok et al. Cell Microbiol. 2008;10(3):807–20.
Human Natural Killer Cells show direct cytotoxic effects against Candida albicans filaments

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Introduction: Human Natural Killer Cells (NKC) are innate immune cells expressing a repertoire of activating and inhibiting receptors. After target recognition they are able to provoke apoptosis in the target cell. Candida albicans is the most common cause of sepsis.

Objectives: Up to now there is only little data on the interaction of human NKC with the pathogenic fungus Candida albicans. While the antifungal relevance of murine NKC could be confirmed, we investigated if there are direct or indirect effects of human NKC against Candida albicans.

Methods: Viable or heat-inactivated Candida albicans SC5314 were used for confrontation with primary human NKC. The immune cells were isolated from peripheral blood of healthy donors and primed with a cytokine cocktail (IL-2, IL-15, IFN-alpha, IFN-beta). Experiments were performed for 4 h at a MOI of 0.5. Furthermore, cells were treated with blocking antibodies (anti-Fasl, anti-TRAIL), strontium chloride (inducing granula release) or concanamycin A (perforin inhibitor) to analyze the mechanism of NKC mediated fungal killing. Reduction of fungal viability was determined by XTT assay. Bioplex or ELISAs were used for quantification of secreted markers. Receptor surface expression was quantified by FACS-analysis.

Results: After priming of primary human NKC by cytokines an enrichment of the cytotoxic granules Perforin and Granzyme B could be measured. Using these activated NKC, XTT-based killing assays showed a significant decrease of fungal viability after confrontation. While a marked increase of the degranulation marker CD107a as well as secretion of NKC specific cytokines was detected, the co-incubation of NKC and C. albicans resulted in down-regulation of Toll-like receptors (TLR2, TLR4), cellular adhesins (CD11a, CD54) and activation markers/ITAM-Bearing Receptors (CD25, CD314, CD335). Activation of NKC was most prominent for C. albicans filaments. Degranulation was significantly reduced for inactivated yeast forms, whereas heat-inactivated filaments resulted in levels comparable to viable wild-type C. albicans. Blocking experiments suggest that cytotoxic effects are not mediated via Fasl/TRAIL receptor pathway. In parallel to these direct effects, NKC may interact with other immune cells since cytokine release shows a secretion of neutrophil activating cytokines like TNF-alpha and MIP1.

Conclusions: Our efforts suggest that human NKC may play an important role in the direct first line defense of C. albicans infections. In addition, the supporting role of NKC as adjuvant bystanders is currently further investigated.

Confocal laser endomicroscopy to assess gastric and intestinal mucosal microcirculation: a quantitative analysis in a porcine model of septic shock

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Introduction: Microcirculatory alterations play a central role in the pathophysiology of sepsis. Even if systemic hemodynamic parameters appear to be adequate, septic patients may suffer from significant intestinal microcirculatory alterations. It has been suggested that these changes play a central role in regard to morbidity and mortality.

Objectives: The present experimental study assessed the feasibility of in vivo detection of mucosal microcirculation in different segments of the gastrointestinal tract in an animal model of septic shock using probe-based confocal laser endomicroscopy (pCLE) (Mauna Kea Technologies, Paris, France).

Methods: Anesthetized and mechanically ventilated pigs were observed over 8 h. Septic shock was triggered by inducing fecal peritonitis (0.75 g autologous feces per kg body weight). Mucosal microcirculation was assessed simultaneously using pCLE in stomach, duodenum, terminal ileum and rectum at baseline, 4 h after induction of septic shock as well as 2 h after treatment of the condition. Four to six areas were examined for each site and images were analysed in a blinded fashion offline thereafter. Mean capillary diameter, capillary length and functional capillary density (FCD) were measured quantitatively.

Results: Two hours after induction of sepsis, FCD was markedly decreased in the duodenal (−20.8 ± 8.5%, p < 0.001), the ileal (−13.4 ± 7.3%, p < 0.001), the gastric (−11.9% ± 5.0%, p < 0.001), and in the rectal mucosal beds (−5.5 ± 3.9%, p < 0.01). After administration of 30 mg kg⁻¹ of gelatine (30 kDa) FCD increased in all mucosal compartments to 90.0% (duodenum), 94.4% (ileum), 95.4% (gastric) and 97% (rectum) of baseline values. Interestingly, mean vessel diameter was unchanged in all compartments investigated.

Conclusions: During the early phase of septic shock, pCLE may be able to quantify microcirculatory alterations in the gastrointestinal mucosa. Fluid resuscitation improves but does not completely restore intestinal microcirculation in this septic shock model.
Gram-positive lipoproteins: Fourier-transform infrared spectroscopy, small-angle X-ray scattering with synchrotron radiation, isothermal titration calorimetry, and Förster resonance energy transfer spectroscopy. The biological techniques comprise as in vitro assay the endotoxin-induced cytokine secretion in human mononuclear cells and various assays of cytotoxicity, and in vivo assays mouse models of endotoxemia and of infection by bacteria, and of cecal ligation and puncture.

Results: The inhibition of the LPS-induced cytokine (TNFα) production takes already place at a slight molar excess ratio SALP:LPS, and in vivo experiments with mice show that these are significantly protected against lethal endotoxemia at a SALP:LPS ratio of 30:1. The in vitro data show an inhibition of the cytokine production when the SALP are administered considerably before LPS addition (prophylactical approach) as well as on addition of the SALP considerably after LPS addition (therapeutical approach). The latter holds true also in the mouse model of endotoxemia. Furthermore, cytotoxic effects against mammalian cells occur at concentrations more than 100-times higher than the therapeutic dose. The biophysical data show a multilamellarization of the LPS aggregates, the compensation of their negative head group charges, and a high binding constant in the range 10^{-9} M. The peptides exhibit clear membrane activity leading to a blocking of cell surface receptors such as TLR4. Furthermore, in a Salmonella model in mice these are protected against the lethal action of the bacteria by a combination of classical antibiotics (ciprofloxacin, tetracyclin) with the SALP. When in mice cecal ligation and puncture is induced, the animals are protected already by the SALP alone.

Conclusions: The data obtained so far indicate a promising agent in the anti-sepsis fight. Presently, in two animal models the cytotoxicity is evaluated according to regulation ICH M3, before next year a FIM (first in man) study is started.

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Infection 2011

Micro-Raman Spectroscopy: a novel method for identification of Sepsis pathogens

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Introduction: Focus control is of outstanding significance for effective sepsis therapy. Early pathogen identification and timely application of appropriate antibiotics could greatly support positive outcome, in particular in patients with resistant pathogens not covered by initial calculated therapy.

Objectives: Here we present a novel method of pathogen identification that is considerably more rapid than current methods. Since single bacterial cells are subjected to analysis, there is no need for time-consuming culture-based techniques.

Methods: We use a micro-Raman setup to measure Raman spectra of single bacterial cells. Since Raman spectroscopy mirrors all molecules present in a sample both in quality and quantity, this method provides easy access to the characteristic composition of the bacteria. The evaluation of these data with statistical computational algorithms such as linear discriminant analysis (LDA) opens up the possibility to identify the bacteria based on the characteristic spectral information.

We investigated Gram-negative bacteria frequently encountered in Sepsis, namely Pseudomonas spp. and E. coli. For proof of principle we used non-pathogenic strains only. Some of the bacteria were stressed during growth by the addition of different antibiotics in subinhibitory concentrations.

Results: Raman spectra of all of the investigated strains were obtained in reasonable quality with an acquisition time of less than 20 s. The resulting spectra were divided into a training data set and an independent test data set. The training data comprised spectra of unstressed bacteria and of bacteria that were treated with Ampicillin. They were used to set up a LDA model to differentiate between the various species.

The model then was challenged with the independent test data set. The model predicted the identity of the bacteria correctly for more than 95% of the data. Spectra of bacteria that were grown with other antibiotics are still identified correctly with an accuracy of more than 90%.

Conclusions: With our method we are able to rapidly and correctly identify different species of Pseudomonas and E. coli even under the influence of various antibiotics. Future work will extend the model to pathogenic representatives of the investigated species as well as other bacteria, in particular resistant strains prevalent in Sepsis.

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Infection 2011

The anti-inflammatory potential of Protein C concentrate: inhibition of leukocyte recruitment during inflammation and improvement of survival during sepsis

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Introduction: Anti-inflammatory and cytoprotective properties of protein C concentrate (PC) are poorly studied compared to activated protein C (APC), although PC is suggested to be safer in clinical use.

Objectives: To elucidate the anti-inflammatory potential of PC we investigated how PC interferes with leukocyte recruitment during acute inflammation in vivo and its efficacy during murine endotoxemia.

Methods: Using intravital microscopy, we explored the impact of PC on the multistep cascade of leukocyte recruitment in different mouse models of acute inflammation with regard to its optimal dosing and timing, and in comparison to APC infusion. In addition, we investigated survival of PC treated and control mice during LPS (40 mg/kg i.p.) induced sepsis. To uncover underlying mechanisms we performed immunohistochemistry, flow cytometry, APC capture assay, used thrombomodulin (TM) mutant and ICAM-1 deficient mice exploring the endothelial protein C receptor (EPCR) dependent signaling and leukocyte adhesion pathways in our inflammatory models.

Results: We found that similar to APC infusion, intravenous PC application reduced leukocyte recruitment in inflamed tissues in a dose and time dependent manner. During both TNFalpha and trauma-induced inflammation of the cremaster muscle, intravital microscopy revealed that leukocyte adhesion and transmigration, but not rolling, were profoundly inhibited by 100 U/kg PC. PC also blocked
leukocyte emigration into the bronchoalveolar space during LPS induced acute lung injury. PC (100 U/kg; 30 min, 8 and 24 h after LPS challenge) was efficiently activated in a murine endotoxemia model, reduced leukocyte infiltration of organs and strongly improved survival (75 vs. 25% of control mice). Dependent on the inflammatory model, PC provoked a significant inhibition of leukocyte recruitment as early as 1 h after administration. PC-induced inhibition of leukocyte recruitment during acute inflammation critically involves thrombomodulin-mediated PC activation, subsequent EPCR and PAR-1-dependent signaling, and downregulation of ICAM-1 leading to reduced endothelial inflammatory response.

**Conclusions:** We conclude that during acute inflammation and sepsis PC is a fast acting and effective therapeutic approach to block leukocyte recruitment and improve survival.

### 029 Infection 2011

**Distinct different contributions of the alternative and classical complement activation pathway for the innate host response during sepsis**

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**Introduction:** During acute inflammation, the complement system can be activated by three well-known pathways: the classical, the lectin and the alternative pathway. Complement activation represents a crucial innate defence mechanism against invading microorganisms, but there is an evident lack of understanding of the separate contribution of the complement activation pathways to the host response during sepsis.

**Objectives:** The project aimed to dissect the different contribution of the alternative and classical complement activation pathway for the innate host response and outcome during experimental sepsis.

**Methods:** To investigate different innate host immune responses during sepsis we used the cecal ligation and puncture (CLP) induced sepsis in mice lacking the alternative (fD$^{-/-}$) or classical (C1q$^{-/-}$) complement pathway. The C5a concentration in plasma was analyzed by ELISA, bacterial load in organs using microbiological methods were determined. To visualize organ damage we conducted histological examinations. To distinguish the host response we quantified cytokine levels and isolated, cultured and analyzed the NF-kB activation of murine leukocytes.

**Results:** Both knockout strains showed a reduced survival when compared to control mice. Surprisingly, fD$^{-/-}$ mice demonstrated a compensated bacterial clearance capacity at 6 h post CLP, whereas C1q$^{-/-}$ mice were overwhelmed by bacteria. This compensate bacterial clearance of fD$^{-/-}$ mice was accompanied by a strongly increased granulocyte presents in blood and lungs. At 24 h post CLP, fD$^{-/-}$ mice failed to clear bacteria in a way comparable to control mice. We demonstrated that despite normal bacterial clearance capacity during the onset of sepsis, fD$^{-/-}$ mice displayed increased inflammatory cytokine generation when compared to control and C1q$^{-/-}$ mice, indicating a loss of control over these immune responses. Furthermore, in vitro experiments confirmed these findings and revealed an increased NF-kB activation capacity in isolated neutrophils from fD$^{-/-}$ mice.

**Conclusions:** These results provide evidence for the new concept that the alternative complement activation pathway exerts a distinctly different contribution to the innate host response during sepsis when compared to the classical pathway. These observations are in striking contrast to older findings within other inflammatory models, which elaborated an amplifying role of the alternative pathway for overall complement activation.

### 035 Infection 2011

**Intravenous glucose administration induces leukocyte recruitment during inflammation in a stimulus-dependent manner in vivo**

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**Introduction:** It is well known that hyperglycemia aggravates the outcome of septic patients. Since pro-inflammatory effects of glucose are still controversially discussed, we investigated the leukocyte recruitment cascade after intravenous injection of glucose in different models of inflammation.

**Objectives:** Aim of this study was to examine the inflammatory effects of hyperglycemia during sepsis.

**Methods:** Using intravital microscopy we observed glucose-dependent leukocyte recruitment in two different cremaster muscle models of inflammation. While surgical preparation of the tissue leads to trauma-induced inflammation within 15 min, the investigated muscle is stimulated with 500 ng TNF$\alpha$ for 3 h in the TNF$\alpha$ model. In both models intravenous bolus injection of glucose (0.5 g/kg) was compared to normal saline. To investigate underlying mechanisms we additionally blocked CXCR2-signaling or used intercellular adhesion molecule 1 (ICAM-1) knockout mice in our experimental setting.

**Results:** We demonstrated that a single injection of 0.5 g/kg glucose rapidly increased blood glucose levels in our inflammatory models. Notably, during the long-term model of TNF$\alpha$-induced inflammation leukocyte recruitment was not influenced by glucose administration. In contrast, glucose injection profoundly augmented adhesion and transmigration of leukocytes into inflamed tissue in the short-term trauma model. Experiments with ICAM-1 knockout mice or pertussis toxin, an inhibitor of the G$\alpha$-coupled chemokine receptor CXCR2, suggest that ICAM-1- and chemokine CXCL-1-CXCR2 pathways are crucially involved in mediating glucose-dependent leukocyte recruitment during trauma induced inflammation.

**Conclusions:** Administration of glucose leads to increased leukocyte adhesion and transmigration during the short term model of trauma-induced inflammation, but not during 3 h-TNF$\alpha$-stimulation indicating that pro-inflammatory properties of glucose are dependent from kind and duration of stimulation.

### 037 Infection 2011

**Volume resuscitation with balanced crystalloid compared with balanced 6% HES 130/0.4 in lethal LPS induced septic shock in rats**

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Introduction: Volume therapy belongs to the pillars of hemodynamic stabilization for septic shock. However, it is still controversially discussed which type of volume substitution should be used. Aim of the present study is to compare balanced crystalloid solution (Sterofundin® ISO) with balanced colloid 6% HES 130/0.4 (Volulyste (HES)) in its capability of macro- and microhemodynamic stabilization after septic shock in rats.

Methods: After animal care committee approval, 18 Sprague–Dawley rats were anaesthetized, tracheotomized, ventilated and carotid artery as well as jugular vein was cannulated. The cardiac output was measured using thermodilution method. The rats were laparatomized and intravital microscopy was performed to evaluate mesenteric microcirculation. The following groups were randomly assigned: Group 1 control (n = 5), Group 2 LPS (n = 5), Group 3 ISO (n = 4), and Group 4 HES (n = 4). Septic shock was induced by an i.v. injection of LPS (5.0 mg/kg BW). At MAP <60 mmHg 1 ml of either ISO or HES were applied for intravasal fluid resuscitation in Groups 3 and 4 over 3 h. Variance analysis (ANOVA post-hoc Tukey-Kramer test) p < 0.05 MW ± SD was used for statistical analysis.

Results: After 3 h mortality of untreated animals was 100%, whereas all ISO/HES treated rats survived. The microcirculation in Group 2 LPS was significantly reduced compared to the control and Group 4 HES, but no significant difference was observed within the treated Groups 3 and 4. However, HES treated rats tended to have a better microcirculation than Group 3 ISO. MAP and CO in Groups 3 and 4 were significantly decreased in comparison to control, but were significantly better than Group 2 LPS. 2 h post shock induction CVP significantly increased in ISO and HES treated groups compared to other animals. The blood parameters pH, pO2, pCO2, Hb, Hct, lactate, HCO3− and SBE did not show any significant difference between ISO and HES. The overall applied volume needed for hemodynamic stabilization differed significantly with 15 ± 2.3 ml for HES treated rats compared to 23.5 ± 12.3 ml ISO.

Conclusions: No significant differences were seen in macro- and microcirculation by using crystalloids to stabilize the septic shock in rats compared with balanced 6% HES 130/0.4. For adequate volume substitution only 58% more crystalloid solution was required.

Methods: After animal care committee approval 17 male Sprague–Dawley rats were randomized in 3 groups: sham (n = 5), LPS (n = 6) and LPS + PD-4-I (n = 6). Animals were prepared for intensive care set up with mechanical ventilation, continuously invasive arterial-, central venous blood pressure and cardiac output measurement and followed by median laparotomy and investigation of mesenterial microcirculation using invert light microscopy for 6 h. Extravasation of FITC-albumin was analyzed for capillary leakage investigation by in vivo fluorescent microscopy. LPS (5 mg/kg BW) were used to induce septic shock. Blood gas analyses were drawn every hour and at the end of the experimental procedures morphologic alterations of tissue sections of the intestine were analyzed in HE staining. Additionally, immunostaining for tight junction proteins Claudin1, Claudin5 and for adherens junction protein E-cadherin was performed in those sections.

Results: LPS induced mortality of 100% after 3 h, whereas PD-4-I treatment resulted in 50% survival over 6 h. This beneficial effect was associated with stabilization of endothelial barrier properties as revealed by measurements of FITC-albumin extravasated from post-capillary mesenteric venules and stabilization of hematocrit. Accordingly, microcirculatory flow in mesenteric venules was significantly increased following PD-4-I treatment and blood gas analyses indicated improved metabolism compared to LPS alone. These findings indicated that reduced loss of intravascular fluid in PD-4-I-treated animals stabilized blood pressure and cardiac output, while severe side effects of PD-4-I were absent. In parallel, in LPS-treated animals the intestinal epithelial barrier was obviously compromised compromised as revealed by reduced staining of claudin1, claudin5 and E-cadherin at intercellular junctions of enterocytes. In contrast, LPS + PD-4-I treatment resulted in regular staining pattern of both tight- and adherens junction proteins comparable to control conditions.

Conclusions: Taken together these data serve as a basis to suggest a clinically applicable approach to stabilize endothelial and epithelial barriers in systemic hyperinflammation.

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Phosphodiesterase-4 inhibition prevents LPS-induced endothelial and intestinal epithelial barrier breakdown in rats

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Introduction: In sepsis and systemic inflammation increased microvascular permeability and consecutive breakdown of microcirculatory flow significantly contribute to organ failure and death. Evidence points to a critical role of cAMP levels in endothelial and epithelial cells to maintain capillary endothelial and intestinal barrier properties in acute inflammation. Therefore, we tested whether systemic application of phosphodiesterase-4 inhibitor (PD-4-I) to increase cAMP is effective to attenuate capillary leakage and to prevent inflammation-induced breakdown of the intestinal barrier in lethal Lipopolysaccharide (LPS)-induced systemic inflammation in rats.

Methods: After animal care committee approval 17 male Sprague-Dawley rats were randomized in 3 groups: sham (n = 5), LPS (n = 6) and LPS + PD-4-I (n = 6). Animals were prepared for intensive care set up with mechanical ventilation, continuously invasive arterial-, central venous blood pressure and cardiac output measurement and followed by median laparotomy and investigation of mesenterial microcirculation using invert light microscopy for 6 h. Extravasation of FITC-albumin was analyzed for capillary leakage investigation by in vivo fluorescent microscopy. LPS (5 mg/kg BW) were used to induce septic shock. Blood gas analyses were drawn every hour and at the end of the experimental procedures morphologic alterations of tissue sections of the intestine were analyzed in HE staining. Additionally, immunostaining for tight junction proteins Claudin1, Claudin5 and for adherens junction protein E-cadherin was performed in those sections.

Results: LPS induced mortality of 100% after 3 h, whereas PD-4-I treatment resulted in 50% survival over 6 h. This beneficial effect was associated with stabilization of endothelial barrier properties as revealed by measurements of FITC-albumin extravasated from post-capillary mesenteric venules and stabilization of hematocrit. Accordingly, microcirculatory flow in mesenteric venules was significantly increased following PD-4-I treatment and blood gas analyses indicated improved metabolism compared to LPS alone. These findings indicated that reduced loss of intravascular fluid in PD-4-I-treated animals stabilized blood pressure and cardiac output, while severe side effects of PD-4-I were absent. In parallel, in LPS-treated animals the intestinal epithelial barrier was obviously compromised compromised as revealed by reduced staining of claudin1, claudin5 and E-cadherin at intercellular junctions of enterocytes. In contrast, LPS + PD-4-I treatment resulted in regular staining pattern of both tight- and adherens junction proteins comparable to control conditions.

Conclusions: Taken together these data serve as a basis to suggest a clinically applicable approach to stabilize endothelial and epithelial barriers in systemic hyperinflammation.
probability of survival was estimated by Kaplan–Meier survival analysis for up to 192 h. Furthermore, blood and organ samples were collected from trained and untrained mice at 6 h (early phase) as well as 24 h (late phase) after sepsis induction. The concentration of several clinical chemistry parameters, cytokines, blood cells, and oxidative stress markers during sepsis between both groups were analyzed.

**Results:** Six weeks of exercise increased the relative heart mass in trained mice by 10% (p < 0.01). Following sepsis, the probability of survival was significantly improved in trained mice (p < 0.01). In the early phase of sepsis, values of IL-6, IL-10, MCP-1, TNF-α, LDH, liver enzymes, creatinine and urea were lower in trained mice (p < 0.01). After 24 h, untrained mice showed a significantly improved level of creatinine (p < 0.05) and in trend higher values of all other parameters analyzed. Interestingly, the level of oxidative stress measured by the GSSG/GSSH ratio in muscles was significantly higher in untrained mice at both 6 and 24 h post sepsis induction.

**Conclusions:** Physical exercise leads to physiological adaptations which attenuated the inflammatory response and the probability of survival in sepsis. These observations offer prospects for further experimental research to understand the mechanisms involved in the changes of certain parameters during sepsis in trained and untrained mice. Physical exercise has beneficial effects on the host response and outcome in sepsis.

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**048**

**Infection 2011**

**Conformational and morphological investigations of antimicrobial and endotoxin-neutralizing peptides**

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**Introduction:** The septic syndrome, sepsis and septic shock, is still a major threat at intensive care units (ICU) in the developed world despite the deployment of maximal invasive medicine therapies for monitoring and/or applying drugs or fluid resuscitation. Unfortunately, pathogens like bacteria can use artificial respiration devices and catheters as entry gates into the body via the airway and urinary tract as well as intravenous causing a local inflammation and leading to septic syndrome and septic shock with a fulminant course of sepsis. So far there is no adequate drug therapy with a minimum of side effects available. Here, we present first data of a peptide which incorporates antimicrobial and endotoxin-neutralizing properties and is therefore a promising anti-infective agent especially for the Gram-negative induced sepsis.

**Objectives:** The molecular mode of action leading to septic shock is not yet well understood. Our objective is to study the molecular mode of action of the new antimicrobial and endotoxin-neutralizing peptide named LPeP19-2.5 by using free lipopolysaccharide (LPS) as endotoxin promoting an inflammation as well as whole bacteria.

**Methods:** For the morphological investigations Atomic Force Microscopy (AFM) and freeze fracturing combined with imaging via cryo-Transmission Electron Microscopy (cryo-TEM) and Electron Microscopy (EM) were utilized. Structural investigations were performed with Small-Angle X-Ray Scattering (SAXS)-experiments as well as Infrared Spectroscopy (IR) and other biophysical methods.

**Results:** LPS aggregates undergo a structural change from an active conical morphology to an inactive cylindrical shape. Also a shift of the phase transition temperature of lipid systems treated with AMP can be observed. Time-dependent experiments by AFM show a multilamellarization of LPS aggregates after application of AMP in reconstituted lipid membrane systems. AFM-experiments with bacteria incubated with variants of the PEP19 series also show a strong bactericidal effect. The results of the cryo-TEM and -EM experiments support the AFM findings showing multilamellar stacks of LPS/peptide aggregates. Also, SAXS measurements on lipid vesicle solutions show a shift towards multilamellarization. Further biological investigations and experiments show a high inhibiting efficiency of the TNF-α release of mononuclear cells (MNC) as a pro-inflammatory cytokine.
Conclusions: The multimolarization of LPS aggregates is discussed to have a crucial influence on LPS-induced cell signaling leading to a neutralization of the toxic properties of LPS. The results gained so far show a high potency in reducing the inflammatory response of the immune system by neutralizing LPS.

049
Infection 2011

Influence of Candida albicans on barrier function and junctional protein expression in intestinal epithelial cells

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Introduction: The barrier for microorganisms from the gut lumen is formed by a tight monolayer of intestinal epithelial cells (IEC). Paracellular permeability of the intestinal barrier is regulated by claudins and other tight junction transmembrane proteins, which constitute diffusion of macromolecules and small solutes. This gate function can be influenced by microorganisms and other stimuli. The polymorphic fungus Candida albicans, a common commensal of the human gastrointestinal tract, is capable of establishing systemic disease within the host after traversing the intestinal barrier.

Objectives: Here we investigated the role of barrier disruption for invasion of IEC by C. albicans.

Methods: Caco-2 derived C2BBc1 IEC, cultivated on permeable cell culture inserts served as in vitro model of the polarised intestinal mucosal epithelium. Transepithelial electric resistance (TEER) of IEC monolayer was measured with a voltohmeter. Cytotoxicity was determined by LDH release assay. Imaging of fixed samples of Candida-infected IEC was carried out with a confocal microscope.

Results: IEC, grown on membrane inserts for 12 days established polarised monolayers with TEER values of more than 500 Ω cm². Infection of IEC with wild-type C. albicans at a MOI of H 0.5 led to an increase of TEER of approx. 50% with a maximum 8 h after inoculation. IEC decreased afterwards until reaching background levels of plain inserts after H 21 h. Microscopic imaging showed a profound disruption of IEC monolayers at this time point with cytotoxicity levels reaching 30–40%. Additionally, to examine the role of filamentation and active penetration, IEC were infected with the non-filamentous C. albicans mutant efg1/cph1. Here, TEER increased until reaching a two-fold level which continued to the end of the experiment (22 h). Cytotoxicity, however, remained at the level of non-inoculated control. To examine the molecular basis of barrier disruption in wild-type C. albicans infected IEC we looked at the expression levels of junctional proteins. E-cadherin (adherens junction) and JAM-A, occludin, as well as various claudin (tight junction) protein levels showed a strong decrease in infected monolayers. Although imaging of Candida-infected IEC showed decreased E-cadherin staining in the cells surrounding the invasion site, some localized E-cadherin (and actin) accumulation could be detected at penetrating fungal filaments.

Conclusions: The results indicate an active participation of C. albicans in intestinal barrier breakdown. However, the relative impacts of host and fungus on barrier disruption will have to be determined in ongoing work. Future analyses are intended to specify localized versus general effects of junctional protein distribution and/or degradation in C. albicans invasion.

050
Infection 2011

Fast detection of Sepsis pathogens by means of Raman spectroscopy

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Introduction: In cases of Sepsis a fast and reliable identification of the causing pathogens is very important. An immediate identification of the pathogens can help to choose an appropriate initial therapy which will contribute to the decrease of the mortality rate.

Objectives: The aim is to provide a fast and nondestructive method to identify pathogens by using Raman spectroscopy. The advantage is that it can be worked on a single cell level, and therefore, time consuming precultivation steps are not needed after the implementation of a database. However, if the samples contain <10⁶ to 10⁹ pathogens per ml some enrichment and isolation steps have to be done.

Methods: We use a Raman spectrometer which is coupled with a microscope so that the spatial resolution is below one micrometer. This allows to investigate single bacterial cells. Micro-Raman spectroscopy provides a spectroscopic fingerprint of the chemical composition of single bacterial cells and can be combined with chemometrical methods to be used for fast identification of the pathogens.

Results: By using a micro-Raman setup with a 532 nm excitation wavelength we investigated several Sepsis relevant pathogens from different genera like Staphylococci, Streptococci or Enterococci. Applying various chemometrical methods like principal component analysis and linear discriminant analysis it is possible to distinguish between different genera and also between different species of one genus.

Conclusions: Our study shows that we can identify the investigated Sepsis relevant pathogens by using micro-Raman spectroscopy. This suggests that the results can be transferred to other genera what have to be proved. Further investigations will focus on other genera and bacteria which are isolated from different body fluids.

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051
Infection 2011

Normal human epidermal keratinocytes attenuate the inflammatory response in Borrelia burgdorferi-activated peripheral blood mononuclear cells

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Introduction: Borreliosis caused by spirochetes of the Borrelia burgdorferi (BB) sensu lato complex is a common inflammatory multisystemic disorder. If treated insufficiently, borreliosis can lead to
chronic manifestations affecting the joints, heart, central nervous system, and particularly the skin. A characteristic cytokine profile is generated by peripheral blood mononuclear cells (PBMC) under the influence of BB. Of utmost importance in that context are supposed to be monocyte-derived Interleukin (IL)-1 and Interferon (IFN)-γ as well as T cell-derived IL-22. IL-22 has important functions in maintaining epithelial barrier function and can provide tissue protection during bacterial infections. However, the interaction of normal human epidermal keratinocytes (NHEK) and PBMC in the context of BB infection is currently unclear.

Objectives: In a co-culture approach, we aimed at investigating the interaction of NHEK with BB-activated PBMC.

Methods: NHEK from human foreskin were isolated and placed on the bottom of Transwell plates. When NHEK were subconfluent, 6 x 10^6 PBMC from healthy donors (in accordance with ethics committee statement) were placed on the 70 μm Transwell insert. Co-culture plates were incubated with 600,000 live BB (±0.1 Multiplicity of Infection) for 48 h. Supernatants were analyzed for proinflammatory cytokines by ELISA. Total RNA was isolated separately from NHEK and PBMC and analyzed for expression of IFN-γ and IL-22 by PCR. ANOVA was used for testing for significance, p < 0.05.

Results: In co-culture with NHEK, BB-induced mRNA expression of IFN-γ and IL-22 in PBMC was significantly impaired. In line with this, BB-induced secretion of IFN-γ, IL-22, IL-1β and IL-8 was significantly reduced under the influence of NHEK. In contrast, production of antimicrobial proteins S100A7 and human β-defensin (hBD)-2 was strongly upregulated in co-cultured NHEK. Recombinant hBD-2 did not affect BB-induced IFN-γ and IL-22 secretion by PBMC. Finally, expression of anti-inflammatory suppressor of cytokine signaling (SOCS)-3 mRNA was upregulated particularly in co-cultured NHEK.

Conclusions: Employing co-culture with NHEK, we showed that the PBMC-driven expression pattern of proinflammatory cytokines after exposure to BB was significantly altered. Consequently, further studies will aim at identifying NHEK-derived soluble factors which may account for our observations.

052
Infection 2011

The NFKB1 promoter polymorphism (94ins/delATTG) alters nuclear translocation of NF-KB1 in monocytes following lipopolysaccharide stimulation and is associated with increased mortality in sepsis

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Introduction: Since the insertion/deletion polymorphism (94ins/delATTG) in the promoter of NFKB1 could impact upon key mechanisms in sepsis, we tested the hypotheses that insertion/deletion polymorphism (94ins/delATTG) (1) alters nuclear-translocation of NF-KB1 in monocytes following lipopolysaccharide (LPS) stimulation, (2) affects LPS-induced NF-KB1 mRNA expression, TNFα production, and tissue factor activity, and (3) may be associated with increased 30 day mortality in patients with sepsis.

Objectives: Prospective genetic association study

Methods: (1) Following ethics committee approval monocytes from healthy volunteers with the homoygote II or DD genotype (n = 5 each) were isolated from peripheral blood, and incubated with either 10 mg ml^-1 LPS or vehicle. Afterwards, NFκB-immunofluorescence staining was performed and via semi-quantitative analysis, cells were categorized as nuclear NFκB negative, intermediate or positive and fractions were compared between groups (statistics: Mann–Whitney U test). (2) Whole blood samples from 105 healthy volunteers were incubated with LPS at final concentrations of 0, 25, 75, and 225 μg/ml for 4 h at 37°C. Thereafter, samples were subjected to thromboelastometry and mRNA as well as DNA was isolated. TNFα concentration was determined in plasma obtained from whole blood samples incubated with 225 μg/ml LPS (n = 60), (statistics: ANOVA and t test for independent measurements). (3) In a prospective study, adults with severe sepsis (n = 143) were genotyped for the NF-κB insertion/deletion polymorphism (94ins/delATTG). The clinical endpoint was survival over the first 30 days dependent on the NF-κB insertion/deletion polymorphism (94ins/delATTG) (statistics: Kaplan–Meier plots and multivariate proportionate hazard analysis).

Results: Following LPS stimulation of monocytes from healthy blood donors, the DD genotype compared to the II genotype (105 healthy blood donors) was associated with a nearly twofold increase in nuclear translocation of NF-KB1 (p = 0.001), a threefold difference in NF-KB1 mRNA expression (p = 0.001) and a twofold increase in tissue factor expression (p = 0.021) and TNFα concentrations (p = 0.043). Multivariate proportional hazard analysis revealed the deletion allele as an important and independent prognostic factor for 30-day mortality (hazard ratio, HR 2.3; 95% CI 1.13–4.8; p = 0.022). Mortality was 25% for II genotypes but 41% for combined ID/DD genotypes (p = 0.034).

Conclusions: Thus, following LPS stimulation the D allele of the NFKB1 insertion/deletion (94ins/delATTG) polymorphism is associated with increased nuclear translocation of NFKB1, an amplified TNFα response, boosted coagulation, and increased 30-day mortality in patients with severe sepsis.

054
Infection 2011

Role of hydrogen sulfide and its generating enzyme cystathionine-γ-lyase in septic cardiomyopathy

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Introduction: Myocardial dysfunction is a frequent complication of severe sepsis and septic shock. Hydrogen sulfide (H₂S), formerly considered mainly as a toxic gas, is increasingly receiving attention as the third gaseous signaling molecule besides nitric oxide (NO) and carbon monoxide (CO) with therapeutic potential, especially in the heart. H₂S in the cardiovascular system is produced mainly by cystathionine-γ-lyase (CTh). Both endogenous and exogenous H₂S showed protective effects in models of myocardial I/R injury, while its role during shock and inflammation is controversially discussed.

Objectives: In a murine model of severe sepsis we analyzed myocardial expression alterations of the gene (CTh) encoding the H₂S generating enzyme CTh. Furthermore we determined transcriptional
changes of spontaneously beating murine cardiomyocytes (HL-1) in an in vitro sepsis model following exposure to H$_2$S.

**Methods:** C57BL/6J mice received intraperitoneal injection of human fecal suspension (PCI = peritoneal contamination and infection) for sepsis induction or sterile saline solution (sham) or were left untreated (control). Hearts were harvested after 6 or 24 h and transcription of Cth was analyzed by real-time qPCR. HL-1 cardiomyocytes were incubated with GYY4137 (H2S donor) and serum of either untreated or septic mice for in vitro simulation of sepsis. After 6 h transcriptional alterations were studied using microarray technology.

**Results:** Heart tissue of PCI treated mice showed a slight down-regulation of CTH mRNA 6 h after sepsis induction, which became more pronounced after 24 h PCI. HL-1 cardiomyocytes exhibited transcriptional regulation of inflammation related genes after treatment with serum of septic mice. Exposure to the H2S donor GYY4137 resulted in an up-regulation of, e.g. heme oxygenase 1 (HMOX1) mRNA.

**Conclusions:** Reduced synthesis of CTH mRNA in the heart tissue of septic mice stands in contrast to the transcriptional up-regulation of the enzyme observed in ischaemic human hearts. As H2S is described to exert cardioprotective effects during myocardial I/R injury, decreased transcription of Cth in the septic heart might also be relevant for the course of cardiomypathy during sepsis. Transcriptional up-regulation of HMOX1 by H2S in HL-1 cardiomyocytes possibly increases the production of CO, which is known to act in an anti-inflammatory manner, and thus might be beneficial during inflammatory conditions.

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**055**

**Infection 2011**

The involvement of innate immunity pattern recognition receptors Toll-like receptors (TLRs) and NOD-like receptors (NLRs) in adrenal dysfunction during sepsis

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**Introduction:** Sepsis is a major health concern worldwide. Failure of the hypothalamic–pituitary–adrenal (HPA) axis is a cofactor for sepsis outcome leading to an uncontrolled immune response. Toll-like receptors (TLRs) and intracellular NOD-like receptors (NLRs) are major components of innate immunity forming the interface between microbial toxins and host immune defense. To date, little is known about the underlying mechanisms of adrenal dysregulation during sepsis, particularly regarding the involvement of TLRs and NLRs.

**Objectives:** Here, we investigated whether direct pathogen-adrenocortical cell interaction mediated via pattern recognition receptors TLRs and NLRs leads to adrenal dysfunction during sepsis.

**Methods:** After ethical approval, TLR2 knock-out (TLR2/-) and wild-type (WT; C57BL/6) mice were injected i.p. with pure LTA (pLTA; 1 mg/kg; 2 h), ACTH (100 µg/kg; 1 h), saline (2 h) or pLTA/ACTH and saline/ACTH. Then, adrenal tissue and plasma samples were taken. Expression of different TLRs (TLR1, 2, 4 and 6) and NLR isoforms (NLRP1, NLRP3, NLRC4, NOD1 and NOD2), NADPH oxidases (p40phox, p47phox, p67phox, p22phox, NOX1, gp91phox, NOX3 and NOX4) and nitric oxide synthases (iNOS and eNOS), several components of their signaling pathways (Rac1, Rac2, ASC, Caspase1, Caspase3, IL1β and IL18) [rt-PCR], cytokine levels [multiplex assay] and apoptotic rate within the adrenals [TUNEL assay] were determined.

**Results:** Murine adrenals expressed TLR1, 2, 4, 6, NOD1, NOD2, p40phox, p47phox, p67phox, p22phox, gp91phox, NOX4, eNOS, Rac1, Rac2, ASC, Caspase1, Caspase3, IL1β and IL-18. Treatment with pLTA but not with ACTH increased mRNA levels of those genes. pLTA challenge alone or together with ACTH resulted in a strong release of several inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β, -2, -4, -5, -6,-10 and -12. This effect was abolished in TLR2/- mice. The apoptotic rate within the adrenals is markedly higher in pLTA- or pLTA/ACTH-treated WT mice.

**Conclusions:** Our data indicate that the regulation of adrenals during inflammatory conditions involves complex interactions between components of innate immunity, oxidative stress and apoptosis. Impairment of TLR2 leads to diminishment of inflammatory cytokine levels and cell death. The knowledge of the interplays between the different pathways is important for the understanding of sepsis-mediated adrenal dysfunction.

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**065**

**Infection 2011**

Monitoring of monocytic host response by Raman spectroscopy

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**Introduction:** Monocytes play an important role in the patient’s immune response. The identification of reliable biomarkers showing the patient’s contact with pathogens could shorten the time of diagnosis of sepsis associated bacteremia. The rearrangement of membrane lipids, in particular the formation of lipid rafts is a hallmark in the cellular signal transduction during stress response. We want to test Raman spectroscopy, a label-free, non-invasive and non-destructive, but very specific technique to monitor the response of single host’s immune cells in vitro after contact with pathogens or stressors. Marker bands shall be detected within these lipid rafts.

**Objectives:** The aim is to establish a method for the Raman spectroscopic investigation of single living monocytes, identify and depict raft domains and reveal differences between activated and resting cells by Raman spectroscopy. Furthermore, it shall be investigated whether it is possible to use the monocyte’s response to distinguish between different activators.

**Methods:** Living cells of the human monocyte cell line Mono Mac 6 were immobilized with calcium alginate in order to perform Raman spectroscopy. The alginate-cell-suspension was applied on calcium fluoride slides by dip-coating and drop deposition. Raman maps of human monocytes were recorded in RPMI medium with excitation at 785 nm using a water immersion objective. Acquisition time for a single spectrum was one second. With a spatial step size of 0.5 µm this results in a total mapping time for a whole cell of about 1 h. Unsupervised statistical methods were used to visualize changes in the cell upon activation.

**Results:** An immobilization system based on calcium alginate to spatially fix living monocytes was established. This allowed us to collect Raman maps of resting and activated cells. Activation was achieved by applying mechanical stress, the bacterial endotoxin lipopolysaccharide (LPS) and prototypical proinflammatory mediators such as TNF to the monocytes. Raman spectra of activated cells differ considerably from resting ones. Raft domains were identified and visualized with clustering methods. Reference measurements of single
The short time impact of different HES-solutions on the microcirculation of the mesentery is not different from crystalloids in colon ascendens stent peritonitis-induced experimental sepsis

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Introduction: Fluid resuscitation plays a crucial role in the therapy of severe sepsis and septic shock. There is an ongoing discussion whether to use crystalloids or colloids.

Objectives: The aim of this study was to evaluate the effects of different HES-solutions parenteral fluids administration on the mesenteric microcirculation in experimental sepsis.

Methods: Male Lewis rats (n = 64) underwent sham (SHAM) or CASP surgery (colon ascendens stent peritonitis insertion of a plastic tube in the intestinal wall inducing continuous feces outflow and peritonitis). Sixteen hours later, during 1 h, the rats received one of the following fluids intravenously: 16 ml/kg Ringer’s solution (SHAM16, n = 8), 16 ml/kg Ringer’s (CASP16, n = 8), 64 ml/kg Ringer’s (CASP64, n = 8), 16 ml/kg Volulyte® (130/0.4 HES) (V-CASP16, n = 10), 16 ml/kg Tetraspan® (130/0.4 HES) (T-CASP16, n = 10), 16 ml/kg Hemohes® (200/0.5 HES) (H-CASP16, n = 10), 16 ml/kg HAES-Steril® (200/0.5 HES) (HS-CASP16, n = 10).

Intravital microscopy was performed before and after fluid administration. In the mesenteric venules plasma extravasation was measured (ratio of fluorescence intensity outside (Ip) versus inside (Iv) of the mesenteric venule under the study), and leukocyte endothelial interactions were studied. Plasma levels of tumor necrosis factor-alpha, IL-1β, IL-6, and IL-10 were also measured.

Results: There were no significant differences in plasma extravasation between the groups receiving crystalloids or colloids. The fluorescence ratios (Ip/Iv) in CASP animals were as follows (mean ± SD): CASP16: 1.08 ± 0.09; CASP64: 1.14 ± 0.13; V-CASP16: 1.04 ± 0.07; T-CASP16: 1.10 ± 0.16; H-CASP16: 1.08 ± 0.11 HS-CASP16: 1.07 ± 0.11. However, the fluorescence ratios of the CASP animals were significantly increased as compared to sham operated animals (SHAM16: 0.87 ± 0.08; p < 0.01). The number of firmly adhering leukocytes was significantly increased as compared to SHAM (99.54 ± 72.74; p < 0.001) animals in all CASP groups treated with colloids or crystalloids (e.g., H-CASP16: 520.3 ± 205.9 to CASP64: 650.6 ± 232.9). The type of infused colloid did not significantly influence the microcirculation in this experiment.

Conclusions: The impact of different HES-solutions on the mesenteric intestinal microcirculation did not differ from crystalloid administration in this short time treatment model of experimental sepsis.

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Infection 2011

S1P promotes thymus involution during sepsis

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Introduction: T cell depletion is a marker of the hypo-inflammatory phase of sepsis. We propose that thymus involution is one mechanism causing blood T cell loss, thus contributing to immune paralysis. Usually, thymic T cell egress is mediated by a sphingosine-1-phosphate gradient. That is T cells leave the thymus towards an increased S1P-level in the periphery (blood/lymph). A disruption of this S1P-gradient blocks T cell emigration. One cytokine identified to promote acute thymus involution is interleukin-6 (IL-6), whose expression is also induced in response to S1P. Thus, we assumed that during sepsis the thymic S1P-level increases and hence induces IL-6 expression. This may initiate thymus involution and concomitantly provokes T cell depletion in the blood.

Objectives: To prove our hypothesis we analyzed thymic involution in a murine polymicrobial sepsis model.

Methods: We induced polymicrobial sepsis in mice by cecal ligation and puncture (CLP). Thymus involution was determined by analyzing T cell count of single positive mature (CD4+CD8− vs. CD4−CD8+), double positive late immature (CD4+CD8+) and double negative early immature cells by FACS analysis. T cells from Sphk1−/− or Sphk2−/− mice, which produce less S1P, were used to characterize whether T cells in these mice have a higher rate of emigration compared to control mice. Thymic as well as serum S1P levels were quantified by LC–MS/MS and the peripheral T cell number in spleen and blood were analysed by FACS. Expression of thymic IL-6 was analyzed by qPCR.

Results: We showed that thymus involution is induced by polymicrobial sepsis. The thymus of septic mice showed more CD3 positive cells but a decreased number of CD4/CD8 double positive T cells, pointing to a thymic retention of single positive mature T cells. In line with our assumption S1P-levels increased in the thymus and consequently decreased in serum following CLP. The knockout of the S1P producing sphingosine-kinases Sphk1 or Sphk2 restores T cell egress as indicated by an increase of double positive immature T cells compared to wild type mice. Moreover in Sphk knockout mice thymic IL-6 expression is inhibited further supporting our working hypothesis.

Conclusions: Our data suggest that during sepsis inhibition of S1P generation restores thymic T cell egress, which might improve septic outcome. Therefore, understanding mechanisms of thymus involution during sepsis could lead to the design of a therapy to reconstitute thymic function, finally improving immune reactions in sepsis patients.

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Infection 2011

Micromanipulation of sepsis relevant bacteria with dielectrophoresis

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**Introduction:** In our research group we develop a highly sensitive, rapid and label-free culture independent test to estimate the resistance of sepsis pathogens with regard to antibiotics. This requires the development of modern techniques for micromanipulation. One tool which has gained large interest in recent years is dielectrophoresis, which makes use of the interaction of high frequent non-uniform electrical fields with dielectric nano- and microparticles, such as bacteria. This interaction leads to a resulting force, which can be used to trap bacteria within a small, well-defined region for further investigations.

**Objectives:** The goal of the presented work is the successful collection and controlled positioning of different kinds of bacteria in a microelectrode array for further optical non-invasive characterization. This requires an understanding of the frequency-dependent behaviour of the bacteria in suspension due to an electrical field.

**Methods:** We use a function generator to apply a sinusoidal voltage in the frequency range of several kHz to MHz to a lithographically produced microelectrode array with gap sizes of a few micrometres on which we put a droplet of bacteria suspension. With an optical microscope we monitor the process.

**Results:** We determine parameters which enable the collection of bacteria in micrometer sized regions and show the successful trapping over time. Furthermore we show the different behaviour of the bacteria for different frequency ranges.

**Conclusions:** Dielectrophoresis is a highly promising method for the precise trapping and controlled positioning of bacteria in suspension for investigations which need the collection of the objects of interest within a defined, micrometer sized region. This is a pre-requisite for optical culture independent sepsis pathogen detection.

**Results:** The pra1p strain supported neutrophil migration to a lower extent than did the parental wild-type strain, whereas neutrophils showed enhanced adhesion and migration to the Pra1p-overexpression strain. The used fungal strains did not significantly differ in their capacity to trigger the release of neutrophil extracellular traps. While inactivated hyphae of the Pra1p-overexpressing mutant enhanced the production and release of reactive oxygen species, myeloperoxidase, lactoferrin, and interleukin 8 by neutrophils, such host cell responses were reduced when stimulated with the pra1p strain. Live Pra1p-overexpressing hyphae, however, also caused a reduced neutrophil activation, indicating that Pra1p released by the fungal cells can block receptor sites and inhibit the activation of neutrophils. Similarly, recombinant Pra1p bound to neutrophils via CD11b/CD18, and inhibited the enhanced neutrophil responses caused by Pra1p-overexpressing fungal cells. Fungal cells lacking Pra1p were more efficiently killed by neutrophils.

**Conclusions:** Surface-exposed Pra1p plays a role in the recognition of C. albicans, especially hyphal cells, by human neutrophils and enhances neutrophil antifungal responses. However, the fungus can suppress some of these defense mechanisms by releasing Pra1p and blocking CD11b/CD18 on neutrophils.

**071 Infection 2011**

**Hepatic Induction of Cholesterol Biosynthesis Reflects a Remote Adaptive Response to Pneumococcal Pneumonia**

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**Introduction:** Community-acquired pneumonia caused by Streptococcus pneumoniae frequently progresses into sepsis, contributing to significant morbidity and mortality. Various serotypes of S. pneumoniae exhibiting different virulence profiles trigger variable host responses. Local and in particular remote organ response patterns underlying the eventual disease progression are incompletely understood.

**Objectives:** We aimed to comprehensively address local and remote events underlying eventual disease progression.

**Methods:** Female C57BL/6 mice were intra-tracheally infected with either serotype 19 S. pneumoniae (causing lobar pneumonia) or serotype 2 S. pneumoniae (causing septic pneumococcal disease). Samples of lung, liver and blood were collected at 6 and 24 h post-infection and subjected to microarray and metabolomic analyses. Significant candidate markers were analyzed for enriched functional categories.
**Results:** We observed a serotype-specific differential regulation of signalling and metabolic pathways in local (lung) and remote (liver) compartments, including hepatic induction of cholesterol biosynthesis during focal pneumonia leading to increased plasma cholesterol (vehicle-treatment: 1.84 mmol/l, S2: 2.35 mmol/l, S19: 2.91 mmol/l, p < .05 compared to vehicle-treatment and S2). This induction seemed to be dependent on the major pneumococcal virulence factor pneumolysin as a pneumolysin-deficient strain of serotype 19 (S19APly) failed to induce cholesterol biosynthesis (S19APly: 1.95 mmol/l). Preincubation of pneumolysin with plasma from hypercholesterolemic mice prior to intra-tracheal instillation protected against lung barrier dysfunction and alveolar macrophage cytotoxicity.

**Conclusions:** The observed increased cholesterol biosynthesis in mice infected with serotype 19 but not with serotype 2 *S. pneumoniae*—which is lost in pneumolysin-deficient mutants of pneumococci—indicates an adaptive host response to pneumococcal pneumonia. We assume that this mechanism enables the host to prevent escape of pneumococci from local lung compartments to cause invasive pneumococcal disease. Mechanistically, we propose that increased lung permeability developing in response to serotype 19 *S. pneumoniae* infection along with the hepatic induction of cholesterol biosynthesis may increase the intra-alveolar bioavailability of cholesterol to facilitate the neutralization of locally released pro-inflammatory mediators and thus prevent septic disease progression.

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**072**

**Infection 2011**

**Combined therapy with dehydroepiandrosterone-orthovanadate may influence intestinal leukocyte activation and increase capillary perfusion in experimental endotoxemia in rats**

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**Introduction:** Sepsis is a condition accompanied by compromised immune response and disturbed microcirculation. Dehydroepiandrosterone (DHEA) has immunomodulatory and vasodilatory effects, while sodium orthovanadate (an inhibitor of tyrosine phosphatase; SOV), may augment vascular tone. Both agents could theoretically be beneficial as therapy in sepsis.

**Objectives:** We examined whether DHEA alone or in combination with SOV could affect intestinal microcirculation in an endotoxemia model of sepsis in rats (induced by lipopolysaccharide from *E. coli* LPS).

**Methods:** For the DHEA-only study, where animals received DHEA 28 mg/kg i.m., and then LPS 10 mg/kg i.v., male Lewis 1A rats were separated into four groups, each of ten: controls, DHEA28, LPS10 and LPS10 + DHEA28. In a subsequent experiment, the combination of DHEA (25 or 50 mg/kg) with SOV (7.5 mg/kg) was evaluated also. In this second study, six groups (each 10 animals) were used: controls; ethanol (solvent) treated controls; DHEA50 + SOV controls; LPS15 (15 mg/kg LPS); DHEA50 + SOV +LPS15; DHEA25 + SOV +LPS15. Two hours after LPS challenge intravital fluorescence microscopy of the intestinal wall was performed and leukocyte adhesion and functional capillary density (FCD) were examined.

**Results:** DHEA-only study: LPS (10 mg/kg) challenge increased leukocyte adhesion in intestinal submucosal venules significantly. DHEA (28 mg/kg) treatment of endotoxemic animals only reduced leukocyte adhesion in collecting venules of the intestinal submucosa. FCD was strongly influenced by DHEA therapy. The number of dysfunctional capillaries was reduced in the longitudinal muscular layer (DHEA28 + LPS10 vs. LPS10, cm/cm², mean, SD: 15.26 ± 13.79 vs. 30.85 ± 18.94; P < 0.01) and in the mucosa (DHEA28 + LPS10 vs. LPS10: 8.78 ± 15.79 vs. 51.09 ± 33.66; P < 0.001). In the combined study, administration of DHEA (both dosages) with SOV resulted in a reduced number of adhering leukocytes in collecting and postcapillary venules of the intestinal submucosa (e.g., postcapillary venules: DHEA25 + SOV + LPS15 vs. LPS15, n/mm², mean, SD: 1.245 ± 223 vs. 43 ± 140; P < 0.05). The mucosal FCD was increased (e.g. DHEA25 + SOV + LPS15 vs. LPS15, cm²/cm², mean, SD: 178.23 ± 69 vs. 30.1 ± 68; P < 0.05).

**Conclusions:** DHEA administration alone strongly improved capillary perfusion while combined treatment, with sodium orthovanadate, produced additional reduction in leukocyte activation. Concomitant administration of SOV permitted to reduce DHEA dosage and prevent potential vasodilation without affecting anti-inflammatory DHEA action.

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**074**

**Infection 2011**

**Following pathogen-host interaction of *C. albicans* in vivo**

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**Introduction:** Sepsis is still mainly viewed as caused by bacteria. However, the importance of fungal pathogens in sepsis, especially caused by *Candida albicans*, is steadily increasing. As a model organism for many pathogenic yeasts, the molecular biology and genetics of *C. albicans* are well investigated. This makes *C. albicans* an optimal model for investigating the fundamental aspects and basic processes of fungal sepsis, especially in the early stages.

**Objectives:** We aim to use intra vital microscopy in a murine liver model of *C. albicans* infections to mimic and investigate the early stages of fungal sepsis. To this end, we created and optimized *C. albicans* strains expressing fluorescent proteins, which can be visualized in tissue with fluorescence microscopy. Using these strains, we aim to follow the pathogen-host interaction of *C. albicans* in the liver to determine the kinetics of hematogenously disseminated fungal cells attaching to their target organs. Of special interest to us is the binding of *C. albicans* to the host endothelia, and the subsequent activation and recruitment of immune effector cells.

**Methods:** We tested different fluorescent proteins (green fluorescent protein, GFP, and a red fluorescent protein deriviate, mCherry) under the control of different promotors to determine the combination best suited for intravitral microscopic analysis. Strains were tested in vitro for fluorescence intensity and in vivo in a murine model to determine the applicability for intra vital microscopy.

**Results:** We have created a *C. albicans* strain expressing the red fluorescent mCherry protein under the control of the highly active *TDH3* promotor. While the normally used green fluorescent protein is difficult to detect in murine liver due to the high autofluorescence of the surrounding tissue, this strain is readily visible using fluorescence microscopy. Our preliminary experiments show that even dead *C. albicans* yeast cells can bind to liver vascular endothelium within seconds to minutes after central catheter infections. Additionally, we found a distinct spatial distribution of this pathogen-host interactions, which will be followed in more detail in the future.

**Conclusions:** We have established a red fluorescent strain of *C. albicans* suitable for intravitral microscopy which can be used for...
infections with live or dead fungal cells. With this strain, we have begun to determine the kinetics of *C. albicans* to murine liver endothelia and are now starting to follow the temporal and spatial dynamics of the host–fungus interaction in more detail.

**075**

**Infection 2011**

Cannabinoid receptor modulation affects the microcirculation of the rat iris in experimental endotoxemia

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**Introduction:** The microcirculation of the iris can be studied non-invasively by intravital microscopy (IVM). This represents a unique opportunity to study changes in the microcirculation under physiological and pathological conditions. The endo-cannabinoid system (ECS) is upregulated during local and systemic inflammation, e.g. sepsis [1]. Functional outcomes of modulating cannabinoid receptor 1/2 (CB1R/CB2R) response during sepsis are currently unclear [2, 3].

**Objectives:** Aim of this study was to evaluate ECS-related changes in leukocyte activation in the iridial microcirculation during experimental endotoxemia (ETX).

**Methods:** Six groups of Lewis rats were studied (n = 6/group): placebo controls; ETX (20 mg LPS/kg i.v.); ETX + CB1R agonist (1 mg/kg WIN55212-2 + 2.5 mg AM630 to block the CB2 action); ETX + CB1R antagonist (2.5 mg/kg AM281); ETX + CB2R agonist (2.5 mg/kg HU308) and ETX + CB2R antagonist (2.5 mg AM630). All treatments were given intravenously 15 min after LPS administration. Intravital microscopy of the iridial microcirculation was performed at 0, 1, and 2 h post-LPS/placebo administration. Leukocyte adhesion was measured offline in a blinded fashion (ImageJ, NIH, US).

**Results:** We observed a significant increase in the number of adhering leukocytes in endotoxemic animals at 2 h in both vessels <25 μm in diameter and >25 μm in diameter (p < 0.001 and p < 0.01, respectively). In comparison to untreated animals treatment with HU308 resulted in a significant attenuation of leukocyte adhesion at the 2 h time point. The drug treatment group AM281 also showed a significant decrease in leukocyte adhesion (p < 0.01).

**Conclusions:** The data suggests that the endocannabinoid system plays a functional role in leukocyte activation during experimental sepsis. Activation of the CB2R by HU308 reduced leukocyte activation in the iridial microcirculation. AM281, a CB1R antagonist, also significantly reduced leukocyte adhesion in the iris microcirculation. Drugs targeting either the CB1R or CB2R may have therapeutic potential in inflammatory diseases such as sepsis.

**078**

**Infection 2011**

Assessing key transcriptional regulators and predicting interventional targets in the murine sepsis model of peritoneal contamination and infection

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**Introduction:** During septic disease progression a cascade of molecular inflammatory events often lead to multiple organ failure. Drugs aimed at blocking pivotal mediators of inflammation failed to demonstrate significant beneficial effects in clinical investigations. Thus, sepsis remains a major health problem without specific cure and persistently high mortality rates. Systems biology has widened the understanding of the complex disease and can discover interventional targets.

**Objectives:** The identification of a transcriptional response, which is evident in all organs and evolves during septic disease progression, shall guide towards a detailed understanding of the molecular mechanisms involved in multiple organ failure and help to identify molecular targets for individualized sepsis therapy.

**Methods:** We examined the transcriptional responses in lung, liver, spleen and circulating leukocytes using the murine sepsis model of Peritoneal Contamination and Infection (PCI). Samples were collected at 6 and 24 h (n = 4) after intraperitoneal injection of C57BL/6 mice with a human faeces suspension and subjected to...
transcriptional analysis using pangenomic microarrays. Gene set enrichment and promoter analysis were used to investigate the transcriptional organ-wide patterns of differentially expressed genes. We applied our developed Weighted Exactly Aligned Sequence Tracker (WEAST)-method to find specific sequence features within the promoter region of these genes.

**Results:** We found a biphasic pattern of differentially expressed genes regulated equally in all organs. These genes were coding for proteins of significantly enriched functional categories including inflammation, regulation of transcription, apoptosis and zinc coordination. Sequence features within the proximal and distal promoters of early upregulated genes, mainly associated with proinflammatory response, could be determined. Using the WEAST-method, we could link the overrepresented sequence pattern of the majority of early upregulated genes to IRF, NFkB and STAT proteins in agreement with results obtained by other methods.

**Conclusions:** The WEAST-method is excellently suited for integrative data analysis in a systems biology approach. Using this sequence analysis we could identify families of synchronously regulated genes during sepsis in all organs. The exactly aligned sequence patterns harbor the possibility to specifically administer synthetic oligonucleotides and transcription factors.

**079**

**Infection 2011**

*Candida albicans* unknown-function genes upregulated in vivo contribute to interaction with host cells and virulence in complex infection models

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**Introduction:** *Candida albicans* is a common fungal pathogen in humans and able to cause a variety of clinical infections. Several *C. albicans* unknown-function genes were found to be transcriptionally upregulated during infection (infection associated genes). However, it is yet unclear if these genes contribute to the infectious process.

**Objectives:** We aimed to identify *C. albicans* infection associated genes with unknown function which contribute to virulence.

**Methods:** Based on transcriptional profiling from oral infections, liver invasion and incubation in human blood, we selected a subset of infection-associated genes with unknown function for further analysis. Isogenic deletion mutants were constructed and subsequently tested in extensive in vitro screens, interaction with epithelial and endothelial cells, in an alternative infection model based on chicken embryos and in a murine sepsis model.

**Results:** Twenty-four isogenic deletion mutants were constructed. Sixteen of these mutants were impaired in their ability to damage monolayers of human endothelial and/or epithelial cells. Within this subset, seven mutants additionally displayed decreased stress resistance in vitro. Only two mutants showed filamentation defects. To determine whether the deleted genes influence virulence in more complex infections models, all 24 mutants were tested in ovo for their ability to kill chicken embryos infected on the chorio-allantoic membrane. We recently showed that mortality in this model depends on the fungal ability to invade the membrane and that the pro-inflammatory host response likely contributes to pathogenesis. Surprisingly, only seven mutants were attenuated in this model, of which five were also attenuated in damaging endothelial cells in vitro. Three of the mutants which were attenuated in ovo, and one mutant which was attenuated in damaging endothelial and epithelial cells but fully virulent in chicken embryos, were subsequently analyzed in a murine *C. albicans* sepsis model. Virulence in mice mirrored the results obtained in ovo.

**Conclusions:** Our results demonstrate that some of the *C. albicans* unknown-function genes which are transcriptionally upregulated during infection are essential for causing full damage of epithelial or endothelial cells in vitro. However, not all of them are crucial for full virulence in complex infection models. We conclude that either the damage potential observed in cell culture is not critical for full virulence in the complex host situation or that the greater complexity of the environment in animal models stimulates additional fungal regulatory networks leading to compensation of the defect.

**080**

**Infection 2011**

**Novel rat model of sepsis induced acute kidney insufficiency**

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**Introduction:** Acute kidney insufficiency (AKI) is an unsolved and common problem in septic patients, which rises mortality up to 67%. The pathomechanism of AKI is still poorly understood. The aim of this study was to establish a practicable animal model for the investigation of sepsis induced AKI.

**Methods:** After animal committee approval 16 male Sprague–Dawley rats (302 ± 25 g) were randomized in following groups control (n = 4), sham (n = 5) and sepsis (n = 6). Rats were anesthetized using 2.0 Vol.% Isoflurane and N2O2. The carotid and jugular vein were cannulated for continuous measurement of blood and central venous pressure over 24 h. Sham and sepsis groups received median laparotomy and the colon ascendens was mobilized. Sepsis was induced by insertion of a specific modified straight suction catheter (Ch. 10) 1.5 cm above the ileocaecal valve at the antimesenteric site. 2 ml of NaCl 0.9% were used to flush the stent and for distribution of feces. 24 h after laparotomy animals were re-anaesthetized with fentanyl/midazolam, tracheotemised and controlled ventilated. Cardio output was measured by thermodilution method. To evaluated kidney function inulin and cystatin c clearance were performed. At the end of experiment serum was drawn to determine kidney function parameters creatinine, urea, cystatin C and neutrophil gelatinase-associated lipocalin (NGAL). Variance analysis (ANOVA post-hoc Duncan test p < 0.05 MW ± SD) was used for statistical analysis.

**Results:** CASP treated rats showed clinical signs of sepsis and intraabdominal infection. All rats survived during experiment and we were haemodynamic (MAD >70 mmHG) stable. Heart rate was significantly increased in CASP animals compared to control and sham. CI and CVP revealed no significant differences between the groups. Serum creatinine (mg/ml), urea (mg/ml), cystatin C (mg/ml) and NGAL (mg/ml) increased significantly in CASP group (0.67 ± 0.22; 84.3 ± 10.17; 2.0 ± 0.29; 18.63 ± 4.15) compared to sham (0.38 ± 0.1; 51.3 ± 14.58; 1.58 ± 0.17; 3.5 ± 1.04) and control (0.43 ± 0.06; 61.33 ± 1.91; 1.67 ± 0.13; 9.85 ± 4.34). Inulin-Clearance (ml/min) was also significantly reduced in CASP rats (0.24 ± 0.23) compared to sham (0.6 ± 0.23).

**Conclusions:** We established a novel modified CASP induced septic AKI model in rats. The experimental set up can be widely used to investigate the pathomechanisms of septic induced acute kidney failure and the impact of pharmacological agents on kidney function in sepsis.
Detection of immune markers for an infection of human whole blood with *Candida albicans*

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**Introduction:** Sepsis is a life-threatening systemic infection with high mortality rates. The most important fungal pathogen in sepsis is *Candida albicans*. Its diagnosis is still difficult and mainly relies on the detection in blood cultures.

**Objectives:** Using a human whole blood model of infection we are investigating the pathophysiological processes in the development and spreading of Candida sepsis in a situation similar to in vivo. These analyses will allow identifying immune markers which might be potentially useful in clinical diagnostics.

**Methods:** Fungal cells were added to human whole blood in different concentrations and patterns of immune activation were monitored in time course analyses. Initial assays included the quantification of humoral markers of immune activation (cytokines/chemokines, antimicrobial peptides, complement system) as well as FACS staining to humoral markers of immune activation (cytokines/chemokines, anti-inflammatory activities in leukocytes by the GC receptor (GR)). The GR regulate gene expression by acting as a homodimer binding to DNA of genes or as a monomer interacting with pro-inflammatory transcription factors. Our study explored, which cells and which regulatory mechanism of the GR mediate the beneficial effects of GCs in SIRS.

**Objectives:** Our study explored, which cells and which regulatory mechanism of the GR mediate the beneficial effects of GCs in SIRS.

**Methods:** Conditional and functions selective GR knockout mice were subjected cecal ligation and puncture and LPS bolus injection. Survival, hormone and cytokine release and gene expression were analyzed.

**Results:** We show that endogenous GCs fulfill their protective function during SIRS by different mechanisms: repression of cytokine expression in macrophages, up-regulation of leptin in white adipose tissue, and facilitation of leptin signaling in the hypothalamus. Disruption of any of these GC effects by impairment of dimerization of the GR in GDrim mice, lowering GR gene dose heterozygous GR knockout mice, ablation of GR expression in macrophages (GRLysMCre mice) and in forebrain neurons (GRCaMKCreERT2 mice) leads to a strong suppression of energy metabolism, low oxygen consumption, severe hypothermia, and increased lethality when treated at the same conditions as GR mutant mice. Importantly, the cytokine response in GRCaMKCreERT2 mice and ob/ob mice during SIRS is lower than in wild type littermates.

**Conclusions:** Our data indicate that an exaggerated cytokine response is not the only mechanism in SIRS pathogenesis controlled by GCs. In addition a precise regulation of energy metabolism is required for survival in systemic inflammatory response.

Glucocorticoids control systemic inflammatory response by regulation of energy metabolism and cytokine expression

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**Introduction:** Systemic inflammatory response (SIRS) is a life-threatening syndrome, which is characterized by an overwhelming activation of the immune system and dramatic alterations of metabolism. Endogenous glucocorticoids (GCs) are essential for survival in SIRS. So far, their effect was attributed exclusively to anti-inflammatory activities in leukocytes by the GC receptor (GR). The GR regulate gene expression by acting as a homodimer binding to DNA of genes or as a monomer interacting with pro-inflammatory transcription factors. Our study explored, which cells and which regulatory mechanism of the GR mediate the beneficial effects of GCs in SIRS.

**Objectives:** Our study explored, which cells and which regulatory mechanism of the GR mediate the beneficial effects of GCs in SIRS.

**Methods:** Conditional and functions selective GR knockout mice were subjected cecal ligation and puncture and LPS bolus injection. Survival, hormone and cytokine release and gene expression were analyzed.

**Results:** We show that endogenous GCs fulfill their protective function during SIRS by different mechanisms: repression of cytokine expression in macrophages, up-regulation of leptin in white adipose tissue, and facilitation of leptin signaling in the hypothalamus. Disruption of any of these GC effects by impairment of dimerization of the GR in GDrim mice, lowering GR gene dose heterozygous GR knockout mice, ablation of GR expression in macrophages (GRLysMCre mice) and in forebrain neurons (GRCaMKCreERT2 mice) leads to a strong suppression of energy metabolism, low oxygen consumption, severe hypothermia, and increased lethality in SIRS. Similarly to the GR mutant mice leptin deficient ob/ob mice demonstrate a strong suppression of energy metabolism, severe hypothermia and increased lethality when treated at the same conditions as GR mutant mice. Importantly, the cytokine response in GRCaMKCreERT2 mice and ob/ob mice during SIRS is lower than in wild type littermates.

**Conclusions:** Our data indicate that an exaggerated cytokine response is not the only mechanism in SIRS pathogenesis controlled by GCs. In addition a precise regulation of energy metabolism is required for survival in systemic inflammatory response.
Objectives: The study aimed in the functional analysis of small non-coding RNAs in a cell culture model of sepsis.

Methods: THP-1 monocytes were stimulated with 10 ng/ml LPS [4 h] to produce a so called conditioned medium. This conditioned medium was used in a further step for the stimulation of HUVEC or HPMEC. After stimulation for 16 h changes in miRNA expression were analyzed by miRNA array analysis. MiR-146a, miR-146b, and miR-155 were selected for further analysis due to their different expression pattern after stimulation. Altered expression of proinflammatory cytokines were investigated after transfection of HUVECs with inhibitory miRNAs.

Results: Up to 1,900 microRNAs were analysed after stimulation of HUVECs and HPMECs by a conditioned medium. Array analysis identified among others a twofold upregulation of miR-146a, miR-146b, and miR-155. In order to identify the functional properties of the selected miRNAs inhibitors of miR-146a, miR-146b, and miR-155 were used. Inhibition of miR-146a resulted in a 68% diminished expression of IL6 and a 64% reduced expression of IL8. In addition, expression of IL6 and IL8 was reduced by inhibition of miRNA-146b at 49 and 26%, respectively. Inhibition of miR-155 resulted in a reduced expression of IL6 of 31% after stimulation of HUVEC miR-155 inhibited cells.

Conclusions: In conclusion, miR-146a, miR-146b, and miR-155 as well, seem to be highly involved in the regulation of inflammatory cytokines, especially IL6 and IL8.

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Modulation of immune functions in polymorphonuclear neutrophils induced by physostigmine, but not neostigmine, independent of cholinergic nerves
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Introduction: Cholinesterase inhibitors (Ch-I) inhibit the release of pro-inflammatory cytokines from macrophages and improve survival in experimental sepsis consistent with activation of the cholinergic anti-inflammatory pathway (CAP) [1, 2]. So far, less is known about whether Ch-I have a direct immunomodulatory effect on immune cells under in vitro conditions, i.e. in the absence of the vagal nerve or cholinergic nerves.

Objectives: Since polymorphonuclear neutrophils (PMNs) and their production of reactive oxygen derivatives (oxidative Burst) appear to be a central component in the inflammatory injury of sepsis [3], we investigated in this comparative study the concentration–response effect of physostigmine and neostigmine on the oxidative burst activity by human PMN induced by phorbol-12-myristate-13-acetate (PMA) under in vitro conditions.

Methods: After obtaining approval of the local Ethics Committee, leucocytes from ten healthy volunteers were isolated from heparinized whole blood using Histopaque®-1077 and incubated with 2,4, 24.2, 96.8 μM physostigmine-hemisulfate (Biozol Diagnostica, Eching, Germany) or 2.9, 29.9, 149.5 μM neostigmine-metilsulfate (Rotemmedica, Trittau, Germany). Afterwards the generation of oxidative free radicals was induced by incubation with 100 nM PMA and finally quantified flow-cytometrically by production of fluorescent rhodamine 123 [4]. The amount of intracellular H2O2 production was compared between both groups. Statistics: Kruskal–Wallis test, all data were presented as molecules of equivalent soluble fluorochrome (MESF) units (mean values ± standard deviation).

Results: Incubation with PMA induced the generation of oxidative free radicals in PMN (1,821.1 ± 406.6 MESF). Prior exposure of physostigmine-hemisulfate resulted in a dose dependent suppression of H2O2 production by PMN: 96.8 μM physostigmine-hemisulfate reduced the effect of PMA to 655.5 ± 142.0 MESF (vs. initial value, p < 0.05). On the contrary, neostigmine-metilsulfate, in the concentration range tested, had no significant effect on neutrophil oxidative burst activity (1,869.8 ± 346.2 MESF with 149.5 μM neostigmine).

Conclusions: While the peripheral Ch-I neostigmine has no effect on PMN H2O2 production, the lipid-soluble, tertiary amine Ch-I physostigmine shows a dose-dependent reduction in oxidative burst activity. This in vitro study is the first investigation which demonstrates a direct modulation of immune functions in PMN by physostigmine, independent of the CAP. The underlying mechanism can probably be explained by the lipid-solubility of physostigmine consistent with intracellular effects and is object of current investigations.

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The impact of immunosuppression on cytokine levels in the swine experimental model of sepsis
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Introduction: Sepsis is a major cause of morbidity and mortality in surgical patients. Moreover, immunosuppression increases the risk of infection and the development of sepsis significantly. The pathophysiology of sepsis in immunosuppressed patients is still poorly understood.

Objectives: The aim of the study was to determine the dynamics of immunopathological cytokine response in polymicrobial sepsis caused by stercoral peritonitis, and possible changes caused by immunosuppression. The findings could be very useful in diagnosing sepsis in immunosuppressed patients.

Methods: 28 minipigs used in this study were divided into 3 groups. In the experimental (immunosuppressed) and control group (without immunosuppression) stercoral peritonitis was surgically induced by CLP (cecal ligation and puncture method). In sham group only laparotomy was performed. Blood samples were drawn from all the animals in defined time intervals and plasmatic concentrations of cytokines (IL1β, IL4, IL6, IL8, IL10, IL18, IFNg, TNFa) and CRP were measured by ELISA method. The kinetics of plasmatic concentrations of cytokines and CRP was analysed using nonparametric Kruskal–Wallis and Mann–Whitney tests.

Results: Since the plasmatic concentrations of all monitored cytokines differed significantly between sham group and animals with peritonitis, CLS proved to be a reliable cause of sepsis followed by appropriate cytokine response. Comparing immunosuppressed and naive group of animals after CLS, a clear difference was found between the plasmatic levels of IL-6. The differences between levels of other measured cytokines did not reach statistical significance.
Conclusions: According to our results, IL-6, the earliest marker of sepsis, represents the main difference in the cytokine profile of sepsis caused by immunosuppression. Because CLP proved to be a realistic model to study the immunopathology of polymicrobial sepsis in experimental settings, we conclude, that IL-6 could serve as a reliable early diagnostic marker of sepsis in immunosuppressed patients in clinical practice.

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Refining the first exon architecture of HMOX1: a link to human sepsis

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Introduction: Heme oxygenases (HO-1 and HO-2) break down heme into carbon monoxide, free iron (II) and biliverdin, which is subsequently metabolized to bilirubin by biliverdin reductases. In experimental models induction of HMOX1 (the gene coding for HO-1) protects from inflammation, making the gene a promising target in sepsis and organ failure. Genetic variations of HMOX1 (single nucleotide polymorphisms (SNP) rs2071746 and (GT)n-microsatellite) in the currently annotated promoter region have recently been found to affect the outcome of severe human sepsis (see accompanying poster by Sponholz et al.). In silico analyses of HMOX1 transcripts give hints to a more complex gene structure placing these polymorphisms in a rather intronic than promoter position.

Objectives: Our analyses re-evaluate the currently accepted HMOX1 gene model and the potential effects of the sepsis-associated polymorphisms on alternative splicing of the 5‘-untranslated region (5‘-UTR).

Methods: RT-PCR/cloning and sequence analysis was used to detect transcripts with elongated 5‘-UTRs. Minigenes representing variable haplotypes of the SNP rs2071746 and (GT)n-microsatellite were set up to evaluate their effect on 5‘-UTR splicing. Quantification of alternative splice-isoforms frequencies has been done by transfecting minigenes to human hepatic cell lines and a subsequent RT-PCR approach using 5‘-6-carboxyfluorescein (FAM)-labeled oligonucleotides before capillary sequencing.

Results: We were able to detect novel transcripts showing elongated 5‘ UTRs and extended to current HMOX1 gene model. A newly indentified first exon placing the SNP rs2071746 and (GT)n-microsatellite in a rather intronic 5‘-UTR than promoter position. Evaluation of alternative 5‘-UTR splicing in the context of several haplotypes revealed a dependency of splice-isoform frequencies and (GT)n-microsatellite length. Splicing of an alternative splice-acceptor in the novel first intron creates a predominant fraction of transcript carrying a short upstream open reading frame (ORF).

Conclusions: Alternative 5‘-UTR splicing has already been shown to control translation efficiency by introducing short upstream ORFs. We could show a dependency of 5‘-UTR alternative splice-isoform frequencies and (GT)n-microsatellite length. A correlation of (GT)n-microsatellite length and HMOX1 mRNA and protein level has already been reported. Therefore, we assume that (GT)n-microsatellite dependent 5‘-UTR splicing is involved in HMOX1 transcriptional and translational control.

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Assessing key transcriptional regulators and predicting interventional targets in the murine sepsis model of peritoneal contamination and infection

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Introduction: During septic disease progression cascades of molecular inflammatory events often lead to multiple organ failure. Drugs aimed at blocking pivotal mediators of inflammation failed to demonstrate significant beneficial effects in clinical investigations. Thus, sepsis remains a major health problem without specific cure and persistently high mortality rates. Systems biology has widened the understanding of the complex disease and can discover interventional targets.

Objectives: The identification of a transcriptional response, which is evident in all organs and evolues during septic disease progression, shall guide towards a detailed understanding of the molecular mechanisms involved in multiple organ failure and help to predict molecular targets for individualized sepsis therapy.

Methods: We examined the transcriptional responses in lung, liver, spleen and circulating leukocytes using the murine sepsis model of Peritoneal Contamination and Infection (PCI). Samples were collected at 6 and 24 h after intraperitoneal infection of C57BL/6mice with a human faeces suspension and subjected to transcriptional analysis using pangenomic microarrays. Gene set enrichment and promoter analyses were used to investigate the transcriptional organ-wide patterns of differentially expressed genes. We applied our Weighted Exactly Aligned Sequence Tracker (WEAST)-method to find specific sequence features within the proximal and distal promoter regions of these genes.

Results: We found a biphasic pattern of differentially expressed genes regulated equally in all organs. These genes were coding for proteins of significantly enriched functional categories including inflammation, regulation of transcription, apoptosis and zinc coordination. Using the WEAST-method, we could link the overrepresented sequence patterns of the majority of early upregulated genes, mainly associated with the proinflammatory response, to IRF, NFkB and STAT proteins in agreement with results obtained by other methods.

Conclusions: The WEAST-method is excellently suited for integrative data analysis in a systems biology approach. Using this sequence analysis we could identify families of synchronously regulated genes during sepsis in all organs. The exactly aligned sequence patterns harbor the possibility to administer synthetic oligonucleotides and transcription factors.

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Systems biology of multiple organ dysfunction: formation of ceramide-enriched macro-domains during host response

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Introduction: Generation of bioactive lipids such as ceramide (Cer) and the formation of Cer-enriched macrodomains with subsequent reorganization of receptor complexes are regarded as a hallmark of stress-induced signalling. A broad panel of compounds used routinely
in ICU care or prior to admission to ICU are acting as (long lasting) inhibitors of Cer-formation.

**Objectives:** Therefore, we addressed the question whether the plasma activity of the secreted isofrom of the Cer-forming enzyme sphingomyelinase (SMPD1) is involved in the orchestration of the host response and eventual development of organ failure.

**Methods:** We measured plasma activity in patients with various degrees of SIRS/sepsis of different origin, elucidated the role of the enzyme in a murine model of polymicrobial infection including a loss of function model and finally determined the effect of its inhibition by bona fide inhibitors in a retrospective clinical observational study.

**Results:** Plasma secreted activity of SMPD1 was found significantly elevated in critically ill patients compared to age matched controls, where threshold levels of SMPD1 activity helped to predict outcome. A severity dependent increase was also observed in patients with MODS following SIRS due to elective cardiac surgery. Beyond immunological detection of increased pSMPD1 in septic patients, we found an increase in Cer-enriched macrodomains in cultured endothelial cells after stimulation with patients’ plasma, wherein an enrichment of Fas was also observed. In a loss of function model we identified 315 transcripts differentially regulated in circulating white blood cells, liver and lung as well as in the cytokine pattern/organ function parameters following poly-microbial sepsis due to peritoneal cavity infection. Furthermore, host responses in ko-mice were more pronounced with respect to bacterial load in lung, liver and blood, plasma cytokine levels, thrombocytopenia as well as delayed migration of neutrophils into hepatic tissue, which might be caused by a reduced leukocyte–endothelium interaction either subsequent to pharmacological inhibition or due to genetic deficiency. Most surprisingly, in our retrospective analysis, we found that a series of compounds used in ICU-care may function as inhibitors of SMPD1 and that administration of such compounds prior to or during ICU stay is associated with significantly improved outcome compared to medications without inhibitory properties against SMPD1.

**Conclusions:** In conclusion, the results provide support to the notion of a bio-functional relevant activity of SMPD1 resulting in altered signal transduction in SIRS and sepsis, which may contribute to the development and resolution of MODS.

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**Infection 2011**

**Passive immunotherapy with modified intravenous immunoglobulin (IVIg) preparations improves survival in experimental sepsis by attenuating inflammation, complement and coagulation pathways**

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**Introduction:** IVIg preparations are known to have broad immunomodulating and anti-inflammatory activities, but the results from their use in sepsis patients have been so far largely disappointing.

**Objectives:** We have previously shown that the exposure of some IgG antibodies to ferrous ions or to a pH 4.0 buffer enhances their antigen-binding polyspecificity that includes a newly-acquired binding to at least one pro-inflammatory cytokine [1, 2].

**Methods:** A commercially available IVIg, exposed in vitro to Fe(II) ions, was used for passive immunotherapy of mice with experimental sepsis induced by the injection of bacterial lipopolysaccharide (LPS), of live E.coli, of zymosan or by the colon puncture and ligation technique. A commercially available IVIg, exposed in vitro to Fe(II) ions or to a pH 4.0 buffer enhances their antigen-binding polyspecificity that includes a newly-acquired binding to at least one pro-inflammatory cytokine [1, 2].

**Results:** A single dose of the Fe(II)-modified preparation, but not of the unmodified IVIg significantly increased survival in all sepsis models. A single dose of the pH 4.0-treated IVIg had the same effect in LPS-sepsis. The mechanisms of the observed protective activity of the ferrous ions-modified IVIg were studied in detail in LPS-induced septic shock. Its therapeutic effect was not due to a more efficient LPS neutralization and was still present when administered as late as 6 hours after LPS. The serum levels of several pro-inflammatory molecules were decreased, IL10 levels were increased, complement exhaustion was diminished and the coagulation abnormality was overcome. The Fe(II) ions treatment induced structural changes in the IgG molecules, demonstrated by fluorescent spectroscopy, as well as by kinetic and thermodynamic analyses of antigen binding. These changes were mild and did not result in full-blown IgG denaturation as the modified preparation still met the strict Pharmacopoeia requirements for human therapeutic IVIg.

**Conclusions:** We suggest that immunoglobulin preparations with additionally enhanced polyspecificity, caused by a brief exposure to ferrous ions, have a clinical potential in sepsis and other variants of the severe inflammatory response syndrome (e.g. post-traumatic, in avian flu, etc.).

**References:**[1] Dimitrova et al, J. Biol. Chem. 2006 281:439–446. [2] Djoumerska-Alexieva et al, FEBS Journal 2010 277:3039–3050.

Clinical Sepsis Research: Diagnostics

007

**Infection 2011**

**NT-pro-brain natriuretic peptide a sensitive and specific prognostic marker in elderly septic patients**

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**Introduction:** Cardiac dysfunction is more frequent in septic patients than commonly supposed. Earlier studies showed that heart failure might be a considerable factor in determining outcome of critically ill patients [1]. Cardiac troponin and N-terminal pro-brain natriuretic peptide (NT-proBNP) are highly predictive for heart failure and probably sensitive in predicting death in septic and heterogeneous other groups of intensive care patients [2]. But the critical date for sampling and the cut off point for testing is still under discussion.

**Objectives:** The aim of this prospective study was to evaluate sensitivity, specificity as well as positive and negative prognostic values of NT-proBNP as an independent prognostic parameter. These results were compared to the outcome markers troponin I and SAPS-II-Score.

**Methods:** The study was carried out at Bayreuth Central Hospital Intensive Care Unit (ICU). 40 consecutive elderly patients (23 male, 17 female; mean ± SD 74.7 ± 6.6 year) who developed severe sepsis after abdominal surgery were included in the study. Day 1 was the day when sepsis diagnosis was established according to the guidelines of the German AWMF. Clinical and laboratory data were recorded daily. The primary end point of the study was either transfer to general ward or death in ICU. Threshold of NT-proBNP plasma level was 6,000 pg/ml, Troponin I 0.2 ng/ml, SAPS II Score 50 points. Microsoft Excel and SPSS software were used for statistical analysis.

**Results:** On day 5 the sensitivity (Se) of NT-proBNP was 65% (95% CI 48–79%), the specificity (Sp) 95% (95% CI 83–99%), the positive predictive value (PPV) 92.8% (95% CI 80–98%) and the negative predictive value (NPV) 72.1% (68–85%). The corresponding data are: Troponin I Se 35% (21–52), Sp 95% (83–99), PPV 87.5% (73–96), NPV 59.3% (43–75); SAPS II Score Se 64.7% (48–79), Sp 69% (53–83), PPV 68.75% (51–82), NPV 64.7% (48–79). Positive
Conclusions: Clinical evaluation of the patient’s status is still the gold standard in determining the therapy in an end of life situation, but NT-pro BNP, taken on day 5, can be helpful as an additional prognostic marker that is superior to other established predictors of outcome.

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014
Infection 2011

Presepsin (soluble CD14 subtype) as a new sepsis marker: first results of the diagnostic and prognostic validity

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Introduction: CD14 is a glycoprotein expressed on the membrane surface of monocytes/macrophages and serves as a receptor for complexes of lipopolysaccharides (LPS) and LPS binding protein (LBPB) activating the toll-like receptor 4 (TLR4) specific pro-inflammatory signaling cascade against infectious agents. Presepsin (soluble sCD14 subtype, sCD14-ST) is a circulating molecule fragment derived from CD14. Plasma presepsin levels are associated with systemic inflammation triggered by bacterial infections. First evidence suggested that presepsin may be beneficial as sepsis marker.

Objectives: The aim of the study was to examine the diagnostic efficacy and the prognostic value in patients presenting with sepsis.

Methods: In 140 septic patients admitted to the emergency room and in 119 healthy persons presepsin, procalcitonin (PCT) and the Acute Physiology and Chronic Health Evaluation II (APACHE) score were determined at admission and after 24 and 72 h. Presepsin was determined using the PATHFAST Presepsin assay (Mitsubishi Medience, Tokyo, Japan). PCT was measured using a luminescence immune assay (BRAHMS, Hennigsdorf, Germany). Primary endpoint was death within 30 days. The combined endpoint major adverse event (MAE) consisted of at least one of the primary or the secondary endpoints need of intensive care, mechanical ventilation or dialysis.

Results: Mean values of presepsin were 159 (90% CI 148–171) pg/ml in the control group and 2.563 (90% CI 1,458–3,669) pg/ml in the patient group. In contrast to PCT, presepsin values differed highly significant between patients with sepsis and severe sepsis or septic shock (p < 0.0001) which was comparable to the clinical scores. The 30-day mortality was 16.4% and increased from the 1st to the 4th quartile of presepsin from 2.7 to 39.4%. Presepsin demonstrated superior prognostic accuracy for 30-day risk of death. The area under the receiver operating characteristics curve (AUC) was 0.878 (95% CI 0.801–0.934) compared to 0.668 (95% CI 0.570–0.757) and 0.815 (95% CI 0.709–0.895) for PCT and APACHE score, respectively. During the first 72 h patients with MAEs showed an increasing tendency of presepsin values, whereas the values of patients without MAEs were decreasing.

Conclusions: Presepsin demonstrated a strong relationship with disease severity and outcome. Presepsin values were related to the course of the disease. In contrast to PCT, presepsin enabled more reliable prognostic and early risk prediction of 30-day mortality already at admission.

015
Infection 2011

PCR dependent pathogen detection shortens time to antifungal therapy in patients with invasive Candida infection

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Introduction: Invasive Candida infection is associated with a high mortality rate. A long time to antifungal therapy considerably increases the risk of death. PCR driven application of antifungals improves outcome in patients after allogenic stem cell transplantation (Hebart H, Bone Marrow Transplantation 2009). PCR dependent pathogen detection might shorten time to diagnosis of invasive fungal infection in ICU patients and, therefore, time to antifungal therapy.

Objectives: To compare the time to antifungal therapy between patients where either the PCR or the BC gives the first indication of an invasive Candida infection.

Methods: Retrospective analysis on patients submitted to the ICU between 2004 and 2010, where either blood cultures (BC) or EDTA blood samples for endpoint PCR was taken for suspected sepsis. A multiplex PCR-based assay was used (VYOO®, SIRS-Lab GmbH, Germany) for pathogen detection since 2009. Only patients, where either BC or PCR tested positive for Candida spp., were included into the analysis. Patients with ongoing antifungal therapy were excluded. Data are given as medians and 95% confidence intervals.

Results: 11 patients with a PCR and 32 patients with a blood culture positive for Candida spp. were identified. In 1 out of 11 positive PCRs, Candida was confirmed in a concomitant BC. Both groups were similar regarding age (BC: 65.8 [53.6–70] years, PCR: 69 [66–71.5] years, p = 0.28) and SAPSII-score (BC: 49.5 [39.8–63.5], PCR: 38 [33–48.5], p = 0.09). Median time to antifungal therapy was 67.5 (52.4–90) h after the BC was taken and 31.0 (28–37.5) h after the PCR was taken (p < 0.01). 3 (27%) patients of the PCR group died compared to 17 (53%) patients of the BC group (p = 0.14).

Conclusions: PCR significantly reduces time to antifungal therapy. This was associated with a trend in lower ICU mortality. Whether PCR driven application of antifungal therapy truly improves outcome in ICU patients, needs to be tested in a larger study.

020
Infection 2011

The Intensive Care Infection Score (ICIS): a new approach for discrimination between sepsis and non-infectious systemic inflammation

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Introduction: Sepsis is a leading cause of death in critically ill patients. Rapid and accurate diagnosis and immediate treatment of sepsis are of crucial importance. However, differentiating sepsis from systemic inflammatory response syndrome (SIRS) can be challenging in spite of many diagnostic approaches based on surrogate markers which have been proposed until today. Because of the complexity of the pathophysiology during systemic inflammation and sepsis it is unlikely that a single
parameter will have sufficient diagnostic accuracy for sepsis. A diagnostic score combining meaningful markers derived from the white blood cell count seems to be a promising approach. Until today various scores are already established for risk prognosis and quantification of severity in SIRS and sepsis. However, to date a score for differentiating SIRS from sepsis is not available.

Objectives: The aim of this study was to develop and evaluate a blood-cell derived score for the diagnosis of systemic infection as early in time as possible.

Methods: Following informed consent, a total of 70 consecutive patients admitted to the Intensive Care Unit (ICU) of the University Medical Center Hamburg-Eppendorf were enrolled in this study. Patients of either sex between 19 and 88 years who fulfilled at least two SIRS criteria within the first 48 h after ICU admission were classified as SIRS patients with or without infection according to the ACCP/SCCM consensus conference criteria. Sepsis was diagnosed either in case of positive blood culture test or positive microbiological findings from catheters. Laboratory data were collected and analyzed from whole blood samples daily. A hematological immune-response based infection score, the intensive care infection score (ICIS), was established from five parameters involved in the early innate immune response: mature neutrophils count, immature neutrophils count, antibody-secreting cells count, detection of neutrophils and monocytes/macrophages activation. All ICIS comprised parameters were measured on a modified fluorescence flow cytometer (Sysmex, Kobe, Japan). The performance of the ICIS score for discrimination between infected and non-infected patients was analyzed. ICIS was compared to five parameters: C-reactive protein (CRP), lipopolysaccharide-binding protein (LBP), Erythropoietin (EPO), interleukin-6 (IL-6) and tumor-necrosis factor-alpha (TNF-alpha).

Results: Each component of the ICIS score in itself is correlated with the occurrence of infection, particularly within the first 48 h. To generate the best sensitivity and specificity to detect infection the ICIS score was developed by using weighting values from 1 to 4 for each parameter derived from area under curve (AUC) performance as well as sensitivity and specificity cut-off values. A mean ICIS value of <5 (lower cut-off level) indicated the absence of infection whereas the score did not fall below a value of 6 in infected patients throughout the observation time. A reliable discrimination between infected and non-infected patients by ICIS was obtained within the critical first 48 h of ICU stay. Further, ICIS was compared to other clinical variables such as CRP, LBP, EPO, IL-6 and TNF-alpha. The AUC for ICIS was found to be highest within the first 48 h after two or more SIRS-criteria had been fulfilled (AUC = 0.851, P < 0.0001) and during the subsequent 3 days (AUC = 0.701, P < 0.0001) when compared to all other examined sepsis markers. With respect to positive and negative predictive values, ICIS was superior to all other measured variables in the crucial first 48 h (PPV = 80%, NPV = 75%).

Conclusions: The immune response of early admitted ICU patients is different in septic versus non-septic patients. The present study shows that the presence or absence of infection in ICU patients with SIRS can be determined using the ICIS score at an early time point following admission. ICIS components are involved during the early inflammatory response and in the bone marrow production of innate immune cells. Moreover, the ICIS score can be provided in real-time without sample preparation and is independent from inter observer variability. The proposed ICIS Score will tentatively help in refining bedside diagnostic tools to efficiently diagnose sepsis after further validation.

021

Infection 2011

Revisiting the white blood cell count: immature granulocytes as a diagnostic marker in adult sepsis

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Introduction: Sepsis is a serious disease condition and a major cause of intensive care unit (ICU) admission. Its diagnosis in critically ill patients is complicated. To diagnose an infection rapidly, and to accurately differentiate systemic inflammatory response syndrome (SIRS) from sepsis, is challenging yet early diagnosis is vital for early induction of an appropriate therapy. The aim of this study was to evaluate whether the immature granulocyte (IG) count is a useful early diagnostic marker of sepsis compared to other markers.

Objectives: To evaluate whether the immature granulocyte (IG) count can be used as an early diagnostic marker of sepsis.

Methods: A total of 70 consecutive patients admitted to the surgical ICUs of the University Medical Center Hamburg-Eppendorf were assessed. IGs were measured using an automated hematology analyzer (XE 2100, Sysmex, Kobe, Japan) from whole blood samples. C reactive protein (CRP), lipopolysaccharide binding protein (LBP) and interleukin 6 (IL 6) concentrations were also determined. The observation period was a maximum of 21 days and ended with the patients discharge from ICU or death. Receiver operating characteristic (ROC) analyses were conducted and area under the curve (AUC) was calculated to determine sensitivities and specificities for the parameters.

Results: We found that the IG count significantly discriminates between infected and non-infected patients (P < 0.0001) with a sensitivity of 89.2% and a specificity of 76.4%, particularly within the first 48 h after SIRS onset. Regarding the discriminative power for infection, the IG count was more indicative than other clinical parameters such as CRP, LBP and IL 6, which had a sensitivity of less than 68%. Additionally, the highest diagnostic odds ratio (DOR) with 26.7 was calculated for the IG count within the first 48 h. During the course of the disease ROC curve analyses showed a superior positive predictive value of the IG count compared to the other measured parameters during the first 5 days following the fulfilment of SIRS criteria. However, the number of IGs was not correlated with ICU mortality.

Conclusions: The total number of IG in peripheral blood from ICU patients is a good marker to discriminate infected and non-infected patients very early during SIRS. However, the IG count is not suitable as a prognostic marker for mortality. Routine and serial measurement of IGs may provide new possibilities for rapid screening of SIRS patients on ICU with suspected infections.

030

Infection 2011

Prognostic impact of procalcitonin in severe sepsis and septic shock results of the VISEP-study

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Introduction: Serum procalcitonin (PCT) levels have been shown to increase with increasing severity of sepsis and organ dysfunction.
Recently, Jensen and colleagues [1] demonstrated that in critically ill patients mortality risk increases for every day that procalcitonin increases. Using a PCT guided algorithm based on this results, the authors reported organ-related harm and prolonged admission to the intensive care unit in a subsequent randomized controlled study [2].

**Objectives:** To investigate the prognostic impact of PCT levels on 28-day all-cause mortality in patients with severe sepsis or septic shock.

**Methods:** This retrospective study was a sub-analysis of 537 patients with severe sepsis and septic shock admitted to 18 academic tertiary hospitals participating in a large multicenter randomized controlled trial conducted in Germany [3]. Since PCT measurements were not available for every day and patient, the measurements were aggregated by computation of 2-day median values derived for days 0–1, 2–3, 4–5 and so on, where day 0–1 denotes the baseline.

**Results:** A total of 255 patients were included in the final analysis: insulin therapy: 128 conventional (CIT), 127 intensive (IIT) or fluid resuscitation: 130 Ringer’s lactate (RL), 125 hydroxy starch (HES), respectively. The baseline characteristics between the treatment groups were comparable except renal dysfunction as reported in the original study. Overall, the overall 28-day mortality for patients was 21.2% (54/255). There was no significant difference in the mortality rates between the treatment groups, 26% in CIT versus 24.7% in IIT, \( P = 0.739 > 0.05 \) in RL versus 26.7% in HES, \( P = 0.484 > 0.05 \). The intra-individual PCT-levels decreased over the time both among the survivors and nonsurvivors \( (P < 0.0001, \text{each}) \). At baseline, the median PCT levels between nonsurvivors and survivors were comparable: 4.4 ng/ml [IQR 1.7–12.5] versus 4.8 ng/ml [1.4–23.7], \( P = 0.861 > 0.05 \). However, from day 4 onwards, median PCT levels in nonsurvivors were significantly higher, when compared to survivors: Days 4–5: 1.3 ng/ml [0.5–3.7] versus 2.7 ng/ml [1.0–5.7], \( P = 0.010 < 0.05 \); days 6–7: 1.9 ng/ml [0.7–4.2] versus 0.7 ng/ml [0.4–2.3], \( P = 0.006 < 0.05 \); days 8–9: 1.6 ng/ml [0.7–3.2] versus 0.6 ng/ml [0.3–2.1], \( P < 0.001 < 0.0001 \); days 10–11: 1.6 ng/ml [0.6–3.2] versus 0.6 ng/ml [0.3–2.0], \( P = 0.0055 < 0.05 \); days 12–13: 1.7 ng/ml [0.9–7.3] versus 0.5 ng/ml [0.3–2.0], \( P = 0.0011 < 0.05 \). There were no differences in the corresponding serum levels of C-reactive protein over time.

**Conclusions:** In patients with severe sepsis or septic shock PCT levels discriminate between surviving and non-surviving patients from day 4 onwards. This may be important for designing further interventional studies.

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### 033

**Infection 2011**

**Prognostic value of procalcitonin and its relation to organ failure in sepsis**

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**Introduction:** Sepsis, which often develops into life threatening shock, is a systemic clinical situation caused by toxic substances released from microorganisms during infection. Early institution of an appropriate antimicrobial regimen in infected patients is associated with better outcome and hence early diagnosis of sepsis is of utmost importance. Sepsis suffers from a lack of specific clinical symptoms and thus there is always a need for an effective and accurate marker to support, or exclude, the diagnosis of infection. Procalcitonin (PCT) has been proposed as a novel biomarker of bacterial infection.

**Objectives:** This study was performed to evaluate the prognostic value of PCT and its relation to sequential organ failure assessment (SOFA) scores in patients with sepsis.

**Methods:** Total number of 50 patients admitted to the intensive care unit of Sir Ganga Ram hospital, New Delhi, India, from July 2010 to January 2011 with a fresh episode of sepsis were included in the study. PCT levels were analyzed and SOFA scores were calculated on 0, 24 and 72 h. Patients were followed up for 28 days and were then grouped as survivors and non survivors.

**Results:** During the observation period of 28 days, 37 patients survived and rest 13 expired. The median age of survivors and non survivors were 58.5 and 62.5, respectively. The mortality was 26%. PCT levels decreased by 66% \( (p = 0.01) \) in survivors and increased by 64% \( (p = 0.851) \) in non survivors. There was no significant association between level of serum PCT and grade of sepsis \( (p > 0.05) \). Higher SOFA levels were associated with significantly \( (p = 0.000) \) higher PCT concentrations \( \text{SOFA} 1–6: \text{PCT} 1.49 \text{ng/ml}; \text{SOFA} 7–12: \text{PCT} 5.32 \text{ng/ml}; \text{SOFA} 13–18: \text{PCT} 36 \text{ng/ml}. \)

**Conclusions:** These observations indicate that PCT concentration helps in determining the prognosis of the patient and is also significantly associated with the severity of multi organ dysfunction syndrome. Hence, routine use of PCT as a monitoring tool might improve the management and, consequently, the survival of patients with sepsis.

### 042

**Infection 2011**

**Automated extraction of microbial DNA from whole blood for the universal PCR detection of pathogens**

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**Introduction:** Timely diagnosis of the etiological agents of sepsis is demanded in order to adjust therapy to specific antibiotic treatment. Blood culture is the gold standard of diagnosis taking at least 2 days until identification of the pathogen. Universal rRNA gene PCR and sequencing are considered a promising way to the rapid identification of bacteria and fungi in blood samples. Among other parameters contained automation is desirable to avoid contamination. Here we present results obtained with a new automated system, SepsiTest SelectNA®. It comprises a pre-treatment of the blood for the removal of human DNA and the extraction and isolation of pathogen DNA. We investigated the influence of the blood volume processed for DNA extraction on the sensitivity of detection of pathogens by universal 16S/18S rRNA gene PCR.

**Objectives:** A new PCR test for microbial pathogens in whole blood, SepsiTest SelectNA®, was studied in terms of detection sensitivity as related to sample volume analyzed.

**Methods:** Universal 16S/18S rDNA Real-Time PCR-test, SepsiTest SelectNA® (Molzym, Bremen); spatial separation/room concept; three room concept—lab 1: laminar flow (extraction), lab 2: PCR cabinet (mastermix preparation), lab 3: universal 16S/18S PCR; Commercial sequencing (gatc GmbH, Constance, Germany, over night); Routinely used databases, NCBI Blast (http://www.ncbi.nlm.nih.gov/BLAST).
nml.nih.gov/BLAST/) or SepsiTest-Blast (http://www.sepsitest-blast.net); Analytical sensitivity determination: spiked whole blood; pre-evaluation: 21 clinical samples from 17 patients with systemic inflammatory response syndrome (SIRS), sepsis, or neutropenic fever.

**Results:** Analytical sensitivity (spike experiments): *Staphylococcus aureus*: <6 cfu/ml (10 ml blood), *Escherichia coli*: <12 cfu/ml (10 ml blood). PCR detection of pathogens appears most sensitive when using large blood volume: 79% (11/14) of clinical samples was positive using 5 ml compared to 50% (7/14) using 1 ml. PCR positives from likely contaminants accounted for 5% corresponding to other results, although another 19% of likely infections had a contaminant background. Handling was recently discussed as major probable source of contamination.

**Conclusions:** PCR was powerful in detecting probable infections (28%) where cultures did not supply the results. Thus PCR may give clues to the diagnosis of difficult-to-grow infections for adequate therapy.

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**047**

**Infection 2011**

Multiple polymerase chain reaction for pathogen detection in septic patients

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**Introduction:** Severe sepsis is one of the main causes of death in hospitalized patients. Early detection of pathogens allows initiation of appropriate antimicrobial therapy that strongly correlates with positive clinical outcomes. Polymerase chain reaction (PCR) techniques enable more rapid and sensitive detection of pathogens compared to conventional blood cultures (BC).

**Objectives:** To test a multiplex polymerase chain reaction (PCR) technique for detection of organisms in septic patients with and without antimicrobial pre-treatment and to evaluate the potential clinical benefit utilizing this PCR method.

**Methods:** In a prospective study 180 adult patients with sepsis were investigated. Fifty-three of them were pre-treated with antibiotics (two doses maximum). At admission, EDTA-blood samples for PCR (VYOO®, SIRS-Lab GmbH, Jena, Germany) and whole blood samples for blood culture (BC) analysis were collected. PCR results were compared to blood and other culture results. The potential impact of PCR results on adequacy of antimicrobial therapy was analysed.

**Results:** Excluding results attributable to contaminants, pathogenic microorganisms were detected in 35 patients (20%) when the findings of the two tests were combined. PCR detected 27 (15%) cases in contrast to 20 (11%) cases which were detected by BC; in detail 16 cases were identified only by PCR, 10 cases only by BC, and 9 cases by both methods. Related to the 53 patients with prior antimicrobial therapy, PCR identified pathogens in 11 cases (21%) in contrast to one case (2%) in BC. Among those 11 cases BC failed to detect *S. maltophilia*, *E. faecium*, *N. meningitidis*, *E. coli*, and Candida in five single cases and *P. aeruginosa*, *S. aureus* and *S. pneumoniae* in the six other cases. In these patients PCR results allowed deescalating the initial empiric antimicrobial therapy.

**Conclusions:** In patients without antimicrobial pre-treatment PCR detected microorganisms that were not found by BC and vice versa. Combined detection rate of both methods was higher compared with PCR or BC alone. PCR detected significantly more microorganisms (21 vs. 2%) in patients with prior antimicrobial treatment than BC and allowed deescalating initial empiric antimicrobial therapy. Despite limitations of both methods, PCR could serve as an adjunct to BC to facilitate early detection of causal microorganisms in sepsis, especially in patients with prior antimicrobial treatment. In these cases PCR may have the potential to facilitate treatment decisions for adequate antimicrobial therapy.

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**058**

**Infection 2011**

BK virus reactivation in critically ill surgical patients with shock: a prospective observational study

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**Introduction:** Infections with polyomavirus BK virus (BKV) are a common cause of renal dysfunction after renal transplantation and may also be harmful in patients with other forms of immunodeficiency, such as hemorrhagic or septic shock.

**Objectives:** To determine the frequency of BKV reactivation in critically ill patients with septic or hemorrhagic shock, and, if reactivation is detectable, whether reactivation may be associated with renal dysfunction.

**Methods:** A total of 125 plasma samples from 44 critically ill postoperative/posttraumatic patients with septic or hemorrhagic shock were tested by real-time polymerase chain reaction (PCR) for BKV DNA during their stay on the intensive care unit (ICU).

**Results:** BKV reactivation occurred in four patients, i.e. in three of the septic and in one of the hemorrhagic shock group. There was no association between virus reactivation and renal dysfunction. All positive samples contained a low viral load (<5 × 102 copies/ml).

**Conclusions:** BKV reactivation was rarely found in critically ill postoperative/posttraumatic patients with shock. Due to the low viral load detected in the surgical patients with shock, it is very unlikely that BKV reactivation results in nephropathy later on.

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**059**

**Infection 2011**

NT-proBNP and HMGB1 in surgical critically ill patients with hypovolemic or septic shock

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**Introduction:** Cardiovascular dysfunction occurs in surgical critically ill patients in volume-deficiency/hemorrhagic (hypovolemic) shock and in septic shock.

**Objectives:** The aim of the present study was to compare the course of NT-proBNP and HMGB1 plasma concentrations in surgical critically ill patients with hypovolemic shock with those with septic shock.

**Methods:** In a prospective observational single-centre study in critically ill surgical patients admitted to an University adult ICU, from 07/2008 to 01/2009, 26 consecutive patients with hypovolemic shock and 18 patients with septic shock were longitudinally monitored. At time points before shock, with <0.1, >0.1 and <1.0, >1.0 μg/ kg × min noradrenaline and/or adrenaline during shock, after shock,
before demission or before death, NT-proBNP and HMGB1 serum concentrations were determined.

**Results:** NT-proBNP serum concentrations were higher in patients with septic shock than with hypovolemic shock at $>0.1$ and $<1.0 \mu g/\text{kg} \times \text{min noradrenaline}$ in surviving patients. During the stay on the ICU, NT-proBNP and HMGB1 serum concentrations declined, remained stable or increased in both patient groups. In the non-survivors, NT-proBNP concentrations were always beyond the normal range in both groups.

**Conclusions:** Severity of cardiovascular dysfunction was associated with higher NT-proBNP values in septic than in hypovolemic shock patients. NT-proBNP concentrations did not separate survivors from non-survivors at the beginning and during ICU stay.

### 063 Infection 2011

**Screening for bacteraemia in sepsis using cartridges for hemodialysis**

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**Introduction:** Blood culture is considered as the gold standard for diagnosis of bacteraemia. However, only 15% of all blood culture samples were found to be positive during sepsis. Renal failure is a common organ dysfunction in patients with sepsis. During renal replacement therapy, conditions of extracorporal blood flow and its contact with a membranous surface over time might represent a risk factor for the development of biofilms, which might be suitable for detection of pathogens in used dialyzer cartridges.

**Objectives:** To study whether discarded dialysis cartridges after conventional use can be used for detection of bacteraemia in patients with sepsis and renal failure.

**Methods:** A total number of 16 ICU patients with sepsis were enrolled. Used hemodialyzers were processed within 1 h after disconnection: residual blood and dialysis fluid were incubated with tryptic soy broth/TSB for 18 h at $37^\circ$C and subsequently to rinsing, eluted culture media were separately incubated at $37^\circ$C. After draining, membranes were incubated with TSB supplemented with protease inhibitor K ($100-200$ microgram/ml, $60$ min). The media were eluted and finally, filters were dehisced under sterile conditions. The extracted capillaries were also incubated with TSB. All samples were incubated for 8 days. Suspicious samples were plated on blood agar and analysed by an experienced microbiologist.

**Results:** Despite the use of antibiotics in 87.5% of all patients at the day of replacement therapy, a positive detection rate of 31.3% (5 out of 16) interpreted as true infection were obtained. In detail two Streptococcus and three Enterococcus positive samples were detected. In 25% contaminations, Bacillus or Staphylococcus epidermidis were observed. The rate of true positive blood cultures as gold standard amounted to only $10.0\%$ (80 samples, 23 positive, 8 true positive) over the whole length of stay.

**Conclusions:** The present data demonstrate the capability to cultivate and to identify pathogenic microorganisms using dialysis cartridges obtained from patients with sepsis. Even under the circumstance that broad spectrum/multiday antimicrobial therapy was performed in most of the cases and only the growth of aerobic bacteria was tested, pathogenic microorganisms were detectable in nearly one-third of sepsis patients. Further studies should extend the spectrum to anaerobic pathogens and test the capability for determination of resistance profiles. Further, a comparison between blood culture- and PCR based results should be performed.

### 064 Infection 2011

**Heart rate variability changes predict sub-acute post-stroke infections**

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**Introduction:** Immune depression after stroke increases the susceptibility to systemic infection, one of the most relevant complication in stroke patients. Changes in the activity of the autonomic nervous system encoded in HRV indices are also potential early predictors of post-stroke infections. These indices can be obtained from the ECG during mandatory stroke monitoring, providing a means of early diagnosis and therapy with implications that could change clinical practice.

**Objectives:** Activity of the autonomic nervous system seems to control post-stroke immunodepression. We investigated heart rate variability (HRV) indices that reflect autonomic readjustments as predictors of post-stroke infection.

**Methods:** Forty-three patients with acute ischemic stroke were enrolled in a prospective study. The predictability of sub-acute infections (day $4 \pm 1$ after admission) was investigated in 34 patients without acute infection by means of HRV indices obtained in the acute period ($48$ h after admission). Associations between HRV indices and post-stroke infection were examined in the sub-acute period.

**Results:** Sub-acute infection could be predicted in patients without clinical or paraclinical (white blood cell count and C-reactive protein) signs of infection in the acute period by: (1) an increased HF$_{\text{norm}}$ (normalized high frequency power), but a reduced LF$_{\text{norm}}$ (normalized low frequency power) as well as a reduced LF/HF ratio during the day for all parameters, and (2) a reduced LF (low frequency power) and VLF (very low frequency power) at night (all changes $p < 0.05$).

**Conclusions:** HRV indices, readily available from routine stroke monitoring systems were found to be associated with sub-acute post-stroke infections, and allow an early diagnosis of a developing infection preceding analysis of routine blood samples. Thus, an HRV-based early diagnosis of post-stroke infection should be investigated in more detail since the method may have implications as a novel tool for a timely and appropriate treatment. We propose that in the present work these changes can be considered as markers of decreased vagal activity. Thus, a parasympathetic deficit can be assessed as a risk factor for infection.

### 076 Infection 2011

**Predictive value of endothelial glyocalyx shedding in septic shock**

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**Introduction:** The endothelial glyocalyx is a recently discovered structure at the luminal side of blood vessels consisting of proteoglycans and glycosaminoglycans which plays an important role in vascular barrier function and cell adhesion. With increasing severity of sepsis, endothelial glyocalyx develops a dysfunction syndrome which is characterized by its shedding from the endothelium and an
increase of its components, e.g. hyaluronan and syndecan, in plasma. This process is triggered by hypoxia and a multitude of humoral mediators like tumor necrosis factor-α.

**Objectives:** Since the process of glyocalyx shedding could be responsible for the clinical course of sepsis, we raised the question if glyocalyx plasma values in septic shock are of predictive magnitude.

**Methods:** This clinical prospective study—approved by the local ethics committee—was performed to assess plasma levels of glyocalyx components by enzyme-linked immunosorbent assay (ELISA) technique in 28 patients with septic shock which was defined according to the guidelines of the ACCP/SCCM Consensus Conference Committee. Patients were included within 24 h after onset of infection signs. Blood was drawn immediately after inclusion (baseline) and on days 1 and 4. Informed consent for blood withdrawal was obtained from all patients or their legal representatives, respectively.

**Results:** 8 of 28 patients did not survive septic shock; 7 of the 8 non-survivors died within the first week of their intensive care unit (ICU) stay. Plasma levels of hyaluronan and syndecan at baseline, on days 1 and 4 were significantly higher in non-survivors than in survivors (all \( p < 0.05 \)). The distance in hyaluronan plasma levels between both groups elevated with time because hyaluronan plasma levels showed a further increase in non-survivors and decreased in survivors during the first 4 ICU days. Surviving septic shock patients who left the ICU within 10 days showed lower hyaluronan plasma levels at baseline and on day 1 than patients with an ICU stay of more than 10 days (both \( p < 0.05 \)). No correlation between the glyocalyx parameters in plasma and duration of hospital stay was found.

**Conclusions:** Shedding of endothelial glyocalyx seems to be a prognostic factor for outcome of sepsis and its component hyaluronan appears to predict survival of septic shock and length of ICU stay in survivors.

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**Abs:** S118

**Abstracts**

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**085 Infection 2011**

**Hemophagocytic and/or macrophage activation syndromes may explain thrombocytopenia, leukopenia and anemia in critically ill postoperative/posttraumatic patients**

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**Introduction:** In patients with septic shock, complicating bicytopenia or tricytopenia occurs, being refractory to substitution with blood products. In children with bi- or tricytopenia, hemophagocytosis is known as hemophagocytic lymphohistiocytosis (HLH).

**Objectives:** To find out whether cytopenia in adult patients with septic shock may be explained by hemophagocytosis and/or macrophage activation syndrome.

**Methods:** In patients with bi- or tricytopenia besides daily measurement of counts of leukocytes, erythrocytes and platelets, immunophenotyping was performed using flow cytometry. Inflammatory cytokines as well as soluble interleukin-2-receptor (sCD25) were measured by chemiluminescence technology. Cell cultures of the adherent fraction were set up from blood cells isolated by Ficoll gradient and morphologically and functionally characterised. Since HLH in children is associated with viral diseases and functional defects of natural killer cells (NK), multiplex-PCR was established as sensitive detection method for Epstein-Barr virus (EBV).

**Results:** In 4/10 patients with cytopenia, immunological characteristics of HLH and/or macrophage activation syndrome were detected. Besides significantly elevated sCD25 concentrations, elevated plasma concentrations of triglycerides were documented. Based on cultivated adherent cell populations, phagocytosis of autologous erythrocytes was proven in vitro, also. Despite the fact that viral load was not elevated in plasma, EBV-specific genes (EBNA-1 and LMP-1) were detected within the hemophagocytizing cells.

**Conclusions:** Hemophagocytic and/or macrophage activation syndromes reflect a serious complication in adult postoperative/posttraumatic intensive care patients. They may be triggered by defective or profoundly reduced NK cell activity. This may be detected early by measuring sCD25 in blood of these patients.
089  
**Infection 2011**

Effect of Drotrecogin alfa (activated) on Cytokeratin-18 levels in blood of septic patients

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**Introduction:** Apoptosis of the epithelium is deemed to play a pivotal role in the pathogenesis of sepsis. Apoptosis induced by either death-inducing receptors or other stimuli leads to activation of specific caspases. Cytokeratin-18 is a type I intermediate filament protein and the major component of single-layer and glandular epithelial cells.

**Objectives:** To evaluate total Cytokeratin-18 levels during the time course of septic patients treated with or without Drotrecogin alfa (activated).

**Methods:** We measured Cytokeratin-18 serum levels in septic patients on day 1, 3 and 5 of sepsis with ELISA-method. We compared Cytokeratin-18 levels of septic patients treated with Drotrecogin alfa (activated) \((n = 9)\) and in nine septic patients without treatment (control group) on day 1, 3 and 5 of sepsis. Statistical analysis was performed with ANOVA.

**Results:** Cytokeratin-18 serum levels increased from day 1 to day 5 of sepsis in patients treated with Drotrecogin alfa (activated) (day 1: MW = 0.372 ng/ml ± SEM = 0.05 ng/ml; day 5: MW = 0.599 ng/ml ± SEM = 0.14 ng/ml). In septic patients not treated with Drotrecogin alfa (activated) Cytokeratin-18 levels decreased from day 1 to day 5 (day 1: MW = 0.654 ng/ml; day 5: MW = 0.480 ng/ml ± SEM = 0.07 ng/ml).

**Conclusions:** We can show that Drotrecogin alfa (activated) decreases Cytokeratin-18 serum levels during the time course of septic patients. This observation strongly suggests that Drotrecogin alfa (activated) plays an important role in regulating the cellular damage during sepsis. Further in vitro and in vivo investigations are needed to confirm these results.

093  
**Infection 2011**

The correlation between the neuron specific enolase and Glasgow coma scale in patients with sepsis and septic shock

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**Introduction:** Sepsis is often complicated by an acute and reversible deterioration of mental status.

**Objectives:** We investigated whether there was any relation between serum levels of neuron-specific enolase (NSE) and Glasgow Coma Scale (GCS) to evaluate cerebral injury of patients with sepsis and septic shock.

**Methods:** This study was performed between 01.06.2010 and 01.04.2011 at Selcuk University Meram Medicine Faculty Hospital Emergency Department. 64 patients with a diagnosis of severe sepsis and septic shock were recruited. Age and gender-matched 30 healthy volunteers were involved to the study. The following features were evaluated in hospital stay duration, mortality or discharge and prognosis of the disease, C-Reactive Protein (CRP), Procalcitonin and PTX-3 levels were checked on admission to the Critical Care Unit (0 h) and following 24 h.

**Results:** PTX-3 and Procalcitonin (0 h), and Glasgow Coma Score (GCS), but not CRP, were significantly correlated with mortality. There was significant relation between hospital stay duration and PTX-3 (24 h) \((p < 0.001)\). There was also significant relation between Procalcitonin (24 h) and GCS \((p < 0.001)\).

**Conclusions:** The results suggest PTX-3 proved to be a specific independent prognostic biomarker in severe sepsis and septic shock. Further studies are needed to support the validity of this evidence.

090  
**Infection 2011**

The role of pentraxin-3 in patients with severe sepsis and septic shock

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**Introduction:** Pentraxin-3 (PTX-3) is an acute phase protein secreted by various cells, including leukocytes and endothelial cells. The role of plasma PTX-3 in septic shock patients is unknown.

**Objectives:** The aim of the study was to investigate the relation between PTX-3 and mortality in patients with severe sepsis and septic shock.

**Methods:** This study was performed between 01.06.2010 and 01.04.2011 at the Selcuk University Department of Emergency Medicine. 64 patients with a diagnosis of severe sepsis and septic shock were recruited. Age and gender-matched 30 healthy volunteers were involved to the study. The following features were evaluated in hospital stay duration, mortality or discharge and prognosis of the disease, C-Reactive Protein (CRP), Procalcitonin and PTX-3 levels were checked on admission to the Critical Care Unit (0 h) and following 24 h.

**Results:** There were no statistically significant relation between GCS duration of hospital stay and NSE duration of hospital stay \((p > 0.05)\). We also found that no statistical correlation existed between NSE and GCS \((p > 0.05)\), neither.

**Conclusions:** NSE can't be used to evaluate cerebral injury for patients with sepsis and septic shock. Further studies are needed to support the validity of this evidence.
094 Infection 2011

Soluble Urokinase-type Plasminogen Activator Receptor as a marker of outcome in severe sepsis.
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Introduction: Urokinase-type Plasminogen Activator Receptor (uPAR) is a multidomain glycoprotein anchored to the cell membrane with a GPI. uPAR is present on various immunologically active cells including white blood cells, endothelial, and smooth muscle cells. Proteolytic receptor cleavage results with shedding of the soluble forms of uPAR (suPAR). The soluble molecule is stable in the bloodstream and can serve as a biomarker of inflammation. The elevated serum concentration of suPAR has been associated with poor prognosis and increased mortality of patients with sepsis.

Objectives: Our objective was to analyze changes in serum concentration of suPAR and to correlate them with clinical symptoms of sepsis, with other laboratory markers, and severity scores.

Methods: The study has been conducted in the Department of Anaesthesiology and Intensive Therapy, Medical University, Wroclaw, Poland. Patients diagnosed with severe sepsis were included into the study. Blood samples for suPAR measurements were collected upon admission and after 5 days of treatment. After centrifugation at 3,000 g at 4°C for 10 min serum aliquots were stored at −80°C for further analysis. The concentration of suPAR was measured with an immunoassay (ViroGates, Denmark). Patients diagnosed with malignant neoplastic disease have been excluded from the study.

Results: 16 surgical patients with severe sepsis were included. All patients had microbiologically confirmed infection. Patients status had been described with APACHE II score on admission and with SOFA score daily. Patients’ data, clinical information, laboratory parameters, and culture results (blood and other specimens) were correlated with suPAR. Concentration of suPAR was markedly elevated in all patients with severe sepsis. Furthermore, suPAR level was markedly higher in those who died from multigorgan failure, compared to the survivors.

Conclusions: Elevated suPAR level can be associated with both gram-negative and gram-positive infection. Based on the preliminary data, high concentration of suPAR correlates with poor clinical outcome therefore monitoring of suPAR in patients with severe sepsis may be a useful prognostic tool. More detailed studies comparing suPAR with other biochemical sepsis markers during the course of severe sepsis are needed.

097 Infection 2011

The diagnosis of bloodstream infections with a new molecular method.
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Introduction: Current microbiological diagnosis of sepsis is based on blood culture. However several factors such as lack of rapidity, prior antibiotic treatment, the presence of difficult to culture pathogens have negative impact on the diagnostic yield of microbiological methods. A well-designed non-culture-based molecular technology for identification of microorganisms in blood of patients with sepsis has been made available. RT-PCR (real time polymerase chain reaction) provides improvement in sensitivity and specificity of pathogen detection, comparing to the culture methods.

Objectives: Our objective was to evaluate a novel multiplex real-time PCR method for detection of bloodstream infections in patients diagnosed with sepsis and septic shock. We hypothesize that the accuracy, specificity and sensitivity of the molecular method will be better than blood culture, and the results will be available much earlier.

Methods: This preliminary, observational study was conducted in the Intensive Care Unit, Medical University, Wroclaw, Poland. Patients admitted to the intensive care unit, diagnosed with sepsis, severe sepsis or septic shock were included to the study. Whole blood samples for PCR testing were collected at the same time as for blood culture. The system we used was an innovative Multiplex real-time PCR method, with a real amplicon detection and dual priming oligonucleotide technology (Magicplex™ Sepsis real-time Test, Seegene). It provides improvement in sensitivity and specificity of pathogen detection, comparing to the conventional real time-PCR methods. Test screens for more than 90 pathogens (73 Gram (+) bacteria, 12 Gram (−) bacteria, 6 fungi), as well as for 3 drug resistance markers (mecA, vanA and vanB) from whole blood samples.

Results: Demographic data (age, sex), and relevant medical history had been collected upon admission. Underlying cause of sepsis (abdominal, cardiovascular, pulmonary, catheter related, urinary, tissue infection following trauma) was recorded. The clinical status of patients had been evaluated with APACHE II score upon admission and with SOFA score daily. The procedure of extraction, amplification and screening for 93 pathogens took 6–7 h and the identification of 27 pathogens on a species level took 1 h. PCR results were compared to the blood and other culture results. Detection of bacterial and fungal pathogens with Magicplex system was accurate in all analyzed samples, and all the PCR results were confirmed by the positive culture results of blood or other specimens. Out of all RT-PCR positive samples, only 48% were positive by blood culture.

Conclusions: Based on the preliminary data Magicplex test is more sensitive than blood culture method. The test is not influenced by ongoing antibiotic therapy and with clinical signs and additional laboratory results it may be a useful tool for the rapid diagnosis of bacteremia and fungemia at the ICU.

099 Infection 2011

Gene polymorphisms in the heme degradation pathway affect outcome of patients with severe sepsis
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Introduction: Heme oxygenases (HMOX) break down heme into carbon monoxide, free iron(II) and biliverdin, which is further metabolized to bilirubin by biliverdin reductases (BLVR). Regulatory elements in both, HMOX and BLVR genes, were shown to affect outcome in various clinical entities, including inflammatory diseases.

Objectives: Here, we tested whether single nucleotide polymorphisms (SNP) in HMOX1 and BLVRA/B genes and a highly polymorphic (GT)n-microsatellite in HMOX1 were associated with outcome of sepsis.
Methods: Two cohorts (n = 430 and 398 patients) of patients with severe sepsis were genotyped for SNPs and/or the microsatellite in both genes. Results from genotyping were correlated to clinical and laboratory findings regarding outcome of patients.

Results: Based on mean-SOFA scores patients homozygous for the rs2071746 A-allele or medium length (GT)n-microsatellites (27–33 units) of HMOX1 showed higher 28-day mortality (p = 0.047 and p = 0.033) in one cohort compared to the other genotypes. The T-allele was less frequently observed in both cohorts than would be expected according to Hardy–Weinberg equilibrium. SNPs within BLVRA/B showed no association with outcome. Furthermore, a novel HMOX1 alternative first exon upstream of the currently annotated ex01 and alternative splice variants that might be influenced by the length of the (GT)n repeat were observed.

Conclusions: Short (GT)n repeats that are in linkage disequilibrium with the T-allele of rs2071746 in HMOX1 are associated with favourable outcome, while no association with gene variants of BLVRA/B were noticed. Evidence to support a gene model for HMOX1 with a new first exon shifting the (GT)n-microsatellite and rs2071746 to an intronic position, need to be further elucidated.

101 Infection 2011

Correlation of Immulite® detectable inflammatory mediators with NETosis and autophagy in phagocytes of patients in septic shock

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Introduction: Bacterial endotoxin may induce an important pathogen specific immune response known as NETosis (neutrophil extracellular traps) 1. NETs are extracellular chromatin fibers capturing and killing microbes. Moreover, sepsis is associated with extensive autophagic vacuolization due to cellular stress.2. We were interested to investigate neutrophilic and eosinophilic granulocytes and monocytes undergoing autophagy/NETosis and its relationship to therapeutic management such as catecholamine or extensive antibiotic treatment.

Objectives: Patients were immune monitored to predict early infections and sepsis following a major surgical trauma. In blood smears leukocyte subpopulations can be monitored according to characteristics of apoptosis and autophagy as well as NETosis. In a short time frame these results are available and can be studied in comparison with clinical signs and other inflammatory markers such as CRP and cytokines.

Methods: Patients with neutropenia were immune monitored for G-CSF treatment. Blood smears of patients on ICU (n = 6) during severe sepsis and septic shock were evaluated using phase contrast light microscopy of Wright’s Giemsa-stained specimen. The relative amount of phagocytes undergoing autophagy was determined in 10^4 nucleated cells using flow cytometry and LC3 staining or were quantified using morphological examination of Giemsa-stained blood smears. In addition, the numbers of cells undergoing NETosis were determined in blood smears. The correlation of the two events were evaluated by comparing the individual patients courses. To prove a possible correlation between autophagy, NETosis, inflammation and infection, the following cytokines were determined: IL-1β, IL-6, IL-8 and TNF-α. Moreover, Ferritin, soluble CD25 and lipopolysaccharide binding protein (LBP) were quantified. Results were documented on the basis of positive blood cultures, catecholamine administration during episodes of septic shock, and the antibiotic treatment

a) Results: During their stay on the ICU, all patients neutrophils had high relative numbers with autophagy and NETosis. Autophagy was found in 32–100% of the neutrophils (median 91%), the number of granulocytes with NETosis ranged between 3 and 28 (median 11) as counted in a single blood smear. (a) In five out of six patients (83%) there was a correlation between the amount of autophagy and the number of NETosis. (b) In three out of five patients (60%) there was a correlation between the incidence of NETosis and the detection of gram-negative bacteria. In one patient there was no correlation, in another doubtful because of high variability in the charts. (c) In one out of four patients (25%) there was a correlation between the incidence of NETosis and the administration of catecholamines. (e) There was no correlation between autophagy and catecholamine administration in all four patients.

Conclusions: The investigation of NETosis in ICU patients clearly demonstrates that this pathogen-specific immune response correlates with positive blood cultures and gram negative infections. Remarkably, NETosis was found up to 48 h before increases of CRP and clinical signs which motivated the caring physician to either initiate or change antibiotic treatment. Therefore we see a demanding necessity to study the inflammatory cytokines in addition to LBP in this patient population. Further, the increased relative amount of autophageous granulocytes may explain mitochondrial dysfunction in severe sepsis and septic shock. The simple and low cost evaluation of routine convention blood smears using phase contrast microscopy may be applied to monitor infections early and to guide antioxidant and therapeutic regimen in these patients.

103 Infection 2011

Quality of blood culture testing: a survey in intensive care units and microbiological laboratories from four European countries

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Introduction: Blood culture (BC) testing before initiation of antimicrobial therapy is recommended as a standard of care in international sepsis guidelines [1] and has been shown to reduce mortality, ICU-stay and antibiotic overuse in patients with severe sepsis [2, 3]. Whereas microbiological laboratory practice has been highly standardized, shortfalls in the preanalytic procedures in the ICU (i.e. indication, time to incubation, blood volume and numbers of BCs) may have a significant effect on the diagnostic yield.

Objectives: To evaluate adherence with recommendations regarding BC-testing.

Methods: 140 structured interviews in four European countries (Italy, UK, France and Germany) between September and November 2009 in 30 microbiological laboratories (MLabs) and 60 intensive care units (ICUs). The survey was carried out by an international agency (Advention BP, London) on behalf of BD Diagnostics.
Spleenectomy predisposes to life-threatening infectious complications also called overwhelming post-splenectomy infection (OPSI). According to retrospective studies mostly conducted 20–50 years ago, this complication is most frequent in the 3 years following the removal of the spleen and is caused in more than 50% of the cases by pneumococci. These epidemiologic data, however, have been challenged by a recent studies that documented only a marginal role for pneumococci in OPSI patients. In general, there is a lack of data on the incidence, burden and major pathogens of OPSI in today’s patient population. So far, no prospective, well-designed clinical trial on the epidemiology and pathogenesis of the OPSI syndrome has been conducted.

Objectives: (1) To rigorously assess the causative microbial pathogens in OPSI; (2) To determine the clinical characteristics and outcome of OPSI; (3) To evaluate the role of co-morbidity, immunosuppression and vaccination status as risk factors for the development of OPSI; (4) To analyse B-cell immunology and pneumococcal immunity of OPSI patients to identify immunological risk factors that identify patients at increased risk for OPSI; (5) To build a registry of OPSI patients for the study of the long-term outcome of OPSI survivors.

Methods: The SPLEEN OFF study is a prospective, multicenter observational cohort study with controls. SPLEEN OFF is a collaborative initiative of the Integrated Research and Treatment Centers for Chronic Immunodeficiency in Freiburg (CICI) and for Sepsis Control and Care in Jena (CSCC); supported by the German Competence Network Sepsis (SepNet). A projected total of 450 German intensive care units (ICU) will screen adult patients with community-acquired severe sepsis or septic shock for previous splenectomy for 12 months (cases). A consecutive patient on the same ICU with community-acquired severe sepsis or septic shock and no history of splenectomy matched for age and sex will serve as control. Patient demographics, Charlson comorbidity index, immunosuppressive medication, vaccination status and indication and timing of splenectomy will be documented at baseline. On study entry, blood cultures will be drawn and processed locally. In addition, universal 16S rRNA PCR for the detection of bloodstream pathogens, pneumococcal urinary antigen ELISA and an ELISA for capsule-specific antipneumococcal antibodies will be performed by the study laboratory. The APACHE II score will be recorded at baseline and the SOFA score will be determined on day 0, 1, 3 and 7. In addition, 28-day mortality, length of hospital and ICU stay and sepsis focus will be documented. After completion of the study on day 28, patients will be transferred into the study register for long-term follow up and specialized care provided by the CCI and the CSCC.

Conclusions: Our ultimate aim is to determine clinical and immunological risk factors that identify patients at increased risk for OPSI and in need for improved preventive measures. In addition, the study may help to unravel the immunological basis for the increased risk for sepsis after splenectomy.

Acknowledgement: Supported by the Paul-Martini Research Group (Clinical Septomics), funded by the Ministry of Thuringia (ProExcellence; PE 108-2), the Thuringian Foundation for Technology, Innovation and Research (STIFT), the German Sepsis Society (GSS) and the Center of Sepsis Control & Care (CSCC); funded by the German Ministry of Education and Research (BMBF).

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SPL[E]Nectomy, Overwhelming inFection and pneumococcal immunity. A nationwide cohort and case Finding study (SPLEEN OFF): study synopsis

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Introduction: Spleenectomy predisposes to life-threatening infectious complications also called overwhelming post-splenectomy infection (OPSI). According to retrospective studies mostly conducted 20–50 years ago, this complication is most frequent in the 3 years following the removal of the spleen and is caused in more than 50% of the cases by pneumococci. These epidemiologic data, however, have been challenged by a recent studies that documented only a marginal role for pneumococci in OPSI patients. In general, there is a lack of data on the incidence, burden and major pathogens of OPSI in today’s patient population. So far, no prospective, well-designed clinical trial on the epidemiology and pathogenesis of the OPSI syndrome has been conducted.

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Methods: The SPLEEN OFF study is a prospective, multicenter observational cohort study with controls. SPLEEN OFF is a collaborative initiative of the Integrated Research and Treatment Centers for Chronic Immunodeficiency in Freiburg (CICI) and for Sepsis Control and Care in Jena (CSCC); supported by the German Competence Network Sepsis (SepNet). A projected total of 450 German intensive care units (ICU) will screen adult patients with community-acquired severe sepsis or septic shock for previous splenectomy for 12 months (cases). A consecutive patient on the same ICU with community-acquired severe sepsis or septic shock and no history of splenectomy matched for age and sex will serve as control. Patient demographics, Charlson comorbidity index, immunosuppressive medication, vaccination status and indication and timing of splenectomy will be documented at baseline. On study entry, blood cultures will be drawn and processed locally. In addition, universal 16S rRNA PCR for the detection of bloodstream pathogens, pneumococcal urinary antigen ELISA and an ELISA for capsule-specific antipneumococcal antibodies will be performed by the study laboratory. The APACHE II score will be recorded at baseline and the SOFA score will be determined on day 0, 1, 3 and 7. In addition, 28-day mortality, length of hospital and ICU stay and sepsis focus will be documented. After completion of the study on day 28, patients will be transferred into the study register for long-term follow up and specialized care provided by the CCI and the CSCC.

Conclusions: Our ultimate aim is to determine clinical and immunological risk factors that identify patients at increased risk for OPSI and in need for improved preventive measures. In addition, the study may help to unravel the immunological basis for the increased risk for sepsis after splenectomy.

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Microbiological burden during the late phase of sepsis

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Introduction: Recent pathophysiology models of sepsis and ex vivo data are speculating about various distinct phases during sepsis. Clinical data regarding survival and microbiological burden are in the time course of sepsis are largely missing.

Objectives: Aim of this study was to determine the significance of the ‘late phase’ of sepsis with respect to survival, microbiological findings and patients’ characteristics.
Methods: In a retrospective trial, 16,041 patient charts from a 50-bed intensive care unit during 2006–2009 were screened, and 999 patients were identified as qualifying for severe sepsis or septic shock. Three phases were established according to the observed mortality peaks which were separated by two distinct nadirs: phase I (days 1–5), phase II (days 6–15) and phase III (days 16–150). All patient charts were analyzed for survival/death rates as well as results of obtained blood cultures over the course of hospital stay.

Results: From the 999 enrolled patients, 308 died during the course of sepsis presenting a characteristic mortality rate (31%) with three distinct mortality peaks. The first peak occurred at around day 2 after diagnosis, the second at day 7 and the last one at day 17. Of all deaths, 37% occurred in the early phase (phase I) and 63% during the later phases (phase II + III). In total, 2,117 blood cultures were drawn. In phase I, 882 blood cultures were taken, representing a sampling rate of 88% with a positive rate of 15%. In phase II, 461 samples were drawn (52%) and a positive rate of 11.3%. Within phase III, 524 samples were obtained (66%) with a positive rate of 15%, which was significantly higher compared to the positive rate of phase II and similar to phase I. The rate of typically opportunistic bacteria rose significantly from 9% in phase I up to 18% in phase III. The same was true for the rate of Candida spp. (phase I 13%, phase III 27%).

Conclusions: During severe sepsis and septic shock a distinct mortality pattern with a majority of deaths after day 5 was observed. The later phase of sepsis is associated with a significant re-raise of positive blood culture results. The rates of opportunistic bacteria and Candida spp. increased over the length of stay, which might underscore the significance of the proposed concept of an immune suppression phase at stage of admission to the ICU, which distinctly differ from patients developing stream infections present with pathophysiological phenotypes already at stage of admission to the ICU, which distinctly differ from patients developing bacterial infections at all time points (I, II and III). The cumulative dosage of norepinephrine was also lower at time point III in patients with fungal infections (16,200 vs. 29,475 μg). Similarly, levels of fibrinogen and aPTT time were slightly lower but within normal range at time point III as well as arterial P02 pressure at time point I.

Conclusions: Our data indicate that patients developing fungal blood stream infections present with pathophysiological phenotypes already at stage of admission to the ICU, which distinctly differ from patients developing bacterial infections. Especially a certain degree of liver damage/liver dysfunction appears to be an independent risk factor hinting at a possible underlying pathogenesis of fungal blood stream infections.

Differences between fungal and bacterial sepsis

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Introduction: Invasive fungal infection is a frequent, but often underestimated cause for sepsis with a strong impact on clinical course and outcome. Furthermore, positive fungal blood cultures were frequently a late finding, since diagnosis is often delayed and time consuming.

Objectives: To identify discriminators in patients with severe infection of fungal versus bacterial origin using various laboratory parameters, treatment procedures and underlying patients characteristics.

Methods: In a retrospective trial, 999 patients from a university ICU developing severe sepsis or septic shock were screened according to identified underlying pathogens. Patients presenting with positive blood cultures results of prototypical bacteria, i.e. E. coli, Enterobacter cloacae, Enterococcus faecalis, E. faecium and S. aureus as well as patients with a positive finding for fungal infection, i.e. various Candida spp., were enrolled. Comparative analyses were performed including patients’ characteristics and laboratory markers at different time points: (I) day of admission on ICU, (II) day of diagnosis of sepsis and (III) day of positive blood culture finding. Chi-square test was used for comparison of categorical data. Continues data across multiple groups were compared by ANOVA testing. P values <0.05 were considered significant.

Results: Positive blood cultures for the pre-selected pathogens were found in 168 cases. No differences were found in patients’ characteristics such as age, BMI, LOS and severity of disease measured by SOFA-, APACHE II- or SAPS-Score and ICU mortality. Interestingly, even at the early time point (I) patients developing a fungal infection presented with significantly increased laboratory markers monitoring liver damage when compared to those, developing bacterial infections (ASAT 656 vs. 155 U/L; ALAT 305 vs. 81 U/L; GLDH 424.2 vs. 25 U/L). These elevated levels were still present or even increased over time (II and III). Additionally, we also found differences in GCS levels at time point II (6 vs. 8 patients) which was in a line with a higher level of days of sedation (53 vs. 30%) potentially due to higher rates of mechanical ventilation (80 vs. 67%) in patients with fungal infections defined with a cut-off value of three patients in RAMSAY-Score. Furthermore, glucose levels were significantly lower, but within normal range, in patients developing fungal infections at all time points (I, II and III). The cumulative dosage of norepinephrine was also lower at time point III in patients with fungal infections (16,200 vs. 29,475 μg). Similarly, levels of fibrinogen and aPTT time were slightly lower but within normal range at time point III as well as arterial P02 pressure at time point I.

Conclusions: Our data indicate that patients developing fungal blood stream infections present with pathophysiological phenotypes already at stage of admission to the ICU, which distinctly differ from patients developing bacterial infections. Especially a certain degree of liver damage/liver dysfunction appears to be an independent risk factor hinting at a possible underlying pathogenesis of fungal blood stream infections.

Identification of new biomarkers for risk stratification of patients with neutropenic fever (LAB-ALERTS pilot study): method validation and first preliminary results

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Introduction: Neutropenia is a common side effect of chemotherapy and associated with an increased risk of infection. Risk classification of neutropenic patients presenting with fever regarding the development of sepsis/septic shock is mainly based on clinical symptoms. Thus identification of new laboratory markers that allow for risk stratification of these patients is of high impact. Recent advances in mass spectrometry based clinical proteomics in particular MALDI/SELDI-TOF-MS have lead to great promise in the search for
Objectives: Development and validation of mass spectrometric (MS) protocols for the investigation of human blood plasma. Collection of preliminary data from blood samples of 47 neutropenic patients. Identification of biomarker candidates for discrimination of neutropenic patients with regard to the development of sepsis/septic shock.

Methods: Collection of blood plasma of neutropenic patients from remaining routine material. After Ca²⁺ induced coagulation one part of the sample was directly prepared for SELDI-TOF analysis. From the second part peptides were extracted using C18 packed pipette tips. The eluted peptides were analyzed with SELDI-TOF and MALDI-TOF. Inter-day variability was calculated by processing of aliquots of a pooled plasma on 10 days. The intra-day variability was investigated by processing ten aliquots in parallel. Number of detected peaks, mean and distribution of CV values were calculated.

Results: Workflows for the analysis of peptides and proteins after artificial clotting of blood plasma were generated. Validation showed that the MALDI protocol produced the highest amount of signals but also the highest variance (138 peaks, mean CV 40.7% in the intra-day measurement). Protocols involving SELDI measurements gave a lower number of peaks and also a lower variance (100 peaks, mean CV 19.1% for the direct SELDI protocol). Preliminary data from a subset of 47 patients are presented and discussed.

Conclusions: A set of MS methods involving MALDI-TOF and SELDI-TOF analyses has been implemented and validated for clinical proteomics. With regard to method imprecision the SELDI-TOF protocol shows the lowest CV. Application of the developed protocols and analysis of a first set of 47 patients gave first hints to possible new biomarker candidates that have to be further validated in ongoing studies.

Acknowledgement: Supported by the Paul-Martini Research Group (Clinical Septomics), funded by the Ministry of Thuringia (ProExcellence; PE 108-2), the Thuringian Foundation for Technology, Innovation and Research (STIFT), the German Sepsis Society (GSS) and the Center of Sepsis Control & Care (CSCC); funded by the German Ministry of Education and Research (BMBF).
A novel FISH test for rapid pathogen identification in positive blood cultures

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Objectives: Evaluation of SepsiTtest™ and UMD Tissue™ for the diagnosis of the etiological agents of IE.

Methods: The study included 30 patients who were classified into 20 definitive IE and 10 non-IE cases according to the modified Duke criteria. Whole-blood (WB) and heart valve (HV) samples were analysed according to the SOP of the laboratory (Vitek 2; bio-Mérieux). Real-Time PCR analyses were performed according to the instructions of the manufacturer. In case of positive samples, the amplicons were sequenced (GATC, Constance) and strains identified by using BLAST search tools (http://www.ncbi.nlm.nih.gov/BLAST; http://www.sepsitest-blast.de).

Results: The sensitivity of PCR (85%) was nearly twice as high as that of culture (45%), which in 10/20 IE cases presumably stayed negative because of growth inhibition of the pathogens by antibiotics. PCR provided the basis for reclassification of 5/10 non-IE cases into IE cases. Culture-negative infections were identified by PCR, including single infections due to streptococci and Gram-negative bacteria (Escherichia coli, Haemophilus parainfluenzae) and mixed infections involving two Gram-positive bacteria or Candida spp. with Gram-positive bacteria.

Conclusions: The commercial tests proved to be of value for the rapid diagnosis of IE, particularly in cases of culture-negative infections.

*The results have been published elsewhere (J. Clin. Microbiol. doi: 10.1128/JCM.00830-11)

Identification of conidia-associated surface proteins in the human pathogenic fungus Aspergillus fumigatus

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Introduction: The saprophytic fungus Aspergillus fumigatus is one of the most important human pathogenic fungi that causes severe invasive lung infections in immunocompromised patients, such as individuals with haematologic malignancies. Recent data show an increasing incidence of invasive aspergillosis (IA) in critically ill patients without malignancy. Only a few molecules and genes associated with the virulence of this fungus have been elucidated yet. The asexual reproduction of the mould A. fumigatus leads to the formation of sexual structures and conidia. These are known to be produced in the human lung. The mechanism of the interaction of conidia with lung tissue is not known.

Results: Between April and June 2010, 157 isolates (58 gram-negatives and 98 grampositives) were identified by conventional methods from 152 positive blood cultures analyzed by FISH. 11/157 microorganisms (7%) were not included in the FISH panels. Of the remaining 146 microorganisms, 140 were identified correctly, 3 were misidentified, 1 was identified only to the family instead to the species level and 2 were not identified at all by FISH. In comparison to conventional methods, the FISH test sensitivity was 95.9% and the specificity was 97.9%. The clinical sensitivity and specificity was 89.2 and 98.1%, respectively.

Conclusions: These data suggest that this novel FISH test accurately identifies blood stream pathogens in positive blood cultures and may considerably reduce the time to result.
of spores (conidia) which are released into the atmosphere. Based on their small size they are inhaled by humans and can easily reach the lung alveoli. Hence, conidia are the fungal entity which have the initial contact with the host’s immune system. Besides cell wall polysaccharides the conidial surface proteins are the first molecular structures which are recognised by the host’s immune system.

**Objectives:** In our study we aimed at characterizing the composition of the conidial surface proteome of an A. fumigatus wild-type strain (WT) and a mutant strain lacking conidial pigmentation (PkSP) and exhibiting reduced virulence. This approach may reveal new important conidial surface structure with immunomodulatory function.

**Methods:** We released conidial, particularly glycosylphosphatidylinositol-anchored surface proteins (GPI) by HF-pyridine extraction and subsequent LC–MS/MS analysis. Spectral counting was applied for relative quantification.

**Results:** We identified 210 different proteins, 50 of which showed a signal peptide for secretion and 9 a C-terminal GPI anchor attachment signal. The most abundant surface proteins of conidia of the WT strain represented the surface hydrophobin protein RodA and a so-far uncharacterised protein. To elucidate the role of the conidial melanin layer on the composition of the conidial surface proteome we included the melanin-free pksP mutant in our study. Our data suggest, that the lacking melanin layer of the pksP mutant may influence the composition of conidial surface proteome, because 11 proteins were not present in the HF-pyridine extract of the white spores-producing mutant strain in comparison to the WT.

**Conclusions:** Our results revealed, that the HF-pyridine extraction is an appropriate method for the release of GPI-anchored proteins. Further studies will try to elucidate the biological role of the most promising candidates and their putative function during the interaction with the host’s immune system.

**Clinical Sepsis Research: Therapy**

**001**

**Infection 2011**

**Effects of a metabolic optimized fast track concept (MOFA)**

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**Introduction:** Fast track concepts using thoracic epidural anesthesia (EDA) and perioperative patient conditioning with omega-3 fatty acids (n3FA), glucose (GC) control and on-demand fluid therapy, respectively, showed beneficial effects.

**Objectives:** The MOFA-study evaluated the effects of combining the mentioned components in patients undergoing colon and liver surgery.

**Methods:** After BFAM and IRB approval 101 patients (61 ± 12 years; 173 ± 8 cm; 83 ± 16 kg) were enrolled in this prospective RCT. All patients received EDA. In addition, the MOFA group preoperatively received 0.2 g/kg fish oil (Omegaven, Fresenius-Kabi, Bad Homburg, Germany) followed by a 3-day continuous infusion of 0.2 g/kg/day n3FA. Further, intraoperative fluids were restricted to 4 ml/kg/h and GC was kept below 8 mmol/L. Pre- and postoperatively energy drinks (ProvideExtra) were administered.

**Results:** With the MOFA concept an earlier onset of bowel function by 12 h (p = 0.018) could be shown. The disease severity (SAPS II score) of patients in the MOFA group recovered faster (p = 0.021) under a comparable deployment of resources (TISS 28 score). Undesirable effects of n3FA on coagulation or bleeding disorders did not occur, in particular after liver surgery. The synthesis capacity for fibrinogen was significantly improved in the MOFA-group (p = 0.034). At the same time overwhelming humoral and cellular immune response could be contained by the MOFA approach still preserving the physiological pro-inflammatory reaction. On the third postoperative day levels of TNFα were reduced in the MOFA-group (p = 0.03). The leukocyte response was blunted in the MOFA group (p = 0.024). Similarly, the more moderate course of the interleukin 6/interleukin 10-ratio after liver surgery demonstrated the avoidance of excessive pro-inflammatory response (25 ± 42 days ctrl. vs. 18 ± 16 MOFA p = 0.028).

**Conclusions:** Due to the dampening of an overwhelming metabolic stress- and inflammation response by the MOFA concept a prompt postoperative decrease in SAPS II values can be shown followed by a more rapid achievement of defined therapeutic goals.

**Acknowledgment:** Die Studie wurde gefördert durch Fresenius-Kabi, Bad Homburg.

**002**

**Infection 2011**

**Outcome predictors of patients with hematologic malignancies requiring admission to the intensive care unit: a retrospective analysis**

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**Introduction:** The admission of patients (pts) with cancer to an intensive care unit (ICU) still remains a matter of substantial controversy. Nevertheless, these patients account for up to 15% of all ICU admissions in larger studies. The identification of factors that potentially influence patients outcome can help ICU professionals make appropriate decisions.

**Objectives:** To evaluate the characteristics and outcomes of patients with hematologic malignancies.

**Methods:** The clinical courses of 91 patients (113 ICU admissions) were evaluated retrospectively for day 30 and day 100 mortality. The main hematologic malignancies were leukemia’s (45.1%, including 9 patients with allogenic stem cell transplantation) and high-grade lymphomas (52.7%).

**Results:** The median SAPS II score at ICU admission was 51 (survivors 45 vs. non-survivors 62). The overall mortality rates at day 30 and 100 were 46.2 and 57.1%, respectively. The most common cause of death was sepsis (40%). Factors associated with an increased mortality were: need for mechanical ventilation and vasopressors, number of SAPS II score points and prolonged stay in ICU. Each additional day on ICU or each additional SAPS II point increased the mortality risk by 4.8% (±4.6%) and 5.9% (±3.1%), respectively. Other factors as age, gender, leucopenia, GCS score at admission and the underlying malignancy were not significantly associated with mortality.

**Conclusions:** This retrospective analysis supports the hypothesis that mortality was mostly dependent on the severity of organ failures, need of mechanical ventilation and vasopressors rather than cancer-related characteristics.
004
Infection 2011

Etomidate versus Ketamine for rapid sequence intubation (RSI) in septic patients: influence on hemodynamic and vasopressor support: a pilot study

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Introduction: Critically ill and septic patients often require intubation. It is well known that etomidate is associated with a reversible adrenal insufficiency which potentially increases the in-hospital mortality. Moreover, standard anestheticization with vasoplastic substances might severely aggravate shock symptoms during RSI. Ketamine with its known stabilizing properties on hemodynamics might be a reasonable alternative anesthetic induction in septic patients.

Objectives: This non-randomised, observational pilot study focuses on the influence of ketamine-based versus an etomidate-based anesthesia on hemodynamic parameters during RSI.

Methods: 56 patients (20 etomidate, 36 ketamine) were monitored with invasive blood pressure (IBP) and USCOM measurements during RSI. The levels of vasopressors required prior to and after RSI were recorded.

Results: Patients with sepsis (median SAPS II score at ICU admission: 51) were intubated either with etomidate- (E) or ketamine-based regimens (K). Noradrenalin demand and mean arterial pressure (MAP) prior to RSI were comparable (E 0.3 mg/h, MAP 88 mmHg; K 0.4 mg/h, MAP 87 mmHg) between the two groups. The lowest MAP during RSI were 82 (E) and 79 (K) mmHg, respectively. The maximum use of noradrenalin, though, was considerably higher within the etomidate group compared to the ketamin group (7.6 vs. 2.6 mg/h).

Conclusions: This pilot study shows that a ketamine-based anesthetic induction regimen is a safe and valuable alternative to etomidate for RSI in septic patients who primarily require vasopressors.

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Infection 2011

A 77-year old patient with liver cirrhosis and recurrent episodes of fever and bacteremia

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Introduction: Endoscopic treatment with N-butyl-2-cyanoacrylate, a substance that polymerizes immediately when getting in contact with blood, is regarded as a highly efficient therapy to achieve haemostasis in case of gastric variceal bleeding [1]. Both, local complications like mucosal ulceration and systemic complications like thromboembolic events or chest pain may occur evoked by injection of N-butyl-2-cyanoacrylate [2]. Recurrent septicemia is only reported in few cases [3, 4]. The underlying pathogenetic mechanisms of this complication remain unclear.

Objectives: We report one patient with recurring fever and septicemia after injection therapy with N-butyl-2-cyanoacrylate.

Methods: Case presentation. A 77-year-old man with nutritive-toxic liver cirrhosis was referred to our hospital with recurrent episodes of fever and shivering attacks for about 5 weeks. Three months before the current admission he underwent gastroscopy with local injection of N-butyl-2-cyanoacrylate in order to control gastric variceal bleeding in another hospital. Two months later he developed a purulent left-sided endophthalmitis. An extensive search by chest X-ray, abdominal and thyroidal sonography, urological and ENT-evaluation, transesophageal echocardiography and thoracoabdominal computer tomography did not reveal an infectious focus. Blood cultures grew extended spectrum beta-lactamase (ESBL) producing Escherichia coli with additional resistance to fluoroquinolones. Upper gastrointestinal endoscopy showed necrotic ulcerations at the site of N-butyl-2-cyanoacrylate injection and the ESBL-producing E. coli strain was cultured in the cyanoacrylate-plug.

Results: In our case, isolated E. coli from blood cultures and the cyanoacrylate plug showed an identical resistance profile, supporting our assumption that the plug itself had been the primary source of bacteremia and sepsis. This case confirms that any implanted material, even cyanoacrylate used to control gastric bleeding, can serve as a focus for bacteremia and should be considered when searching for a focus of bacteremia, particularly because this focus can be mechanically removed.

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006
Infection 2011

Sepsis in geriatric patients: process oriented knowledge management

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Introduction: Within the demographic shift elderly people are increasingly part of the intensive care patient clientele. Sepsis has a high rate of mortality in this patient collective. Guidelines and recommendations on treating sepsis exist, but the group of geriatric patients has not yet been observed in an isolated manner.

Objectives: The investigation’s objective was to apply initial sepsis treatment with geriatric patients and identify the positive effects.

Methods: Patients over the age of 70 diagnosed with sepsis were included in a retrospective analysis of 2,000 patient cases at a medical
Results: 221 patients ages 70 and over who were diagnosed with sepsis were identified during the period of observation. The age distribution was similar in both comparison groups (average age of 80.3 prior to the SOP; 79.7 after the SOP). Sepsis was diagnosed in 22% of the cases prior to the SOPs introduction and in 57% of the cases after its introduction. The diagnosis of severe sepsis declined from 42 to 17%. The initial volume therapy was conducted in 64% (11% prior to the SOP); samples of blood cultures were taken prior to the initial administration of antibiotics in 67% (in 5% prior to the SOP). The lactate measurement was documented to evaluate tissue perfusion in 77% (11% prior to the SOP). A central venous catheter was inserted for volume management and measuring the central venous saturation in 89% (68% prior to the SOP) and the target central venous pressure was achieved in 64% (47% prior to the SOP). The initial administration of antibiotics was applied within the first hour as of admission in 73% (32% prior to the SOP). In this observed patient population ICU mortality was reduced about 2% (25.3 vs. 20.1%), hospital mortality about 6.4% (53.3 vs. 46.9%).

Conclusions: The application of the standard operation procedure (SOP sepsis) exhibited positive results in implementation in critically ill geriatric patients. Under differentiated observation, the guidelines of acute medicine are quite transferrable to geriatric patients. The matrix is suitable for accompanying structural and process quality as well as the overall constructive change in culture in the treatment of geriatric patients.

008
Infection 2011

Influence of hydroxyethyl starch (HES) 130/0.4 on hemostasis measured by thrombelastography: a systematic review

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Introduction: The plasma-expander hydroxyethyl starch (HES) is the most frequently used colloid in critically ill patients. These patients often have an increased risk for bleeding. HES effects on coagulation can be assessed by thrombelastography, a global test of hemostasis in whole blood. Due to the recent retraction of numerous studies a re-evaluation of published evidence is necessary.

Objectives: To assess effects of HES 130/0.4 on hemostasis compared to crystalloid or albumin control fluids in published studies using thrombelastography.

Methods: Searched databases included Medline, EMBASE, and the Cochrane Library. Only studies which provided statistical comparisons between study fluids were included. Data was independently extracted by two reviewers. HES effects which differed significantly from control fluids were noted. Hypocoagulation was defined as (A) prolonged clotting time and (B) impaired clot formation.

Results: The final sample included 24 studies (17 in vitro and 7 in vivo hemodilution studies) which used Sonoclot (SCR), Thrombelastography (TEG), or Rotation thromboelastometry (ROTEM). Only two studies reported quality control of the thromboelastographic measuring device. HES 130/0.4 showed a markedly consistent and uniform effect on hemostasis in all 24 studies. 66% of all 279 measured variables were significantly abnormal and indicated hypocoagulation. Only one variable (0.4% of all measured variables) showed a hypercoagulatory change. Clot formation was significantly impaired in 79% of measured variables (n = 72). HES effects on hemostasis were already apparent in low doses. In vitro studies, which investigated higher dilutions up to 80%, showed a dose-relation effect. In contrast, in vivo studies were fewer and with one exception did not investigate doses >40 ml/kg. Studies which simultaneously assessed HES 200/0.5 (n = 7) found no major difference between the two HES solutions in comparison to control fluids. Four retracted papers were found. Their results differed considerably from the results of the included 24 studies.

Conclusions: Thrombelastography confirms that HES specifically impairs hemostasis compared to crystalloids or albumin. HES dilution results in a weaker and smaller clot.

009
Infection 2011

Systematic analysis of hydroxyethyl starch (HES) reviews: proliferation of low quality reviews overwhelms the results of well-performed meta-analyses

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Introduction: HES is a widely used colloid plasma expander in intensive care although less expensive fluids with fewer side effects are available. A closer look at HES reviews may provide an explanation.

Objectives: To examine the relationship between review quality, hypothetical claims [1], potential conflict of interest (pCOI) statements and HES recommendation.

Methods: Reviews and meta-analyses on HES in hypovolemia in English, German and French were identified through searches in MEDLINE, EMBASE and Cochrane Library. Data were abstracted independently by two authors. Categories of HES recommendation were developed according to authors' own words. Review quality was assessed by OQAQ score (Overview Quality Assessment Questionnaire [2], 7 points denote top quality). An additional internet search was done for pCOI statements from top-publishing authors.

Results: From 1975 to 2010, 165 published reviews contained recommendations for or against HES use. From the 1990s, favorable reviews increased from two to eight per year and HES’s share of the artificial colloid market tripled from 20 to 60%. Only 7% (12/165) of HES reviews contained meta-analyses while 93% (153/165) did not. Meta-analyses had high OQAQ scores [median (range) of 6.5 (3–7)] and 83% (10/12) recommended against HES use. Reviews without meta-analysis (122/153) had lower scores [2 (1–4); p < 0.001] and 80% recommended HES (p < 0.0001). Meta-analyses favoring HES had lower OQAQ scores than unfavorable meta-analyses [3 (3–3) vs. 7 (4–7); p = 0.02]. 14 authors published the majority (70/124) of positive reviews and 9/14 had or developed a pCOI with HES manufacturers. These lower quality positive reviews used hypothetical statements such as HES has manageable side effects or beneficial effects beyond plasma expansion (both, p < 0.005).

Conclusions: The majority of HES reviews were positive and low quality. Most of these were written by a small group of authors with ties to industry. Their conclusions differed from high-quality meta-analyses by independent groups such as Cochrane Reviews. The proliferation of positive HES reviews was associated with increased use of an expensive therapy despite lack of evidence for meaningful clinical benefit and increased risks. Physicians need to be made more aware of marketing efforts disguised as scientific literature.
Introduction: Surviving sepsis depends on the implementation of the severe sepsis bundles and their elements. In Africa we find a number of challenging clusters in medicine and the general health system endangering this successful implementation.

Objectives: Only a minority of these clusters have a pure medical background. We need to identify and address them, because the burden of sepsis in resource limited countries cannot be lifted otherwise.

Methods: The cluster bomb destroying the implementation of sepsis bundles can only be dismantled from inside Africa. The material presented here is based on the authors experiences with sepsis in different hospitals of low income countries, their responsibility for anaesthesia in one of the busiest hospitals in Africa, informal discussions on the challenges of sepsis in three of the four central hospitals of Malawi and its comparison to literature.

Results: The most important of the non medical clusters (C) and subclusters threatening the sepsis bundles:

The Cultural Cluster:
1. The meaning of timelines and diseases
2. No preemptive culture in medicine and a different prioritization
3. Prolonged traditional treatment and witchcraft

The Management C:
1) Procurement, maintenance, establishment and administration as a threat
2) A syndrome of an overwhelming number of deadly problems to tackle at the same time.
3) Rotation, renumeratation, career paths and drained brains of our staff

The Personal C:
1) The sick health professional working in times of AIDS
2) Negligence, low value of women, no basic education in the villages
3) Great Souls still suffer quietly

The Money C:
1) Low budgets with impenetrable general finances
2) Direct and indirect costs for patients to reach the hospital
3) No money for sepsis in ICU despite costs through better funded specialties

The Education C:
1) Quality of training versus quantity of health personnel
2) Effectiveness and sustainability of training programmes
3) Communication patterns unable to cope

Conclusions: The lifesaving effects of the Surviving Sepsis Campaign are threatened in Africa by clusters of problems which are currently hardly addressed in sepsis research. Concentration on the medical aspects will fall short of being successful in Africa. The knowledge of these clusters is mandatory to design a successful rescue strategy to secure the benefits of modern medicine for the African patient.
Objectives: To compare outcomes of three different nutrition strategies, i.e. enteral (EN) versus parenteral (PN) versus mixed nutrition (EN + PN) in patients with severe sepsis or septic shock and prolonged intensive care unit (ICU) stay.

Methods: Secondary analysis of the prospective, randomized-controlled, multicenter Intensive Insulin Therapy and Pentastarch Resuscitation in Severe Sepsis (VISEP) trial. 353 patients with severe sepsis or septic shock and length of ICU stay >7 days were included in the analysis. Categorical outcome data were reported as absolute or relative frequencies and tested by Chi-square test or Fisher’s Exact test, as appropriate. Continuous data were presented by mean and standard deviation or median and interquartile range and compared by t test, ANOVA, Mann–Whitney U test or Kruskal–Wallis H test.

Results: Patients were classified into three groups by type of nutrition therapy with the majority (68.5%) receiving EN + PN, 24.4% receiving exclusively EN and 7.1% exclusively PN. Overall, the median daily caloric intake was 1,199 kcal/day with a protein intake of 42.4 g/day. In the EN group, median caloric intake was 918 kcal/day while it was 1,210 kcal/day in the PN and 1,343 kcal/day in the EN + PN group (p < 0.001). This resulted in a mean daily ratio of caloric intake to calculated basal energy expenditure of 0.9 in the patients with EN + PN compared to EN (0.6) and PN (0.8) (p < 0.001). The rate of renal replacement therapy, secondary infections and duration of mechanical ventilation were significantly higher in patients with EN + PN as was hospital mortality compared to EN alone (41.3 vs. 26.7%, p = 0.048). After adjustment for severity of illness and baseline characteristics, the differences in secondary infections (hazard ratio [HR] = 1.91, 95% confidence interval [CI] 1.29–2.82, p = 0.005) and mortality (HR = 1.86, 95% CI 1.16–2.98, p = 0.034) remained between the EN and EN + PN group.

Conclusions: The use of EN alone albeit hypocaloric was associated with improved clinical outcome compared to EN + PN with enhanced caloric intake in patients with severe sepsis or septic shock and prolonged ICU stay. Thus, the concept of EN alone withholding PN may be more appropriate in this patient population.

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022

Infection 2011

Clinical analyses programs for optimizing antibiotics prescription

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Introduction: The threat of the microbial resistance to the antibiotics (AB) makes the clinicians to look in the Clinical Analytic Programs of AB-prescribing as well as for monitoring of the microbial resistance. This activity is a very important part of the Antimicrobial Stewardship Policy all over the world. That is a very strong reason for organizing the Universal Electronic Data-bases in order to improve the decision making process at the Clinical Situations with AB-prescribing.

Objectives: The decision-making process of AB-prescribing depends on the many factors: the etiology, severity, stage, phase of the definite infectious situation, individual features of the patients and so on. These situations should be systematized and described by definite criteria. The principles of systematization and quantization of the Informative Continuum of Clinical Events of the infectious process were created on the base of Classification of Sepsis (International Guidelines and National Recommendations for Sepsis-The ACCP/SCCM Consensus Conference Committee Report.). The applicability of such approach for constructing the rules of AB-prescribing for using them in electronic data-bases was tested.

Methods: Quantization of the Clinical Events. Systematization of the Clinical Situations. Construction the Protocols of AB-prescribing. Implementation of the ideology of the AB-prescribing protocols and technology of monitoring with electronic data-base.

Results: 5-cubits segmentation of information about Clinical Events. 5 Classes of Clinical Situations with definite criteria allowed to create a structuring order in the decision making process of AB-prescribing and to use it in electronic data-base. The improvement in the quality of Clinical Practice gave a substantial saving (50%) in the lives of the adult patients with severe pneumonia.

Conclusions: The first experience of modeling the Patterns for decision making AB-prescribing on the base of principle of the 5-cubits segmentation of information, showed its applicability for structuring Clinical Protocols and organizing the Clinical electronic data-bases for monitoring the quality of AB-prescribing.

023

Infection 2011

Dramatic course of septic vasculitis with rhabdomyolysis caused by bilateral pneumonia

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Introduction: We report a case of unusual dermal lesions as a sign of underlying septic pneumonia.

Objectives: Dermal lesions in septic patients need rapid diagnostic procedures followed by adequate antibiotic and supportive therapy.

Methods: Case report

Results: We saw a 46-year-old caucasian male transferred from an outside hospital suspected of autoimmune disease of unknown origin, respiratory partial insufficiency with noninvasive ventilation and...
fever (41°C). Impressive livido racemosa and reticularis (Figs. 1, 2), combined with massive, painful edema of the limbs.

Fig. 2 Livido racemosa lower limb

A community acquired infection of the lower airways was treated before doxycycline. The chest CT-scan shows large bilateral pulmonary infiltrates and mediastinal lymphomas in combination with interstitial lung edema caused by capillary leakage (Figs. 3, 4). The MRT scan of the legs showed diffuse polymyositis and inflammation of the fascia without evidence of muscular necrosis or a compartment syndrome, the clinical finding was extreme pain in the edematous lower limbs accompanied by rhabdomyolysis. A biopsy from the lower leg showed septic vasculitis with thrombotic vessel occlusions and wide-spread epithelial necrosis. The muscle revealed only rare signs of inflammation. A broad laboratory and microbiological analysis found no evidence for the reason of the underlying septic disease.

Fig. 3 Chest X-ray bilateral pulmonary infiltrates + interstitial edema

With the clinical findings of severe sepsis and MODS (impaired kidney function, respiratory failure, thrombopenia) caused by pneumonia, we started a broad antibiotic regimen intravenously—ampicillin + sulbactam, clarithromycin—with additional penicillin G administration because a meningococcosis without meningitis could clinically not be excluded. An additional corticoid administration was already started outside. In the following hours the wide spread dermal lesions declined rapidly, the biochemical markers of infection declined also, but later. The kidney function and the platelet count returned to normal in the next days. The use of catecholamines could by avoided by aggressive volume therapy monitored by a PICCO system. Because of the pulmonary capillary leakage with interstitial edema a non invasive ventilation (modus: CPAP/ASB) was necessary for several days.

Conclusions: Skin lesions and abnormalities, combined with sepsis, need a rapid diagnostic and therapeutic approach including skin–muscle biopsy. The calculated initial therapy should be effective for meningococcosis even if there is no evidence of meningitis and for a necrotizing fasciitis.

Abstracts

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Effects of chronic inflammation and non-steroidal anti-inflammatory drugs on outcome in sepsis

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Introduction: Anti-platelet drugs such as acetylsalicylic acid (ASA) or clopidogrel are widely used for prevention of arterial thrombosis and ischaemic events in atherosclerotic patients. Retrospective studies have shown that pre-hospital use of anti-platelet drugs was associated with a markedly decreased frequency of organ failure and mortality in critically ill patients (Winning et al. 2009, 2010), probably due to the anti-thrombotic and anti-inflammatory action of the drugs. However, one cannot exclude that the moderate chronic inflammation associated with atherosclerosis can contribute to the benefit of anti-platelet drugs in critical ill patients.

Objectives: To evaluate the history of chronic inflammation and the effects of non-steroidal anti-inflammatory drugs (NSAID) on the outcome in sepsis.

034
Infection 2011

Effects of chronic inflammation and non-steroidal anti-inflammatory drugs on outcome in sepsis

Boettel J, Otto GP, Sossdorf M, Winning I, Löschke W
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Introduction: Anti-platelet drugs such as acetylsalicylic acid (ASA) or clopidogrel are widely used for prevention of arterial thrombosis and ischaemic events in atherosclerotic patients. Retrospective studies have shown that pre-hospital use of anti-platelet drugs was associated with a markedly decreased frequency of organ failure and mortality in critically ill patients (Winning et al. 2009, 2010), probably due to the anti-thrombotic and anti-inflammatory action of the drugs. However, one cannot exclude that the moderate chronic inflammation associated with atherosclerosis can contribute to the benefit of anti-platelet drugs in critical ill patients.

Objectives: To evaluate the history of chronic inflammation and the effects of non-steroidal anti-inflammatory drugs (NSAID) on the outcome in sepsis.
Methods: Data of 985 patients with severe sepsis/septic shock were analysed for the influence of NSAID and inflammation on ICU mortality.

Results: 450 patients had a history of chronic inflammation (CI) according to ICD-10 codes (ischemic heart disease: n = 319, cerebrovascular disease: n = 191, atherosclerosis: n = 84, inflammatory polyarthropathy and systemic connective tissue disorders: n = 26). 331 patients were on NSAID during their ICU stay (ASA: n = 274, ibuprofen: n = 45, diclofenac: n = 56). Lowest mortality was found in patients on NSAID but without CI history (n = 80; mortality 20.0%), followed by patients with NSAID and CI history (n = 250; 26.8%). Patients without both NSAID and CI history (n = 435) had a mortality of 36.6%, and those without NSAID but with CI history (n = 220) had a mortality of 35.4%.

Conclusions: NSAID medication rather than CI history was associated with a reduced mortality in patients with severe sepsis or septic shock. It remains an open question whether the anti-inflammatory or the anti-platelet action of NSAID have the higher impact on the benefit of these drugs.

039
Infection 2011
Sepsis diagnosis in organ donation
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Introduction: On account of the persistent organ shortage in Germany and the whole Eurotransplant area more and more organs are offered under extended criteria.

Objectives: The German Organ Transplant Foundation East Region examined how many donors with the diagnosis sepsis were involved in the donation process in this area.

Methods: We examined all donation processes with the diagnosis sepsis in our region (tachycardia, leukocytosis, temperature rise/drop, organ dysfunction) from the year 2006 to 4/2011. We recognized 41 cases. They resulted in 11 explantations. Furthermore we registered the proved germs and the antibiotic treatment on the intensive care unit. We pursued how many organs were transplanted.

Results: In the years from 2006 to 4/2011 41 cases with the diagnosis sepsis were reported in the East Region. The age of donors reached from 20 to 86 years. In 11 cases organs were explanted. The causes of death were: hypoxia after resuscitation (2), cranioencephalic injury (2), media infarction (1), meningitis (3), cerebral bleeding (3). The strain spectrum (from blood culture, liquor) of all reports showed predominantly gram-positive germs and mycosis. In 21% of the cases no germ could be found under antibiotic therapy. Spectrum: Staphylococcus aureus 14, no germ proof 10, Streptococcus pneumoniae 5, Escherichia coli 5, Mycosis 4, Pseudomonas 2, Acinetobacter 1. Most patients received antibiotics: ß-lactam antibiotics 9, nitromidazole 3, cephalosporin 7, glycyrine inhibitors 4, antymycotics 4, penicillin 4, other substances like tetracycline, rifampicin, glycopen, linezolid, lincosamide 8. From 41 reports 11 donations could be realized. 20 kidneys, 6 livers and 3 hearts could be transplanted. Inquiries in the transplantation centres proved that no infections were transferred by donor organs.

Conclusions: In 11 cases organs could be transplanted successfully in spite of known sepsis and proved germs. This is only possible if all findings are available for the surgeons performing the transplantation. The prospect of success of such transplantations can be estimated by the experience of the recovering surgeon and the regime of the transplantation centre validly.

043
Infection 2011
The frequency of physiotherapeutic interventions affects the ICU mortality rate among patients with severe sepsis and septic shock
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Abstracts

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Introduction: Physiotherapists are routinely involved in the management of critically ill patients. Their assessment and treatment are focused on deconditioning (physical inactivity, muscle weakness, joint stiffness) and respiratory conditions (atelectasis, retained airway secretions, respiratory muscle weakness). The precise role and impact of physiotherapeutic interventions (PTI) in critical ill patients, however, are insufficiently investigated, especially in patients with sepsis.

Objectives: In this retrospective study we investigated: (1) the implementation of physiotherapy in our ICU and (2) the effect of PTI on the ICU mortality rate among patients with severe sepsis or septic shock.

Methods: Data records of 16,041 critically ill patients admitted to the Intensive Care Unit (ICU) of the Jena University Hospital from 2006 to 2009 were extracted from patient-data management system (PDMS) and retrospectively analyzed. Physiotherapeutic treatment, ICU mortality rate, clinical and demographic characteristics as well as co-morbid conditions were evaluated. For comparable quantification, the index relative numbers of PTI (RNPTI index) was calculated. The value RNPTI, given in percent, was calculated by dividing the absolute number of PTI during the length of stay (LOS) in the ICU by the ICU LOS for each patient. Cox regression analyses were performed with following predictive variables: age, gender, body-mass index (BMI), SOFA score, APACHE II score, sedation index, RNPTI index as well as co-morbid conditions. The hazard ratios (HR) of all test variables were calculated and adjusted for established risk factors.

Results: In the observation period from 2006 to 2009, 16,041 critically ill patients were admitted to the ICU of the Jena University Hospital. In this cohort, 999 patients fulfilled criteria for severe sepsis and septic shock resulting in an incidence rate of 6.2%. The ICU mortality rate for patients with severe sepsis or septic shock was 30.8% and the median ICU LOS was 12 days. In total, 9,476 PTI were recorded for all patients with severe sepsis or septic shock. The multivariate Cox regression analysis with adjusted covariates revealed the RNPTI index as a strong predictor variable of the ICU mortality rate (HR 0.984; 95% CI 0.975–0.992; p < .001).

Conclusions: The frequency of PTI is associated with an improved ICU survival rate among patients with severe sepsis and septic shock. Prospective studies are necessary to confirm this favorable impact.

044
Infection 2011

Low-dose aspirin: a novel therapeutic approach in critically ill patients?

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Introduction: Blood platelets have been shown to contribute to the development of organ failure in critically ill patients. There is some evidence from animal and retrospective clinical studies that platelet drugs may reduce organ failure.

Objectives: To test the hypothesis that low-dose aspirin (≥320 mg/day) as used in the prevention of arterial thrombosis in cardiovascular patients improves the outcome in critically ill patients.

Methods: We analysed the data from three cohorts of critically ill patients with respect to the effect of pre- and/or intra-hospital aspirin medication on the development of organ failure and in-house mortality.

Results: Study I included patients who were admitted to hospital for community acquired pneumonia. Patients with statins were excluded. 37 (17%) of the 237 patients were on pre-hospital aspirin. Compared to patients without aspirin, aspirin users were markedly older (57.6 ± 7.3 vs. 57.6 ± 13.6 years), but did not significantly differ in SOFA score at day of admission. However, they had a significantly shorter stay in hospital (16.8 ± 5.7 vs. 16.8 ± 10.1 days, p < 0.05) and needed less frequently ICU treatment (8.1 vs. 25.5% as an indication for organ failure. Study II included 590 mixed ICU admissions, and 129 of them (21.9%) were on pre-hospital aspirin, but without statins. Patients on aspirin were markedly older (69.1 ± 9.3 vs. 52.2 ± 20.4 years) and more seriously ill (APACHE II score at admission 26.1 ± 9.3 vs. 19.4 ± 8.5). Using a model of logistic regression with APACHE II score, age, gender and pre-hospital aspirin as independent variables we found a significant reduction in mortality by aspirin indicated by an odds ratio (OR) and 95% confidential interval of 0.20 (0.12–0.35). The benefit of aspirin was also evident in patients with active bleeding or increased bleeding risk.

In study III we analysed data from 834 patients who were admitted to ICU with severe sepsis or septic shock, irrespective of the medical background. 187 of these patients (22.4%) were on pre- and/or intra-hospital aspirin, but did not receive any other non-steroidal anti-inflammatory drugs. In a model of logistic regression with APACHE II score, age, and use of aspirin, we found again a significant reduction in mortality by aspirin [OR = 0.55 (0.38–0.81)].

Conclusions: The use of low-dose aspirin as a novel therapeutic approach in critically ill patients, including those with serious infection and severe sepsis/septic shock, should be tested in prospective intervention studies.

045
Infection 2011

The obesity paradox in surgical intensive care unit patients

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Introduction: The increasing prevalence of obesity and its associated health effects have become a major concern for health care providers, struggling to serve a growing number of obese patients. Management of obese patients, particularly in the intensive care unit (ICU) is often demanding and complications are common [1]. Previous studies have given conflicting results as to whether or not obesity increases the risk of death in critically ill patients [2, 3].

Objectives: To investigate the possible impact of obesity, as assessed by the body mass index (BMI), on 60-day mortality and other outcomes in surgical intensive care unit patients.

Methods: Prospectively collected data from all consecutive adult patients admitted to our ICU between January 2004 and January 2009 were analysed retrospectively. BMI was calculated using the formula: BMI = body weight/height^2 (kg/m^2) and patients were grouped as underweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), obese (30–39.9 kg/m^2), and very obese (≥40 kg/m^2).
Results: Among the 12,938 patients who were admitted to our ICU during the study period, 9,935 (76.8%) had complete height and weight data and constituted the study group. The mean BMI was 27.1 ± 5.0 kg/m². Overall, 34.4% of the study population had a normal BMI, 1.8% were underweight, 41.2% were overweight, 20.8% obese, and 1.8% were very obese. The ICU mortality rate was similar among BMI subgroups, but hospital mortality was higher in underweight patients than in patients with normal BMI (17.8 vs. 11.1%, P = 0.006). In a multivariate Cox regression analysis, being overweight [hazard ratio (HR) 0.86: 95% CI 0.74–0.99, P = 0.047] or obese (HR = 0.83: 95% CI 0.69–0.99, P = 0.047) were independently associated with lower 60-day in-hospital mortality, with normal BMI as the reference category.

Conclusions: In surgical ICU patients, being overweight or obese was not associated with an increased risk of hospital mortality. Indeed, being overweight or obese may be associated with a lower in-hospital 60-day mortality, compared to having a normal BMI. Obesity should not be considered as a risk factor in these patients and should not influence decision making.

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053
Infection 2011

Sepsis and SIRS: a survey based on the data of the ICU and IMC-ward of Klinikum Lippe-Detmold in 2011

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Introduction: Klinikum Lippe-Detmold is a community based hospital equipped for maximum care except heart–lung machine and ECMO. The Intensive Care Unit (ICU, 12 beds) and the Intermediate Care Ward (IMC, 5 beds) cares for app. 3,100 patients a year. SIRS and sepsis are important diseases in this setting with a high demand of human and non-human resources.

Objectives: The management of SIRS and sepsis needs special skills of the doctors and nurses, and these diseases will have important influence on health care economics and financial challenges in the next years. Our prospective survey for the year 2011 will help us to manage the logistic and financial burden caused by the special needs in the care and treatment of septic patients in our community in the future.

Methods: Our prospective, guideline based survey (ICU and IMC patients) comprised all cases of SIRS and sepsis in the year 2011. A follow-up will be done on day 30 and 60.

Results: From 01.01 to 15.06.2011 we treated a total number of 177 patients with SIRS and/or sepsis (102 male, 75 female); the age ranges from 20 to 94 years (average 70 years). The SIRS criteria were fulfilled by 32 patients (18%), the sepsis criteria by 145 patients (42%). There was a subgroup of 42 patients (24%) with severe sepsis and another subgroup of 28 patients (16%) with septic shock (Fig. 1).
4 patients died. The overall mortality within the first 60 days was 23% (42 patients). In the subgroup of severe sepsis the mortality was higher (33%, 14 of 42 patients, and—as expected—the highest mortality occurred in the subgroup “septic shock” (57%, 16 of 28 patients).

Conclusions: There are lots of patients with sepsis, severe sepsis and septic shock. This emphasizes the strong need for implementation of standardized diagnostic and therapeutic procedures in the clinical all-day setting to reduce the high mortality in septic patients. Our data will help us to establish human-resources management with regard to standardized procedures fitting for our hospital. This will reduce mortality, will provide well-trained doctors and nurses and may reduce costs by cutting down the length of stay in hospital.

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Physical and mental health in survivors of severe sepsis and their relatives: a dyadic perspective on long-term sequelae

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Introduction: A critical illness like sepsis is an inherently stressful event for the patient. Recent systematic reviews have shown a considerable impairment of mental health and quality of life after surviving a critical illness. Apart from the patients, family members may also witness a life-threatening crisis during the time of critical illness that negatively affects their mental health. Although psychological consequences of surviving critical illness are investigated well, studies examining long-term sequelae of severe sepsis, both in patients and in relatives, are rare.

Objectives: The present study aimed to investigate the physical and mental long-term sequelae in survivors of severe sepsis and in their relatives. Moreover, we focussed on the examination of the dyadic relation of physical and mental health.

Methods: Patients and relatives who contacted the German Sepsis Aid were asked to answer a questionnaire comprising of aspects of their current medical condition, health-related quality of life, mental health (anxiety, depression, post-traumatic stress disorder), and ICU experiences. Inclusion criterion for the dyads was the patients’ survival of sepsis associated with intensive care.

Results: To date, N = 50 dyads replied. Among the dyads, there were n = 46 patient–partner dyads, n = 2 patient–parent dyads, and n = 2 patient–child dyads. Mean age of the patients was 60.3 years (SD 13.4), 62% were male. Relatives’ mean age was 60.1 years (SD 12.1), 36% were male. On average, patients suffered from sepsis 55 months ago (SD 31), and experienced a mean ICU stay of 44.6 days (SD 45.4).

Conclusions: Because the data collection is still ongoing further results and conclusions will be presented at the conference.

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Development of critical organ failures in immune-mediated diseases

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Introduction: Some of the affected organs of systemic immune-mediated diseases may cause critical conditions. These could be direct manifestation of the underlying disease, accompanying complications, treatment complications of the autoimmune disease, intercurrent or coincidental diseases.

Objectives: This study was conducted to examine retrospectively patients who suffered from critical stadium of autoimmune diseases.

Methods: Here we report on 8 patients, who were treated in our department between 1 January 2007 and 31 December 2010. In order to establish the diagnosis we determined how many of the vital organs were affected, which of the organs and to what extent were dysfunctional and whether the organ dysfunction was part of the underlying disease or it was its complication. The following diseases were considered most frequently at differential diagnosis: infection/organ failures were observed: respiratory insufficiency in two cases, severe sepsis in three cases (4/5 organ failures), hemorrhagic diathesis in one patient, and intoxication in one patient. The underlying disease was known in only one patient at admission: HELLP. On average, 7.12 ± 5.4 days were required in order to establish the final diagnosis. The following organ failures were observed: respiratory insufficiency (pleuritis, ARDS) in seven patients; cardiovascular failure (endothelial dysfunction) in seven patients; acute renal failure and renal arterial-venous thrombosis in five and one patient, respectively; cognitive dysfunction (psychosis, stroke) in 6/1 patients; coagulation dysfunction (thrombocytopenia, consumption coagulopathy) in six patients. The main treatment steps were the followings: in addition to the treatment of the underlying disease the damaged organ functions were substituted or supported, the intercurrent infection were prevented, and the potential complications were controlled. Four patients died due to the critical organ failure caused by the underlying immune-mediated disease, its infectious or thrombosis embolism complications.

Conclusions: The systemic immune-mediated diseases are associated with a wide spectrum of clinical manifestations, which may cause critical condition in any organ system. In this stage, the underlying disease presents difficult differential diagnostic problems. The recognition and adequate treatment of such conditions are an interdisciplinary task and therefore require comprehensive diagnostic/treatment arsenal.

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A review of activated Protein C therapy
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Introduction: According to the 2001 PROWESS study, the absolute mortality of sepsis decreased by 6.1% int the patient group treated with a PC. Following the Barcelona Declaration, The Hungarian Association of Anesthesiology and Intensive Care issued a directive about the aPC treatment for severe sepsis/septic shock patients.

Objectives: The objective of our examination was to review the practice of handling septic patients with activated protein C in view of the European recommendation.

Methods: Retrospective study of 436 septic patients/septic shock patients. We have examined the time lag between the beginning of infection/organ failure and start of treatment, and the number of organ failures. We have examine whether the first 6–24 h treatment of patients fulfils the requirements declared in the sepsis bundles. We have assessed their clinical state at the end of the 12th h and recorded the first appearance of irreversible organ failures. Based upon the results, we have determined the number of patients treated and the number of patients who did not receive activated protein C.

Results: The number of organ failures: failure of 1 organ (107 patients) 24%, 2 or more organ failure (153) 35%/septic shock (176) 40.3%. The first appearance of the infection/organ failures were followed by admission to the intensive care unit within 79.6 ± 39.4/14.3 ± 5.6 h. During the first 6 h of treatment 1 sepsis bundles criteria was completed in 26%, 2 sepsis bundle criteria in 29%, 4 criteria in 30% and four criteria in 15% of the patients. During the first 24 h of treatment 1 sepsis bundle criteria was followed in 12%, 2 criteria in 42%, 3 criteria in 39%, 4 criteria in 5% of the patients. After the first 24 h of treatment, the state of patients with 2 or more organ failures improved in 109 (71%), and deteriorated in 44 (28%) of the patients. Regarding the group of septic shock patients, 83 (47%) have improved and 46 (26%) stagnated. 47 patients suffered irreversible organ failure. During our comprehensive study, we found that 108 (44 + 64) patients would have been indicated for an aPC treatment, which was carried out in 27 patients.

Conclusions: The starting of aPC treatment was delayed by: (a) the too late admission to the intensive care unit, (b) by failing to follow the sepsis bundles criteria, (c) and by unsatisfactory treatment of the sepsis within the first 6–12–24 h.

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Required fluid volume ratios in patients with severe sepsis receiving only crystalloids or synthetic colloids plus crystalloids
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Introduction: It is commonly stated that fourfold volumes or more of crystalloid than of colloid solutions are necessary to achieve effective resuscitation. Previous clinical volume trials with 5% albumin or 6% HES 130/0.4 and crystalloid controls have recently found much lower crystalloid-colloid ratios around 1.4–1.5 in critically ill or septic patients [1, 2].

Objectives: We intended to analyse the crystalloid-colloid volume ratio for HES 130/0.4 or gelatin versus crystalloids in a large cohort of patients with severe sepsis.

Methods: Controlled before-and-after study of 1,046 patients with severe sepsis on a surgical ICU. In the period from 2004 to 2006, patients received predominantly 6% HES 130/0.4 (HES group, n = 360) plus crystalloids. In 2006–2008, standard colloid was changed to 4% gelatin (Gel group, n = 352). Between 2008 and April 2010, patients received only crystalloids (Crys group, n = 334). Fluid balances and total fluid input (all i.v. solutions including parenteral feeding) was determined for the first seven treatment days after onset of sepsis. Data is shown by median [interquartile range].

Results: Groups were comparable at baseline concerning age, renal function and SOFA scores. From day 0 to day 7, total fluid requirement was 319 [237–417] ml/kg bodyweight (BW) in the HES group (p = 0.001), 321 [233–429] ml/kg BW in the Gel group (p = 0.05) and 311 [203–412] ml/kg BW in the Crys group. Median cumulative colloid doses were 56 [29–91] ml/kg BW HES and 26 [15–44] ml/kg BW gelatin. Cumulative crystalloid doses were 142 [98–216] ml/kg BW for the HES group (p < 0.001), 219 [145–302] ml/kg BW for the Gel group (p = 0.367) and 230 [150–323] ml/kg BW for the Crys group. Daily fluid input was significant lower only on day 0 in the HES group (HES 22 [10–36] ml/kg BW vs. Crys 41 [18–67] ml/kg BW; p < 0.001) and on day 1 in both colloid groups (HES 57 [39–82] ml/kg BW, p < 0.001; Gel 65 [46–95] ml/kg BW, p = 0.041; Crys 72 [49–106] ml/kg BW). Crystalloid–colloid volume ratio from day 0 to day 4 was 1.14–1 for HES and 1.05–1 for Gel. Total fluid balance was −43 [−113 to 30] ml/kg BW for HES (p < 0.001), −4 [−67 to 68] ml/kg BW for Gel (p = 0.448) and −2 [−77 to 49] kg/ml BW for Crys. ICU and hospital mortality did not differ significantly between groups.

Conclusions: Patients with severe sepsis who receive only crystalloid solutions do not have a much higher fluid need than patients receiving synthetic colloids.

References: [1] Finfer et al. N Engl J Med. 2004;350: 2247–56. [2] Bayer O, et al. Crit Care Med. 2011;39: 1335–42.

Acknowledgement: Supported by the Paul-Martini Research Group (Clinical Septomics), funded by the Ministry of Thuringia (ProExcellence; PE 108-2), the Thuringian Foundation for Technology, Innovation and Research (STIFT), the German Sepsis Society (GSS) and the Center of Sepsis Control & Care (CSCC); funded by the German Ministry of Education and Research (BMBF).

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SSC guideline limit in surgical patients with septic shock

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Introduction: The Surviving Sepsis Campaign (SSC) guidelines aimed to reduce heterogeneity of conventional therapy and mortality under the scope of evidence-based medicine. A 100% fulfillment of these guidelines might be suspected as ideal to lower mortality.

Objectives: To find out: (1) the degree of fulfillment of the 2008 SSC guidelines in surgical ICU patients with septic shock; (2) factors resulting in fulfillment of less than 100%; (3) whether degree of fulfillment is associated with survival; (4) and to demonstrate that despite high degree of fulfillment, guidelines will reach a limit in their benefit, i.e., the guideline limit.

Methods: 98 surgical patients in septic shock treated from 01.01.2008 until 30.06.2009 were analysed regarding the association between item fulfilled or not-fulfilled concerning 34 items of the 6-and 24-h bundles of the 2008 SSC guideline and outcome.

Results: The degree of fulfillment fulfilled of the 34 items varied between 0 and 97%. Besides the categories fulfilled and not-fulfilled, additional categories partially fulfilled, not-applicable and unknown have to be assessed. No item was essential for survival, solely. Patients with septic shock on admission (n = 68) had significantly
higher SOFA scores (degree of organ dysfunctions) compared to patients developing septic shock on the ICU (n = 30). Patients with the item urinary output (0.5 ml/kg h) not-fulfilled (n = 20) demonstrated significantly higher SAPS 3 values (degree of severity of disease) at the beginning of septic shock (median 59; 41–85) than patients with this item fulfilled (n = 64; median 49.5; 31–67), without difference in mortality.

Conclusions: Factors contributing to degree of fulfillment, prognosis and guideline limit are: severity of organ dysfunctions and disease, and structures (type of ward, number and quality of staff). In the appraisal of the degree of fulfillment of guidelines, a huge amount of confounders has to be taken into account, resulting in the guideline limit, i.e., the benefit of the guidelines reaches a maximum lower than 100% despite high degree of fulfillment. There, each item of a guideline in addition will increase the effort profoundly to increase survival rate only marginally, and may even decrease the survival rate due to increasing rate of therapeutic side effects and complications. Under the scope of cost-benefit analysis, with the limitation of the benefit, effort in addition or even harm would not justify the effort and costs.

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Case report: co-infection of influenza B and Pneumocystis jirovecii as the cause of severe pneumonia with ARDS in a man with B cell non-Hodgkin's lymphoma of the central nervous system in complete remission
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Introduction: Pulmonary co-infections with Influenza A virus and Streptococcus pneumoniae is a known risk factor for increased morbidity and mortality. In contrast to Influenza A, Influenza B is typically associated with a lower morbidity and mortality. It hypothesized that the bacterial infection in such cases represents a secondarily obtained infection. Pneumocystis jirovecii pneumonia (PCP) is a life-threatening opportunistic infection which occurs in immunocompromised patients such as organ transplant recipients, patients treated with corticosteroids or chemotherapy or patients with acquired immunodeficiency syndrome (AIDS). The co-infection with influenza virus (A/B) and other pulmonary pathogens in such patients increased mortality risk.

Objectives: Here we report a case of pneumonia co-infected with influenza B and pneumocystis jirovecii pneumonia associated with ARDS in a patient with a B-NHL of CNS after chemotherapy and radiation who was, however, in complete remission. B-NHL of the central nervous system had been diagnosed in this 69 years old man in 11/2010. The patient was in complete remission after three courses of chemotherapy with rituximab, methotrexate, ifosfamide and dexamethasone administered 11/2010–12/2010. Treatment was consolidated by irradiation of the brain and dexamethasone until 3/2011. In May 2011 the patient was hospitalized with influenza-like symptoms including cough, dizziness, headache and fever (39°C). In addition, the serum lactate dehydrogenase was increased and a CT of the chest revealed ground-glass opacities. The patient required intubation and mechanical ventilation due to severe hypoxemia and severe bilateral pneumonia. The Horovitz index (PaO2/FiO2), which, if below 200 has been used as marker for ARDS was at 138. Microbiological analysis of tracheal fluid identified influenza B infection, but not influenza A in a bed side test as well as by PCR. The same sample of tracheal fluid, however, also contained pneumocystis jirovecii detected by PCR and microscopical analysis (Grocott stain). Treatment with oseltamivir and trimethoprim/sulfamethoxacol was instituted for 10 days and resulted in a resolution of the infiltrations and full recovery.

Methods: Case Report
Results: Following anti B-cell antibody and chemotherapy treatment and subsequently high dose corticosteroids have to be considered as risk factors for this life threatening co-infection with influenza B and pneumocystis jirovecii. Suggesting that these patients are still immunocompromised even after weeks following the completion of chemotherapy. It is known that CD27 positive memory B cells were significantly reduced after treatment with rituximab. The risk for PCP under such conditions is well known but an influenza B pneumonia has been rarely reported. This is one of the first descriptions of a co-infection with Pneumocystis jirovecii and influenza B virus.

Conclusions: Also, these results demonstrate that NHL patients treated with rituximab-containing regimens may be at particular risk for infection even at long-standing complete remission. Whether prophylactic treatment for PCP and Influenza infections are necessary for certain patients needs to be determined. However, diagnostic strategies needs to be developed in order to identify such patients.

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The Jena Sepsis Registry: a prospective observational registry for patients with severe sepsis or septic shock, supported by primary care
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Introduction: There is a lack of prospective registries, observing long-term mortality, sequelae, costs and quality of care for patients with severe sepsis or septic shock. The awareness that improving quality of care is an important task for daily clinical practice, has led to an increasing number of clinical registries. However, there are only few sepsis registries worldwide. Most of the existing sepsis registries are limited to ICU treatment and hospital mortality. This might be due to difficulties in accessing patient follow-up data after hospital discharge. The Jena Sepsis Registry will be the first sepsis registry ensuring secure long-term follow-up data from the primary care setting.

Objectives: Aim of the project is the development of the Jena Sepsis Registry, a prospective, observational registry for patients with severe sepsis or septic shock, supported by primary care.

Methods: Every patient admitted to the ICU at Jena University Hospital will be screened for international criteria of severe sepsis or septic shock. The Center for Sepsis Control and Care (CSCC) Core Data Set, based on ICU parameters, will be completed at time of diagnosis and hospital discharge. Primary care data will be collected during follow-up providing better knowledge of long-term post-sepsis mortality and morbidity.

Results: We expect the Jena Sepsis Registry to collect long-term data regarding mortality, sequelae, prognosis and costs and to improve quality of care for patients with severe sepsis or septic shock. We started the registry pilot study in January 2011 and will present first pilot data.

Conclusions: The development of the Jena Sepsis Registry will be relevant to ensure long-term post-sepsis follow up data and to improve quality of care for patients with severe sepsis or septic shock.
Acknowledgement: The Jena Sepsis Registry is supported by the Center for Sepsis Control and Care (CSCC), funded by the German Federal Ministry of Education and Research (BMBF) grant no. 01 E0 1002, the Paul-Martini Clinical Sepsis Research Group, funded by the Thuringian Ministry of Education, Science and Culture, grant no. PE 108-2, the Thuringian Foundation for Technology, Innovation and Research (STIFT) and the German Sepsis Society (GSS).

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Sepsis survivors monitoring and coordination in outpatient health care (Smooth): study synopsis

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Introduction: Postsepsis morbidity has long been ignored: Despite the considerable medical and health economic impact of sepsis, little is known on its long-term burden. Sepsis sequelae include critical illness polyneuropathy, myopathy, wasting, posttraumatic stress disease, depression and chronic pain. In addition, most sepsis patients are older adults and have complicated co-morbidities. This may result in a prolonged period of rehabilitation. Due to a lack of tailored post-discharge strategies, lack of awareness and specific training in primary care and rehabilitation facilities, sepsis survivors do not receive adequate long-term care. This controlled intervention study is the first trial to evaluate the effects of a postacute disease management program on long-term clinical outcome of patients after severe sepsis.

Objectives: Aim of the study is to explore whether the quality of life and other clinical outcomes of sepsis patients can be improved by a specific follow-up in terms of a Disease Management Program (DMP).

Methods: In a two-armed multicentric intervention study patients will be followed actively and in a structured way for 24 month after surviving a sepsis episode, in cooperation with their General Practitioners (GP). Following the structure of a Disease Management Program the intervention is divided into three areas: (1) Discharge management with structured information between inpatient and outpatient care, (2) Training of GPs and patients in sepsis-related illnesses with evidence based treatment options and guidelines, (3) Continuous monitoring of patients through specially designed telephone interviews regarding the main complications of sepsis. The central point in the intervention is the Case Manager as an attendant of the patients. There is also a liaison physician available as a contact for the GP, to review and provide the results of the monitoring.

Outcome: Primary endpoint of the study is the health related quality of life after 6 months, measured by the SF 36 questionnaire. Secondary outcome variables include the assessment of rehospitalisations, physical activity, level of pain, depression symptoms, cognitive deficits or neuropathic symptoms by other established instruments—6, 12 and 24 months after discharge from intensive care.

Results: We expect the intervention to improve significantly clinical outcomes in severe sepsis survivors compared to standard care. We started in February 2011 and will present first experiences of the study development.

Conclusions: An effective longterm care for sepsis survivors would be of clinical relevance for the individual patient and for the public, as most of these patients are treated in primary care.

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Early use of immunoglobulin in septic shock

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Introduction: In addition to the interventions suggested by the guidelines of the Surviving Sepsis Campaign, in the recent years other therapeutic options have been proposed and tested for patients with septic shock. Intravenous polyclonal IgM-enriched immunoglobulins (IgGAM) have been recently re-evaluated as a valid therapeutic option. The exact mechanism of action of IgGAM is still unclear, but the antitoxin and anti-inflammatory properties may influence and modulate the host immune response.

Objectives: In this retrospective study we assessed the effects of the early use of polyclonal intravenous immunoglobulins enriched of IgA-M (IgGAM) in addition to standard therapies on clinical outcome of patients with septic shock.

Methods: In January 2008, IgGAM has been introduced in our ICU protocol for the management of septic shock patients (within 24 h after shock appearance and at the dosage of 5 ml/kg for 3 days). From January 2008 to December 2009, we studied 100 consecutive patients with septic shock admitted in our ICU. In each patient we recorded the SAPS II and SOFA scores, the compliance to 6- and the 24-h sepsis bundles, the compliance to IgGAM use, the length of stay in ICU and the 30 days and hospital mortality rate.

Results: Due to low compliance of medical staff to protocol application, IgGAM has been used only in 57 patients (IgGAM group). At ICU admission, the severity scores were similar in patients with and without (Control group) IgGAM therapy (SAPS II: control 61 ± 17, IgGAM: 58 ± 16; p > 0.05 and SOFA: control 9 ± 3; IgGAM 9 ± 3; p > 0.05). The compliance to 6 and 24 h bundles, was also similar in the two groups, in the control group 30.2%±(13/43) versus 26.3% (15/57) for 6 h bundles and 30.2%(13/43) versus 49.1%(28/57) for the 24 h bundles. The length of stay in ICU was 18 ± 17 days in the IgGAM group and 17 ± 24 days in Control group (p > 0.05); the 30-day mortality was similar (p > 0.05) in IgGAM group (28.1%) than in Control group (44%), while the Hospital mortality was significantly higher in the Control group (53.1%) than in the IgGAM group (40.4%).

Conclusions: These preliminary data indicate that the early use of IgGAM, associated to interventions proposed by the SSC guidelines, seems to reduces mortality of patients with septic shock.
Introduction: In decompensated liver failure extracorporeal liver support (ELS) by albumin dialysis provides a therapeutic option in critically ill patients. However, indications, contraindications and timing of ELS remain unclear and large prospective clinical trials are still missing.

Objectives: The aim of this retrospective analysis was therefore to identify possible indications and risks factors associated with negative outcome for ELS in patients presenting with decompensated liver failure in our institution.

Methods: Retrospective age and SAPS-II matched pair analysis of patients presenting with acute decompensated liver failure on a single center surgical intensive care unit (ICU), receiving either ELS + standard medical treatment (SMT) or SMT alone. Univariate analysis and binary multiple regression analysis is used to compare groups.

Results: Between 2004 and 2009 70 patients received ELS at our institution. 66 age and SAPS-II matched pairs (acute liver failure treated with SMT) could be found and were eligible for further analysis. APACHE-II, urea, bilirubin, ammonia and blood glucose levels showed significant differences between ELS + SMT and SMT group (n = 28 per group) in univariate analysis and were therefore included in binary multiple regression analysis. Here, APACHE-II, bilirubin and ammonia were identified as independent factors for clinical indication of ELS in our patients. To describe possible contraindications for ELS outcome on ICU after ELS served as dependent variable (n = 66). In univariate analysis age, platelet count, CRP, need for norepinephrine, PCT level, SOFA-, APACHE-II-, SAPS-II-Score and lactate difference were independent risk factors for negative outcome in ICU (0 RF: 100% ICU survival, 1 RF: 70%, 2 RF: 30%, 3 RF: 10%, 4 RF: 0%). Lower APACHE-II and SOFA-II scores were associated with a higher survival rate after ELS. Admission lactate was identified as independent risk factor for higher mortality (n = 326) were PSA beta-lactams (PSA-penicillin: n = 178; PSA-cephalosporin: n = 21; PSA-carbapenem: n = 155). In 408 patients the beta-lactam was combined with the one of following drugs: aminoglycoside (11.4%, of these 73.8% were PSA-BL); quinolone (16.8%, of these 62.2% were PSA-BL); glycopeptide (10.6%, of these 87.7% were PSA-BL); macrolide (11.5%, of these 56.5% were PSA-BL); metronidazole (25.7%, of these 52.9% were PSA-BL). Altogether 18.8% patients were treated with 2 PSA-active drugs (quinolone: 10.4%; aminoglycoside: 8.4%). 70% of antimicrobial therapies were administered empirically and 29% were guided by pre-existing culture results and 1% prophylactic.

Conclusions: Most patients received a beta-lactam based combination treatment. Combination partners reflect that in most patients the beta-lactam was combined with the one of following drugs: aminoglycoside (11.4%, of these 73.8% were PSA-BL); quinolone (16.8%, of these 62.2% were PSA-BL); glycopeptide (10.6%, of these 87.7% were PSA-BL); macrolide (11.5%, of these 56.5% were PSA-BL); metronidazole (25.7%, of these 52.9% were PSA-BL). Altogether 18.8% patients were treated with 2 PSA-active drugs (quinolone: 10.4%; aminoglycoside: 8.4%). 70% of antimicrobial therapies were administered empirically and 29% were guided by pre-existing culture results and 1% prophylactic.
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Capnocytophaga canimorsus a rare cause of septic shock

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Introduction: We report on a case of a septic shock following a small dog bite in the right hand, obviously caused by Capnocytophaga canimorsus. This bacterium is known as a common commensal in the oral microflora of the dogs (1). A 42-year-old woman came to hospital with head ache, pain of the limbs, shivering, fever and general fatigue for 3 days. She reported a bite of her own dog in the right hand. The wound, essentially more scratch than a deep bite was cleaned by an ambulant surgery before. In half a day the patient deteriorated to an overwhelming septic shock with consecutive respiratory failure, acute renal failure, DIC, ARDS as well as acute liver failure. In the blood culture Capnocytophaga canimorsus was detected, which was interpreted as the cause of septic shock. Broadspectrum antibiotic therapy with piperacillin/tazobactam, combined with moxiflaxacin was initiated (according to the culture testing results) and continued until day 14 as well as a second surgical debridement was performed. Under supportive intensive care including goal directed hemodynamic therapy, renal replacement, mechanical ventilation according the ARDSNet criteria, prone positioning and DIC therapy the patient was stabilized in 3 days. However, weaning from the respirator was prolonged due to recurrent vagal cardiac arrests, a secondary bronchopneumonia due to acinetobacter, respiratory failure after extubation (3 times), and a postseptic delirium. After placement of a temporary pacemaker and dilatative tracheostomy (Cigliola) the patient was successfully weaned, and transferred to the ward on day 35. 3 months after admission the patient visited our ICU with restitution ad integrum.

Conclusions: Capnocytophaga canimorsus is probably a very rare reason for septic shock in humans, but due to its commonness in the saliva of dogs and its virulence it has to be considered in patients with injuries due to dog bites. In patients with no clear cause of sepsis a close contact to dogs has to be evaluated by a careful anamnestic interview.

Reference: [1] Oehler RL, et al. Lancet Infect Dis. 2009;9:439–47.

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Integration of the clinical data management system OpenClinica into the research infrastructure of the Center for Sepsis Control and Care (CSCC)

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Introduktion: The CSCC is a research institute of the Jena University Hospital (JUH) and the School of Medicine of the Friedrich Schiller University of Jena. The CSCC covers all aspects of sepsis, from risk prediction to long-term sequelae and health economy. The sepsis data unit develops a research database (RDB), which will aggregate and provide data from all CSCC projects. Data is collected via Electronic Data Capture (EDC) of the Clinical Request Forms (CRF) by a clinical data management system.

Objectives: The RDB integrates routine data, data from a sepsis registry and trial data from different trial centers, e.g. multi-centric studies with up to 40 centers. Routine data from the JUH comprises data from HIS (Hospital Information System, Laboratory and Patient Data Management System). The sepsis registry acquires baseline data from JUH sepsis patients at different time points. Wherever adequate and possible this data should only be raised once and will be reused in the study CRF. Routine data will not be uploaded into the CDMS, but will be merged with the trial data at a later stage in the research database. Data capture, storage and retrieval are performed according to the legal requirements for data protection.

Methods: Our requirements for the EDC system were: platform independent web-interface, simple eCRF modeling, support for monitoring, query management and reporting, support for standards like Web-Services and the CDISC Operational Data Model interchange format, full GCP Part 11 conformance and low license costs. OpenClinica from Akaza Research fulfilled these requirements and offers an open source Community Edition, which can be used free of charge. Nevertheless, OpenClinica is not a Clinical Trial Management System: ID-management, patient-management, tracking of CRFs, regulatory timelines or support for adverse event reporting is not covered. The aggregation of data from different sources requires a data privacy and data security concept. The data privacy concept is based on the generic concepts for data privacy of the Technologie- und Methodenplattform (TMF). A tool was developed to generate the master-patient-index (PID), the generation of a trial specific subject code (SIC) and the subject registration for the EDC system in one step. Further tools support validation rule generation, patient management and tracking.

Results: OpenClinica showed to be a simple and robust tool for EDC and clinical data management. As the internal database structure for the eCRF resembles rather an EAV (entity–attribute–value) scheme then the classical relational model, direct data access via SQL is intractable. Our aim for the CSCC is to establish a long-term Sepsis research database which allows comprehensive analysis of research and routine data, regardless of source and scope. This demands an effective way to harmonize data across studies; e.g. the use of metadata-repository (MDR) tools.

Acknowledgement: Supported by the Paul-Martini Research Group (Clinical Septomics), funded by the Ministry of Thuringia (ProExcellence; PE 108-2), the Thuringian Foundation for Technology, Innovation and Research (STIFT), the German Sepsis Society (GSS) and the Center of Sepsis Control & Care (CSCC); funded by the German Ministry of Education and Research (BMBF).
Introduction: Current guidelines recommend a short duration between diagnosis of septic shock and first application of antimicrobial therapy. Standard educational programs can reduce time to antimicrobial therapy (TTA) but TTA usually remains clearly above the recommended 1 h.

Objectives: To assess the efficacy of a multifaceted compared to a conventional educational program with focus on improving diagnosis of sepsis and shortening the time to adequate antibiotic therapy to reduce mortality and TAA in patients with severe sepsis or septic shock.

Methods: In this cluster randomized trial (CRT), hospitals are randomly allocated to a control group, which receives conventional educational materials only, or an intervention group. The intervention group is offered a specific program with benchmarking of quality parameters such as TTA, length of stay, and mortality, training in change management, implementation of local change teams, and materials to increase vigilance about sepsis. All patients with new onset of severe sepsis or septic shock in the prehospital setting, in the emergency department, in the operating theatre, on the regular ward, or on the intensive care unit (ICU) are documented in both groups. Patients are excluded if sepsis therapy was started on a non-study site, if the patients are not submitted to the ICU, or if there is no commitment to full medical support. Study duration is 2 years. Primary endpoint is 28 days mortality. The CRT is preceded by a 5 months observational phase to obtain baseline values for all hospitals. The hospitals switch groups after the CRT is finished to study sustainability in the former intervention group and apply the intervention in the former control group.

Results: 44 centers have been recruited to participate in this study. Recruitment rate in the observation period was 200 patients per month. A monocentric study would need 1,572 patients to detect a decrease in 28-day mortality from 42 to 35% with an alpha of 5% and a statistical power of 80%. For the CRT, a design effect of 1.3–1.6 was estimated to control for the individual cluster size. This results in a sample size of 2,000–2,400 patients.

Conclusions: For the first time, efficacy of specific training methods to shorten TTA is tested by a CRT. The study is sufficiently powered to proof whether a shorter TTA will affect outcome of the patients.

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Infection 2011

Efficacy and safety of gelatin for fluid therapy in hypovolemia: a systematic review and meta-analysis

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Introduction: Gelatin is frequently used as volume expander. There are growing concerns about safety.

Objectives: To systematically assess clinical evidence concerning mortality, coagulation and renal function.

Methods: Systematic review of randomised controlled trials (RCT) on gelatin in hypovolemia in comparison to any other fluid with comprehensive search strategy [Ovid Medline (1948-May 2011), EMBASE (1947-May 2011), Cochrane Library]. Data were independently extracted and risk of bias assessed using the 2010 Cochrane tool. Primary outcome was overall mortality. Secondary outcomes were number of patients exposed to allogeneic transfusion, frequency of renal replacement therapy (RRT) or acute renal failure (ARF).
Albumin and crystalloid solutions were defined as “suitable”, other synthetic colloids as “unsuitable” control fluids since they carry similar risk of side effects. Relative risks (RR) and weighted mean differences with 95% confidence intervals (CIs) were calculated. Data were pooled using a random-effects model (RevMan 5.1, Cochrane Collaboration).

**Results:** The search yielded 1,288 citations, 210 reports were read in full. The final sample consisted of 73 RCT in English, German, French and Italian, published between 1975 and 2010, with 5,915 patients overall, 2,538 of which received gelatin. Median sample size in the gelatin groups was 20 patients (range 10–249). In 54 RCT (74%), the study period was ≤24 h. Total gelatin dose was 20 ml/kg (median, range 6–62). Only 39 RCT (53%) used “suitable” control fluids. 49 RCT (67%) investigated elective surgical patients, mostly from cardiac surgery (33 RCT, 465). 9 RCT (12%) investigated critically ill patients, 7 RCT (10%) were in emergency patients and 7 RCT (10%) were in children. Risk ratio (RR) for mortality was 1.02 (CI 0.87–1.19, data from 23 RCT with 2,694 patients which reported mortality). Numbers of patients exposed to alloengenic transfusions were provided in 12 RCT, n = 1,193 patients and RR was 1.15 (0.94–1.41). When only studies with “suitable” control fluids were included, RR for mortality was 1.13 [0.88–1.46, 10 RCT, 1,392 patients] and risk for transfusion exposure was 1.24 (0.87–1.79, 8 RCT, n = 702), tending towards control. Only six RCT (n = 662 patients) reported the occurrence of RRT or ARF, five of them in comparison to HES solutions. 3 RCT reported anaphylactoid events.

**Conclusions:** Most published studies on gelatin are small and short-time, use unsuitable control fluids and report too few events to reliably assess the safety of gelatin.

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Infection 2011

Delay in antibiotic therapy is associated with mortality only in non-ICU acquired infections

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**Introduction:** Severe Sepsis is one of the most common conditions in intensive care with a great contribution to morbidity, mortality and costs. Delay in antibiotic therapy is associated with higher mortality.

**Objectives:** To analyze time to antibiotic treatment of severe sepsis in a single tertiary care centre and its relation to patients’ outcome.

**Methods:** Patients of four intensive care units (72 beds in total) caring for adult patients in a tertiary care university hospital were screened daily for new onset of severe sepsis as part of the ongoing MEDUSA trial between 2010 December and 2011 May. Our criteria for severe sepsis were a suspected or proven infection and at least one newly developed organ dysfunction (ODF) supposed to be caused by the infection. Patients were excluded if sepsis therapy was started in a referring hospital, if patients were not submitted to the ICU, or if there was no commitment to full medical support. Only the first episode of severe sepsis was documented for every patient.

**Results:** 111 patients with a new onset of severe sepsis (68% male, median age 69 years) were identified. ICU mortality was 30%, their 28-day mortality was 37% and hospital mortality was 42%. 76 patients received new antibiotics after the onset of severe sepsis with a median delay of 196 min (IQR 92–254 min). 25 patients were treated with new antibiotics for a suspected infection and developed severe sepsis after the start of treatment with a median time interval of 155 min (IQR 276–65 min.). 10 patients, who already were under antibiotic treatment for 1–5 days, had no change of their antibiotic treatment with the onset of severe sepsis. The 76 patients who received new antibiotics after the onset of severe sepsis a higher 28-day mortality (45% vs. 20%, p = 0.017) compared to the 25 patients who received new antibiotics before the onset of severe sepsis. They had also higher SAPS II scores (median 50 [IQR 41–61] vs. median 44 [IQR 34–51] p = 0.007) and higher SOFA scores (median 11 [IQR 8–13] vs. median 8 [IQR 6.5–10.5], p = 0.002) compared to the 25 patients who received new antibiotics before the onset of severe sepsis. In the subgroup (n = 61) of patients with infections acquired outside the ICU and receiving antibiotics after the development of severe sepsis the delay between the onset of a new ODF and the start of antibiotic treatment was correlated with 28-day mortality. This effect was still significant (p = 0.037) after correction for SAPS II and SOFA scores. This effect could not be shown for patients with ICU acquired infections.

**Conclusions:** This study confirms that delay in antimicrobial therapy is correlated with outcome for patients who acquired severe sepsis or septic shock outside of the ICU. Why patients with ICU acquired severe sepsis did not profit from early antibiotic therapy needs further research.

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Infection 2011

Direct costs related to severe sepsis in Germany: an update

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**Introduction:** Representative data on the costs of severe sepsis in Germany is missing. Based on data from an older (1997–2000) retrospective chart analysis in three German adult ICUs of three university hospitals, direct per patient costs for severe sepsis treatment (restricted to the ICU stay) has been estimated to 23,297 € on average. Linking the direct costs per patient with estimated incidence data (44,000–95,000 patients per year), the total direct costs for severe sepsis in Germany per year had been estimated to range from 1,025 to 2,214 million € [1]. Since severe sepsis and septic shock had not been adequately addressed in the International Statistical Classification of Diseases and Related Health Problems (ICD) system, the German Sepsis Society proposed a new coding system (R65 codes), which was subsequently integrated in the ICD-GM code in 2005 and recently, in the international ICD coding system.

**Objectives:** To determine the incidence, hospital mortality and related direct costs per year based on the new R65.1 coding system of severe sepsis as reported to one of the largest German health insurance fund agencies [kindly provided by Bundesverband der Betriebskrankenkassen (BKK), Essen, Germany] and to calculate the national
frequency, hospital mortality and direct (hospital) costs according to the incidence data of a large, representative national study [2].

Methods: For year 2007, data of 12,611,111, for 2008 of 13,907,820 and for 2009 of 13,532,530 medically insured German citizens were analyzed based on the routine hospital discharge records for the presence of the ICD-code R65.1 ("systemic inflammatory response syndrome of infectious origin with organ dysfunction"; i.e. severe sepsis). Attributable direct (hospital) costs were calculated according to the actual costs for the health insurance fund agency (BKK). Only data from year 2009 are presented in this preliminary report.

Results: In 2009, n = 9,153 patients were discharged with a diagnosis of severe sepsis. Hospital mortality was 45.54% (n = 4,168 patients). Related direct hospital costs were 0.523 billion € on average (57.098 € per case; 53.299 € for non-survivors and 60.477 € for survivors). The average per case costs for patients without severe sepsis were only 2.886 €. Extrapolating this data according to the more representative results of the German SepNet Prevalence Study (2), n = 14,886 patients are discharged annually with a diagnosis of severe sepsis with an in-hospital mortality of 55% (n = 8,187). The related direct costs are estimated to be 0.850 billion €. Based on these data, a projection for all German citizens revealed an incidence of 86,900 severe sepsis cases per year and related direct costs of 4.962 billion €.

Conclusions: Due to the introduction of the R65 codes in the international ICD coding system a more accurate estimate of the direct costs related to the burden of severe sepsis in hospitals is potentially possible. For Germany, the current direct costs for in-hospital treatment of severe sepsis are up to fourfold higher compared to estimates from the 1990s.

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121 Infection 2011

Delayed appropriate antibiotic therapy in Staphylococcus aureus bloodstream infection and influence on outcome

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Objectives: In patients with septic shock, delayed treatment with appropriate antimicrobials has been shown to increase mortality and hence early, aggressive treatment has been advocated. However, in Staphylococcus aureus bloodstream infections (SAB) a delay of antimicrobial therapy may not be that critical. In a prospective epidemiological study we examined whether treatment delay influences outcome in patients with SAB.

Methods: 258 patients from 10 study centers were enrolled in the prospective preSABATO study and followed for 3 months. Predisposing factors, clinical features, diagnostic procedures, antimicrobial therapy, and outcome were recorded. Empiric antimicrobial therapy was considered delayed when appropriate antimicrobial therapy was initiated more than 24 h after drawing the initial positive blood culture; appropriateness of antimicrobial therapy was based on in vitro activity against S. aureus and drug dosage.

Results: From 244 patients data for the complete follow-up period could be obtained. In 135 (55%) patients SAB was considered hospital acquired. The most prevalent portals of entry were catheter-related infections (75, 31%), skin and soft-tissue infections (21, 9%), pneumonia (20, 8%), and infective endocarditis (21, 9%); in 50 (37%) patients the portal of entry could not be identified. MRSA was present in 46 (19%) patients. Appropriate antimicrobial therapy was administered with delay in 98 (40%) patients. There was no significant difference in mortality (30-, 90-day mortality) and relapse rate between early appropriate and delayed therapy (Table 1).

Conclusions: In our study, delayed appropriate antimicrobial treatment did not adversely affect outcome in SAB. However, the influence of potential confounders and interacting variables needs to be addressed in a larger study.

|                | Total (n = 244) | Delay of antibiotic therapy | p* |
|----------------|----------------|-----------------------------|----|
|                | <24 h (n = 146) | ≥24 h (n = 98)              |    |
| 30-day case fatality | 40 16.4 | 25 17.1 | 15 15.3 | 0.7 |
| S. aureus related | 18 7.4 | 12 8.2 | 6 6.1 | 0.6 |
| 90-day case fatality | 68 27.9 | 44 30.1 | 24 24.5 | 0.4 |
| S. aureus related | 25 10.2 | 15 10.3 | 10 10.2 | 1 |
| Late complications | 11 4.5 | 7 4.8 | 4 4.1 | 1 |

*Pearson’s Chi-squared test
Abstracts

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Introduction: Self-help has developed to an important field in the society. Information about the severe illness, the exchange of experiences in all their phases or the turn to sb. for comfort—the demand of contact to other cases is enormous. Germany has more than 70,000 active health—and social-related self-help groups and societies. The first and actually only structured self-help and counseling institutions for sepsis patients and their relatives worldwide is the German Sepsis Aid, founded in 2007.

Objectives: The main objective of the German Sepsis Aid is to inform patients, relatives, and citizens interested in Sepsis and to foster contacts among interested parties, i.e. medical scientific societies, health care providers and public funding agencies. Therefore the nation-wide number +49-700-737747-00 was established. Volunteers of the German Sepsis Aid, ex-patients and relatives, were trained and are now at the callers disposal.

Results: About 2,500 national and international counseling interviews were performed, not only via phone but also via e-mail. Three main categories of the interviews and frequently asked questions (FAQ) can be summarized: (1) subacute physical and mental longterm effects/chronical problems after ICU-care and their rehabilitation—an about 40%; (2) acute sepsis-illness, an about 35%; (3) counseling in bereavement and finally need for help due to medical errors, an about 10%. The service of different telephonists every day of the week ensues according to a special plan of action. The forwarding happens automatically direct to the unpaid helpers with the sympathetic ear.

Conclusions: The feedback is throughout positive. The one-on-one interviews, the listening and the prompt forwarding of problems to the office of the German Sepsis Aid in Jena characterize the responses. A double effect the conversations have: they are helping the Volunteer Counsellors with the own processing of the sepsis. Kind and frequency of the contacts show an urgent need for the support of self-help groups and experienced institutional services for advice and help in patients with severe sepsis—a fact which is largely overseen by intensive care institutions and medical societies.

Fig. 1 Counselors of the German Sepsis Aid

Pediatric Sepsis Research

010

Infection 2011

A population at risk for pediatric sepsis in Africa and its preoperative identification

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Introduction: In Malawi we see a lot of malnourished children in theatres and ICU. The interdependence of sepsis and malnutrition in children is well established. It is not difficult to recognize affected children by anthropometric measurements. Reality in Africa sees us struggling in theatres even with MUAC. Easy to use the MUAC-tape is no solution because frequently it is out of stock, broken, lost, sold, locked away or stolen.

Objectives: We tried to find a realistic way to prevent malnourished children from being exposed to the risk of postoperative sepsis after elective surgery. Awareness had to be raised with the surgeons and the anaesthetists that these children need treatment in the NRU instead of an operation—in order to minimize the risk of sepsis in our setting.

Methods: Our idea was to train nurses as gatekeepers. In front of and in the theatre they should use a tool to recognize these sepsis prone children, which even in the poorest African country is always available, cannot be lost, sold or stolen and—which is for free.

Results: 145 nurses and nursing students were trained how to identify a MUAC < 11 cm with their own hands and fingers. Four different gauze bandages with a circumference of 9, 10, 11, and 13 cm were used as test-substitutes for the sick children.

Conclusions: We were able to show that our method identifies malnourished children in a few seconds without any tool. This can prevent children from being put in danger of sepsis in elective surgery. Moreover we raised the awareness of the (mostly non-physician) anaesthetist for further septic or immunological problems the child may present in Africa at the same time—like Malaria or AIDS.

012

Infection 2011

Tackling peripartum and posttraumatic sepsis in Malawi by providing training to non-physicians

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Introduction: Postpartum sepsis and sepsis after trauma are major causes of death in developing countries. The cadres dealing with these problems in the districts are coming from different professional backgrounds (clinical officers, medical assistants, nurses, midwives) having in common not to be doctors and to face a multitude of medical and structural problems.

Objectives: We aimed at integrating the therapeutic approach to sepsis on three different levels. Medically the severe sepsis bundles have to be combined with the aim to treat emergencies already initially in a way that sepsis does not develop. Staffwise non-physicians...
have to be trained in the absence of doctors. Finally the infrastructure of the districts has to be integrated into that of the central hospitals through adequate referrals and training for HDU pel in the absence of ICUs.

Methods: In order to tackle these challenges we incorporated them into a course for health professionals of three Malawian districts (Chiradzulu, Thyolo, Blantyre). The course the HOT-course was held 16 times from 2005 to 2010. The participants were trained in 3 days on sepsis treatment, shock, obstetric emergencies, trauma care and the role of triage, referrals and high dependency units. A formal evaluation was carried out with 32 participants through a questionnaire, focused interviews and case reviews. Maternal Mortality and Trauma Mortality Rates (MMR and TMR) were compared with Mangochi district.

Results: 391 participants completed the course successfully. 44 of them could be identified as future instructors on the courses. Evaluation revealed improved practice for sepsis management and indirectly sepsis related parameters like airway management, stabilization of post partum haemorrhage, resuscitation and prioritization. During the 6 years our course was running we could show that the MMR in at least two districts were reduced overproportional in comparison with the not included but comparable Mangochi district. Data for TMR were not robust enough to show the same.

Conclusions: Our participants felt a substantial benefit for their daily work with septic patients. Despite the fact that other interventions are going on to reduce MMR we do feel that our courses can be credited with having played a beneficial role in this reduction.

073
Infection 2011
Managing neonatal severe sepsis: a national survey of current practices in Germany
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Introduction: In 2002 and 2007 the American College of Critical Care Medicine (ACCCM) provided clinical guidelines for hemodynamic support of pediatric and neonatal patients in septic shock. In 2008 the Surviving Sepsis Campaign Guidelines Committee offered up-to-date clinical guidelines for management of severe sepsis and septic shock in adults and in pediatric patients. Implementation of guideline-oriented care has shown to improve mortality and length of hospital stay.

Objectives: To assess the standard of care of neonates with sepsis, severe sepsis and septic shock in German NICUs with regard to variability in management and guideline-conformity.

Methods: 199 pediatric clinics in Germany were asked to describe their management of neonatal sepsis patients in an anonymous telephone survey. The questionnaire that was used for the survey was designed based on the ACCCM clinical guidelines.

Results: A total of 90 (45%) surveys were completed and analyzed. Table 1 shows the results of respondent hospitals.

| Management of neonatal sepsis patients | 90 hospitals (%) |
|----------------------------------------|------------------|
| **Diagnosis**                           |                  |
| Cultures obtained prior to administration of antibiotics | 100              |
| Determine capillary refill time >2 s | 91               |
| Lumbar puncture | 16               |
| Laboratory parameters                   |                  |
| WBC | 92               |
| CRP | 99               |
| Procalcitonin | 27               |
| II-6 | 61               |
| II-8 | 4                |
| Differential diagnostics                |                  |
| Heart ultrasound | 77               |
| Ammoniac | 51               |
| Lactate | 79               |
| BGA | 97               |
| Hemodynamic support and adjunctive therapy |              |
| Initial fluid therapy                   |                  |
| Crystalloids | 96               |
| Colloids | 0                |
| Initial vasopressor                     |                  |
| Dopamine | 47               |
| Dobutamine | 44               |
| Epinephrine | 10               |
| Norepinephrine | 13              |
| Other | 0                |
| Immuno additive therapy                 |                  |
| Hydrocortisone | 42               |
| Recombinant human activated protein c | 1                |
| Human protein c | 1                |
| Pentoxifylline | 2                |
| Immunoglobulin | 20               |
| Glucose control | 100              |

Conclusions: Management of sepsis, severe sepsis and septic shock in neonates varies between health care centers. These findings raise concerns as to the standardized care for sepsis of neonates. Implementation of guideline-based protocols are mandatory.
Sepsis Prevention and Control

003
Infection 2011

Knowledge and practice regarding universal precaution among nurses working in Medical–Surgical Units of BPKIHS, Dharan, Nepal

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Introduction: Universal precautions are simple measures of infection prevention and control that reduce the risk of transmission of blood borne pathogens through exposures to blood and body fluids. It is especially important for the nurses since they are always in contact with the patients.

Objectives: To assess the knowledge and practice of universal precaution among the staff nurses and ANMS working in Medical–Surgical Units of BPKIHS.

Methods: A descriptive, cross- sectional study design was adopted. Using census methods of sampling, 39 staff nurses and 18 ANMS out of the total 58 nurses were selected for the study. A structured questionnaire was used to assess the knowledge and self report practice. An observation checklist was also used to assess the practice and the available facilities in the wards. A focus group discussion was organized to explore the various aspects of universal precaution. Descriptive statistics (medium and inter quartile e range) and inferential statistics (Mann–Whitney U test and Spearman’s correlation) were used for data analysis. The P value was estimated at 95% confidence level and 5% permissible error.

Results: The study results showed that the median percentage of overall knowledge was 57.5% and that of self reported practice was 92.9%. The median percentage of observed practice was 62.5%. This shows that there is knowledge deficit among nurses working in the Medical–Surgical wards but relatively the practice is better. They also claim that they practice universal precaution properly (92.7%) but the observed practice is not the same (62.5%). The results also show that there is no significant difference between knowledge and practice of universal precaution with P value at 0.933. The qualitative data focus group discussion (FGD) also reveals decreased knowledge among the nurses but facilities seem to be quite adequate on observation.

Conclusions: If we increase knowledge by training and inservice education, the practice can also be improved. Adequate facilities in the ward, constant supervision, continuous nursing education and proper strict policies regarding universal precaution can drastically improve the current scenario.

016
Infection 2011

Understaffing, overcrowding, inappropriate nurse-to-ventilated patient ratio and nosocomial infections which parameter reflects deficits best?

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Introduction: Intensive care units (ICU) are known to have a high risk for the transmission of methicillin-resistant staphylococcus aureus (MRSA). In all Charité university hospital ICUs, MRSA surveillance has been implemented as a means to assess MRSA colonization rates and to control MRSA transmission. Among the 16 Charité ICUs, a nephrology unit was found to have a particularly high MRSA transmission rate of 5.5/1,000 patient days in 2009.

Objectives: We investigated the impact of multiple interventions to reduce MRSA transmission in this nephrology ICU.

Methods: MRSA was defined as ICU-acquired when isolated after the third ICU day in patients initially screened negative for MRSA on admission. Phase 1 (starting January 2010): Daily washings with 0.1% chlorhexidine were established for all ICU-patients regardless of MRSA status. In addition, mandatory training on MRSA transmission for ICU physicians and nursing staff (n = 81) was carried out between January and March 2010. Phase 2 (starting July 2010): weekly joint rounds of ICU physicians, head nurses, microbiologists and infection control specialists were implemented with systematic analysis of device use, MRSA decolonisation practices and antibiotic therapy.
Results: In 2009, 24 patients acquired MRSA colonisation of infection in the ICU, corresponding to the high MRSA transmission rate of 5.5/1,000 patient days. Following the implementation of phase 1 measures, there were another eight MRSA transmissions within 6 months. After establishing the additional phase two interventions, only two MRSA transmissions were observed. Overall, MRSA transmission rate declined considerably to 1.8/1,000 patient days in 2010.

Conclusions: Systematic interventions lead to a successful reduction of MRSA transmission rates in the Charité ICU. The additional decline of MRSA transmission observed during phase 2 demonstrates that a bundle consisting of several different approaches is more promising than single measures like chlorhexidine washings alone.

Objective: To evaluate the effect of the multimodal intervention on resource use and hospital costs (COST-ALERTS; health-economic evaluation study); (3) To identify new candidate markers and biologic signatures of the innate immune system that may help to improve the early detection of HAIRelated sepsis (LAB-ALERTS; nested laboratory-based case–control study); (4) To develop and validate an innovative clinical prediction tool to identify patients at high risk of HAIRelated sepsis by linking clinical, immunologic and microbiological determinants and biomarkers (RISK-ALERTS; nested case–control study).

Methods: So far, the incidence density of HAIIs i.e. Central Line-Associated Blood Stream Infections (CLABSI), Surgical Site Infections (SSIIs), Catheter-Associated Urinary Tract Infections (CAUTIs) and Ventilator-Associated Pneumonia (VAP)] and its different systemic conditions (i.e. localized infections without a systemic response to infection, sepsis, severe sepsis and septic shock) has not been systematically studied in a hospital wide setting. In a first step we will therefore perform a surveillance study in 26 wards and 4 intensive care units of the Jena University Hospital (JUH) over a 12-month period in order to document the incidence for HAIIs. Beginning in June 2011 a hospital-wide computerized screening tool for HAIIs was implemented at the JUH. This screening tool is based on the daily information whether an antimicrobial therapy was initiated or changed in a patient with a hospital stay >48 h. If so, the ALERTS Study-Team visits the patient and evaluates whether an HAI is present. After this first study period a pragmatic, multimodal infection control programme aimed at a bundle of measures for the prevention of HAIIs will be implemented hospital-wide. This programme focuses on the prevention of the four most prevalent HAIIs, namely CLABSI, SSI, CAUTI and VAP. A second focus will be on education of proper hand hygiene, according to the WHO programme Clean care is safer care. Following this 6-months intervention period, a 12 months surveillance period will be performed to evaluate the effectiveness of the intervention. Data from our Hospital Information System show that each year 32,000 patients 25,000 of whom with an hospital stay of >48 h are treated. Thus 25,000 patients will be at risk for developing HAIIs.

Conclusions: Our ultimate aim is to serve as a powerful instrument of change toward safer, more efficient, and effective delivery of care within and outside Germany. Better patient outcome, through prevention of HAI-related sepsis, is an eminently reachable goal.

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